Overview information for

Benzene

BENZENE REFERENCES					
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TOXICOLOGICAL PROFILE FOR BENZENE

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

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DISCLAIMER

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

UPDATE STATEMENT

A Toxicological Profile for Benzene, Draft for Public Comment was released in August 2005. This edition supersedes any previously released draft or final profile.

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

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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

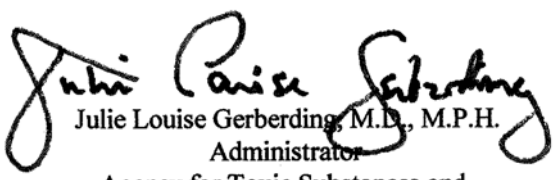
(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

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 is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.



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The 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 December 7, 2005 (70 FR 72840). 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); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999 (64 FR 56792); October 25, 2001 (66 FR 54014); and November 7, 2003 (68 FR 63098). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

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

Primary Chapters/Sections of Interest

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

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

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

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

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

Other Sections of Interest:

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

ATSDR Information Center

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

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

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

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

Other Agencies and Organizations

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

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

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

Referrals

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

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

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

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

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

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

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

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PEER REVIEW

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

1. Dr. Jeffrey Fisher, Professor and Department Head, Department of Environmental Health Science, University of Georgia, Athens, Georgia;

2. Dr. Tee Guidotti, Chair and Professor, Department of Occupational and Environmental Health, School of Public Health and Sciences, The George Washington University Medical Center, Washington, DC; and

3. Dr. Rogene Henderson, Senior Scientist, Lovelace Respiratory Research Institute, Albuquerque, New Mexico.

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

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

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

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about benzene and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Benzene has been found in at least 1,000 of the 1,684 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which benzene is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

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

If you are exposed to benzene, many factors will determine whether you will 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 any other chemicals you are 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 approximately 60 parts of benzene per million parts of air (ppm) and recognize it as benzene at 100 ppm. Most people can begin to taste benzene in water at 0.5–4.5 ppm. One part per million is approximately equal to one drop in 40 gallons. Benzene is found in air, water, and soil. Benzene comes from both industrial and natural sources.

Industrial Sources and Uses. Benzene was first discovered and isolated from coal tar in the 1800s. Today, benzene is made mostly from petroleum. 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 in the manufacturing of some types of rubbers, lubricants, dyes, detergents, drugs, and pesticides.

Natural Sources. Natural sources of benzene, which include gas emissions from volcanoes and forest fires, also contribute to the presence of benzene in the environment. Benzene is also present in crude oil and gasoline and cigarette smoke. For more information on characteristics and uses of benzene, see Chapters 4 and 5.

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 be elevated by emissions from burning coal and oil, benzene waste and storage operations, motor vehicle exhaust, and evaporation from gasoline service stations. Tobacco smoke is another source of benzene in air, particularly indoors. Industrial discharge, disposal of products containing benzene, and gasoline leaks from underground storage tanks 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 also be deposited on the ground by rain or snow.

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 5 and 6.

1.3 HOW MIGHT I BE EXPOSED TO BENZENE?

Everyone is exposed to a small amount of benzene every day. You are exposed to benzene in the outdoor environment, in the workplace, and in the home. Exposure of the general population to benzene mainly occurs 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 national exposure to benzene. About half of the exposure to benzene in the United States 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 amount is about 10 times the average daily intake of benzene by nonsmokers.

Measured levels of benzene in outdoor air have ranged from 0.02 to 34 parts of benzene per billion parts of air (ppb) (1 ppb is 1,000 times less than 1 ppm). 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 may be exposed to higher levels of benzene in air by living near hazardous waste sites, petroleum refining operations, petrochemical manufacturing sites, or gas stations.

For most people, the level of exposure to benzene through food, beverages, or drinking water is not as high as through air. Drinking water typically 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 that contain 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 include coke oven workers in the steel industry, 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 6.

1.4 HOW CAN BENZENE ENTER AND LEAVE MY BODY?

Benzene can enter your body through your lungs, gastrointestinal tract, and across your skin. When you are exposed to high levels of benzene in air, about half of the benzene you breathe in passes through the lining of your lungs and enters your bloodstream. When you are exposed to benzene in food or drink, most of the benzene you take in by mouth passes through the lining of your gastrointestinal tract and enters your bloodstream. 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 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 3.

1.5 HOW CAN BENZENE AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory

animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

After exposure to benzene, several factors determine whether harmful health effects will occur, as well as the type and severity of such health effects. These factors include the amount of benzene to which you are exposed and the length of time of the exposure. Most information on 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 (10,000–20,000 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 are no longer 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.

Long-term exposure to benzene 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). The Department of Health and Human Services has determined that benzene is a known carcinogen (can cause cancer). Both the International Agency for Cancer Research and the EPA have determined that benzene is carcinogenic to humans.

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.

We do not know what human health effects might occur after long-term exposure to food and water contaminated with benzene. In animals, exposure to food or water contaminated with benzene can damage the blood and the immune system and can cause cancer. See Chapters 2 and 3 for more information on the health effects resulting from benzene exposure.

1.6 HOW CAN BENZENE AFFECT CHILDREN?

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

Children can be affected by benzene exposure in the same ways as adults. Benzene can pass from the mother's blood to a fetus. It is not known if children are more susceptible to benzene poisoning than adults.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO BENZENE?

If your doctor finds that you have been exposed to substantial amounts of benzene, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Gasoline and cigarette smoke are two main sources of human exposure to benzene. Benzene exposure can be reduced by limiting contact with these sources. People are exposed to benzene from both active and passive second-hand smoke. Average smokers take in about 10 times more benzene than nonsmokers each day. Families are encouraged not to smoke in their house, in enclosed environments, or near their children.

Benzene is a major component of gasoline and used in many manufacturing processes. Increased levels of benzene can be found at fueling stations, and in air emissions from manufacturing plants and hazardous waste sites. Living near gasoline fueling stations or hazardous waste sites may increase exposure to benzene. People are advised not to have their families play near fueling stations, manufacturing plants, or hazardous waste sites.

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

Several tests can show whether 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, because benzene rapidly disappears in the blood, measurements may be useful 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*-phenylmercapturic acid 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, because phenol is present in the urine from other sources (diet, environment). Measurements of muconic acid or *S*-phenylmercapturic acid in the urine are more sensitive and reliable indicators 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. Blood counts of all components of the blood and examination of bone marrow are used to determine 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 3 and 7.

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

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

Regulations and recommendations can be expressed as “not-to-exceed” levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans.

Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

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

EPA has set 5 ppb as the maximum permissible level of benzene in drinking water. EPA has set a goal of 0 ppb for benzene in drinking water and in water such as rivers and lakes because benzene can cause leukemia. EPA estimates that 10 ppb benzene in drinking water that is consumed regularly or exposure to 0.4 ppb in air over a lifetime could cause a risk of one additional cancer case for every 100,000 exposed persons. EPA recommends 200 ppb as the maximum permissible level of benzene in water for short-term 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.

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 ppm. Because benzene can cause cancer, NIOSH recommends that all workers wear special breathing equipment when they are likely to be exposed to benzene at levels exceeding the recommended (8-hour) exposure limit of 0.1 ppm. For more information on federal regulations, see Chapter 8.

1.10 WHERE CAN I GET MORE INFORMATION?

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

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

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

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

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

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

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO BENZENE IN THE UNITED STATES

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. Smoking has been identified as the single most important source of benzene exposure for the estimated 40 million U.S. smokers. Smoking accounts for approximately half of the total benzene exposure of the general population. Individuals employed in industries that make or use benzene, or products containing benzene, are probably exposed to the highest concentrations of atmospheric benzene. In addition, benzene is a common combustion product, providing high inhalation exposure potential for firefighters. Of the general population, those residing around certain chemical manufacturing sites or living near waste sites containing benzene or near leaking gasoline tanks 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.

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. 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. Showering and bathing with benzene-contaminated water 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. No causal relationship has been proposed for health conditions identified in the base subregistry or continued follow-up of the population.

2.2 SUMMARY OF HEALTH EFFECTS

The carcinogenicity of benzene is well documented in exposed workers. Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute myelogenous leukemia (AML). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power, but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor.

In vivo and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic. Chromosomal aberrations (hypo- and hyperdiploidy, deletions, breaks, and gaps) in peripheral lymphocytes and bone marrow cells are the predominant effects seen in humans.

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. Animal data support these findings.

The most characteristic systemic effect resulting from intermediate and chronic benzene exposure is arrested development of blood cells. Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. 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 and in animals. Benzene-associated cytopenias vary and may involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood.

Benzene also causes a life-threatening disorder called aplastic anemia in humans and animals. This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow, leading to fibrosis, an irreversible replacement of bone marrow. Benzene has also been associated with acute non-lymphocytic leukemia in humans, and aplastic anemia may be an early indicator of developing acute non-lymphocytic leukemia in some cases.

Limited information is available on other systemic effects reported in humans and is associated with high–level benzene exposure. Respiratory effects have been noted after acute exposure of humans to benzene vapors. Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the

cause of death in fatal exposures to benzene vapor. Gastrointestinal effects have been noted in humans after fatal inhalation exposure (congestive gastritis), and ingestion (toxic gastritis and pyloric stenosis), of benzene. Myelofibrosis (a form of aplastic anemia) was reported by a gasoline station attendant who had been exposed to benzene by inhalation, and probably also through dermal contact. Myalgia was also reported in steel plant workers exposed to benzene vapors. Reports of renal effects in humans after benzene exposure consist of kidney congestion after fatal inhalation exposure. Dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapors. Swelling and edema have been reported to occur in a human who swallowed benzene. Studies in animals show systemic effects after inhalation exposure, including cardiovascular effects. Oral administration of benzene to animals has yielded information concerning hepatic effects. 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.

Neurological effects have been commonly reported in humans following high-level exposure to benzene. Fatal inhalation exposure has been associated with vascular congestion in the brain. Chronic inhalation exposure has been associated with distal neuropathy, difficulty in sleeping, and memory loss. Oral exposure results in symptoms similar to inhalation exposure. Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain, loss of involuntary reflexes and narcosis, decrease in hind-limb grip strength and tremors, and narcosis, among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behavior. No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals.

Acute inhalation and oral exposures of humans to high concentrations of benzene have caused death. These exposures are also associated with central nervous system depression. Chronic low-level exposures have been associated with peripheral nervous system effects. Abnormalities in motor conduction velocity were noted in four of six pancytopenic individuals occupationally exposed to adhesives containing benzene.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association. Some animal studies provide limited evidence that benzene affects reproductive organs following inhalation exposure. 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. Results are conflicting or inconclusive as to whether

inhalation of benzene vapors reduces the number of live fetuses and/or the incidences of pregnancy. Other studies are negative for effects on reproductive competence.

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. Results of inhalation studies conducted in animals are fairly consistent across species and demonstrate that, at levels >47 ppm, benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. Benzene has also been shown to reduce pup body weight in mice. A persistent decrease in the number of erythroid precursors was found in mice exposed *in utero*. Benzene has not been shown to be teratogenic, but has been shown to be fetotoxic in animals at high concentrations that are maternally toxic.

Cancer. The strongest evidence for the leukemogenic potential of benzene comes from series of cohort mortality studies on workers exposed to benzene in Ohio (the Pliofilm study) and China (the NCI/CAPM study). The Pliofilm study investigated workers exposed to benzene in three rubber hydrochloride ('Pliofilm') manufacturing plants. Mortality from all leukemias was increased but declined after additional years of follow-up, suggesting that the excess risk diminished with time since exposure. Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously. AML accounted for most of the increased leukemia, and the risk of AML increased with increasing cumulative exposure above 200 ppm-years.

The NCI/CAPM study, a collaboration between the National Cancer Institute and the Chinese Academy of Preventive Medicine, evaluated lymphohematopoietic malignancies and other hematologic disorders in 74,828 benzene-exposed workers employed in 672 factories in 12 cities in China. Findings included increased risks for all leukemias, acute nonlymphocytic leukemia (ANLL), and combined ANLL and precursor myelodysplastic syndromes. These risks were increased at average exposure levels of 10–24 ppm and cumulative exposure levels of 40–99 ppm-years, and tended to increase with increasing average and cumulative levels of exposure.

The results of the Pliofilm and NCI/CAPM studies are consistent with epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent. No significant increases in leukemia or other lymphohematopoietic malignancies were found in chemical industry workers or petroleum industry workers exposed to lower levels of benzene.

Possible associations between occupational exposure to benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma have been suggested. The risk for mortality from NHL increased with increasing level and duration of benzene exposure the NCI/CAPM study. The significance of this finding is unclear because NHL mortality was not significantly elevated in the cohort overall, concerns regarding the adequacy of the data have been raised, and increases in NHL were not found in other cohort mortality studies or in case-control studies of benzene-exposed workers.

The risk of mortality from multiple myeloma was increased in one of the early assessments of the Pliofilm cohort. The implication of this finding is unclear because the risk declined to non-significant levels in subsequent follow-up studies, and was not supported by the findings of other cohort mortality studies. Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of multiple myeloma. A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene-containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents, petroleum, or petroleum products.

Animal studies provide supporting evidence for the carcinogenicity of benzene. Benzene has been shown to be a multiple site carcinogen in rats and mice following inhalation and oral exposure. Tumors that were increased in rats that were exposed to 200 or 300 ppm benzene by inhalation for 4–7 hours/day, 5 days/week for up to 104 weeks included carcinomas of the Zymbal gland and oral cavity. Mice that were exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life developed a variety of tumors, including thymic lymphomas, myelogenous leukemias, and Zymbal gland, ovarian, and lung tumors.

In oral bioassays conducted by the National Toxicology Program, benzene was administered to rats and mice by gavage at dose levels of 25–200 mg/kg/day on 5 days/week for 103 weeks. Tumors that were induced in the rats included Zymbal gland carcinomas and squamous cell papillomas and carcinomas of the oral cavity and skin. In the mice, benzene caused tumors that included malignant lymphomas, Zymbal gland carcinomas, lung alveolar/bronchiolar adenomas and carcinomas, Harderian gland adenomas, preputial gland squamous cell carcinomas, and mammary gland carcinomas. Similar effects occurred in rats exposed to 50–500 mg/kg/day benzene by gavage on 4–5 days/week for up to 104 weeks and observed for life; induced tumors included carcinomas of the Zymbal gland, oral cavity, forestomach,

nasal cavity, and skin. Mice that were similarly exposed to 500 mg/kg/day for 52 or 78 weeks developed Zymbal gland carcinomas, mammary carcinomas, and lung adenomas.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most of the dermal studies were inadequate for cancer evaluation. Many dermal carcinogenicity studies of other chemicals used benzene as a vehicle and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. Two studies classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies. Based on human leukemia data, EPA derived a range of inhalation unit risk values of $2.2x10^{-6}$–$7.8x10^{-6}$ $(\mu g/m^3)^{-1}$ for benzene. For risks ranging from $1x10^{-4}$ to $1x10^{-7}$, the corresponding air concentrations range from 13.0–45.0 $\mu g/m^3$ (4–14 ppb) to 0.013–0.045 $\mu g/m^3$ (0.004–0.014 ppb), respectively.

The consensus conclusion that benzene is a human carcinogen is based on sufficient inhalation data in humans supported by animal evidence, including the oral studies in animals. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic (myelocytic) leukemia, whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk range. The oral slope factor ranges from $1.5x10^{-2}$ to $5.5x10^{-2}$ $(mg/kg/day)^{-1}$, and for cancer risks from $1x10^{-4}$ to $1x10^{-7}$, the corresponding dose levels are $6.7x10^{-3}$–$1.8x10^{-3}$ to $6.7x10^{-6}$–$1.8x10^{-6}$ mg/kg/day, respectively.

Hematological Effects. Both human and animal studies have shown that benzene exerts toxic effects on various parts of the hematological system. All of 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. 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.

Numerous earlier studies of benzene-exposed workers demonstrated that chronic exposure to benzene air concentrations of 10 ppm or more resulted in adverse hematological effects, which increased in severity with increasing benzene exposure levels. Animal studies support the findings in humans. Significantly reduced counts for all three blood factors (white blood cells [WBCs], red blood cells [RBCs], and platelets); and other evidence of adverse effects on blood-forming units (reduced bone marrow cellularity, bone marrow hyperplasia and hypoplasia, granulocytic hyperplasia, decreased numbers of colony-forming granulopoietic stem cells and erythroid progenitor cells, damaged erythrocytes and erythroblast-forming cells) have been observed in animals at benzene concentrations in the range of 10–300 ppm and above.

Several more recent epidemiological studies have demonstrated hematological effects (including significant reductions in WBC, RBC, and platelet counts) in workers chronically exposed to benzene levels below 10 ppm, and even as low as 1 ppm or less. Results of one of these studies served as the basis for a chronic-duration inhalation MRL for benzene. Other reports demonstrated the lack of clinical signs of hematotoxicity following long-term, low-level occupational exposure to benzene levels below approximately 0.5 ppm (8-hour time-weighted average [TWA]). These investigators utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values considered clinically abnormal or adverse may reduce the sensitivity of a particular study to detect meaningful changes at the population level.

Only one study was found that described hematological effects in humans after oral exposure to benzene. 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.

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.

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 and decreases in levels of circulating antibodies in workers exposed to benzene at 3–7 ppm. Other studies have shown decreases in human lymphocytes and other blood elements after exposure; these effects have been seen at occupational exposure levels as low as 1 ppm or less. Animal data support these findings. Both humans and rats 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, intermediate, or chronic periods 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. 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 10^4 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. Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods, and in rats and mice exposed for chronic time periods. 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. Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours/day for 30 days. The acute-duration inhalation MRL was based on a study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day for 6 days. The intermediate-duration inhalation MRL was based on a study showing delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day, 5 days/week for a total of 20 exposures.

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 who 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 cause-and-effect relationship between acute inhalation of very high concentrations of benzene and symptoms indicative of central nervous system toxicity. 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. Comparable toxicity in humans has been reported following ingestion of benzene at doses of 125 mg/kg and above. 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). 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 hypothalamus-pituitary-adrenal axis. Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive.

Animal studies provide additional support that benzene affects the nervous system following acute inhalation and oral exposures, albeit at extremely high acute exposure levels. Effects reported include narcosis, nervous system depression, tremors, and convulsions. 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. 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. The rats exposed to benzene exhibited a significantly impaired learning ability when tested on problems of the closed-field, maze-learning task. This sign of neurotoxicity 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. 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 days 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. Mice exposed to 3 ppm for 2 hours/day for 30 days exhibited increased levels of acetylcholinesterase in the brain. *In vitro* studies suggest that benzene may have a direct effect on brain cells. 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 lower and not likely to be of concern.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (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 dermal exposure.

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

Inhalation MRLs

- An MRL of 0.009 ppm has been derived for acute-duration inhalation exposure (14 days or less) to benzene.

The acute-duration inhalation MRL of 0.009 ppm was derived from a lowest-observed-adverse-effect level (LOAEL) value of 10.2 ppm for reduced lymphocyte proliferation following mitogen stimulation in mice (Rozen et al. 1984). The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6 hours/24 hours to correct for less than a full day of exposure. The resulting adjusted LOAEL, 2.55 ppm, was then converted to a human equivalent concentration (HEC) according to EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) as follows:

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

where:

$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), blood:gas partition coefficients for benzene in mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{[HEC]}$ is equivalent to the $LOAEL_{[ADJ]}$.

Therefore:

$LOAEL_{HEC} = LOAEL_{ADJ} = 2.55$ ppm

The resulting $LOAEL_{(HEC)}$ of 2.55 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) to yield the MRL value of 0.009 ppm (see Appendix A). An increased number of micronucleated polychromatic erythrocytes (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 hours/day for 6 days. 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 colony-forming granulopoietic stem cells (CFU-E) after exposure to 10 ppm benzene for 6 hours/day for 5 days (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, 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 no-observed-adverse-effect level (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 0.006 ppm has been derived for intermediate-duration inhalation exposure (15–364 days) to benzene.

The intermediate-duration inhalation MRL of 0.006 ppm was derived from a LOAEL value of 10 ppm for significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction following the exposure of male C57Bl/6 mice to benzene vapors 6 hours/day, 5 days/week for 20 exposure days (Rosenthal and Snyder 1987). The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10 ppm) by 6 hours/24 hours to correct for less than a full day of exposure and by 5 days/7days to correct for less than a full week of exposure. The resulting adjusted LOAEL, 1.8 ppm, was then converted to a HEC according to EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) as follows:

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

where:

$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), blood:gas partition coefficients for benzene in mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{[HEC]}$ is equivalent to the $LOAEL_{[ADJ]}$.

Therefore:

$LOAEL_{HEC} = LOAEL_{ADJ} = 1.8$ ppm

The resulting $LOAEL_{(HEC)}$ of 1.8 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) to yield the MRL value of 0.006 ppm (see Appendix A).

Results of several studies support the choice of Rosenthal and Snyder (1987) as the basis for the intermediate-duration inhalation MRL for benzene (Baarson et al. 1984; Green et al. 1981a, 1981b), although the supporting studies employed a single exposure level, which precluded consideration as critical studies for MRL derivation. Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week caused significant depressions in numbers of lymphocytes (ca. 30% lower than controls) as early as exposure day 32; this effect was also noted at the other scheduled periods of testing (exposure days 66 and 178) (Baarson et al. 1984). Splenic RBCs were significantly reduced (ca. 15% lower than controls) at exposure days 66 and 178. The failure of the erythrons of benzene-exposed mice to support normal red cell mass was illustrated by the significant reduction in peripheral red cell numbers in these animals at 66 and 178 days of benzene exposure. Green et al. (1981a, 1981b) exposed male CD-1 mice to benzene vapors at concentrations of 0 or 9.6 ppm for 6 hours/day, 5 days/week for 50 days and assessed the effects of exposure on cellularity in the spleen, bone marrow, and peripheral blood. Exposure-related effects included a 90% increase in numbers of multipotential hematopoietic stem cells (CFU-S) (Green et al. 1981a), approximately 25% increase in spleen weight and total splenic nucleated cellularity (Green et al. 1981b), and 80% increase in nucleated RBCs (Green et al. 1981b).

One intermediate-duration inhalation study reported increased rapid response to an electrical shock in mice at 0.78 ppm (Li et al. 1992). However, this study was not selected as the critical study for deriving an intermediate-duration inhalation MRL for benzene due to apparent discrepancies between reported and actual benzene exposure levels. Other animal studies identified neurological effects only at much higher exposure levels (Carpenter et al. 1944; Dempster et al. 1984; Evans et al. 1981; Frantik et al. 1994; Green et al. 1978).

- An MRL of 0.003 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to benzene.

This MRL is based on statistically significantly decreased counts of B-lymphocytes in workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b), using a benchmark dose (BMD) analysis. The 250 benzene-exposed workers had been employed for an average of 6.1±2.9 years. Controls consisted of 140 age- and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1–<10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term, low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup of the <1-ppm group for which exposure to other solvents was negligible.

As shown in Table A-1 of Appendix A, exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for BMD modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a, 2004b) identified a LOAEL in the absence of a NOAEL.

As discussed in detail in Appendix A, the BMD analysis selected a point of departure ($BMCL_{0.25sd}$) of 0.1 ppm, which was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) using the default occupational minute volume (EPA 1994b). The resulting $BMCL_{0.25sdADJ}$ of 0.03 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the chronic-duration inhalation MRL value of 0.003 ppm.

Several recent epidemiological studies provide supporting information to the Lan et al. (2004a, 2004b) findings of hematotoxicity in workers chronically exposed to relatively low levels of benzene (Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Ward et al. 1996). Qu et al. (2002, 2003a, 2003b) compared hematologic values among 105 healthy workers (51 men, 54 women) in industries with a history of benzene usage (Tianjin, China) and 26 age-and gender-matched workers in industries that did not use benzene. A LOAEL of 2.26 ppm was identified for significantly reduced total WBCs, neutrophils, and RBCs; there was also an indication of benzene-induced changes in some hematological values at exposure levels lower than the current industry 8-hour TWA of 1 ppm. Rothman et al. (1996a, 1996b) identified an 8-hour TWA LOAEL of 7.6 ppm for a group of 11 benzene-exposed workers in a cross-sectional study of 44 healthy workers in Chinese (Shanghai) industries with a history of benzene usage and age- and gender-matched workers in industries that did not use benzene. In a nested case-control study of a cohort of workers in the Pliofilm production departments of a rubber products manufacturer in Ohio (Ward et al. 1996), a strong exposure-response relationship was correlated with low WBC count. A weak positive exposure-response relationship was observed for RBCs, which was significant for cumulative exposure up until the blood test date. The study authors noted that there was no evidence for a threshold for hematologic effects and suggested that exposure to benzene levels <5 ppm may result in hematologic suppression.

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 were ingested. The only lower doses in the acute oral database are 50 mg/kg/day, at which pregnant rats experienced alopecia of hindlimbs and trunk (Exxon 1986), an end point not suitable for MRL derivation for benzene because the toxicological significance is not clear; and 88 mg/kg/day, a concentration resulting in slight non-dose-related central nervous system depression 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) should be considered for derivation of an intermediate-duration oral MRL. Adult male CD-1 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 (leukopenia, erythrocytopenia, lymphopenia, enhanced splenic lymphocyte proliferation) 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.

- An MRL of 0.0005 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to benzene.

No human data are available to evaluate hematological effects following oral exposure to benzene. No adequate oral data were located from which a chronic-duration oral MRL for benzene could be derived, although a 2-year carcinogenesis bioassay of orally-exposed rats and mice is available (NTP 1986). 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 database. Therefore, the threshold for hematological and immunological effects of benzene was not identified.

However, results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. Therefore, the point of departure for the chronic-duration inhalation MRL for benzene, namely the $BMCL_{0.25sdADJ}$ of 0.03 ppm for decreased B cell counts in benzene-exposed workers (Lan et al. 2004a, 2004b), serves as the point of departure for deriving the chronic-duration oral MRL as well.

The point of departure (in ppm) was converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25 °C and 760 mm Hg:

$$BMCL_{0.25sdADJ} \text{ of } 0.03 \text{ ppm} \times 78.11/24.45 = 0.096 \text{ mg/m}^3$$

The $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 for inhaled benzene was converted to an equivalent $BMDL_{0.25sdADJ}$ for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 versus 100%, respectively) as follows:

$$BMDL_{0.25sdADJ} = BMCL_{0.25sdADJ} \text{ of } 0.096 \text{ mg/m}^3 \times 20 \text{ m}^3\text{/day} \times 0.5 \div 70 \text{ kg} = 0.014 \text{ mg/kg/day}$$

A total uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation) was applied to the $BMDL_{0.25sdADJ}$ of 0.014 mg/kg/day; the resulting chronic-duration oral MRL is 0.0005 mg/kg/day.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 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.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

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

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

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

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

3.2.1 Inhalation Exposure

Although occupational or environmental exposure to benzene or benzene-containing materials may include inhalation, oral, and dermal exposure routes, the inhalation and dermal routes are usually of primary concern in such scenarios. Data regarding occupational or environmental exposure in which inhalation is considered to have been the primary exposure route are summarized in this section (Section 3.2.1). Information regarding adverse health effects following oral or dermal exposure to benzene or benzene-containing materials is summarized in Sections 3.2.2 and 3.2.3, respectively.

3.2.1.1 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). No studies were located regarding noncancer-related mortality in humans following long-term inhalation exposure to benzene. Cancer-related mortality data for chronic-duration human occupational exposure to benzene are presented in Section 3.2.1.7.

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-1 mice exposed by inhalation to doses of benzene up to 4,862 ppm, 6 hours/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/day, 5 days/week for 50 days) of male CD-1 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/day, 5 days/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/week, 6 hours/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 3-1 and plotted in Figure 3-1.

3.2.1.2 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 3-1 and plotted in Figure 3-1.

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 Collom 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 >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 Collom 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/week, 6 hours/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).

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	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	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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	
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 F	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)			

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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 wk 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 and leukocytes)	Cronkite et al. 1982	
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 (C57BL/6BNL)	8 d 6 hr/d	Hemato		3000 F (decreased marrow cellularity)		Cronkite et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
20	Mouse (DBA/2J)	5 d 6 hr/d	Hemato		10 M (50% decrease in CFU-E numbers)		Dempster and Snyder 1991	
21	Mouse (C57B1/6)	2-8 d 24 hr/d	Hemato			100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	
22	Mouse (CD-1)	5 d 6 hr/d	Hemato	9.9 M		103 M (decreased marrow cellularity; granulocytopenia, lymphocytopenia; decreased polymorphonucleucytes)	Green et al. 1981b	
			Bd Wt	4862 M				
23	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	
24	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d	Hemato	300 F	900 F (CFU-E depression)		Plappert et al. 1994a	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
25	Mouse (C57Bl/6J)	6 d 6 hr/d	Hemato		10.2 M (depressed lymphocyte counts; elevated RBCs)		Rozen et al. 1984	
26	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	
27	Mouse (NMRI)	1 wk	Hemato	14 M			Toft et al. 1982	
28	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	
29	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				

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
30	Mouse (Swiss-Webster)	5 d 6 hr/d	Hemato	3 M	25 M (decrease in WBC count)		Wells and Nerland 1991	
31	Rabbit	Gd 7-20 24 hr/d	Bd Wt	156.5 F	313 F (reduced maternal weight gain)		Ungvary and Tatrai 1985	
Immuno/ Lymphoret								
32	Rat (Wistar)	7 d 8 hr/d		50 F	100 F (leukopenia; increased leukocyte alkaline phosphatase)		Li et al. 1986	
33	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d		200 M	400 M (29% reduction in total splenic cells, 28% lower thymus weight)		Robinson et al. 1997	
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 and thymus weights and WBC count)		Aoyama 1986	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
36	Mouse (DBA/2)	2 wk 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	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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. 1981a	
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			10.2[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	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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; bone marrow hypoplasia; histopathological lesions in spleen, thymus, selected lymph nodes)	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	
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	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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 and blink reflex; pupillary contraction and 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	
60	Mouse (CF-1)	Gd 6-15 7 hr/d		500 F			Murray et al. 1979	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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 F	100 F (decreased fetal weight)		Coate et al. 1984	
64	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d			100 F (increased incidence of missing sternebrae)		Green et al. 1978	
65	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d		10 F	50 F (decreased fetal weight)		Kuna and Kapp 1981	
66	Rat (CFY)	Gd 7-14 24 hr/d			125 F (decreased mean fetal weight; increased fetal weight retardation; skeletal retardation; 17% decrease in mean placental weight)		Tatrai et al. 1980a	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
67	Rat (CFY)	Gd 7-14 24 hr/d			47 F (decreased fetal weight; signs of skeletal retardation)	141 F (significant increase in fetal mortality)	Tatrai et al. 1980b	
68	Mouse (Swiss-Webster)	Gd 6-15 6 hr/d		10 F	20 F (decreased circulating erythroid precursors, elevation of granulocytic precourser cells in neonates and 6-week old offspring)		Keller and Snyder 1988	
69	Mouse (CF-1)	Gd 6-15 7 hr/d			500 F (decreased fetal body weight; delayed skeletal ossifications)		Murray et al. 1979	
70	Mouse (CFLP)	Gd 6-15 12 hr/d			156.5 F (decreased fetal body weight and skeletal retardation)		Ungvary and Tatrai 1985	
71	Rabbit (New Zealand)	Gd 6-18 7 hr/d			500 F (increased minor skeletal variants)		Murray et al. 1979	
72	Rabbit (New Zealand)	Gd 7-20 24 hr/d		156.5 F	313 F (decreased fetal weight; increased minor anomalies)		Ungvary and Tatrai 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Death								
73	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (death)	Maltoni et al. 1983, 1985	
74	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (97% mortality)	Cronkite 1986	
75	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (deaths in males during exposure; deaths in females shortly after exposure)	Cronkite et al. 1989	
76	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (11/125 died during first 9 months after initiation of exposure)	Farris et al. 1993	
77	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (50% mortality)	Green et al. 1981b	
Systemic								
78	Human	4 mo- 1 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	
79	Human	4 mo -1 yr (occup)	Hemato			210 (pancytopenia, hypocellular to hypercellular bone marrow)	Aksoy et al. 1972	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
80	Human	1 yr (occup)	Hemato		40 (decrease in WBC counts in first 4 months)		Cody et al. 1993	
81	Human	3.5 mo- 19 yr (occup)	Hemato			29 (aplastic anemia)	Yin et al. 1987c	
82	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d	Hemato			500 (decreased WBC and lymphocytes; increased RBC and 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	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
86	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed peripheral red blood cells, CFU-E)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
87	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed splenic red cells)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
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 (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	
91	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato		300 M (granulocytic hyperplasia in bone marrow)		Farris et al. 1993	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
92	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d	Hemato	10 M	100 M (decreased numbers of differentiating and maturing hematopoietic bone marrow cells)		Farris et al. 1997a	
93	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d	Hemato			302 M (decreased WBC, RBC; altered RBC morphology)	Green et al. 1981b	
			Bd Wt	302 M				
94	Mouse (CD-1)	50 d 5 d/wk 6 hr/d	Hemato	9.6 M			Green et al. 1981b	
			Bd Wt	9.6 M				
95	Mouse (Kunming)	30 d 6 d/wk 2 hr/d	Hepatic	12.52 M			Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
			Renal	12.52 M				
			Bd Wt	12.52 M				
96	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	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
97	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d	Hemato	100 F	300 F (slight anemia; BFU-E and CFU-E depression in bone marrow)		Plappert et al. 1994a	
98	Mouse (Hybrid)	8 wk 5 d/wk 6 hr/d	Hemato			300 F (decreased Hgb, Hct, erythrocyte counts)	Plappert et al. 1994b	
99	Mouse (BDF1)	8 wk 5 d/wk 6 hr/d	Hemato		100 F (BFU-E and CFU-E depression)		Seidel et al. 1989b	
100	Mouse (NMRI)	8 wk	Hemato	14 M			Toft et al. 1982	
101	Mouse (Hybrid)	6 or 7 wk 5 d/wk 6 h/d	Hemato			300 F (decreased CFU-C, BFU-E and CFU-E)	Vacha et al. 1990	
102	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				
103	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d	Hemato		88 (leukopenia)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
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, and increased erythroid cells)			Dow 1992	
Immuno/ Lymphoret										
106	Human	4 mo- 1 yr (occup)					210	(pancytopenia, hypoplastic to hyperplastic bone marrow, enlarged spleen)	Aksoy et al. 1972	
107	Human	1 yr (occup)			40	(decreased lymphocytes)			Cody et al. 1993	
108	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d					500	(decreased myeloid and lymphoid cells)	Dow 1992	
109	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		200 M	400 M	(29% reduction in total splenic cells, 28% lower thymus weight)			Robinson et al. 1997	
110	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		30	300	(leukopenia and lymphopenia)			Ward et al. 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
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	The use of one dose precludes a dose-response assessment.
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 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
116	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d			300 M (granulocytic hyperplasia)		Farris et al. 1993	
117	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced numbers of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, peripheral blood leukocytes and RBCs)		Farris et al. 1997a	
118	Mouse (B6C3F1)	8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced lymphocyte and total nucleated cell counts)		Farris et al. 1997b	
119	Mouse (C57B1/6)	6 wk 5 d/wk 6 hr/d				1000 (leukopenia, granulocytopenia, lymphocytopenia)	Gill et al. 1980	
120	Mouse (CD-1)	50 d 6 hr/d 5 d/wk			9.6 M (increased splenic CFU-S)		Green et al. 1981a	The use of one exposure level precludes usefulness for dose-response assessment.

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
121	Mouse (CD-1)	26 wk 6 hr/d 5 d/wk				302 M (reduced marrow and spleen cellularity; decreased spleen weight)	Green et al. 1981a	
122	Mouse (CD-1)	50 d 5 d/wk 6 hr/d			9.6 M (increased spleen weight, total splenic nucleated cellularity and NRBC)		Green et al. 1981b	The use of one exposure level precludes usefulness for dose-response assessment.
123	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (lymphocytopenia, anemia, decreased spleen weight, decreased spleen and marrow cellularities)	Green et al. 1981b	
124	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	Exposures conducted under static conditions; measured only on first 3 of 30 days.

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
125	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d		100 F	300 F (increased T4/T8 ratio)		Plappert et al. 1994a	
126	Mouse (C57B1/6)	20 d 5 d/wk 6 hr/d			10[c] M (delayed splenic lymphocyte reaction to foreign antigens evaluated in in vitro mixed lymphocyte reaction)		Rosenthal and Snyder 1987	
127	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	
128	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	
129	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (leukocyte & lymphocyte depression; bone marrow hypoplasia; histopathologic lesions in spleen and selected lymph nodes)	Ward et al. 1985	
130	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
131	Rabbit (NS)	243 d 5 d/wk 7 hr/d			80 (leukopenia)		Wolf et al. 1956	
132	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	
Neurological								
133	Rat (Wistar)	3 wk 3-4 x 4 hr				929 M (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
134	Mouse (H)	3 wk 3-4 x 2 hr				856 F (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
135	Mouse (Kunming)	30 d 6 d/wk 2 hr/d			0.78 M (increased rapid response)		Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
Reproductive								
136	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d		300 F			Kuna et al. 1992	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
137	Rat (Wistar)	93 d 5 d/wk 7-8 hr/d			6600 M (testicular weight increase)		Wolf et al. 1956	
138	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	
139	Gn Pig (NS)	32 or 269 d 5 d/wk 7-8 hr/d			88 M (testicular weight increase)		Wolf et al. 1956	
140	Rabbit (NS)	243 d 5 d/wk 7-8 hr/d			80 M (degeneration of germinal epithelium in testes)		Wolf et al. 1956	
Cancer								
141	Human	3.5 mo- 19 yr (occup)				29 (CEL: humanlymphocytic leukemia)	Yin et al. 1987c	
142	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a, 1983, 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
143	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	
144	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				100 M (CEL: leukemia)	Cronkite 1986	
145	Mouse (C57BL/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300 (CEL: thymic and non-thymic lymphoma)	Cronkite et al. 1984, 1985	
146	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung)	Cronkite et al. 1989	
147	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				100 M (CEL: hepatomata, lymphomatous and myelogenous neoplasms)	Cronkite et al. 1989	
148	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (CEL: lymphoma in 12%)	Farris et al. 1993	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
149	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								
150	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (61% died, versus 46% in controls)	Maltoni et al. 1982a	
151	Rat (Sprague-Dawley)	691 d 5 d/wk 6 hr/d				300 M (median lifespan 51 weeks versus 65 weeks in controls)	Snyder et al. 1978a	
152	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d				300 M (median lifespan 11-41 weeks versus 39-75 weeks in controls)	Snyder et al. 1978a, 1980	
153	Mouse (CD-1)	222 d 5 d/wk 6 hr/d				300 M (decreased survival)	Snyder et al. 1982	
Systemic								
154	Human	4 mo- 15 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
155	Human	14 yr (Occup)	Hemato	0.55			Collins et al. 1997	The NOAEL is for abnormal hematological values, not significantly altered values that would still fall within the range of normal values.
156	Human	1-3 yr (occup)	Hemato			3 (anemia, lymphocytosis, thrombocytopenia, leukopenia, leukocytosis)	Doskin 1971	
157	Human	NS (occup)	Hemato			24 (pancytopenia, hypoplastic bone marrow)	Erf and Rhoads 1939	
158	Human	3-29 yr (occup)	Hemato		25 M (increased mean corpuscular volume)		Fishbeck et al. 1978	
159	Human	0.5-5 yr (occup)	Hemato			11 (anemia, macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
160	Human	1-25 yr (occup)	Hemato	20		75 (anemia and leukopenia)	Kipen et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
161	Human	6.1 yr (avg) (Occup)	Hemato		0.57[d] (reduced WBC and platelet counts, approximately 7-18% lower than control values)		Lan et al. 2004a	
162	Human	4.5-9.7 yr (mean duration)	Hemato		2.26 (reduced neutrophils and RBC counts, approximately 12% lower than controls)		Qu et al. 2002, 2003	
163	Human	6.3 yr (avg) (Occup)	Hemato		7.6 (reduced absolute lymphocyte count, approximately 16% lower than controls)		Rothman et al. 1996a, 1996b	
164	Human	1-21 yr (occup)	Hemato	0.53 M			Tsai et al. 1983	
165	Human	>1 yr (occup)	Hemato		0.69 (leukopenia)		Xia et al. 1995	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
166	Human	>1 yr (occup)	Resp		33[e] M (sore throat; nasal irritation)		Yin et al. 1987b	
					59 F (sore throat; nasal irritation)			
			Hemato	33[e] M				
				59 F				
			Renal	33[e] M				
				59 F				
			Ocular		33[e] M (eye irritation)			
					59 F (eye irritation)			
167	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)			

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
168	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)		
169	Mouse (CD-1)	222 d 5 d/wk 6 hr/d	Hemato		300 M (decreased RBCs and lymphocytes)		Snyder et al. 1982	
			Bd Wt		300 M (reduced body weight gain)			
Immuno/ Lymphoret								
170	Human	3-5 yr (occup)				11 (macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
171	Human	1-25 yr (occup)		20	75 (leukopenia)		Kipen et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
172	Human	>1 yr (occup)			0.69 (leukopenia)		Xia et al. 1995	
173	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d			100 M (decreased lymphocyte counts, splenic hyperplasia)		Snyder et al. 1978a, 1984	
174	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d			100 M (lymphocytopenia, bone marrow hypoplasia)		Snyder et al. 1978a, 1980	
175	Mouse (C57BL, CD-1)	lifetime 6 hr/d 5 d/wk			300 M (lymphopenia)		Snyder et al. 1988	
Cancer								
176	Human	4-15 yr (occup)				150 (CEL: leukemia)	Aksoy and Erdem 1978	
177	Human	28 mo- 40 yr				1 (CEL: leukemia, lymphoma)	Aksoy et al. 1987	
178	Human	1-10 yr (occup) (occup)				10 M (CEL: leukemia)	Infante 1978; Infante 1977	
179	Human	18 mo (occup)				0.3 M (CEL: leukemia)	Ott et al. 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
180	Human	1-14 yr (occup)				16 M (CEL: leukemia)	Rinsky et al. 1981; Infante et al. 1977a	
181	Human	1-30 yr (occup)				200 (CEL: leukemia)	Vigliani and Forni 1976	
182	Human	>1 yr (occup)				2 (CEL: chronic erythroid leukemia)	Yin et al. 1989	
183	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a	
184	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1983, 1985	
185	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d				100 M (CEL: Zymbal gland carcinoma, myelogenous leukemia, liver tumors)	Snyder et al. 1984	
186	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 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
187	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 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.009 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

c Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.006 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and 5 days/7 days and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

d Study results used to derive a chronic-duration inhalation minimal risk level (MRL) of 0.003 ppm for benzene, as described in detail in Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure and divided by an uncertainty factor of 10 for human variability. Study results also used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts in benzene-exposed workers to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

e Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

AChE = acetylcholinesterase; Bd Wt = body weight; BFU-E = burst-forming units - erythroid; 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; CFU-S = colony-forming units - spleen; CNS = central nervous system; d = day(s); F = female; Gd = gestational day; Hct = hematocrit; Hemato = hematological; Hgb = hemoglobin; hr = hour(s); LC50 = 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; NRBC = nucleated red blood cells; NS = not specified; occup = occupational exposure; RBC = red blood cell; WBC = white blood cell; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation

Acute (≤14 days)

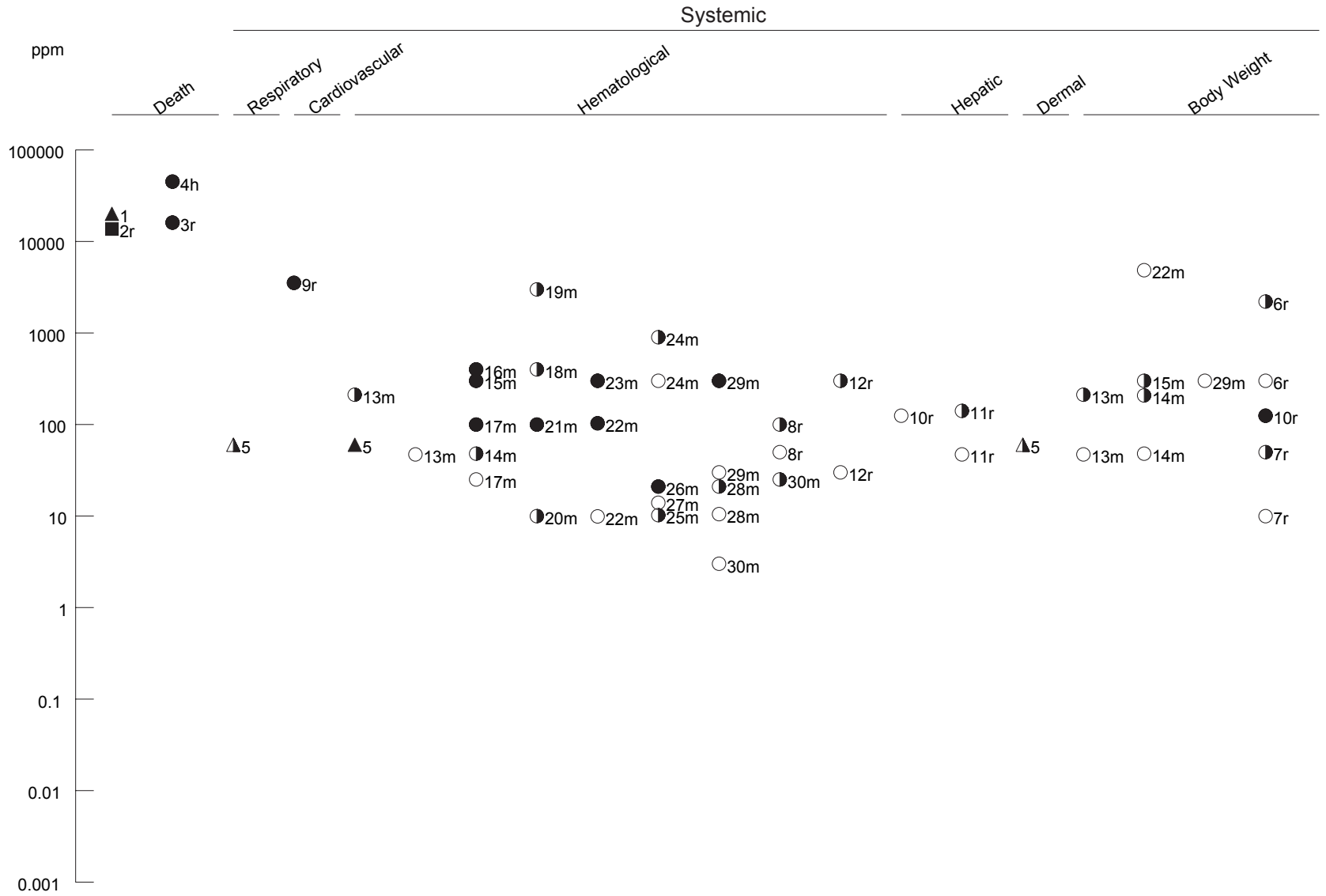


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Acute (≤14 days)

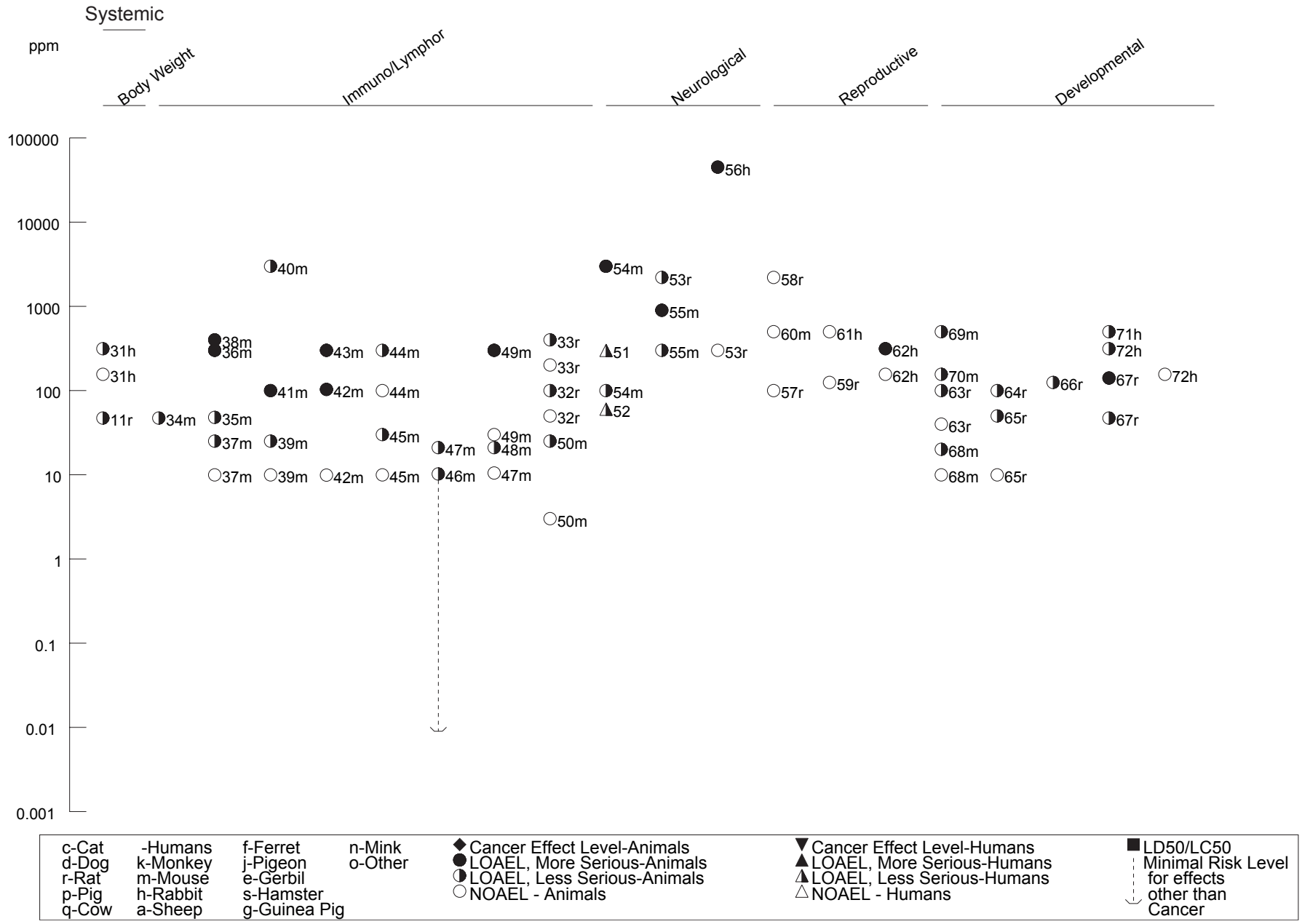


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)

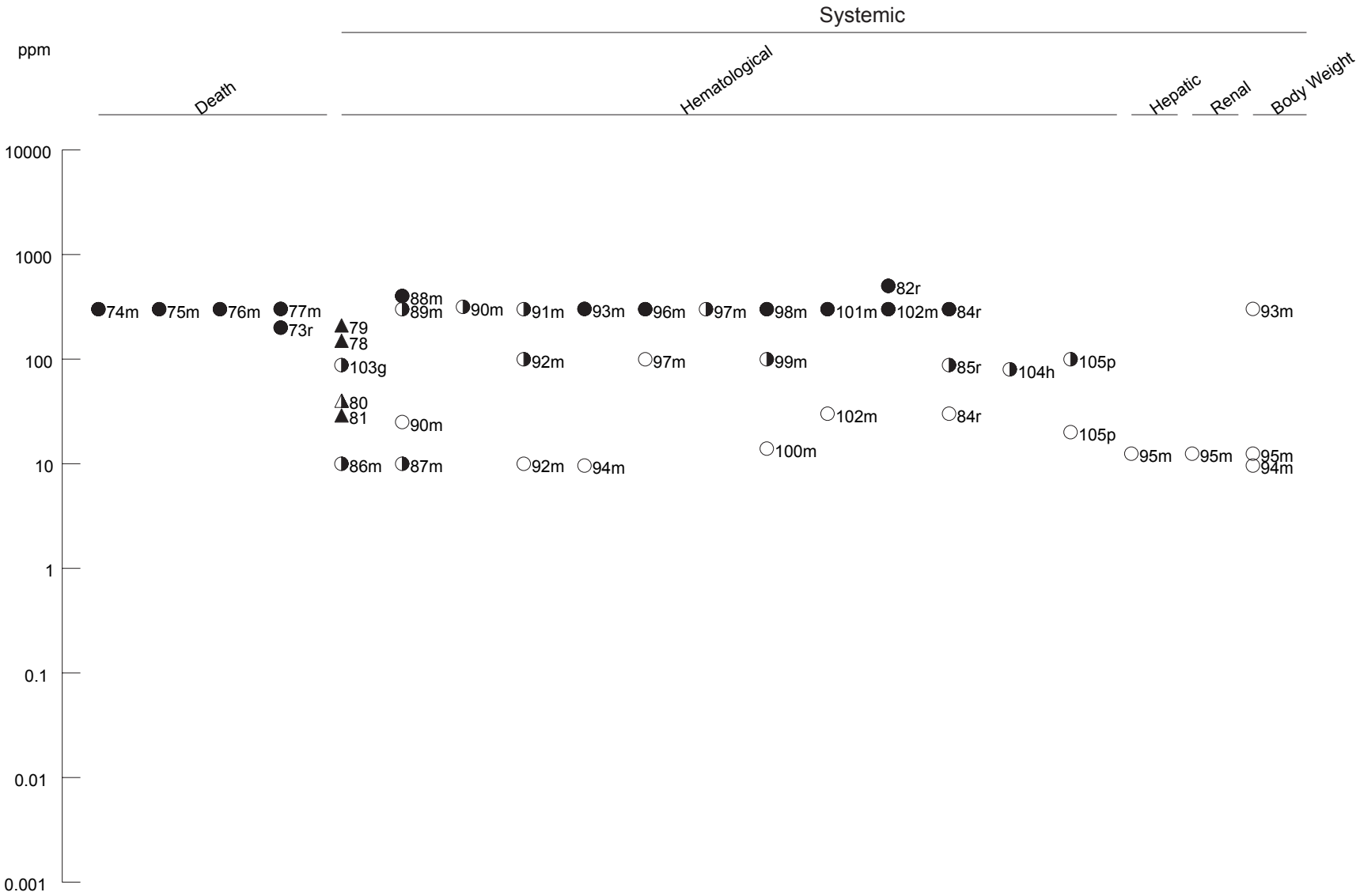
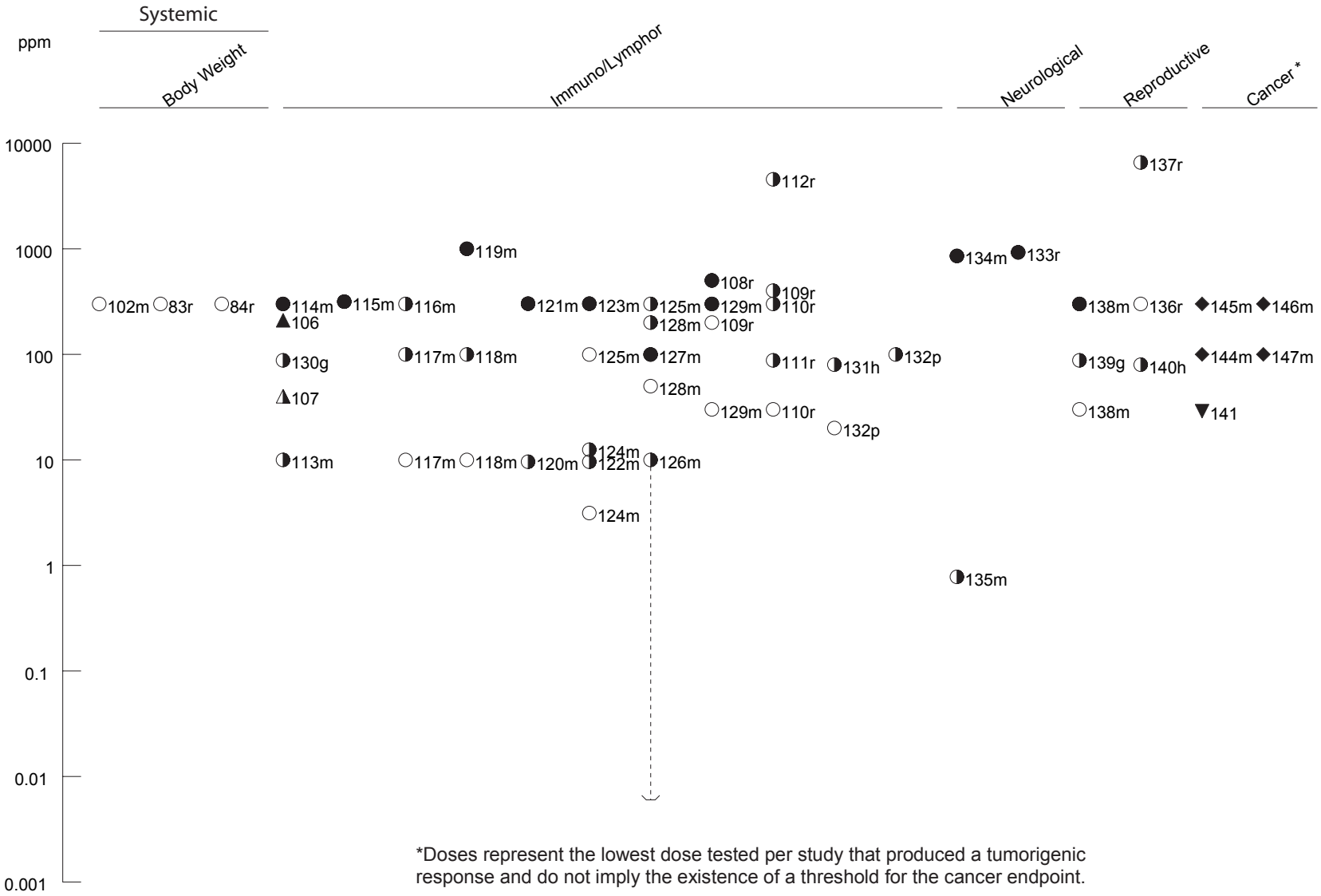


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)

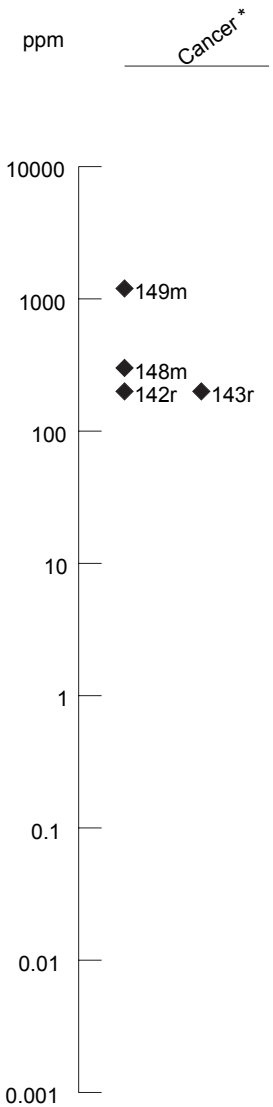


*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)



*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 endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	¦ Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Chronic (≥365 days)

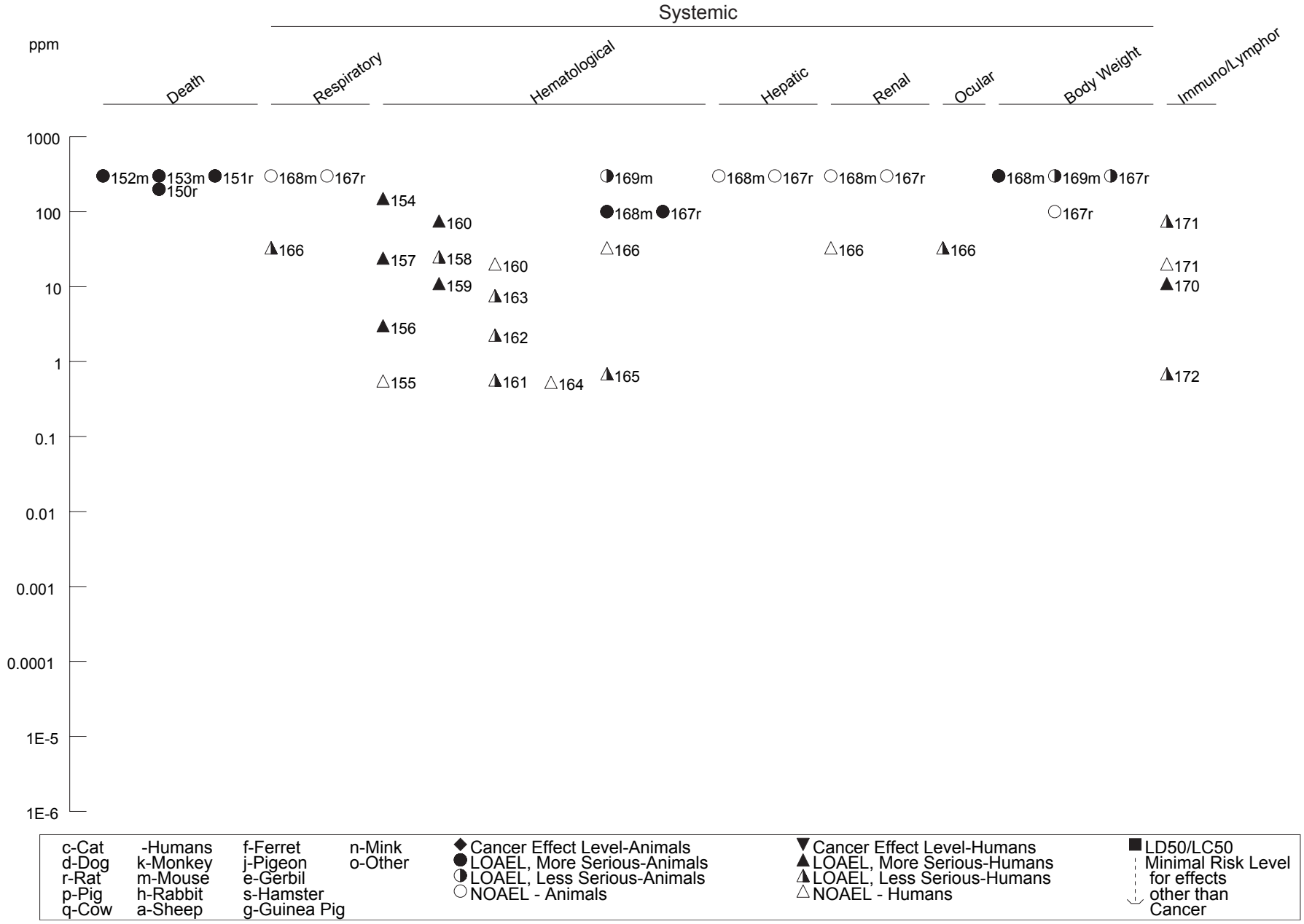
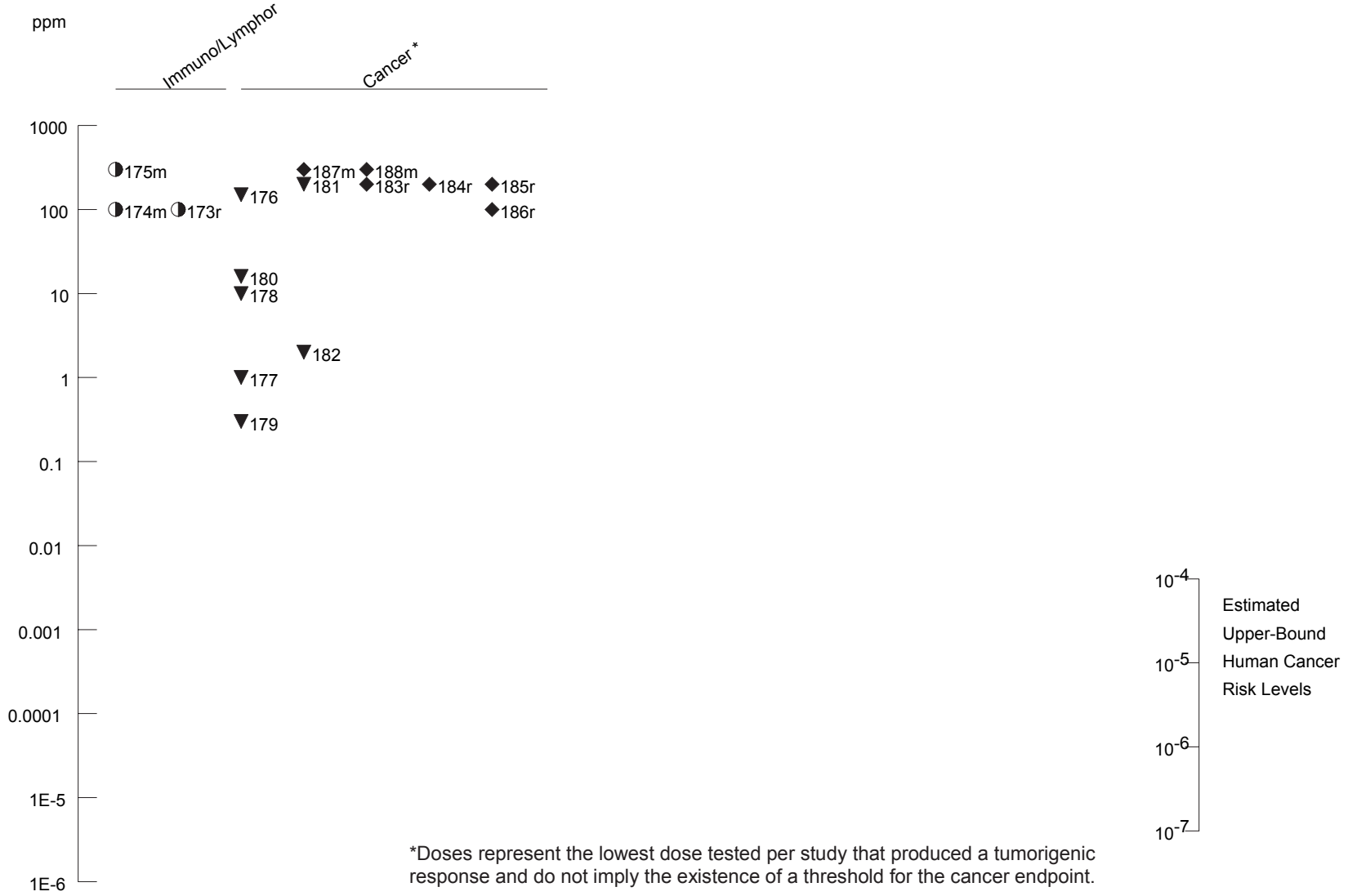


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Chronic (≥365 days)



*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 endpoint.

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

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

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

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

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 concentrations 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. These findings suggest that the arrhythmias were caused by catecholamine release and sympathetic discharge. 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 18-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 of 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. Deficiencies in most of these studies include uncertainty in estimates of historical exposure levels, concomitant exposure to other chemicals, and lack of appropriate control groups. However, sufficient data are available to show that the hematopoietic system is a critical target for benzene toxicity. Studies that were conducted well and that show effects linked to specific exposure levels are presented in Table 3-1 and Figure 3-1. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Inhalation exposure to benzene levels in excess of regulated workplace limits (8-hour TWA of 1 ppm) for several months to several years can result in deficits in the relative numbers of circulating blood cells, which may be severe enough to be considered clinical pancytopenia. Continued exposure to benzene can

also result in aplastic anemia or leukemia (Aksoy et al. 1974; EPA 1995; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1987c, 1996a, 1996b).

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 (Erf and Rhoads 1939). Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia (Aksoy 1980), which is discussed in Section 3.2.1.7.

Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. For example, statistically significantly decreased total red blood cells (RBCs), white blood cells (WBCs), absolute lymphocyte count, platelets, and hematocrit were reported for a group of 44 healthy subjects exposed to benzene in the workplace (median 8-hour time-weighted average [TWA] of 31 ppm; minimal exposure to other solvents) for an average of 6.3 years in China (Rothman et al. 1996a, 1996b). Age- and gender-matched workers with no history of occupational exposure to benzene served as controls. Among the 22 workers whose mean 5-day benzene exposure levels did not exceed 31 ppm (median 8-hour TWA of 13.6 ppm), significantly depressed absolute lymphocyte count, RBCs, and platelets were noted. Only absolute lymphocyte count was significantly decreased in a subgroup of 11 workers with no 8-hour TWA exceeding 31 ppm (median 8-hour TWA of 7.6 ppm).

Qu et al. (2002, 2003a, 2003b) compared hematology values in a group of 130 chronically exposed workers in China with those obtained from 51 age- and gender-matched subjects without occupational exposure to benzene. Statistically significant trends for depressed RBCs, WBCs, and neutrophils were observed in the benzene-exposed workers (average measured 4-week benzene exposure levels ranged

from 0.08 to 54.5 ppm just prior to blood testing). A subgroup of 73 of these workers, whose average 4-week benzene exposure level was 2.26 ppm, exhibited significantly depressed RBCs and neutrophils. This study provided exposure-response data, but apparent discrepancies in reported low-concentration results render the study of limited value for MRL derivation.

One recent cross-sectional study (Lan et al. 2004a, 2004b), performed on 250 workers exposed to benzene in shoe manufacturing industries in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene, was of sufficient quality to serve as the basis for deriving a chronic-duration inhalation MRL for benzene (see footnote to Table 3-1 and Appendix A). The benzene-exposed workers had been employed for an average of 6.1±2.9 years. Controls consisted of 140 age-and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (controls, <1, 1–<10, and ≥10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1, 1–<10, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset

who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible. Lan et al. (2004a, 2004b) also presented information on the effect of benzene on colony forming progenitor cells (data were only presented for the mid- and high-exposure groups). Benzene exposure was associated with a concentration-dependent decrease in colony formation and progenitor cells were suggested to be more sensitive than circulating cells.

As described above, several epidemiology studies compared hematological variables for benzene-exposed workers to gender- and age-matched controls and noted hematological effects at relatively low concentrations (well below 1 ppm in some exposure groups) (Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b). An alternative approach was used by Collins et al. (1991, 1997) and Tsai et al. (1983, 2004). This approach utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. Collins et al. (1991) found no significant correlations between benzene exposure and the prevalence of abnormal hematological values among 200 workers exposed to benzene at estimated concentrations ranging from 0.01 to 1.4 ppm, relative to the prevalence of abnormal hematological values obtained from 268 unexposed workers in the same plant. In a more recent evaluation, Collins et al. (1997) found no significant correlation between exposure to benzene at an 8-hour TWA of 0.55 ppm and prevalence of clinically-defined lymphopenia (or other measures of hematotoxicity including mean corpuscular volume [MCV], and counts of WBCs, RBCs, hemoglobin, and platelets) among a group of 387 workers exposed for ≥5 years. Tsai et al. (1983) measured benzene levels in a Texas refinery at one time point (mean benzene concentration of 0.53 ppm) and found that hemoglobin, hematocrit, RBCs, WBCs, and thrombocytes of workers exposed for up to 21 years were within the range of normal values. Tsai et al. (2004) found no clinically adverse hematotoxic effects among a large group of 1,200 petrochemical employees with mean 8-hour TWA benzene exposure levels of 0.6 ppm from 1977 to 1988 and 0.14 ppm from 1988 to 2002. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values

considered clinically abnormal or adverse may reduce the sensitivity of the study to detect meaningful changes at the population level.

Many reports of hematological effects in benzene-exposed workers involve estimated exposure levels well in excess of 1 ppm. A series of studies conducted on Turkish workers exposed to benzene-containing adhesives in various occupations 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 (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 such as leukopenia, thrombocytopenia, eosinophilia, and pancytopenia. 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.

Leukopenia, lymphocytosis, a biphasic leukocyte response, and bone marrow hypercellularity were reported for workers in a Russian industry who were exposed to a complex of hydrocarbons that included benzene, cyclohexane, and 1,3-butadiene (Doskin 1971). Benzene levels were estimated at 3.2–12.8 ppm, which are 2–8 times the Russian standard. 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. Enlarged RBCs, transient anemia, reduced hemoglobin concentrations, and clinically increased MCV were reported in workers in a chemical factory who were exposed to over 25 ppm of benzene in the workplace for an average of 9 years (Fishbeck et al. 1978).

Dosemeci et al. (1996) assessed the relative risk of abnormal hematological values with increasing benzene levels in a study of workers employed in 672 rubber and rubber glue application facilities in China between 1949 and 1987. Compared to workers with estimated exposures <5 ppm, relative risks of abnormal hematological values (indicating benzene poisoning) by estimated exposure intensity at 1.5 years prior to clinically-diagnosed benzene poisoning were 2.2 (95% confidence interval [CI] 1.7–

2.9), 4.7 (95% CI 3.4–6.5), and 7.2 (95% CI 5.3–9.8) for exposures in the ranges of 5–19, 20–39, and >40 ppm, respectively.

Examination of individual hematological records, demographic data, and chronological work histories of 459 workers employed at a rubber products manufacturing plant in Ohio between 1940 and 1975 revealed significant decreases in WBC and RBC counts and hemoglobin for the period of 1940–1948 when the exposure was 75 ppm (Kipen et al. 1989). The trend was not apparent during later years (1949–1975) when the exposure was decreased to 15–20 ppm. A more focused study of many of the same workers during their first year of employment revealed significantly lower WBC and RBC counts in employees exposed to benzene levels greater than the median (estimated at 40–54 ppm), compared to those with lower estimated exposure levels (Cody et al. 1993). Using a nested case-control approach at the same rubber products manufacturing plant to assess a possible exposure-response relationship between benzene and the risk of developing a low WBC or RBC count in workers for whom hematologic screening data were available, Ward et al. (1996) reported a strong correlation between low WBC counts and benzene exposure and a weak correlation between RBC count and benzene exposure.

More severe effects, including preleukemia or acute leukemia, were observed in 26 out of 28,500 benzene workers exposed to 210–650 ppm for 1–15 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 <1 ppm) paralleled the decrease in the number of hematological abnormalities reported for benzene-exposed workers.

Another study revealed effects, ranging from mild to severe, of benzene exposure in factory workers in China (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, 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.

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 11–1,060 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).

Animal studies have been designed to characterize exposure-response relationships of benzene hematotoxicity; the results provide support to the human data. 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. However, 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. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Repeated acute-, intermediate-, and chronic-duration inhalation exposure of laboratory animals (mainly mice, but also rats, rabbits, and guinea pigs) to benzene vapor concentrations ranging from 10 to >300 ppm demonstrate significant decreases in blood values that include RBCs, total WBCs, lymphocytes, granulocytes, hematocrit, and hemoglobin (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982; Farris et al. 1997a, 1997b; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1986; Rozen and Snyder 1985; Rozen et al. 1984; Snyder et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956). Pancytopenia was noted in the study of Ward et al. (1985).

Short-duration exposure of mice to benzene has been shown to cause both concentration- and duration-related reductions in bone marrow cellularity (number of nucleated cells) and the number of colony-forming granulopoietic stem cells (CFU-C), and an increased frequency of micronucleated polychromatic erythrocytes (MN-PCE) (Toft et al. 1982). Mice that were exposed continuously at benzene concentrations ≥21 ppm in air for 4–10 days showed significant changes in all three parameters. Intermittent exposure (8 hours/day, 5 days/week for 2 weeks) to 21 ppm significantly reduced the number of CFU-C and elevated the frequency of MN-PCE, but did not affect bone marrow cellularity. Intermittent exposure at concentrations ≥50 ppm caused significant changes in all three 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 for 2 weeks, decreased cellularity and CFU-C per tibia were observed at 95 ppm after 6–8 hours/day exposure, whereas at 201 ppm benzene, decreased cellularity (but no effect on CFU-C) was noted after 2 hours/day exposure. A decrease in cellularity and CFU-C was observed after 4–8 hours/day exposure to 201 ppm benzene.

Results of numerous additional studies of laboratory animals (mainly mice, which are particularly sensitive to benzene hematotoxicity, but also rats and pigs) support findings of benzene-induced effects on bone marrow cellularity (hyper- and/or hypocellularity) and colony-forming stem cells, as well as granulocytic hyperplasia, following repeated acute-, intermediate-, or chronic-duration inhalation exposure to benzene vapors at concentrations ranging from 10 to 500 ppm (Baarson and Snyder 1991; Baarson et al. 1984; Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris et al. 1993, 1997b; Neun et al. 1992, 1994; Plappert et al. 1994a, 1994b; Snyder et al. 1978a, 1980, 1982; Vacha et al. 1990). For example, Dempster and Snyder (1991) observed a 50% reduction in CFU-E (erythroid progenitor cells) in bone marrow of DBA/2 mice exposed to 10 ppm benzene for 6 hours/day for 5 days. Farris et al. (1997b) reported benzene-induced decreased numbers of total bone marrow forming cells, progenitor cells, and differentiating hematopoietic cells in mice exposed to benzene vapor concentrations ≥200 ppm, 6 hours/day, 5 days/week for up to 8 weeks. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period, presumably as compensation for cytotoxicity. Granulocytic hyperplasia was detected in the bone marrow of mice exposed to 300 ppm benzene in air for 6 hours/day, 5 days/week for 16 weeks, and held 18 months after the last exposure (Farris et al. 1993). Prolonged exposure to lower levels had a greater hematotoxic effect than exposure to higher levels for a shorter period of time. Recovery from hematotoxicity has been demonstrated following the cessation of exposure (Cronkite et al. 1982, 1985, 1989) and may be most closely associated with rate of exposure, since longer-term exposure of mice to

316 ppm of benzene caused irreparable hematologic injury, while the hematotoxic effects caused by shorter-term exposure to 10 times higher benzene concentrations were reversible (Cronkite et al. 1989).

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/day) for either 5 days/week (Regimen 1) or 3 days/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. 1989). A substantial decrease in erythroid colony-forming units and smaller decreases in erythroid burst-forming units occurred in BDF_1 mice intermittently exposed to 100 ppm of benzene for 8 weeks (6 hours/day, 5 days/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.

Benzene-induced hematotoxicity was also demonstrated in the spleen of rats and mice following intermediate- or chronic-duration repeated inhalation exposure (Snyder et al. 1978a, 1984; Ward et al. 1985). Snyder et al. (1978a, 1984) reported benzene-induced increased extramedullary hematopoiesis in the spleen. Ward et al. (1985) noted that the finding of hemosiderin in the spleen of benzene-exposed rats could be due to erythrocyte hemolysis.

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 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 at airborne concentrations ranging from 150 to 650 ppm.

CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) and toluene (265 ppm) for 24 hours/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/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 a 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/week, 6 hours/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 are 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/week, 6 hours/day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57B1/6J 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 burns on the face, trunk, and limbs (Avis and Hutton 1993). Skin irritation has been noted at occupational exposures of >60 ppm for up to 3 weeks (Midzenski et al. 1992). These effects are due to direct contact of the skin with the vapor, and other dermal effects resulting from direct contact of the skin are discussed in Section 3.2.3.2.

Ocular Effects. Three hundred solvent workers who had inhalation exposures for >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/day, 5 days/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 3.2.3.2.

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-1 mice exposed to 300 ppm benzene for 13 weeks (Ward et al. 1985). No significant decrease in body weight was observed in CD-1 mice exposed to doses up to 4,862 ppm for 6 hours/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/day, 5 days/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/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/day, 6 days/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 gestation days (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/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/day, 6 days/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/day, 5 days/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/day, 5 days/week for life had decreased weight gain at 300 ppm (Snyder et al. 1978a, 1980).

3.2.1.3 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. However, since the workers were exposed to multiple solvents, the specific role of benzene is uncertain.

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). In these studies, benzene levels in workplace air ranged from 1 to 1,060 ppm. Hematological effects reported in these studies are described in Section 3.2.1.2. 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 (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased white blood cell counts and with changes in bone marrow activity. 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 1972–1987 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 exposure to benzene at a concentration of 25 ppm, 6 hours/day for 5 days, the same exposure concentration 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 B- and 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-duration inhalation MRL of 0.009 ppm (see footnote to Table 3-1 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 inhalation 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/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); decreased leukocytes in mice exposed to 300 ppm for 10 days (Ward et al. 1985); bone marrow hyperplasia, lymphocytopenia, and anemia in mice repeatedly exposed to 100 ppm or higher for a lifetime (Snyder et al. 1978a, 1980); and leukopenia and lymphopenia in rats repeatedly exposed to 100 ppm for life (Snyder et al. 1978a, 1984). Aoyama (1986) noted decreased spleen and thymus weights and decreased levels of B- and T-lymphocytes in blood and spleen in mice exposed 6 hours/day to approximately 48 ppm of benzene for 14 days. Exposures of mice to benzene vapor concentrations ≥100 ppm, 6 hours/day, 5 days/week resulted in significantly reduced numbers of lymphocytes (ranging from 25 to 43% lower than controls) in the spleen, thymus, and femur as early as 1 week following the initial exposure, and persisting throughout an 8-week exposure period (Farris et al. 1997a). In rats, exposure to benzene vapors at a concentration of 400 ppm (but not 200 ppm) for 6 hours/day, 5 days/week for 4 weeks resulted in reduced thymus weight and decreased splenic lymphocytes (Robinson et al. 1997).

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 peripheral blood and 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/day for 5 days. Intermediate studies consisted of two different exposure regimens and concentrations: 6 hours/day, 5 days/week for 10 weeks (50 days) to a mean concentration of 0 or 9.6 ppm benzene; or 6 hours/day, 5 days/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 ≥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 et al. 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/week, 6 hours/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/day, 5 days/week for 3 weeks (Dow 1992). Exposure to 500 ppm resulted in significant decreases in total white blood cells, T-cells, peripheral blood lymphocytes, and proportion of myeloid cells in bone marrow counts.

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 1986; Cronkite et al. 1985). 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 two groups of male CBA/Ca mice exposed to a total of 6,000 ppm of benzene by inhalation using two 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. This suggests that, at relatively high exposure levels, repeated or prolonged exposure is more potent than the magnitude of exposure in causing lymphopenia. 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 BDF_1 mice (C57BL/6xDBA/$2F_1$ hybrids) exposed to 0, 300, or 900 ppm benzene 6 hours/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. 1994a). No concentration dependency was observed. Granulocytic hyperplasia of the spleen was observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993).

Leukopenia, granulocytopenia, and lymphocytopenia were observed in C57BL/6 mice exposed to 1,000 ppm benzene for 6 hours/day, 5 days/week for up to 6 weeks (Gill et al. 1980). Decreased leukocyte counts were also noted in Sprague-Dawley rats and CD-1 mice exposed to 300 ppm benzene 6 hours/day, 5 days/week for up to 13 weeks (Ward et al. 1985). 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 (Songnian et al. 1982). Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week, for 24 weeks caused depressions in the numbers of splenic nucleated red cells and lymphocytes (Baarson et al. 1984).

Li et al. (1992) observed a 26% decrease in spleen weight in male Kunming mice exposed to 12.52 ppm benzene 2 hours/day, 6 days/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. These results indicate a benzene-induced delay in immune response to *L. monocytogenes*. Concentrations of 200 or 400 ppm for 4–5 weeks (5 days/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/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. This study is the basis for the intermediate-duration inhalation MRL of 0.006 ppm (see footnote to Table 3-1 and Appendix A). A similar *in vitro* observation was made using T-cells from mice exposed to 100 ppm of benzene 5 days/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).

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

3.2.1.4 Neurological Effects

Following acute inhalation of benzene, humans exhibit 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, include 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 nonspecific 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 removed 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; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of eight patients (six with aplastic anemia and two with preleukemia) with previous occupational exposure to adhesives and solutions containing 9–88% benzene, four of the six 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. 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 6–15.6 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.

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-1 and C57BL/6 mice were exposed to 300 or 900 ppm of benzene 6 hours/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/day, 6 days/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 was observed. However, the exposure levels used by Li et al. (1992) were more than 10-fold lower than those eliciting signs of neurotoxicity in other animal studies, an indication that the actual exposure levels in the study of Li et al. (1992) may have been higher than indicated. Uncertainty regarding actual exposure levels renders this study of little value for purposes of risk assessment.

All reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 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 due to uncertainties in exposure assessment and the limited data collected. 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.

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 cases in 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.

Reproductive competence of male workers in two organic chemical factories in France was evaluated by 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: <5 ppm (low) and ≥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 either pure air or benzene (125 ppm) for 24 hours/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/day on Gd 6–15 (Coate et al. 1984). Pregnant rabbits exposed 12 hours/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 modern industrial environment (Ward et al. 1985; Wolf et al. 1956). In an intermediate-duration inhalation study, groups of male and female CD-1 mice were exposed to benzene vapor concentrations of 0, 1, 10, 30, or 300 ppm, 5 days/week, 6 hours/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/day, 5 days/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 one or two 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 10–15 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 3-1 and plotted in Figure 3-1.

3.2.1.6 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 sternebrae 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/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/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/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/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 sternebrae was significantly increased in the 100 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 sternebrae.

Kuna and Kapp (1981) found decreased fetal weight after exposure to 50 ppm. From a group of 151 pups examined after *in utero* exposure to 500 ppm benzene, a single pup exhibited exencephaly. In the same study, of 98 pups examined for skeletal effects after *in utero* exposures of 500 ppm, a single 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/day (Tatrai et al. 1980b) or 100 ppm for 10 days, 6 hours/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/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/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 *in utero* 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 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute nonlymphocytic leukemia (ANLL), particularly the myeloid cell type (acute myelogenous leukemia, AML) (EPA 1985b, 1998; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b). Some of the studies also provide suggestive evidence of associations between benzene exposure and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997;

Rinsky et al. 1987). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power (due to small numbers of cases), but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor. Many of the earlier studies are additionally limited by a lack of information on leukemia cell types other than AML, because leukemia used to be considered a single diagnostic category for epidemiological purposes, due in part to historical nomenclature, small numbers of deaths by cell type, and unavailability of cell-type-specific rates for comparison.

Two series of studies on workers exposed to benzene in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987, 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) have yielded particularly strong data on the leukemogenic potential of benzene and are summarized below. The studies of the Pliofilm cohort provide the best set of data for evaluating human cancer risks from benzene exposure because, in comparison to other published studies, the Pliofilm workers had the fewest reported co-exposures to other potentially carcinogenic substances and experienced a greater range of estimated exposures to benzene (EPA 1998). The NCI/CAPM Chinese study is one of the largest of its type ever undertaken and evaluated many thousands of benzene-exposed workers, enabling detection of significantly elevated risks at unusually low levels of exposure.

A number of studies were performed on a cohort of workers exposed to benzene in three rubber hydrochloride (‘Pliofilm’) manufacturing plants in Ohio (Collins et al. 1997; Finkelstein 2000; Infante 1978; Infante et al. 1977; Ireland et al. 1997; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987, 2002; Schnatter et al. 1996a; Wong 1995). Analyses using various expansions and updates of the cohort, statistical methods, and exposure estimates have consistently shown a significant relationship between exposure to benzene and excess mortality from leukemia. In the report by Rinsky et al. (1987), a cohort of 1,165 white males employed between 1940 and 1965 and followed through 1981 experienced increased mortality from all leukemias (9 observed versus 2.7 expected; standardized mortality ratio [SMR]=3.37, 95% CI 1.54–6.41) and multiple myeloma (4 observed versus 1 expected; SMR=4.09, 95% CI 1.10–10.47). Death rates for age-matched U.S. white males during the same calendar period were used for comparison with death rates observed in the cohort. Assessment after an additional 15 years of follow-up showed declines in the SMRs for both leukemias (2.56, 95% CI 1.43–4.22) and multiple myeloma (2.12, 95% CI 0.69–4.96), suggesting that the excess risks diminished with time since exposure (Rinsky et al. 2002). Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously (Finkelstein 2000). AML accounted for most of the increased leukemia

(SMR=5.03, 95% CI 1.84–10.97) (Schnatter et al. 1996a; Wong 1995). The risk of all leukemias and AML, but not multiple myeloma, significantly increased with increasing cumulative exposure above 200 ppm-years (Rinsky et al. 1987, 2002; Wong 1995). Analysis of 4,417 workers from one of the Pliofilm plants showed no clear evidence that the risks of all leukemias, ANLL, multiple myeloma, or other lymphohematopoietic cancers increased with increasing exposure at lower levels of cumulative exposure (1–72 ppm-years), and the number of peak exposures over 100 ppm for ≥40 days was a better predictor of risk for all leukemias and multiple myeloma (Collins et al. 2003; Ireland et al. 1997). No clear associations between exposure to benzene and mortality from NHL or Hodgkin's disease were reported (Collins et al. 2003; Ireland et al. 1997; Rinsky et al. 2002).

A collaborative study between the National Cancer Institute and the Chinese Academy of Preventive Medicine (NCI/CAPM) evaluated incidence rates (occurrence of disease and cause of death) for lymphohematopoietic malignancies and other hematologic disorders in a cohort of 74,828 benzene-exposed and 35,805 nonexposed workers employed in 672 factories in 12 cities in China (Hayes et al. 1996, 1997, 2001; Linet et al. 1996). The joint NCI/CAPM study is an expansion of earlier studies performed by CAPM alone (Yin et al. 1987a, 1987b, 1987c, 1989). The workers were employed from 1972–1987, followed for an average of nearly 12 years, and worked in various job categories using benzene as a solvent for paints, varnishes, glues, coatings, and other products. The derivation of the cohort from many different factories and job types suggests that the members were concurrently exposed to many other chemicals. Findings in the exposed workers included significantly increased relative risk (RR) for all hematologic neoplasms (RR=2.6, 95% CI 1.4–4.7), all leukemias (RR=2.5, 95% CI 1.2–5.1), ANLL (RR=3.0, 95% CI 1.0–8.9), and combined ANLL and precursor myelodysplastic syndromes (ANLL/MDS; RR=4.1, 95% CI 1.4–11.6) (Hayes et al. 1997). Increases were also observed for other (unspecified) leukemias (RR=2.0, 95% CI=0.7–5.4), but the results were not statistically significant. Analysis by level of average benzene exposure (<10, 10–24, and ≥25 ppm) and cumulative exposure (<40, 40–99, and ≥100 ppm-years) indicated that the risk for all hematologic neoplasms was significantly increased at <10 ppm (RR=2.2, 95% CI 1.1–4.2) and <40 ppm-years (RR=2.2, 95% CI 1.1–4.5), respectively. The risks for all leukemias, ANLL, and ANLL/MDS were increased at exposure levels of 10–24 ppm (average) and 40–99 ppm-years (cumulative). The elevated risks showed weak tendencies to increase with increasing average and cumulative levels of exposure. Analysis by duration of exposure (<5, 5–9, or ≥10 years) did not show increased risk with increasing exposure duration. Analysis by occupational group (coatings, rubber, chemical, shoe, other/mixed) showed that the increased risks for ANLL and ANLL/MDS were consistent across the spectrum of industries studied, implying that the associations were due to the common exposure to benzene rather than other industry-specific exposures.

The results of a study of shoe factory workers in Italy are similar to those of the Pliofilm and NCI/CAPM studies in showing that the risk of leukemia increases with increasing exposure to benzene (Costantini et al. 2003; Paci et al. 1989). The cohort was followed from 1950 to 1999 and consisted of 891 men and 796 women who were exposed to estimated benzene concentrations ranging from 0 to 92 ppm, and had mean cumulative exposures of 71.8 and 43.4 ppm-years, respectively (exposure durations not reported). Leukemia risk was significantly increased in both sexes in the highest of four exposure categories and most apparent in the men. For cumulative exposures of <40, 40–99, 100–199, and >200 ppm-years, the leukemia SMR values for the men were 1.4 (95% CI 0.2–5.0), 3.7 (95% CI 0.1–20.6), 3.0 (95% CI 0.4–10.9), and 7.0 (95% CI 1.9–18.0), respectively. Leukemia subtypes were not evaluated. The findings are consistent with earlier epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent (Aksoy et al. 1974, 1987; IARC 1982; Vigliani and Forni 1976).

No significant increases in leukemia were found in chemical industry workers (Bloemen et al. 2004; Bond et al. 1986; Ott et al. 1978) or petroleum industry workers (Lewis et al. 1997; Raabe and Wong 1996; Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c; Tsai et al. 1983) exposed to lower levels of benzene. Cause-specific mortality was determined in a prospective study of 2,266 chemical workers who were exposed to benzene in various Dow Chemical Company manufacturing processes between 1938 and 1970 (Bloemen et al. 2004). The workers were followed from 1940–1996 and had an average duration of exposure, intensity of exposure, and cumulative exposure of 4.8 years, 9.6 ppm, and 39.7 ppm-years, respectively. There were no significant increases in risk for any lymphohematopoietic malignancies, including all leukemias, ANLL, chronic lymphatic leukemia (CLL), NHL, and multiple myeloma. A previous investigation of this cohort found a significantly increased risk of 'myelogenous' leukemias (SMR=4.44, 95% CI not reported) (Bond et al. 1986; Ott et al. 1978), but this result is less reliable than those of the current study because it is based on a much smaller number of deaths.

A meta-analysis was conducted on 19 cohorts of petroleum workers in the United States and the United Kingdom that were pooled into a single database for cell-type-specific leukemia analysis (Raabe and Wong 1996). The combined cohort consisted of 208,741 workers, mainly refinery employees who contributed more than 4.6 million person-years of observation. Benzene exposures were mainly from handling gasoline and the estimated mean and cumulative exposures for the most exposed jobs were <1 ppm and <45 ppm-years, respectively. No increased risks were found for mortality from AML, chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), or CLL. Analyses limited to

studies of refinery workers or studies with at least 15 years of follow-up yielded similar results. The negative results of this meta-analysis are consistent with those of case-control studies of the common leukemia cell types in petroleum workers (Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c). A case-control study of hairy cell leukemia, a rare B-lymphoid chronic leukemia, found no association between reported exposure to benzene and the risk of this cancer in workers from various occupations with generally low levels of exposure (Clavel et al. 1996).

A possible association between occupational exposure to benzene and NHL is suggested by results of the most recent analysis (Hayes et al. 1997) of the cohort from the NCI/CAPM Chinese study summarized above. The relative risk for mortality from NHL in the whole cohort was 3.0 (95% CI 0.9–10.5), an increase that was not statistically significant. However, the risk for NHL did significantly increase at the highest level and duration of benzene exposure. For exposure to average concentrations of <10, 10–24, and ≥25 ppm, the RR values were 2.7 (95% CI 0.7–10.6), 1.7 (95% CI 0.3–10.2), and 4.7 (95% CI 1.2–18.1), respectively (P for trend=0.04). For cumulative exposures of <40, 40–99, and ≥100 ppm-years, the RR values were 3.3 (95% CI 0.8–13.1), 1.1 (95% CI 0.1–11.1), and 3.5 (95% CI 0.9–13.2), respectively (P for trend=0.02). Additionally, the risk for NHL significantly increased with increasing duration of exposure among workers exposed for <5 years (RR=0.7, 95% CI 0.1–7.2), 5–9 years (RR=3.3, 95% CI 0.7–14.7), and >10 years (RR=4.2, 95% CI 1.1–15.9) (P for trend=0.01).

Although the results of the NCI/CAPM Chinese study (Hayes et al. 1997) indicated a possible association between exposure to benzene and NHL, other cohort mortality studies found no significant increases in NHL mortality. These studies include the entire Pliofilm cohort (Rinsky et al. 2002); a total of 4,417 workers from one of the three Pliofilm plants assessed for cumulative exposures of 0, <1, 1–6, and >6 ppm-years (Collins et al. 2003); a cohort of 2,266 chemical manufacturing workers exposed to a cumulative benzene level of 39.7 ppm-years (Bloemen et al. 2004); and a meta-analysis of 26 cohorts of petroleum workers from the United States and five other countries with expected low exposures to benzene (Wong and Raabe 2000). Furthermore, case-control studies provide no indications of an association between benzene exposure and risk of NHL (Schnatter et al. 1996b; Wong and Raabe 2000), and the adequacy of the data from the NCI/CAPM Chinese study (Hayes et al. 1997) has been questioned (Dosemeci et al. 1994; Wong 1999; Wong and Raabe 2000).

Other evidence suggests a possible association between occupational exposure to benzene and increased risk of multiple myeloma. Mortality from multiple myeloma was initially elevated in the Pliofilm cohort, but appeared to diminish with increasing duration of observation. Based on an evaluation of 1,165 white

male workers followed through 1981, Rinsky et al. (1987) found an SMR for multiple myeloma of 4.09 (95% CI 1.10–10.47), an increase that was statistically significant. Assessment of the cohort after an additional 6 and 15 years of follow-up yielded lower SMRs of 2.91 (95% CI 0.79–7.45) (Wong 1995) and 2.12 (95% CI 0.69–4.96) (Rinsky et al. 2002), respectively, risks that were no longer statistically significant. The risk of multiple myeloma did not increase with increasing level of cumulative exposure to benzene or with duration of employment, but this finding is particularly limited by the small number of deaths. No increases in mortality from multiple myeloma were found in other cohort mortality studies, including the NCI/CAPM study of Chinese workers (no observed deaths) (Yin et al. 1996a, 1996b), the Bloemen et al. (2004) study of chemical workers (SMR=0.72, 95% CI 0.15–2.10), and the Paci et al. (1989) study of shoe factory workers (no observed deaths in males; SMR=1.11 in females, 95% CI not calculated) summarized above. Additionally, mortality from multiple myeloma was not increased in a meta-analysis of 22 other cohorts of petroleum workers with potential exposure to benzene or benzene-containing petroleum products (Wong and Raabe 1997). The combined cohort consisted of 250,816 workers (from the United States, Canada, the United Kingdom, and Australia) who were observed over a period of 55 years from 1937 to 1991. The overall SMR for multiple myeloma was 0.93 (95% CI=0.81–1.07), and analyses by type of facility/industrial process (refinery, distribution, and crude oil production and pipeline workers) and duration of observation showed no differences in risk. The individual cohorts used in the meta-analysis also had no increased risk of multiple myeloma.

As summarized above, one of the early assessments of the Pliofilm cohort found an increased risk of mortality from multiple myeloma (Rinsky et al. 1987). The implications of this finding are unclear because the risk declined to non-significant levels in subsequent follow-ups (Rinsky et al. 2002; Wong 1995), and was not supported by the findings of other cohort mortality studies (Bloemen et al. 2004; Paci et al. 1989; Wong and Raabe 2000; Yin et al. 1996a, 1996b). Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of mulitple myeloma (Bezabeh et al. 1996; Heineman et al. 1992; Linet et al. 1987; Schnatter et al. 1996b; Sonoda et al. 2001; Wong and Raabe 1997). A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene-containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents (summary odds ratio [OR]=0.74, 95% CI 0.60–0.90), petroleum (summary OR=1.11, 95% CI 0.96–1.28), or petroleum products (summary OR=1.08, 95% CI 0.89–1.33) (Sonoda et al. 2001).

Studies in animals provide supporting evidence for the carcinogenicity of inhaled benzene. As summarized below, inhalation exposure to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice.

Carcinomas of the Zymbal gland and oral cavity were clearly increased in Sprague-Dawley rats that were exposed to 200–300 ppm benzene for 4–7 hours/day, 5 days/week for up to 104 weeks (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989). The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Marginal increases in nasal cavity carcinomas, mammary tumors, and hepatomas were also observed. The significance of these findings is unclear because statistical analyses were not performed and there were no clearly increased incidences of Zymbal gland carcinoma or tumors at other sites in Sprague-Dawley rats that were similarly exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for life (Snyder et al. 1978b, 1984).

Benzene was carcinogenic in mice exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993). These exposures consistently induced a variety of tumors, including leukemia (mainly thymic lymphoma) and Zymbal gland and ovarian tumors in C57BL/6 mice, and myelogenous leukemias, malignant lymphomas, and Zymbal gland and lung tumors in CBA/Ca mice. Incidences of lymphocytic lymphoma with thymic involvement were significantly increased in C57BL/6 mice similarly exposed to 300 ppm benzene for life (Snyder et al. 1980). Intermittent lifetime exposure to 300 ppm benzene (6 hours/day, 5 days/week on every third week) was more tumorigenic (in the Zymbal gland and lungs) to CD-1 and C57BL/6 mice than short-term exposure to 1,200 ppm (6 hours/day, 5 days/week for 10 weeks) followed by lifetime observation, although neither of these exposures induced leukemia (Snyder et al. 1988).

The cancer effect levels (CELs) for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services (NTP 2005) determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. IARC (1987, 2004, 2007) classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA (IRIS 2007) classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal

studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). Based on the Rinsky et al. (1981, 1987) human leukemia data, EPA derived a range of inhalation unit risk values of $2.2x10^{-6}$–$7.8x10^{-6}$ $(\mu g/m^3)^{-1}$ for benzene (IRIS 2007). For risks ranging from $1x10^{-4}$ to $1x10^{-7}$, the corresponding air concentrations for lifetime exposure range from 13.0–45.0 $\mu g/m^3$ (4–14 ppb) to 0.013–0.045 $\mu g/m^3$ (0.004–0.014 ppb), respectively. These risk levels are presented in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 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, 30–35 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/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 all reliable LOAEL values for death in each species following acute and chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

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

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

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 chronic-duration 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 ≤200 mg/kg/day (male

Table 3-2 Levels of Significant Exposure to Benzene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	once				126 (death)	Thienes and Haley 1972	
2	Rat (Sprague-Dawley)	once (G)				930 M (LD50)	Cornish and Ryan 1965	
3	Rat (Wistar)	once (GO)				5600 M (LD50)	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 and 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 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
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	
INTERMEDIATE EXPOSURE								
Death								
12	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				250 F (9/35 died)	Maltoni et al. 1983, 1985	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
13	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)	Bd Wt	50	250 (body weight decreased 19%)		Maltoni et al. 1983, 1985	
14	Rat (F-344/N)	<1 yr 5 d/wk (GO)	Hemato		50 M (lymphocytopenia and leukocytopenia)		NTP 1986	
					25[b] F (lymphocytopenia and leukocytopenia)			
			Bd Wt	100	200 M (body weight decreased 11% or more in 25 weeks)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	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[b] (dose-related leukopenia at 120 days)			
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
			Bd Wt		200 (body weight decreased 14% in males and 16% in females)			
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	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
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				
21	Mouse (B6C3F1)	<1 yr 5 d/wk (GO)	Hemato		25 (lymphocytopenia)		NTP 1986	
			Bd Wt	100				

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
22	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986	
			Cardio	600				
			Gastro	600				
			Hemato	200 F	50[b] M (dose-related leukopenia)			
				25	400 F (dose-related leukopenia)			
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
			Bd Wt	600				
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 intake)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
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)		Huff et al. 1989; NTP 1986	
					25 F dose-related lymphopenia at 120 days)			
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)			27 M (decreased number of splenocytes and 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	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
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[b] 200 F	50 M[b] (dose-related lymphopenia) 400 F		Huff et al. 1989; NTP 1986	
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	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
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	
39	Mouse (B6C3F1)	17 wk 5 d/wk (GO)		600			NTP 1986	
Cancer								
40	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/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 1 x/d (GO)				50 (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989	
						250 (CEL: nasal cavity carcinoma, fore-stomach liver angiosarcoma; Zymbal gland carcinoma)		

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
42	Mouse (RF/J)	52 wk 4-5 d/wk (GO)				500 (CEL: mammary pulmonary leukemias)	Maltoni et al. 1989	
CHRONIC EXPOSURE								
Death								
43	Rat (F-344/N)	2 yr 5 d/wk (GO)				200 M (30/50 died) 50[b] F (14/50 died)	NTP 1986	
44	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				100 (41/50 males died, 35/50 females died)	NTP 1986	
Systemic								
45	Human	6.1 yr (avg) (Occup)	Hemato		0.29[c] (reduced WBC and platelet counts, approximately 7-18% lower than control values)		Lan et al. 2004a	Route-to-route extrapolation from the reported LOAEL of 0.57 ppm for occupational exposure was used by ATSDR to estimate equivalent oral dose.

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
46	Rat (F-344/N)	2 yr 5 d/wk (GO)	Resp	200 M 100[b] F			Huff et al. 1989; NTP 1986	
			Cardio	200 M 100[b] F				
			Gastro	100	200 M (hyperkeratosis and acanthosis in nonglandular forestomach)			
			Hemato		50 M (lymphocytopenia and leukocytopenia) 25[b] F (lymphocytopenia and leukocytopenia)			
			Musc/skel	200 M 100[b] F				
			Hepatic	200 M 100[b] F				
			Renal	200 M 100[b] F				
			Endocr	200 M 100[b] F				
			Dermal	200 M 100[b] F				
			Ocular	200 M 100[b] F				

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
47	Rat (F-344/N)	2 yr 5 d/wk (GO)	Bd Wt	100 M		200 M (body weights decreased 23% in 103 weeks)	Huff et al. 1989; NTP 1986	
48	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 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
49	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	50 M 25[b] F	100 M 50[b] F (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 weeks to 19% in 103 weeks in males; decreased 14-15% in week 99-103 in females)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
50	Rat (F-344/N)	2 yr 5 d/wk (GO)			50 M (lymphoid depletion of spleen and thymus) 25[b] F (lymphoid depletion of spleen and thymus)		Huff et al. 1989; NTP 1986	
51	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)			500 (decreased WBCs after 84 weeks)		Maltoni et al. 1983, 1985	
52	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								
53	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 100[b] F			NTP 1986	
54	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		100			NTP 1986	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
55	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 50[b] F		100 F (endometrial polyps)	NTP 1986	
56	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25 (preputial gland hyperplasia in males; ovarian hyperplasia and senile atrophy in females)		NTP 1986	
Cancer								
57	Rat (F-344/N)	2 yr 5 d/wk (GO)				50 M (CEL: squamous cell papillomas and carcinomas of the oral cavity)	Huff et al. 1989; NTP 1986	
						25[b] F (CEL: Zymbal gland carcinomas)		
58	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: Zymbal gland carcinoma; oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1983, 1985	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
59	Rat (Wistar)	104 wk 4-5 d/wk 1 x/d (GO)				500	(CEL: Zymbal gland and oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1989	
60	Rat (Sprague-Dawley)	104 wk 5 d/wk (GO)				50 M	(CEL: Zymbal gland carcinoma)	Maltoni et al. 1989	
61	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				25	(CEL: harderian gland adenoma, lymphoma in males; CEL: lymphoma in females)	Huff et al. 1989; NTP 1986	
62	Mouse (Swiss)	78 wk 4-5 d/wk 1 x/d (GO)				500	(CEL: mammary tumors, lung tumors, Zymbal gland)	Maltoni et al. 1989	

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

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

c Study results used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

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 oil; Hemato = hematological; 5-HT = 5-hydroxystryptamine; LD50 = 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 = vanillin mandelic acid; (W) = water; WBC = white blood cells; wk = week(s); yr = year(s); x = times

Figure 3-2 Levels of Significant Exposure to Benzene - Oral

Acute (≤14 days)

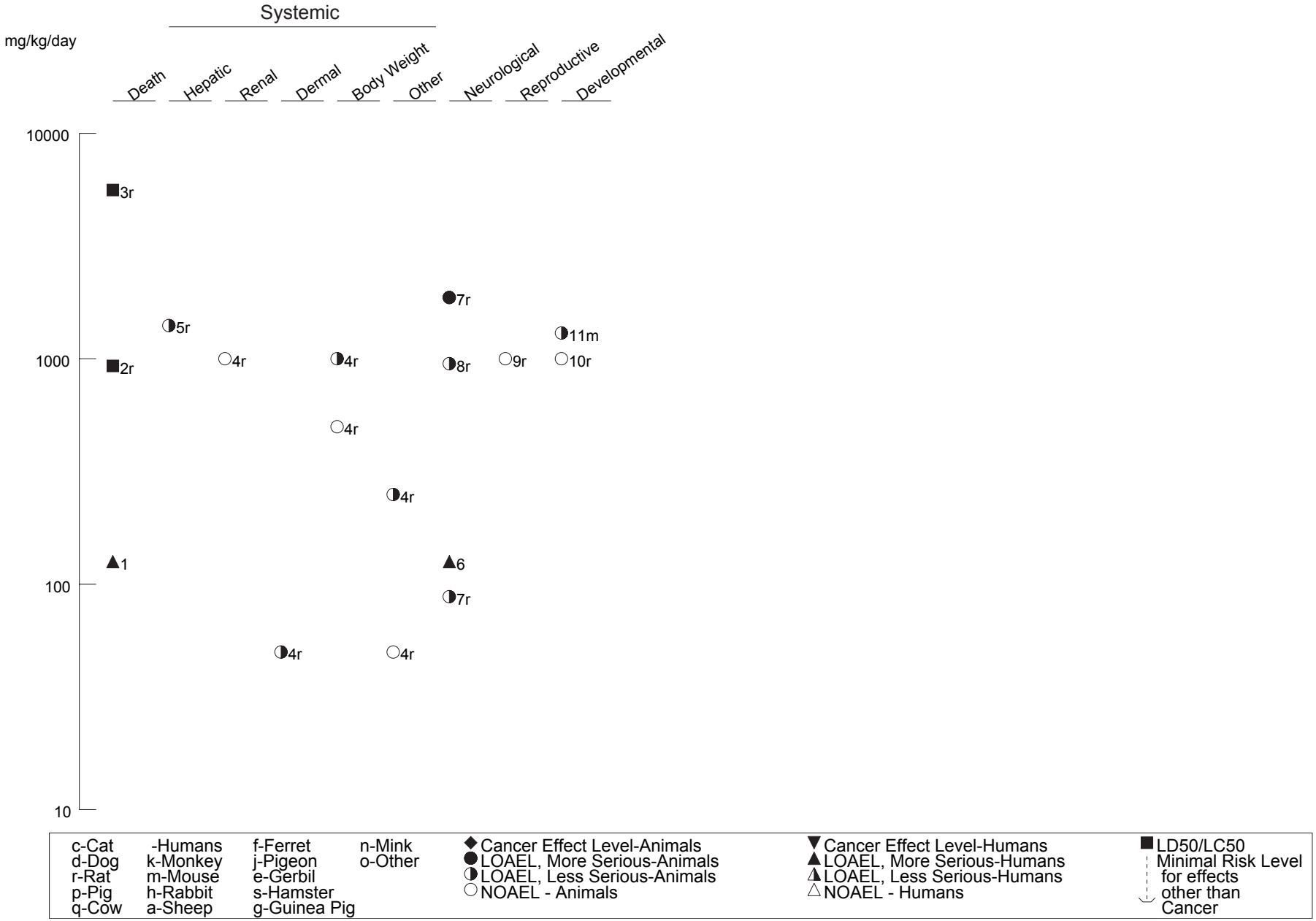


Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Intermediate (15-364 days)

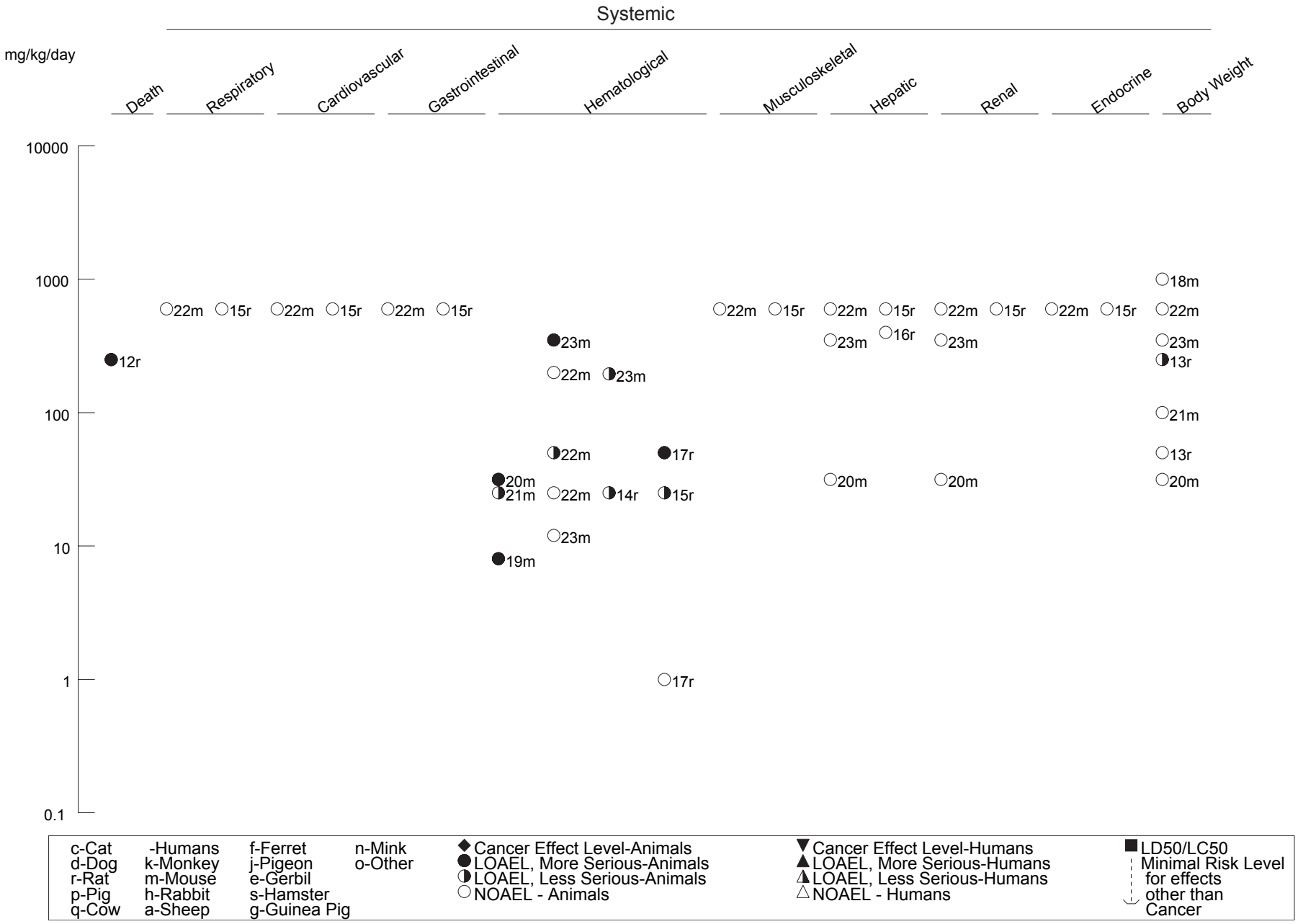
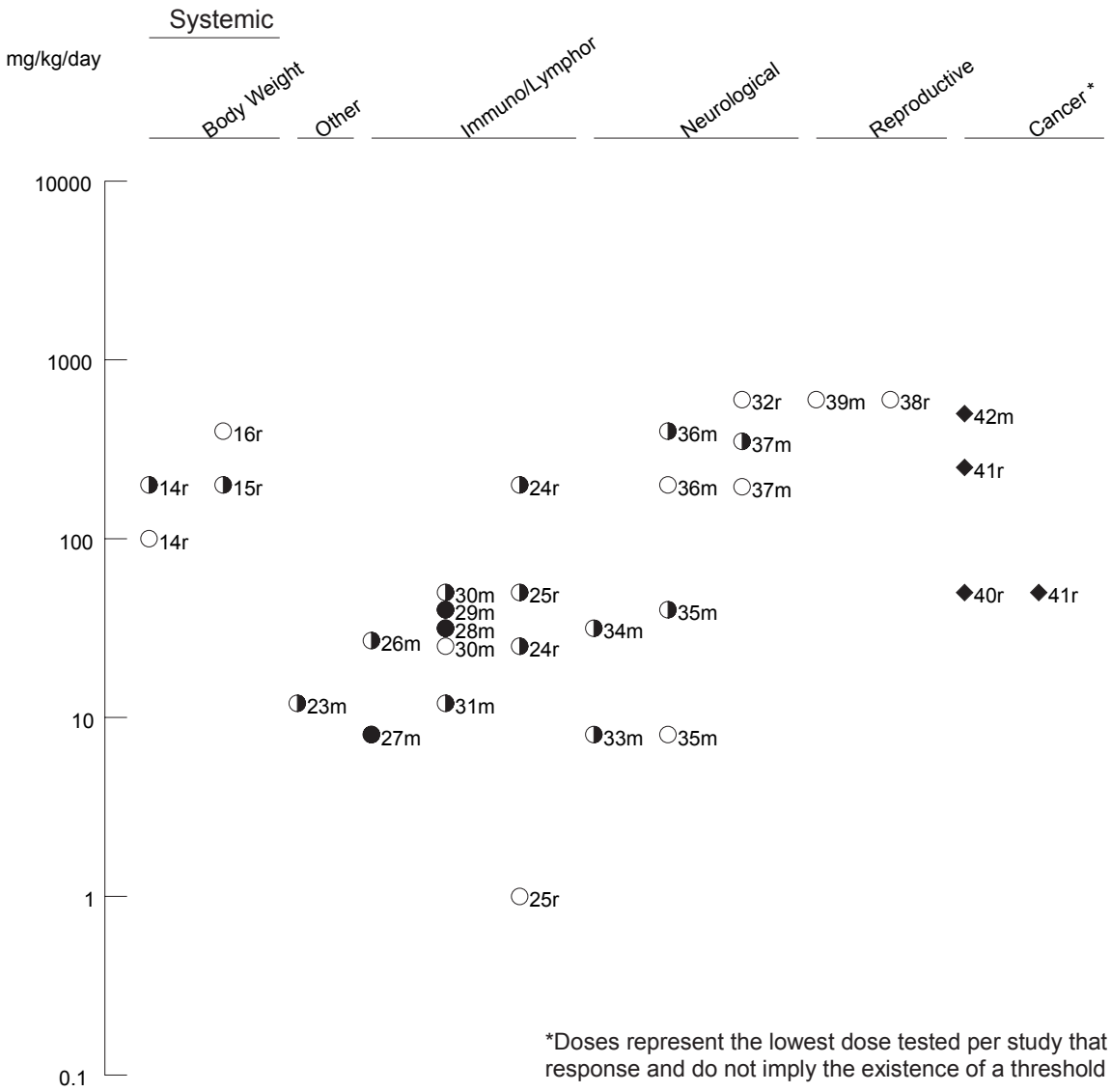


Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Intermediate (15-364 days)



*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 endpoint.

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

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

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

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

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Chronic (≥365 days)

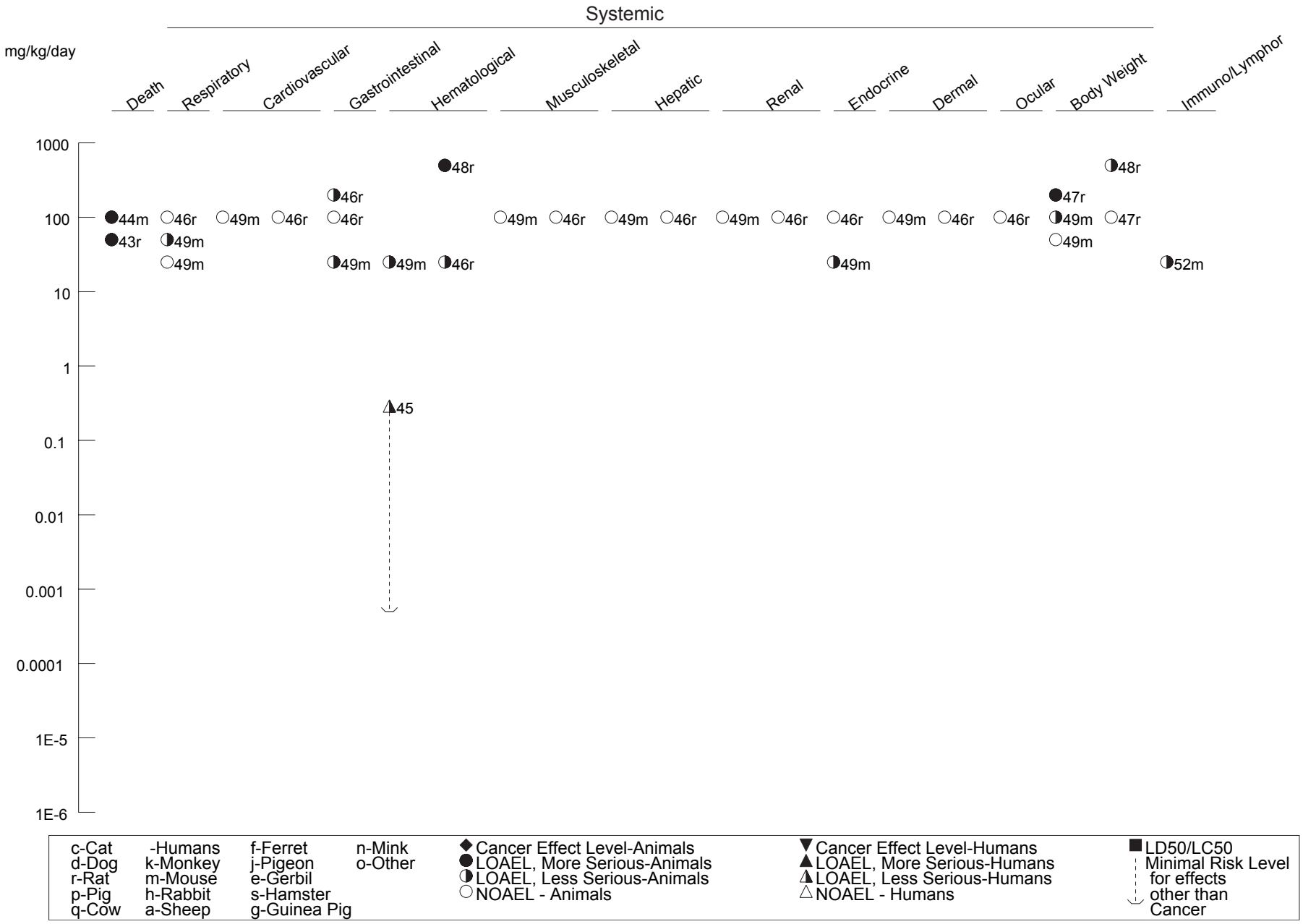
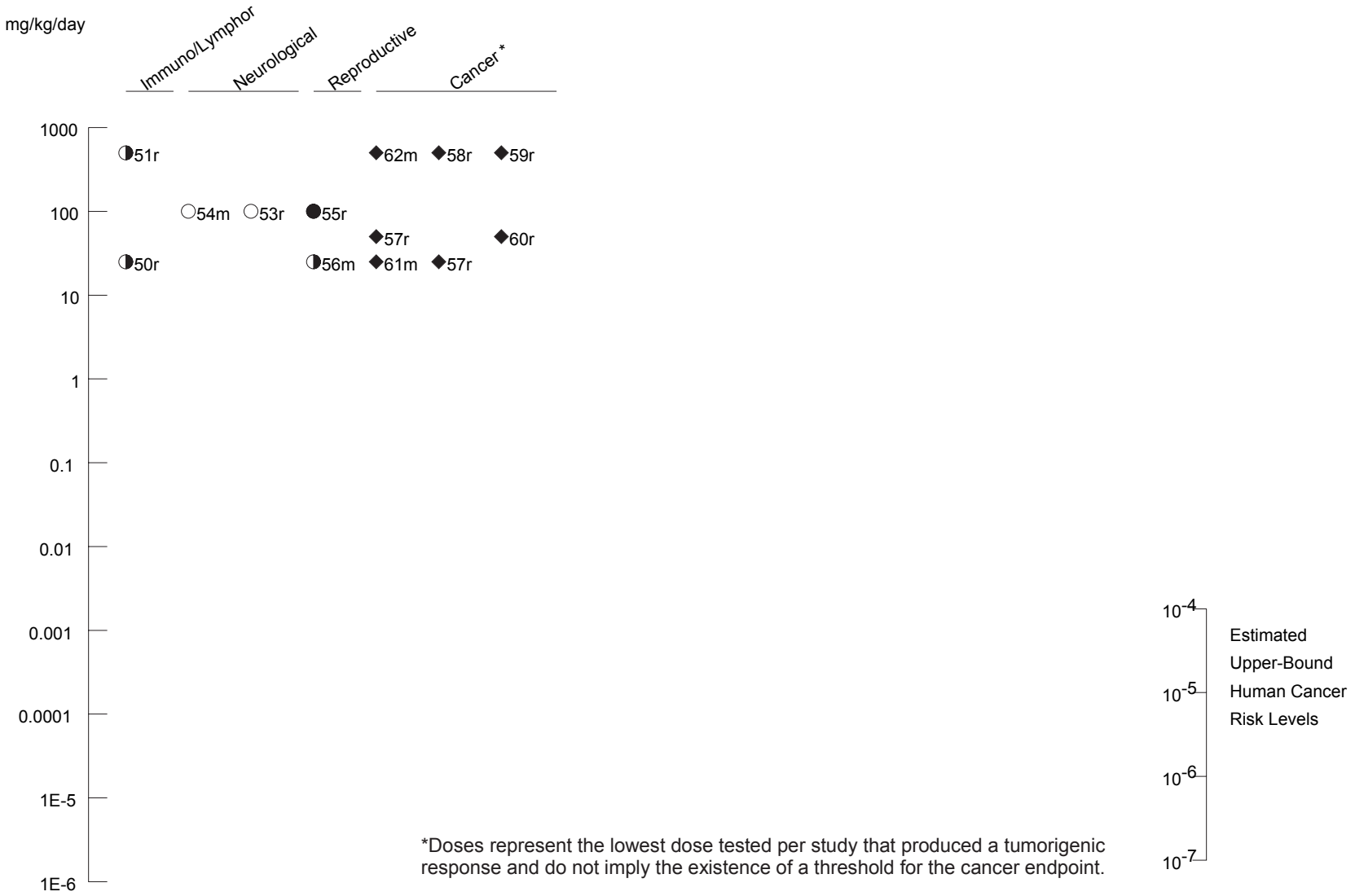


Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Chronic (≥365 days)



rats) or ≤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 nonglandular forestomach at 200 mg/kg/day, and mice exhibited epithelial hyperplasia and hyperkeratosis in the forestomach at 25 mg/kg/day (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/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 3–18 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/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased erythrocytes and leukocytes 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 supernatant 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/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-1

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-1 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 3.2.2.7). 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/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-1 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/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/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.

3.2.2.3 Immunological and Lymphoreticular Effects

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

Oral administration of benzene to CD-1 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. Selected mice of the 27 mg/kg/day dose group were sacrificed on postexposure days 7, 14, and 21 in order to assess the postexposure time course of the benzene toxicity. 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-cells 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 were significantly 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 release 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/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 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

In humans, symptoms of central nervous system toxicity (including euphoria, 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-dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, 3-methoxy-4-hydroxy-phenylglycol (MHPG), serotonin, and 5-hydroxyindoleacetic 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-1 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 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 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 3.2.2.7). 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 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 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 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Essentially no information was located regarding the oral carcinogenicity of benzene in humans. Lymphatic and hematopoietic cancers were increased in vehicle maintenance workers who occasionally siphoned gasoline by mouth (Hunting et al. 1995), but the skin and lungs were the main routes of exposure (see Section 3.2.3.7).

Benzene has been shown to be a multiple site carcinogen by the oral route in animals (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986). In bioassays conducted by the NTP, benzene in corn oil was administered groups of 50 F344/N rats and 50 $B6C3F_1$ of each sex by gavage on 5 days/week for 103 weeks (Huff et al. 1989; NTP 1986). The male rats were exposed to dose levels of 0, 50, 100, or 200 mg/kg/day, and female rats and mice of both sexes were exposed to 0, 25, 50, or 100 mg/kg/day. In the rats, benzene caused significantly increased incidences of Zymbal gland carcinomas in males at ≥100 mg/kg/day and females at ≥25 mg/kg/day, oral cavity squamous cell papillomas and carcinomas in males at ≥50 mg/kg/day and females at ≥25 mg/kg/day, and skin squamous cell papillomas and carcinomas in males at 200 mg/kg/day. In the mice, benzene mainly caused significantly increased incidences of malignant lymphomas in both sexes at ≥25 mg/kg/day, Zymbal gland carcinomas in males at ≥50 mg/kg/day and females at 100 mg/kg/day, lung alveolar/bronchiolar adenomas and carcinomas in males at ≥100 mg/kg/day and females at ≥50 mg/kg/day, Harderian gland adenomas in males at ≥25 mg/kg/day, preputial gland squamous cell carcinomas in males at ≥50 mg/kg/day, and mammary gland carcinomas in females at ≥50 mg/kg/day. NTP (1986) concluded that there was clear evidence of carcinogenicity of benzene in male and female F344/N rats and $B6C3F_1$ mice under the conditions of these studies.

Maltoni et al. (1983, 1985, 1989) assessed carcinogenicity in groups of 30–50 rats and mice of each sex that were exposed to benzene in olive oil by gavage on 4–5 days/week for up to 104 weeks and observed for life. Sprague-Dawley rats were exposed to 0, 50, or 250 mg/kg/day for 52 weeks or 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland carcinomas in

females at ≥50 mg/kg/day, oral cavity carcinomas in females at 250 mg/kg/day and both sexes at 500 mg/kg/day, forestomach carcinomas in females at 500 mg/kg/day, nasal cavity and skin carcinomas in males at 500 mg/kg/day, and liver angiosarcomas in both sexes at 500 mg/kg/day. Wistar rats were exposed to 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland, nasal cavity, and oral cavity carcinomas. Swiss mice were exposed to 0 or 500 mg/kg/day for 78 weeks; effects included increased incidences Zymbal gland carcinomas in males, mammary carcinomas in females, and lung adenomas in both sexes. RF/J mice were exposed to 0 or 500 mg/kg/day for 52 weeks; effects included increased incidences of mammary carcinomas in females and lung adenomas in both sexes.

As discussed in Section 3.2.1.7, there is a consensus that benzene is a human carcinogen (IARC 1987, 2004, 2007; IRIS 2007; NTP 2005). This conclusion is based on sufficient inhalation data in humans supported by animal evidence, including the oral studies summarized above. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic leukemia, whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk range (IRIS 2007). The oral slope factor ranges from $1.5x10^{-2}$ to $5.5x10^{-2}$ $(mg/kg/day)^{-1}$, and for cancer risks of $1x10^{-4}$–$1x10^{-7}$, the corresponding dose levels range from $6.7x10^{-3}$–$1.8x10^{-3}$ to $6.7x10^{-6}$–$1.8x10^{-6}$ mg/kg/day, respectively. These risk levels are presented in Figure 3-1.

3.2.3 Dermal Exposure

3.2.3.1 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 three 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.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, or body weight effects in humans or animals after dermal exposure to benzene. Available data pertaining to hematological, 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 3-3.

Hematological 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 burns 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 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 hours). 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 1 time/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 10–300 ppm benzene for 6 hours/day, 5 days/week (Shell 1980). Moderate conjunctival irritation and transient corneal damage were observed in rabbits subsequent to placement of 2 drops of benzene onto the eyeball (Wolf et al. 1956).

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Systemic								
Human	1-21 d 2.5-8 hr/d	Dermal		60 M ppm	(mucous membrane anc skin irritation)		Midzenski et al. 1992	
Rabbit (NS)	once	Ocular		2 Unknown	(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 M ppm	10 M ppm	(lacrimation)		Shell 1980	
CHRONIC EXPOSURE								
Systemic								
Human	>1 yr (occup)	Ocular		33 M ppm	(eye irritation)		Yin et al. 1987b	
				59 F ppm	(eye irritation)			

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

3.2.3.3 Immunological and Lymphoreticular Effects

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

3.2.3.4 Neurological Effects

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979 and who developed myelofibrosis. 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.

3.2.3.5 Reproductive Effects

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

3.2.3.6 Developmental Effects

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

3.2.3.7 Cancer

A cohort mortality study was conducted to estimate the relative risk of hematological cancer among 335 male vehicle maintenance workers who were employed for at least 1 year between 1977 and 1989 and followed through 1991 (Hunting et al. 1995). The workers were car and mobile equipment mechanics who regularly used gasoline to clean parts and wash their hands. Exposure to gasoline also occurred via inhalation, and some of the workers occasionally siphoned gasoline by mouth. Since most of the cohort consisted of non-white (race data were missing for 9.5% of the cohort) vehicle maintenance workers who lived within the District of Columbia, death rates for age- and calendar-matched non-white males in the District of Columbia were used to calculate expected deaths for the cohort. Three deaths due to lymphatic and hematopoietic cancer were observed, yielding SMRs of 3.63 (95% CI 0.75–10.63) in the whole cohort and 4.22 (95% CI 0.87–12.34) in a subgroup of 297 workers with the highest potential for

exposure. Neither of these SMRs were significant, although analysis by cancer subtype showed a significantly elevated risk for leukemia and aleukemia in the high exposure subgroup (two cases, SMR=9.26, 95% CI 1.12–33.43). Aleukemia refers to leukemias in which the blood has normal or near-normal white blood cell counts, but few numbers of young leukocytes. Two additional cases of leukemia were identified among workers who were not included in the cohort (one died after the end of the follow-up period and another was still alive). Mortalities from causes other than lymphatic/hematopoietic cancer were not significantly increased.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most studies were inadequate for evaluation. As summarized by IARC (1982, 1987), many dermal carcinogenicity studies of chemicals other than benzene used benzene as a vehicle, and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

3.3 GENOTOXICITY

The genotoxic effects of benzene have been studied extensively. The *in vivo* and *in vitro* data are summarized in Tables 3-4 and 3-5, respectively. In chronically-exposed humans, benzene and/or its metabolites primarily cause chromosomal aberrations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Major et al. 1992, 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). Chromosomal aberrations in humans are frequently demonstrated in peripheral blood lymphocytes and bone marrow. Although inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans, available *in vivo* human data 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. However, Qu et al. (2003a, 2003b) noted a concentration-related increase in chromosomal aberrations across a wide range of exposure concentrations, including workers with relatively low-level benzene exposure. Limitations of many of the occupational studies include lack of accurate exposure data, possible coexposure to other chemicals, and lack of appropriate control groups.

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Prokaryotic cells:			
Escherichia coli (host mediated DNA repair)	DNA synthesis	–	Hellmér and Bolcsfoldi 1992a
Invertebrate animal cells:			
Drosophila melanogaster	Sex-linked recessive lethal	–	Kale and Baum 1983
Spermatocytes	Recombination	–	
Spermatogonia	Recombination	+	
Spermatocytes	Heritable translocation	–	
Mammalian cells:			
Mouse (bone marrow)	Chromosomal aberrations	+	Giver et al. 2001; Shelby and Witt 1995; Siou et al. 1981
Mouse (bone marrow)	Chromosomal aberrations	+[a]	Meyne and Legator 1980
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1980; 1982
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991; Rithidech et al. 1987
Mouse (lymphoid cells, myeloid cells)	Chromosomal aberrations	+	Giver et al. 2001
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1990; Hoechst 1977; Philip and Jensen 1970; Styles and Richardson 1984
Rat (bone marrow)	Chromosomal aberrations	+[b]	Anderson and Richardson 1981[b]
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; 1973
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Bogadi-Šare et al. 1997; Ding et al. 1983; Forni et al. 1971a; Kašuba et al. 2000; Picciano 1979; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Smith et al. 1998; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Zhang et al. 1998a, 1999
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	–	Bogadi-Šare et al. 1997; Jablonická et al. 1987
Mouse (bone marrow)	Micronuclei	+	Shelby and Witt 1995; Shelby et al. 1993
Mouse (bone marrow PCEs)	Micronuclei	+[c]	Ciranni et al. 1988
Mouse[d] (bone marrow PCEs)	Micronuclei	+[e]	Suzuki et al. 1989

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Mouse (bone marrow PCEs)	Micronuclei	+	Au et al. 1990; Barale et al. 1985; Chen et al. 1994; Diaz et al. 1980; Erexson et al. 1986; Farris et al. 1996; Harper et al. 1984; Hite et al. 1980; Siou et al. 1981; Toft et al. 1982
Mouse (bone marrow PCEs)	Micronuclei	+[a]	Meyne and Legator 1980
Mouse (bone marrow PCEs)	Micronuclei	+[f]	Eastmond et al. 2001
Mouse (bone marrow NCEs)	Micronuclei	+	Farris et al. 1996
Mouse (bone marrow NCEs)	Micronuclei	+[f]	Eastmond et al. 2001
Mouse (pregnant/bone marrow PCEs)	Micronuclei	(+)	Ciranni et al. 1988
Mouse (peripheral blood)	Micronuclei	+	Hayashi et al. 1992; Healy et al. 2001
Mouse (peripheral blood PCEs)	Micronuclei	+	Farris et al. 1996
Mouse (peripheral blood PCEs)	Micronuclei	+[c,g]	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei	+	Barale et al. 1985; Choy et al. 1985; Farris et al. 1996; Rithidech et al. 1988
Mouse (peripheral blood NCEs)	Micronuclei	+[c,f]	Luke et al. 1988a
Mouse (lung fibroblasts)	Micronuclei	+	Ranaldi et al. 1998
Mouse (fetus/liver cells)	Micronuclei	+	Ciranni et al. 1988
Rat (lymphocytes)	Micronuclei	+	Erexson et al. 1986
Chinese hamster (bone marrow)	Micronuclei	+	Siou et al. 1981
Human (lymphocytes)	Micronuclei	+	Robertson et al. 1991
Human (occupational exposure/lymphocytes)	Micronuclei	+	Liu et al. 1996
Human (occupational exposure/lymphocytes)	Micronuclei	–	Pitarque et al. 1996; Surrallés et al. 1997
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980; 1982
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
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	+	Popp et al. 1992
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	–	Kašuba et al. 2000; Pitarque et al. 1997; Seiji et al. 1990; Yardley-Jones et al. 1988
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (lung tissue)	Mutations	+	Mullin et al. 1998
Mouse embryo (premelanocytes)	Mutations (deletions)	+	Schiestl et al. 1997
Human (bone marrow)	Mutations (gene-duplicating)	+	Rothman et al. 1995

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Human (bone marrow)	Mutations (gene-inactivating)	–	Rothman et al. 1995
Mouse (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Mouse (white blood cells)	DNA adducts	+	Lévay et al. 1996
Mouse (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Mani et al. 1999; Turteltaub and Mani 2003
Rat (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Rat (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lutz and Schlatter 1977; Mani et al. 1999; Mazzullo et al. 1989; Turteltaub and Mani 2003
Mouse (peripheral blood lymphocytes)	DNA strand breaks	+	Tuo et al. 1996
Rat (lymphocytes, bone marrow, spleen, liver)	DNA strand breaks	+	Lee et al. 2005
Human (occupational exposure/lymphocytes)	DNA strand breaks	+	Andreoli et al. 1997; Nilsson et al. 1996; Sul et al. 2002
Mouse (peripheral blood lymphocytes, bone marrow)	DNA damage	+	Chang et al. 2005
Human (occupational exposure/lymphocytes)	DNA repair efficiency	_	Hallberg et al. 1996
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Human (occupational exposure/lymphocytes)	DNA oxidative damage	+	Liu et al. 1996
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
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
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]Males affected to a significantly greater degree than females.
[d]Two strains of mouse were tested; Ms/Ae and CD-1. The result applies to both strains.
[e]This result was observed following both oral and intraperitoneal exposure; however, oral exposure produced the greater effect.
[f]Increase in micronuclei was exposure duration-dependent.
[g]Increase in micronuclei was exposure duration-independent.

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

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
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	Kaden et al. 1979; Seixas et al. 1982
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	Hellmér and Bolcsfoldi 1992b
Plasmid DNA ΦX-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	+	Eastmond et al. 1994; Morimoto 1976
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Chinese hamster (ovary cell culture)	Micronuclei	–	–	Douglas et al. 1985
Human (whole blood cells)	Micronuclei	–	–	Zarani et al. 1999
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Douglas et al. 1985; Gulati et al. 1989
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
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Rat (hepatocytes)	DNA breaks	No data	–	Bradley 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+[a]	Lakhanisky and Hendricks 1985
Chinese hamster (V79 cell culture)	DNA breaks	–	–	Swenberg et al. 1976
Mouse (L5178Y cell culture)	DNA breaks	No data	–	Pellack-Walker and Blumer 1986
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Human (lymphocyte cell culture)	DNA repair	No data	_	Hallberg et al. 1996
Rat liver epithelial cells	DNA hyperphos-phorylatioin	No data	+	Dees and Travis 1994
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert et al. 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	–	Probst and Hill 1985; Williams 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

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat, rabbit (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

Chromosomal aberrations observed in workers chronically exposed to benzene include hypo- and hyperdiploidy, deletions, breaks, and gaps. For example, analysis of peripheral lymphocytes of workers exposed to benzene vapors at a mean concentration of 30 ppm revealed significant increases in monosomy of chromosomes 5, 7, and 8 (but not 1), and tri- and/or tetrasomy of chromosomes 1, 5, 7, and 8 (Zhang et al. 1998b, 1999). In another series of epidemiological studies in workers chronically exposed to benzene, nonrandom effects were apparent in chromosomes 1, 2, 4, and 9; nonrandom breaks in chromosomes 2, 4, and 9 were twice as prevalent in benzene-exposed workers versus controls; and chromosomes 1 and 2 were nearly twice as prone to gaps (Sasiadek and Jagielski 1990; Sasiadek et al. 1989). Twenty-one people with hematological signs of chronic benzene poisoning exhibited significantly more chromosomal abnormalities than controls (Ding et al. 1983). A significant increase in dicentric chromosomes and unstable aberrations was noted in 36 female workers exposed to benzene in a shoe factory for up to 32 years (Kašuba et al. 2000). Significant increases in hyperploidy of chromosomes 8 and 21 and translocations between chromosomes 8 and 21 were observed in workers exposed to benzene vapors at a mean TWA of 31 ppm (Smith et al. 1998).

DNA repair efficiency was evaluated in blood lymphocytes collected from exposed or unexposed workers in a petrochemical plant (Hallberg et al. 1996). Plasmids ($pCMV_{CAT}$) were irradiated with UV light (254 nm) to induce thymidine dimers and were then transfected into blood lymphocytes from workers. Transfected plasmids that were repaired in the lymphocytes would express the chloramphenicol actetyltransferase reporter gene (CAT) product whereas unrepaired plasmids would not. Lymphocytes from exposed or unexposed workers did not show significant differences in their ability to repair light-damaged DNA; however, the authors suggest that the sample population was too small to detect any differences given the large individual variations in repair capacity (Hallberg et al. 1996).

Oxidative DNA damage was assessed in workers (n=87) exposed to benzene by conducting measurements of 8-hydroxy-2-deoxyguanosine (8-OHdG) in peripheral blood lymphocytes (Liu et al. 1996). 8-OHdG is formed by hydroxy radical (OH·) addition at the C-8 position of deoxyguanosine (Kasai and Nishimura 1986) and appears to be a biomarker of oxidative DNA damage (Liu et al. 1996). The exposure to benzene was classified as low, medium, or high (mean benzene levels of 2.46, 103, or 424 mg/m^3, respectively [corresponding to 0.78, 32.2, or 133 ppm]). Levels of 8-OHdG in exposed workers rose in a concentration-related manner although the increases were significant only in the medium and high exposure groups. Toluene, also detected in the workplace air, did not alter levels of 8-OHdG. Formation of micronuclei, a measure of DNA damage, increased in a concentration-related manner to levels that were significantly higher than those in controls. The levels of urinary *trans,trans*-

muconic acid, a biomarker of benzene exposure, were well correlated with levels of 8-OHdG which, in turn, were well correlated with levels of lymphocyte micronuclei. The findings of Liu et al. (1996) are supported by the results of a study by Nilsson et al. (1996) in which concentration-related increased urinary levels of 8-OHdG were reported in male workers (n=30) at a gasoline station. Breathing zone benzene concentrations ranged from 0.003 to 0.6 ppm (mean 0.13 ppm). Significant concentration-related increases in DNA single-strand breaks were also noted in these workers. Collectively, the results of Liu et al. (1996) and Nilsson et al. (1996) provide suggestive evidence that benzene metabolites may induce reactive oxygen species, which could result in oxidative DNA damage and formation of hydroxylated bases such as 8-OHdG (Liu et al. 1996).

Rothman et al. (1995) used the glycophorin A (GPA) gene loss mutation assay to assess the nature of DNA damage in workers heavily exposed to benzene. The GPA assay measures the frequency of variant cells that have lost the expression of the M form of the GPA gene in the peripheral blood of heterozygous (MN) subjects. The variant cells possess the NN phenotype (double-copy expression of the N allele and no expression of M) or the NØ phenotype (single-copy expression of the N allele and no expression of M). The NN variants are thought to arise from mitotic recombination, chromosome loss, and reduplication, or gene conversion. The NØ variants appear to be associated with point mutations, deletions, or gene inactivation. Rothman et al. (1995) demonstrated a significant increase in the frequency of NN cells in benzene-exposed workers (compared with unexposed control subjects) in the absence of a significant effect on the frequency of NØ cells. These results suggest that benzene induces gene-duplicating, but not gene-inactivating, mutations at the GPA locus in benzene-exposed humans.

Sister chromatid exchange was not found to be a significant effect of benzene exposure in humans (Kašuba et al. 2000; Seiji et al. 1990; Yardley-Jones et al. 1988); however, the selection of control subjects in the studies by Seiji et al. (1990) and Yardley-Jones et al. (1988) was poor. Refer to Table 3-4 for a further summary of these results.

In vivo animal studies provide convincing evidence of benzene's genotoxicity (Table 3-4). Furthermore, the finding that male mice are more sensitive than females to benzene-induced chromosomal damage is consistent among reports (Armstrong and Galloway 1993; Barale et al. 1985; Choy et al. 1985; Ciranni et al. 1988; Hatakeyama et al. 1992; Meyne and Legator 1980; Siou et al. 1981). Consistently positive findings for chromosomal aberrations in bone marrow and lymphocytes in animals support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Micronucleus assays 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 micronucleus assays (Luke et al. 1988a) investigated the effects of different inhalation exposure durations on polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) in peripheral blood. PCEs are newly formed erythrocytes that contain mRNA and as a result, exhibit staining when RNA staining reagents are used. NCEs are mature erythrocytes that lack mRNA and are not stained under the same conditions. 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. 1990a). 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). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Arfellini et al. (1985) noted that binding to RNA and proteins was more prevalent than binding to DNA. Lévay et al. (1996) observed dose- and time-dependent formation of two DNA adducts in white blood cells and bone marrow cells of mice administered benzene for 7 days via intraperitoneal injection. Turteltaub and Mani (2003) consistently observed DNA and protein binding in mice at intraperitoneal doses as low as 5 µg/kg body weight. 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 3-4.

Molecular mechanisms of benzene-induced genotoxicity have been studied to some extent in laboratory animals. Schiestl et al. (1997) noted benzene-induced reversion of the mouse pink-eye unstable mutation in premelanocytes of mouse embryos following intraperitoneal injection of the chemical into pregnant dams; the reversions resulted from genomic DNA deletions. Results of Chen et al. (1994) and Eastmond et al. (2001) indicate that benzene-induced micronuclei in mouse bone marrow erythrocytes are formed predominantly from chromosome breakage, but also from aneuploidy.

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 *trans*,*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.

Several studies revealed a possible connection between certain benzene metabolites and DNA damage via the formation of oxygen radicals. Lewis et al. (1988) found that both 1,2,4-benzenetriol and hydroquinone produce DNA strand breaks, 1,2,4-benzenetriol to a greater degree than hydroquinone. Consistent with these findings was the observation that 1,2,4-benzenetriol generates a greater concentration of oxygen radicals than hydroquinone. The study concluded that 1,2,4-benzenetriol damages DNA by producing oxygen radicals, while hydroquinone probably exerts its genotoxic effects by some other mechanism (Lewis et al. 1988). However, results of Li et al. (1995b) indicate that much of the benzene-mediated DNA damage may result from the generation of reactive oxygen species via a copper-redox cycling mechanism involved in the oxidation of the benzene metabolite, hydroquinone. Increased recombination, which can lead to adverse genetic changes, was observed in Chinese hamster ovary cells exposed to phenol, catechol, or benzoquinone (Winn 2003). Benzene had no effect on recombination. The observed increases in recombination were abolished by the addition of catalase to the cells, suggesting that the effect of the benzene metabolites was elicited by oxidative stress (Winn 2003).

Oxidative damage (manifested as DNA strand breaks) was observed in HL60 cells treated with 1,4-benzoquinone or 1,4-hydroquinone (Hiraku and Kawanishi 1996). 1,4-Benzoquinone and hydroquinone induced DNA strand breaks in Chinese hamster ovary cells while phenol, catechol, 1,2,4-benzenetriol, *trans,trans*-muconic acid, and *S*-phenyl mercapturic acid did not (Sze et al. 1996). No synergism between hydroquinone and the other metabolites was observed.

Phenol, catechol, hydroquinone, and benzene induced morphological transformation and gene mutations in Syrian hamster embryo cells (Tsutsui et al. 1997). Chromosomal aberrations, sister chromatid exchange, and unscheduled DNA synthesis were increased by the benzene metabolites while aneuploidy was observed in cells treated with benzene or catechol (Tsutsui et al. 1997). There was no significant increase ($p>0.005$) in DNA fragmentation in human respiratory epithelial cells exposed to an atmosphere of 5 mg benzene/m^3 for 8 hours, although there was evidence of an inflammatory response (Gosepath et al. 2003). Chromosomal breaks and hyperdiploidy were observed in human lymphocytes after exposure to hydroquinone *in vitro* (Eastmond et al. 1994). Aneusomy of chromosomes 7 and 8 were observed in human umbilical cord blood cells treated with hydroquinone (2, 10, or 50 μM) for 72 hours (Smith et al. 2000). Monosomy 7 and trisomy 8 are two common clonal aberrations observed in myeloid leukemias (Smith et al. 2000). Hydroquinone (26–49 μM) also induced monosomy of chromosomes 5, 7, and 8 in a human lymphoblast cell line (Stillman et al. 1997). Hydroquinone (10–100 μM) and 1,2,4-benzenetriol (10–50 μM) significantly increased monosomy 5 and 7 in human lymphocytes and long-arm deletions in chromosomes 5 and 7 (Zhang et al. 1998b). Benzene metabolites have been shown to form DNA adducts in human bone marrow and HL-60 cells (Bodell et al. 1993; Lévay and Bodell 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 in a dose-related manner. 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). Benzene (1–5 mM) did not elicit micronuclei formation in whole blood cells treated for 48 hours with or without metabolic activation with 10% S9 rat liver fraction for 2 hours (Zarani et al. 1999). The assay was conducted with blood collected from four subjects. 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. More recently,

Eastmond et al. (2001) demonstrated decreased activity of topoisomerase II activity in nucleated bone marrow cells of mice administered benzene by oral gavage for subchronic durations.

Available *in vitro* data 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 3-5 for the results of these and other *in vitro* studies.

In summary, 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. 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 3.4) 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.

3.4 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. Benzene is readily absorbed following inhalation or oral exposure. Although benzene is also readily absorbed from the skin, a significant amount of a dermal application evaporates from the skin surface. Absorbed

benzene is rapidly distributed throughout the body and tends to accumulate in fatty tissues. The liver serves an important function in benzene metabolism, which results in the production of several reactive metabolites. Although it is widely accepted that benzene toxicity is dependent upon metabolism, no single benzene metabolite has been found to be the major source of benzene hematopoietic and leukemogenic effects. At low exposure levels, benzene is rapidly metabolized and excreted predominantly as conjugated urinary metabolites. At higher exposure levels, metabolic pathways appear to become saturated and a large portion of an absorbed dose of benzene is excreted as parent compound in exhaled air. Benzene metabolism appears to be qualitatively similar among humans and various laboratory animal species. However, there are quantitative differences in the relative amounts of benzene metabolites.

3.4.1 Absorption

3.4.1.1 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. 1994; 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; Yu and Weisel 1996). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects who 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%(Nomiyama and Nomiyama 1974a). In a similar study, three healthy nonsmoking 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). Yu and Weisel (1996) measured the uptake of benzene by three female subjects exposed to benzene in smoke

generated by burning cigarettes, which resulted in airborne benzene concentrations in the range of 32–69 ppm. Average absorption for exposure periods of 30 or 120 minutes was 64% and did not appear to be influenced by exposure duration.

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 work phases were measured at five Finnish garages. Blood samples from car mechanics (eight 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 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six 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. 1994). 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 6 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 levels of benzene measured 758–1,670 µg/m^3 (235–518 ppb) in the shower stall at 18–20 minutes, 366–498 µg/m^3 (113–154 ppb) in the bathroom at 10–25 minutes, 81–146 µg/m^3 (25–45 ppb) in the bedroom at 25.5–30 minutes, and 40–62 µg/m^3 (12–19 ppb) in the living room at 36–70 minutes. The individual who took the 20-minute shower had estimated inhalation doses of 79.6, 105, and 103 µg (mean=95.9 µg) for the 3 consecutive sampling days. These doses were estimated 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 [99 ppb]) gave a total average shower-related inhalation dose of 113 µg. An average dermal dose of 168 µg was estimated 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/hour, an exposure duration of 0.33 hours, and a unit conversion factor of 11/1,000 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. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

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 as 110.9 and 116.4 ng/L, respectively for nonsmokers, and 328.8 and 405.4 ng/L, respectively for smokers. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations ranging from 0.2 to 4.9 mg/L in eight 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 µg/L (range ND–0.055 µg/L), 0.18 µg/L (range 0.063–1.1 µg/L), and 0.066 µg/L (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.

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 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/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.1.2 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 (in corn oil) 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. 1994). Although no information was located regarding the extent of oral absorption of benzene in aqueous solutions, it is reasonable to assume that oral absorption from water solutions would be nearly 100%.

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.

3.4.1.3 Dermal Exposure

Studies conducted *in vivo* in humans and *in vitro* using human skin indicates that benzene can be absorbed dermally. 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 (Lodén 1986).

In vivo experiments on four 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 work phases were measured at five Finnish garages. Blood samples from car mechanics (eight 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 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

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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 (Lodén 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. The study author indicated that evaporation of benzene did not exceed 5%. 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 theoretical 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 (unoccluded) 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.

McDougal et al. (1990) estimated permeability constants of 0.15 and 0.08 cm/hour for rat and human skin, respectively, based on the appearance of benzene in the blood of rats dermally exposed to benzene vapors at a concentration of 40,000 ppm for 4 hours. A physiologically based pharmacodynamic (PBPK) model was used to estimate the permeability of the vapor in rat and human skin. These results indicate that dermal absorption of benzene may be greater in rats than humans. Therefore, results in rats may provide a conservative estimate of dermal absorption of benzene in humans.

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 to retain 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. 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 for humans exposed to benzene to be 0.037. 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.

3.4.2 Distribution

3.4.2.1 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). Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Furthermore, benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b; Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998) and mice (McDonald et al. 1994). The relatively widespread distribution of benzene and its metabolites to other tissues and organs indicates that

protein adduct formation in the blood does not adversely affect distribution, although no confirming studies were located.

Results from animal studies indicate that absorbed benzene is distributed among 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 of pregnant mice to 2,000 ppm benzene, parent compound and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in well-perfused 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/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 1–3 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/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).

Benzene was detected in the liver, lung, and blood of rats and mice examined immediately following a 6-hour exposure to benzene vapors at a concentration of 50 ppm (Sabourin et al. 1988). Sabourin and coworkers (Sabourin et al. 1987, 1988) also examined effects exposure concentration, exposure rate, and route of administration on the comparative metabolism of benzene in rats and mice. Results of these studies are summarized in Section 3.4.3 (Metabolism).

3.4.2.2 Oral Exposure

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

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.

3.4.2.3 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 soil-adsorbed

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 three groups, <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 remaining carcass.

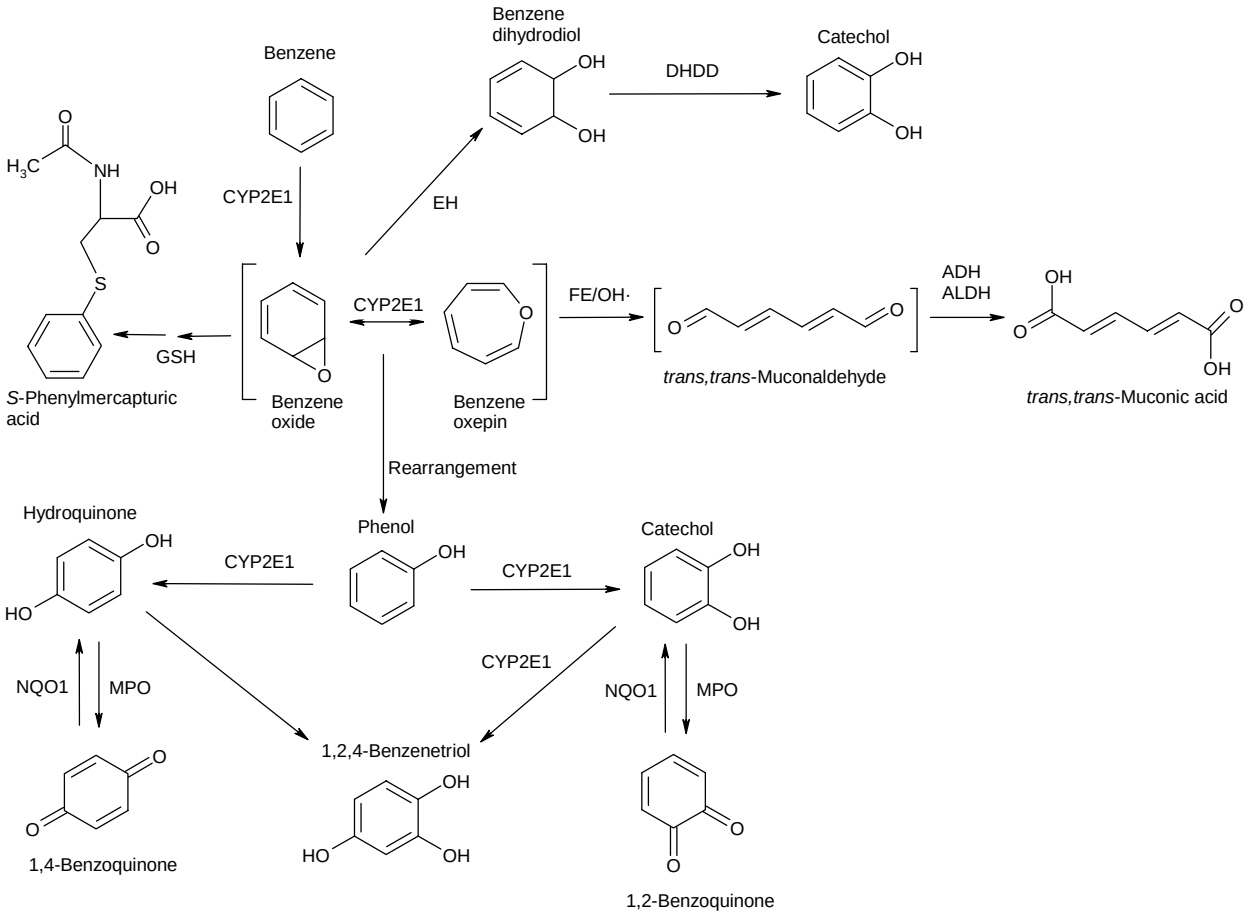
3.4.3 Metabolism

Although the metabolism of benzene has been studied extensively, the steps leading to benzene toxicity are not yet fully understood. It is generally understood that both cancer and noncancer effects are caused by one or more reactive metabolites of benzene. Available data indicate that metabolites produced in the liver are carried to the bone marrow where benzene toxicity is expressed. Benzene metabolism may occur, at least in part, in the bone marrow. Benzene metabolism has been demonstrated in isolated perfused rabbit lung preparations (Powley and Carlson 2002). As discussed in detail in Section 3.5, available evidence suggests that multiple benzene metabolites may collectively be responsible for the expression of benzene toxicity.

Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites (but also as parent compound in small amounts) 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).

The metabolic scheme shown in Figure 3-3 is based on results of numerous mechanistic studies of benzene metabolism (see Henderson et al. 1989; Huff et al. 1989; and Ross 1996, 2000 for comprehensive reviews of benzene metabolism). The first step is the cytochrome P-450 2E1 (CYP2E1) catalyzed oxidation of benzene to form benzene oxide (Lindstrom et al. 1997), which is in equilibrium with its oxepin (Vogel and Günther 1967). Several pathways are involved in the metabolism of benzene oxide. The predominant pathway involves nonenzymatic rearrangement to form phenol (Jerina et al. 1968), the major initial product of benzene metabolism (Parke and Williams 1953a). Phenol is oxidized in the presence of CYP2E1 to catechol or hydroquinone, which are oxidized via myeloperoxidase (MPO) to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively (Nebert et al. 2002). The reverse

Figure 3-3. Metabolic Pathways for Benzene



ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQ01 = NAD(P)H:quinone oxidoreductase

Source: adapted from Nebert et al. 2002; Ross 2000

reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NAD(P)H:quinone oxidoreductase (NQ01) (Nebert et al. 2002). Both catechol and hydroquinone may be converted to the reactive metabolite 1,2,4-benzenetriol via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalyzed conversion to catechol (Nebert et al. 2002; Snyder et al. 1993a, 1993b). Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation (Nebert et al. 2002; Schrenk and Bock 1990); the conjugates of phenol and hydroquinone are major urinary metabolites of benzene (Sabourin et al. 1989a; Wells and Nerland 1991). Other pathways of benzene oxide metabolism include: (1) reaction with glutathione (GSH) to form S-phenylmercapturic acid (Nebert et al. 2002; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al. 1993), and (2) iron-catalyzed ring-opening conversion to *trans*,*trans*-muconic acid, presumably via the reactive *trans*,*trans*-muconaldehyde intermediate (Bleasdale et al. 1996; Nebert et al. 2002; Ross 2000; Witz et al. 1990b, 1990c, 1996).

Results of several studies provide strong evidence for the involvement of CYP2E1 in the oxidation of benzene. For example, no signs of benzene-induced toxicity were observed in transgenic CYP2E1 knockout mice (that do not express hepatic CYP2E1 activity) following exposure to benzene vapors (200 ppm, 6 hours/day for 5 days) that caused severe genotoxicity and cytotoxicity in wild-type mice (Valentine et al. 1996a, 1996b). Pretreatment of mice with CYP inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity (DNA damage as assessed by the alkaline comet assay) in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986). Immunoinhibition studies in rat and rabbit hepatic microsomes provide additional support to the major role of CYP2E in benzene metabolism (Johansson and Ingelman-Sundberg 1988; Koop and Laethem 1992). Occupationally exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene hematotoxicity than workers not expressing this phenotype (Rothman et al. 1997). *In vitro* studies using human liver microsomes demonstrate a positive correlation between benzene metabolism and CYP2E1 activity (Nedelcheva et al. 1999; Seaton et al. 1994). Although CYP2E1 appears to be the major catalyzing agent in initial benzene metabolism, other CYPs, such as CYP2B1 and CYP2F2, may also be involved (Gut et al. 1996a, 1996b; Powley and Carlson 2000, 2001; Sheets and Carlson 2004; Sheets et al. 2004; Snyder et al. 1993a, 1993b).

CYPs involved in benzene metabolism are found in all tissues. However, the predominant repository is the liver, which is considered to be the primary site of benzene metabolism. By demonstrating that partial hepatectomy diminished both the rate of metabolism of benzene and its toxicity in rats exposed to benzene via subcutaneous injection, Sammett et al. (1979) provided suggestive evidence that one or more benzene metabolites formed in the liver are necessary for toxicity. *In vitro* studies have demonstrated that pulmonary microsomes of humans and laboratory animals are capable of metabolizing benzene, which appears to be catalyzed by both CYP2E1 and CYP2F2 (Powley and Carlson 1999, 2000; Sheets et al. 2004). There is some indication that CYP2E1-catalyzed benzene metabolism may also occur in bone marrow, a major target tissue of benzene toxicity. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) 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. Similar studies have been conducted in mice (Ganousis et al. 1992). 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 are known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mice to benzene-induced hematotoxicity (Zhu et al. 1995). Bernauer et al. (1999, 2000) recently noted the presence of CYP2E1 in bone marrow samples of mice (several strains), rats, rabbits, and humans. However, although Irons et al. (1980) demonstrated that the isolated perfused rat femur was capable of metabolizing a very small amount of benzene (approximately 0.0002% of ^{14}C-benzene was recovered as metabolites), neither benzene oxide nor phenol were detected in a test of benzene metabolism using microsomal preparations of bone marrow from rats (Lindstrom et al. 1999), indicating that bone marrow is not a likely source of initial metabolic oxidation for benzene. No studies were located the potential for human bone marrow tissue to metabolize benzene.

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-*trans,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*-muconaldehyde, 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. 1990c). 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. 1990c). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; Sabourin et al. 1989a; 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±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±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).

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. The analysis of Kenyon et al. (1995) 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. Kenyon et al. (1995) 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).

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 CYP2E1 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 CYP2E1, which also metabolizes alcohol and aniline. CYP2E1 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. 1991; Parke 1989; Snyder et al. 1993a, 1993b). It is possible that hydroxy radical formation by CYP2E1 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 CYPs 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.

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5 and 26%, respectively, in rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. These results suggest 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).

The ultimate disposition and metabolic fate of benzene depends on animal species, dose, and route of exposure. 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. 1990b, 1990c). The effect of species differences in metabolism of inhaled benzene 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). Species differences in benzene metabolism following oral exposure were elucidated in rats and mice 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 rats and 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 supernatant 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).

The effect of dose rate 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 (200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced. Bois and Paxman (1992) used a PBPK model to assess effects of dose rate on the disposition of benzene metabolites. Simulations were performed for rats exposed either for 15 minutes to 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs). The amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level. Differences between the model predictions (Bois and Paxman 1992) and the empirical data of Sabourin et al. (1989a, 1989b) may be related, at least in part, to the higher benzene exposure levels (50, 150, and 600 ppm) used by Sabourin and coworkers.

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 form covalent adducts with nucleic acids and proteins in rats and mice (Norpoth et al. 1988; Rappaport et al. 1996); 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; Creek et al. 1997; Longacre et al. 1981a, 1981b; Sun et al. 1990); bind to proteins in perfused bone marrow preparations (Irons et al. 1980) and in rat and mouse liver DNA *in vivo* (Creek et al. 1997; Lutz and Schlatter 1977); and bind to DNA in rabbit and rat bone marrow mitochondria *in vitro* (Rushmore et al. 1984). Exposure-related increases in blood levels of

albumin adducts of benzene oxide and 1,4-benzoquinone were noted among workers occupationally exposed to benzene air concentrations ranging from 0.07 to 46.6 ppm (Rappaport et al. 2002a, 2002b). Several reactive metabolites of benzene have been proposed as agents of benzene hematotoxic and leukemogenic effects. These metabolites include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans*,*trans*-muconaldehyde. See Section 3.5.2 for a discussion of mechanisms of benzene toxicity.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Available human data indicate that 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). In six male and female volunteers exposed to 52–62 ppm benzene for 4 hours, respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 17%; no gender-related differences were observed (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects who 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 1–200 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 urine samples. Kok and Ong (1994) report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively, in nonsmokers, and 328.8 and 405.4 ng/L, respectively in smokers. 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*-phenylmercapturic acid 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 3.4.3, 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 ($p=0.001$), and nonpregnant ($p=0.001$) 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±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 female smokers (1.13±0.12 mg/g creatinine compared to 1.82±0.14 mg/g creatinine). Benzene levels ranging from 0.01 to 0.18 µg/kg have been detected in samples of human breast milk (Fabietti et al. 2004).

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 an 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 10–1,000 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 11–130 ppm in mice), <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/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.4.2 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 1953a). 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 trihydroxy-benzene (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 1953a).

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.

Mathews et al. (1998) reported similar results following oral (gavage) administration of ^{14}C-benzene to rats, mice, and hamsters in single doses from as low as 0.2 mg/kg and up to 100 mg/kg. For example, >95% of a 0.5 mg/kg dose was recovered in the urine of rats; a small amount (3%) was recovered in expired air. At benzene doses of 10 and 100 mg/kg, elimination in the breath rose to 9 and 50%, respectively, indicating the likely saturation of benzene metabolism. Excretion in the feces was minimal at all dose levels. Similar results were noted for mice and hamsters. Both dose and species differences were noted in the composition of urinary metabolites. Phenyl sulfate was the major metabolite in rat urine at all dose levels, accounting for 64–73% of urinary radioactivity. Phenyl sulfate (24–32%) and hydroquinone glucuronide (27–29%) were the predominant urinary metabolites in mice. At a dose of 0.1 mg/kg, mice produced a considerably higher proportion of muconic acid than rats (15 versus 7%). In hamsters, hydroquinone glucuronide (24–29%) and muconic acid (19–31%) were the primary urinary metabolites. Two additional metabolites (1,2,4-trihydroxybenzene and catechol sulfate) were recovered from the urine of hamsters, but not rats or mice.

3.4.4.3 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 1g 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).

3.4.4.4 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).

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

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

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

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, 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) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

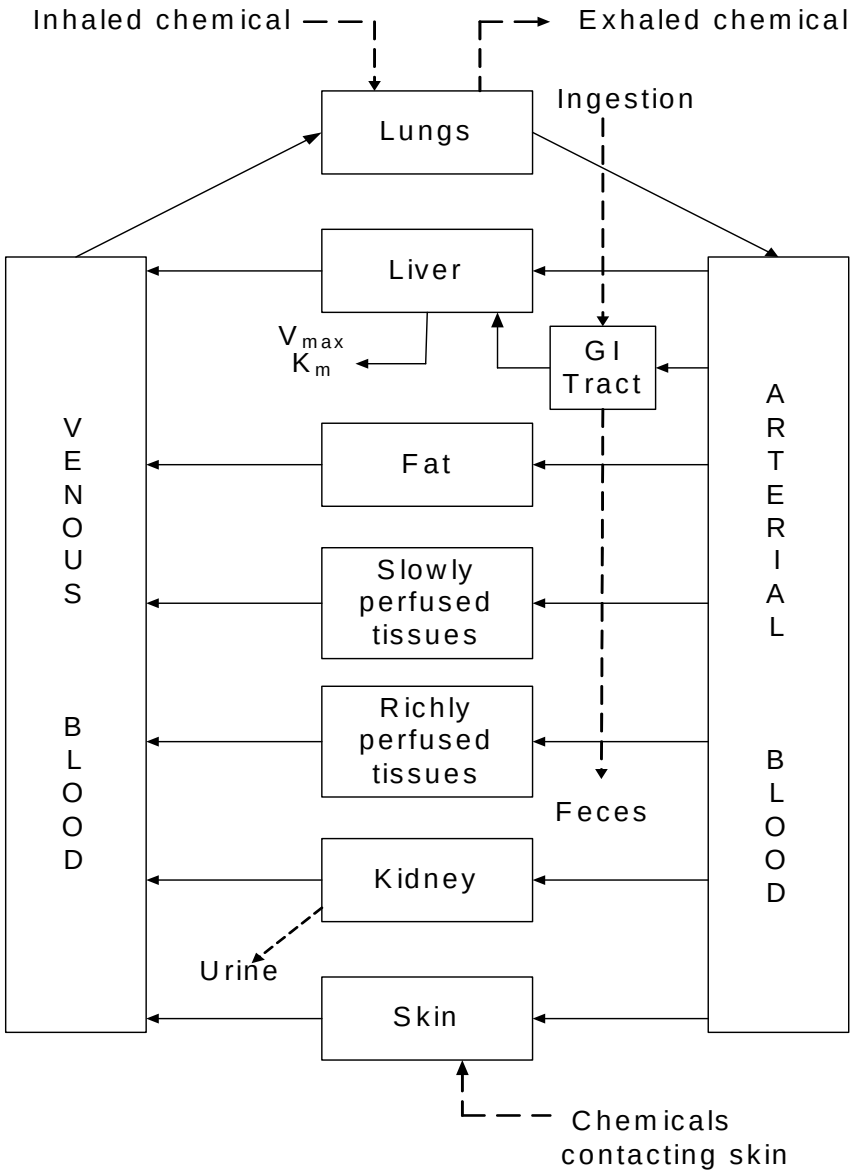
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in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

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

Several PBPK models have been developed that simulate the disposition of benzene in humans (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Medinsky et al. 1989c; Sinclair et al. 1999; Travis et al. 1990), mice (Cole et al. 2001; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990), and rats (Bois et al. 1991a; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990). A comparative summary of the models is provided in Table 3-6. All of the models have the same general structure (Figure 3-5). Most of the models simulate inhalation and oral exposures; one model provides a simulation of dermal absorption (Sinclair et al. 1999). Physiological parameters and partition coefficients for simulating benzene biokinetics of human females were reported for the Brown et al. (1998) and Fisher et al. (1997) models. Flow-limited exchange of benzene between blood and tissues is assumed in all models, with excretion of benzene in exhaled air and, in one case, to breast milk (Fisher et al. 1997). All models include simulations of blood, fat, liver, lung, and lumped compartments representing other slowly-perfused tissues (e.g., skeletal muscle) and rapidly-perfused tissues (e.g., kidneys, other viscera). Simulation of bone marrow, the primary target for benzene toxicity, is included in the models reported by Bois et al. (1991a, 1996), Sinclair et al. (1999), and Travis et al. (1990).

Simulations of metabolism in the various models vary in complexity. In the simplest representation, metabolic elimination of benzene is simulated as a single capacity-limited process, represented with

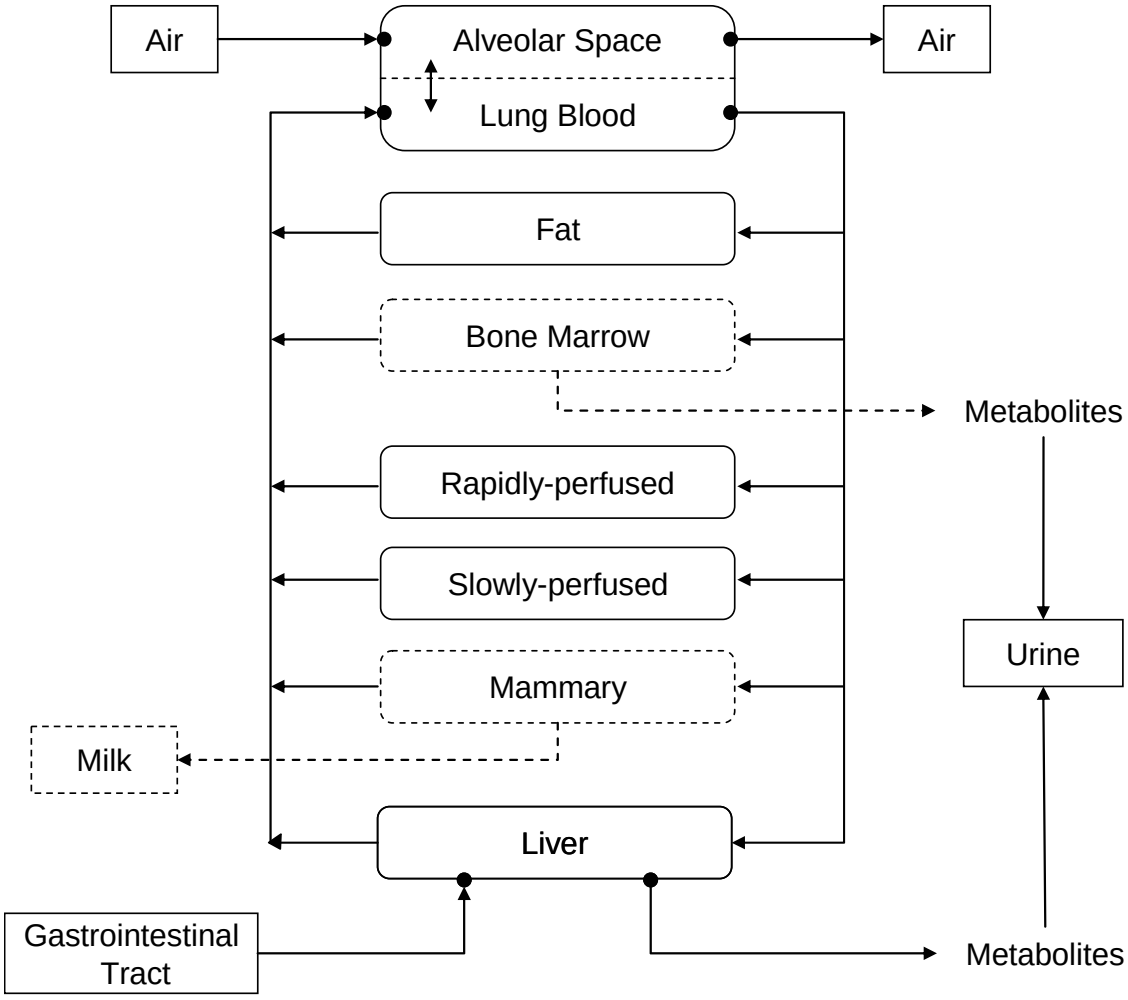
Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



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

Source: adapted from Krishnan and Andersen 1994

Figure 3-5. General Structure of Physiologically Based Pharmacokinetic Models of Benzene*



*Tissues shown with dashed lines are not simulated in all models. Flow-limited exchange of benzene between blood and tissues is assumed. Metabolism is simulated to varying degrees of complexity (see Table 3-6 for model comparison).

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Reference	Simulated areas: Species[a]	Absorption pathways[b]	Tissues[c]	Metabolic pathways[d]		Excretion pathways[e]		Comment
Bois et al. 1991a	R	IH, OR	BL, BM, FA, LI, LU, RP, SP	BM, LI:	BZ→BO(c) BO→BG(c) BO→PH(f) BO→GSH(c) BG→DI(c) PH→HQ(c) PH→CA(c)	EH: UR:	BZ PH	Simulates metabolic pathways in bone marrow, and phenol conjugation in lung and gastrointestinal tract
				BM, LU, LU, GI:	PH→PHCO(c)			
Bois et al. 1996	H	IH	BL, BM, FA, LU, LI, RP, SP	BM, LI:	BZ→M_{tot}(c)	EH:	BZ	Simulates metabolic pathways in bone marrow, and endogenous production of phenolic metabolites
				LI:	PHX_{end}→PH(z)	UR:	M_{tot} PH	
Brown et al. 1998	H (m,f)	IH	BL, FA, LI, LU, RP, SP	LI:	BZ→M_{tot}(f)	EH:	BZ	Simulates males or females
Cole et al. 2001	M	IH, OR	BL, FA, LI, LU, RP, SP	LI:	BZ→BO(c) BO→PH(f) BO→PMA(f) BO→MA(f) PH→HQ(c) PH→PHCO(c) PH→CA(c) CA→THB(c) HQ→HQCO(c)	EH: UR:	BZ CA MA PHCO PMA HQCO THB	All metabolism is assigned to the liver
Fisher et al. 1997	H	IH	FA, LU, LI, RP, SP, MI	LI:	BZ→M_{tot}(c)	EH: MI:	BZ BZ	Simulates transfer of benzene to breast milk
Medinsky et al. 1989a, 1989b, 1989c	H, M, R	IH, OR	FA, LI, LU, RP, SP	LI:	BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c)	EH:	BZ	All metabolism is assigned to the liver

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Reference	Simulated areas: Species[a]	Absorption pathways[b]	Tissues[c]	Metabolic pathways[d]		Excretion pathways[e]		Comment
Sinclair et al. 1999	H	IH, OR, DE	BL, BM, LI, LU, MU, RP	BM, LI:	$BZ \rightarrow M_{tot}$(c)	EH:	BZ	Simulates dermal exposure and absorption
						UR:	M_{tot}	
							PH	
Sun et al. 1990	M, R	IH, OR	BL, FA, LI, LU, RBC, RP, SP	LI:	BZ→BO(c)	EH:	BZ	Simulates formation of hemoglobin adducts in red blood cells derived from benzene oxide
					BO→PHCO(c)			
					BO→PMA(c)			
					BO→HQCO(c)			
					BO→MA(c)			
				RBC:	BO→HBA(c,f)			
Travis et al. 1990	H, M, R	IH, OR	BL, BM, FA, LI, LU, MU, RP	BM, LI:	$BZ \rightarrow M_{tot}$(c)	EH:	BZ	Total metabolism of benzene in the bone marrow and liver

[a]Species simulated: H=human; M=mouse; R=rat; m=male; f=female
[b]Absorption pathways simulated: IH=inhalation; OR=oral; DE=dermal
[c]Tissues simulated: BL=blood; BM=bone marrow; FA=fat; LI=liver; LU=lung; MU=muscle; RBC=red blood cells; RP=other rapidly-perfused tissues; SP=other slowly-perfused tissues
[d]Metabolic pathways simulated: BZ=benzene; BD=benzene diols; BO=benzene oxide; BG=Benzene glycol; CA=catechol; HBA=hemoglobin adduct; HQ=hydroquinone; HQCO=ydroqinione conjugates; MA=muconic acid; M_{tot}=total metabolites; PH=phenol; PHCO=phenol conjugates; PMA=phenylmercapturic acid; THB=trihydroxybenzene; PHX_{end}=endogenous phenolic metabolites; (c)=capacity-limited; (f)=first-order; (z)= zero-order
[e]Excretion pathways simulated: EH=exhalation; MI=breast milk; UR=urine

Michaelis-Menten function of benzene concentration in tissue (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Sinclair et al. 1999; Travis et al. 1990). In the more complex representations, the major pathways of metabolism of benzene, including conjugation reactions, are simulated as capacity-limited or first-order processes (Bois et al. 1991a; Cole et al. 2001; Medinsky et al. 1989a, 1989b, 1989c; Sun et al. 1990). In most of the models, all metabolic pathways are attributed to the liver; however, four of the models include simulations of metabolism in bone marrow (Bois et al. 1991a, 1996; Sinclair et al. 1999; Travis et al. 1990), and one model includes simulations of the formation of sulfate and glucuronide conjugates of phenol in the gastrointestinal and respiratory tracts (Bois et al. 1991a). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. In models that simulate the disposition of the metabolites, metabolites are assumed to be excreted in urine either at a rate equal to their formation (Cole et al. 2001), or in accordance with a first-order excretion rate constant (Bois et al. 1991a, 1996; Sinclair et al. 1999); the difference being, in the latter, the mass balance for formation and excretion of metabolites is simulated, allowing predictions of metabolite levels in tissues. All of the models use typical parameters and values for species-specific blood flows and tissue volumes.

Brief summaries of the models presented in Table 3-6 are provided below, with emphasis on unique features that are applicable to risk assessment.

Medinsky et al. 1989a, 1989b, 1989c

Description of the Model. The Medinsky et al. (1989a, 1989b, 1989c) model simulates absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process; absorption and excretion of benzene in the lung are assumed to be flow-limited. Exchange of benzene between blood and tissues is assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of benzene to benzene oxide as a function of the concentration of benzene in liver. Conversion of benzene oxide to phenol conjugates, phenylmercapturic acid, hydroquinone conjugates, and muconic acid are simulated as parallel, capacity-limited reactions in liver. The model simulates rates of formation of metabolites, but not the disposition (e.g., excretion) of metabolites. Metabolism parameter values (V_{max}, K_m) for the mouse and rat models were estimated by optimization of the model to observations of total metabolites formed in mice and rats exposed by inhalation or oral routes to benzene (Medinsky et al. 1989b; Sabourin

et al. 1987). Human metabolism parameter values were derived from allometric scaling of the values for mice (Medinsky et al. 1989c).

Risk Assessment. The model has been used to predict the amounts of benzene metabolites formed in rats and mice after inhalation or oral exposures (Medinsky et al. 1989a, 1989b). For inhalation concentrations up to 1,000 ppm, mice were predicted to metabolize at least 2–3 times more benzene than rats. For oral doses >50 mg/kg, rats were predicted to metabolize more benzene on a kg-body weight basis than mice. The model also predicts different metabolite profiles in the two species: mice were predicted to produce primarily hydroquinone glucuronide and muconic acid, metabolites linked to toxic effects, whereas rats were predicted to produce primarily phenyl sulfate, a detoxification product. These predictions agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

Validation of the Model. The model was calibrated with data from Sabourin et al. (1987). Bois et al. (1991b) compared predictions made to observations of benzene exhaled by rats following exposures to 490 ppm benzene, reported by Rickert et al. (1979), as well as the data from which the model was calibrated (Sabourin et al. 1987). In general, the model tended to overestimate observations to which it was not specifically fitted.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as amounts of metabolites formed. It does not simulate concentrations of metabolites in these tissues. It does not simulate bone marrow, a target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations of mice, rats, and humans (Medinsky et al. 1989a, 1989b, 1989c).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rodents ranging from 1 to 1,000 ppm and oral gavage doses ranging from 0.1 to 300 mg/kg (Bois et al. 1991b; Medinsky et al. 1989a, 1989b, 1989c).

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Medinsky et al. 1989a, 1989b, 1989c).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested (single dose) benzene, including rates and amounts of major metabolites formed in mice, rats, and humans. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model attributes all metabolism to the liver; (3) the model does not simulate the fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites (e.g., muconaldehyde) in tissues; and (4) the model does not simulate bone marrow, a major target tissue for benzene metabolites.

Sun et al. 1990

Description of the Model. The Sun et al. (1990) model is an extension of the mouse and rat models developed by Medinsky et al. (1989a, 1989b, 1989c). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. Adduct formation is represented as the sum of capacity-limited and first-order functions of the concentration of benzene oxide in the liver. Parameter values were estimated by optimization to measurements of hemoglobin adduct formations in rats and mice exposed to single oral gavage doses of benzene (Sun et al. 1990).

Risk Assessment. The model has been applied to predicting the levels of hemoglobin adducts in mice and rats following inhalation or oral exposures to benzene. This approach could be potentially useful for predicting exposure levels that correspond to measured hemoglobin adduct levels, for use of adducts as an exposure biomarker.

Validation of the Model. The model was calibrated against measurements of hemoglobin adduct formation in mice and rats that received single oral gavage doses of benzene ranging from 0.008 to 800 mg/kg (Sun et al. 1990). The model was evaluated by comparing predictions to observations of amounts of hemoglobin adducts formed in mice and rats exposed to benzene vapor concentrations of 5, 50, or 600 ppm for 6 hours (Sabourin et al. 1989a).

Target Tissues. The model predicts hemoglobin adduct formation after oral and inhalation exposure (Sun et al. 1990).

Species Extrapolation. The model has been applied to simulations for mice and rats.

High-low Dose Extrapolation. The model was calibrated with observations made in mice and rats exposed to single gavage doses ranging from 0.1 to 10,000 μmol/kg (0.008–800 mg/kg), and evaluated for predicting observations in mice and rats exposed by inhalation to 600 ppm benzene.

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. Strengths of the model are that it extends the Medinsky et al. (1989a, 1989b, 1989c) models to simulate hemoglobin adduct formation secondary to formation of benzene oxide. A limitation of the adduct model is that it simulates production of adducts as a function of benzene oxide concentration in liver and does not consider other potential pathways of adduct formation through hydroquinone, phenol, or muconaldehyde.

Travis et al. 1990

Description of the Model. The Travis et al. (1990) model simulates the absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited, as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in bone marrow and liver. The model simulates rates of metabolic elimination of benzene, but not the rates of formation of specific metabolites or their disposition (e.g., excretion). For the purpose of comparing model predictions to observations, 80% of the total metabolite formed in 24 hours (and excreted in urine) was assumed to be phenol. Metabolism parameter values (V_{max}, K_m) were estimated by optimization of the model to observations of total metabolites formed (i.e., excreted in urine) in humans, mice, and rats exposed to benzene by inhalation or oral routes to benzene. The V_{max} for metabolism in bone marrow in humans was assumed to be 4% of that of liver, consistent with optimized values for rodents.

Risk Assessment. This model has been used to predict the amounts of benzene in expired air, concentrations of benzene in blood, and total amount of benzene metabolized following inhalation exposures to humans and inhalation, intraperitoneal, oral gavage, or subcutaneous exposures in mice or

rats (Travis et al. 1990). Cox (1996) applied the model to derive internal dose-response relationships for benzene in humans.

Validation of the Model. The model was evaluated by comparing predictions with observations made in mouse and rat inhalation studies (Rickert et al. 1979; Sabourin et al. 1987; Sato et al. 1975; Snyder et al. 1981); mouse oral gavage studies (Sabourin et al. 1987); mouse subcutaneous injection studies (Andrews et al. 1977); and rat intraperitoneal injection studies (Sato and Nakajima 1979). Predictions of benzene in expired air and/or blood concentrations were also compared to observations made in humans who inhaled concentrations ranging from 5 to 100 ppm benzene (5 ppm: Berlin et al. 1980; Sherwood 1972; 25–57 ppm: Sato et al. 1975; Sherwood 1972; Nomiyama and Nomiyama 1974a, 1974b; 99–100 ppm: Sherwood 1972; Teisinger and Fiserova-Bergerova 1955). Further evaluations of predictions of benzene in workers are reported in Sinclair et al. (1999) and Sherwood and Sinclair (1999), who compared model predictions with observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; and amounts of metabolites formed in liver and bone marrow. It does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for mice, rats, and humans (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans ranging from 1 to 1,110 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990). Evaluations of predictions in rodents included observations made during inhalation exposures that ranged from 11 to 1,000 ppm and oral gavage doses that ranged from 0.5 to 300 mg/kg.

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by these routes (Travis et al. 1990).

Strengths and Limitations. Strengths of the model are that it simulates (1) disposition of inhaled and ingested (single dose) benzene in mice, rats, and humans; and (2) concentrations of benzene, and

rates and amount of benzene metabolized in bone marrow, a target tissue for benzene metabolites. Limitations of the model include: (1) the model simulates metabolic elimination of benzene, but not the rates of formation of major metabolites; and (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues.

Fisher et al. 1997

Description of the Model. The Fisher et al. (1997) model extends the model reported by Travis et al. (1990) to include a simulation of lactational transfer of benzene to breast milk in humans. Other tissues simulated include blood, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited, as is excretion of benzene in breast milk. The lactational transfer model includes simulations of breast milk production and loss from nursing; the latter is represented as a first-order process. Estimates of blood:air and blood:milk partition coefficients during lactation (from which the milk:blood partition coefficient could be calculated) were measured in nine lactating subjects (Fisher et al. 1997). The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites and their disposition (e.g., excretion) are not simulated. Metabolism parameter values (K_m,V_{max}) and tissue:blood partition coefficients were derived from Travis et al. (1990).

Risk Assessment. This model has been used to predict benzene concentrations in breast milk and lactational transfers to breast feeding infants (Fisher et al. 1997). Exposures to the threshold limit value (TLV) (10 ppm, 8 hours/day, 5 days/week) were predicted to yield 0.053 mg of benzene in breast milk per 24 hours. This approach has potential applicability to assessing lactational exposures to infants resulting from maternal exposures.

Validation of the Model. The lactation model was evaluated (Fisher et al. 1997) by comparing predictions for perchloroethylene (not benzene) with those predicted by a perchloroethylene model developed by Schreiber (1993). Other components of the biokinetics model were derived from the Travis et al. (1990) model, which has undergone evaluations against data obtained from studies in humans.

Target Tissues. The model simulates concentrations of benzene in blood, breast milk, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic

elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans (Fisher et al. 1997).

High-low Dose Extrapolation. The lactational model has not been evaluated for simulating inhalation exposures to benzene in humans; therefore, applicability to high-low dose extrapolations cannot be assessed.

Interroute Extrapolation. The model was developed to simulate inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates the disposition of inhaled benzene in females during lactation, including transfers of benzene to breast milk and nursing infants; concentrations of benzene in blood and tissues; and rates of metabolic elimination of benzene metabolized. Limitations of the model include that the model does not simulate rates of formation of major metabolites and that the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Sinclair et al. 1999

Description of the Model. The Sinclair et al. (1999) model is an extension of the human model developed by Travis et al. (1990) to include a simulation of first-order urinary excretion of total metabolites and phenol, and dermal absorption of benzene.

Risk Assessment. The model has been applied to predicting the levels of benzene in exhaled air and phenol in urine in workers exposed to benzene (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Validation of the Model. The model was evaluated against measurements of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; rates of metabolic elimination of benzene in liver and bone marrow; and excretion of total metabolites formed and phenol.

Species Extrapolation. The model has been applied to simulations for humans (Sinclair et al. 1999).

High-low Dose Extrapolation. The model was evaluated against observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Interroute Extrapolation. The model simulates inhalation, oral, and dermal exposures.

Strengths and Limitations. Strengths of the model are that it extends the Travis et al. (1990) model to include simulation of dermal absorption of benzene.

Bois et al. 1991a

Description of the Model. The Bois et al. (1991a) model simulates absorption and disposition of benzene and the benzene metabolite, phenol, in the rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene and phenol are simulated as a first-order function for dose. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene and phenol between blood and tissues. Excretion of phenol is simulated as a first-order transfer to urine. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in bone marrow, liver, gastrointestinal tract, and respiratory tract (see Table 3-6). All pathways are assumed to be capacity-limited reactions, except for the spontaneous hydrolysis of benzene oxide to form phenol, which is simulated as a first-order process. The model simulates rates of formation of metabolites and first-order excretion of phenol; however, disposition (e.g., excretion) of other metabolites is not simulated. Parameter values, including metabolism parameter values, were optimized to a reference set of observations of metabolites formed in rats exposed by inhalation or to single gavage doses of benzene (see below).

Risk Assessment. This model has been used to predict amounts of benzene and phenol metabolites formed in rats during oral gavage exposures to benzene equivalent to those administered in NTP (1986) and to inhalation exposures equivalent to the OSHA PEL (Bois and Paxman 1992; Bois et al. 1991a). Model simulations indicate that dose rate may be an important factor in benzene toxicity. For example, when the model was applied to simulations for rats exposed either for 15 minutes to a benzene vapor concentration of 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs), the amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level (Bois and Paxman 1992). These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). These types of analyses, if extended to humans, would be applicable to evaluations of the adequacy of short-term exposure limits.

Validation of the Model. The model was calibrated (Bois and Paxman 1992; Bois et al. 1991a) with observations made in rats exposed to single oral gavage doses of benzene, or to inhalation exposures of 13–870 ppm (Sabourin et al. 1987, 1989b), in rats administered single parenteral doses of phenol (Cassidy and Houston 1984), and in *in vitro* metabolism studies (Sawahata and Neal 1983). Further evaluations against data not used in the calibration were not reported.

Target Tissues. The model simulates amounts and concentrations of benzene and phenol in bone marrow, a target tissue for benzene metabolites, as well as in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. The model also simulates amounts of specific metabolites formed and urinary excretion of the major urinary metabolite, phenol. It does not simulate concentrations of metabolites, other than phenol, in these tissues.

Species Extrapolation. The model has been applied to simulations for rats. A human model has been developed that implements a scaled-down version of the rat metabolism model (see Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rats ranging from 13 to 870 ppm and oral gavage doses ranging from 15 to 300 mg/kg.

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene (and phenol), including rates and amounts of most of the major metabolites formed in rats. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) although the model simulates the fate of benzene and phenol, it does not simulate the fate of other metabolites formed and, therefore, cannot be used to predict concentrations of these metabolites in tissues; and (3) the model, as configured in Bois et al. (1991a), does not simulate benzene disposition in humans.

Bois et al. 1996

Description of the Model. The Bois et al. (1996) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates metabolic elimination of benzene as a single capacity-limited (i.e., Michaelis-Menten) reaction, occurring in bone marrow and liver. Endogenous formation of phenolic metabolites is also simulated as a zero-order process occurring in liver. The model simulates first-order excretion of total metabolites and the phenol fraction (approximately 80% of total). Parameter values (physiological and chemical) were estimated by Bayesian optimization techniques (Markov Chain Monte Carlo analysis) using reference observations of benzene concentration in blood and urinary excretion of phenol in human subjects who were exposed to benzene in air (Pekari et al. 1992).

Risk Assessment. The model has been used to predict rates and amounts of benzene metabolized in human populations (Bois et al. 1996). The population model (population geometric means and standard deviations of parameter values) was derived using Markov Chain Monte Carlo analysis with observations from three human subjects serving as the reference data for inter-individual variability (from Pekari et al. 1992). The population model predicts probability distributions of model outputs (for example, rates or amounts of benzene metabolized for a given exposure). This approach could be used to evaluate uncertainty factors in risk assessments intended to account for uncertainties in our understanding of benzene pharmacokinetics variability.

Validation of the Model. The model was calibrated with observations of benzene concentrations in blood and urinary phenol levels, made in three human subjects who were exposed to 1.7 or 10 ppm

benzene for 4 hours (Pekari et al. 1992). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in bone marrow, a target of benzene toxicity, as well as blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. Amounts of total metabolites formed and excreted are simulated; however, the model does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for humans (Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in human subjects ranging from 1.7 to 10 ppm (Bois et al. 1996).

Interroute Extrapolation. The model simulates inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene and rates of total metabolism in humans. Limitations include that the model has not been evaluated for multiple exposures and that the model simulates total metabolism of benzene, and not the rates of formation of the major metabolites of benzene of toxicological interest.

Brown et al. 1998

Description of the Model. The Brown et al. (1998) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites, or their disposition (e.g., excretion), are not simulated. For the purpose of comparing model predictions to observations, 80% of the total metabolites formed and excreted in urine (i.e., amount of benzene eliminated by metabolism) in 24 hours was assumed to be phenol. The K_m parameter for metabolism was derived from Travis et al. 1990; the V_{max} was estimated by optimization of the model to observations of blood concentrations of

benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Sato et al. 1975). Partition coefficients for males and females were derived from vial equilibrium studies conducted on blood and/or tissues from males and females (Fisher et al. 1997; Paterson and Mackay 1989).

Risk Assessment. This model has been used to predict the benzene concentrations in blood and amounts of benzene metabolized in females and males who experience the same inhalation exposure scenarios. Females were predicted to metabolize 23–26% more benzene than similarly-exposed males. This difference was attributed, in part, to a higher blood:air partition coefficient for benzene in females.

Validation of the Model. The model was calibrated by comparing predictions of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Brown et al. 1998; Sato et al. 1975). Further evaluations against data not used in the calibration were not been reported.

Target Tissues. The model simulates concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans.

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans. Evaluations of predictions included observations made during inhalation exposures to 25 ppm (Brown et al. 1998; Sato et al. 1975).

Interroute Extrapolation. The model simulates inhalation and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by this route (Brown et al. 1998). Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene in female and male humans as well as the concentrations of benzene and rates of metabolic elimination of benzene metabolized. Limitations of the model include: (1) the model does not simulate

rates of formation of major benzene metabolites; (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues; and (3) the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Cole et al. 2001

Description of the Model. The Cole et al. (2001) model simulates absorption and disposition of benzene in the mouse. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in liver (see Table 3-6). Capacity-limited reactions in bone marrow and liver include benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, catechol to trihydroxybenzene, and conjugation of phenol and hydroquinone. First-order reactions in liver include conversion of benzene oxide to phenol, muconic acid, and phenylmercapturic acid. The model simulates rates of formation of metabolites, tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. Capacity-limited metabolism parameter values were estimated from *in vitro* studies of mouse liver (Lovern et al. 1999; Nedelcheva et al. 1999; Seaton et al. 1995); first-order parameters were estimated by optimization of model output to observations of metabolites formed in mice exposed by inhalation or to single gavage doses (Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Blood:tissue partition coefficients for benzene and metabolites were derived from Medinsky et al. (1989a) or estimated based on the n-octanol-water partition coefficient (Poulin and Krishnan 1995).

Risk Assessment. This model has been used to predict amounts of benzene exhaled and amounts of benzene metabolites produced in mice during inhalation exposures or following oral gavage exposures to benzene (Cole et al. 2001).

Validation of the Model. The model was calibrated with observations made in mice exposed to single oral gavage doses of benzene, or to inhalation exposures (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly perfused and slowly perfused tissues; rates of formation of metabolites; tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. It does not simulate concentrations of metabolites in bone marrow, a target tissue for benzene metabolites.

Species Extrapolation. The model has been applied to simulations for mice (Cole et al. 2001).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice (50 ppm) and oral gavage doses ranging from 0.1 to 100 mg/kg (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988).

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Cole et al. 2001).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene, including rates and amounts of major metabolites. Most of the metabolism parameter values were derived empirically from *in vitro* studies, rather than by model optimization. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model does not simulate the metabolism of benzene in bone marrow, a major target of benzene toxicity; (3) the model, as configured in Cole et al. (2001), does not simulate benzene disposition in humans.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Benzene is readily absorbed via all natural routes of exposure (inhalation, oral, and dermal) and distributed throughout the body via the blood. Based on physical properties such as slight water solubility, high lipid solubility, and nonpolarity, benzene is expected to enter the blood via passive diffusion from gut, lungs, and skin. Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Being moderately lipophilic, benzene tends to accumulate in fatty tissues. However, benzene metabolism is relatively rapid and required for hematopoietic and leukemogenic effects to be expressed. Multiple reactive metabolites appear to be involved in benzene toxicity. As discussed in detail in Section 3.5.2, potential candidates include benzene oxide, phenolic metabolites (phenol,

catechol, hydroquinone, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone), and *trans*,*trans*-muconaldehyde. Both human and animal data demonstrate the importance of CYP2E1 in benzene metabolism (see Section 3.4.3 for a detailed discussion). Metabolism is assumed to take place primarily in the liver, with some secondary metabolism in the bone marrow, the site of characteristic benzene toxicity. Processes involved in transport of hepatic metabolites of benzene to the critical toxicity target (bone marrow) are not known, although some degree of covalent binding of reactive benzene metabolites to blood proteins is expected. At relatively low exposure levels, urinary excretion of conjugated benzene derivatives represents the major excretory pathway for benzene. Biliary excretion represents a minor excretory pathway.

3.5.2 Mechanisms of Toxicity

Numerous mechanistic studies have been conducted in an effort to elucidate mechanisms of benzene-induced hematotoxic and leukemogenic effects, widely recognized as the most critical effects of benzene exposure. Conversely, benzene-induced effects on reproduction, development, and the nervous system have not been studied in sufficient detail to assess mechanisms of toxicity for these end points. The database of information for benzene-induced hematotoxic and leukemogenic effects has been reviewed extensively (e.g., Bird et al. 2005; Irons 2000; Morgan and Alvares 2005; Ross 1996, 2000, 2005; Schnatter et al. 2005; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). It is generally believed that reactive hepatic metabolites of benzene are transported to the major toxicity target (bone marrow). Additional metabolism likely occurs in bone marrow. Phenolic metabolites (phenol, hydroquinone, catechol, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone) appear to play a major role in benzene toxicity. Smith (1996a, 1999b) noted that the phenolic metabolites can be metabolized by bone marrow peroxidases, such as myeloperoxidase (MPO), to highly reactive semiquinone radicals and quinones that stimulate the production of reactive oxygen species. These steps lead to damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself (clastogenic effects such as strand breakage, mitotic recombination, chromosome translocations, and aneuploidy). Damage to stem or early progenitor cells would be expressed as hematopoietic and leukemogenic effects.

Results of several mechanistic studies demonstrate that benzene hematotoxicity is dependent upon metabolism (see Section 3.4.3 for a detailed discussion). Inhibition of benzene metabolism reduced its toxicity (Andrews et al. 1977). Partial hepatectomy decreased both benzene metabolism and toxicity (Sammett et al. 1979). Pretreatment with inducers of metabolism increased both benzene metabolism and

toxicity (Gad-El-Karim et al. 1985, 1986). Inhibition of CYPs (enzymes that catalyze oxidation pathways in benzene metabolism) reduced benzene-induced genotoxicity (Tuo et al. 1996). Mice lacking CYP2E1 or microsomal epoxide hydrolase expression were not susceptible to benzene levels known to cause myelotoxicity and cytotoxicity in wild type mice (Bauer et al. 2003; Valentine et al. 1996a, 1996b). Occupationally-exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene toxicity than those expressing slow CYP2E1 metabolism (Rothman et al. 1997). The enzyme NAD(P)H:quinone oxidoreductase (NQ01), which maintains quinones in reduced form where they are more readily conjugated and excreted (Nebert et al. 2002), is another example of the importance of metabolism in benzene hematotoxicity. Between 22% (Caucasian) and 45% (Asian) of the population is homozygous for an NQ01 allele whereby NQ01 production is negligible. Rothman et al. (1997) found that workers homozygous for an NQ01 allele whereby NQ01 production is negligible (wild type) exhibited a 2.4-fold increased risk for benzene hematotoxicity than workers with the normal genotype. Greater than 7-fold increased risk of benzene hematotoxicity was noted in workers who expressed both rapid CYP2E1 metabolism and the NQ01 wild type (Rothman et al. 1997).

Benzene metabolism involves the production of reactive metabolites that may act directly on cellular macromolecules (proteins and DNA). No single metabolite has been implicated; effects are probably due to many metabolites, which include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans*,*trans*-muconaldehyde. Evidence that benzene oxide may play a role in benzene toxicity includes findings that benzene oxide is a product of oxidative benzene metabolism in mouse, rat, and human liver microsomes (Lovern et al. 1997), benzene oxide can be released from the liver into the blood (Lindstrom et al. 1997), benzene oxide-protein adducts have been found in the blood and bone marrow of mice exposed to benzene (McDonald et al. 1994), and benzene oxide hemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998).

Urinary *trans,trans*-muconic acid has been detected in humans and animals following benzene exposure, although its purported reactive precursor (*trans,trans*-muconaldehyde; see Figure 3-3) has not been detected *in vivo*. The highly reactive *trans,trans*-muconaldehyde, which has been found in mouse hepatic microsomes (Latriano et al. 1986), can undergo reductive and oxidative metabolism (Goon et al. 1992) and has been shown to be hematotoxic (Witz et al. 1985). A small amount (<0.05%) of parenterally-administered *trans,trans*-muconaldehyde to mice reached the bone marrow (Zhang et al. 1997). Rivedal and Witz (2005) found *trans,trans*-muconaldehyde to be a strong inhibitor of gap junction intercellular communication in rat liver epithelial cells.

Phenolics (phenol, catechol, hydroquinone; 1,2,4-benzenetriol; 1,2- and 1,4-benzoquinone) are major metabolites of benzene that have been shown to persist in bone marrow following inhalation exposure (Rickert et al. 1979; Sabourin et al. 1988). Hydroquinone induces chromosomal damage in lymphocytes *in vitro* in a manner similar to that observed in lymphocytes of benzene-exposed workers (Eastmond et al. 1994; Stillman et al. 1997; Zhang et al. 1998b). Kolachana et al. (1993) demonstrated that both hydroquinone and 1,2,4-benzenetriol cause oxidative damage to DNA in mouse bone marrow (*in vivo*) and human myeloid cells (*in vitro*). Glutathione adducts of 1,4-benzoquinone have been shown to be hematotoxic in bone marrow of mice exposed to benzene (Bratton et al. 1997). Additional evidence that phenolic metabolites may play an important role in benzene toxicity includes the finding that MPO, an enzyme found in high concentration in bone marrow (Bainton et al. 1971), catalyzes the oxidation of polyphenols to reactive quinones, semiquinones, and oxygen radicals (Nebert et al. 2002). This can lead to strand breaks and inhibition of topoisomerases and microtubule assembly, which could result in chromosome damage (Chen and Eastmond 1995; Eastmond et al. 2001; Irons and Neptune 1980; Smith 1996a, 1996b). Eastmond et al. (2005) demonstrated that hydroquinone can be readily activated to a potent topoisomerase II inhibitor in the presence of human MPO and H_2O_2 and that partial inhibition occurs at hydroquinone concentrations as low as 50 nM. Irons and Neptune (1980) suggested that benzene-derived hydroquinone may inhibit cell replication by covalently binding to tubulin, a protein essential for spindle formation in mitosis.

Benzene-induced effects on DNA have been studied in some detail. Schwartz et al. (1985) demonstrated that benzene metabolites inhibit mitochondrial DNA polymerase. Hydroquinone has been shown to inhibit ribonucleotide reductase, a key step in DNA synthesis (Li et al. 1997, 1998). Rushmore et al. (1984) found that benzene metabolites covalently bind to mitochondrial DNA and inhibit RNA synthesis. Benzene metabolites bound to hepatic DNA have been observed in animals following inhalation exposure to radiolabeled benzene (Lutz and Schlatter 1977). However, the reported levels of DNA adduct formation appear to be low. For example, Creek et al. (1997) observed adducts to both protein and DNA in the range of nanograms per kilogram in mice given radiolabeled benzene.

Benzene-induced DNA damage may result from the oxidation of DNA by reactive oxygen species that are produced during benzene metabolism. Both 1,4-benzoquinone and hydroquinone are known to increase superoxide, nitric oxide, and hydrogen peroxide in HL-60 cells (Rao and Snyder 1995). Chen et al. (2004) observed nitric oxide-derived benzene metabolites, namely nitrobenzene, nitrophenyl, and nitrophenol isomers in the bone marrow of mice 1 hour following intraperitoneal injection of a 400 mg/kg

dose of benzene. These nitro metabolites were either not detected in other tissues or were present in much smaller concentrations, indicating that they were most likely produced in bone marrow. Brunmark and Cadenas (1988) described a metabolic pathway from hydroquinone leading to the production of glutathionyl-benzenetriol, which can undergo autoxidation leading to superoxide formation. Rao (1996) suggested that benzene-induced DNA damage is mediated by the release of free iron in the bone marrow (probably by polyphenolic metabolites), followed by the chelation of iron by hydroquinone or benzenetriol to yield a reactive oxygen-generating species such as superoxide, which causes oxidative damage to DNA.

The expression of benzene toxicity may involve multiple benzene metabolites. All of the known unconjugated metabolites of benzene, with the exception of phenol and 1,2,4-benzenetriol, have been shown to decrease erythropoiesis (Snyder and Hedli 1996). In mice, the combination of phenol and hydroquinone resulted in exacerbated loss of bone marrow cellularity (Eastmond et al. 1987), increased peroxidatic activation of hydroquinone (Subrahmanyam et al. 1989, 1990), and increased DNA damage (Lévay and Bodell 1992; Marrazzini et al. 1994). Combinations of either phenol and hydroquinone, or phenol and catechol, were more hematotoxic than any of the metabolites given alone (Guy et al. 1991). The combination of hydroquinone and muconaldehyde was the most potent in inhibiting erythropoiesis (Snyder et al. 1989). Catechol was found to stimulate the peroxidase-mediated activation of hydroquinone and produced a synergistic genotoxic effect in lymphocytes (Robertson et al. 1991).

3.5.3 Animal-to-Human Extrapolations

Pathways of benzene metabolism are generally similar among various rodent and nonhuman primate species. However, species differences exist regarding capacity to metabolize benzene and relative proportions of various benzene metabolites formed.

Species differences exist in absorption and retention of benzene. For example, following 6-hour exposures to low concentrations (7–10 ppm) of benzene vapors, mice retained 20% of the inhaled benzene, whereas rats and monkeys retained only 3–4% (Sabourin et al. 1987, 1992). Mice exhibit a greater overall capacity to metabolize benzene, compared to rats. An Inhalation exposure to 925 ppm resulted in an internal dose of 152 mg/kg in mice, approximately 15% of which was excreted as parent compound, and an internal dose of 116 mg/kg in rats, approximately 50% of which was excreted unchanged (Henderson et al. 1992; Sabourin et al. 1987).

The proportions of benzene metabolites produced depend on both species and exposure concentration. Hydroquinones and muconic acid (potential sources of benzene toxicity) were detected in much higher concentrations in the blood, liver, lung, and bone marrow of mice than rats, following a 6-hour inhalation exposure to benzene at a concentration of 50 ppm (Sabourin et al. 1988). It is generally understood that metabolic profiles of benzene in mice and humans are more similar than those of humans and rats. Sabourin et al. (1989a) noted increased production of detoxification metabolites (phenylglucuronide and prephenylmercapturic acid) and decreased production of potentially toxic metabolites (hydroquinones and muconic acid) in both mice and rats exposed to benzene at much higher concentrations (600 ppm in air or 200 mg/kg orally), which indicates that extrapolation of toxicological results from studies using high exposure concentrations to low exposure scenarios may result in an underestimation of risk.

Recent PBPK models have tried to address benzene metabolism in an effort to derive animal-to-human extrapolations (Bois et al. 1991a, 1996; Cole et al. 2001; Medinsky 1995; Medinsky et al. 1989a, 1989b, 1989c; 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 models are not sufficiently refined to allow them to accurately predict human metabolism.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to

the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No information was located to indicate that benzene may adversely affect the endocrine system.

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage

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

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta and can be found in cord blood at concentrations that equal or exceed those of maternal blood (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al.

1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk than adults for benzene toxicity. Results of Infante-Rivard et al. (2005) indicate that maternal exposure to benzene during pregnancy or from 2 years before pregnancy up to birth does not result in increased risk of childhood acute lymphoblastic leukemia (ALL), a frequent form of childhood cancer (Infante-Rivard et al. 2005). However, no information was located regarding the risk of childhood AML, the form of leukemia most frequently associated with exposure to benzene.

Children could potentially be at increased risk for significant benzene exposure via the inhalation route based on higher activity levels and ventilation rates than adults. However, no information was located to indicate that children are at increased risk for benzene toxicity. Age-related differences in benzene metabolism could potentially affect susceptibility. Results of one human study indicate that CYP2E1, a major enzyme involved in benzene metabolism, is not present in the fetus, but appears in rapidly increasing concentrations during early postnatal development (Vieira et al. 1996). This suggests that fetuses and neonates may be at decreased risk of benzene toxicity due to a reduced metabolic capacity. No information was located regarding potential age-related differences in pharmacodymanic processes such as benzene-target interactions in the hematopoietic system.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

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 (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, 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 3.8.1.

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

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

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Benzene

Several biomarkers of exposure to benzene have been reported in the literature. Unmetabolized benzene can be detected in the expired air and urine of humans exposed to benzene vapors (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001). Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene (OSHA 1987), and urinary phenol levels appear to be correlated with exposure levels (Astier 1992; Inoue et al. 1986, 1988b; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992).

Urinary *trans*,*trans*-muconic acid has been widely studied as a biomarker of exposure to benzene (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Urinary *S*-phenylmercapturic acid levels have also been correlated with occupational exposure to benzene

(Boogaard and van Sittert 1995, 1996; Farmer et al. 2005; Inoue et al. 2000; Jongeneelen et al. 1987; Popp et al. 1994; Qu et al. 2005). Significant exposure-response trends for urinary *trans,trans*-muconic acid and *S*-phenylmercapturic acid levels have been demonstrated in occupationally-exposed subjects at exposure levels of ≤1 ppm (Qu et al. 2005). The American Conference of Governmental Industrial Hygienists (ACGIH) has established 25 µg *S*-phenylmercapturic acid/g creatinine in the urine and 500 µg *trans,trans*-muconic acid/g creatinine in the urine as Biological Exposure Indices (BEIs) for benzene exposure in the workplace (ACGIH 2006). The BEI is primarily an index of exposure and not a level at which health effects might occur from exposure to benzene. Positive correlations have been made between benzene workplace air levels and urinary catechol and hydroquinone in exposed workers (Inoue et al. 1988a, 1988b; Rothman et al. 1998).

Hemoglobin and albumin adducts of the benzene metabolites, benzene oxide and 1,4-benzoquinone, have been used as biomarkers of exposure to benzene (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Smith and Rothman 2000; Yeowell-O'Connell et al. 1998, 2001). Furthermore, DNA adducts with benzene metabolites have been found after benzene exposure (Hedli et al. 1991; Lutz and Schlatter 1977; Reddy et al. 1989).

Ong et al. (1995) evaluated 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).

The biomarkers discussed in the preceding paragraphs appear to be adequate indicators of exposure to benzene at relatively high occupational exposure levels, and may serve as biomarkers in acute exposure scenarios involving relatively high levels of benzene. However, some of these biomarkers do not appear to be reliable indicators of environmental exposure to benzene (concentrations below the common industrial standard of 1 ppm TWA). For example, 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 (Inoue et al. 1988a). 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. In a study of pharmacy workers exposed to benzene levels measured in the parts per billion (ppb) range, there

was no significant difference in urinary levels of *trans*,*trans*-muconic acid between subjects exposed to 1.5 ppb and those exposed to 2.5 ppb (Sanguinetti et al. 2001). Recent reports indicate that urinary benzene may serve as the most sensitive biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Several additional factors must be taken into account when assessing the reliability of biomarkers of exposure to benzene. High and variable background levels of phenol and its metabolites 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 been found in persons with no known exposure to benzene (NIOSH 1974). Although muconic acid is used as a marker for benzene exposure, muconic acid in the urine can also result from ingestion of sorbic acid, a common food preservative (Ducos et al. 1990). Inoue et al. (1989b) suggested that individual urinary *trans*,*trans*-muconic acid content was not a useful index of benzene exposure due to large variations in measured individual background urinary *trans*,*trans*-muconic acid values.

In summary, several benzene metabolites may serve as biomarkers of exposure to benzene. Urinary benzene appears to be the most sensitive biomarker for low-level exposure to benzene. Refer to Tables 7-1 and 7-2 for information regarding analytical methods for determining benzene in biological samples and benzene metabolites in urine.

3.8.2 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 1987). 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. Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased

white blood cell counts and changes in bone marrow activity. 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. 1991; 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 cells are being used. 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. (1991) 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 of benzene exposure. Work by other researchers also suggests that monitoring DNA adducts or products of DNA damage might be useful (Bodell et al. 1993; Chen et al. 1994; Lagorio et al. 1994a; Reddy et al. 1989).

3.9 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. Interactions reported in *in vivo* studies occurred at relatively high benzene exposure levels, which would not likely be encountered near hazardous waste sites.

Ethanol and benzene induce the formation of the hepatic cytochrome P-450 isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingelman-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). Administration of ethanol (5 or 15% in drinking water, 4 days/week for 13 weeks) to mice exposed to benzene vapors at a concentration of 300 ppm, 6 hours/day, 5 days/week for 13 weeks) resulted in greater severity of benzene-induced hematological effects (anemia, lymphocytopenia, bone marrow aplasia, transient increases in normoblasts and peripheral blood atypia) relative to benzene-exposed mice not given ethanol (Baarson et al. 1982). The modulating effects of benzene were dose dependent. The enhancement of the hematotoxic effects of benzene by ethanol may be of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985), although the interactions demonstrated in the mice occurred at much higher benzene exposure concentrations than would likely be experienced in workplace air. 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 high levels of 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/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 (35–112.8 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).

3.10 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 result in reduced detoxification or excretion of benzene, or compromised function of organs affected by benzene. Populations who are at greater risk due to their unusually high exposure to benzene are discussed in Section 6.7, Populations with Potentially High Exposures.

Variability in human susceptibility to benzene toxicity may be related, at least in part, to genetic polymorphisms associated with metabolic processes. As discussed in Section 3.4.3, the flavoenzyme, NAD(P)H:quinone oxidoreductase (NQ01), catalyzes the reduction of 1,2- and 1,4-benzoquinone (reactive metabolites of benzene) to catechol and hydroquinone, respectively (Nebert et al. 2002), thus protecting cells from oxidative damage by preventing redox cycling. The NQ01*1 (wild type) allele codes for normal NQ01 enzyme and activity. An NQ01*2 allele encodes a nonsynonymous mutation that has negligible NQ01 activity. Approximately 5% of Caucasians and African Americans, 15% of Mexican-Americans, and 20% of Asians are homozygous for the NQ01*2 allele (Kelsey et al. 1997; Smith and Zhang 1998). Rothman et al. (1997) demonstrated that workers expressing negligible NQ01 activity were at increased risk of benzene poisoning (Rothman et al. 1997). In the same group of workers, those expressing rapid CYP2E1 activity were also at increased risk of benzene poisoning. Those workers with polymorphisms for both negligible NQ01 activity and rapid CYP2E1 activity exhibited greater than 7-fold increased risk of benzene poisoning than workers not expressing these polymorphisms. These

results indicate that individuals expressing rapid CYP2E1 activity may also be at increased risk for benzene toxicity.

Polymorphisms have been described for many of the glutathione genes (Cotton et al. 2000; Hengstler et al. 1998; Strange and Fryer 1999), the myeloperoxidase gene (Williams 2001), and the epoxide hydrolase gene (Omiecinski et al. 2000), which are known to be involved in benzene metabolism. However, the potential involvement of such polymorphisms in benzene toxicity have not been demonstrated in benzene-exposed workers.

Individuals with medical conditions that include reduced bone marrow function or decreased blood factors would be at increased risk for benzene toxicity. Treatments for certain medical conditions might result in decreases in particular blood factors, which could lead to increased susceptibility to benzene poisoning.

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.

Gender-related differences in susceptibility to benzene toxicity have been observed in animals. For example, Kenyon et al. (1998) exposed male and female mice to benzene vapor concentrations of 100 or 600 ppm and found increased benzene metabolism and associated genotoxicity in males, relative to females. Brown et al. (1998) used a PBPK modeling approach to assess potential gender-related differences in susceptibility to benzene. Their results suggest that women exhibit a higher blood/air partition coefficient and maximum velocity of benzene metabolism than men, and that women metabolize 23-26% more benzene than men under similar exposure scenarios. However, gender-related differences in susceptibility among benzene-exposed workers were not located in available reports.

At early stages of human development, metabolic pathways may not be fully functional, which might result in a lower level of susceptibility. Young children might experience increased susceptibility to benzene by inhalation due to increased breathing rates and potential for increased absorption, relative to

adults. However, no definitive human or animal data were located regarding age-related differences in susceptibility to benzene.

3.11 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 texts provide specific information about treatment following exposures to benzene:

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th ed. Stamford: Appleton & Lange, 1384-1398.

Haddad LM, Shannon MW, Winchester JF. 1998. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B Saunders Company, 940-941.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to benzene can occur by inhalation, oral, or dermal routes. 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 et al. 1998; HSDB 2007; 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 et al. 1998; 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 et al. 1998; HSDB 2007; 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; Ellenhorn and Barceloux 1988; Haddad et al. 1998; 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 (Ellenhorn and Barceloux 1988); however, other sources do not recommend the use of emetics because of the risk of aspiration pneumonitis, especially once the patient loses consciousness (Bronstein and Currance 1988; Haddad et al. 1998). Some sources recommend gastric lavage if indicated (Haddad et al. 1998; Kunisaki and Augenstein 1994).

While lavage may be useful, induction of emesis should be regarded with great caution because the rapid onset of central nervous system depression may lead to aspiration. 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 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Diazepam and phenytoin may be helpful in controlling seizures (Bronstein and Currance 1988; HSDB 2007; Stutz and Janusz 1988). Administration of epinephrine or other catecholamines has not been recommended because of the possibility of myocardial sensitization and subsequent arrhythmia (Haddad et al. 1998; HSDB 2007; Nahum and Hoff 1934).

3.11.2 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, accumulation of 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.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

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-1, a cytokine essential for hematopoiesis (Renz and Kalf 1991). External administration of recombinant IL-1 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-1α and IL-1β 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 six 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 (six 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.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 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.

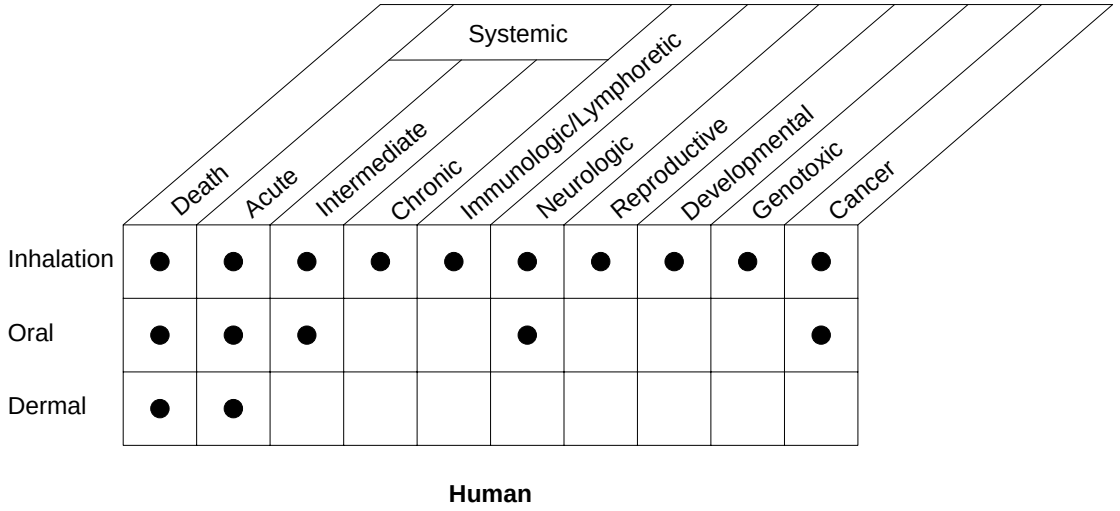
3.12.1 Existing Information on Health Effects of Benzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to benzene are summarized in Figure 3-6. 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* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

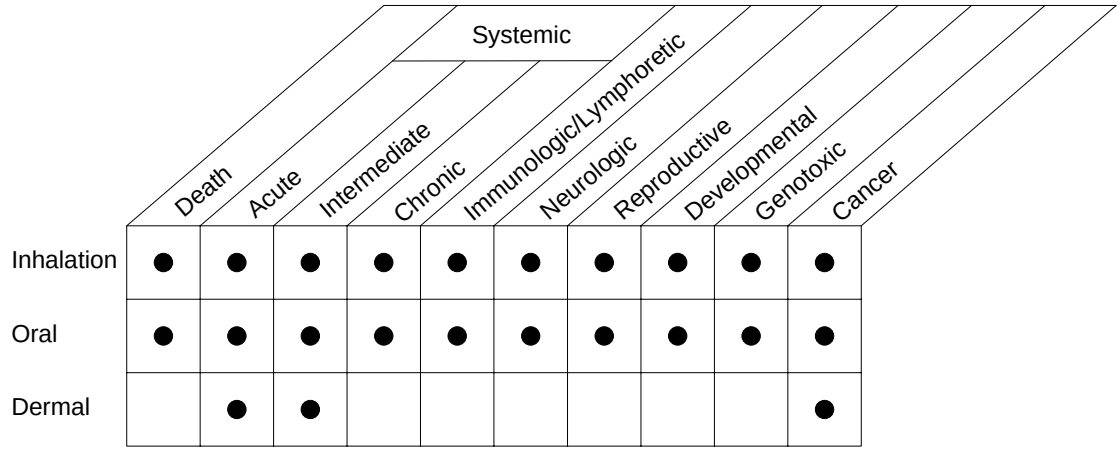
Virtually all of the information regarding health effects in humans comes from studies of workers exposed to benzene-containing solvents and/or adhesives. Exposures to benzene occurred at rotogravure printing shops; at shoe, rubber, and raincoat manufacturing plants; and during chemical manufacturing 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 3-6, 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

Figure 3-6. Existing Information on Health Effects of Benzene



Human



Animal

● Existing Studies

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.

3.12.2 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 of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Data for hematological effects in animals after acute-duration inhalation 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; Corti and Snyder 1996; 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-duration inhalation

MRL of 0.009 ppm was determined based on the LOAEL for immunologic effects in the mouse (Rozen et al. 1984) (discussed below under Immunotoxicity). No acute-duration oral studies were suitable for deriving MRLs. Additional studies that include dose-response information on hematological effects following acute oral exposure could be designed to provide information that could be useful in deriving an acute-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. 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 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-duration exposures to benzene in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1972). 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 and immunological effects (e.g., Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Farris et al. 1993, 1997a, 1997b; Green et al. 1981a, 1981b; Luke et al. 1988b; Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1987; Seidel et al. 1989; Snyder et al. 1978a, 1980; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wolf et al. 1956). An intermediate-duration inhalation MRL of 0.006 ppm was derived, based on a LOAEL for immunologic effects in the mouse (Rosenthal and Snyder 1987) (discussed below under Immunotoxicity). 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 no intermediate-duration oral MRL could be derived because the threshold for hematological (and immunological) effects could not be identified. Additional studies that include dose-response information on hematological effects following intermediate-duration oral exposure could be designed to provide information that could be useful in deriving an intermediate-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Intermediate-duration 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. Hematotoxicity was reported in studies of humans chronically exposed to benzene in the workplace air (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974, 1987; Cody et al. 1993; Dosemeci et al. 1996; Doskin 1971; Erf and Rhoads 1939;

Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Ward et al. 1996; Yin et al. 1987c). Several studies of occupational inhalation exposure to benzene did not find clinically-defined hematological effects (Collins et al. 1991, 1997; Tsai et al. 1983, 2004), but they had a high degree of uncertainty regarding estimations of benzene exposure levels. The study of workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b) identified the lowest LOAEL for hematotoxicity and was selected as the principal study for deriving a chronic-duration inhalation MRL of 0.003 ppm for benzene. Chronic-duration animal studies are available for hematological effects following inhalation exposure and provide support to the human data (Snyder et al. 1978a, 1980, 1982, 1984). No human data are available to evaluate hematological effects following oral exposure. Although chronic duration oral animal studies are available for hematological effects (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986), the most extensive study (NTP 1986) did not conclusively define a NOAEL or less serious LOAEL for end points that could be used to derive an MRL. However, a chronic-duration oral MRL of 0.0005 mg/kg/day was derived for benzene based on route-to-route extrapolation of the same results (Lan et al. 2004a, 2004b) that served as the basis for the chronic-duration inhalation MRL. Additional chronic-duration oral animal data could be designed to provide support to the chronic-duration oral MRL and to assist in defining threshold levels for populations living near hazardous waste sites. Dermal data for humans and animals were not available. However, chronic-duration 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.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen based on sufficient data in humans supported by animal evidence (IARC 2004; IRIS 2007; NTP 2005). Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and the occurrence of acute myelogenous leukemia (AML) (Hayes et al. 1997; IARC 1982, 1987; EPA 1995, 1998; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b), as well as is suggestive evidence of associations between benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997; Rinsky et al. 1987). Studies of workers in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987; 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) provide the strongest data on the leukemogenic potential of benzene, including exposure-response information, and data from the Pliofilm study was used as the basis of inhalation and oral cancer risk values for benzene (EPA 1998; IRIS 2007). Additional studies on these and other cohorts could better characterize exposure level and exposure duration relationships for benzene

and leukemia, particularly at low levels of exposure, and clarify the potential of benzene to induce NHL and multiple myeloma.

Benzene is a multiple site carcinogen in rats and mice following inhalation exposure (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; Snyder et al. 1980) and oral exposure (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986), inducing lymphomas and other neoplasms in numerous tissues not affected in humans. Although contributing to the weight of evidence for carcinogenicity, the animal studies do not identify a suitable model for leukemia in humans. An appropriate animal model would help to provide a better understanding of how benzene causes cancer, particularly the mechanism of benzene leukemogenesis. The exact mechanism of benzene carcinogenicity is not known, but 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. The clastogenic properties of benzene may play a role in its carcinogenicity. 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 benzene metabolites produce greater-than-additive effects, determination of the critical target genes, whether aplastic anemia is essential to the development of leukemia, and determination of the role of cytokines and growth factor pathways in benzene toxicity.

Genotoxicity. Evidence for the genotoxicity of benzene in humans comes from studies of chronically-exposed populations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hallberg et al. 1996; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Liu et al. 1996; Major et al. 1992, 1994; Nilsson et al. 1996; Picciano 1979; Pitarque et al. 1996, 1997; Popp et al. 1992; Qu et al. 2003a, 2003b; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). 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. 1991; Chen et al. 1994; Chang et al. 2005; Eastmond et al. 2001; Erexson et al. 1986; Farris et al. 1996; Fujie et

al. 1992; Healy et al. 2001; Lee et al. 2005; Kolachana et al. 1993; Ranaldi et al. 1998; Siou et al. 1981; Toft et al. 1982; Ward et al. 1992). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). 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 al.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).

The genotoxicity of benzene has been extensively studied and demonstrated in both humans and laboratory animals. Although some information is available regarding possible mechanisms of benzene genotoxicity and carcinogenicity, additional mechanistic studies would be helpful to more completely characterize mechanisms responsible for these effects.

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. 1992; 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. 1994b; 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; Songnian et al. 1982), and chronic (Snyder et al. 1980, 1984, 1988) periods. An acute-duration 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-duration inhalation MRL has been derived based on this study. An intermediate-duration inhalation study found delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm (Rosenthal and Snyder 1987), and an intermediate-duration 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; 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). An intermediate-duration inhalation study found increased rapid response in mice at 0.78 ppm (Li et al. 1992), but this study was limited due to apparent discrepancies between reported and actual benzene exposure levels, since neurological effects reported in other animal studies occurred only at much higher exposure levels. Acute-duration oral exposures of 950 mg/kg benzene altered neuro-transmitter levels in the brains of rats (Kanada et al. 1994). Intermediate-duration 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).

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 benzene-exposed 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-duration 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 epidemiological data comes from occupational studies and associated analysis (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974; Baslo and Aksoy 1982; Ciccone et al. 1993; Cody et al. 1993; Dosemeci et al. 1994, 1996; Doskin 1971; EPA 1986, 1995, 1998; Erf and Rhoads 1939; Goldwater 1941; Greenburg et al. 1939; Hayes et al. 1997; IARC 1982, 1987; Infante 1978; Infante et al. 1977; IRIS 2007; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Ott et al. 1978; Paustenbach et al. 1992; Paxton et al. 1994a, 1994b; Qu et al. 2002, 2003a, 2003b; Rinsky et al. 1981, 1987, 2002; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Travis et al. 1994; Utterback and Rinsky 1995; Ward et al. 1996; Yin et al. 1987c, 1989, 1994, 1996a, 1996b). 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 (Agency for Toxic Substances and Disease Registry 2001). No causal relationship has been proposed for health conditions identified in the base subregistry (Agency for Toxic Substances and Disease Registry 1995) or continued follow-up of the population (Agency for Toxic Substances and Disease Registry 2001).

Few well-conducted epidemiological studies of exposed populations exist. An epidemiological 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 history of materials deposited, 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 studies include analyses of workers in China (Dosemeci et al. 1994, 1996; Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Yin et al. 1987c, 1994), reanalysis of data from the Pliofilm cohort (Paxton et al. 1994a, 1994b; Utterback and Rinsky 1995; Ward et al. 1996), and assessment of clinical manifestations of benzene hematotoxicity in U.S. industries with potential for benzene exposure (Collins et al. 1991, 1997; Tsai et al. 1983, 2004). A publication by Lioy and Pellizzari (1995) outlines an appropriate framework for the National Human Exposure Assessment Survey, using benzene as a candidate compound. Other 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.

Studies of DNA damage have primarily been performed on workers with preexisting hematological effects and cancer (Ding et al. 1983; Forni et al. 1971a; Picciano 1979; Yardley-Jones et al. 1988) or as biomonitoring of benzene exposure (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Hallberg et al. 1996; Kašuba et al. 2000; Liu et al. 1996; Nilsson et al. 1996; Pitarque et al. 1996; Sul et al. 2002; Surrallés et al. 1997; Zhang et al. 1999). Additional data on benzene-induced DNA damage could be useful in

monitoring possible adverse effects in individuals living near hazardous waste sites if more quantitative dose-response data were available for clastogenicity.

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, including unmetabolized benzene in expired air and urine (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001), urinary phenol levels (Astier 1992; Inoue et al. 1986, 1988b; Jongeneelen et al. 1987; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992), and urinary *trans*,*trans*-muconic acid and *S*-phenylmercapturic acid levels (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Farmer et al. 2005; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine (Hammond and Hermann 1960). However, urinary sulfate levels are variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effect. Furthermore, benzene-derived adducts with hemoglobin and albumin may be useful as biomarkers (Bechtold et al. 1992a, 1992b; Bechtold and Henderson 1993; Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Hedli et al. 1991; Lutz and Schlatter 1977; Nomiyama and Nomiyama 1974a, 1974b; Pekari et al. 1992; Reddy et al. 1989; Schad et al. 1992; Sherwood 1988; Smith and Rothman 2000; Srbova et al. 1950; Waidyanatha et al. 2001; Yeowell-O'Connell et al. 1998, 2001).

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*-phenylmercapturic acid, the proposed urinary BEI (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. 1989b; Lee et al. 1993; Melikian et al. 1993; Ong and Lee 1994; Witz et al. 1990b). 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. 1989b). 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). Recent reports indicate that urinary benzene may serve as a sensitive

biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Effect. Monitoring of benzene workers has included monthly blood counts. Toxic effects occur in the bone marrow and arise 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).

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; Yu and Weisel 1996). 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 1953a; 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 a preferential substrate of CYP2E1, which also metabolizes alcohol. The induction of CYP2E1 by benzene (and some of its metabolites) with subsequent generation of reactive metabolites, oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Irons 2000; Parke 1989; Ross 1996, 2000; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). CYP2E1 is not confined to the liver, but has also been detected in bone marrow. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier

et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) 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. Similar studies have been conducted in mice (Ganousis et al. 1992). Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of CYP2E1 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 benzene-induced 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.

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.

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 3.12.3).

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. Related lines of investigation include the use of non-steroidal anti-inflammatory drugs to block prostaglandin and prostaglandin synthetase-mediated activity after benzene exposure, and the role of IL-1 cytokine activity in preventing depression of hematopoiesis.

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

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al. 1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk than adults for benzene toxicity. Children could potentially be at increased risk for benzene toxicity via the inhalation exposure route based on higher activity levels and ventilation rates than adults. Age-related differences in benzene metabolism could potentially affect susceptibility. Well-designed animal studies should be performed to adequately assess the potential for age-related increased susceptibility to benzene.

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

3.12.3 Ongoing Studies

Ongoing studies pertaining to benzene have been identified and are shown in Table 3-7 (FEDRIP 2005).

Table 3-7. Ongoing Studies on the Health Effects of Benzene

Investigator	Affiliation	Research description	Sponsor
Conaway CC	University of California Lawrence Livermore National Laboratory	DNA binding of radiolabeled benzene	National Center for Research Resources
French JE	National Institute of Environmental Health Sciences	Carcinogen inactivation of tumor suppressor genes in p53 mice	National Institute of Environmental Health Sciences
French JE	National Institute of Environmental Health Sciences	Mechanisms of leukemogenesis in genetically-altered mice	National Institute of Environmental Health Sciences
Sabri MI	Oregon Health and Science University	Biomarkers of exposure and effect in neurotoxicants including neurotic benzene derivatives	National Institute of Environmental Health Sciences
Monks TJ	University of Texas at Austin	Benzene metabolites and hematotoxicity	National Institute of Environmental Health Sciences
Ross D	Texas A&M University System	Protective effect of NQ01 against benzene toxicity	National Institute of Environmental Health Sciences
Smith MT	University of California Berkeley	Biomarkers of benzene exposure and genotoxicity	National Institute of Environmental Health Sciences

Source: FEDRIP 2005

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4. CHEMICAL AND PHYSICAL INFORMATION

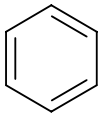
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of benzene is located in Table 4-1. 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.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of benzene is located in Table 4-2. 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 2007).

Table 4-1. Chemical Identity of Benzene

Characteristic	Information	Reference
Chemical name	Benzene	HSDB 2007
Synonym(s)	Annulene, benzeen (Dutch), benzen (Polish), benzol, benzole; benzolo (Italian), coal naphtha, cyclohexatriene, fenzen (Czech), phene, phenyl hydride, pyrobenzol, pyrobenzole	HSDB 2007
Registered trade name(s)	Polystream	IARC 1982
Chemical formula	C_6H_6	Budavari et al. 2001
Chemical structure		Budavari et al. 2001
Identification numbers:		
CAS registry	71-43-2	HSDB 2007
NIOSH RTECS	CY-1400000	HSDB 2007
EPA hazardous waste	NA	
OHM/TADS	No Data	
DOT/UN/NA/IMCO shipping	UN1114; IMO3.2	HSDB 2007
HSDB	35	HSDB 2007
NCI	C55276	HSDB 2007
Merck	1066	Budavari et al. 2001

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

Table 4-2. Physical and Chemical Properties of Benzene

Property	Information	Reference
Molecular weight	78.11	Budavari et al. 2001
Color	Clear, colorless liquid	Budavari et al. 2001
Physical state	colorless to light yellow liquid	HSDB 2007
Melting point	5.5 °C	Budavari et al. 2001
Boiling point	80.1 °C	Budavari et al. 2001
Density at 15 °C, g/cm^3	0.8787	Budavari et al. 2001
Odor	Aromatic	NFPA 1994
Odor threshold:		
Water	2.0 mg/L	HSDB 2007
Air[a]	Detection range: 34–119 ppm (geometric mean: 61 ppm) Recognition: 97 ppm	AIHA 1989
Taste threshold:	0.5–4.5 mg/L	HSDB 2007
Solubility:		
Water at 25 °C	w/w: 0.188%	Budavari et al. 2001
Organic solvents	Alcohol, chloroform, ether, carbon disulfide, acetone, oils, carbon, tetrachloride, glacial acetic acid	Budavari et al. 2001
Partition coefficients:		HSDB 2007; Karickhoff 1981; Kenaga 1980
Log K_{ow}[b]	2.13	
Log K_{oc}[c]	1.8–1.9	
Vapor pressure at 20 °C	75 mm Hg	NFPA 1994
Henry's law constant at 25 °C	5.5x10^{-3} atm-m^3/mol	Mackay and Leinonen 1975
Autoignition temperature	498 °C	NFPA 1994
Flashpoint	-11 °C (closed cup)	Budavari et al. 2001
NFPA hazard classification:		HSDB 2007
Health	2.2	
Flammability	3.3	
Reactivity	0.0	
Flammability limits in air	1.2% (lower limit; 7.8% (upper limit)	NFPA 1994
Conversion factors	1 ppm=3.26 mg/m^3 at 20 °C and 1 atm pressure; 1 mg/m^3=0.31 ppm	HSDB 2007
Explosive limits	1.4% (lower limit); 8% (upper limit)	HSDB 2007

[a]Odor threshold values considered by AIHA (1989) to be acceptable based on review of peer-reviewed reports of odor thresholds for benzene (range 0.78–100 ppm).
[b]K_{ow} = octanol-water partitioning coefficient
[c]K_{oc} = soil adsorption coefficient

NFPA = National Fire Protection Association

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

5.1 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 byproducts of the ethylene manufacturing process (Fruscella 1992).

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 1987). 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 1988). 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 cyclodehydrogenation. 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 byproduct 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 (Fruscella 1992; IARC 1982a). In the toluene hydrodealkylation process, toluene or toluene/xylene mixtures are reacted with hydrogen at temperatures ranging from 500 to 595 °C with usual pressures of 4–6 mPa (40–60 atm), 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 (Fruscella 1992). 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; Fruscella 1992). New coking, gasification, and

liquefaction processes for coal are all potential sources of benzene (IARC 1982a). Of the total U.S. production capacity of 3.109 billion gallons in 2004, catalytic reformats constituted 45%, toluene and xylene 30%, pyrolysis gasoline 23%, and coke oven <2% (SRI 2004).

Benzene ranks in the top 20 most abundantly produced chemicals in the United States (C&EN 1994; Kirschner 1995; Reisch 1994). Production data from 1984 to 1994 indicate that the production of benzene increased by about 4% annually (C&EN 1995).

According to the Toxics Release Inventory (TRI), 2,528 facilities in the United States produced or processed benzene in 2004 (TRI04 2006). Table 5-1 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 5-1 should be used with caution since only certain types of facilities were required to report. Therefore, this is not an exhaustive list.

SRI (2004) lists the companies producing benzene in the United States in 2004. This list is summarized in Table 5-2. Facilities listed as being responsible for producing the top 50% of regional capacity in the United States include: Exxon Mobil Chemical Company, 518 million gallons (16%); Equistar Chemicals LP, 330 million gallons (11%), Dow Chemical Company, 300 million gallons (10%); Flint Hills Resources LP, 250 million gallons (8%), and BP Oil Company 230 million gallons (7%) (SRI 2004).

5.2 IMPORT/EXPORT

Benzene is imported and exported to the United States as both the pure chemical and as a mixture of mineral fuels. The import of pure benzene into the United States is dependent on domestic production and demand. Imports of benzene (from mineral fuels and pure benzene) into the United States were approximately 5,252 million L (10,176 million pounds) in 2004, 6,024 million L (11,672 million pounds) in 2003, and 5,912 million L (11,455 million pounds) in 2002 (USITC 2005). The largest exporters of benzene to the United States during 2002-2004 were Canada, Iraq, Israel, Kuwait, Venezuela, and Saudi Arabia (USITC 2005).

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 2002–2004 data indicate an increase in export volumes for benzene during this period. Exports of benzene (both pure benzene and benzene derived from mineral fuels) to other countries were approximately 11 million L (21 million pounds) in 2002, 150 million L

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

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	26	0	99,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12
AL	85	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
AR	24	100	9,999,999	1, 2, 3, 4, 6, 8, 9, 11, 12, 13
AS	1	100,000	999,999	9
AZ	30	100	9,999,999	1, 2, 4, 5, 7, 9, 10, 11, 12, 13, 14
CA	172	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
CO	23	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13
CT	8	1,000	9,999,999	1, 4, 6, 7, 9, 10, 12
DE	13	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
FL	36	0	499,999,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14
GA	33	0	49,999,999	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
GU	6	100	9,999,999	2, 3, 4, 7, 9, 12
HI	15	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14
IA	21	0	10,000,000,000	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14
ID	3	10,000	9,999,999	7, 9, 12
IL	102	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
IN	80	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
KS	59	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
KY	66	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
LA	212	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MA	15	100	99,999,999	2, 4, 7, 8, 9
MD	20	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
ME	9	100	9,999,999	2, 3, 4, 7, 9, 12, 13
MI	108	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MN	36	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MO	30	0	9,999,999	1, 5, 7, 8, 9, 11, 12
MP	7	0	999,999	2, 3, 4, 7, 9
MS	43	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
MT	27	10,000	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
NC	34	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
ND	9	0	49,999,999	1, 2, 3, 4, 5, 6, 9, 12, 13
NE	11	0	9,999,999	1, 7, 10, 12, 13
NH	9	0	999,999	1, 2, 7, 9, 12, 13
NJ	61	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
NM	32	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
NV	5	100	99,999	1, 2, 4, 8, 9, 12, 13
NY	48	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
OH	101	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

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

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
OK	48	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
OR	12	1,000	9,999,999	1, 2, 3, 4, 5, 7, 9, 12, 13
PA	100	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
PR	52	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
RI	9	10,000	9,999,999	1, 2, 4, 7, 8, 9
SC	26	0	9,999,999	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13
SD	8	100	999,999	2, 3, 4, 7, 8, 11, 12, 13, 14
TN	53	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
TX	361	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	42	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VA	36	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VI	8	10,000	499,999,999	1, 2, 3, 4, 6, 7, 8, 9
WA	57	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
WI	27	0	9,999,999	1, 4, 5, 7, 8, 9, 11, 12, 13, 14
WV	32	0	99,999,999	1, 2, 4, 5, 6, 7, 8, 9, 12, 13
WY	37	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14

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

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

Source: TRI04 2006 (Data are from 2004)

Table 5-2. Current U.S. Manufacturers of Benzene[a]

Company	Location (annual capacity millions of gallons)
ATOFINA Petrochemicals, Inc.	Port Arthur, Texas (87)
BASF FINA Petrochemicals, Inc.	Port Arthur, Texas (34)
BP America, Inc.	Texas City, Texas (230)
Ceveron Phillips Chemical Company, LP	Pascagoula, Mississippi (150)
Olefins and Polyolefins Business Unit	Port Arthur, Texas (35)
CITGO Petroleum Corporation	Corpus Christi, Texas (78) Lake Charles, Louisiana (55) Lemont, Illinois (19)
Conoco Phillips Refining Marketing and Transportation Division	Alliance Louisiana (65) Sweeny, Texas (39) Wood River Illinois (60)
Dow Chemical USA	Freeport, Texas (80) Plaquemine, Louisiana (317)
Equistar Chemicals, LP	Alvin, Texas (137) Channelveiw, Texas (90) Corpus Christi, Texas (103)
ExxonMobil Chemical Company	Baton Rouge Louisiana (100) Baytown, Texas (180) Beaumont, Texas (182) Chalmette, Louisiana (56)
Flint Hills Resources, LP	Corpus Christi, Texas (250)
Frontier El Dorado Refining Company	El Dorado, Kansas (15)
HOVENSA, LLC	St. Croix, Virgin Islands (60)
Huntsman, LLC	Port Arthur, Texas (90)
Lyondell-Citgo Refining, LP	Houston, Texas (50)
Marathon Ashland Petroleum, LLC	Catlettsburg, Kentucky (58) Texas City, Texas (3)
Motiva Enterprises, LLC	Delaware City, Delaware (15)
NOVA Chemicals Corp.	Bayport, Texas (15)
The Premcor Refining Group Inc.	Lima, Ohio (145)
Shell Chemical Company	Deer Park, Texas (180)
Sunoco, Inc.	Marcus Hook, Pennsylvania (60) Philadelphia, Pennsylvania (24) Toledo Ohio (20) Westville, New Jersey (18)
Valero Energy Corporation	Corpus Christi, Texas (82) Three Rivers, Texas (20)
Eagle-Pitcher Industries, Inc.	Lenexa, Kansas
Eagle-Pitcher Technologies, LLC	
Chem Syn Laboratiories (^{14}C-benzene)	

[a]Derived from Stanford Research Institute (SRI) 2004, receipt where otherwise noted. SRI reports production of chemicals produced in commercial quantities (defined as exceeding 5,000 pounds or $10,000 in value annually) by the companies listed.

(290 million pounds) in 2003, and 75 million L (145 million pounds) in 2004. These numbers are up from 15 million L (29 million pounds) in 2001 and 3.3 million L (6.4 million pounds) in 1993 (USITC 2005). The largest importers of benzene from the United States are Canada, the Netherlands, Taiwan, Spain, and Korea (USITC 2005).

5.3 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 2007). 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 1–2% (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 2007). 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 products such 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 1978; 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% 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 >5% benzene, and paint solvents and thinners containing <10% 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 than its potential usefulness.

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 1977).

5.4 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 2007). Regulations governing the treatment and disposal of benzene-containing wastes are presented in Chapter 8. Waste byproducts from benzene production processes include acid and alkali sludges, liquid-solid slurries, and solids (EPA 1982b; Saxton and Narkus-Kramer 1975). In the past, landfilling and lagooning have been the major methods of disposal of benzene-containing industrial wastes (EPA 1982b). 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 820–1,600 °C and residence times of seconds for liquids and gases, and hours for solids; and 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 2007). Since benzene burns 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 1–2 days is sufficient for total destruction (HSDB 2007).

Underground injection also appears to be an important disposal method in some states. Approximately 436,000 pounds of benzene (6% of the total environmental release) was disposed of by underground injection. This disposal via underground injection in 2006 was higher than the amount (356,000 pounds) released in 1992 (TRI90 1992), but lower than the release in 1990 (654,000 pounds) (TRI90 1992) and 2002 (692,000 pounds) (TRI02 2005). In addition, 24,000 pounds of benzene (0.3% of the total environmental release) was disposed of via land disposal (TRI04 2006). The amount discharged to soil in 2002 was less than one quarter of the amount (724,000 pounds) discharged in 1990 (TRI90 1992) and less than one third of the amount (340,000 pounds) discharged in 1992 (TRI92 1994).

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 1994a; IRPTC 1985). Full-scale chemical treatability studies have demonstrated 95–100% reductions in benzene concentrations for industrial waste waters receiving biological treatments (HSDB 2007). 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).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

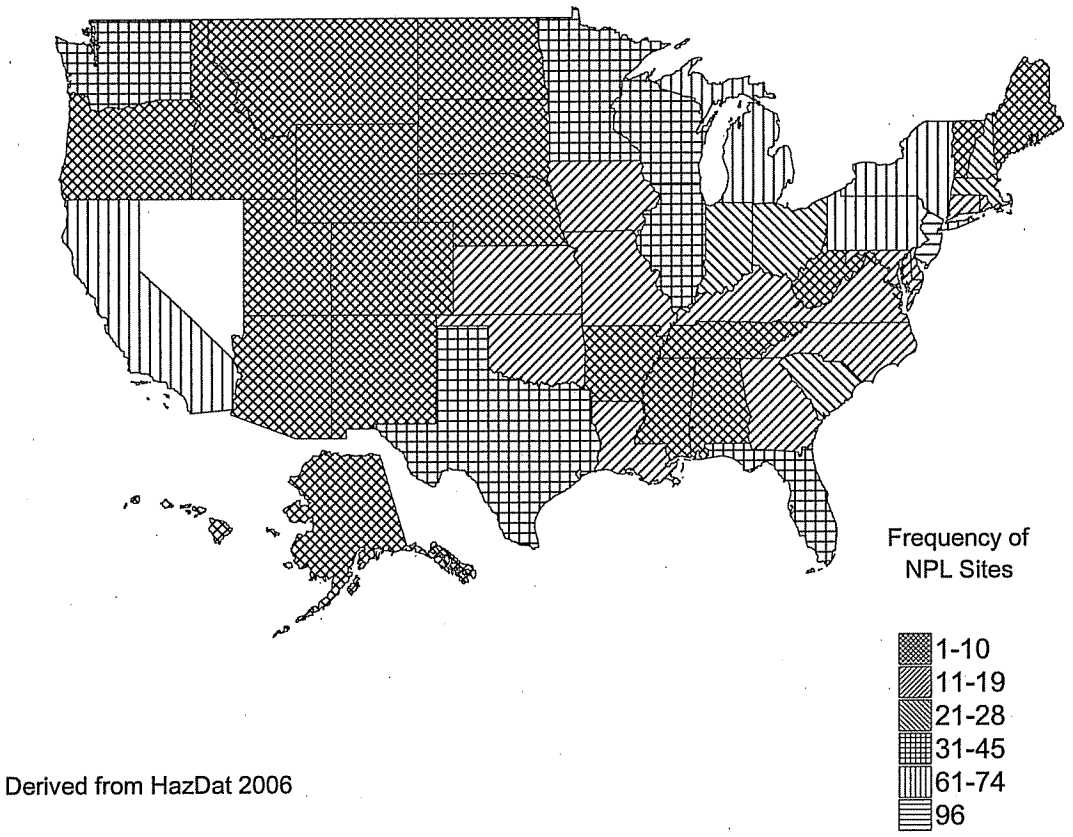
Benzene has been identified in at least 1,000 of the 1,684 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2006). However, the number of sites evaluated for benzene is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 995 are located within the United States, 3 are located in the Commonwealth of Puerto Rico (not shown), and 2 are located in the Virgin Islands (not shown).

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 1989). According to the Toxics Release Inventory, releases to the air from manufacturing and processing facilities were about 6,7 million pounds (3,055 metric tons) in 2004 (TRI04 2006). Releases to air accounted for about 93% of the total industry-related releases to the environment (TRI04 2006). 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 an 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 accounting for >99% of the total daily intake of benzene (Hattemer-Frey et al. 1990; MacLeod and MacKay 1999). 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 and air around landfills and hazardous waste sites that contain benzene are additional sources of exposure. Exposure to benzene can

Figure 6-1. Frequency of NPL Sites with Benzene Contamination



Derived from HazDat 2006

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 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 smoking tobacco products, from gasoline filling stations, and from auto exhaust. Smoking was found to be the largest anthropogenic source of direct human exposure to benzene (Duarte-Davidson et al. 2001; Hattemer-Frey et al. 1990).

6.2 RELEASES TO THE ENVIRONMENT

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

6.2.1 Air

Estimated releases of 6.7 million pounds (~ 3,055 metric tons) of benzene to the atmosphere from 968 domestic manufacturing and processing facilities in 2004, accounted for about 93% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases are summarized in Table 6-1.

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

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

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	7	19,568	19	0	375	0	19,962	0	19,962
AL	15	290,660	290	0	280	2	290,955	277	291,232
AR	8	33,137	66	0	0	0	33,203	0	33,203
AZ	13	4,910	0	0	9	0	4,915	4	4,919
CA	65	57,611	129	228	1,292	598	57,970	1,888	59,858
CO	11	19,630	0	0	10	36	19,630	46	19,676
CT	9	2,552	2	0	0	2	2,554	2	2,556
DE	5	3,425	6,006	0	26	0	9,431	26	9,457
FL	26	396,187	41	0	0	157	396,228	157	396,385
GA	15	36,893	128	0	9	0	37,026	4	37,030
GU	3	7,106	1	0	0	401	7,107	401	7,508
HI	9	11,171	30	0	12	0	11,201	12	11,213
IA	14	42,671	1	0	0	0	42,672	0	42,672
ID	2	1,732	No data	0	0	0	1,732	0	1,732
IL	35	171,104	102	0	2,152	104	172,227	1,235	173,462
IN	30	211,476	805	14,001	4,654	29	227,079	3,886	230,965
KS	19	69,823	164	231	259	0	70,226	251	70,477
KY	22	77,345	799	0	766	20	78,904	26	78,930
LA	62	605,128	1,075	122,723	1,688	968	729,109	2,473	731,582
MA	10	5,556	55	0	26	537	5,611	563	6,173
MD	11	12,644	8	0	184	1	12,821	15	12,836
ME	5	3,027	23	0	0	467	3,049	467	3,516
MI	41	345,255	8	0	127	2,295	345,363	2,322	347,686
MN	16	16,614	2,724	0	131	1,561	19,360	1,670	21,030
MO	17	98,498	0	0	0	0	98,498	0	98,498
MP	2	710	0	0	0	0	710	0	710
MS	13	59,076	25	0	14	0	59,102	13	59,115
MT	5	17,242	5	0	15	0	17,248	14	17,262
NC	12	17,290	0	0	5	48	17,295	48	17,343
ND	2	6,400	0	0	1	0	6,400	1	6,401
NE	9	1,333	No data	0	0	6	1,333	6	1,339
NJ	20	51,054	443	0	150	169	51,510	306	51,816
NM	9	38,730	No data	7	2	0	38,739	0	38,739
NV	3	1,005	No data	0	0	0	1,005	0	1,005
NY	44	60,682	56	0	10	1,335	60,738	1,345	62,084
OH	52	183,972	67	5	730	82	184,049	807	184,856
OK	11	114,701	14	0	278	71	114,984	80	115,064

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

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
OR	6	4,651	8	0	41	0	4,659	41	4,700
PA	39	234,647	419	0	345	11,389	235,066	11,733	246,800
PR	10	49,879	7	0	0	0	49,886	0	49,886
RI	2	1,841	4	0	0	820	1,845	820	2,665
SC	6	64,627	6	0	250	1,023	64,883	1,023	65,906
SD	11	272	1	0	0	0	273	0	273
TN	17	73,926	63	0	280	0	73,989	280	74,269
TX	150	2,681,863	621	298,595	8,326	1,713	2,935,539	55,579	2,991,118
UT	11	31,314	750	0	809	233	32,069	1,038	33,107
VA	17	183,748	787	0	26	0	184,535	26	184,561
VI	3	20,576	0	0	9	0	20,576	9	20,585
WA	18	50,667	14	0	95	48	50,770	54	50,824
WI	14	141,183	0	0	251	63	141,183	314	141,496
WV	5	76,556	284	0	393	0	76,840	393	77,233
WY	7	24,986	0	0	1	0	24,986	1	24,987
Total	968	6,736,655	16,051	435,790	24,033	24,179	7,147,049	89,659	7,236,707

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

RF = reporting facilities; UI = underground injection

Source: TRI04 2006 (Data are from 2004)

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 include automobiles, petrochemical industries, waste water treatment plants, and petroleum industries (Edgerton and Shah 1992).

Industrial and automotive sources of benzene are well monitored. EPA (1989) estimates of nationwide benzene atmospheric emissions, in metric tons/year (kkg/year), from various industrial sources include: (1) coke byproduct 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 byproduct recovery plants (4,600 kkg/year), (5) benzene storage vessels (620–1,290 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). Benzene is present in passenger car tailpipe emissions at compositions ranging from 2.9 to 15% of the total tailpipe hydrocarbon composition (Black et al. 1980). The 2002 benzene industrial emission inventory for California totaled 266 metric tons/year (CARB 2005); these numbers did not include motor vehicle exhaust,which accounted for 71% of emissions in 1984 (Cal EPA 1987). 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 areas and mobile sources combined, with major stationary point sources excluded (EPA 1994d). Benzene is also released by off-gassing from particle board (Glass et al. 1986), vaporization from oil spills, and emissions from landfills (Bennett 1987; Wood and Porter 1987). While all of these sources release more benzene into the environment, a large percentage of the benzene inhaled by humans comes from cigarette smoke. Exhaled breath of smokers contains benzene (Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986).

According to the Texas Natural Resource Conservation Commission (TNRCC), of the 93 districts in Texas reporting benzene emissions from industrial sources, in 1980, 1985, and 1988, 10 districts reported 0 tons/year, 79 districts reported 0.1–100 tons/year, 4 districts reported 100–400 tons/year, 2 districts reported 400–1,000 tons/year, and 1 district had emissions projected at above 1,000 tons/year (Pendleton 1995).

The natural sources most monitored for benzene released to air are fires (Austin et al. 2001; Lowry et al. 1985). A study of nine municipal fires in Canada found a mean concentration of 3.45 ppm of benzene

and had very high relative concentration of other volatile organic compounds (VOCs) monitored (Austin et al. 2001).

There is a potential for atmospheric release of benzene from hazardous waste sites. Benzene has been detected in air in 200 of the 1,684 current and former NPL sites where it has been detected in some medium (HazDat 2006).

6.2.2 Water

Estimated releases of 16,051 pounds (~7 metric tons) of benzene to surface water from 968 domestic manufacturing and processing facilities in 2004, accounted for about 0.2% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released to water from the discharges of both treated and untreated industrial waste water, gasoline leaks from underground storage tanks, accidental spills during marine transportation of chemical products, and leachate from landfills and other contaminated soils, (CDC 1994; Crawford et al. 1995; EPA 1979; 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 other chemicals. The seepage from this site contaminated nearby surface water (EPA 1993). 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).

There is a potential for release of benzene to water from hazardous waste sites. Benzene has been detected in groundwater samples collected at 832 of the 1,684 current and former NPL sites and in surface water samples collected at 208 of the 1,684 sites (HazDat 2006).

6.2.3 Soil

Estimated releases of 24,033 pounds (~11 metric tons) of benzene to soils from 968 domestic manufacturing and processing facilities in 2004, accounted for about 0.3% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). An additional 435,000 pounds (~197 metric tons), constituting about 6% of the total environmental emissions, were released via underground injection (TRI04 2006). These releases are summarized in Table 6-1.

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).

There is a potential for release of benzene to soil from hazardous waste sites. Benzene has been detected in soil samples collected at 436 of the 1,684 sites, and in sediment samples collected at 145 of the 1,684 sites where benzene has been detected in some medium (HazDat 2006).

6.3 ENVIRONMENTAL FATE

6.3.1 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 moderately soluble in water, with a solubility of 1,780 mg/L at 25 °C, and the Henry's law constant for benzene ($5.5x10^{-3}$ atm-m^3/mole at 25 °C) indicates that benzene partitions readily to the atmosphere from surface water (Mackay and Leinonen 1975). Since benzene is 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. The soil organic carbon sorption coefficient (K_{oc}) for benzene has been measured with a range of 60–83 (Karickhoff 1981; Kenaga 1980), indicating that benzene is highly mobile in soil and readily leaches into groundwater. Other parameters that influence leaching potential include the soil type (e.g., sand versus clay), amount of rainfall, depth of the groundwater, and 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 soil 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). The rate of volatilization and leaching are the principal factors that determine overall persistence of benzene in sandy soils (Tucker et al. 1986).

Studies suggest that benzene does not bioaccumulate in marine organisms. 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). Benzene has a relatively low octanol/water partition coefficient (log K_{ow}=2.13 or 2.15) (Gossett et al. 1983; HSDB 2007). In the alga, *Chlorella,* a bioaccumulation factor of 30 was determined experimentally (Geyer et al. 1984). An experimental bioconcentration factor (BCF) of 4.27 was measured in goldfish reared in water containing 1 ppm of benzene (Ogata et al. 1984). Based on these measured values, bioconcentration/bioaccumulation of benzene in the aquatic food chains does not appear to be important. These results are consistent with the fact that benzene has a relatively low octanol/water partition coefficient (Gossett et al. 1983; HSDB 2007), suggesting relatively low bioaccumulation. There is no evidence in the literature of biomagnification of benzene in aquatic food chain.

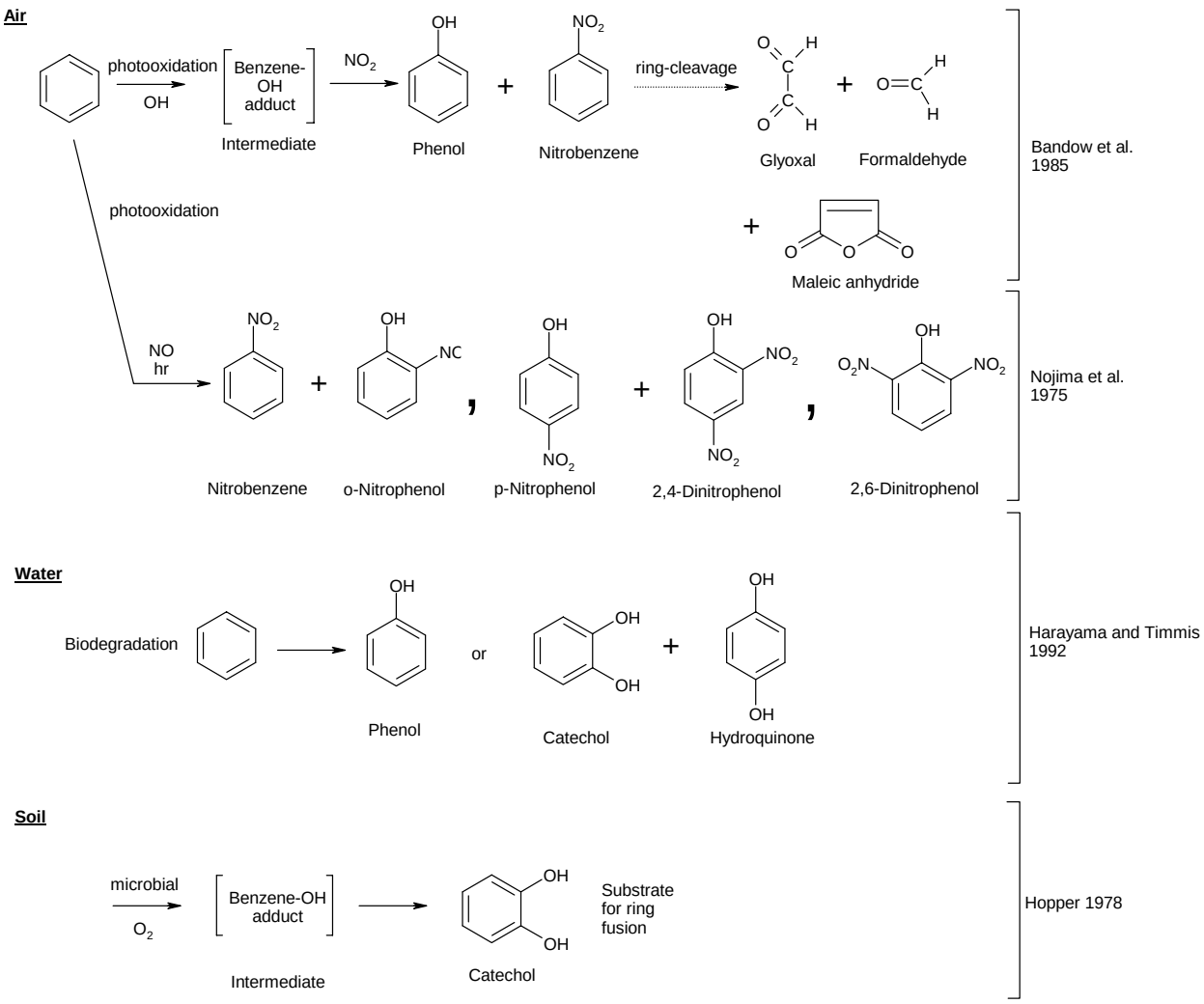
Evidence exists for the uptake of benzene by cress and barley plants from soil (Scheunert et al. 1985; Topp et al. 1989). BCFs for barley plants after 12, 33, 71, and 125 days were 17, 2.3, 2.9, and 4.6, respectively. BCFs for cress plants after 12, 33, and 79 days were 10, 2.3, and 1.9, respectively. The relative decrease in the BCFs with time was attributed to growth dilution (Topp et al. 1989). Since benzene exists primarily in the vapor phase, air-to-leaf transfer 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% was from root uptake (Hattemer-Frey et al. 1990).

Benzene also accumulates in leaves and fruits of plants. After 40 days, plants grown in benzene-rich environments showed bioaccumulation in the leaves and fruit that were greater than the air portioning coefficient of benzene in the atmosphere. Blackberries exposed to 0.313 ppm and apples exposed to 2.75 ppm contained about 1,000 and 36 ng/g of benzene, respectively (Collins et al. 2000).

6.3.2 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 6-2.

Figure 6-2. Environmental Transformation Products of Benzene in Various Media



6.3.2.1 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 $1.3x10^{-12}$ cm^3/molecule-second, which corresponds to a residence time of 8 days at an atmospheric hydroxyl radical concentration of $1.1x10^6$ molecules/cm^3 (Gaffney and Levine 1979; Lyman 1982). With a hydroxyl radical concentration of $1x10^8$ molecules/cm^3, corresponding to a polluted atmosphere, the estimated residence time is shortened to 2.1 hours (Lyman 1982). Benzene may also react with other oxidants in the atmosphere such as nitrate radicals and ozone; however, the rate of degradation is considered insignificant compared to the rate of reaction with hydroxyl radicals. 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 $7x10^{-23}$ cm^3/molecule-second (Pate et al. 1976) and atmospheric concentrations for O_3 of $9.6x10^{11}$ molecules/cm^3 (rural) and $3x10^{12}$ 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). 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 10–100 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); however, these species also have relatively short atmospheric lifetimes, which should limit the potential exposure to these compounds. Photooxidation of benzene in a nitrogen monoxide/nitrogen dioxide-air system formed formaldehyde, formic acid, maleic anhydride, phenol, nitrobenzene, and glyoxal (ethane-1,2-dione) (Bandow et al. 1985).

Direct photolysis of benzene in the atmosphere is not likely because the upper atmosphere effectively filters out wavelengths of light <290 nm, and benzene does not absorb wavelengths of light >260 nm (Bryce-Smith and Gilbert 1976).

6.3.2.2 Water

Benzene is subject to indirect photolysis in sunlit surface water, but does not undergo direct photolysis. For direct photolysis to occur, a substance must absorb photons of light >290 nm. During indirect photolysis, light energy is absorbed by other constituents (photosensitizers) of the media (water, soil) and the excited species can then transfer energy to benzene (indirectly promoting it to an excited electronic state), or lead to the formation of reactive species, such as singlet oxygen or hydroxy radicals, which react with benzene. Humic and fulvic acids are well-known photosensitizing agents and are practically ubiquitous in natural waters. 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).

Benzene is readily degraded in water under aerobic conditions. 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 8% (4 mg/L) of the initial 50 mg/L dose of benzene remained (Davis et al. 1981). Water from a petroleum production site was successfully biotreated for complete removal of benzene using a flocculated culture of *Thiobacillus denitrificans* strain F and mixed heterotrophs (Rajganesh et al. 1995).

Microbial degradation of benzene in aquatic environments is influenced by many factors including microbial population, dissolved oxygen, nutrients, other sources of carbon, inhibitors, temperature, pH, and initial concentration of benzene. Vaishnav and Babeu (1987) 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, however, biodegradation did occur, with a half-life of 8 days, in surface water enriched with both nutrients and microbes (Vaishnav and Babeu 1987). At very high levels, as may be the case of a petroleum spill, benzene (and other compounds contained in petroleum) is toxic to microorganisms and the rate of degradation is slow compared to low initial starting concentrations. 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 1 mg/kg to 14 days for an initial benzene concentration of 10 mg/kg. Under acidic 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).

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. Nitrogen enhanced the biodegradation rate of benzene 4.5-fold, over innoculum-enriched water alone. More than 95% of the benzene in groundwater was removed through microbial action within 73.5 hours (Karlson and Frankenberger 1989).

Benzene biodegradation under anaerobic conditions does not readily occur. When dissolved oxygen is depleted, an alternative electron acceptor such as nitrate, carbonate, or iron(III) must be available, and microbes capable of using the alternative electron acceptor to degrade the benzene must be present (McAllister and Chiang 1994). Using aquifer material obtained from a landfill from Norman, Oklahoma, 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 and 99% of the benzene was degraded after 120 weeks (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).

6.3.2.3 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). After acclimation, *Norcardia* species and *Pseudomonas* species, effectively degraded benzene to carbon dioxide within 7 days (45–90%). A strain of *Rhodococcus* isolated from 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 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). The biodegradation of 2 mg of radiolabeled benzene in 100 g of soil with a mixed microbial population transformed 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). According to this model, most of the benzene that would be leaked in soil would either volatilize (67%) or move into groundwater (29%).

Salanitro (1993) summarized the aerobic degradation rates for BTEX in laboratory subsoil-groundwater slurries and aquifers. The data indicate that decay rates for benzene are highest (19–52% per day) for benzene concentrations <1 ppm when initial dissolved oxygen levels are about 8 ppm. Rates are significantly reduced (0–1.1% per day) when benzene levels are 1–2 ppm, and no degradation was observed when benzene levels were >2 ppm. This is particularly relevant in the case of petroleum spills as high concentrations of petroleum compounds are toxic to organisms and decrease the rate of biodegradation.

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, benzene was the only semicontinuously supplied energy sources in the defined mineral medium (Grbić-Galić and Vogel 1987).

After an initial acclimation time of 11 days, at least 50% of the substrate was converted to CO_2 and methane. 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 Grbić-Galić 1992). Similar studies using only benzene at initial concentrations ranging from 40 to 200 μM showed degradation rates ranging from 0.36 to 3.7 μM/day depending upon substrate concentration and the presence of other carbon sources (Edwards and Grbić-Galić 1992).

6.3.2.4 Other Media

Twenty-day-old spinach leaves placed in a hermetic chamber containing vapors of ^{14}C-labeled benzene were shown to assimilate benzene, which was subsequently metabolized to various nonvolatile organic acids (Ugrekhelidze et al. 1997).

6.4 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. Concentrations of benzene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on benzene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring benzene in a variety of environmental media are detailed in Chapter 7.

6.4.1 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 6-2 lists benzene levels in outdoor air from various cities in the United States.

Table 6-2. Benzene Levels in Air Samples

Location	Concentration (ppb)	Comments	References
Outdoor air			
San Francisco, California	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, California	0.38±0.39[a]	Data from remote coastal area in 1984; n=21	Wester et al. 1986
Houston, Texas	1.2–18.7 (range); 37.5[b]	EPA survey, urban area; n=14; summer 1986	EPA 1987a[c]
Houston, Texas	0.55–6.3; 1.73[b]	Semirural area; n=22; 1990–1991	Kelly et al. 1993
St. Louis, Missouri	0.63–12.1 (range); 1.85[b]	EPA survey, urban area; n=18; summer 1985	EPA 1987a[c]
Denver, Colorado	3.0–6.6 (range); 4.1[b]	EPA survey, urban area; n=13; summer 1986	EPA 1987a[c]
Philadelphia, Pennsylvania	0.32–3.0 (range); 1[b]	EPA survey, urban area; n=14; summer 1985	EPA 1987a[c]
New York (Manhattan), New York	0.88–5.3 (range); 1.75[b]	EPA survey, urban area; n=12; summer 1986	EPA 1987a[c]
Chicago, Illinois	0.63–5.05 (range); 3.45[b]	EPA survey, urban area; n=14; summer 1986	EPA 1987a[c]
Boston, Massachusetts	0.69–3.1; 1.06[b]	Urban area; n=22; 1990–1991	Kelly et al. 1993
New York (Staten Island), New York	0.1–34 (range); 4.4±6.6[a]	Urban area; spring 1984	Singh et al. 1985
73 km northwest of Denver, Colorado	0.02–0.85 (range)	Rural area; May 1981–December 1982	Roberts et al. 1985
Elizabeth and Bayonne, New Jersey	Night 2.7[d], 28.5 (max); day, 3.0[d], 13.8 (max)	Night, n=81–86; day, n=86–90; fall 1981	Wallace et al. 1985
Personal air			
Elizabeth and Bayonne, New Jersey	Night 9.7[d], 159.6 (max); day, 8.5[d], 84.5 (max)	Night, n=346–348; day, n=339–341; fall 1981	Wallace et al. 1985
Elizabeth and Bayonne, New Jersey	Winter 5.2, summer 2.7, fall 4.8		Rappaport and Kupper 2004
Los Angles, California	Winter 4.5, spring 2.2, fall 2.8		Rappaport and Kupper 2004

[a]Average±standard deviation
[b]Median
[c]Concentrations reported as ppb carbon; values presented in table represent conversion equivalents as ppb benzene.
[d]Weighted average

EPA = Environmental Protection Agency; max = maximum; N = number of samples

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). 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 a 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.

Ambient air samples from 44 sites in 39 U.S. urban areas were collected from 6 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 0.8 to 6 ppb, with the overall median being 2.1 ppb (estimated detection limit ~0.04 ppb carbon; conversion equivalent ≈0.007 ppb benzene). The data indicated that mobile sources were the major source of benzene in the vast majority of samples (EPA 1987a). Benzene concentrations were in the range of >1–<5 ppbv for all of the 13 sites tested in a 1996 study. The sites included: Baton Rouge, Louisiana; Brownsville, Texas; Brattleboro, Vermont; Burlington, Vermont; Camden, New Jersey; El Paso, Texas; Garyville, Louisiana; Galveston, Texas; Hanville, Louisiana; Port Neches, Texas; Rutland, Vermont; Underhill, Vermont; and Winooski, Vermont (Mohamed et al. 2002).

The following daily median benzene air concentrations were reported in 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 while the indoor air data represent 30 cities in 16 states (Shah and Singh 1988). In a NHEXAS study of six states in the Great Lakes region, benzene was found in 99.7% of the 386 personal air samples taken with an average concentration of 2.35 ppb. Benzene was also found in 100% of both indoor and outdoor samples with concentrations averaging 2.25 and 1.13 ppb, respectively (Clayton et al. 1999).

An EPA study of the concentration of various pollutants in 1990 showed an average concentration of benzene of 0.948 ppb. The source of benzene included manufacturing facilities and manufacturers that emit benzene to the environment (17%), mobile (60%), and background (23%) sources (Morello-Frosch et al. 2000).

In California, motor vehicle exhaust accounted for over 70% of the nonsmoking population’s exposure to ambient benzene (Cal EPA 1987). The 1984 population-weighted average benzene concentration in California was estimated to be 3.3 ppb (Cal EPA 1987). Benzene emissions in a Los Angeles roadway tunnel were measured at a concentration of 382 mg/L (118,420 ppm) (Fraser et al. 1998).

Industrial sites that produce benzene are monitored often. Analysis of ambient air samples collected in industrial areas showed benzene levels ranging from 0.13 to 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).

An analysis of 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 1.2 ppb (Bennett 1987). Maximum estimated levels of benzene in air near uncontrolled (Superfund) hazardous waste sites were 59.5 ppb at the Kin-Buc Landfill (Edison, New Jersey) and 162.8 ppb in Love Canal basements (Niagara Falls, New York) (Bennett 1987; Pellizzari 1982).

According to the National Cancer Institute, the average concentration of benzene in 1998 was 0.58 ppb in metropolitan areas (NCI 2003). A study of New York City air found mean values of 0.80, 1.87, and 1.47 ppb for outdoor (home), indoor (home), and personal air, respectively (Kinney et al. 2002). According to the New York Department of Health, the air from about 50% of oil fuel heated homes between the years 1997 and 2003 contained benzene concentrations ≥0.69 ppb inside the home, and 0.47 ppb in the area outside their homes. New York City was not included in this study (NYSDOH 2005). Concentrations of VOCs 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 Assessment Methodology (TEAM) study. The overall mean personal exposure was about 4.7 ppb, compared to an overall mean outdoor concentration of only 1.9 ppb (Wallace 1989a). The study also reported that the median level of benzene in 185 homes without smokers was 2.2 ppb, and the median level of benzene in 343 homes with one or more smokers was 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 8.08–11.3 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 1.21 ppb with a maximum of 5.93 ppb. In 25 smoking homes, the mean benzene concentration was 1.73 ppb, with a maximum of 8.43 ppb. However, benzene was not significantly correlated or associated with 3-ethenylpyridine, a proposed vapor phase environmental tobacco smoke marker (Heavner et al. 1995).

A British study measured benzene concentrations from both rural and urban areas in 1995. The highest concentrations of benzene were in Southampton urban center, London roadside, and Liverpool, which contained measured benzene levels of 2.53, 1.69,, and 1.60 ppb, respectively. The lowest measured benzene concentrations were in rural Hartwell and urban Edinborough, which contained 0.4 and 0.69 ppb of benzene, respectively (Duarte-Davidson 2001).

6.4.2 Water

Data from EPA's STOrage and RETrieval (STORET) database (2003–2005) showed that benzene was positively detected in 38% of the surface water samples collected at 571 observation stations ranging from concentrations too low to quantify to 100 μg/L (100 ppb) in one Utah test site. 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 (EPA 2005f).

Benzene was found in 904 of the 8,200 samples tested by the U.S. Geological Survey across the United States and Canada (USGS 2005). The EPA found benzene in 13,919 and 1,119 groundwater and surface water samples, respectively (EPA 2001).

Benzene is found in both polluted and unpolluted waters and rainwater. Measured benzene levels in open ocean from the Gulf of Mexico in 1977 in relatively unpolluted and polluted waters were 5–15 ng/kg (5–15 parts per trillion [ppt]), and 5–40 ng/kg (5–40 ppt), respectively (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 Thorburn 1980).

Benzene levels in water in the vicinity of five industrial facilities using or producing benzene ranged from <1 ppb to a high of 179 ppb found in plant effluent. In general, benzene in plant effluents quickly dispersed in rivers or streams to levels of 1–2 ppb or less (EPA 1979). 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). At detection limits ranging from 0.2 to 1.0 ppb, benzene was detected at a concentration of 2 ppb in only one sample of 182 samples of bottled drinking water (Page et al. 1993). In six states of the Great Lakes region, unspecified amounts of benzene were found in 5.7% of the 247 drinking water sites tested (Clayton et al. 1999).

Because the New Jersey Department of Environmental Protection identified serious water issues in a 930 km^2 area within metropolitan Philadelphia, tests have been done to find other water sources such as the Kirkwood-Cohansey aquifer system. A study of 57 water samples taken from the shallow ground water supply at Kirkwood Cohansey aquifer in New Jersey showed that the majority of samples (50) contained between 0.2 and 1 ppbv of benzene, three samples contained <0.1 ppbv, and four samples contained >1 ppbv (Baehr et al. 1999).

6.4.3 Sediment and Soil

A Canadian study estimated that benzene is absorbed by the soil at a rate of 100 tons/year (MacLeod and MacKay 1999). Data from EPA's STORET database (2003–2005) showed that benzene had been positively detected in sediment samples taken at 9% of 355 observation stations with a median level of <5 ppb (EPA 2005f). The concentration of benzene in soil near factories where benzene was produced or used ranged from 2 to 191 ppb (IARC 1982).

Benzene levels ranging from <2 to 191 ppb were recorded in the vicinity of five industrial facilities using or producing benzene (EPA 1979). 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 about 1,000 feet from that storage terminal contained benzene gas at a concentration of 1,500 ppm at a depth of 10 feet (Mushrush et al. 1994).

6.4.4 Other Environmental Media

Benzene has been detected in a variety of food. Benzene has been reported to occur in fruits, fish, vegetables, nuts, dairy products, beverages, and eggs (EPA 1982a). 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). 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, 1982a). A 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 (e.g., imitation strawberry preserves, taco sauce, and duck sauce) ranged from <1 to 38 ng/g. In many foods, the presence of benzene is likely to be due to uptake from the air (Grob et al. 1990). This conclusion was supported by the fact that the uptake decreased with a decrease in exposed surface area of foods and contact time with air (Grob et al. 1990).

The U.S. Food and Drug Administration sponsored a 5-year study to determine the amount of volatile organics in food from 1996 to 2000. The results are shown in Table 6-3 (Fleming-Jones and Smith 2003). Benzene was found in cheddar cheese, cream cheese, margarine, butter, sour cream, ground beef, bologna, hamburger, cheeseburger, pork, beef frankfurters, tuna canned in oil, chicken nuggets, chocolate

Table 6-3. Benzene in Food[a]

Food	Number of cases found (n=14)	Concentration minimum-maximum in ppb
Cheddar cheese	2	20–47
Mixed nuts	3	1–6
Ground beef	12	9–190
Pork bacon	6	2–17
Banana, raw	13	11–132
Frankfurters, beef	4	2–11
Cream cheese	3	1–17
Chocolate cake icing	2	2–23
Tuna canned in oil	7	4–13
Fruit flavored cereal	5	2–21
Eggs scrambled	4	2–40
Peanut butter	5	2–25
Avocado, raw	10	3–30
Popcorn, popped in oil	3	4–22
Blueberry muffin	3	3–8
Strawberries, raw	1	1
Cola carbonated	3	1–138
Orange, raw	2	11–15
Coleslaw with dressing	14	11–102
Sweet roll danish	1	3–3
Potato chips	2	2–7
Fruit flavored sherbet	3	3–61
Quarter pound hamburger cooked	11	4–47
Margarine	1	7
Sandwich cookies	3	1–39
Butter	6	4-22
Chocolate chip cookies	2	1–8
Sour cream	2	3–15
Apple pie fresh/frozen	4	2–11
Chicken nuggets fast food	4	2–5
Graham crackers	2	1–9
French fries fast food	3	2–56
Cheeseburger quarter pound	8	5–44
Cheese pizza	2	1–2
Bologna	4	2–44
Cheese and pepperoni pizza	4	8–30
Olive/safflower oil	6	1–46
Sugar cookies	3	8–30
Cake doughnuts with icing	2	3
Popscicle	4	1–10

[a]Derived from Fleming-Jones and Smith 2003

cake icing, sandwich cookie, chocolate chip cookies, graham crackers, sugar cookies, cake doughnuts with icing, french fries, apple pie, cola carbonated beverages, sweet roll Danish, potato chips, cheese pizza, cheese and pepperoni pizza, mixed nuts, fruit-flavored cereal, fruit flavored sorbet, popsicles, olive/safflower oil, scrambled eggs, peanut butter, popcorn popped in oil, blueberry muffins, coleslaw with dressing, raw banana, avocado, oranges, and strawberries. American cheese was the only food tested that did not contain benzene. Foods with the greatest maximum concentration of benzene included ground beef (maximum 190 ppb), raw bananas (maximum 132 ppb), carbonated cola (maximum 138 ppb), and coleslaw with dressing (maximum 102 ppb).

As part of a program to identify possible exposures that may be important in the high incidence of lung cancer among women in Shanghai, China, 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.

Cigarette smoke remains an important source of human exposure to benzene. 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). Benzene has been found in vapor from cigarette smoke in concentrations ranging from 3.2 to 61.2 µg/cigarette depending on the brand of cigarette. The amount of tar in the cigarette was not directly related to the benzene concentration (Darrall et al. 1998).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

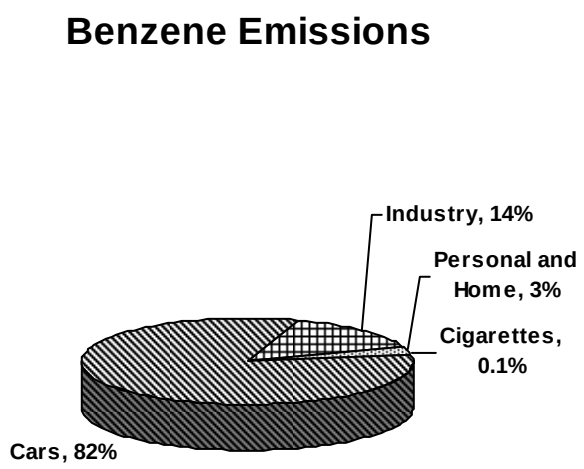
Exposure to low levels of environmental benzene is unavoidable due to the ubiquitous presence of benzene in the environment from a variety of anthropogenic sources. Benzene can be detected in blood samples. In a study designed to establish reference blood concentrations of benzene and other selected volatile organic compounds in the general population of the United States, Ashley et al. (1994) measured blood benzene levels in 883 people (not occupationally exposed) in the United States who had participated in the Third National Health and Nutrition Examination Survey (NHANES III). Within this group of nonoccupationally exposed subjects, the mean and median blood benzene levels were 0.13 and 0.061 ppb, respectively. Seven nonsmoking subjects from this study group were assessed for blood benzene levels just prior to entering examination vans with limited potential for benzene exposure and at

the end of a 3-hour period in this environment. Mean blood benzene levels prior to van entry and after 3 hours in the van measured 0.046 ppb (range from not detected to 0.061 ppb) and 0.033 ppb (range from not detected to 0.055 ppb), respectively. Lemire et al. (2004) reported a median blood benzene level of 0.047 ppb in a subgroup of 546 nonsmoking and nonoccupationally exposed subjects from the NHANES III study group. Elevated blood benzene levels may be expected among subjects with potential for elevated exposure to benzene, such as smokers, commuters, gas station attendants, and people who use products that emit benzene.

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 nearly 85% of atmospheric benzene in outdoor air is produced by cars burning petroleum products and the remaining 15% is produced by industry. Despite the fact that petroleum products contribute to the majority of benzene in the atmosphere, half of the total national personal 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 6-3. 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 $\mu g/m^3$ (4.7 ppb), almost 10 times the level of 1.5–2 $\mu g/m^3$ (0.47–0.63 ppb) observed in more than 300 nonsmokers (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. This suggests that smoking represented an additional source of benzene above that of outdoor ambient air (Wester et al. 1986). In 10 of 11 homes inhabited by tobacco smokers,

Figure 6-3. Benzene Emissions and Exposures



Note: A comparison of benzene emission sources (home sources include paints and petroleum products; personal activities include driving and use of consumer products that contain benzene). Data taken from Wallace et al. (1991).

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) estimated 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 two packs of cigarettes per day will have an additional daily intake of 1,200 µg.

A British study of rural and urban environments suggests that benzene exposure is greatly affected by proximity to smokers (Duarte-Davidson et al. 2001). Air concentrations of benzene at an urban center in South Hampton averaged about 2.5 ppb, while in a rural location in Hartwell, the average amount of benzene in the air was 0.41 ppb. Air at a smoky pub was found to contain 22 ppb of benzene. Comparing the daily doses of rural nonsmokers, urban nonsmokers, urban passive smokers, and urban smokers, very little difference between the rural nonsmokers' 24 ppb daily dose and the urban nonsmokers' 30 ppb daily dose was found. Passive urban smokers, on average, have a daily benzene exposure dose of 38 ppb of benzene while smokers have a daily exposure dose of benzene of 163 ppb. On average, nonsmokers in urban and rural environments have estimated benzene intakes of 1.15 and 1.5 µg/kg body weight/day.

Women tend to intake more of benzene per kg body weight than men. Passive smokers' estimated daily intake averages are 2.10 and 1.74 µg/kg body weight/day for women and men, respectively. Urban women and men smokers' estimated intakes are estimated at 9.00 and 7.46 µg/kg body weight/day, respectively; this is equivalent to an atmospheric concentration of 8.2 ppb (Duarte-Davidson et al. 2001).

In 1990, a study in Germany analyzed factors that predicted people's exposures to VOCs and found that while smoking was the most significant determinant of benzene exposure, automobile-related activities, such as refueling and driving, were also significant (Hoffmann et al. 2000). 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 1–2% 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 μg/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 μg/day (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 μg/L (median=0.12 μg/L). Blood benzene levels of 0.29, 0.14, and 0.58 μg/L were measured in one female and two male smoking commuters, respectively. In 12 nonsmoking male car repair workers, blood benzene levels ranged from 0.11 to 0.98 μg/L (median=0.19 μg/L); in 8 smoking male car repair workers, levels ranged from 0.17 to 0.67 μg/L (median=0.42 μg/L). Three nonsmoking male gasoline attendants had blood benzene levels ranging from 0.32 to 0.47 μg/L (median=0.36 μg/L) (White et al. 1993).

Pumping gasoline can also be a significant source of exposure to benzene. A study conducted between July 1998 and March 1999 that comprised of 39 customers of gasoline self service stations from North Carolina, measured the benzene level in the air around the station as well as the levels of benzene in customers' breath prior to and immediately after fueling (Egeghy et al. 2000). Benzene levels in the air around the station ranged from <0.02 to 11.16 ppm, with a mean (±1 standard deviation) of 0.91 (±1.8) ppm. The range of benzene levels in the breath of customers prior to fueling was <0.001–0.022 ppm with a mean (±1 standard deviation) of 0.0027 (±0.0034) ppm while the range of benzene levels in the breath of customers after re-fueling was <0.001–0.434 ppm with a mean (±1 standard deviation) of 0.05 (±0.081) ppm (Egeghy et al. 2000). Another study reported a benzene concentration of 1 ppm at the breathing-level of a person pumping gas (EPA 1986). Using this concentration and an estimated 70 minutes/year of time spent pumping gasoline, a benzene intake of 10 μg/day has been calculated (Wallace 1989a). In a group of 26 subjects who were not occupationally exposed to benzene, but were exposed to benzene during refueling in Fairbanks, Alaska, median blood benzene levels prior to and immediately following refueling were 0.19 ppb (range 0.08–0.65 ppb) and 0.54 ppb (range 0.13–1.70 ppb), respectively (Backer et al. 1997).

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 0.94 to 61.3 ppb. 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).

A study of human exposure to benzene in the California South Coast Air Basin showed that benzene exposure in that area has decreased greatly since 1989. Adult smokers saw a 28% decrease in benzene exposure from 1989 to 1997, while adult nonsmokers and adolescents saw a decrease in benzene exposure of 67 and 55%, respectively. Where people were being exposed to benzene also changed during that time. Ambient air was the source of 49% of the benzene that nonsmokers were exposed to in 1997, which is less than the 59% from 1987. For smokers in the region, 85% of their benzene exposure was from smoking and 4% was from the ambient air in 1997. This shows an increase in benzene exposure from smoking since 1987 (78%) and a decrease in exposure from the ambient air (12%) (Fruin et al. 2001). The main sources credited with this decrease in exposure are reformulated gasoline and stricter air emission laws as well as smoking control measures.

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 10–20% by weight of total non-methane hydrocarbons (Barrefors and Petersson 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 2007). According to another estimate, the daily intakes of benzene for a nonsmoking individual (not exposed to secondary smoke) are 1–550 µg (Fishbein 1992).

While most human exposure to benzene is believed to be through inhalation, studies show that benzene can permeate skin with a permeability factor of about 0.14–0.18 cm/hour at 25 °C. The permeability factor was not affected by moisturizer, baby oil, or insect repellent; however, it was affected by temperature (50 °C) and sunscreen with the permeability factors increasing to 0.26 and 0.24 cm/hour, respectively (Nakai et al. 1997).

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, dieticians, pharmacists, and janitors (NIOSH 1990). 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 15-minute period is 5 ppm (OSHA 2003). The NIOSH recommended exposure limit is 0.1 ppm for an 8-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 6-4) (OSHA 1987). 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 nonsmoking workers versus 0.25 µg/L

Table 6-4. 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 1987
[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

for nonsmoking controls). OSHA requires the use of engineering controls and/or respiratory protection in situations where compliance with the TWA is not feasible (OSHA 1987).

Certain jobs, such as gasoline station workers, firefighters, and dry cleaners, are believed to put people at a higher risk of benzene exposure. In an analysis of literature, it was estimated that workers in the area of crude petroleum and natural gas are exposed to 0.04 ppm benzene, while workers in petroleum refining, gas stations, and crude petroleum pipelines are exposed to 0.22, 0.12 and 0.25 ppm benzene, respectively. This study also showed that fire fighters are exposed to an average of 0.38 ppm benzene (van Wijngaarden and Stewart 2003). Workers from four different dry cleaning facilities in Korea had mean benzene air concentrations ranging from 2.7 to 3.2 ppb. Their exposure to benzene was dependent upon the type of solvent used for cleaning (Jo and Kim 2001). Benzene concentrations of 25.46 and 1,331.29 ppb were found near the kiln and at the rotary line, respectively, inside a hazardous waste incinerator in Turkey (Bakoglu et al. 2004).

A study comparing workers who were exposed to benzene regularly at work to people who were not exposed to benzene at work showed that while the general population in Italy had average blood benzene concentration of 165 ng/L, the people who were exposed to high benzene levels at work had an average benzene blood concentration of 186 ng/L. Immediately following their shift, the average benzene blood level samples from of benzene-exposed workers was 420 ng/L. The average blood benzene levels for smoking and nonsmoking occupationally exposed workers were 264 and 123 ng/L respectively (Brugnone et al. 1998).

6.6 EXPOSURES OF CHILDREN

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

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths,

sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children can be subject to increased benzene exposure by inhalation of second-hand smoke. In a study of nonsmoking rural families, urban families, and urban smoking families, infant exposure to benzene was estimated at doses of 15.3, 19.7, and 25.9 µg/day, respectively, with daily intakes of 1.68, 2.16, and 2.55 µg/kg body weight/day, respectively. For children of the same classification, benzene exposure was measured at doses of 29.3, 37.6, and 49.3 µg/day, respectively, with daily intakes of 0.71, 0.91, and 1.20 µg/kg bodyweight/day, respectively. For all infants and children, benzene exposure predominantly comes from the indoors (Duarte-Davidson et al. 2001).

Depending on a child's living environment, they may have higher exposure to benzene than adults. In a study of two lower-income areas of Minneapolis, children were found to have average personal benzene exposures of 0.66 and 0.53 ppb in the winter and spring, respectively. The highest concentration of benzene in their environment came from the home with winter and spring concentrations of 0.69 and 0.66 ppb respectively, while the outdoor and school benzene concentrations were 0.41 and 0.19 ppb, respectively (Adgate et al. 2004). In Italy, concentrations of the benzene metabolite, *trans,trans*-muconic acid (MA), was measured in the urine of children from both urban areas in Naples and rural areas in Pollica. The mean urinary concentrations of MA detected for rural and urban children were 48.4 and 98.7 µg/L (Amodio-Cocchieri et al. 2001). These studies also found no strong link between passive smoking and MA levels. The only factor that affected levels of MA in urine samples was how close the family lived to the road. A study in Rouen, France, compared benzene exposure and concentrations in nonsmoking parents and their children. Despite the fact that the children were exposed to slightly less benzene 3.47 ppb (11.1 µg/m^3) than their parents 4.51 ppb (14.4 µg/m^3), there was no significant correlation between exposure means and urinary metabolite levels (Kouniali et al. 2003).

Benzene concentrations in women and their tissue as well as breast milk have been a major concern. In a study of South Korean housewives living near service stations, indoor, outdoor and breath benzene concentrations averaged 5.73, 3.63, and 3.29 ppm, respectively (Jo and Moon 1999). Benzene has been found in mother's milk (Pellizzari 1982). Benzene was found at mean concentrations of 0.06 µg/kg in 23 samples of breast milk taken from a children's hospital in Rome (Fabietti et al. 2004). While this may provide a mechanism by which infants are exposed to benzene, these concentrations are lower than in other foods.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

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 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. 1994). 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 6 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 total benzene dose resulting from the shower was estimated to be approximately 281 µg, with 40% via inhalation and 60% via the dermal pathway (Lindstrom et al. 1994).

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). In a study in Germany, the mean benzene concentrations for frequent smokers and nonsmokers were 6.1 and 2.4 ppb, respectively (Hoffmann et al. 2000).

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 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 the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. 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, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2004, became available in May of 2006. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Benzene is one of the top 20 highest volume chemicals produced in the United States. In 1994, the U.S. production volume of benzene was 14.7 billion pounds (C&EN 1995). The production volume during the 1984–1994 period has increased by 4% annually (C&EN 1995). The United States currently has a benzene production capacity of 11.8 billion liters (SRI 2004). Imports of benzene into the United States have generally ranged from 10,176 to 11,672 million pounds during 2002–2004 (USITC 2005). Exports increased from 21 million pounds in 2002 to 290 million pounds in 2003, but decreased to 145 million pounds in 2004 (USITC 2005). 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, 1987), and as an anti-knock agent in unleaded gasoline (Brief et al. 1980; EPA 1985a). 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 1978; 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 2007). 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. 1994; Lodén 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 2006; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1994; Mannino et al. 1995; Nomiyama and

Nomiyama 1974a; Ong and Lee 1994; Ong et al. 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 (Bennett 1987; Black et al. 1980; Brief et al. 1980; Cal EPA 1987; Edgerton and Shah 1992; EPA 1989, 1994d; Glass et al. 1986; Graedel 1978; Mayer et al. 1994; TRI02 2005; Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986; Wood and Porter 1987), drinking water (CDC 1994; EPA 1979), and soil (HazDat 2006; Mushrush et al. 1994; TRI02 2005). 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 appears to be needed.

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

Benzene is widely distributed in the environment and has been detected in air (Clayton et al. 1999; EPA 1987a; Mohamed et al. 2002; Morello-Frosch et al. 2000), water (EPA 2001, 2005f; Sauer 1981, USGS 2005), soil (EPA 1979; Ferrario et al. 1985; MacLeod and MacKay 1999; Staples et al. 1985), sediment, and some foods (EPA 1980b, 1982a; Fleming-Jones and Smith 2003). 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. 1984); 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. 1989b; Karacic et al. 1987; OSHA 1987; van Wijngaarden and Stewart 2003). 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. 1989b). 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.

Exposures of Children. Benzene levels have been monitored in children and the environments in which they live. This information gives levels found for infants and children in rural and urban area as well as the levels found for children in homes of parents who smoke (Duarte-Davidson et al. 2001). There have been many studies relating oil and petroleum exposure to childhood leukemia and other diseases; however, the majority of these studies have not recorded benzene levels. More information about the exposures of children, particularly those subject to high exposures such as smoking, busy roads, and gasoline stations, are needed.

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

Exposure Registries. In 2001, 1,143 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 was carried out by the Exposure and Disease Registry Branch (EDRB), Division of Health Studies (DHS), ATSDR. The

data became part of public-user data files maintained by ATSDR. The statistical analyses of the baseline data was completed and published (Agency for Toxic Substances and Disease Registry 1995). The information that was amassed in the National Exposure Registry will be used to facilitate the epidemiological research needs to assess adverse health outcomes that may be related to the exposure to this substance.

6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2005) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These studies are summarized in Table 6-5.

Table 6-5. Ongoing Studies on the Potential for Human Exposure to Benzene[a]

Investigator	Affiliation	Research description	Sponsor
Cho, CY	CHA Corporation; Laramie, Wyoming	Study of the use of microwave technology for superfund site remediation	NIEHS
Fischer, LJ		Understanding potential health hazards from groundwater and soil contamination of chemicals commonly found at hazardous waste sites	USDA
Greenberg, A		Study of critical steps in the ring-opening metabolism of the human carcinogen benzene to muconaldehyde	USDA
Gurian, PL	University of El Paso Texas; El Paso, Texas	Modeling of organic compounds in drinking water	National Institute of General Medical Sciences
Nylander-French, LA	University of North Carolina; Chapel Hill, North Carolina	Assessment of dermal exposure to benzene and naphthalene	NIEHS
Rothman, N		Study of occupational and environmental exposures	Division of cancer epidemiology and genetics
Scow, KM	University of California; Davis, California	Molecular characterization of aquifer microbial communities	NIEHS
Stenzel, PS	National Institute of Health	Study of the link between occupational exposure of carcinogens and cancer	Division of cancer epidemiology and genetics
Thrall, KD	Oregon Health and Science University; Portland, Oregon	Assessment of human volatile organic compounds exposure near Superfund sites	NIEHS
Weisel, CP		Study of the modulation of benzene metabolism by exposure to environmental mixtures	USDA

[a]FEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; USDA = U.S. Department of Agriculture

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7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, 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.

7.1 BIOLOGICAL MATERIALS

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 7-1.

Breath samples are collected on a solid sorbent (EPA 1987b; Gruenke et al. 1986; Pellizzari et al. 1988; Wallace et al. 1985), in canisters (Thomas et al. 1991), or 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 (EPA 1987b; Pellizzari et al. 1988; Wallace et al. 1985). Techniques involving headspace analysis of benzene adsorbed on silica gel have 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 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

Table 7-1. 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
Blood	Centrifugation; dialysis; precipitation; acidic hydrolysis; Sep Pak cartridge column purification	HPLC	20 pmol/g globin	NR	Hanway et al. 2000
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 = hydrochloric 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

used for analyzing benzene (Michael et al. 1980; Pellizzari 1982). 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 Bourne 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/FID. Care must be taken when preparing benzene metabolite samples from urine and bodily fluids in order to protect against enzymatic and oxidative degradation. These samples are often treated to denature enzymes and avoid oxidation by hydroquinone. 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 GC/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 7-2. 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%) (IARC 1988). Sulfate and glucuronide conjugates 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.

Table 7-2. 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	IARC 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
Urine (phenol)	Enzymatic reaction	HPLC/fluoro-metric 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 et al. 1995
Urine (S-phenyl-mercapturic acid	Solid-phase extraction; hydrolysis; derivatization	HPLC	1 µg/L	NR	Einig and Dehnen 1995
Urine (*trans,trans*-muconic acid)	Cleanup on anion exchange resin	HPLC/UV	5 ppb	NR	Weaver et al. 2000

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

Analysis of urinary *trans,trans*-muconic acid 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. 1989b). The detection of low levels of *trans,trans*-muconic acid in urine was difficult by earlier methods because of low recovery of *trans,trans*-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 *trans,trans*-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). Weaver et al. (2000) used an anion exchange column to extract the urine sample, which was then analyzed using HPLC-UV; detection limits were around 5 ppb. The relative standard deviation of the method was 5% in the concentration range 1–20 ng/L. *trans,trans*-muconic acid has been determined directly by HPLC/UV with similar sensitivity (detection limit=0.1 mg/L) (Inoue et al. 1989b). The detection limit and specificity for the determination of urinary *trans,trans*-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 *trans,trans*-muconic acid at concentrations above 40 µg/L, but GC/MS is capable of detecting the metabolite at concentrations below 40 µg/L (Bartczak et al. 1994).

The metabolite, S-phenyl-mercaptic acid, 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 (Einig and Dehnen 1995). After purification by reverse phase cartridge chromatography, S-phenyl cysteine was detected with sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000).

7.2 ENVIRONMENTAL SAMPLES

Methods exist for determining benzene in air (ambient, occupational, and industrial), water, sediment, 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 7-3 summarizes several of the methods that have been used to analyze for benzene in environmental samples.

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 1979, 1980a; Fung and Wright 1986; Gruenke et al. 1986; Harkov et al. 1985; Reineke

Table 7-3. 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	EPA 1985d
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 1979
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
Air (consumer products)	Sample trapped on solid sorbent; thermal desorption	GC/MS	NR	NR	Bayer et al. 1988

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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)	10 ppb	NR	NIOSH 1994
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 1992a
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 1979
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
Water	Purge and trap on adsorbent column; thermal desorption	GC/PID (EPA Method 602)	0.2 µg/L	106	NEMI 2005a
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

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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
Waste water	Purge and trap onto adsorbent column; thermal desorption	GC/MS (EPA Method 624)	4.4 µg/L	113	NEMI 2005b
Waste water	Addition of isotopically labeled benzene analog; purge and trap onto adsorbent column; thermal desorption	GC/IDMS (EPA Method 1624)	10 µg/L	65–141	NEMI 2005c
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	EPA 1984b
Landfill leachate	Extract sample with pentane	GC/MS	1,000–10,000 µg/L	NR	Schultz and Kjeldsen 1986
Solid wastes	Purge and trap, direct injection, vacuum distillation, or headspace	PID (EPA Method 8021B)	0.009 µg/L	99	EPA 1994c
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 1979
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
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

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Mainstream cigarette smoke	Collection on filters and impingers; [2H6]-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

and Bächmann 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 1988a; 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 (EPA 1985c; Kowalski et al. 1985; Nutmagul and Cronn 1985; Reineke and Bächmann 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 (DOE 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, and 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 (Gonzalez 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 Thorburn 1980; DOI 1984; EPA 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Lysyj et al. 1980; Michael et al. 1988; Pereira and Hughes 1980; Sporstol et al. 1985). Benzene is usually isolated from aqueous media by the purge-and-trap method (Brass et al. 1977; Colenutt and Thorburn 1980; DOI 1984; EPA 1979, 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Michael et al. 1988). 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 (≈70%) (EPA 1979, 1984; Michael et al. 1988) to very good (≥90%) (Colenutt and Thorburn 1980; EPA 1984, 1992; Hammers and Bosman 1986). Detection limits in the sub-ppb to ppt range may be attained with HRGC/MS techniques (EPA 1992a); Michael et al. 1988). Liquid-liquid extraction procedures (Harrison et al. 1994; Schultz and Kjeldsen 1986; Sporstol 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 1979, 1994c; 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 1994c). 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.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 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 the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. 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 Bourne 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 test for 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; IARC 1988; Jen and Tsai 1994; Jongeneelen et al. 1987; 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 *trans,trans*-muconic acid

(detection limit 10 µg/L) (Bechtold et al. 1991; Ruppert et al. 1995). Since urinary *trans,trans*-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; EPA 1992b; Weaver et al. 2000). 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. S-phenyl-mercapturic acid has also been useful in biological monitoring of benzene exposure in humans and animals. S-phenyl-mercapturic acid can be measured with a sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000). S-phenyl-mercapturic acid levels can also be correlated to environmental benzene exposure (Popp et al. 1994), which may indicate its utility as a biomarker.

Effect. 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 would be useful.

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 1979, 1984; 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.

7.3.2 Ongoing Studies

The information in Table 7-4 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2005).

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, 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 give detection limits in the low parts per trillion (ppt) range.

Table 7-4. Ongoing Studies on Benzene, Analytical Methods[a]

Investigator	Affiliation	Research description	Sponsor
Fales, HM		Analysis of proteins, peptides, and metabolites for carcinogen exposure using mass spectrometry	USDA
Kalman, DA	University of Washington Seattle, Washington	Study of the use of human dosimetry for assessment of exposure to volatile compounds	NIEHS
Kavanaugh, TJ		Study of glutathione biosynthesis as a biomarker of toxic exposure	NSF
O'Brien, RJ		Assessment Environmental Health Breath using PF-GC	NIEHS
Pavel, K	VOC Technologies Inc.; Portland, Oregon	Assessment Environmental Health Breath using PF-GC	NIEHS
Rappaport, SM	University of North Carolina; Chapel Hill, North Carolina	Development and application of biomarkers in the study of exposure to carcinogens	NIEHS
Sabri, MI	Oregon Health Science University; Portland, Oregon	Study of biomarkers of neurotoxicant exposure and neurodegeneration	NIH
Smejtek, PK	VOC Technologies Inc.; Portland, Oregon	A PF-GC for Environmental Health Breath Assessment	NIEHS
Smith, MT	University of California at Berkeley	Study of biomarkers of benzene exposure and genotoxicity	NIEHS
Spencer, PS	Oregon Health Sciences University; Portland, Oregon	Study of neurotoxic chemicals and biomarkers at superfund sites	NIH
Stewart, P		Use of biomarkers to study occupational exposure of carcinogens in order to enhance exposure assessment	Division of cancer epidemiology and genetics
Turtletaub, KW	University of California at Berkeley	Study and development of biomarkers for exposure using accelerator mass spectrometry	NIEHS
Weaver, VM		Use of biomarkers to study benzene exposure in inner city residents	USDA
Wiencke, JK		Use of molecular biomarkers of occupational benzene exposure	USDA

[a]FEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health; NSF = National Science Foundation; USDA = U.S. Department of Agriculture

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8. REGULATIONS AND ADVISORIES

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

ATSDR has derived an acute-duration inhalation MRL of 0.009 ppm for benzene based on a LOAEL of 10.2 ppm for immunological effects in mice exposed for 6 hours/day for 6 consecutive days (Rozen et al. 1984). The LOAEL of 10.2 ppm was adjusted from intermittent to continuous exposure ($LOAEL_{ADJ}$=2.55 ppm) and converted to a human equivalent concentration ($LOAEL_{HEC}$=2.55 ppm) using EPA (1994b) methodology for a category 3 gas; an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 to protect sensitive individuals) was applied.

ATSDR has derived an intermediate-duration inhalation MRL of 0.006 ppm for benzene based on a LOAEL of 10 ppm for significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction following the exposure of male C57Bl/6 mice to benzene vapors for 6 hours/day, 5 days/week for 20 exposure days (Rosenthal and Snyder 1987). The concentration was adjusted from intermittent to continuous exposure (LOAELADJ=1.8 ppm) and converted to a human equivalent concentration ($LOAEL_{HEC}$=1.8 ppm) using EPA (1994b) methodology for a category 3 gas; an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) was applied.

ATSDR has derived a chronic-duration inhalation MRL of 0.003 ppm for benzene based on the results of benchmark dose (BMD) modeling of B cell counts in workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a). The resulting $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from intermittent to continuous exposure ($BMCL_{0.25sdADJ}$=0.03 ppm) using EPA (1994b) methodology; an uncertainty factor of 10 (to protect sensitive individuals) was applied.

ATSDR has derived a chronic-duration oral MRL of 0.0005 mg/kg/day for benzene based on estimation of equivalent chronic-duration oral dosing that would result in effects similar to those observed in the occupationally-exposed workers assessed by Lan et al. (2004a, 2004b). The $BMCL_{0.25sdADJ}$ of 0.03 ppm (0.096 mg/m^3) was converted to an equivalent $BMDL_{0.25sdADJ}$ of 0.014 mg/kg/day for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure;

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 1[a]	IARC 1987, 2004, 2007
WHO	Air quality guidelines	$6x10^{-6}$ unit risk	WHO 2000
	Drinking water quality guidelines	0.01 mg/L[b]	WHO 2004
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (TWA)	0.5 ppm[c]	ACGIH 2006
	STEL	2.5 ppm[c]	
EPA	Hazardous air pollutant	Yes	EPA 2004b 42 USC 7412
NAS/NRC	AEGL-1[d]		EPA 2005a
	10 minutes	130 ppm	
	30 minutes	73 ppm	
	60 minutes	52 ppm	
	4 hours	18 ppm	
	8 hours	9.0 ppm	
	AEGL-2[d]		
	10 minutes	2,000 ppm[e]	
	30 minutes	1,100 ppm	
	60 minutes	800 ppm	
	4 hours	400 ppm	
	8 hours	200 ppm	
	AEGL-3[d]		
	10 minutes	9,700 ppm[f]	
	30 minutes	5,600 ppm[e]	
	60 minutes	4,000 ppm[e]	
	4 hours	2,000 ppm[e]	
	8 hours	990 ppm	
NIOSH	REL (10-hour TWA)	0.1 ppm[g]	NIOSH 2005
	STEL	1.0 ppm[g]	
	IDLH	500 ppm[g]	
OSHA	PEL (8-hour TWA) for general industry	1 ppm	OSHA 2005b, 2005e 29 CFR 1910.1000 29 CFR 1910.1028
	PEL (8-hour TWA) for construction industry	1 ppm	OSHA 2005d, 2005f 29 CFR 1926.55, Appendix A 29 CFR 1926.1128

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
OSHA	PEL (8-hour TWA) for shipyard industry	1 ppm	OSHA 2005a,2005b 29 CFR 1915.1000 29 CFR 1915.1028
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	Yes	EPA 2005b 40 CFR 116.4
	Drinking water standards and health advisories		EPA 2004a
	1-day health advisory for a 10-kg child	0.2 mg/L	
	10-day health advisory for a 10-kg child	0.2 mg/L	
	DWEL	0.1 mg/L	
	10^{-4} Cancer risk	0.1 mg/L	
	National primary drinking water standards		EPA 2002a
	MCLG	Zero	
	MCL	0.005 mg/L	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act	10 pounds	EPA 2005c 40 CFR 117.3
	Water quality criteria for human health consumption of:		EPA 2002b
	Water + organism	2.2 µg/L[h]	
	Organism only	51 µg/L[h]	
c. Food			
FDA	Bottled drinking water	0.005 mg/L	FDA 2004 21 CFR 165.110
d. Other			
ACGIH	Carcinogenicity classification	A1[i]	ACGIH 2006
	Biological exposure indices (end of shift)		
	S-phenylmercapturic acid in urine	25 µg/g creatinine	
	t,t-Muconic acid in urine	500 µg/g creatinine	
EPA	Carcinogenicity classification	Group A[j]	IRIS 2007
	Oral slope factor	$1.5x10^{-2}$–$5.5x10^{-2}$ per (mg/kg)/day	
	Inhalation unit risk	$2.2x10^{-6}$–$7.8x10^{-6}$ per µg/m^3	
	RfC	0.03 mg/m^3	
	RfD	$4x10^{-3}$ mg/kg/day	

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Superfund, emergency planning, and community right-to-know		
	Designated CERCLA hazardous substance		EPA 2005d 40 CFR 302.4
	Reportable quantity	10 pounds[k]	
	RCRA hazardous waste number	U019	
	Effective date of toxic chemical release reporting	01/01/87	EPA 2005e 40 CFR 372.65
NTP	Carcinogenicity classification	Known human carcinogen	NTP 2005

[a]Group 1: carcinogenic to humans
[b]For substances that are considered to be carcinogenic, the guideline value is the concentration in drinking water associated with an upper-bound excess lifetime cancer risk of 10^{-5} (one additional cancer per 100,000 of the population ingesting drinking water containing the substance at the guideline value for 70 years). Concentrations associated with upper-bound estimated excess lifetime cancer risks of 10^{-4} and 10^{-6} can be calculated by multiplying and dividing, respectively, the guideline value by 10.
[c]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[d]AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.
[e]Values denoted as having safety considerations against the hazard of explosion, whereas the Lower Explosive Limit (LEL) =14,000 ppm and each value should be ≥10% LEL.
[f]Value denoted as having extreme safety considerations against the hazard of explosion must be taken into account, whereas the LEL =14,000 ppm and each value should be ≥50% LEL.
[g]NIOSH potential occupational carcinogen
[h]This criterion is based on carcinogenicity of 10^{-6} risk.
[i]A1: confirmed human carcinogen
[j]Group A: known human carcinogen
[k]Designated CERCLA hazardous substance pursuant to Section 311(b)(2) and 307(a) of the Clean Water Act, Section 112 of the Clean Air Act, and Section 3001 of RCRA.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = Acute Emergency Exposure Guideline Levels; CERCLA = Comprehensive Environmetnal Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NAS/NRC = National Academy of Sciences/National Research Council; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term expsoure limit; TLV = threshold limit values; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

an uncertainty factor of 30 (10 to protect sensitive individuals and 3 for uncertainty in route-to-route extrapolation) was applied.

EPA (IRIS 2007) derived an inhalation reference concentration (RfC) for benzene of 0.03 mg/m^3 (0.009 ppm) based on the results of BMD modeling of absolute lymphocyte (ALC) data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m^3 and adjusted from intermittent to continuous exposure ($BMCL_{ADJ}$=8.2 mg/m^3); a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies) was applied.

EPA (IRIS 2007) derived an oral reference dose (RfD) for benzene of 0.004 mg/kg/day, based on the results of BMD modeling of ALC data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m^3 and adjusted from intermittent to continuous exposure ($BMCL_{ADJ}$=8.2 mg/m^3). Route-to-route extrapolation methodology was applied to convert from inhalation to equivalent oral exposure, resulting in an equivalent oral dose rate of 1.2 mg/kg/day. This value was divided by a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies).

The International Agency for Research on Cancer (IARC) classifies benzene as a Group 1 carcinogen (carcinogenic to humans) (IARC 2004). EPA classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). 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 2005).

The EPA has a current maximum contaminant level (MCL) of 0.005 mg/L for benzene in drinking water (EPA 2002a). The World Health Organization (WHO) has established a guideline value of 0.01 mg/L for benzene in drinking water (WHO 2004).

Benzene is on the list of chemicals in "The Emergency Planning and Community Right-to-Know Act of 1986" (EPA 2005d). 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 an employer can document that benzene is used in the workplace <30 days/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 (8-hour TWA). 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 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 2005c).

ACGIH limits exposure to benzene to 0.5 ppm (8-hour TWA) (ACGIH 2006). The National Institute for Occupational Safety and Health (NIOSH 2005) has established a recommended exposure level (REL) of 0.1 ppm (15-minute ceiling limit).

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10. GLOSSARY

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

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

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

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

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

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

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

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

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

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

Carcinogen—A chemical capable of inducing cancer.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Immunological Effects—Functional changes in the immune response.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

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

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Environmental Medicine, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation [ ] Oral
Duration: [x] Acute [ ] Intermediate [ ] Chronic
Graph Key: 46
Species: Mouse

Minimal Risk Level: 0.009 [ ] mg/kg/day [x] ppm

Reference: 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.

Experimental design: Male C57BL/6J mice (7–8/group) were exposed to benzene (0, 10.2, 31, 100, or 301 ppm) in whole-body 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. The 10.2 ppm exposure level resulted in significant depression of femoral lipopolysaccharide-induced B-colony-forming ability in the absence of a significant depression of total numbers of B cells. At 31 ppm, splenic phytohemagglutinin-induced blastogenesis was significantly depressed without a concomitant significant depression in numbers of T-lymphocytes. Peripheral lymphocyte counts were depressed at all exposure levels. These results demonstrate that short-term inhaled benzene even at low exposure concentrations can alter certain immune associated processes.

Effect noted in study and corresponding doses:

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.

Dose and end point used for MRL derivation:

[ ] NOAEL [x] LOAEL

Uncertainty Factors used in MRL derivation: 300

[ ] 1 [ ] 3 [x] 10 (for use of a LOAEL)
[ ] 1 [x] 3 [ ] 10 (for extrapolation from animals to humans using dosimetric conversion)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: 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 $LOAEL_{ADJ}$ is 2.55 ppm.

According to current EPA (1994b) methodology for calculating a human equivalent concentration (HEC) for extrarespiratory effects of a category 3 gas (such as benzene):

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

where:

$LOAEL_{HEC}$ = The LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = The LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = The ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$ = 2.55 ppm.

Was a conversion used from intermittent to continuous exposure? 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 $LOAEL_{ADJ}$ is 2.55 ppm.

Other additional studies or pertinent information that lend support to this MRL: Increased number of micronucleated polychromatic erythrocytes (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. C57BI/6J mice were exposed to 0, 10.2, 31, 100, and 301 ppm benzene for 6 days at 6 hours/day. 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 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 erythroid progenitor cells (CFU-E) after exposure to 10 ppm benzene for 5 days, 6 hours/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 end points were not monitored in this study. These data support the choice of Rozen et al. (1984) as a critical study.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation [ ] Oral
Duration: [ ] Acute [x] Intermediate [ ] Chronic
Graph Key: 126
Species: Mouse

Minimal Risk Level: 0.006 [ ] mg/kg/day [x] ppm

Reference: Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 88:35-43.

Experimental design: Male C57Bl/6 mice were exposed to 10, 30, or 100 ppm of benzene by inhalation 6 hours/day, 5 days/week for 20 exposure days. The number of lymphocytes and their functional capacities were evaluated in spleens of exposed mice. Following the 20 days of exposure, functional capacity of splenic lymphocytes was evaluated in two *in vitro* assays: mixed-lymphocyte culture (MLC) and 51Cr-release cytotoxicity assay. Measured mean daily benzene concentrations in the 10, 30, and 100 ppm groups were 11.1 (±1.5) ppm, 29.5 (±4.4) ppm, and 99.7 (±7.0) ppm, respectively. No changes were observed in the relative proportions of splenic leukocytes, in the percentage of T-cell subsets or in the ratio of T-helper and T-suppressor cells, even at the highest exposure level (100 ppm). Therefore, the functional assays could be normalized for particular lymphocyte populations by using equal numbers of splenic cells. MLC is an *in vitro* measure of alloreactivity (capacity to mount an immune response against foreign antigens). The MLC activity of spleen lymphocytes from 10- and 100-ppm mice was delayed on days 2–4 of culture (relative to air-exposed controls), indicating that benzene exposure causes impaired in vitro alloreactivity (data for the 30-ppm mice were not included in the reported results). This delayed alloreactivity was not due to spleen suppressor cells. The lymphocyte cytotoxic function evaluated in the 51Cr-release assay was also altered; splenic lymphocytes from 100-ppm mice had a significantly reduced lysing capacity. The results indicate that inhalation exposure of mice to benzene has an immunodepressive effect on *in vitro* alloreactivity and cytotoxicity of splenic lymphocytes.

Effect noted in study and corresponding doses:

10 ppm = Significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction (less serious LOAEL).

30 ppm = Results not reported.

100 ppm = Significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction.

Dose and end point used for MRL derivation:

[ ] NOAEL [x] LOAEL

Uncertainty Factors used in MRL derivation: 300

[ ] 1 [ ] 3 [x] 10 (for use of a LOAEL)
[ ] 1 [x] 3 [ ] 10 (for extrapolation from animals to humans using dosimetric conversion)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: According to current EPA (1994b) methodology for calculating a human equivalent concentration (HEC) for extrarespiratory effects of a category 3 gas (such as benzene):

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

where:

$LOAEL_{HEC}$ = The LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = The LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = The ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$.

Was a conversion used from intermittent to continuous exposure? The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10 ppm) by 6 hours/24 hours to correct for less than a full day of exposure and 5 days/7 days to correct for less than a full week of exposure. The resulting $LOAEL_{ADJ}$ is 1.8 ppm.

Other additional studies or pertinent information that lend support to this MRL: Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week caused significant depressions in numbers of lymphocytes (ca. 30% lower than controls) as early as exposure day 32; this effect was also noted at the other scheduled periods of testing (exposure days 66 and 178) (Baarson et al. 1984). Splenic red blood cells were significantly reduced (ca. 15% lower than controls) at exposure days 66 and 178. The failure of the erythrons of benzene-exposed mice to support normal red cell mass was illustrated by the significant reduction in peripheral red cell numbers in these animals at 66 and 178 days of benzene exposure. Green et al. (1981a, 1981b) exposed male CD-1 mice to benzene vapors at concentrations of 0 or 9.6 ppm for 6 hours/day, 5 days/week for 50 days and assessed the effects of exposure on cellularity in the spleen, bone marrow, and peripheral blood. Exposure-related effects included a 90% increase in numbers of multipotential hematopoietic stem cells (CFU-S) (Green et al. 1981a), approximately 25% increase in spleen weight and total splenic nucleated cellularity (Green et al. 1981b), and 80% increase in nucleated RBCs (Green et al. 1981b). The results of Baarson et al. (1984) and Green et al. (1981a, 1981b) are limited for purposes of quantitative risk assessment because a single exposure level was employed. However, they support the choice of Rosenthal and Snyder (1987) as the critical study, which serves as the basis for the intermediate-duration inhalation MRL.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 161
Species: Human

Minimal Risk Level: 0.003 [ ] mg/kg/day [x] ppm [ ] mg/m^3

Reference: Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

Experimental design: A cross-sectional study was performed on 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene. The benzene-exposed workers had been employed for an average of 6.1±2.9 years. Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Post-shift urine samples were collected from every worker. Urinary benzene concentrations were highly correlated with mean individual air levels. Benzene was not found (detection limit 0.04 ppm) in workplace and home air samples of control workers taken at three different time periods. Study subjects were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1–<10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured twice during the month prior to phlebotomy. Of the 250 exposed workers, 109 were exposed to <1 ppm benzene. Each of these individuals worked at the larger of the two facilities included in the study. Exposure concentrations were generally higher at the smaller facility due to a less adequate ventilation system. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively (see Table A-1). An evaluation of potential confounding factors showed that age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index were associated with at least one hematological end point. The values in Table A-1 represent values that were adjusted to account for these variables. All types of white blood cells (WBCs) and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of

the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible.

Table A-1. Significantly Reduced Blood Values in Workers Exposed to Benzene in Tianjin, China (Adapted from Lan et al. 2004a)

	Mean exposure level in ppm[a] (number of subjects)			
End point	<0.04 (140)	0.57±0.24 (109)	2.85±2.11 (110)	28.73±20.74 (31)
WBCs[b]	6,480±1,710	5,540±1,220[d]	5,660±1,500	4,770±892
Granulocytes[b]	4,110±1,410	3,360±948[d]	3,480±1,170	2,790±750
Monocytes[b]	241±92	217±97[d]	224±93	179±74
Lymphocytes[b]	2,130±577	1,960±541[d]	1,960±533	1,800±392
CD4+-T cells[b]	742±262	635±187[d]	623±177	576±188
CD4+/CD8+ ratio	1.46±0.58	1.26±0.41[d]	1.22±0.45	1.09±0.35
B cells[b]	218±94	186±95[d]	170±75	140±101
Platelets[c]	230±59.7	214±48.8[d]	200±53.4	172±44.8

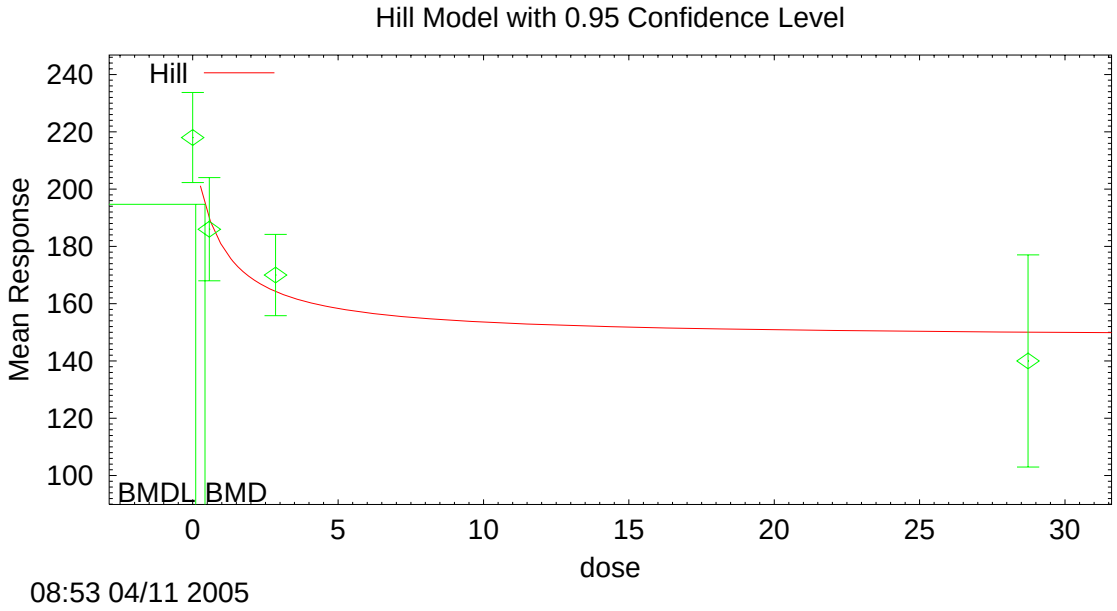
[a]Arithmetic mean of an average of two measurements per subject collected during the month prior to phlebotomy
[b]Mean cell numbers per microliter blood±standard deviation
[c]Mean number of platelets ($x10^3$)
[d]Statistically significantly lower than controls ($p<0.05$) by linear regression on ln of each end point

Effect noted in study and corresponding doses: As shown in Table A-1, exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for benchmark dose (BMD) modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a) identified a LOAEL in the absence of a NOAEL.

Dose and end point used for MRL derivation: 0.10 ppm ($BMCL_{0.25sd}$) for decreased B cell count.

All continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to the B cell count data shown in Table A-1. Visual inspection of the plots of observed versus expected values for B cell counts indicated that the Hill model provided the only adequate fit of the data set (see Figure A-1). A benchmark response (BMR) of 0.25 sd below the control mean B cell count was selected because it resulted in a $BMC_{0.25sd}$ of 0.42 ppm and its lower 95% confidence limit ($BMCL_{0.25sd}$) of 0.10 ppm (Figure A-1), which are below the mean exposure level of the lowest exposure group (0.57 ppm) for which a statistically significant decrease in mean B cell count (186 versus 218 in controls, see Table A-1) was observed. Although Lan et al. (2004a, 2004b) noted significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the 0.57 ppm exposure group, this subgroup could not be included in the BMD analysis because the study authors did not include the means and standard deviations for the decreased blood factors, nor did they provide quantitative information regarding the remaining 70 subjects in the 0.57 ppm exposure group (n=109). Assuming that the 0.29 ppm exposure level may represent a minimally adverse exposure level, it seems reasonable to accept the $BMCL_{0.25sd}$ of 0.10 ppm as the point of departure for deriving a chronic-duration inhalation MRL for benzene.

Figure A-1. Observed and Predicted B Cell Counts in Human Subjects Occupationally Exposed to Benzene. BMD=$BMC_{0.25sd}$=0.42 ppm; BMDL=$BMCL_{0.25sd}$=0.10 ppm



The computer output for fitting of the Hill model to B cell counts in human subjects occupationally exposed to benzene (Lan et al. 2004a) follows.

```
==========================================================================
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\ATSDR\BENZENE\BMD FILES\BENZENELANBCELLS.(d)
        Gnuplot Plotting File:  C:\ATSDR\BENZENE\BMD
FILES\BENZENELANBCELLS.plt
                                          Mon Nov 20 09:27:13 2006
 ==========================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = MEAN
   Independent variable = ppm
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	8224.64	
rho =	0	Specified
intercept =	218	
v =	-78	
n =	0.572459	
k =	1.5675	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	1	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	8027.09	1
rho	0	1
intercept	217.113	1
v	-69.0144	1
n	1	NA
k	0.878186	1

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	140	218	94	217	89.6	0.0099
0.57	109	186	95	190	89.6	-0.0441
2.85	110	170	75	164	89.6	0.063
28.73	31	140	101	150	89.6	-0.113

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)

```
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


Degrees of freedom for Test A1 vs fitted <= 0

                      Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
              A1       -1947.632025       5    3905.264050
              A2       -1943.411648       8    3902.823297
           fitted      -1948.162584       4    3904.325168
              R        -1962.157799       2    3928.315597

 Test 1:  Does response and/or variances differ among dose levels
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous (A1 vs A2)
 Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df     p-value

   Test 1             37.4923          6          <.0001
   Test 2             8.44075          3         0.03773
   Test 3             1.06112          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is less than .05.  Consider running a
non-homogeneous variance model

NA - Degrees of freedom for Test 3 are less than or equal to 0.  The Chi-
Square test for fit is not valid

Benchmark Dose Computation
Specified effect =          0.25

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMC =       0.42196

            BMCL =      0.104163
```

Although Test 3 (mean fit) produced an invalid Chi-Square test (degrees of freedom ≤0), visual inspection of the observed vs expected B cell counts from the Hill model output (Figure A-1) resulted in the determination that the predicted B cell counts adequately reflect the observed values and that the

associated $BMCL_{0.25sd}$ of 0.104163 provides an appropriate point of departure for deriving a chronic-duration inhalation MRL for benzene.

[ ] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation: 10

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[ ] 1 [ ] 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? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? The $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) as follows:

$BMCL_{0.25sdADJ} = BMCL_{0.25sd}$ x (8 hours/24 hours) x (6 days/7 days)

Therefore:

$BMCL_{0.25sdADJ}$ = 0.10 ppm x (8 hours/24 hours) x (6 days/7 days)

$BMCL_{0.25sdADJ}$ = 0.03 ppm

Other additional studies or pertinent information that lend support to this MRL: Lan et al. (2004a, 2004b) was selected as the critical study for derivation of a chronic-duration inhalation MRL because it (1) was well designed, (2) provided adequate exposure-response information, (3) employed individual exposure monitoring data collected for up to 16 months prior to blood testing, (4) demonstrated effects that did not appear to be significantly influenced by previous high-level exposures, and (5) included larger numbers of subjects than previous studies (Qu et al. 2002, 2003; Rothman et al. 1996a, 1996b; Ward et al. 1996). In addition, Lan et al. (2004a, 2004b) measured lymphocyte subsets and colony formation from hematopoietic progenitor cells as measures of toxicity.

Previously conducted epidemiology studies provide support to the findings of Lan et al. (2004a). Qu et al. (2002, 2003) compared hematologic values among 105 healthy workers (51 men, 54 women) in industries with a history of benzene usage (Tianjin, China) and 26 age- and gender-matched workers in industries that did not use benzene. Benzene-exposed workers were chosen based on at least 3 years of exposure history. The mean duration of occupational exposure to benzene was 9.7 years (SD=6.2 years). At the time of the study, benzene exposure was monitored by individual organic vapor monitors at 1-week intervals for 4 weeks prior to collection of blood samples for analysis. Measured benzene levels were averaged for each individual to produce a 4-week mean exposure level. Exposure-response relationships were assessed according to ranges of benzene levels (unexposed, >0–5, >5–15, >15–30, and >30 ppm). Benzene hematotoxicity was assessed by mechanical counts of total WBCs, red blood cells (RBCs), and platelets. The WBC differential was hand-counted on a total of 900 cells. Calculations of the numbers of various WBC types were based on total WBCs and differential counts. The mean 4-week benzene level in the control group was 0.004±0.003 ppm. Among all the benzene-exposed workers, the mean 4-week benzene exposure level was 5.2±7.3 ppm. Within the >0–5, >5–15, >15–30, and >30 ppm exposure categories, mean 4-week benzene levels were 2.26±1.35, 8.67±2.44, 19.9±3.1, and

51.8±43.3 ppm, respectively. A significant exposure-related reduction in the numbers of neutrophils (ranging in magnitude from 12% in the 2.26 ppm exposure group to 31% in the 51.8 ppm exposure group) was observed in all four groups of benzene-exposed workers, relative to controls. Significantly reduced numbers of RBCs (approximately 11–16% lower than controls) were also noted in all benzene-exposed groups. Significantly reduced total WBCs were seen in the highest (>30 ppm) exposure group. The study authors identified a subgroup (within the >0–5 ppm exposure group) of 16 women with no measured exposure levels exceeding 0.5 ppm (4-week mean benzene exposure level of 0.14±0.04 ppm) and reported significantly reduced total WBCs, neutrophils, and RBCs in this subgroup as well. However, these results are based on a small number of workers within the larger group and the reduced counts of total WBCs, neutrophils, and RBCs within this subgroup are –much greater in magnitude than those reported for the main (>0–5 ppm) exposure group, rendering the results in this subgroup of questionable value for purposes of risk assessment. Qu et al. (2002, 2003) clearly identified a LOAEL of 2.26 ppm for significantly reduced total WBCs, neutrophils, and RBCs, and provided indication of benzene-induced changes in some hematological values at exposure levels lower than the current industry 8-hour TWA of 1 ppm.

Rothman et al. (1996a, 1996b) performed a cross-sectional study in 1992 on 44 healthy workers (23 males, 21 females) in Chinese (Shanghai) industries with a history of benzene usage and 44 age- and gender-matched workers in industries that did not use benzene. The mean duration of occupational exposure to benzene was 6.3 years (SD=4.4 years). At the time of the study, benzene exposure was monitored by individual organic passive dosimetry badges on 5 separate days during 1 to 2 weeks prior to the collection of blood and urine samples for analysis. Benzene hematotoxicity was assessed by mechanical counts of total WBCs, absolute lymphocytes (ALC), RBCs, and platelets, as well as hemoglobin value and mean corpuscular volume (MCV). The WBC differential was also hand-counted on 100 cells. Abnormal counts were reviewed by hand. Mean (geometric mean of the five exposure samples) 8-hour TWAs for the benzene-exposed workers ranged from 1 to 238 ppm (median 8-hour TWA of 31 ppm). Benzene-exposed workers exhibited statistically significantly reduced numbers of total WBCs, ALC, RBCs, and platelets (approximately 12, 21, 6, and 23% lower, respectively) and significantly increased MCV (approximately 3% higher), relative to unexposed workers. The results were comparable in both men and women. Among the benzene-exposed workers whose mean exposure levels were >31 ppm (median 8-hour TWA of 91.9 ppm; n=22), all measured blood parameters were significantly different from controls; only ALC, RBCs, and platelets were significantly lower in benzene workers with mean exposures of <31 ppm (median 8-hour TWA of 13.6; n=22), compared with controls. In a subgroup of benzene-exposed workers whose measured benzene exposure levels did not exceed 31 ppm on any of the five sampling days (median 8–hour TWA of 7.6 ppm; n=11), significantly reduced ALC (approximately 16% lower than controls) was noted.

In a nested case-control study of a cohort of workers in the Pliofilm production departments of a rubber products manufacturer in Ohio (Ward et al. 1996), incident cases were defined as the first occurrence of a low WBC or RBC count, and matched controls were chosen from those tested within approximately 6 months of the case's blood test date. Hematologic screening data were available for 657 of 1,037 individuals employed at the plant from 1939 through 1976. A total of 21,710 blood test records were identified; the number of blood tests per individual ranged from 1 to 354, but the majority of subjects had five or fewer blood tests. All blood tests were taken from 1940 through 1975, the majority of which were routine hematological screening tests. Benzene exposures were estimated using a job exposure matrix developed by Rinsky et al. (1987). The effects of benzene exposure in the 30, 90, and 180 days prior to the blood test date, as well as cumulative exposure up until the blood test date, were examined using conditional logistic regression. A total of 78 cases and 5,637 controls were included in the WBC analysis and 105 cases and 8,489 controls in the RBC analysis, all of whom had worked only within the rubber hydrochloride departments during the 180 days prior to the selected blood sample date. The maximum daily benzene exposure estimate was 34 ppm. A strong exposure-response relationship

was noted for WBCs, and all of the exposure metrics selected showed a significant relationship with low blood count. A weak positive exposure-response relationship was observed for RBCs, which was significant for the dose metric of cumulative exposure up until the blood test date. The study authors noted that there was no evidence for a threshold for hematologic effects and suggested that exposure to benzene levels <5 ppm may result in hematologic suppression.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 45
Species: Human

Minimal Risk Level: 0.0005 [x] mg/kg/day [ ] ppm

Reference: Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

Experimental design: The chronic-duration oral MRL for benzene is based on route-to-route extrapolation of the results of benchmark dose analysis of a hematological endpoint (B cell count) assessed in 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene. See the MRL worksheet for the chronic-duration inhalation MRL for details of study design.

Effect noted in study and corresponding doses: As described in the MRL worksheet for the chronic-duration inhalation MRL (see also Table A-1), exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for benchmark dose (BMD) modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a) identified a LOAEL in the absence of a NOAEL.

Dose and end point used for MRL derivation: $BMCL_{0.25sdADJ}$ of 0.014 mg/kg/day for decreased B cell count, resulting from route-to-route extrapolation of the $BMCL_{0.25sdADJ}$ of 0.03 ppm described in the MRL worksheet for the chronic-duration inhalation MRL.

Results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. Therefore, the point of departure for the chronic-duration inhalation MRL for benzene, namely the $BMCL_{0.25sdADJ}$ of 0.03 ppm for decreased B cell counts in benzene-exposed workers (Lan et al. 2004a, 2004b), serves as the point of departure for deriving the chronic-duration oral MRL as well.

The point of departure (in ppm) was converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25 °C and 760 mm Hg:

$BMCL_{0.25sdADJ}$ of 0.03 ppm x 78.11/24.45 = 0.096 mg/m^3

The $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 for inhaled benzene was converted to an equivalent $BMDL_{0.25sdADJ}$ for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body

weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 versus 100%, respectively) as follows:

$BMDL_{0.25sdADJ}$ = $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 x 20 m^3/day x 0.5 ÷ 70 kg = 0.014 mg/kg/day

[ ] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation: 30

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [x] 10 (for human variability)
[ ] 1 [x] 3 [ ] 10 (for uncertainty in route-to-route extrapolation)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? The $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) as follows:

$BMCL_{0.25sdADJ}$ = $BMCL_{0.25sd}$ x (8 hours/24 hours) x (6 days/7 days)

Therefore:

$BMCL_{0.25sdADJ}$ = 0.10 ppm x (8 hours/24 hours) x (6 days/7 days)

$BMCL_{0.25sdADJ}$ = 0.03 ppm

Other additional studies or pertinent information that lend support to this MRL: Results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. See the chronic-duration inhalation MRL worksheet for additional information that supports the selection of the principal study and critical effect for deriving the chronic-duration inhalation MRL.

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

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

LEGEND

See Sample Figure 3-1 (page B-7)

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

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

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

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

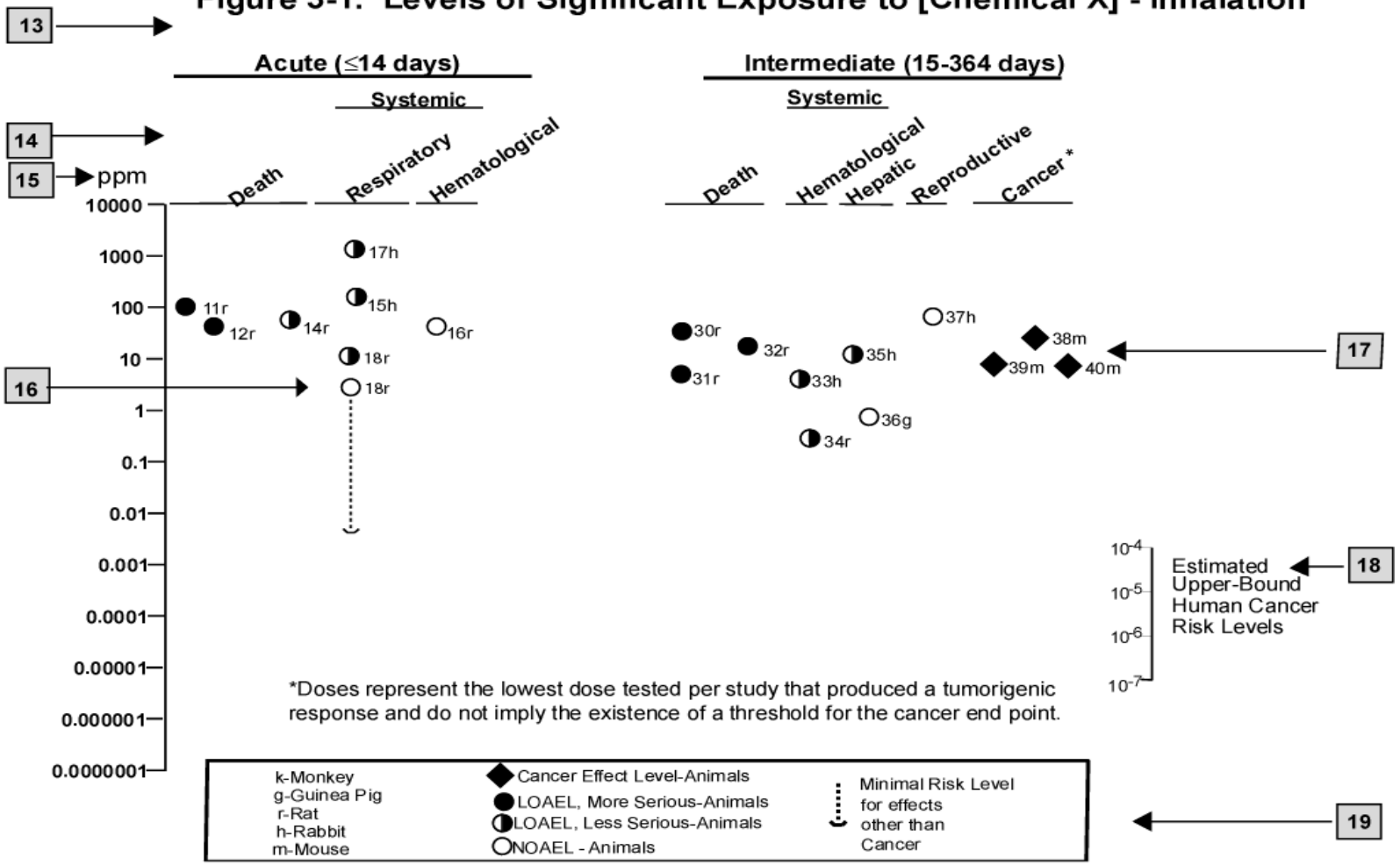
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer					11		
						↓		
	38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/Intergovernmental Maritime Dangerous Goods Code

DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor

MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon

PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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APPENDIX D. INDEX

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renal effects 13, 82, 129
reproductive effects 92, 94, 135, 140, 225
respiratory effects 32, 105
retention 204
solubility 159, 161, 200, 258
spermatogonia 145
spermatozoa 94, 225
systemic effects 12, 32, 105, 131, 138, 219, 221, 222
T4 88
thrombocytopenia 12, 73, 77, 221
thyroid 129, 166

toxicokinetic 29, 154
tremors 5, 13, 20, 91, 134, 228
tumors 15, 16, 18, 89, 103, 141, 165, 228, 231
vapor phase 259, 261, 269
vapor pressure 258
volatility 258
volatilization 249, 251, 258

BENZENE

Benzene was considered by previous IARC Working Groups in 1981 and 1987 (IARC, 1982, 1987). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 71–43–2
Chem. Abstr. Serv. Name: Benzene
IUPAC Systematic Name: Benzene



C6H6
Relative molecular mass: 78.1
From O'Neil (2006) and Lide (2008), unless otherwise stated
Description: Clear, colourless, volatile, highly flammable liquid
Solubility: Slightly soluble in water; miscible with acetone, chloroform, diethyl ether and ethanol; soluble in carbon tetrachloride
Octanol/water partition coefficient: log K_{ow}, 2.13 (Hansch *et al.*, 1995)
Conversion factor: ppm = 0.313 × mg/m^3

1.2 Uses

Historically, benzene has been used as a component of inks in the printing industry, as a solvent for organic materials, as starting material and intermediate in the chemical and drug industries (e.g. to manufacture rubbers, lubricants, dyes, detergents, pesticides), and as an additive to unleaded gasoline (NTP, 2005; ATSDR, 2007; Williams *et al.*, 2008).

The primary use of benzene today is in the manufacture of organic chemicals. In Europe, benzene is mainly used to make styrene, phenol, cyclohexane, aniline, maleic anhydride, alkylbenzenes and chlorobenzenes. It is an intermediate in the production of anthraquinone, hydroquinone, benzene hexachloride, benzene sulfonic acid and other products used in drugs, dyes, insecticides and plastics (Burridge, 2007). In the United States of America, the primary use of benzene is in the production of ethylbenzene, accounting for 52% of the total benzene demand in 2008. Most ethylbenzene is consumed in the manufacture of styrene, which is used in turn in polystyrene and various styrene copolymers, latexes and resins. The second-largest use of benzene in the United States of America (accounting for 22% of demand) is in the manufacture of cumene (isopropylbenzene), nearly

Table 1.1 Estimated numbers of workers exposed to benzene in the European Union (top 10 industries)

Industry, occupational activity	
Personal and household services	942500
Wholesale and retail trade and restaurants and hotels	248300
Land transport	42800
Manufacture of plastic products	17000
Iron and steel basic industries	14900
Manufacture of other chemical products	12700
Manufacture of industrial chemicals	12500
Manufacture of machinery, except electrical	9600
Construction	8300
Education services	7400
TOTAL	1367800

all of which is consumed in phenol production. Benzene is also used to make chemical intermediates: cyclohexane, used in making certain nylon monomers (15%); nitrobenzene, an intermediate for aniline and other products (7%); alkylbenzene, used in detergents (2%); chlorobenzenes, used in engineering polymers (1%); and miscellaneous other uses (1%) (Kirschner, 2009). Benzene occurs naturally in petroleum products (e.g. crude oil and gasoline) and is also added to unleaded gasoline for its octane-enhancing and anti-knock properties. Typically, the concentration of benzene in these fuels is 1–2% by volume (ATSDR, 2007).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to benzene occurs via inhalation or dermal absorption of solvents in the rubber, paint (including paint applications) and parts-manufacturing industries. It also occurs during crude-oil refining and chemical manufacturing, a large component of which entails exposure to gasoline. Workers involved in the transport of crude oil and gasoline and in the dispensing of gasoline at service stations, as well as street workers, taxi drivers and others employed at workplaces with exposure to exhaust gases from motor vehicles also experience exposure to benzene (Nordlinder & Ramnäs, 1987).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for benzene in the EU by industry for the top-10 industries (CAREX, 1999). Exposure to benzene is defined as inhalation or dermal exposure at work to benzene likely to exceed significantly non-occupational exposure due to inhaling urban air or filling in gasoline stations (long-term exposure usually below 0.01 ppm)].

From the US National Occupational Exposure Survey (1981–1983), it was estimated that approximately 272300 workers (including 143000 women) were potentially exposed to benzene in the United States of America. Industries where potential exposure occurred included agricultural services, oil and gas extraction, construction (includes general building and special trades

contractors), food products, tobacco manufacturing, textile mills, lumber and wood, printing and publishing, chemical and allied products, petroleum and coal products, rubber manufacturing, leather manufacturing, transportation, and health services (NIOSH, 1990).

van Wijngaarden & Stewart (2003) conducted a critical review of the literature on occupational exposures to benzene in the 1980s in the USA and Canada. The data indicated that workers in most industries experienced exposure levels below the regulatory limit (1 ppm) of the US Occupational Safety and Health Administration (OSHA), with a weighted arithmetic mean of 0.33 ppm across all industries. It was noted that little information was available on exposure levels and their determinants for many industries with potential exposure.

Williams *et al.* (2008) summarized the values of the benzene content of selected petroleum-derived products based on published literature between 1956 and 2003. A total of 22 studies were identified, which contained 46 individual data sets and evaluated potential occupational exposure to benzene in the USA during the handling or use of these petroleum-derived products. All mean (or median) airborne concentrations were less than 1 ppm, and most were < 0.1 ppm. Table 1.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table1.2.pdf) summarizes airborne benzene concentrations from studies and governmental reports published between 1981 and 2006.

Capleton & Levy (2005) tabulated typical benzene-exposure levels in different occupational groups in various areas in Europe and North America (Table 1.3). The values are similar to those reported by van Wijngaarden & Stewart (2003) and Williams *et al.* (2008) for exposures of 1 hour or more.

Williams *et al.* (2005) reviewed available industrial-hygiene data describing exposure during the marine transport of benzene-containing products. Although there were differences in sampling strategies and in benzene content of the liquids being transported, typical benzene concentrations in air (personal time-weighted average) were in the range of 0.2–2.0 ppm during closed loading and 2–10 ppm during open loading-operations.

Liang *et al.* (2005) reviewed and tabulated benzene exposures by industry in the People's Republic of China, using data published between 1960 and 2003. The five industries with the highest reported exposures were those producing leather products, electronic devices, machinery, shoes, and office supplies and sports equipment. Median ambient concentrations in these industries were, respectively: 124.8 mg/m^3, 98.7 mg/m^3, 75.4 mg/m^3, 50.4 mg/m^3, and 50.3 mg/m^3. [The Working Group noted that all data were collected with sampling methods of very short duration (1–20-minute time-weighted averages). In addition, a considerable part of the surveys were follow-up studies of benzene poisonings. Therefore, these data cannot be considered as representative and cannot be compared with the information reported from the USA.] Levels of short-term exposure to benzene varied considerably between industries (Table 1.4) and showed generally a downward trend over time (Fig. 1.1).

Urinary *trans,trans*-muconic acid (*t,t*-MA) and *S*-phenylmercapturic acid (*S*-PMA) are sensitive markers for recent exposure to benzene at low levels (Qu *et al.*, 2005).

1.3.2 Non-occupational exposure

The major sources of benzene in the atmosphere are anthropogenic and include fixed industrial sources, fuel evaporation from gasoline filling-stations and automobile exhaust. Benzene has been measured in outdoor air at various locations in the USA at concentrations ranging from 0.02 ppb (0.06 µg/m^3) in a rural area, to 112 ppb (356 µg/m^3) in an urban area. Exposure to benzene is highest in areas of heavy motor-vehicle traffic and around gasoline filling-stations. Based on

Table 1.3 Typical benzene exposure levels in different occupational groups/areas in Europe and North America[a]

Occupational group/area	Year	Long-term exposure levels (mg/m^3)					Short-term exposure levels (mg/m^3)					Reference
		N	AM	GM	Min	Max	N	AM	GM	Min	Max	
Upstream petrochemical industry												Verma *et al.* (2000)
Conventional oil/gas	1985–96	198	0.206	0.036	0.003	7.78	23	0.662	0.021	< 0.004	7.954	
Conventional gas	1985–96	608[b]	0.089	0.024	0.006	6.868	40	2.328	0.144	< 0.02	35.2	
Heavy oil processing	1985–96	236	0.112	0.051	< 0.003	1.60	24	0.056	0.027	< 0.017	0.731	
Pipeline	1985–96	8	0.392	0.350	0.160	1.540	-	-	-	-	-	
Downstream petrochemical industry												CONCAWE (2000, 2002), Merlo *et al.* (2001)
Refinery												
On-site operators	1993–98	97	0.22	-	0.008	7.88	-	-	-	-	-	
	1999–2001	-	-	-	-	-	6	1.0	0.9	0.8[c]	1.4[c]	
Off-site operators	1993–98	321	0.32	-	0.008	23.3	49	2.19	-	0.08	11.8	
	1999–2001	-	-	-	-	-	7	0.7	0.7	0.6[c]	0.8[c]	
Maintenance workers	1993–98	373	0.41	-	0.008	18.1	7	2.62	-	0.23	8.6	
Laboratory technicians	1993–98	628	0.30	-	0.0015	5.0	5	1.93	-	0.28	4.6	
Marine and rail car loading												
Deck crew, open loading	1993–98	41	0.56	-	0.08	5.4	4	0.23	-	0.23	0.3	
Deck crew, closed loading	1993–98	2	0.56	-	0.51	0.6	-	-	-	-	-	
Marine loading	1993–98	32	0.51	-	0.023	3.7	2	0.7	-	0.23	1.2	
Jetty staff	1993–98	46	0.37	-	0.023	1.7	24	0.79	-	0.23	5.8	
Rail car terminal operators (toploading with VR)	1999–2001	21	0.5	0.4	0.2[c]	0.7[c]	3	0.5	-	0.5	0.5	
Road tanker distribution												
Terminal supervisors	1993–98	151	0.36	-	0.001	3.1	8	2.20	-	0.23	11.2	
Drivers, bottom loading & VR	1999–2001	33	0.6	0.4	0.2[c]	1.2[c]	15	1.8	1.4	0.5[c]	3.8[c]	
Drivers, delivery	1999–2001	-	-	-	-	-	7	0.7	0.4	0.2[c]	1.6[c]	

Table 1.3 (continued)

Occupational group/area	Year	Long-term exposure levels (mg/m³)					Short-term exposure levels (mg/m³)					Reference
		N	AM	GM	Min	Max	N	AM	GM	Min	Max	
Service station												CONCAWE (2000, 2002), Merlo *et al.* (2001) Contd.
Attendants	1999–2000	78	0.102	-	0.0115	0.478	-	-	-	-	-	
Cashiers	1993–98	268	0.05	-	0.001	1.92	-	-	-	-	-	
Miscellaneous workers	1999–2001	6	0.2	0.1	0.1[c]	0.2[c]	-	-	-	-	-	
Gasoline pump maintenance	1993–98	2	0.55	-	0.16	0.93	6	3.8	-	0.19	11.8[d]	
Coke oven industry												Hotz *et al.* (1997)
Coke plant	1994–95	19	0.13[e]	-	ND[f]	1.76[f]	-	-	-	-	-	
Coke plant	1994–95	17	1.79[e]	-	0.52[f]	23.82[f]	-	-	-	-	-	
By-product plant	1994–95	21	1.17[e]	-	0.20[f]	5.30[f]	-	-	-	-	-	
Motor mechanics	1994–98	243	0.362	-	< 0.005	13.0	8	10.15[g]	-	1.2	46	CONCAWE (1986), Nordlinder & Ramnäs (1987), Popp *et al.* (1994), Hotz *et al.* (1997), Javelaud *et al.* (1998)
	1981	-	-	-	-	-	-	0.52	-	0.33	1.50	
Aviation												CONCAWE (2000), Egeghy *et al.* (2003)
Civilian airport operators	1993–98	10	0.10	-	0.008	0.60	-	-	-	-	-	
Military fuel maintenance workers	2003	114	0.252[e]	-	0.006	6.63	-	-	-	-	-	
Military fuel handling, distribution, recovery & testing workers	2003	38	0.007[e]	-	0.001	1.85	-	-	-	-	-	
Firefighters	1991–2002	43	-	-	< 0.37[h]	6.14[h]	22[i]	-	-	< LOD	68.25	Jankovic *et al.* (1991), Bolstad-Johnson *et al.* (2000), Caux *et al.* (2002)
							96[j]	1.24	-	0.228	6.468	

Table 1.3 (continued)

Occupational group/area	Year	Long-term exposure levels (mg/m³)					Short-term exposure levels (mg/m³)					Reference
		N	AM	GM	Min	Max	N	AM	GM	Min	Max	
Urban workers												Fustinoni *et al.* (1995), Carrer *et al.* (2000), Crebelli *et al.* (2001), Merlo *et al.* (2001)
Traffic police/wardens	1994–2000	236	0.020	-	0.009	0.316	-	-	-	-	-	
Bus drivers	1998–2000	152	0.0238	-	0.003	0.092	-	-	-	-	-	
Office workers	1994–2000	289	0.016	-	0.002	0.115	-	-	-	-	-	

[a] When selecting typical benzene exposure values, preference has been given to studies published within the previous 10 years and for which greater than 10 subjects were sampled. Where appropriate, data sets have been combined to give an overall mean exposure.
[b] Data for which an arithmetic mean was available
[c] 10th and 90th percentile values.
[d] The mean was strongly influenced by one high exposure level of 46 mg/m3, if this is excluded the mean exposure is 5.03 mg/m3 (range:1.2–14.0 mg/m3).
[e] Median value.
[f] 5th and 95th percentile values.
[g] Small spillage associated with the highest result.
[h] Exposure estimated from biological monitoring.
[I] Exposure during the knockdown phase of fire fighting.
[j] Exposure during the overhaul phase of fire fighting.
AM, arithmetic mean; GM, geometric mean; Max, maximum; Min, minimum; N, number of samples; VR, vapour recovery
From Capleton & Levy (2005)

Table 1.4 Comparison of the average benzene concentrations (mg/m³) by industry

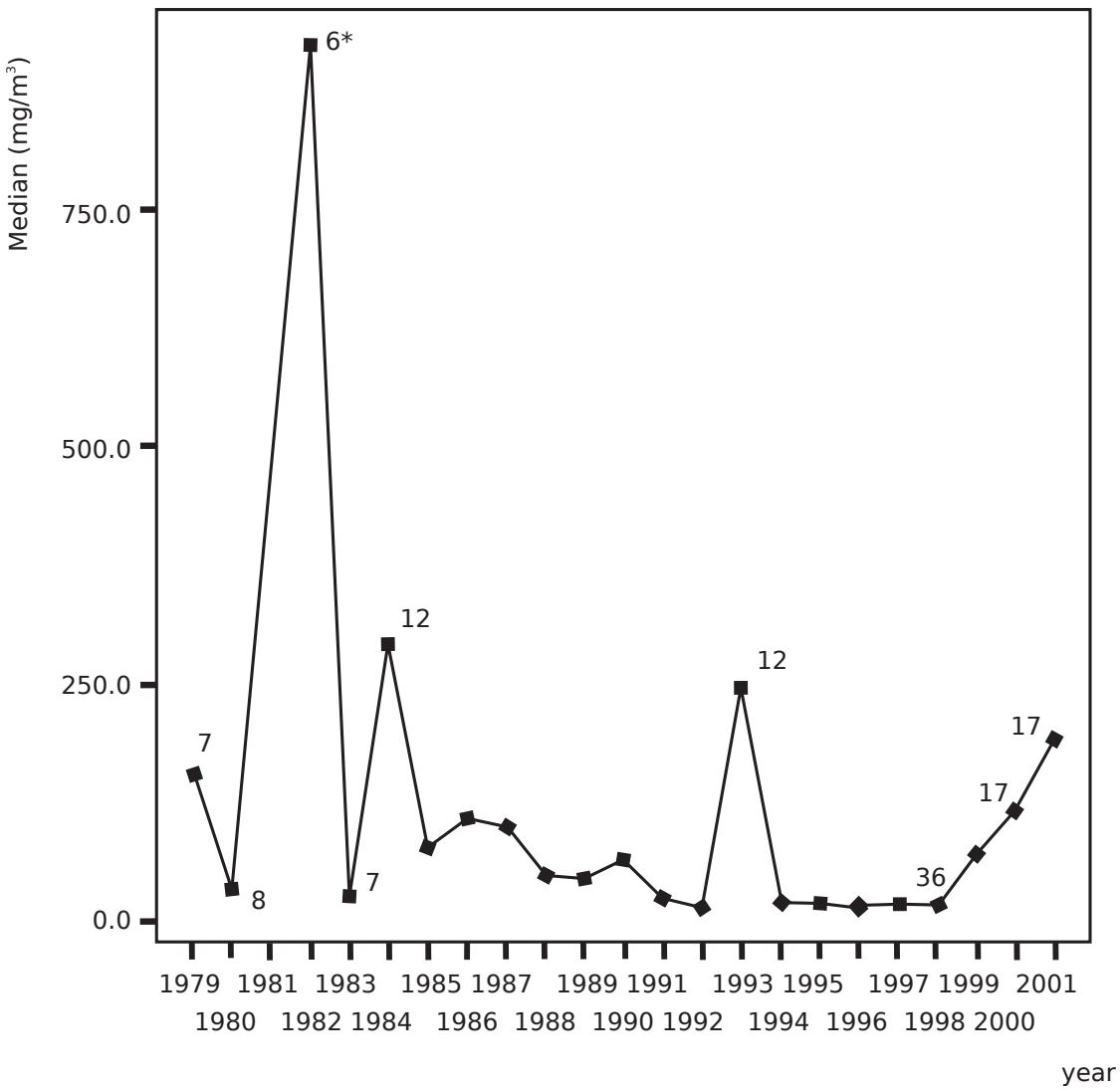
Type of industry	No. of sets	No of samples	Median	Average (range)
Leather products[a]	18	1487	124.8	124.1 (3.7–267.8)
Electronic devices manufacturing[a]	6	1930	98.7	120.2 (4.5–254.9)
Machinery manufacturing[a]	6	6815	75.4	75.6 (4.2–152.7)
Shoes manufacturing, leather[a]	70	12 197	50.4	149.9 (1.3–1488.6)
Office supplies and sports equipment[a]	6	106	50.3	79.4 (10.7–256.0)
Spray painting	29	1186	39.8	53.4 (0–226.8)
Furniture manufacturing	8	618	39.3	36.6 (2.0–72.0)
Misc. electronic parts manufacturing	7	197	33.6	50.5 (3.0–105.6)
Automobile manufacturing	6	3478	32.8	56.8 (0–196.1)
Organic chemical industry	19	650	23.8	39.3 (12.8–130.5)
Rubber products manufacturing	15	182	22.9	114.6 (0.1–633.6)
Other industries	10	6799	18.5	23.8 (2.2–85.5)
Paint manufacturing	37	525	13.2	23.9 (1.0–127.5)
Chemical industry	18	859	7.6	19.3 (0–123.9)
Printing industry	8	6416	6.5	7.2 (0–23.6)
Metal-based products processing	10	77	1.4	7.5 (0–38.0)
Toy manufacturing	2	2531	132.9	132.9 (1.5–264.3)
Coal products manufacturing	3	23	96.0	79.8 (12.8–130.5)
Crude oil processing	3	992	62.6	54.4 (7.4–93.2)
Petroleum & geological prospecting	3	22	57.2	41.9 (5.8–62.6)
Other textile industries/printing & dyeing	1	178	26.2	26.2
Civil engineering & construction	3	137	20.3	122.2 (1.2–345.2)
Pottery & porcelain products manufacturing	3	26	20.2	22.4 (7.1–40.0)
Electronic circuit manufacturing	3	26	20.2	22.4 (7.1–40.0)
Plastic products manufacturing	2	1216	15.2	15.2 (2.3–28.2)
Other precision instruments manufacturing	2	44	14.3	14.3 (8.7–19.9)
Household metal hardware manufacturing	1	1139	2.3	2.3

[a] The top five industries with more than six measurement sets in an individual industry. Industries following the blank space (after Metal-based processing) are those for which fewer than six data sets were available.
From Liang *et al.* (2005)

an average benzene concentration of 12.5 ppb (40 μg/m³) in the air and an exposure of 1 hour per day, the daily intake of benzene from driving or riding in a motor vehicle is estimated to be 40 μg. Exposure is higher for people who spend significant time in motor vehicles in areas of congested traffic (NTP, 2005; ATSDR, 2007).

The primary sources of exposure to benzene for the general population are ambient air containing tobacco smoke, air contaminated with benzene (for example, in areas with heavy traffic, around gasoline filling-stations), drinking contaminated water, or eating contaminated food. The median level of benzene was 2.2 ppb (7 μg/m³) in 185 homes without smokers and 3.3 ppb (10.5 μg/m³) in 343 homes with one or more smokers. Amounts of benzene measured per cigarette ranged from 5.9 to 75 μg in mainstream smoke and from 345 to 653 μg in sidestream smoke. Benzene intake from ingestion of water and foods is very low, compared with intake from ambient air (ATSDR, 1997; NTP, 2005). Residential exposure to benzene can also occur from leaking underground gasoline-storage tanks. Benzene concentrations in homes from such exposures have been estimated to

Fig. 1.1 Overall trend in median benzene exposure in Chinese industry, 1979–2001. The star indicates the number of measurement sets in the database



From Liang *et al.* (2005)

Table 1.5 Benzene in breath, blood and urine samples in the general population without occupational or known exposure to benzene[a]

Country	Analyte	Median/Mean	Reference
People's Republic of China	Urine	120 ng/L	Kim *et al.* (2006a)
People's Republic of China	Urine	69 ng/L	Waidyanatha *et al.* (2001)
People's Republic of China and Malaysia	Urine	1.49 ng/L	Ong *et al.* (1995)
Estonia	Blood Breath Urine	12 nmol/L 7 nmol/L 0.1 nmol/L	Kivistö *et al.* (1997)
Italy	Blood	110 ng/L (NS) 219 ng/L (S)	Brugnone *et al.* (1998)
Italy	Urine	1155 ng/L	Gobba *et al.* (1997)
Mexico	Blood	0.63 µg/L (service attendants) 0.30 µg/L (street vendors) 0.17 µg/L (office workers)	Romieu *et al.* (1999)
Singapore	Blood Urine	1.27 nmol/L 1.29 nmol/L	Ong *et al.* (1996)
Thailand	Blood	65.6 ppt	Navasumrit *et al.* (2005)

[a] Including control workers
NS, non-smoker; S, smoker
From Johnson *et al.* (2007)

range from 0–42 ppm (1–136 mg/m^3) (Patel *et al.*, 2004).

Duarte-Davidson *et al.* (2001) assessed human exposure to benzene in the general population of the United Kingdom. It was estimated that infants (< 1 year old), the average child (11 years old), and non-occupationally exposed adults receive average daily doses of benzene in the range of 15–26 µg, 29–50 µg, and 75–522 µg, respectively. These values correspond to average airborne benzene concentrations in the range of 3.40–5.76 µg/m^3, 3.37–5.67 µg/m^3, and 3.7–41 µg/m^3 for these three groups, respectively.

Benzene concentrations in breath, blood and urine samples collected among the general populations (without occupational or known exposure to benzene) in Asia, Europe and North America are presented in Table 1.5 (Johnson *et al.*, 2007).

2. Cancer in Humans

In *IARC Monographs* Volume 29 (IARC, 1982) the Working Group concluded there was *sufficient evidence* in humans for the carcinogenicity of benzene, noting that a series of cohort and case–control studies showed statistically significant associations between occupational exposure to benzene and benzene-containing solvents and leukaemia (predominantly myelogenous leukaemia). In *IARC Monographs* Supplement 7 (IARC, 1987) benzene was classified as a Group-1 carcinogen, citing additional evidence of an increased incidence of acute nonlymphocytic leukaemia (ANLL) in workers exposed to benzene in three cohort studies, including an update of a cohort cited in Volume 29 (IARC, 1982). Since 1987, there have been numerous reports from cohort studies in populations exposed to benzene, including updates of earlier reports, and new case–control studies of leukaemia or its subtypes, non-Hodgkin lymphoma (NHL), multiple myeloma, and to a

lesser extent other tumours in adults. There have also been several case–control studies of childhood leukaemia with data on benzene, solvents, gasoline, and other related exposures. In addition, several meta-analyses have been published of one or more tumour sites.

[The Working Group decided to restrict its review to those case–control studies of paediatric cancers that included estimates of environmental benzene exposure, rather than surrogate exposures such as proximity to petrol stations or traffic. Also, the Working Group weighed more heavily the findings from studies with estimates of occupational exposure to benzene rather than broader measures (e.g. to solvents) in case–control studies. It was also decided not to rely in general on case–control studies where exposure assessment was limited to asking study subjects directly if they had been exposed to particular chemicals. Furthermore, the Working Group did not consider cohort studies of workers in synthetic rubber-manufacturing due to the difficulty of separating out effects from benzene *vs* those of other chemicals that may cause haematological malignancies. The Working Group decided not to take into consideration a series of meta-analyses of studies of petroleum workers (Wong & Raabe, 1995, 1997, 2000a, b). There were methodological concerns about the expansion from paper to paper of additional studies, cohorts, and countries, and the overall approach may dilute out the risks associated with relatively highly exposed subgroups of these populations that in general were not identified. In addition, an increased risk of ANLL – or the alternative classification, Acute Myelogenous Leukaemia (AML), which is more restrictive but still constitutes most of ANLL – was not detected in the initial meta-analysis by Wong & Raabe (1995), this body of work was not considered relevant for assessing what additional cancers may be associated with exposure to benzene beyond ANLL/AML. Abd finally, the Working Group noted that some meta-analyses of the same tumour came to opposite conclusions, which could be due to different inclusion/exclusion criteria, focusing on different subgroups of the study populations, or to different approaches to selecting risk estimates for inclusion (e.g. Lamm *et al.*, 2005; Steinmaus *et al.*, 2008), thus complicating the overall assessment of the literature. The Working Group therefore decided not to rely in general on results of meta-analyses in its evaluations.]

2.1 Leukemias and lymphomas

2.1.1 Acute non-lymphocytic leukaemia/ acute myelogenous leukaemia

Since 1987, additional analyses of previously published cohort studies (e.g. results in Crump (1994) and Wong (1995), based on the cohort study described in Infante *et al.* (1977) and Rinsky *et al.* (1981, 1987), which reported an excess risk for combined (mostly acute) myelogenous and monocytic leukaemia) and new cohort studies with quantitative data on benzene exposure have shown evidence of a dose–response relationship between exposure to benzene and risk for ANLL/AML in various industries and in several countries (Hayes *et al.*, 1997; Rushton & Romaniuk, 1997; Divine *et al.*, 1999b; Guénel *et al.*, 2002; Collins *et al.*, 2003; Glass *et al.*, 2003; Bloemen *et al.*, 2004; Gun *et al.*, 2006; Kirkeleit *et al.*, 2008; see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.1.pdf). It was also noted that the NCI-CAPM cohort study found evidence of an increased risk for the combined category of ANLL and myelodysplastic syndromes (Hayes *et al.*, 1997). Case–control studies do not add substantively to these conclusions (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.2.pdf). In one case–control study an increased risk for childhood ANLL was found for maternal self-reported occupational exposure to benzene (Shu *et al.*, 1988; see Table 2.1 online). One case–control

study of childhood cancer in Denmark did not find an association of estimates of environmental benzene exposure from air pollution with an increased risk for ANLL (Raaschou-Nielsen *et al.*, 2001).

2.1.2 Acute lymphocytic leukaemia

Acute Lymphocytic Leukaemia (ALL) is now considered one subtype of NHL in the WHO-classification of lymphomas. In multiple cohorts there was a non-significantly increased risk for ALL, but the numbers of cases were small (Rushton, 1993; Wong *et al.*, 1993; Satin *et al.*, 1996; Divine *et al.*, 1999b; Lewis *et al.*, 2003; Kirkeleit *et al.*, 2008; Yin *et al.*, 1996; Guénel *et al.*, 2002; Gun *et al.*, 2006; see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.3.pdf). [The Working Group noted that the magnitude of the risk-estimate in the NCI-CAPM cohort (Yin *et al.*, 1996) was similar to the risk observed for ANLL in the same study, which was statistically significant. This approach has been suggested when attempting to interpret the association between occupational exposure to benzene and hematological subtypes that are less common than AML (Savitz & Andrews, 1997).]

In one case–control study in adults in Shanghai, a significant increased risk for ALL was found for the group with 15 or more years of self-reported occupational exposure to benzene (Adegoke *et al.*, 2003); another study in the USA had only three exposed cases (Blair *et al.*, 2001; Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.4.pdf). In a case–control study of childhood ALL no association was found with maternal self-reported occupational exposure to benzene, but a borderline significant association was noted with exposure to gasoline (Shu *et al.*, 1988; see Table 2.4 online). No association with self-reported maternal exposure to benzene was found in a large study of childhood ALL in the USA (Shu *et al.*, 1999; see Table 2.4 online). A case-control study of childhood cancer in Denmark did not find an association of estimated environmental exposure to benzene from air pollution with ALL (Raaschou-Nielsen *et al.*, 2001).

2.1.3 Chronic myelogenous leukaemia

Several studies in the petroleum industry and in other settings show non-significantly increased risks for CML, whereas other studies show no evidence of an association, including two that had quantitative estimates of exposure to benzene but no dose–response relationship (Rushton & Romaniuk, 1997; Guénel *et al.*, 2002; see Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.5.pdf). Case–control studies have shown inconsistent results, with both increased risks (exposure for > 15 years was associated with an OR of 5.0 (1.8–13.9; Adegoke *et al.*, 2003) and no increase in risk (Björk *et al.*, 2001) reported (see Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.6.pdf).

2.1.4 Chronic lymphocytic leukaemia

Chronic Lymphocytic Leukaemia (CLL) – also referred to as small lymphocytic lymphoma (SLL) – is now considered as a subtype of NHL in the WHO-classification of lymphomas. CLL can be an indolent disease of the elderly, which raises questions about cohorts that are not followed up until the study population is relatively old and about studies that use mortality instead of incident data. In addition, the diagnosis of CLL was less frequently made in the past, until complete blood counts were routinely obtained in recent decades.

Several cohort studies in the petroleum industry showed mixed results, with some non-significantly increased risks reported and other studies showing no association (see Table 2.7 available at http://monographs.iarc.fr/ENG/

Monographs/vol100F/100F-19-Table2.7.pdf). In a nested case–control study in the Australian petroleum industry an increasing risk for CLL was detected with increasing exposure to benzene over a relatively small range of ppm–years, but the increase was not significant (Glass *et al.*, 2003). Similarly, in a nested case–control study within a cohort of French gas and electrical utility workers, a non-significant increase in risk with increasing years of benzene exposure was detected (Guénel *et al.*, 2002). Some evidence of risk with increasing benzene exposure was also found in a cohort study among petroleum workers in the United Kingdom, but the trends were not clear and interpretation is difficult as white- and blue-collar workers were mixed in the analysis and interactions may have been present (Rushton & Romaniuk, 1997). Updates of two cohort studies in the Southern US found an increased risk for CLL, which was significant in one cohort for workers hired before 1950, but not in the other (Huebner *et al.*, 2004).

A case–control study in Italy showed evidence of a dose–response relationship between the extent of benzene exposure with the number of years worked with benzene (Costantini *et al.*, 2008) and in a large multicentre international study in Europe a significant excess in risk for CLL was found with increasing exposure to benzene, but the dose–response was not significant (Cocco *et al.*, 2010; see Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.8.pdf). Blair *et al.* (2001) conducted a study in the Midwestern USA and found no association with benzene exposure although there were only three cases in the high-exposure category. In a study of women in Connecticut, a non-significantly increased risk for CLL was found with increasing exposure to benzene (Wang *et al.*, 2009; see Table 2.8 online).

2.1.5 Non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of histological subtypes, and the definition of both NHL and its subtypes has evolved over the last several decades with the application and discontinuation of several classification schemes, which complicates the assessment of exposure to benzene and risk for NHL. For example, CLL – now classified by the WHO as a subtype of NHL – has generally not been combined with other types of NHL in reports from cohort studies of benzene-exposed workers or in earlier case–control studies of NHL. Further, given the indolent nature of some NHL subtypes, cohorts with only mortality data may underestimate associations with NHL. In most cohort studies an increased risk for NHL was not detected, one particular exception being the NCI-CAPM cohort study in China (Hayes *et al.*, 1997; Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.9.pdf). An excess of NHL was not detected in the Pilofilm cohort (Rinsky *et al.*, 2002) or in the Australian Health Watch study in an analysis of NHL combined with multiple myeloma (two-thirds of which were NHL cases) (Glass *et al.*, 2003).

Of 14 independent case–control studies that were considered informative, five showed evidence of increased risk with benzene exposure, two (Fabbro-Peray *et al.*, 2001; Dryver *et al.*, 2004) for NHL as a whole (Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.10.pdf). Data on histological subtypes of NHL have generally not been reported in publications of occupational cohort studies of benzene-exposed workers, but they have been mentioned in some case–control studies. For various benzene-exposure metrics, slightly increased, but non-significant risks for NHL were found in a case–control study among women in Connecticut, as well as higher risks – also non-significant – for follicular lymphoma

and diffuse large B-cell lymphoma (DLBCL), two common NHL subtypes (Wang *et al.*, 2009). Cocco *et al.* (2010) conducted an analysis of a large multicentre case–control study of NHL in Europe and found no significant increase in risk for B-cell NHL or DLBCL, but an elevated risk, albeit not statistically significant, for follicular lymphoma associated with exposure to benzene (see Table 2.10 online), and a significant association between combined exposure to benzene/toluene/xylene and follicular lymphoma. Other case–control studies showed increased, non-significant risks for one or both of these histological subtypes, and in one study in Italy a significant association was found between medium/high exposure to benzene and the risk for diffuse lymphoma (Miligi *et al.*, 2006; OR = 2.4, 95%CI: 1.3–1.5).

2.1.6 Multiple myeloma

Most cohort studies showed no association with multiple myeloma (MM) (Table 2.11 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.11.pdf). However, there was a statistically significant excess of MM reported for the Pliofilm cohort (SMR 4.1; 95%CI: 1.1–10.5, based upon four deaths) (Rinsky *et al.*, 1987), which did not persist in the most recent update (Rinsky *et al.*, 2002; see Table 2.11 online). In a cohort study among chemical workers at the Monsanto chemical company suggestive evidence was found of a dose–response relationship (Collins *et al.*, 2003), while in a cohort study of Norwegian workers in the upstream petroleum industry (i.e. the phases of oil extraction and initial transportion, which entail extensive exposure to crude oil) a significant increased risk for MM was found (Kirkeleit *et al.*, 2008).

Case–control studies of MM with estimates of exposure to benzene largely show no association (Table 2.12 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.12.pdf). An exception was an early study in which a significant association was found between risk for MM and the proportion of cases and controls with "solvent/benzene" exposure (La Vecchia *et al.*, 1989). In another study, borderline significant effects were detected (Costantini *et al.*, 2008). In a large multicentre case–control study of NHL in Europe there was no association of benzene exposure with MM (Cocco *et al.*, 2010).

A meta-analysis by Infante (2006) analysed data from seven well defined "benzene cohorts" outside of petroleum refining and found a statistically significant increase in risk for MM (RR 2.1; 95%CI: 1.3–3.5).

2.1.7 Hodgkin disease

There are sparse data on Hodgkin disease in studies of benzene-exposed cohorts, with most studies having very small numbers of cases and showing no association (see Table 2.13 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.13.pdf). Overall, there is no evidence of an increased risk. The relatively few case–control studies in adults also show no association (see Table 2.14 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.14.pdf). In a case–control study of childhood cancer in Denmark, an increased risk for Hodgkin disease was detected in association with estimated environmental exposures to benzene (Raaschou-Nielsen *et al.* (2001) (see Table 2.14 online).

2.2 Cancer of the lung

Cohort studies with information on potential or estimated benzene exposure and lung cancer are shown in Table 2.15 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.15.pdf). Although most studies show no association, in two cohorts with quantitative exposure-assessment evidence of a dose–response relationship was found (Hayes *et al.*, 1996; Collins

et al., 2003) and in two others statistically significant increases in risk were observed (Lynge *et al.*, 1997; Sorahan *et al.*, 2005). A case–control study from Canada showed no association of exposure to benzene with lung cancer overall or with the major histological subtypes (Gérin *et al.*, 1998; see Table 2.16 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.16.pdf).

2.3 Cancer of the kidney

Cohort studies with results on kidney cancer are shown in Table 2.17 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.17.pdf). Results generally do not show any association. In a case–control study among males in Germany an association was found between exposure to benzene and an increased risk for kidney cancer (Pesch *et al.*, 2000), but in a study in Montreal, Canada, there was little evidence of an association (Gérin *et al.*, 1998) (see Table 2.18 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.18.pdf).

2.4 Other cancers

In the evaluation of the cohort studies that provided data on the cancer sites considered above, it was apparent that associations have occasionally been found with other cancer sites including malignant melanoma (Schnatter *et al.*, 1996; Consonni *et al.*, 1999; Lewis *et al.*, 2003), nose and stomach cancer (Fu *et al.*, 1996) and prostate cancer (Collingwood *et al.*, 1996), but overall there was no consistency across the cohorts.

3. Cancer in Experimental Animals

Studies on the carcinogenesis of benzene in rats and mice after exposure by inhalation, intragastric gavage, skin application, and by intraperitoneal or subcutaneous injection have been reviewed in *IARC Monographs* Volume 29 and in Supplement 7 (IARC, 1982, 1987). In Supplement 7 it was concluded that there is *sufficient evidence* in experimental animals for the carcinogenicity of benzene. Results of adequately conducted carcinogenicity studies reported before and after 1987 are summarized in Tables 3.1, 3.2, 3.3, 3.4.

Exposure to benzene by inhalation increased the incidence of Zymbal gland carcinomas, liver adenomas, and forestomach and oral cavity carcinomas in female rats (Maltoni *et al.*, 1982a, c, 1983, 1985, 1989). It also increased the incidence of lymphohaematopoietic (lymphoma, myelogenous) neoplasms in male and female mice (Snyder *et al.*, 1980; Cronkite *et al.*, 1984, 1989; Farris *et al.*, 1993), and Zymbal gland carcinomas, squamous cell carcinomas of the preputial gland, and lung adenomas in male mice (Snyder *et al.*, 1988; Farris *et al.*, 1993).

Oral administration of benzene increased the incidence of Zymbal gland carcinomas and oral-cavity papillomas and carcinomas in rats of both sexes, of carcinomas of the tongue, papillomas and carcinomas of the skin and of the lip and papillomas of the palate in male rats, of forestomach acanthomas in both sexes of the rat, and of forestomach carcinomas in female rats (Maltoni & Scarnato, 1979; Maltoni *et al.*,1982b, 1983, 1988, 1989; NTP, 1986; Maronpot, 1987; Huff *et al.*, 1989; Mehlman, 2002). Given by the oral route, benzene also increased the incidence of Zymbal gland carcinomas, forestomach papillomas, lymphomas, and lung adenomas and carcinomas in mice of both sexes, of liver carcinomas, adrenal gland pheochromocytomas, harderian gland adenomas and preputial gland squamous cell carcinomas in male mice,

Table 3.1 Carcinogenicity studies in experimental animals exposed to benzene by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley, (M, F) 150 wk Maltoni *et al.* (1982a, c, 1983, 1985, 1989)	Three different treatment groups (n = 54–75) and 2 controls (breeder controls, n = 60; embryo controls, n = 149–158). Pregnant breeders (Group 1) and embryos exposed transplacentally (Group 2) were exposed 4 h/d, 5 d/wk for 7 wk at 200 ppm; then postpartum breeders and offspring were exposed 7 h/d, 5 d/wk for 12 wk during weaning at 200 ppm; after weaning, breeders and offspring were exposed 7 h/d, 5 d/wk for 85 wk at 300 ppm. Group 3 were embryos exposed 4 h/d, 5 d/wk for 7 wk at 200 ppm transplacentally then 7 h/d, 5 d/wk for 8 wk at 200 ppm. Therefore, the embryos were exposed transplacentally during pregnancy and the offspring were exposed by inhalation and possibly by ingestion via milk.	Zymbal's gland carcinomas Group 1 (breeders 104 wk): F–1/60 (controls), 3/54 Group 2 (embryos 104 wk): M–2/158 (controls), 6/75 F–0/149 (controls), 8/65 Group 3 (embryos 15 wk); M–2/158 (controls), 4/70 F–0/149 (controls), 1/59 Liver adenomas Group 1: F–0/60, 1/54 Group 2: M–1/158, 2/75 F–0/149, 5/65 Group 3: M–1/158, 2/70 F–0/149, 5/59 Oral cavity carcinomas Group 1: F–0/60, 2/54 Group 2: M–0/158, 1/75 F–0/149, 10/65 Group 3: M–0/158, 2/70 F–0/149, 6/59	[NS] [NS] [significant] [NS] [NS] [NS] [NS] [significant] [NS] [significant] [NS] [NS] [significant] [NS] [significant]	Breeders were 13 wk old at the start of exposure; embryos were 12 days old at the start of the exposures

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley, (M, F) 150 wk Maltoni *et al.* (1982a, c, 1983, 1985, 1989) Contd.		Forestomach carcinomas (in situ) Group 1: F–0/60, 0/54 Group 2: M–0/158, 0/75 F–0/149, 3/65 Group 3: M–0/158, 0/70 F–0/149, 0/59	 [NS] [NS] [significant] [NS] [NS]	
Mouse, C57BL/6J (M) Lifetime Snyder *et al.* (1980)	0 (filtered air) or 300 ppm benzene, 6 h/d, 5 d/wk 40/group	Total lymphohaematopoietic: 2/40, 8/40 - Lymphocytic lymphoma: 2/40, 6/40 - Plasmocytoma 0/40, 1/40 - Leukaemia 0/40, 1/40	$P < 0.005$, X^2-test $P < 0.001$, X^2-test NS NS	Purity unspecified Exposed mice had body weight gain depression relative to the controls throughout the study. Exposed mice had a median survival of 41 wk vs 75 wk for the controls.
Mouse, CD-1 Lifetime Goldstein *et al.* (1982)	0 or 300 ppm benzene 6 h/d, 5 d/wk 40/group	Myelogenous leukaemia: 0/40, 3/40	NS	Purity unspecified. Sex unspecified Although the incidence of 3/40 is not significantly higher than the 0% incidence observed in control animals ($P = 0.147$), these preliminary findings do give credence to the myeloleukaemogenic effect of benzene due to the lack of observation of spontaneous myeloproliferative disorders in the animal strain under study.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C57Bl/6 BNL (F) Lifetime Cronkite *et al.* (1984)	0 or 300 ppm for 16 wk, 6 h/d, 5 d/wk 88–90/group	Total lymphohaematopoietic malignancies: 0/88, 8/90	[$P < 0.01$]	Purity unspecified
		- Thymic lymphoma: 0/88, 6/90	[$P < 0.05$]	
		- Lymphoma (unspecified): 0/88, 2/90	[NS]	
Mouse, CD-1 (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 1200 ppm benzene, 6 h/d, 5 d/wk for 10 wk 50 exposures total 80/group	Lung adenomas: 17/71, 33/71	$P < 0.001$	Purity unspecified
		Zymbal's gland carcinomas: 0/71, 4/71	$P < 0.05$	
Mouse, CD-1 (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 300 ppm benzene, 6 h/d, 5 d/wk for 1 wk followed by 2 wk of non exposure for life 60/group	Lung adenomas: 3/46, 14/54	$P < 0.005$	Purity unspecified
		Leukaemia/lymphomas: 1/46, 7/54	NS	
		Zymbal's gland carcinomas: 0/46, 2/54	NS	
Mouse, C57Bl (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 1200 ppm benzene, 6 h/d, 5 d/wk for 10 wk 80/group	Zymbal's gland carcinomas: 0/67, 4/68	NS	Purity unspecified
Mouse, C57Bl (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 300 ppm benzene, 6 h/d, 5 d/wk for 1 wk followed by 2 wk of non exposure for life 60/group	Zymbal's gland carcinomas: 0/46, 19/54	$P < 0.001$	Purity unspecified

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, CBA/Ca BNL (M, F) Lifetime Cronkite *et al.* (1989)	0, 100 (M), 300 (M, F) ppm benzene for 16 wk 6 h/d, 5 d/wk. 60 – 85/group	100 ppm Myelogenous neoplasms: 0/70, 2/85 Other neoplasms, other than hepatoma and haematopoietic: 14/70, 38/85 300 ppm Myelogenous neoplasms: M–0/60, 11/57 F–1/60, 6/54 Other neoplasms, other than hepatoma and haematopoietic: M–13/60, 30/57 F–21/60, 43/54	 NS $P < 0.001$ $P < 0.001$ $P = 0.040$ $P < 0.001$ $P < 0.001$	Purity unspecified Medium lifespan in male (510 days) and female (580 days) mice exposed to 300 ppm was significantly reduced versus sham –exposed males (1 030 days) and females (1 100 days). Myelogenous neoplasms included acute myeloblastic and chronic granulocytic leukaemia. Other neoplasms included Zymbal's and Harderian gland tumours, squamous cell carcinoma, mammary gland adenocarcinoma, and papillary adenocarcinoma of the lung.
Mouse, CBA/Ca (M) 22 months Farris *et al.* (1993)	0, 300 ppm benzene 6 h/d, 5 d/wk for 16 wk 125/group	Malignant lymphomas: 2/119, 14/118 Preputial gland (squamous cell carcinomas): 0/118, 71/118 Lung (alveolar/bronchiolar adenomas): 17/119, 42/119 Zymbal's gland (carcinomas): 1/125, 14/125 Forestomach (squamous cell carcinomas): 0/125, 9/125 Harderian gland: 6/125, 7/125	$P < 0.002$ $P < 0.01$ $P < 0.01$ See comments See comments See comments	100% pure Exposure to benzene caused a significant decrease ($P < 0.01$) in survival and was a significant cause ($P < 0.01$) of early mortality during the first 9 mo post exposure. Most (12) of the lymphomas were of the lymphoblastic or lymphocytic type; two (2) were of the mixed type. Zymbal's gland, forestomach and Harderian gland were examined microscopically only when gross lesions were evident.

d, day or days; h, hour or hours; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; ppm, parts per million; wk, week or weeks; yr, year or years

Table 3.2 Carcinogenicity studies in experimental animals exposed to benzene by gavage

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989)	0, 50, 100, 200 mg/kg bw benzene in corn oil (M); 0, 25, 50, or 100 mg/kg bw in corn oil (F) 5 d/wk 60/group	***Zymbal's Gland:***		> 99.7% pure Groups of 10 rats/sex/group were removed at 51 wks for blood sampling and killed at 52 wks. Survival decreased with increasing dose in both sexes; survival of the high-dose females was significantly less than that of the controls; control females had a greater than average survival normally observed for female F344 rats. Final mean body weight of the high dose males was significantly less than that of the vehicle controls. Most of the dosed rats that died before 103 wks had neoplasms.
		Carcinoma: M–2/32, 6/46, 10/42, 17/42 F–0/45, 5/40, 5/44, 14/46	$P < 0.001$, $P = 0.193$, $P = 0.017$, $P < 0.001$ (M); $P < 0.001$, $P < 0.022$, $P < 0.018$, $P < 0.001$ (F)	
		Adenoma/Carcinoma: M–2/32, 7/46, 10/42, 18/42 F–0/45, 5/40, 6/44, 15/46	$P < 0.001$, $P = 0.131$, $P = 0.017$, $P < 0.001$ (M); $P < 0.001$, $P = 0.022$, $P = 0.010$, $P < 0.001$ (F)	
		Palate:		
		Papilloma: M–0/50, 4/50, 4/50, 9/50 F–1/50, 3/50, 5/50, 3/50	$P < 0.001$, $P = 0.064$, $P = 0.057$, $P < 0.001$ (M); $P = 0.103$, $P = 0.240$, $P = 0.053$, $P = 0.183$ (F)	
		Lip:		
		Papilloma: M–0/50, 2/50, 5/50, 5/50 F–0/50, 0/50, 2/50, 2/50	$P < 0.001$, $P = 0.216$, $P = 0.015$, $P = 0.008$ (M)	
		Carcinoma: M–0/50, 0/50, 0/50, 3/50 F–0/50, 0/50, 0/50, 0/50	$P = 0.002$, -,-, $P = 0.035$ (M)	
		Papilloma/Carcinoma: M–0/50, 2/50, 5/50, 8/50 F–0/50, 0/50, 2/50, 2/50	$P < 0.001$, $P = 0.216$, $P = 0.015$, $P = 0.008$ (M)	
		Tongue:		
		Papilloma: M–1/50, 0/50, 2/50, 2/50 F–0/50, 1/50, 1/50, 0/50		
		Carcinoma: M–0/50, 3/50, 4/50, 4/50 F–0/50, 0/50, 4/50, 4/50	$P = 0.039$, $P = 0.133$, $P = 0.051$, $P = 0.028$ (M)	
		Papilloma/Carcinoma: M–1/50, 3/50, 6/50, 6/50 F–0/50, 1/50, 5/50, 4/50	$P = 0.013$, $P = 0.328$, $P = 0.044$, $P = 0.028$ (M)	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989) Contd.		***Oral Cavity*** (overall rates): Papilloma: M–1/50, 6/50, 11/50, 13/50 F–1/50, 4/50, 8/50, 5/50	$P < 0.001$, $P = 0.058$, $P = 0.001$, $P < 0.001$ (M); $P = 0.017$, $P = 0.127$, $P = 0.006$, $P = 0.032$ (F)	
		Carcinoma: M–0/50, 3/50, 5/50, 7/50 F–0/50, 1/50, 4/50, 5/50	$P = 0.001$, $P = 0.133$, $P = 0.030$, $P = 0.001$ (M); $P = 0.003$, $P = 0.468$, $P = 0.047$, $P = 0.010$ (F)	
		Papilloma/Carcinoma: M–1/50, 9/50, 16/50, 19/50 F–1/50, 5/50, 12/50, 9/50	$P < 0.001$, $P = 0.012$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.068$, $P < 0.001$, $P = 0.001$ (F)	
		Skin: Papilloma: M–0/50, 2/50, 1/50, 5/50	$P < 0.001$, $P = 0.216$, $P = 0.451$, $P < 0.005$ (M)	
		Carcinoma: M–0/50, 5/50, 3/50, 8/50 =	$P < 0.001$, $P = 0.032$, $P = 0.098$, $P < 0.001$ (M)	
		Papilloma/Carcinoma: M–1/50, 7/50, 5/50, 12/50	$P < 0.001$, $P = 0.031$, $P = 0.076$, $P < 0.001$ (M)	
		Uterus: Endometrial stromal polyp: F–7/50, 7/50, 7/49, 14/50	$P = 0.001$, $P = 0.468$, $P = 0.420$, $P = 0.003$ (F)	
Rat, Wistar (M, F) Lifetime Maltoni *et al.* (1983, 1988, 1989), Mehlman (2002)	0, (control), 500 mg/kg bw benzene in olive oil once/d, 5 d/wk, 104 wk 40/group/sex	**Zymbal's gland:** Carcinoma: M–0/40, 7/40 F–0/40, 6/40	[$P < 0.01$]; [$P < 0.05$]	99.93% pure Mortality was higher in benzene-treated male and female rats. Benzene treated rats had lower body weights.
		Oral cavity: Carcinoma: M–1/40, 2/40 F–0/40, 4/40	[NS]	
		Nasal cavity: Carcinoma: M–0/40, 2/40 F–1/40, 1/40	[NS]	
		Malignant tumours: M–8/40, 19/40 F–10/40, 21/40	-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) Lifetime Maltoni *et al.* (1983, 1989), Maltoni & Scarnato (1979), Mehlman (2002)	Benzene in olive oil 0 (control), 50 or 250 mg/kg bw once/d, 4–5 d/wk for 52 wk 30 or 35/group	**Leukaemia:** M–0/28, 0/28, 4/33 F–1/30, 2/30, 1/32	[NS]	99.93% pure
		Zymbal's gland (carcinomas): M–0/28, 0/28, 0/33 F–0/30, 2/30, 8/32*	*[$P < 0.005$] (F)	
Rat, Sprague-Dawley (M, F) Lifetime Maltoni *et al.* (1989) Maltoni *et al.* (1983), Mehlman (2002), Maltoni *et al.* (1982b)	Benzene in olive oil 0 (control), 500 mg/kg bw once/d, 4–5 d/wk for 104 wk Controls, 50/group Treated, 40/group	**Leukaemia:** M–3/50, 1/40 F–1/50, 3/40	[NS]	99.93% pure
		Zymbal's gland: Carcinoma: M–1/50, 18/40 F–0/50, 16/40	[$P < 0.0001$] (M, F)	
		Nasal cavity: Carcinoma: M–0/50, 3/40 F–0/50, 1/40	[NS]	
		Oral cavity: Carcinoma: M–0/50, 21/40 F–0/50, 20/40	[$P < 0.0001$] (M, F)	
		Skin: Carcinoma: M–0/50, 9/40 F–1/50, 0/40	[$P < 0.001$] (M)	
		Liver: Hepatomas: M–3/50, 3/40 F–0/50, 1/40	[NS]	
		Angiosarcoma: M–0/50, 2/40 F–0/50, 3/40		
		Forestomach: Acanthoma/dysplasia: M–0/50, 10/40 F–0/50, 7/40	[$P < 0.005$] (M, F)	
		Carcinoma M–0/50, 1/40 F–0/50, 6/40	[$P < 0.01$] (F)	
		Total Malignant tumours: M–12/50, 68/40 F–11/50, 59/40	-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989)	0, 25, 50, or 100 mg/kg bw benzene in corn oil (M, F) 5 d/wk 60/group	***Zymbal's Gland:*** Carcinoma: M–0/43, 1/34, 4/40, 21/39 F–0/43, 0/32, 1/37, 3/31	$P < 0.001$, $P = 0.489$, $P = 0.012$, $P < 0.001$ (M); $P = 0.007$,–, $P = 0.450$, $P = 0.045$ (F)	> 99.7% pure Groups of 10 mice/sex/group were removed at 51 wks for blood sampling and killed at 52 wks. Survival decreased with increasing dose in both sexes; survival of the high dose males and high dose females was significantly less than those of the controls ($P < 0.001$ and $P = 0.004$, respectively). Final mean body weights of the high dose males and females were less than that of the vehicle controls. Increased incidence of neoplasms were observed at multiple sites for male and female mice.
		Preputial Gland: Squamous cell carcinoma: 0/21, 3/28, 18/29, 28/35	$P < 0.001$, $P = 0.225$, $P < 0.001$, $P < 0.001$ (M)	
		Carcinoma NOS: 0/21, 2/28, 1/29, 3/35	$P < 0.019$, $P = 0.359$, $P = 0.445$, $P = 0.043$ (M)	
		Carcinoma (all types): 0/21, 5/28, 19/29, 31/35	$P < 0.001$, $P = 0.091$, $P < 0.001$, $P < 0.001$ (M)	
		Ovary Tubular adenoma: 0/47, 0/44, 3/49, 3/48	$P = 0.008$, -, $P = 0.090$, $P = 0.047$	
		Granulosa cell tumour or carcinoma: 1/47, 1/44, 6/49, 8/48	$P < 0.001$, $P = 0.730$, $P < 0.040$, $P < 0.004$ (F)	
		Benign mixed cell tumour: 0/47, 1/44, 12/49, 7/48	$P < 0.001$, $P = 0.471$, $P < 0.001$, $P < 0.001$ (F)	
		Mammary gland: Carcinoma: F–0/49, 2/45, 5/50, 10/49	$P < 0.001$, $P = 0.202$, $P < 0.026$, $P < 0.001$ (F)	
		Carcinosarcoma: F–0/49, 0/45, 1/50, 4/49	$P < 0.001$, –, $P < 0.495$, $P < 0.017$ (F)	
		Harderian gland: Adenoma: M–0/49, 9/46, 13/49, 11/48 F–5/48, 6/44, 10/50, 6/47	$P < 0.001$, $P = 0.001$, $P < 0.001$, $P < 0.001$ (M); $P = 0.133$, $P = 0.369$, $P = 0.090$, $P = 0.204$ (F)	
		Carcinoma: M–1/49, 2/46, 0/49, 3/48 F–0/48, 0/44, 0/50, 4/47	$P < 0.001$, –, –, $P = 0.020$ (F	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989) Contd.		Adenoma/Carcinoma: M–1/49, 10/46, 13/49, 14/48 F–5/48, 6/44, 10/50, 10/47	$P < 0.001$, $P = 0.002$, $P < 0.001$, $P < 0.001$ (M); $P = 0.009$, $P = 0.369$, $P = 0.090$, $P = 0.017$ (F)	
		Lung (alveolar/bronchiolar): Adenoma: M–6/49, 6/48, 8/50, 12/49 F–4/49, 2/42, 5/50, 9/49	$P < 0.001$, $P = 0.499$, $P = 0.188$, $P = 0.005$ (M); $P = 0.003$, $P = 0.437$N, $P = 0.398$, $P = 0.011$ (F)	
		Carcinoma: M–5/49, 11/48, 12/50, 14/49 F–0/49, 3/42, 6/50, 6/49	$P < 0.001$, $P = 0.052$, $P = 0.017$, $P < 0.001$ (M); $P = 0.002$, $P = 0.084$, $P = 0.010$, $P = 0.004$ (F)	
		Adenoma/Carcinoma: M–10/49, 16/48, 19/50, 21/49 F–4/49, 5/42, 10/50, 13/49	$P < 0.001$, $P = 0.069$, $P = 0.007$, $P < 0.001$ (M); $P = < 0.001$, $P = 0.0.366$, $P = 0.039$, $P < 0.001$ (F)	
		Lymphohaematopoietic: Lymphoma: M–4/49, 9/48, 9/50, 15/49 F–15/49, 24/45, 24/50, 20/49	$P < 0.001$, $P = 0.075$, $P < 0.030$, $P < 0.001$ (M); $P = 0.031$, $P = 0.021$, $P = 0.025$, $P = 0.037$ (F)	
		Leukaemia: M–0/49, 1/48, 1/50, 0/49 F–0/49, 1/45, 2/50, 2/49	NR	
		Lymphoma/leukaemia: M–4/49, 10/48, 10/50, 15/49 F–15/49, 25/45, 26/50, 22/49	$P < 0.001$, $P = 0.048$, $P < 0.018$, $P < 0.001$ (M); $P = 0.014$, $P = 0.014$, $P = 0.012$, $P = 0.017$ (F)	
		Forestomach: Papilloma: M–2/45, 1/42, 2/44, 5/38 F–1/42, 3/40, 6/45, 5/42	$P = 0.003$, $P = 0.567$N, $P < 0.556$, $P < 0.014$ (M); $P = 0.022$, $P = 0.288$, $P = 0.038$, $P = 0.040$ (F)	
		Carcinoma: M–2/45, 1/42, 2/44, 5/38	NR	
		Papilloma/carcinoma: M–2/45, 2/42, 3/44, 5/38	$P = 0.004$, $P = 0.623$, $P = 0.335$, $P = 0.014$ (M)	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989) Contd.		***Liver:*** Adenoma: M–7/49, 11/48, 6/50, 3/47 F–1/49, 8/44, 5/50, 4/49	$P = 0.156$, $P = 0.008$, $P = 0.079$, $P = 0.077$ (F)	
		Carcinoma: M–9/49, 8/48, 17/50, 8/47 F–3/49, 4/44, 8/50, 4/49	$P = 0.072$, $P = 0.589$, $P = 0.028$, $P = 0.293$ (M)	
		Adenoma/Carcinoma: M–15/49, 17/48, 22/50, 11/47 F–4/49, 12/44, 13/50, 7/49	$P = 0.076$, $P = 0.256$, $P = 0.029$, $P = 0.225$ (M); $P = 0.103$, $P = 0.014$, $P = 0.008$, $P = 0.086$ (F)	
		Adrenal Gland: Pheochromocytoma: M–1/47, 1/48, 7/49, 1/46 F–6/49, 1/44, 1/50, 1/48	$P = 0.096$, $P = 0.725$, $P = 0.010$, $P = 0.632$ (M)	
Mouse, A/J (M, F) 24 wk Stoner *et al.* (1986)	0 (control), 24 g/kg bw in tricaprylin vehicle 3x/wk for 8 wk 16/group	**Lung** (adenomas): M–3/15, 8/16 F–2/14, 5/15	NR	Purity NR
		tumours/ mouse: M–0.27 ± 0.59, 0.63 ± 0.72 F–0.14 ± 0.36, 0.53 ± 0.92	$P < 0.05$ NS	
Mouse, Swiss (M, F) Lifetime Maltoni *et al.* (1988)	0 (control), 500 mg/kg bw benzene in olive oil once/d, 5d/wk 40/group	**Mammary gland** (carcinomas): M–1/40, 0/40 F–2/40, 19/40	[$P < 0.0001$] (F)	99.93% pure
		Lung (adenomas): M–3/40, 16/40 F–4/40, 15/40	[$P < 0.01$] (M, F)	
		Zymbal's gland (carcinomas): M–0/40, 4/40 F–0/40, 1/40	[NS]	
		Malignant tumours: M–9/40, 14/40 F–11/40, 28/40	-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, RF/J (M, F) Lifetime Maltoni *et al.* (1989), Mehlman (2002)	0 (control), 500 mg/kg bw benzene in olive oil once/d, 4–5 d/wk for 52 wk Male, 45/group Female, 40/group	**Mammary Gland** (carcinomas): M–0/45, 0/45 F–1/40, 9/40	[$P < 0.05$] (F)	99.93% pure
		Lung: All tumours: M–5/45, 23/45 F–3/40, 15/40	[$P < 0.005$] (M, F)	
		Adenocarcinomas: M–0/45, 0/45 F–0/40, 1/40	[NS]	
		Leukaemia: M–17/45, 26/45 F–14/40, 24/40	[NS]	
Mouse, C57Bl/6-Trp53 (F) 26 wk French & Saulnier (2000)	0 (control), 200 mg/kg bw benzene 5d/wk Controls – 20/group Dosed – 40/group	**Subcutis** (sarcomas): 0/20, 16/39	[$P < 0.001$]	> 99.9% pure vehicle unspecified
		Thymus (lymphomas): 0/20, 3/39	[NS]	
Mouse, haploinsufficient $p16^{Ink4a}/p19^{Arf}$ (M, F) 27 wk NTP (2007)	0 (control), 25, 50, 100, 200 mg/kg bw benzene in corn oil 5 d/wk 15/group	**Malignant lymphomas:** M–0/15, 0/15, 0/15, 0/15, 5/15	$P = 0.021$ (high dose) $P < 0.001$ (trend)	

d, day or days; F, female; i.p., intraperitoneal; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years; bw, body weight

Table 3.3 Carcinogenicity studies in experimental animals exposed to benzene by intraperitoneal injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, A/J (M, F) 24 wk Stoner *et al.* (1986)	0 (control), 480, 1 200, 2 400 mg/kg bw in tricaprylin vehicle 3x/wk for 8 wk 16/group	**Lung adenomas:** M–3/16, 5/15, 8/16, 10/16 F–4/16, 4/15, 4/16, 6/15	NR	Purity NR
		Tumours/ mouse: M–0.25 ± 0.58, 0.53 ± 0.92, 0.63 ± 0.72, 0.69 ± 0.60 F–0.31 ± 0.60, 0.44 ± 0.89, 0.25 ± 0.45, 0.47 ± 0.64	$P < 0.05$ (1 200 and 2 400 mg/kg) (M)	

F, female; M, male; mo, month or months; NR, not reported; wk, week or weeks; bw, body weight

Table 3.4 Carcinogenicity studies in experimental animals exposed to benzene via skin application

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, hemizygous and homozygous Tg.AC (v-Ha-*ras*) (M, F) 20 wk Blanchard *et al.* (1998)	200 µl of acetone: vehicle control 200 µl benzene, neat 2–7x/wk 10 mice/treated group	Skin papillomas: Hemizygous Tg.AC M–6/65, 3/10 F–2/65, 4/10 Homozygous Tg.AC M–NR, 10/10 F–NR, 9/10	 [NS] [$P < 0.01$] - -	Purity NR
Mouse, hemizygous Tg.AC (v-Ha-*ras*) (M, F) 26 wk Holden *et al.* (1998)	G1: Untreated (shaved) G2: acetone 200 µl, 7d/wk, 20 wk G3: 100 µl benzene, 3x/wk, 20 wk G4: 150 µl benzene, 3x/wk, 20 wk 10 mice/group	Skin (papillomas): M–0/10, 0/10, 0/10, 3/10 F–0/10, 0/10, 1/10, 1/10	$P \leq 0.05$, G4 vs negative controls	Purity NR
Mouse, homozygous, FVB/N-Tg.AC (v-Ha-*ras*) (F) 32 wk French & Saulnier (2000)	0 µl/wk: 200 µl acetone 1/d 3x/wk for 20 wk (control) 450 µl/wk: 150 µl in 50 µl acetone 1/d, 3x/wk for 20 wk 800 µl/wk: 200µl neat, 2/d, 2/wk for 20 wk 20 mice/group	Granulocytic leukaemia: 0/19, 4/14*, 11/15*	*$P \leq 0.05$	> 99.9% pure

d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years

and of benign and malignant ovarian tumours, mammary gland carcinomas and carcinosarcomas, and Harderian gland carcinomas in female mice (NTP, 1986; Stoner *et al.*, 1986; Maronpot, 1987; Maltoni *et al.*, 1988, 1989; Huff *et al.*, 1989; Mehlman, 2002).

Increased multiplicity of lung adenomas was observed in male mice after intraperitoneal injection of benzene (Stoner *et al.*, 1986).

Exposure of genetically altered, tumour-prone mice to benzene by oral administration, skin application, or inhalation resulted in increased incidences of skin tumours (Blanchard *et al.* 1998; Holden *et al.*, 1998; French & Saulnier, 2000) and lymphohaematopoietic malignancies (French & Saulnier, 2000; NTP, 2007; Kawasaki *et al.*, 2009).

4. Other Relevant Data

4.1 Genetic and related effects

Benzene induced chromosomal aberrations, micronuclei and sister chromatid exchange in bone-marrow cells of mice, chromosomal aberrations in bone-marrow cells of rats and Chinese hamsters and sperm-head anomalies in mice treated *in vivo*. It induced chromosomal aberrations and mutation in human cells *in vitro* but did not induce sister chromatid exchange in cultured human lymphocytes, except in one study in which high concentrations of an exogenous metabolic system were used. In some test systems, benzene induced cell transformation. It did not induce sister chromatid exchange in rodent cells *in vitro*, but it did induce aneuploidy and, in some studies, chromosomal aberrations in cultured Chinese hamster ovary cells. Benzene induced mutation and DNA damage in some studies in rodent cells *in vitro*. In *Drosophila*, benzene was reported to be weakly positive in assays for somatic mutation and for crossing-over in spermatogonia; in single studies, it did not induce sex-linked recessive lethal mutations or translocations. It induced aneuploidy, mutation and gene conversion in fungi. Benzene was not mutagenic to bacteria (IARC, 1982, 1987). Chromosomal aberrations in human peripheral lymphocytes have been associated with occupational exposure to benzene for decades (Forni, 1979; IARC, 1982; Eastmond, 1993; Zhang *et al.*, 2002; Holecková *et al.*, 2004).

4.2 Leukaemogenic potential of benzene

Benzene is carcinogenic to the bone marrow causing leukaemia and myelodysplastic syndromes (MDS) and probably also to the lymphatic system causing non-Hodgkin lymphoma. Its carcinogenic mechanism of action is likely to be different for these two target tissues and probably multifactorial in nature. The metabolism of benzene will be summarized below and a review is presented of the current state of knowledge on the mechanisms of leukaemia and lymphoma induction by benzene. With regard to leukaemia, probable mechanisms of leukaemogenesis in the myeloid series, mainly acute myeloid leukaemia (AML) and MDS are discussed. Then, potential mechanisms by which benzene could cause acute lymphocytic leukaemia (ALL) in both adults and children are reviewed. Finally, mechanisms for the benzene-induced development of non-Hodgkin lymphoma are summarized, including that of chronic lymphocytic leukaemia (CLL), as it is now classified as a form of lymphoma.

4.2.1 Metabolism of benzene and its relevance to carcinogenicity

Benzene must be metabolized to become carcinogenic (Ross, 2000; Snyder, 2004). Its metabolism is summarized in Fig. 4.1. The initial metabolic step involves cytochrome P450 (CYP)-dependent oxidation to benzene oxide,

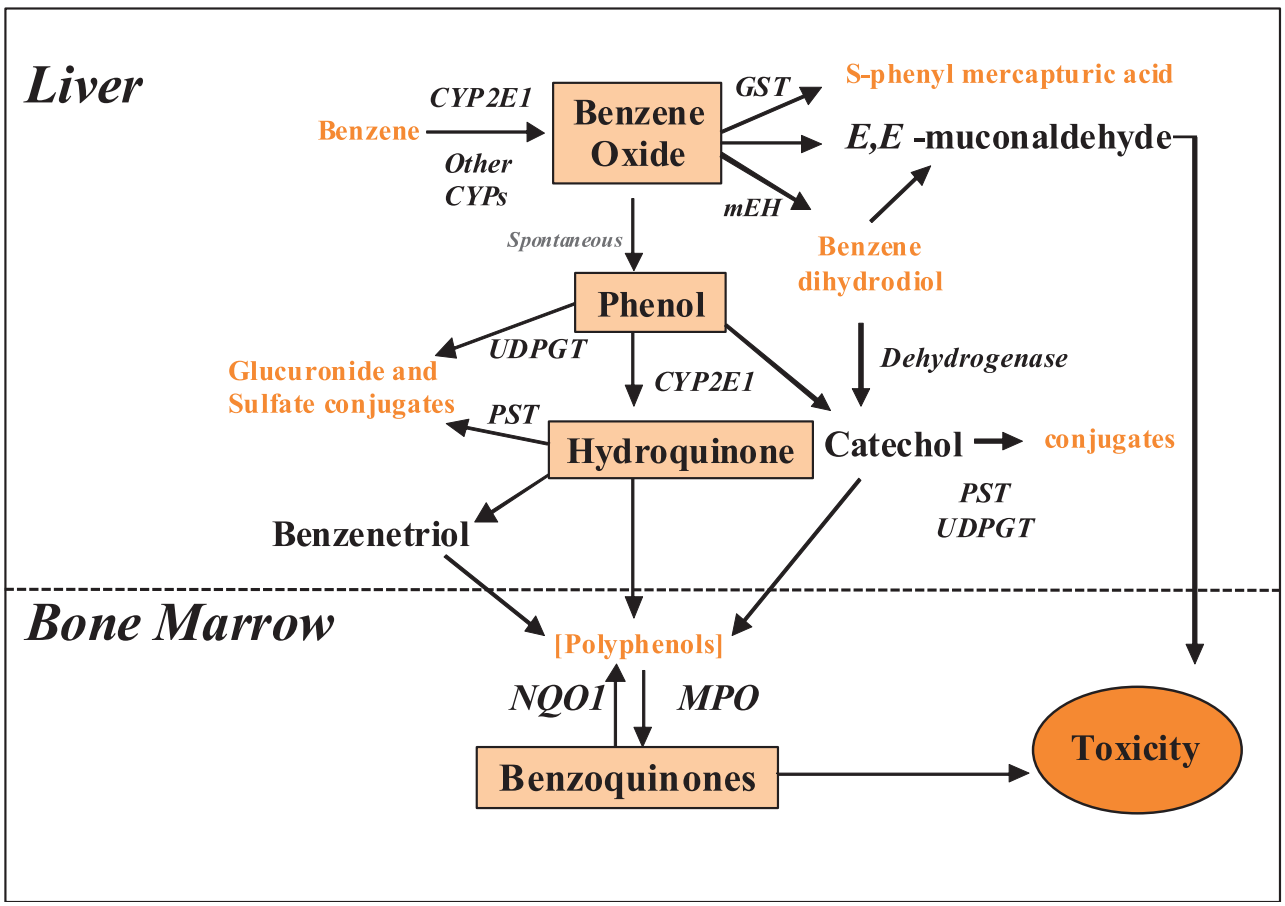
which exists in equilibrium with its tautomer oxepin. Most benzene oxide spontaneously rearranges to phenol, which is either excreted or further metabolized to hydroquinone and 1,4-benzoquinone. The remaining benzene oxide is either hydrolysed to produce benzene 1,2-dihydrodiol (catechol), which is further oxidized to 1,2-benzoquinone, or it reacts with glutathione to produce *S*-phenylmercapturic acid. Metabolism of oxepin is thought to open the aromatic ring, to yield the reactive muconaldehydes and *E,E*-muconic acid. Human exposure to benzene at concentrations in air between 0.1 and 10 ppm, results in urinary metabolite profiles with 70–85% phenol, 5–10% each of hydroquinone, *E,E*-muconic acid and catechol, and less than 1% of *S*-phenylmercapturic acid (Kim *et al.*, 2006b). Benzene oxide, the benzoquinones, muconaldehydes, and benzene dihydrodiol epoxides (formed from CYP-mediated oxidation of benzene dihydrodiol) are electrophiles that readily react with peptides, proteins and DNA (Bechtold *et al.*, 1992; McDonald *et al.*, 1993; Bodell *et al.*, 1996; Gaskell *et al.*, 2005; Henderson *et al.*, 2005; Waidyanatha & Rappaport, 2005) and can thereby interfere with cellular function (Smith, 1996). It remains unclear what role these different metabolites play in the carcinogenicity of benzene, but benzoquinone formation from hydroquinone via myeloperoxidase in the bone marrow has been suggested as being a key step (Smith, 1996). There is considerable evidence for an important role of this metabolic pathway that leads to benzoquinone formation, as the benzoquinone-detoxifying enzyme NAD(P)H:quinone oxidoreductase1 (NQO1) protects mice against benzene-induced myelodysplasia (Long *et al.*, 2002; Iskander & Jaiswal, 2005) and humans against the hematotoxicity of benzene (Rothman *et al.*, 1997). However, this does not rule out adverse effects from other metabolites.

Increased susceptibility to the toxic effects of benzene has been linked to genetic polymorphisms that increase the rate of metabolism of benzene to active intermediates, or decrease the rate of detoxification of these active intermediates (Rothman *et al.*, 1997; Xu *et al.*, 1998; Kim *et al.*, 2004).

Recently it has been shown that benzene is most likely metabolized initially to phenol and *E,E*-muconic acid via two enzymes rather than just one CYP enzyme, and that the putative, high-affinity enzyme is active primarily at benzene concentrations below 1 ppm (Rappaport *et al.*, 2009). CYP2E1 is the primary enzyme responsible for mammalian metabolism of benzene at higher levels of exposure (Valentine *et al.*, 1996; Nedelcheva *et al.*, 1999). CYP2F1 and CYP2A13 are reasonable candidate enzymes that are active at environmental levels of exposure below 1 ppm (Powley & Carlson, 2000; Sheets *et al.*, 2004; Rappaport *et al.*, 2009). These CYPs are highly expressed in the human lung. Despite much research, more work is needed to elucidate the different roles of multiple metabolites in the toxicity of benzene and the pathways that lead to their formation.

A role for the aryl-hydrocarbon receptor (AhR) is also emerging in the haematotoxicity of benzene. AhR is known mainly as the mediator for the toxicity of certain xenobiotics (Hirabayashi & Inoue, 2009). However, this transcription factor has many important biological functions and evidence is emerging that it has a significant role in the regulation of haematopoietic stem cells (Hirabayashi & Inoue, 2009; Singh *et al.*, 2009). It has been hypothesized that AhR expression is necessary for the proper maintenance of quiescence in these cells, and that AhR downregulation is essential for their "escape" from quiescence and subsequent proliferation (Singh *et al.*, 2009). It has been demonstrated that AhR-knockout (KO) ($AhR^{-/-}$) mice do not show any haematotoxicity after exposure to benzene (Yoon *et al.*, 2002). Follow-up studies have shown that mice that had been lethally irradiated and repopulated with marrow cells from AhR-KO mice did not display any sign of benzene-induced

Fig. 4.1 Simplified metabolic scheme for benzene showing major pathways and metabolizing enzymes leading to toxicity. CYP2E1, cytochrome P450 2E1; GST, glutathione-*S*-transferase; NQO1, NAD(P)H:quinone oxidoreductase 1; MPO, myeloperoxidase; UDPGT, Uridine diphosphate glucoronosyl transferase; PST, phenol sulphotransferase; mEH, microsomal epoxide hydrolase



haematotoxicity (Hirabayashi *et al.*, 2008). The most likely explanation for these findings is that the absence of AhR removes haematopoietic stem cells from their quiescent state and makes them susceptible to DNA damage from benzene exposure and subsequent cell death through apoptosis. Further research is needed to examine the effects of benzene and its metabolites on cycling and quiescent haematopoietic stem cells.

4.2.2 Mechanisms of myeloid leukaemia development

(a) General

AML and MDS are closely-related diseases of the bone marrow that arise de novo (without an obvious cause) in the general population or following therapy with alkylating agents, topoisomerase II inhibitors, or ionizing radiation (therapy-related AML and MDS, i.e. t-AML and t-MDS) (Pedersen-Bjergaard *et al.*, 2006, 2008). Occupational exposure to benzene is widely thought to cause leukaemias that are similar to various forms of t-AML and t-MDS (Irons

& Stillman, 1996; Larson & Le Beau, 2005; Zhang *et al.*, 2007). AML and MDS both arise from genetically altered CD34+ stem cells or progenitor cells in the bone marrow (Morgan & Alvares, 2005; Passegué & Weisman, 2005) and are characterized by many different types of recurrent chromosome aberrations (Pedersen-Bjergaard *et al.*, 2006; Mrózek & Bloomfield, 2008). These aberrations have been shown to often develop into the genetic mutations that produce leukaemia. Cytogenetic analysis of chromosome number and structure has therefore become important in diagnosis and treatment of MDS and AML (Pedersen-Bjergaard *et al.*, 2006; Mrózek & Bloomfield, 2008). Recent research has shown that the chromosome aberrations and gene mutations detected in therapy-related and de novo MDS and AML are identical, although the frequencies with which they are observed in different subtypes may differ (Pedersen-Bjergaard *et al.*, 2008). Hence, therapy-related and de novo MDS and AML are considered identical diseases (Pedersen-Bjergaard *et al.*, 2008).

At least three cytogenetic categories of AML and MDS are commonly observed: those with unbalanced aberrations, with balanced rearrangements, and with normal karyotype:

Unbalanced chromosome aberrations comprise primarily the loss of various parts of the long arm or loss of the whole chromosome 5 or 7 (5q–/–5 or 7q–/–7) and gain of a whole chromosome 8 (+8) (Pedersen-Bjergaard *et al.*, 2006, 2007, 2008). These cases often have a complex karyotype and carry point mutations of *TP53* or *AML1*. Unbalanced chromosome aberrations are common after therapy with alkylating agents.

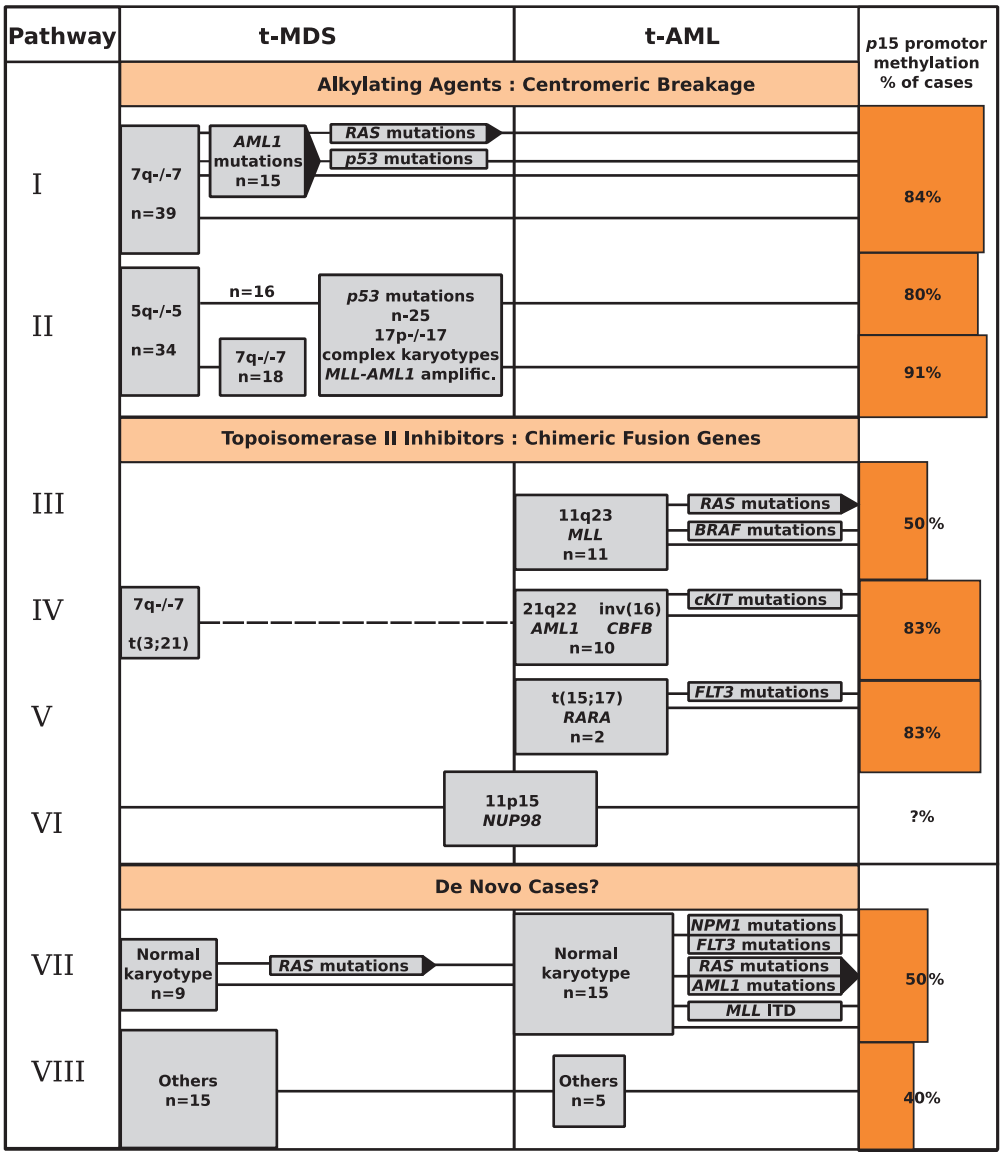
Balanced rearrangements are recurrent balanced translocations [e.g. t(11q23), t(8;21) and t(15;17)] or inversions [e.g. inv(16)], which arise, at least in the therapy-related subset of cases, as illegitimate gene recombinations related to functional inhibition of topoisomerase II (Pedersen-Bjergaard *et al.*, 2006, 2008). Among the most important rearranged transcription-factor genes are the mixed-lineage leukaemia (*MLL*) at 11q23, the *AML1* at 21q22, the retinoic-acid receptor-α *RARA* at 17q21 and the core-binding factor subunit-β (*CBFB*) at 16q22 (Pedersen-Bjergaard *et al.*, 2007).

Cases with a normal karyotype often harbour mutations of the *NPM1* gene (which encodes nucleophosmin), internal tandem duplications of the *FLT3* gene (which encodes fms-related tyrosine kinase), and/or point mutations or an altered methylation status of the *C/EBPα* gene (which encodes CCAAT/enhancer binding protein α) (Cuneo *et al.*, 2002; Pedersen-Bjergaard *et al.*, 2006, 2007, 2008; Hackanson *et al.*, 2008).

Within these three cytogenetic categories there are at least eight different genetic pathways that lead to MDS and AML, as defined by the specific chromosome aberrations present in each (Pathways I –VIII in Fig. 4.2). As more becomes clear about the molecular cytogenetics of leukaemia, it seems likely that many other pathways to AML and MDS will be discovered. For example, recent unbiased high-resolution genomic screens have identified many genes not previously implicated in AML that may be relevant for pathogenesis, along with many known oncogenes and tumour-suppressor genes (Ley *et al.*, 2008; Mardis *et al.*, 2009; Walter *et al.*, 2009).

Another classical pathway to AML is through the transformation of a myeloproliferative disorder (MPD) (Abdulkarim *et al.*, 2009), although there is less evidence for this pathway as a relevant mechanism to benzene-induced AML. MPDs include Philadelphia chromosome (Ph)-positive chronic myelogenous leukaemia (CML) and the Ph-negative conditions *polycythemia vera*, essential trombocythemia and idiopathic myelofibrosis. It is well established that AML may occur as a late complication in all these disorders. Over the first ten years after diagnosis, the incidence of leukaemic transformation is reported to be higher in patients with idiopathic myelofibrosis (8–23%) compared with

Fig. 4.2 Genetic Pathways to Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukaemia



From Pedersen-Bjergaard *et al.* (2006)

patients with essential trombocythemia (0.5–1%) and *polycythemia vera* (1–4%) (Abdulkarim *et al.*, 2009). Thus, benzene may first produce an MPD, which later transforms into AML.

An important role for epigenetic changes is also emerging in association with the development of leukaemia. Functional loss of the CCAAT/enhancer binding protein α (*C/EBPα*) (also known as CEBPA), a central regulatory transcription factor in the haematopoietic system, can result in a differentiation block in granulopoiesis and thus contribute to leukaemic transformation (Fröhling & Döhner, 2004). Recent work has shown that epigenetic alterations of *C/EBPα* occur frequently in AML and that *C/EBPα* mRNA is a target for miRNA-124a (Hackanson *et al.*, 2008). This miRNA is frequently silenced by epigenetic mechanisms in leukaemia cell lines. *C/EBPα* is also capable of controlling *miRNA-223* expression, which is vital in granulocytic differentiation (Fazi *et al.*, 2005). Altered expression of several miRNAs is also observed in some forms of AML (Dixon-McIver *et al.*, 2008; Marcucci *et al.*, 2008).

(b) Mechanisms of benzene-induced myeloid leukaemia development

There is strong evidence that benzene can induce AML via pathways I, II and IV, considerable supporting evidence for pathway V, some evidence for pathway III, but little information regarding pathways VI–VIII (see Fig. 4.2). Exposure to benzene has been associated with higher levels of the chromosomal changes commonly observed in AML, including 5q–/–5 or 7q–/–7, +8, and t(8;21) in the blood cells of highly exposed workers (Smith *et al.*, 1998; Zhang *et al.*, 1999, 2002). The benzene metabolite hydroquinone produces these same changes in cultured human cells, including cultures of CD34+ progenitor cells (Smith *et al.*, 2000; Stillman *et al.*, 2000). This provides strong evidence for the induction by benzene of AML via pathways I, II and IV (see Fig. 4.2).

Pathways III, IV and V are related to the inhibition of the DNA-related enzyme topoisomerase II, which is essential for the maintenance of proper chromosome structure and segregation; it removes knots and tangles from the genetic material by passing an intact double helix through a transient double-stranded break that it creates in a separate segment of DNA (McClendon & Osheroff, 2007; Bandele & Osheroff, 2009). To maintain genomic integrity during its catalytic cycle, topoisomerase II forms covalent bonds between active-site tyrosyl residues and the 5′-DNA termini created by cleavage of the double helix (Bandele & Osheroff, 2009). Normally, these covalent topoisomerase II-cleaved DNA complexes (known as cleavable complexes) are fleeting intermediates and are tolerated by the cell. However, when the concentration or longevity of cleavage complexes increases significantly, DNA double-strand breaks occur (Lindsey *et al.*, 2004). If topoisomerase II–induced double-strand breaks are incorrectly repaired, two unrelated (nonhomologous) chromosomes are fused together to produce translocations or inversions (Deweese & Osheroff, 2009).

There are different types of topoisomerase-II inhibitors. Epidophyllotoxins, such as etoposide, cause chromosome damage and kill cells by increasing physiological levels of topoisomerase II-DNA cleavage complexes (Baker *et al.*, 2001; Felix, 2001; Deweese & Osheroff, 2009). These drugs are referred to as topoisomerase-II poisons to distinguish them from catalytic inhibitors of the enzyme because they convert this essential enzyme to a potent cellular toxin. Other drugs, such as merbarone, act as inhibitors of topo-II activity but, in contrast to etoposide they do not stabilize topoisomerase II-DNA cleavable complexes. Nevertheless, they are potent clastogens both in vitro and in vivo (Wang *et al.*, 2007).

Several studies have shown that benzene in vivo, and its reactive metabolites hydroquinone

and 1,4-benzoquinone in vitro, inhibit the functionality of topoisomerase II and enhance DNA cleavage (Chen & Eastmond, 1995; Frantz *et al.*, 1996; Hutt & Kalf, 1996; Eastmond *et al.*, 2001; Fung *et al.*, 2004; Lindsey *et al.*, 2004, 2005; Whysner *et al.*, 2004). Bioactivation of hydroquinone by myeloperoxydase to 1,4-benzoquinone enhances topoisomerase-II inhibition (Eastmond *et al.*, 2005). Indeed, 1,4-benzoquinone was shown to be a more potent topoisomerase-II inhibitor than hydroquinone in a cell-free assay system (Hutt & Kalf, 1996; Baker *et al.*, 2001). These findings demonstrate that benzene through its reactive quinone metabolites can inhibit topoisomerase II and probably cause leukaemias with chromosome translocations and inversions known to be generated by topoisomerase-II inhibitors, including AMLs harbouring t(21q22), t(15;17) and inv(16) in a manner consistent with pathways IV and V (Andersen *et al.*, 2002; Voltz *et al.* 2004; Mistry *et al.*, 2005; Pedersen-Bjergaard *et al.*, 2007, 2008). The evidence for rearrangements of the mixed lineage leukaemia (*MLL*) gene through t(11q23) via pathway III in benzene-induced leukaemia is less convincing but may occur through an apoptotic pathway (Vaughan *et al.*, 2005).

AML can arise de novo via pathways VII and VIII without apparent chromosome abnormalities, but molecular analysis has revealed many genetic changes in these apparently normal leukemias, including mutations of *NPM1, AML1, FLT3, RAS* and *C/EBPα*. (Fig. 4.2; Cuneo *et al.*, 2002; Falini *et al.*, 2007; Mardis *et al.*, 2009). More work is needed to clarify the ability of benzene and its metabolites to produce mutations of the type found in these leukaemias, along with those found in Ph-negative MPDs such as Janus kinase 2 (*JAK2*), and somatic mutations in the ten-eleven translocation 2 (*TET2*) oncogene, which are found in about 15% of patients with various myeloid cancers (Delhommeau *et al.*, 2009). One potential mechanism for the induction of such mutations is through the generation of reactive oxygen species.

The ability of benzene and/or its metabolites to induce epigenetic changes related to the development of leukaemia, such as altered methylation status of *C/EBPα,* is unclear at this time. Bollati *et al.* (2007) reported that hypermethylation in *p15* (+0.35%; $P = 0.018$) and hypomethylation in the *MAGE-1* gene (encoding the human melanoma antigen) (–0.49%; $P = 0.049$) were associated with very low exposures to benzene (~22 ppb) in healthy subjects including gas-station attendants and traffic-police officers, although the corresponding effects on methylation were very low. Further study of the role epigenetics in the haematotoxicity and carcinogenicity of benzene is warranted, including studies of aberrant DNA methylation and altered microRNA expression.

While benzene and its metabolites are clearly capable of producing multiple forms of chromosomal mutation, including various translocations, deletions and aneuploidies, these are usually insufficient as a single event to explain the induction of leukaemia (Guo *et al.*, 2008; Lobato *et al.*, 2008). Other secondary events, such as specific gene mutations and/or other chromosome changes, are usually required (Guo *et al.*, 2008; Lobato *et al.*, 2008). Thus, benzene-induced leukaemia probably begins as a mutagenic event in the stem cell or progenitor cell and subsequent genomic instability allows for sufficient mutations to be acquired in a relatively short time. Studies have shown that the benzene metabolite hydroquinone is similar to ionizing radiation in that it induces genomic instability in the bone marrow of susceptible mice (Gowans *et al.*, 2005). Recent findings showing the importance of genes involved in DNA repair and maintenance – such as the *WRN* gene encoding the Werner syndrome protein – in determining genetic susceptibility to the toxicity of benzene also support this mechanism (Shen *et al.*, 2006; Lan *et al.*, 2009; Ren *et al.*, 2009).

Haematotoxic effects may also contribute to leukaemogenesis from benzene. Haematopoietic stem cells occupy an ordered environment in the bone marrow and interact with supportive stromal cells and mature lymphocytes. Haematotoxic damage to this ordered stem-cell microenvironment most likely allows for the clonal expansion of the leukaemic stem cells. This dual mode of action for benzene fits with the known ability of benzene metabolites to induce chromosomal mutations and genomic instability in blood stem cells and progenitor cells, and with the fact that haematotoxicity is associated with an increased risk for benzene-induced haematopoietic malignancies (Rothman *et al.*, 1997).

Thus, exposure to benzene can lead to multiple alterations that contribute to the leukaemogenic process. Benzene may act by causing chromosomal damage (aneuploidy, deletions and translocations) through inhibition of topoisomerase II, disruption of microtubules and other mechanisms; by generating oxygen radicals that lead to point mutations, strand breaks and oxidative stress; by causing immune system dysfunction that leads to decreased immunosurveillance (Cho, 2008; Li *et al.*, 2009); by altering stem-cell pool sizes through haematotoxic effects (Irons *et al.*, 1992); by inhibiting gap-junction intercellular communication (Rivedal & Witz, 2005); and by altering DNA methylation and perhaps specific microRNAs. This multimodal mechanism of action for benzene suggests that the effects of benzene on the leukaemogenic process are not singular and can occur throughout the process.

4.2.3 Potential mechanisms of benzene-induced acute lymphocytic leukaemia (ALL) development

Evidence of an association between exposure to benzene from air pollution and childhood leukaemia is growing. The most common form of childhood leukaemia is ALL, with AML being less common at around 15% of the incidence of ALL. The opposite is true for adults where the ratio is reversed, with AML being predominant. Reasons for this difference were suggested to be age-related defects in lymphopoiesis (Signer *et al.*, 2007). Studies with a murine model of chronic myeloid leukaemia – an adult-onset malignancy that arises from transformation of haematopoietic stem cells by the breakpoint cluster region-Ableson (*BCR-ABL*^{P210}) oncogene – demonstrated that young bone-marrow cells transformed with *BCR-ABL*^{P210} initiated both MPD and B-lymphoid leukaemia, whereas *BCR-ABL*^{P210}-transformed old bone-marrow cells recapitulated the human disease by inducing MPD with rare lymphoid involvement (Signer *et al.*, 2007). Thus, if benzene were to induce a leukaemia-related oncogenic mutation in young bone-marrow cells, it could produce either an MPD that transformed to AML, or a B-cell ALL, whereas exposure in an adult would have only a very limited chance of producing ALL.

The long-standing distinction between AML and ALL also has become somewhat blurred in recent years. Both forms of leukaemia arise in pluripotential stem cells or early progenitor cells in the bone marrow. Either disease can occur under conditions that formerly seemed restricted to AML. These include ALL occurring in the acute leukaemia seen in Down Syndrome (Kearney *et al.*, 2009); in secondary leukaemias related to chemotherapy (Lee *et al.*, 2009); and in the blast crisis of chronic myelogenous leukaemia (Calabretta & Perrotti, 2004). Similarly, the Philadelphia chromosome, long considered to be specific to chronic myelogenous leukaemia, is also the most common chromosome rearrangement in adult ALL (Ravandi & Kebriaei, 2009).

Since the genotoxic action of benzene metabolites on pluripotent precursor cells in the bone marrow appears promiscuous, producing multiple genetic abnormalities, it seems probable that exposure to benzene can initiate both AML and ALL by causing the chromosomal rearrangements

and mutations that are on the causal pathway to these malignancies. For childhood ALL and AML it has been shown that the disease is usually initiated *in utero*, since leukaemic translocations and other genetic changes have been detected in blood spots collected at birth (Wiemels *et al.*, 1999; Wiemels *et al.*, 2002; Greaves & Wiemels, 2003; McHale *et al.*, 2003). Thus, exposure of the mother, and perhaps even the father, to benzene could be just as important as exposure of the child in producing childhood AML and ALL, as has been suggested in several epidemiological studies (van Steensel-Moll *et al.*, 1985; McKinney *et al.*, 1991; Shu *et al.*, 1999; Scélo *et al.*, 2009). Supporting this hypothesis is an animal study demonstrating that *in utero* exposure to benzene increases the frequency of micronuclei and DNA recombination events in haematopoietic tissue of fetal and post-natal mice (Lau *et al.*, 2009). Another study showed that oxygen radicals play a key role in the development of *in utero*-initiated benzene toxicity through disruption of haematopoietic cell-signalling pathways (Badham & Winn, 2010). These studies support the idea that genotoxic and non-genotoxic events following exposure to benzene may be initiators of childhood leukaemia *in utero*.

4.2.4 Mechanisms of lymphoma development

(a) General

Lymphoma is a cancer of the immune system that includes over 40 malignant diseases originating from B- and T-lymphocytes and natural killer (NK) cells (Swerdlow *et al.*, 2008). It is therefore not surprising that functional disorders of immune-system cells are associated with a risk for malignant transformation. Immune deficiency is one of the strongest known risk factors for non-Hodgkin lymphoma (NHL) (Hartge & Smith, 2007). The risk for NHL increases with the degree of immune deficiency, and there is no evidence of a threshold (Grulich *et al.*, 2007). Thus, even modest immunosuppression, especially at the local level, may increase the risk for lymphoma.

It is well recognized that lymphomas, like other tumours, develop according to a multistep pathogenic process (Smith *et al.*, 2004). Clonal progression of an initiated cell to a clone of highly malignant cells is well documented. Natural selection of clones already present within oligoclonal expansions gives rise to true monoclonal lymphomas. Thus, it is possible to make generalizations about the type of molecular mechanism responsible for each of the stages involved in lymphomagenesis. For example, a cell may become initiated and genetically unstable through errors in recombination and DNA repair, which could be spontaneous or induced by an exogenous chemical agent. Other early molecular events often inhibit apoptosis and lead to the expansion of an intrinsically genetically unstable population of cells, which is at risk for additional genetic events and tumour progression. An example is the t(14;18) chromosome translocation associated with B-cell lymphoma 2 gene *BCL2* dysregulation, which inhibits apoptosis (Cimmino *et al.*, 2005; Thomadaki & Scorilas, 2006). Normally, one of the key protectors against the selection and progression of malignant clones of cells into full-blown lymphoma is local immunosurveillance in which activated T-cells kill the mutated clones. It is generally accepted that if this immunosurveillance is no longer intact, e.g. in immuno-suppressed individuals, then the malignant cells divide and grow rapidly, collecting more mutations to become aggressive, rapidly growing tumours.

(b) Mechanisms of benzene-induced lymphoma development

From the discussion above, there are at least two probable mechanisms by which exposure to benzene could enhance the incidence of lymphoma, i.e. by inducing chromosome rearrangements associated with NHL, and through

immunosuppression leading to decreased immunosurveillance.

Benzene is well known to produce multiple cytogenetic abnormalities in lymphocytes (Tough & Brown, 1965; Forni, 1971, 1979; Picciano, 1979; Smith & Zhang, 1998; Zhang *et al.*, 2002). Further, benzene induces specific chromosomal changes associated with NHL in human lymphocytes (Zhang *et al.*, 2007). Fluorescence in situ hybridization (FISH) analysis showed increased levels of t(14;18) and del(6q) in benzene-exposed workers, but the higher levels of t(14;18) could not be confirmed in a follow-up study by use of real time-PCR (polymerase chain reaction) (McHale *et al.*, 2008). This may be because the PCR method only detected 50% of t(14;18) translocations or that the FISH method detects non-functional as well as functional translocations. Reduced immunosurveillance is another potential mechanism of NHL induction by benzene. The importance of T-cell immunosurveillance in preventing B-cell neoplasia is well established and is carried out by activated cytotoxic T lymphocytes. The toxic effects of benzene on T-cells is well documented and there appears to be a selective effect on $CD4^+$ T-lymphocytes resulting in a lowering of the $CD4^+/CD8^+$ ratio (Lan *et al.*, 2004). This immunosuppressive pattern is similar to the early onset of acquired immuno-deficiency syndrome (AIDS), and although it is not as severe it may be associated with an increased risk for NHL (Grulich *et al.*, 2007). Thus, benzene, like other leukaemogens including alkylating agents, topoisomerase inhibitors, and ionizing radiation, may cause NHL through a combination of immunosuppression and DNA double-strand break induction that leads to illegitimate recombination and chromosome rearrangements in lymphoid cells.

Thus, the biological plausibility of benzene as a cause of lymphoproliferative disorders has been strengthened in recent years. There are additional studies demonstrating that benzene produces lymphomas in laboratory animals, and a recent study showing that it does so simultaneously with AML in *Tp53*-deficient mice (Kawasaki *et al.*, 2009). Multiple studies show that it produces genotoxicity in the lymphocytes of exposed humans. Accordingly, there is considerable support for the notion that it is biologically plausible for benzene to cause human lymphatic tumours.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of benzene. Benzene causes acute myeloid leukaemia/acute non-lymphocytic leukaemia.

Also, a positive association has been observed between exposure to benzene and acute lymphocytic leukaemia, chronic lymphocytic leukaemia, multiple myeloma, and non-Hodgkin lymphoma.

There is *sufficient evidence* for the carcinogenicity of benzene in experimental animals.

There is strong evidence that benzene metabolites, acting alone or in concert, produce multiple genotoxic effects at the level of the pluripotent haematopoietic stem cell resulting in chromosomal changes in humans consistent with those seen in haematopoietic cancer. In multiple studies in different occupational populations in many countries over more than three decades a variety of genotoxic changes, including chromosomal abnormalities, have been found in the lymphocytes of workers exposed to benzene.

Benzene is *carcinogenic to humans (Group 1).*

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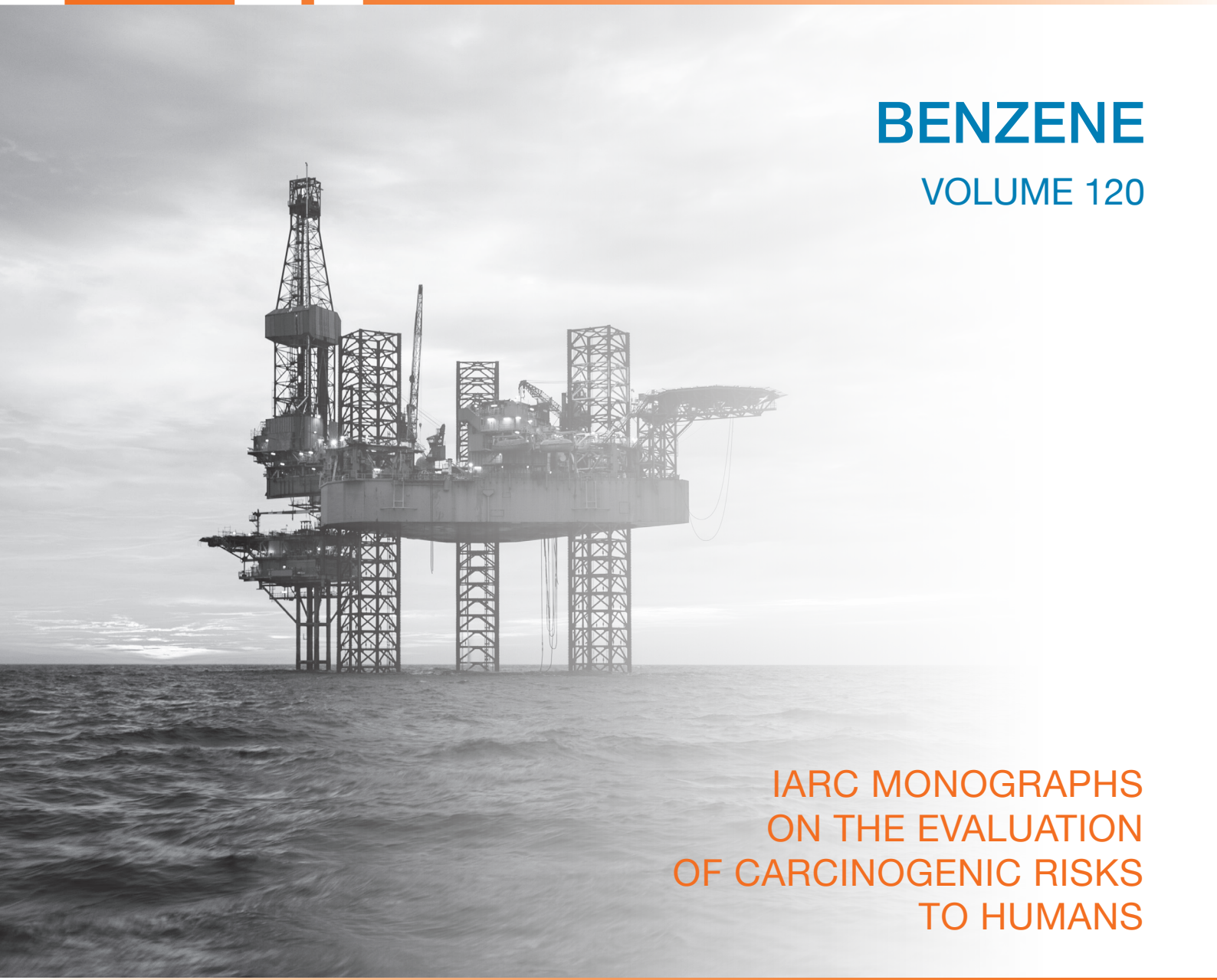
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IARC MONOGRAPHS



BENZENE

VOLUME 120

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS



BENZENE

VOLUME 120

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 10–17 October 2017

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in carcinogenesis and related fields; and to indicate where additional research efforts are needed. The lists of IARC evaluations are regularly updated and are available on the Internet at http://monographs.iarc.fr/.

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NOTE TO THE READER

The term 'carcinogenic risk' in the *IARC Monographs* series is taken to mean that an agent is capable of causing cancer. The *Monographs* evaluate cancer hazards, despite the historical presence of the word 'risks' in the title.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a *Monograph* does not mean that it is not carcinogenic. Similarly, identification of cancer sites with *sufficient evidence* or *limited evidence* in humans should not be viewed as precluding the possibility that an agent may cause cancer at other sites.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the IARC Monographs Group, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the *Monographs* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the IARC Monographs Group, so that corrections can be reported in future volumes.

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[8] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a *Monograph*, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[9] Richard H. Adamson attends as an Observer for the American Beverage Association.
[10] Peter J. Boogaard is employed by Shell International and is also a participant of the Global Employee Share Purchase Programme of Royal Dutch Shell. He attended as an Observer for the Aromatics Producers Association, Belgium.
[11] Alex Forrest attended as an Observer for the Manitoba Professional Firefighters Association, Canada.
[12] Tom K. Grimsrud attended as an Observer for the Cancer Registry of Norway.
[13] Sean Hays consulted for a confidential oil company and consults for a confidential tobacco company (amount not disclosed). His company, Summit Toxicology, serves in litigation involving petroleum products and benzene. He attended as an Observer for the American Petroleum Institute.
[14] Peter F. Infante has served as an expert witness for plaintiffs in litigation involving benzene. He attended as an Observer for Peter F. Infante Consulting, L.L.C.
[15] Veli-Pekka Parkkinen attended as an Observer for the University of Kent, United Kingdom.

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PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a *Monograph* or list of evaluations.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to summarize adequately the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this topic. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended '... that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.' The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. In 1988, the phrase 'of chemicals' was dropped from the title, which assumed its present form, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Through the *Monographs* programme, IARC seeks to identify the causes of human cancer. This is the first step in cancer prevention, which is needed as much today as when IARC was established. The global burden of cancer is high and continues to increase: the annual number of new cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 (Stewart & Kleihues, 2003). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monographs* evaluations, national health agencies have been able, on scientific grounds, to take measures to reduce human exposure to carcinogens in the workplace and in the environment.

The criteria established in 1971 to evaluate carcinogenic risks to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad hoc Advisory Groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991; Vainio et al., 1992; IARC, 2005, 2006).

The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The procedures through which a Working Group implements these principles are not specified in detail. They usually involve operations that have been established as being effective during previous *Monograph* meetings but remain, predominantly, the prerogative of each individual Working Group.

2. Objective and scope

The objective of the programme is to prepare, with the help of international Working Groups of experts, and to publish in the form of *Monographs*, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

In this Preamble, the term 'agent' refers to any entity or circumstance that is subject to evaluation in a *Monograph*. As the scope of the programme has broadened, categories of agents now include specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioural practices, biological organisms and physical agents. This list of categories may expand as causation of, and susceptibility to, malignant disease become more fully understood.

A cancer 'hazard' is an agent that is capable of causing cancer under some circumstances, while a cancer 'risk' is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The *Monographs* are an exercise in evaluating cancer hazards, despite the historical presence of the word 'risks' in the title. The distinction between hazard and risk is important, and the *Monographs* identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

In the *Monographs*, an agent is termed 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Part B, Section 3a) contribute to the judgement that the agent is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

The Preamble continues the previous usage of the phrase 'strength of evidence' as a matter of historical continuity, although it should be understood that *Monographs* evaluations consider studies that support a finding of a cancer hazard as well as studies that do not.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991; Vainio et al., 1992; IARC, 2005, 2006; see also Part B, Sections 4 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged

on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose–response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose–response relationship. A *Monograph* may undertake to estimate dose–response relationships within the range of the available epidemiological data, or it may compare the dose–response information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate Working Group with expertise in quantitative dose–response assessment.

The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning preventive measures, provide effective cancer control programmes and decide among alternative options for public health decisions. The evaluations of IARC Working Groups are scientific, qualitative judgements on the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country and relate to many factors, including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments or other international organizations.

3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme web site (http://monographs.iarc.fr). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data become available on an agent for which a *Monograph* exists, a re-evaluation may be made at a subsequent meeting, and a new *Monograph* published. In some cases it may be appropriate to review only the data published since a prior evaluation. This can be useful for updating a database, reviewing new data to resolve a previously open question or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Part B, Section 6) are more appropriately addressed by a full review.

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section 4). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to epidemiological studies, cancer bioassays, and mechanistic and other relevant data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government agency reports that are publicly available are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Exposure data and other information on an agent under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, published and unpublished sources of information may be considered.

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5. Meeting participants

Five categories of participant can be present at *Monograph* meetings.

(a) The Working Group

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

(b) Invited Specialists

Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues in the section on exposure, such as a general description of data on production and use (see Part B, Section 1). Invited Specialists do not serve as meeting chair

or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) Representatives of national and international health agencies

Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations.

(d) Observers with relevant scientific credentials

Observers with relevant scientific credentials may be admitted to a meeting by IARC in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings (available at http://monographs.iarc.fr).

(e) The IARC Secretariat

The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables and analyses.

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume (Cogliano et al., 2004).

The names and principal affiliations of participants are available on the *Monographs* programme web site (http://monographs.iarc.fr) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano et al., 2005).

All participants are listed, with their principal affiliations, at the beginning of each volume. Each participant who is a Member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year in advance of the meeting of a Working Group, the agents to be reviewed are announced on the *Monographs* programme web site (http://monographs.iarc.fr) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as PubMed. Meeting participants who are asked to prepare

preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, before the meeting, to Working Group Members and Invited Specialists for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the working papers, develop a joint subgroup draft and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days, the Working Group meets in plenary session to review the subgroup drafts and develop the evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections.

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

After the meeting, the master copy is verified by consulting the original literature, edited and prepared for publication. The aim is to publish the volume within six months of the Working Group meeting. A summary of the outcome is available on the *Monographs* programme web site soon after the meeting.

B. SCIENTIFIC REVIEW AND EVALUATION

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The scope of the *IARC Monographs* programme has expanded beyond chemicals to include complex mixtures, occupational exposures, physical and biological agents, lifestyle factors and other potentially carcinogenic exposures. Over time, the structure of a *Monograph* has evolved to include the following sections:

Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and some key issues the Working Group encountered during the meeting.

This part of the Preamble discusses the types of evidence considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations.

1. Exposure data

Each *Monograph* includes general information on the agent: this information may vary substantially between agents and must be adapted accordingly. Also included is information on production and use (when appropriate), methods of analysis and detection, occurrence, and sources and routes of human occupational and environmental exposures. Depending on the agent, regulations and guidelines for use may be presented.

(a) General information on the agent

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host response and clinical disease other than cancer are also presented.

For physical agents that are forms of radiation, energy and range of the radiation are included. For foreign bodies, fibres and respirable particles, size range and relative dimensions are indicated.

For agents such as mixtures, drugs or lifestyle factors, a description of the agent, including its composition, is given.

Whenever appropriate, other information, such as historical perspectives or the description of an industry or habit, may be included.

(b) Analysis and detection

An overview of methods of analysis and detection of the agent is presented, including their sensitivity, specificity and reproducibility. Methods widely used for regulatory purposes are emphasized. Methods for monitoring human exposure are also given. No critical evaluation or recommendation of any method is meant or implied.

(c) Production and use

The dates of first synthesis and of first commercial production of a chemical, mixture or other agent are provided when available; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided when available. In addition, methods of synthesis used in past and present commercial production and different methods of production,

which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d) Occurrence and exposure

Information on the occurrence of an agent in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, plants, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases.

Data that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with date and place. For biological agents, the epidemiology of infection is described.

(e) Regulations and guidelines

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccination and therapy, are described.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part A, Section 4). Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

(a) Types of study considered

Several types of epidemiological study contribute to the assessment of carcinogenicity in humans — cohort studies, case–control studies, correlation (or ecological) studies and intervention studies. Rarely, results from randomized trials may be available. Case reports and case series of cancer in humans may also be reviewed.

Cohort and case–control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the subsequent decrease in risk for lung cancer.

In correlation studies, the units of investigation are usually whole populations (e.g. in

particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports and case series usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure.

The uncertainties that surround the interpretation of case reports, case series and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, these types of study may add materially to the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an agent and disease. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to several aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

First, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Second, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than

those with national rates. Internal comparisons of frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

Third, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well conducted analyses can be considered. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis) and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) (Greenland, 1998).

The advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, procedures of data collection, methods of measurement and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about the potential carcinogenicity of agents.

IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monograph* (see Part A, Section 4). Additionally, as a means of gaining insight from the results of multiple individual studies, ad hoc calculations that combine data from different studies may be conducted by the Working Group during the course of a *Monograph* meeting. The results of such original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important that the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

(d) Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and

time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes (IARC, 1991; Vainio et al., 1992; Toniolo et al., 1997; Vineis et al., 1999; Buffler et al., 2004). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses, of individual susceptibility or host responses, and inference of a mechanism (see Part B, Section 4b). This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites that are thought to be the basis of susceptibility may provide evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons that arise from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f) Criteria for causality

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or that use different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in exposure), and results of studies that are judged to be of high quality are given more weight than those of studies that are judged to be methodologically less sound.

If the risk increases with the exposure, this is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of

multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first that the studies meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of effect of unity for any observed level of exposure, (b) when considered together, provide a pooled estimate of relative risk that is at or near to unity, and (c) have a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn et al., 1986; Tomatis et al., 1989). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio et al., 1995). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (see Part A, Section 4). In all experimental settings, the nature and extent of impurities or contaminants present in the agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, route of exposure, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported. Those studies in experimental animals that are judged to be irrelevant to the evaluation or judged to be inadequate

(e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. OECD, 2002).

Other studies considered may include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); studies in which the end-point was not cancer but a defined precancerous lesion; experiments on the carcinogenicity of known metabolites and derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For studies of mixtures, consideration is given to the possibility that changes in the physicochemical properties of the individual substances may occur during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose–response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar in structure or of a similar virus genus) to that being evaluated is also considered. Such results may provide biological and mechanistic information that is relevant to the understanding of the process of carcinogenesis in humans and may strengthen the biological plausibility that the agent being evaluated is carcinogenic to humans (see Part B, Section 2f).

(a) Qualitative aspects

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age and duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

Considerations of importance in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence (Huff et al., 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo transition to malignancy, the agent

should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship (Hoel et al., 1983; Gart et al., 1986), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto et al., 1980; Gart et al., 1986; Portier & Bailer, 1989; Bieler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed (Sherman et al., 1994; Dunson et al., 2003).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from a given experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of

historical controls, particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals (Haseman et al., 1984; Fung et al., 1996; Greim et al., 2003).

Although meta-analyses and combined analyses are conducted less frequently for animal experiments than for epidemiological studies due to differences in animal strains, they can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

4. Mechanistic and other relevant data

Mechanistic and other relevant data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of the mechanistic and other relevant data depends on the biological activity of the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important; thus, not every available study is cited. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive; thus, the same studies may be discussed in more than one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

(a) Toxicokinetic data

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect dose–response relationships include uptake, deposition, biopersistence and half-life in tissues, protein binding, metabolic activation and detoxification. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be important for the extrapolation of hazards between species and in clarifying the role of in-vitro findings.

(b) Data on mechanisms of carcinogenesis

To provide focus, the Working Group attempts to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to gaps in the data and to data that suggests that more than one mechanism may be operating. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems. Changes in the affected organs, tissues or cells

can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of potentially adverse physiological changes include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal hormones and changes in immune surveillance.

(ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities of enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in cyclin-dependent kinases that govern cell cycle progression, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in the secretion of factors related to the stimulation of DNA replication and transcription and changes in gap–junction-mediated intercellular communication.

(iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis is given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal aberration/aneuploidy to carcinogenesis (Vainio et al., 1992; McGregor et al., 1999). The adequacy of the reporting of sample characterization is considered and, when necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The concentrations employed are given, and mention is made of whether the use of an exogenous metabolic system in vitro affected the test result. These data are listed in tabular form by phylogenetic classification.

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap–junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests

have been published (Montesano et al., 1986; McGregor et al., 1999).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals in vivo indicates that it may have carcinogenic activity. Negative results in tests for mutagenicity in selected tissues from animals treated in vivo provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) (Vainio et al., 1992). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere (Montesano et al., 1986; McGregor et al., 1999).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression, and formation of calculi and other deposits that cause chronic irritation), that evidence is presented and reviewed critically in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. Capen et al., 1999).

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may include descriptions of the pathology of infection, integration and expression of viruses, and genetic alterations seen in human tumours. Other observations that might comprise cellular and tissue responses to infection, immune response and the presence of tumour markers are also considered.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. 'Physical agents' may also be considered to comprise foreign bodies, such as surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(c) Other data relevant to mechanisms

A description is provided of any structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from testing hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret changes in individual end-points (e.g. changes in expression in one gene) without considering the consistency of that finding in the broader context of the other end-points (e.g. other genes with linked transcriptional control). High-output data can be used in assessing mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data, where the number of observations far exceeds the number of end-points measured, their utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem

plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) Susceptibility data

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on toxicokinetics, mechanisms of carcinogenesis and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in-vivo and in-vitro laboratory studies to humans.

(e) Data on other adverse effects

Data on acute, subchronic and chronic adverse effects relevant to the cancer evaluation are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is judged by the same criteria as those applied to epidemiological studies of cancer, but fewer details are given.

5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be found on the *Monographs* programme web site (http://monographs.iarc.fr).

(a) Exposure data

Data are summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given to compare exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

(b) Cancer in humans

Results of epidemiological studies pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) in which an increase in cancer was observed is identified. Dose–response and other quantitative data may be summarized when available.

(c) Cancer in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species, study design and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also mentioned. Negative findings, inverse relationships, dose–response and other quantitative data are also summarized.

(d) Mechanistic and other relevant data

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and the possible mechanism(s) of carcinogenesis (e.g. genetic toxicity, epigenetic effects) are summarized. In addition, information on susceptible individuals, populations and life-stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data that are considered to be important.

6. Evaluation and rationale

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms. The strength of the mechanistic evidence is also characterized.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

An evaluation of the degree of evidence is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of the degree of evidence.

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:**

The Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

***Limited evidence of carcinogenicity*:**

A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence of carcinogenicity*:**

The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

***Evidence suggesting lack of carcinogenicity*:**

There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative

risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:**

The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

***Limited evidence of carcinogenicity*:**

The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

***Inadequate evidence of carcinogenicity*:**

The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

***Evidence suggesting lack of carcinogenicity*:**

Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) Mechanistic and other relevant data

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and toxicokinetics, physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is probably carcinogenic to humans.

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is possibly carcinogenic to humans.

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed,

especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is probably not carcinogenic to humans.

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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GENERAL REMARKS

This one-hundred-and-twentieth volume of the *IARC Monographs* presents an evaluation of the carcinogenic hazard to humans of exposure to benzene.

The conclusions of this volume represent the sixth evaluation of the carcinogenicity of benzene by an *IARC Monographs* Working Group. Successive evaluations published in Volumes 7 (IARC, 1974), 29 (IARC, 1982), and 100F (IARC, 2012) and Supplements 1 (IARC, 1979) and 7 (IARC, 1987) considered progressively larger and more complex volumes of data and yielded consistent, yet steadily broader and more compelling, conclusions about the carcinogenicity of benzene.

The available data were sparse at the time of the first evaluation (IARC, 1974). The Working Group determined that the available evidence from studies of experimental animals did not permit a conclusion to be drawn, but found suggestive evidence from epidemiological case reports and one case–control study that benzene causes leukaemia in humans; the current system of formal classifications of evidence had not yet been introduced at that time (it was introduced in Volume 17). Benzene was reviewed again in Supplement 1, which updated Volumes 1–20. With formal classifications then in place, the evidence in experimental animals was found to be *inadequate*, and the human epidemiological evidence, now supplemented by several occupational cohort studies and case–control studies in addition to case reports, was found to be *sufficient*. In the overall evaluation, benzene was found to be *carcinogenic to humans* (Group 1), a finding that has stood since that time.

Additional data had become available when benzene was reviewed again for Volume 29 (IARC, 1982). The Working Group now found the evidence in experimental animals to be *limited* and concluded that the modestly expanded epidemiological evidence established a causal relationship between exposure to benzene and development of acute myeloid leukaemia.

With further growth of the database during the 1980s, the evidence in experimental animals was found to be *sufficient* when benzene was evaluated again for Supplement 7 (IARC, 1987). Although mechanistic evidence was not yet formally incorporated into overall evaluations at that time, induction of chromosomal aberrations in exposed humans and of chromosomal aberrations and micronuclei in rodents was also noted in the summary report.

The volume of evidence had grown substantially larger and more complex by 2009, when the evaluation of benzene was updated for Volume 100F (IARC, 2012). The Working Group confirmed the previous findings of *sufficient evidence* of carcinogenicity in humans and experimental animals and, for the first time, presented *strong evidence* of multiple genotoxic effects based on a review of extensive mechanistic data. In humans, the Working Group concluded

that benzene causes acute myeloid leukaemia/acute non-lymphocytic leukaemia (both terms were used in epidemiological studies reviewed in that volume) and found *limited* evidence that benzene causes acute lymphocytic leukaemia, chronic lymphocytic leukaemia, non-Hodgkin lymphoma, and multiple myeloma.

The current evaluation was undertaken with two principal goals: (i) to incorporate new epidemiological and experimental evidence, including a large number of mechanistic studies in exposed humans, and (ii) to assess quantitative exposure–response relationships of exposure to benzene with both human cancer risks and relevant biological end-points in exposed humans. Such quantitative evaluations were recommended as an adjunct to future *Monographs* by an Advisory Group on quantitative risk characterization (IARC, 2014).

In the current evaluation, the Working Group again confirmed the carcinogenicity of benzene based on *sufficient evidence* of carcinogenicity in humans, *sufficient evidence* of carcinogenicity in experimental animals, and *strong* mechanistic evidence. The Working Group's evaluation of the accumulated evidence from human epidemiological studies focused on studies in which occupational or environmental exposure to benzene was specifically identified. The findings fully supported the previous conclusion that benzene causes acute non-lymphocytic leukaemia – including acute myeloid leukaemia – in adults, as well as the previous observations of *limited evidence* for chronic lymphocytic leukaemia, non-Hodgkin lymphoma, and multiple myeloma. On the basis of new data available since the last review, the Working Group also found *limited* evidence that benzene causes chronic myeloid leukaemia and lung cancer, and acute myeloid leukaemia in children. The Working Group's review of the large body of mechanistic studies took into account the key characteristics of carcinogens (Smith et al., 2016). The Working Group affirmed the *strong evidence* that benzene is genotoxic, and found that it also exhibits many other key characteristics of carcinogens, including in exposed humans. In particular, benzene is metabolically activated to electrophilic metabolites; induces oxidative stress and associated oxidative damage to DNA; is genotoxic; alters DNA repair or causes genomic instability; is immunosuppressive; alters cell proliferation, cell death, or nutrient supply; and modulates receptor-mediated effects.

The evidence reviewed for this evaluation, the Working Group's conclusions, and their analysis of exposure–response relationships are detailed in this volume. A summary of the key findings has appeared in *The Lancet Oncology* (Loomis et al., 2017).

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1. EXPOSURE DATA

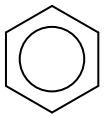
1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 71-43-2
Primary name: benzene
IUPAC systematic name: benzene

1.1.2 Structural and molecular formulae, and relative molecular mass

Structural formula:



From O'Neil (2006) and Lide (2008)

Molecular formula: C_6H_6
Relative molecular mass: 78.1

1.1.3 Chemical and physical properties of the pure substance

From HSDB (2018)

Description: clear, colourless, volatile, highly flammable liquid

Boiling point: 80.1 °C

Melting point: 5.558 °C

Density: 0.8756 g/cm^3

Refractive index: 1.5011 at 20 °C

Solubility: slightly soluble in water (1.8 g/L at 25 °C); miscible with acetic acid, acetone, chloroform, ethyl ether, and ethanol

Viscosity: 0.604 mPa at 25 °C

Vapour pressure: 94.8 mmHg at 25 °C

Stability: benzene is a very stable molecule due to its aromaticity, that is, the delocalization of pi electrons in the benzene molecule creating a resonance; catalysts are often needed to make benzene undergo a chemical reaction; benzene is volatile with a boiling point of 80 °C, and is highly flammable

Flash point: –11.1 °C

Octanol/water partition coefficient: log K_{ow}, 2.13; conversion factor (20 °C, 101 kPa): 1 ppm = 3.19 mg/m^3.

1.1.4 Technical products and impurities

The impurities found in commercial products are toluene, xylene, phenol, thiophene, carbon disulfide, acetylnitrile, and pyridine. Thiophene-free benzene has been specially treated to avoid destroying the catalysts used in reactions with benzene. Refined nitration-grade benzene is free of hydrogen sulfide and sulfur dioxide (HSDB, 2018).

1.2 Production and use

1.2.1 Production

(a) Production process

Benzene was first isolated by Faraday in 1825 from a liquid condensed by compressing oil gas; Mitscherlich first synthesized it in 1833 by distilling benzoic acid with lime. Benzene was first recovered commercially from light oil derived from coal tar in 1849, and from petroleum in 1941 (IARC, 1982).

Benzene can be produced in several ways. One method is by catalytic reforming, which involves the dehydrogenation of cycloparaffins, dehydroisomerization of alkyl cyclopentanes, and the cyclization and subsequent dehydrogenation of paraffins. The feed to the catalytic reformer (platinum-rhenium on an alumina support of high surface area) for benzene is thermally cracked naphtha cut at 71–104 °C. The benzene product is most often recovered from the reformate by solvent extraction techniques (Fruscella, 2002).

Benzene can also be prepared by cracking, a multistep process where crude oil is heated, steam is added, and the gaseous mixture is then briefly passed through a furnace at temperatures of 700–900 °C. The dissolved compounds undergo fractional distillation, which separates out the different components, including benzene (Fruscella, 2002).

Alternatively, benzene can be prepared from toluene by hydrodealkylation. In the presence of a catalyst (chromium, molybdenum, and/or platinum), toluene and hydrogen are compressed to pressures of 20–60 atmospheres and the mixture is heated to temperatures of 500–660 °C. This reaction converts the mixture to benzene and methane, and benzene is separated out by distillation (Fruscella, 2002).

(b) Production volume

Benzene is listed as a high production volume chemical by the Organisation for Economic Co-operation and Development (OECD, 2009). In 2012, global benzene production was approximately 42.9 million tonnes. In the USA, production volumes during 1986–2002 were more than 1 billion pounds [> 450 000 tonnes] (HSDB, 2018). In order of volume produced, the five countries producing the greatest quantities of benzene in 2012 were China, the USA, the Republic of Korea, Japan, and Germany (Merchant Research & Consulting Ltd, 2014). In 2014, the industry reported benzene production and consumption in western Europe (Germany, Belgium, France, Italy, Luxembourg, the Netherlands, Denmark, Ireland, the United Kingdom, Greece, Spain, Portugal, Austria, Finland, and Sweden – the EU-15 – plus Norway and Switzerland) of 6.7 and 7.5 million tonnes, respectively (PetroChemicals Europe, 2015).

The use of benzene for the production of ethylbenzene, cumene, cyclohexane, and nitrobenzene accounts for 90% of annual benzene consumption. In order of volume consumed, China, the USA, and western Europe consume about half of the total benzene produced (IHS Markit, 2017).

The United States Environmental Protection Agency (EPA) report published in February 2017 (Report No. 17-P-0249) reports a total benzene consumption of 57 701 737 237 gallons (equivalent to 1.9×10^8 tonnes; 1 gallon = 3.7858 L, benzene density of 0.879 g/cm^3) for 84 facilities in the USA in 2014 (EPA, 2017).

1.2.2 Uses

Historically, benzene was used as a degreaser of metals, a solvent for organic materials, a starting and intermediate material in the chemical and drug industries (e.g. to manufacture rubbers, lubricants, dyes, detergents, and pesticides), and an additive to unleaded gasoline

(ATSDR, 2007; Williams et al., 2008; NTP, 2016). Benzene use has diminished since its carcinogenic properties became widely publicized (IARC, 1982); however, some countries have continued to use benzene in specific products such as glue (Vermeulen et al., 2004).

Benzene occurs naturally in petroleum products (e.g. crude oil and gasoline), and is also added to unleaded gasoline for its octane-enhancing and anti-knock properties. Typically, the concentration of benzene in these fuels is 1–2% by volume (ATSDR, 2007). Benzene concentration in fuels sold in the European Union must be less than 1.0% by volume (European Commission, 2009).

The percentage of benzene in gasoline has varied with the refinery and time period from which it originated. Until 1931, the benzene content of the gasoline imported into the United Kingdom was 1% v/v (Lewis et al., 1997). In 1971, Parkinson reported that gasoline in the United Kingdom contained 2.8–5.8% benzene v/v (Parkinson, 1971). In Canada in the 1970s and the 1980s, benzene content in fuel was reported as 0.7–3.7% (Armstrong et al., 1996); in Australia, benzene content of 1–5% by weight during 1950–1990 was reported (Glass et al., 2000).

Gasoline can be enriched with benzene by adding benzene-toluene-xylene, which is generated during coke making. Where necessary, sidestream petroleum is added to adjust the octane rating; for example, reformate includes 5–12% benzene (Glass et al., 2000). Before 1950, a small proportion of gasoline enriched with benzene sold in the United Kingdom included up to 36% benzene (Lewis et al., 1997). Gasoline enriched with benzene included up to approximately 10% benzene in Canada during 1914–1938 (Armstrong et al., 1996) and in Australia until around 1970 (Glass et al., 2000).

The primary use of benzene today is in the manufacture of organic chemicals. In Europe, benzene is mainly used to make styrene, phenol, cyclohexane, aniline, maleic anhydride, alkylbenzenes, and chlorobenzenes. It is an intermediate in the production of anthraquinone, hydroquinone, benzene hexachloride, benzene sulfonic acid, and other products used in drugs, dyes, insecticides, and plastics (ICIS, 2010). In the USA, the primary use of benzene is in the production of ethylbenzene, accounting for 52% of the total benzene demand in 2008. Most ethylbenzene is consumed in the manufacture of styrene, which is used in turn in polystyrene and various styrene copolymers, latexes, and resins. The second-largest use of benzene in the USA (accounting for 22% of demand) is in the manufacture of cumene (isopropylbenzene), nearly all of which is consumed in phenol production. Benzene is also used to make chemical intermediates, including cyclohexane, used in making certain nylon monomers (15%); nitrobenzene, an intermediate for aniline and other products (7%); alkylbenzene, used in detergents (2%); chlorobenzenes, used in engineering polymers (1%); and miscellaneous other uses (1%) (Kirschner, 2009).

1.3 Measurement and analysis

1.3.1 Detection and quantification

Common standard methods to assay benzene in air are presented in Table 1.1, along with selected methods for measuring some biomarkers of exposure in urine.

Assays to monitor benzene in air were first developed to measure air concentration in the workplace, including personal exposure of workers, and to assess compliance with occupational limits. Typically, to measure 8-hour exposure, air is pumped through cartridges containing charcoal or other suitable sorbents for the duration of the entire work shift. In the laboratory, benzene is desorbed from sorbent using solvents such as carbon disulfide (NIOSH, 2003, method 1501) or high-temperature thermal desorption (NIOSH, 1996, method 2549), and analysed with either a gas chromatograph equipped with a flame ionization

Table 1.1 Representative methods for the analysis of benzene in air and its main urinary biomarkers

Sample matrix	Analyte	Assay procedure	Limit of detection	Reference
Air	Benzene	Pumping air through solid sorbent tube, solvent desorption, and GC-FID	0.5 µg/sample (sample volume 5–30 L)	NIOSH (2003), method 1501
	Benzene	Pumping air through solid sorbent tube, thermal desorption, and GC-MS	100 ng per tube or less (sample volume 1–6 L)	NIOSH (1996), method 2549
	Benzene	Real-time monitor with FTIR detector	0.32 ppm for a 10 m absorption pathlength	NIOSH(2002), method 3800
	Benzene	Portable GC-PID	0.02 ppm	NIOSH (1994), method 3700
	Benzene	Passive sampling [with solid sorbent device], solvent/thermal desorption, and GC-MS	Variable depending on geometry of sampler and sampling time	EPA (2014)
Urine	t,t-MA	HPLC-UV analysis	5 µg/L	Lee et al. (2005)
	SPMA	SPE LC-MS/MS analysis	0.2 µg/L	NIOSH (2014), method 8326
	Benzene	HS GC-MS analysis	0.025 µg/L	Fustinoni et al. (1999)

FTIR, Fourier-transform infrared spectroscopy; FID, flame ionization detection; GC, gas chromatography; HPLC-UV, high-pressure liquid chromatography, ultraviolet spectroscopy; HS, head space; LC-MS/MS, liquid chromatography, tandem mass spectrometry; MS, mass spectrometry; PID, photoionization detector; SPE, solid phase extraction; SPMA, *S*-phenylmercapturic acid; t,t-MA, *trans*,*trans*-muconic acid

detector (NIOSH, 2003, method 1501) or a mass spectrometer (NIOSH, 1996, method 2549). As an alternative, passive samplers do not need a pump and allow benzene sampling via air diffusion through them; see EPA (2014) for a review of different assays using passive samplers for the determination of volatile organic compounds, including benzene. The sensitivity of both active and passive assays depends on sample volume, desorption method, and instrumental analysis; a higher sampling volume, the use of thermal desorption, and detection by mass spectrometer are associated with greater sensitivity (detection by mass spectrometer also offers high specificity). The design determines the sampling rate for passive samplers; radial geometry warrants a high flow rate and therefore larger sampling volume over a specific sampling time (Cocheo et al., 2000).

A real-time monitor can be used to check for benzene leaks and to measure short-term exposure, especially during critical operations, allowing the simultaneous sampling of air and detection of benzene. Benzene can be separated from other chemicals by portable gas chromatography and detected by photoionization detector (NIOSH, 1994, method 3700), or can be measured by extractive Fourier-transform infrared spectrometry (NIOSH, 2002, method 3800).

The alternative method of measuring benzene exposure by biomonitoring dates to the 1980s (Lauwerys, 1983); the first biomarkers, such as phenol, have been progressively abandoned in favour of biomarkers that are less abundant but more specific. The currently recommended biomarkers for assessment of benzene exposure in the workplace include urinary *trans*,*trans*-muconic acid (t,t-MA), urinary *S*-phenylmercapturic acid (SPMA), and urinary benzene (INRS, 2017).

t,t-MA is a urinary metabolite of benzene accounting for about 4% of the absorbed dose. Formed and excreted in urine with rapid kinetics with a half-life of about 5 hours (Boogaard & van

Sittert, 1995), it is useful for assessment of recent exposure. It is measured using high-performance liquid chromatography with an ultraviolet detector (Lee et al., 2005), and standardized assays are present on the market. Its limitation is poor specificity, as t,t-MA is also produced by the metabolism of the preservative sorbic acid or sorbates contained in food and beverages (Ruppert et al., 1997; Weaver et al., 2000). t,t-MA is recommended when exposure is higher than 0.2 ppm (Kim et al., 2006a), depending on the amount of sorbic acid preservatives in the diet.

SPMA is a urinary metabolite of benzene accounting for less than 1% of the absorbed dose; it is formed and excreted in urine with rapid kinetics (half-life of ~9 hours; Boogaard & van Sittert, 1995). SPMA in urine is a specific biomarker, and is assayed using solid phase extraction followed by liquid chromatography coupled with tandem mass spectrometry (NIOSH, 2014, method 8326). The limitations of the use of this biomarker are the few standardized assays available and the high cost of the equipment to perform the assay. The variability associated with genetic polymorphism of glutathione *S*-transferase enzymes also affects urinary levels of SPMA (see Section 4.1).

Unmetabolized benzene is excreted in urine in a tiny proportion (< 0.1%) and with rapid kinetics (a half-life of a few hours). It is a specific biomarker, being uniquely indicative of exposure to benzene. It is assayed using online headspace sampling followed by gas chromatography or mass spectrometry (Fustinoni et al., 1999). A limitation in the use of urinary unmetabolized benzene is the lack of standardized assays; in addition, the volatility of benzene in urine may cause the loss of the analyte if no precautions are taken during sampling and in the storage of samples.

Both SPMA and urinary benzene are currently the biomarkers of choice to assess exposure to benzene in studies involving the general population (Fustinoni et al., 2005; Lovreglio et al., 2011; Andreoli et al., 2015).

1.3.2 Assessment of occupational exposure in epidemiological studies

A variety of exposure assessment methods have been used in epidemiological studies of workers potentially exposed to benzene; methods are summarized in the following sections. Additional details on exposure assessment methods used in key epidemiological studies evaluated by the Working Group are provided in Section 1.6.

(a) Occupational cohorts compared with the general population

Many early studies of chemical and petroleum industry workers compared mortality and cancer incidence in the workers and in the general population (e.g. Decouflé et al., 1983; Consonni et al., 1999; Divine et al., 1999; Koh et al., 2014) in terms of either standardized mortality ratios and/or standardized incidence ratios. Benzene was known to be present at such facilities, but benzene exposure estimates were not provided and benzene may not have been specifically mentioned in such studies. Where benzene is mentioned, the metrics are usually expressed as exposed/not exposed, sometimes with the duration or era of the exposed job included. In all cases, there could have been individuals occupationally exposed to benzene in the general population (comparison group).

(b) Expert assessment using interviews, personal questionnaires, or job-specific modules

In occupational studies, some investigators have classified workers with respect to benzene exposure from questionnaires, including those that probe for specific determinants of exposure, such as job-specific modules (e.g. Reid et al., 2011). Benzene exposure may be categorized

semiquantitatively, for example, "no exposure" versus "probable exposure", or "high" versus "medium" versus "low" exposure (e.g. Adegoke et al., 2003; Black et al., 2004; Miligi et al., 2006; Krishnadasan et al., 2007; Seidler et al., 2007). The interpretation of such exposure categories varies from one study to another, depending on the era, country, and industry sectors evaluated, for example.

In population-based studies, exposure must be assessed across a range of occupations and industries by evaluating the type and duration of jobs reported by study participants.

(c) Expert assessment using job characteristics with no individual-level measurements

In some studies, experts classify workers within certain employment start-date periods, industry sectors, and/or job or task categories as exposed or not exposed to benzene (e.g. Koh et al., 2011; Linet et al., 2015). These experts are usually from the specific facility, or at least from the industry sector, and are often occupational hygienists. In most studies the exposure groupings appeared to be performed before case identification, for example in cohort studies, or the assessors were case-blind for case–control studies. This methodology can be used for cohort studies (Infante et al., 1977; Wong, 1987a; Koh et al., 2011), or in case–control studies (e.g. Wong et al., 2006). Duration of exposure is a common metric in these types of studies, and provides a semiquantitative dimension to the exposure assessment. The metrics commonly used in these analyses are exposure category (where provided) and duration of exposed job. Broad exposure groupings were based on employment structure in several studies, for example hourly (potentially higher risk of exposure) versus salaried (potentially lower risk of exposure) workers (e.g. Wen et al., 1983; Wongsrichanalai et al., 1989; Honda et al., 1995). Some similar exposure assessments have a semiquantitative element, for example providing an exposure dimension of high, medium, or low for the work area (McMichael et al., 1975; Rushton & Alderson, 1981).

(d) Exposure assessment using quantitative measurements grouped by job characteristics

The strongest exposure estimates are those where measured benzene exposure data from relevant facilities were attributed by experts to individual job titles or work areas (e.g. Dosemeci et al., 1994). Exposure data may have been collected on an industry- or cohort-wide basis and then applied to specific individual participants, notably in nested case–control studies. This methodology has been applied in China in population-based case–control studies (Bassig et al., 2015), where measured exposure data from many industries has been available since the 1950s (e.g. Wong et al., 2010; Friesen et al., 2012).

There will be some imprecision in the application of a (usually) limited number of data points to other individuals, perhaps employed at other facilities or over different timeframes. Exposure may vary between facilities, between workers, and between days for the same worker, regardless of how average exposure data are assigned. It is important to ensure that the measurement data are representative of usual exposure (normal working circumstances), and include jobs for which lower and higher levels of exposure have been measured. The exposure estimates are quantitative and usually expressed as averaged mean benzene intensity (ppm or mg/m^3) or cumulative exposure (ppm-years or (mg/m^3)-years). The exposure grouping may take into account measured exposure data from multiple sites across a range of industry sectors (e.g. Portengen et al., 2016).

Data on personal exposure to benzene were not usually available before 1970, so extrapolations back in time may be needed. Exposure modifiers, for example, historical changes in work processes, percentage of benzene in petrol, or the presence of ventilation, may have been

used to estimate exposure for jobs and for eras where measured data may not be available or applicable (Armstrong et al., 1996; Lewis et al., 1997; Glass et al., 2000). These exposures were usually estimated with the aid of occupational hygienists from within the industry, and are discussed in more detail in Section 1.6.1. Smith et al. (1993) used such methodology to estimate total hydrocarbon exposure, from which Wong et al. (1999) estimated benzene exposure.

1.3.3 Exposure assessment for molecular epidemiology

Several factors should be considered in the design of epidemiological mechanistic studies. These include the congruency in the time period of effect or disease onset relative to exposure, the magnitude of effects observed, and inter- and intraindividual variability in the response.

For studies on cancer, long-term average exposure is relevant. The latency for leukaemia can be relatively short, for example less than 10 years (Finkelstein, 2000; Richardson, 2008), so exposure during this period should be characterized.

Shorter periods of more recent exposure should be considered for other end-points such as leukopenia (Lan et al., 2004), or chromosomal aberrations (Zhang et al., 1998; Marchetti et al., 2012) including genetic damage (Liu et al., 1996; Zhang et al., 2016). To identify changes in leukocyte numbers, for example, exposure to benzene in the 180 days before blood collection is relevant (Ward et al., 1996).

In a cross-sectional study, it is important to collect both exposure and outcome data for the same individuals to account for inter- and intraindividual variability associated with relevant parameters, for example, diet, smoking, shift work, and time-of-day effects. Data describing these factors should be collected systematically and incorporated within the analyses.

In assessing the exposure, a sufficient number of participants are needed to account for the variability in uptake and human metabolism, particularly where the biomarker of effect is labile (e.g. oxidative stress). In addition, repeated measurements to estimate average exposure are advisable to account for day-to-day variability in exposure.

Investigators should use recognized and validated methods of collection and analysis, ensuring quality by taking into account the most relevant parameters, including the limit of detection.

1.4 Occurrence and exposure

1.4.1 Occupational exposure

Benzene is a ubiquitous pollutant that is present in several industries and occupations, including the production and refining of oil and gas, the distribution, sale, and use of petroleum products, coke production, the manufacture and use of chemical products, automobile repair, shoe production, firefighting, and various operations related to engine exhaust. Due to the high volatility of benzene, occupational exposure to benzene mainly occurs via inhalation. Benzene also penetrates skin, but the degree of dermal absorption of benzene will depend upon the exposure scenario. Dermal absorption will vary according to the tasks being performed (e.g. dipping machinery parts, immersion of hands, or using petroleum-based products as degreasing agents), the benzene content of the product, the composition of the product containing benzene, contact time, and the area of the body on which the chemical resides (Kalnas & Teitelbaum, 2000; Williams et al., 2011; Jakasa et al., 2015). In these scenarios, the exposure will not usually be to pure benzene.

The major industries and occupations in which workers are potentially exposed to benzene are reviewed in the following sections. This

summary is not exhaustive, and the interested reader is referred to several reviews of occupational exposure to benzene across industries that have been published for Europe and North America (Runion & Scott, 1985; Nordlinder & Ramnäs, 1987; van Wijngaarden & Stewart, 2003; Capleton & Levy, 2005; Williams et al., 2008) and Asia (Kang et al., 2005; Liang et al., 2005; Navasumrit et al., 2005; Liu et al., 2009; Park et al., 2015). For some industries or applications, information in the literature is limited. For example, the use of pure benzene as a solvent and reagent in chemical laboratories is well known, but no report on exposure level of benzene was found for laboratory technicians apart from in the petroleum industry.

Although not exhaustive, Table 1.2 gives a summary of reported personal full-shift airborne benzene concentrations, while Table 1.3 summarizes biomonitoring data for the industries.

(a) Production, refining, and distribution of petroleum and petroleum-derived products

The petroleum industry can be divided into upstream and downstream segments. The upstream segment refers to conventional exploration, extraction, and production of crude oil and natural gas, described in the following section, as well as unconventional oil and gas development (UOGD). UOGD involves high-volume hydraulic fracturing, commonly referred to as "fracking", which is coupled to (vertical or horizontal) drilling to extract oil and gas from shale formations (i.e. extraction of materials other than crude oil and natural gas). UOGD includes the process of injecting large volumes of water, proppants (often sand), and potentially hazardous chemicals into wellbores at high pressure, fracturing the rock and enabling the outflow of trapped oil or gas from shale formations (EPA, 2013). The downstream segment consists of refinery operations (production and ancillary operations within the refinery and distribution depots, e.g. tank dipping, pump repairs, filter cleaning), distribution (loading of ships, railcars and road tankers, delivery to service stations), and retail of the petroleum fractions (attendant or self-service filling of customer vehicles).

(i) Upstream petroleum industry (conventional oil and gas extraction)

During drilling, the revolving steel bit must be lubricated and cooled, the well requires pressure support, and the rock cuttings must be transported to the surface. Drilling fluid, a complex oil- or water-based mixture, is used for these purposes. The characteristics of the hydrocarbon base oils in the drilling fluids have changed over time. Diesel as a base oil for drilling was gradually replaced in the early 1980s in the United Kingdom and Norway by petroleum-mineral oils with a reduced aromatic content; non-aromatic mineral oils (aromatic content < 0.01%) were used after 1998 (Gardner, 2003; Steinsvåg et al., 2006, 2007; Bråtveit et al., 2012). The mud-handling areas were originally designed for water-based mud that did not generate vapours, with open flow lines and mud pits. Other than measurements of oil mist and oil vapour, there have been very limited attempts to characterize the exposure regarding its composition. Theoretically, however, hydrocarbon and benzene exposure can occur through contamination of the drilling fluid from the geological formation in which it is drilled, or from hydrocarbons that are added to the drilling fluid to improve drilling properties, as in diesel and drilling fluids containing aromatics in the 1980s (Verma et al., 2000; Steinsvåg et al., 2007). With the exception of eight area measurements made during drilling in Canada showing a full-shift concentration of 0.006 mg/m^3 (with a highest measurement of 0.019 mg/m^3 and one personal measurement of < 0.010 mg/m^3), no information on this exposure scenario was available (Verma et al., 2000).

Table 1.2 Occupational exposure to benzene in air: personal measurements

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m^3)[a]	Exposure range (mg/m^3)[a]	Comments/additional data[a]
Upstream petroleum industry, unconventional							
Esswein et al. (2014)	USA, 2013	Flowback operations, workers gauging tanks	17	Full shift (typically 12 h)	AM (SD), 0.25 (0.16) ppm [0.8 (0.51)]	0.01–0.37 ppm [0.032–1.18]	Task-based short-term (2.5–30 min)
		Flowback operations, workers not gauging tanks	18	Full shift (typically 12 h)	AM (SD), 0.04 (0.03) ppm [0.13 (0.096)]	0.004–0.05 ppm [0.013–0.16]	
Upstream petroleum industry, conventional							
Bråtveit et al. (2007)	Norway, 2005	Process operators	35	657 min (range, 450–730 min)	AM (SD), 0.042 (0.132) ppm [0.13 (0.42)] GM, 0.005 ppm [0.016]	< 0.001–0.69 ppm [< 0.003–2.2]	Exposure varied according to tasks performed
		Flotation work	6		AM (SD), 0.221 (0.267) ppm [0.71 (0.85)] GM, 0.114 ppm [0.360]	0.030–0.688 ppm [0.095–2.2]	
		Sampling	11		AM (SD), 0.005 (0.005) ppm [0.16 (0.16)] GM, 0.003 ppm [0.096]	< 0.001–0.014 ppm [< 0.003–0.04]	
		Miscellaneous	18		AM (SD), 0.005 (0.01) ppm [0.16 (0.03)] GM, 0.003 ppm [0.096]	< 0.0010.023 ppm [< 0.003–0.07]	
Steinsvåg et al. (2007)	Norway, 1994–2003	Process and drilling operations (12 installations)	367	12 h	AM (SD), 0.037 (0.099) ppm [0.12 (0.32)] GM (GSD), 0.007 (5.7) ppm [0.22 (18.21)]	< LOD–2.6 ppm [< LOD–8.31]	165 measurements < LOD were set to LOD/√2

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Steinsvåg et al. (2007) (cont.)		Deck workers	29		AM (SD), 0.17 (0.51) ppm [0.54 (1.63)] GM (GSD), 0.010 (14) ppm [0.03 (44.7)]	< LOD–2.6 ppm [< LOD–8.31]	> LOD = 10
		Process operators	204		AM (SD), 0.036 (0.097) ppm [1.15 (0.31)] GM (GSD), 0.008 (5.3) ppm [0.026 (16.93)]	< LOD–0.97 ppm [< LOD–3.1]	> LOD = 101
		Laboratory	40		AM (SD), 0.012 (0.019) ppm [0.038 (0.06)] GM (GSD), 0.006 (3.7) ppm [0.019 (11.82)]	< LOD–0.11 ppm [< LOD–0.35]	> LOD = 13
		Mechanics	78		AM (SD), 0.006 (0.011) ppm [0.019 (0.035)] GM (GSD), 0.002 (4.5) ppm [0.006 (14.37)]	< LOD–0.08 ppm [< LOD–0.26]	> LOD = 37
		Electricians	16		AM (SD), 0.015 (0.017) ppm [0.048 (0.05)] GM (GSD), 0.007 (5.7) ppm [0.019 (18.85)]	< LOD–0.05 ppm [< LOD–0.16]	> LOD = 4
Kirkeleit et al. (2006b)	Norway, 2004	Crude oil production, vessel	139	592 min (range, 43–931 min)	AM, 0.43 ppm [1.37] GM (GSD), 0.02 (12.42) ppm [0.06 (39.7)]	< 0.001–16.75 ppm [< 0.003–53.5]	LOD, 0.001 ppm [0.003]

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Kirkeleit et al. (2006b) (cont.)		Process operators	30	669 min (range, 182–915 min)	AM, 0.39 ppm [1.25] GM (GSD), 0.01 (9.68) ppm [0.03 (30.92)]	< 0.001–7.3 ppm [< 0.003–23.32]	
		Deck workers	47	564 min (range, 43–866 min)	AM, 0.89 ppm [2.84] GM (GSD), 0.02 (19.11) ppm [0.06 (61.04)]	< 0.001–16.75 ppm [< 0.003–53.5]	The high exposure levels represent cleaning and maintenance of crude oil cargo tanks
		Mechanics	31	632 min (range, 257–705 min)	AM, 0.07 ppm [0.22] GM (GSD), 0.007 (12.04) ppm [0.02 (38.4)]	< 0.001–0.51 ppm [< 0.003–1.63]	
		Contractors	31	518 min (range, 190–931 min)	AM, 0.11 ppm [0.35] GM (GSD), 0.05 (4.90) ppm [0.16 (15.65)]	< 0.001–0.42 ppm [< 0.003–1.34]	
Verma et al. (2000)	Canada, 1985–1996	Conventional oil/gas	198	Long-term	AM, 0.206 GM, 0.036	0.003–7.78	For occupational groups see paper
		Conventional gas	838		GM, 0.010	0.006–57.6	
		Pipeline	8		AM, 0.392 GM, 0.350	0.16–1.54	
		Heavy oil processing	236		AM, 0.112 GM, 0.051	< 0.003–1.60	
Oil spill clean-up operations							
Gjesteland et al. (2017)	Norway, 2016	Sampling boats	21	10.8 h (range, 5.2–14.3 h)	AM, 0.43 ppm [1.37] GM (GSD), 0.20 (4.52) ppm [0.64 (14.44)]	0.01–1.52 ppm [0.03–4.86]	Field trial with spill of two fresh oils (22 workers, > 2 d)
		Workers on release ship and oil recovery ship	11	9.8 h (range, 5.2–12.5 h)	AM, 0.05 ppm [0.16] GM (GSD), 0.02 (0.02) ppm [0.064 (0.064)]	0.002–0.10 ppm [0.006–0.32]	
Downstream, petroleum refinery industry							
Almerud et al. (2017)	Sweden, 2009–2011	Process technicians, refinery I	132	Full shift (8 or 12 h)	AM, 0.153	95% CI, 0.01–0.022; maximum, 3.77	
		Maintenance workers, refinery I	67		AM, 0.0059	95% CI, 0.004–0.009; maximum, 1.32	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Almerud et al. (2017) (cont.)		Process technicians outdoor, refinery II	66		AM, 0.0137	95% CI, 0.0083–0.023; maximum, 0.27	
		Laboratory workers, refinery I	25		AM, 0.0046	95% CI, 0.0034–0.0062; maximum, 0.0154	
		Laboratory workers, refinery II	11		AM, 0.0084	95% CI, 0.0034–0.021; maximum, 0.02	
Akerstrom et al. (2016)	Sweden, 2011–2013	Turnarounds, refinery I	43	Full shift (8 or 12 h)	AM, 0.61	95% CI, 0.23–1.60 µg/m³	
		Turnarounds, refinery II	26		AM (SD), 0.96 (1.3) GM (GSD), 0.23 (0.0075)	0.007–4.5	
		Oil harbour workers (jetty workers and dock workers)	34		AM, 0.31	95% CI, 0.08–1.2	
		Sewage tanker drivers	16		AM, 0.36	95% CI, 0.068–1.9	
Widner et al. (2011)	USA, 1977–2005	Refinery and dock workers	406	480–661 min	NR	0.006–15 ppm [0.19–47.9]	GM not calculated because > 50% of measurements < LOD
		Dock connecting crew	179	535–664 min	GM (GSD), 0.023 (11) ppm [0.073 (35.1)]	0.010–15 ppm [0.03–47.9]	
		Contractor–tankerman	38	326–463 min	GM (GSD), 0.25 (8.8) ppm [0.8 (28.1)]	0.010–9.8 ppm [0.03–31.3]	
Kreider et al. (2010)	USA, 1977–2006	Routine operation, all areas and job titles	624	> 180 min	AM, 0.091 ppm [0.29]	Minimum–maximum detected, 0.004–6.0 ppm [0.013–19.2] 75th, 95th percentile, 0.043, 0.31 ppm [0.14–0.99]	GM not calculated because > 50% of measurements < LOD

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Kreider et al. (2010) (cont.)		Start up	50		AM, 0.046 ppm [0.15]	Minimum–maximum detected, 0.015–0.29 ppm [0.048–0.93] 75th, 95th percentile, 0.05, 0.17 ppm [0.16, 0.54]	
		Turnaround	471		AM, 0.17 ppm [0.54] GM (GSD), 0.032 (6.7) ppm [0.1 (21.4)]	Minimum–maximum detected, 0.004–9.200 ppm [0.013–29.4] 75th, 95th percentile, 0.12, 0.68 ppm [0.38, 2.17]	
CONCAWE (2002)	Europe, 1999–2001	Offsite refinery operator	6	451–498 min	AM, 0.3 GM, 0.2	10–90th percentiles, 0.1–0.5	
		Laboratory technician blending test gasoline for research	7	215–487 min	AM, 3.7 GM, 1.6	10–90th percentiles, 0.2–8.3	
CONCAWE (2000)	Europe, 1993–1998	Onsite operators (including catalytic reformer, gasoline blending)	97	Full shift	AM, 0.22	0.008–7.88	91% corresponding to full shift (8 or 12 h)

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
CONCAWE (2000) (cont.)		Refinery offsite operators (tank farm, including dipping, sampling, valve operation, dewatering, loading rail cars)	321		AM, 0.32	0.008–23.3	
		Refinery maintenance workers (pump maintenance, instrument calibration, enclosed equipment)	373		AM, 0.41	0.008–18.1	
		Refinery laboratory technicians (including product analysis, octane rating testing)	628		AM, 0.30	0.0015–5.0	
		Tank cleaners (including sludge cleaning)	49		AM, 2.10	0.008–38.7	
Downstream, distribution							
Lovreglio et al. (2016)	Italy, NR	Fuel tanker drivers	17	8 h	AM (SD), 0.28 (0.248) Median, 0.246	0.0074–1.017	
CONCAWE (2002)	Europe, 1999–2001	Rail car operators, top loading with vapour recovery)	21	64–363 min	AM, 0.5 GM, 0.4	10–90th percentiles, 0.2–0.7	
		Rail car operators, top loading without vapour recovery	16	165–450 min	AM, 4.0 GM, 1.4	10–90th percentiles, 0.3–10	
CONCAWE (2002)	Europe, 1999–2001	Road tanker distribution; drivers, bottom loading with vapour recovery	33	185–555 min	AM, 0.6 GM, 0.4	10–90th percentiles, 0.2–1.2	Pre-2000 specification gasoline

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
CONCAWE (2000)	Europe, 1993–1998	Marine and rail loading; ship deck crew, open loading	41	Full shift	AM, 0.56	0.08–5.4	91% corresponding to full shift (8 or 12 h)
		Ship deck crew, closed loading	2		AM, 0.56	0.51–0.6	
		Ship deck crew, unloading	32		AM, 0.51	0.023–3.7	
		Jetty staff	46		AM, 0.37	0.023–1.7	
CONCAWE (2000)	Europe, 1993–1998	Road tanker distribution Road tanker drivers, top loading	69	Full shift	AM, 2.07	0.04–48.2	
		Road tanker drivers, bottom loading (without vapour recovery)	223		AM, 0.82	0.008–15	
		Road tanker drivers, bottom loading (with vapour recovery)	137		AM, 0.37	0.03–1.99	
		Drivers, other category or unspecified	56		AM, 1.26	0.07–19.2	
		Road tanker terminal rack operators	126		AM, 0.64	0.003–4.2	
		Road tanker terminal supervisors/ operators	151		AM, 0.36	0.001–3.1	
		Road tanker terminal maintenance	52		AM, 0.52	0.001–7.9	
Foo (1991)	Singapore	Petroleum delivery tanker drivers	14	Full shift	AM, 1.10 ppm [3.51] GM, 0.81 ppm [2.59]	0.08–2.37 ppm [0.26–7.57]	21 gasoline stations Short-term exposure (n = 7): AM, 3.1 ppm [9.9], range 0.07–11.6 ppm [0.22–37.05]

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Petrochemical manufacturing							
Sahmel et al. (2013)	USA, 1974–1999	Use of petroleum-based raw materials;	2359	8 h	AM (SD), 0.54 (5.0) ppm [1.72 (15.97)] Median, 0.042 ppm [0.13]	NR	
		Routine employee exposure (all) 1974–1986	1289		AM (SD), 0.885 (6.72) ppm [2.83 (21.47)] Median, 0.12 ppm [0.38]	NR	Median exposure during time periods corresponding to year when OEL changed
		1987–1999	1070		AM (SD), 0.125 (0.676) ppm [0.4 (2.16)] Median, 0.016 ppm [0.051]	NR	
		1974–1983	916		AM (SD), 1.103 (7.739) ppm [3.52 (24.72)] Median, 0.19 ppm [0.61]	NR	Median exposure during time periods stratified according to key process changes
		1984–1991	865		AM (SD), 0.206 (2.024) ppm [0.66 (6.47)] Median, 0.01 ppm [0.03]	NR	
		1992–1999	578		AM (SD), 0.148 (0.578) ppm [0.47 (1.85)] Median, 0.021 ppm [0.067]	NR	
Williams & Paustenbach (2005)	USA, 1976–1987	Petrochemical manufacturing facility (acetic acid); mainly process operators	749	4–10 h	AM (SD), 1.75 (3.8) ppm [5.59 (12.14)]	NR	See paper for mean exposure levels for various production processes/areas
Coke production							
He et al. (2015)	China, NR	Topside, plant A	27	8 h	AM (SD), 0.705 (0.259)	0.268–1.197	Plant A: top charging of coal; no air pollution control
		Topside, plant B	28		AM (SD), 0.290 (0.11)	0.085–0.489	Plant B: stamp charging of coal; bag house for air pollution control

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Bieniek & Łusiak (2012)	Poland, 2005–2010	Cokery workers	202	Full shift	0.15	0.01–1.79	
		Coke oven workers	122		Median, 0.16	5–95th percentile, 0.04–0.60	
		Coke by-products	37		Median, 0.37	5–95th percentile, 0.061–1.39	
		Other workers in the coke plant	43		Median, 0.09	5–95th percentile, 0.011–0.292	Electricians and supervising personnel
Kivistö et al. (1997)	Estonia, 1994	Cokery workers	18	Full shift	AM (SD), 1.3 (2.7) ppm [4.15 (8.62)] Median, 0.4 ppm [1.28]	0.09–11.7 ppm [0.29–37.37]	
		Benzene factory workers	20		AM (SD), 1.6 (3.3) ppm [5.11 (10.54)] Median, 0.6 ppm [1.92]	0.06–14.7 ppm [0.19–46.96]	
Drummond et al. (1988)	UK, 1986	Battery workers	NR	Full shift	AM, 0.31 ppm [0.99]	NR	Each worker measured for 3–5 consecutive shifts
		Refining process of benzene	NR		AM, 1.32 ppm [4.22]	Maximum, 4.3 ppm [13.74]	
Petrol stations							
Campo et al. (2016)	Italy, 2008–2009	Petrol station attendants	89	~5 h	Median, 0.059	5–95% CI, 0.005–0.284	
Lovreglio et al. (2016)	Italy, NR	Filling station attendants	13	8 h	AM (SD), 0.02 (0.015) Median, 0.0138	0.0045–0.0534	
Lovreglio et al. (2014)	Italy, NR	Filling station attendants	24	8 h	AM (SD), 0.023 (0.017) Median, 0.02	0.0045–0.0663	
Bahrami et al. (2007)	Islamic Republic of Iran, NR	Petrol station workers	25	2–4 h	AM (SD), 1.40 (0.80) ppm [4.47 (2.56)]	0.2–3.1 ppm [0.64–9.9]	
Navasumrit et al. (2005)	Thailand, NR	Petrol station attendants	50	8 h	AM (SD), 121.67 (14.37) ppb [0.39 (0.046)] GM, 86.4 ppb [0.28]	2.80–439.9 ppb [0.0089–1.42]	
CONCAWE (2002)	Europe, 1993–1998	Service station attendants, without vapour recovery	26	189–465 min	AM, 0.3 GM, 0.3	10–90th percentile, 0.2–0.5	Pre-2000 specification gasoline

Table 1.2 Occupational (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
CONCAWE (2002) (cont.)		Service station attendants, with vapour recovery	7	288–437 min	AM, 0.1 GM, 0.1	10–90th percentile, 0.1–0.1	
		Service station cashiers	13	235–490 min	AM, 0.2 GM, 0.2	0.1–0.2	
		Service station workers, miscellaneous	6	237–280 min	AM, 0.2 GM, 0.1	0.1–0.2	
CONCAWE (2000)	Europe, 1993–1998	Service station attendants, without vapour recovery	417	Full shift	AM, 0.25	0.001–1.9	91% corresponding to full shift (8 or 12 h)
		Service station cashiers	268		AM, 0.05	0.001–1.92	
		Petrol pump maintenance workers	2		AM, 0.55	0.16–0.93	
		Service station workers, miscellaneous	5		AM, 0.03	0.01–0.10	
Lagorio et al. (1994)	Italy, 1991–1992	Petrol station attendants	27	8 h	AM (SD), 1.73 (5.53)	NR	Alkylated and lead-free gasoline: 2.86% and 2.65% benzene by volume, respectively
Lagorio et al. (1993)	Italy, 1992	Filling station attendants	111	8 h	AM (SD), 0.55 (2.46) GM (GSD), 0.12 (3.82)	0.001–28.02	111 filling stations
Foo (1991)	Singapore, NR	Gasoline kiosk attendants	54	Full shift	AM, 0.20 ppm [0.64] GM, 0.16 ppm [0.51]	0.028–0.71 ppm [0.89–2.27]	21 gasoline stations Short-term exposure (n = 49): AM, 6.6 ppm [21.08]; GM, 1.0 ppm [3.19]; range, 0.064–179 ppm [0.20–571.78]
Runion & Scott (1985)	USA, 1978–1783	Retail service stations	1478	Full shift	AM (SD), 0.06 (0.02) ppm [0.19 (0.06)] GM (GSD), 0.02 (5.4) ppm [0.06 (17.25)]	< 1.0 to > 10 ppm [< 3.19 to > 31.9] Range, NR	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Automobile repair							
Egeghy et al. (2002)	USA, 1998–1999	Mechanics	197	4 h	AM (SD), 0.118 (0.166) Median, 0.0597	< 0.009–1.14	Self-administered sampling; benzene content of gasoline < 1%
Javelaud et al. (1998)	France, 1996	Mechanics	65	8 h	AM (SD), 0.48 (1.49) GM, 0.06 Median, 0.14	< 0.005–9.31	23 garages
Hotz et al. (1997)	Country NR, 1994–1995	Mechanics	156	8 h	Median, 0.01 ppm [0.032]	5–95th percentile, < LOD–0.14 ppm [< LOD–0.45]	
Foo (1991)	Singapore, NR	Motorcar service mechanics	54	Full shift	AM, 0.17 ppm [0.54] GM, 0.10 ppm [0.32]	0.014–1.7 ppm [0.045–5.43]	21 gasoline stations
Nordlinder & Ramnäs (1987)	Sweden, NR	Mechanics, small garage (summer)	> 100	Full shift	AM, 1.6	NR	
		Mechanics, small garage (winter)			AM, 6.8	NR	
		Mechanics, medium and large garages (summer)			AM, 0.4	NR	
		Electricians, medium and large garages (summer)			AM, 1.0	NR	
		Mechanics, medium and large garages (winter)			AM, 0.8	NR	
		Electricians, medium and large garages (winter)			AM, 1.4	NR	
Exposure from engine exhaust							
Arayasiri et al. (2010)	Thailand, 2006	Traffic police	24	8 h	AM (SD, 0.0382 (0.0027) Median, 0.039	(0.0155–0.069)	
		Office police	24		AM (SE), 0.007 (0.0005) Median, 0.0062	0.0036–0.014	
Manini et al. (2008)	Italy, 2005	Traffic police	19	6 h	AM, 0.0061	0.0003–0.012	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Bahrami et al. (2007)	Islamic Republic of Iran, NR	Taxi drivers	60	2–4 h	AM (SD), 0.31 (0.22) ppm [0.99 (0.7)]	0.07–0.95 ppm [0.22–3.03]	
Manini et al. (2006)	Italy, 2004	Taxi drivers	37	24 h	AM (SD), 0.006 (0.0017)	NR	Non-smokers, ambient concentration in taxi during the 12 h shift, 0.0075 (0.0019)
Crebelli et al. (2001)	Italy, 1998–1999	Traffic police	139	7 h	AM (SD), 0.009 (0.011) GM (GSD), 0.0068 (0.002)	0.0013–0.0767	
		Office police	63		AM (SD), 0.0038 (0.0015) GM (GSD), 0.0035 (0.0015)	0.0011–0.0083	
Fustinoni et al. (1995)	Italy, 1994	Traffic wardens, urban and outdoors	20	5 h	AM (SD), 0.053 (0.03)	0.02–0.108	
		Traffic wardens, indoors (clerks)	19		AM (SD), 0.029 (0.008)	0.017–0.044	
Navasumrit et al. (2005)	Thailand, NR	Cloth vendors	22	8 h	AM (SD), 22.61 (1.32) ppb [0.073 (0.004)] Median, 21.1 ppb [0.067]	13.9–40.7 ppb [0.044–0.13]	
		Grilled-meat vendors	21		AM (SD), 28.19 (2.23) ppb [0.09 (0.007)] Median, 24.61 ppb [0.078]	16.8–52.0 ppb [0.054–0.17]	
Shoemaking							
Azari et al. (2012)	Islamic Republic of Iran, NR	Shoemakers, 12 workshops (October)	48	8 h	Mean (SE), 1.10 (0.11) ppm [3.51 (0.35)]	NR	Three consecutive months (October–December), examined effects of climate change and restriction of air flow due to closure of windows and shutdown of general ventilation systems
		Shoemakers, 12 workshops (November)			Mean (SE), 1.37 (0.14) ppm [4.38 (0.45)]	NR	
		Shoemakers, 12 workshops (December)			Mean (SE), 1.52 (0.18) ppm [4.86 (0.57)]	NR	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Estevan et al. (2012)	Spain, 2002–2007	Shoemakers: 2002–2003	329	NR	AM (SD), 0.05 (0.15)	NR	18–26% of samples were ≥ LOD (0.01)
		Shoemakers: 2004–2005	218		AM (SD), 0.07 (0.14)	NR	
		Shoemakers: 2006–2007	302		AM (SD), 0.05 (0.14)	NR	
Zhang et al. (2011)	China, NR	Shoemakers	44	8 h	AM (SD), 44.81 (33.59) GM (GSD), 27.91 (3.29)	2.57–146.11	
Vermeulen et al. (2004)	China, 2000–2001	Large shoe factory (safety shoes): all workers	2667	8 h	AM, 3.46 ppm [11.05] GM (GSD), 1.28 (3.64) ppm [4.09 (11.63)]	10–90th percentiles, 0.20–7.00 ppm [0.64–22.4]	No glues reported to contain benzene
		Large shoe factory (safety shoes): cutting	427		AM, 0.45 ppm [1.44] GM (GSD), 0.34 (2.05) ppm [1.09 (6.55)]	0.17–0.15 ppm [0.54–3.67]	
		Large shoe factory (safety shoes): modelling	735		AM, 2.74 ppm [8.75] GM (GSD), 1.71 (2.81) ppm [5.46 (8.98)]	0.38–6.04 ppm [1.21–19.29]	
		Large shoe factory (safety shoes): fitting	1096		AM, 2.19 ppm [7] GM (GSD), 1.12 (2.98) ppm [3.58 (9.52)]	0.26–4.68 ppm [0.83–14.95]	
		Large shoe factory (safety shoes): finishing	241		AM, 8.35 ppm [26.67] GM (GSD), 2.91 (3.33) ppm [9.3 (10.64)]	0.65–11.69 ppm [2.08–37.34]	
		Large shoe factory (safety shoes): packing	168		AM, 15.55 ppm [49.67] GM (GSD), 7.60 (3.47) ppm [24.28 (11.08)]	1.43–43.06 ppm [4.57–137.55]	
		Small shoe factory (luxury shoes): all workers	116	8 h	AM, 21.86 ppm [69.83] GM (GSD), 14.4 (2.31) ppm [46 (7.38)]	10–90th percentiles, 5.23–50.63 ppm [16.71–161.73]	6 of 7 glues contained benzene (0.60–34% benzene)

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Vermeulen et al. (2004) (cont.)		Small shoe factory (luxury shoes): cutting	41		AM:10.96 ppm [35.01] GM (GSD), 10.24 (1.45) ppm [32.71 (4.63)]	6.53–16.26 ppm [20.86–51.94]	
		Small shoe factory (luxury shoes): modelling	18		AM, 9.04 ppm [28.88] GM (GSD), 7.75 (1.75) ppm [24.76 (5.59)]	4.45–18.69 ppm [14.21–59.7]	
		Small shoe factory (luxury shoes): fitting	47		AM, 29.31 ppm [93.62] GM (GSD), 21.34 (2.34) ppm [68.17 (7.47)]	7.06–65.17 ppm [22.55–208.17]	
		Small shoe factory (luxury shoes): finishing	10		AM, 54.64 ppm [174.54] GM (GSD), 28.03 (3.30) ppm [89.54 (10.54)]	7.62–179.60 ppm [24.34–573.69]	
Printing							
Portengen et al. (2016)	China, 1949 to after 2000	Printing	232	NR	AM, 94.1 GM (GSD), 8.2 (13.0)	NR	40% of measurements < LOD (3.19)
Kang et al. (2005)	Republic of Korea, 1992–2000	Offset printing	4	NR	AM (SD), 0.017 (0.012) ppm [0.0543 (0.038)] GM (GSD), 0.014 ppm [0.0447]	0.008–0.034 ppm [0.0255–0.11]	
Handling of jet fuel							
Smith et al. (2010)	USA, NR	US Air Force personnel					LOD, 0.9 µg/m³ Jet fuel JP-8 (0.004–0.007% benzene)
		All	69	Full shift	GM (GSD), 0.0016 (0.0035)	< LOD–0.0364	
		Group assumed exposed to concentration	25	Full shift	GM (GSD), 0.0029 (0.0034)	< LOD–0.0364	
Egeghy et al. (2003)	USA, NR	US Air Force personnel					Jet fuel JP-8 (0.0002–0.0123 weight% benzene)
		Group exposed to low concentration	140	4 h	Median, 0.0031	< 0.001–0.0613	
		Group exposed to moderate concentration	38	4 h	Median, 0.0074	0.0014–1.85	
		Group exposed to high concentration	114	4 h	Median, 0.252	0.0061–6.63	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Holm et al. (1987)	Sweden, 1983–1984	Swedish National Defence	92	12 h	GM (GSD), 0.06 (4.0)	Maximum, 7.2	Jet fuel MC-77 (equivalent to JP4 (< 1% benzene)
		All samples	46	8 h (TWA)	GM (GSD), 0.06 (4.1)	Maximum, 4.1	
		Jet fuel handling	6		GM (GSD), 0.03 (2.6)	Maximum, 0.1	
		Flight service	28		GM (GSD), 0.08 (3.6)	Maximum, 1.2	
		Workshop service	12		GM (GSD), 0.05 (5.7)	Maximum, 4.1	
		Pure jet fuel exposure	38		GM (GSD), 0.06 (4.2)	Maximum, 4.1	
		Mixed solvents exposure	8		GM (GSD), 0.11 (3.1)	Maximum, 0.5	
Firefighting							
Reinhardt & Ottmar (2004)	USA, 1992–1995	Initial attack (full shift)	45	13.3 h (range, 12–18 h)	GM, 3 ppb [0.096]	Maximum, 24 ppb [0.077]	13 d of initial attack incidents
		Initial attack (at fires)		3.3 h (range, 2–10 h)	GM, 14 ppb [0.045]	Maximum, 43 ppb [0.14]	
		Project wildfires (full shift)	84	13.9 h (range, 4–24 h)	GM, 4 ppb [0.013]	Maximum, 249 ppb [0.8]	17 d at eight separate project wildfires
		Project wildfires (at fires)		10.4 h (range, 2–24 h)	GM, 6 ppb [0.019]	Maximum, 384 ppb [1.23]	
		Prescribed burns (full shift)	200	11.5 h (range, 6–18 h)	GM, 16 ppb [0.051]	Maximum, 58 ppb [0.19]	39 prescribed burns
		Prescribed burns (at fires)		7 h (range, 2–13 h)	GM, 28 ppb [0.089]	Maximum, 88 ppb [0.28]	
Austin et al. (2001)	Canada, NR	Structural fires	9	Short-term	AM (SD), 3.38 (3.45) ppm [10.8 (11.02)]	0.12–10.76 ppm [0.38–34.37]	Area samples (not personal)
Bolstad-Johnson et al. (2000)	USA, 1998	Structural fires	95	Short-term	AM (SD), 0.383 (0.425) ppm [1.22 (1.36)]	0.07–1.99 ppm [0.22– 6.36]	25 fires

AM, arithmetic mean; CI, confidence interval; d, day(s); GM, geometric mean; GSD, geometric standard deviation; h, hour(s); LOD, limit of detection; min, minute(s); *n*, number of measurements; NR, not reported; OEL, occupational exposure limit; ppb, parts per billion; ppm, parts per million; SD, standard deviation; SE, standard error; TWA, time-weighted average

[a] Exposure concentrations and range given in mg/m³, unless indicated otherwise; if published in another unit, the concentration in mg/m³ is given in square brackets

Table 1.3 Summary of selected studies on the biological monitoring of occupational exposure to benzene

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (μg/m³)[a, b]	Urinary t,t-MA (μg/g creatinine)[a,c]	Urinary SPMA (μg/g creatinine)[a,c]	Urinary benzene (μg/L)[a,d]	Other biomarkers[a]
Campo et al. (2016)	Italy, NR	Filling station attendants	89	Median, 59 (5–284)[e]	Median, 127 (27–522)[e] μg/L	Median, 0.19 (< 0.1–1.28)[e] μg/L	Median, 0.339 (0.090–2.749)[e]	NR
		Unexposed workers	90	4 (1–18)[e]	Median, 117 (< 20–509)[e] μg/L	Median, < 0.1 (< 0.1–0.99)[e] μg/L	Median, 0.157 (0.054–2.554)[e]	NR
Lv et al. (2014)	China, NR	Shoe manufacturing workers	55	GM, 6980	NR	GM, 99[e]	NR	NR
Fustinoni et al. (2011)	Poland, NR	Petrochemical refinery workers	71	Median, 190 (50–2310)[e]	NR	Median, 0.65 (0.12–5.3)[e]	Median, 0.55 (0.117–7.487)[e]	NR
		Petrochemical office workers	97	NR	NR	Median, 0.40 (< 0.10–2.29)[e]	Median, 0.32 (0.083–2.316)[e]	NR
Carrieri et al. (2010)	Italy, 2006	Petrochemical workers	29	0.014 (< 0.001–0.280) ppm [45 (< 3–890)]	101 (< 6.86–746)	2.8 (< 0.06–38.59)	NR	NR
Lovreglio et al. (2010)	Italy, NR	Fuel tanker drivers	18	307 (7.4–1017)	134 (16–400)	2.94 (0.25–12.13)	2.96 (0.16–10.4)	Urinary phenol, 19 (5.0–33.0) mg/L
		Filling station attendants	23	23.5 (4.5–66.3)	86 (11–157)	0.79 (0.05–3.33)	0.62 (0.04–2.87)	Urinary phenol, 17.1 (8.0–29.0) mg/L
		Controls	31	4.6 (< 3.0–11.5)	93 (13–734)	0.65 (0.03–4.48)	1.23 (< 0.02–11.4)	Urinary phenol, 18.6 (3.0–36.0) mg/L
Hoet et al. (2009)	NR	Petrochemical workers	110	< 0.1 ppm [< 320]	50 (< 20–980)	0.97 (0.21–12.78)	0.270 (< 0.10–5.35)	Blood benzene, 0.405 (< 0.10–13.58) μg/L
Bråtveit et al. (2007)	Norway, 2004–2005	Petrochemical workers	12	0.042 (< 0.001–0.69) ppm [130 (< 3–2200)]	NR	NR	3.9 (0.5–34) nmol/L	Post shift; blood benzene, 1.8 (1.0–4.0) nmol/L
		Catering operator and office employees	9	NR	NR	NR	1.6 (0.5–4.0) nmol/L	Blood benzene, 1.8 (1.0–4.0) nmol/L

Table 1.3 (continued)

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (μg/m³)[a, b]	Urinary t,t-MA (μg/g creatinine)[a,c]	Urinary SPMA (μg/g creatinine)[a,c]	Urinary benzene (μg/L)[a,d]	Other biomarkers[a]
Bahrami et al. (2007)	Islamic Republic of Iran, NR	Taxi drivers	60	0.31 (0.07–0.95) ppm [990 (220–3030)]	310 (90–1270)	NR	NR	NR
		Petrol station workers	9	1.40 (0.2–3.1) ppm [4470 (640–9900)]	2640 (1200–3280)	NR	NR	NR
		Controls	18	ND	170 (10–350)	NR	NR	NR
Manini et al. (2006)	Italy, 2004	Taxi drivers	21 NS	7.5	122	GM, 2.14	GM, 0.44	NR
			16 S	8.1	154	GM, 3.79	GM, 2.58	NR
Kim et al. (2006a)	China, 2000–2001	Shoemaking factory workers	164 women	Median, 1.28 (0.017–88.9) ppm [4090 (54–284 000)]	Median, 13.5 (0.644–426) μmol/L	Median, 262 (1.50–29 400) nmol/L	Median, 283 (6.21–53 900) nmol/L	NR
			86 men	Median, 1.05 (0.122–50.2) ppm [3350 (390–160 350)]	Median, 10.3 (1.50–370) μmol/L	Median, 137 (3.68–33 000) nmol/L	Median, 216 (19.4–42 600) nmol/L	NR
		Clothes manufacturing workers (controls)	87 women	Median, 3.40 (0.146–21.2) ppb [10.86 (0.47–67.72)]	Median, 1.06 (0.152–6.17) μmol/L	Median, 1.94 (0.591–86.4) nmol/L	Median, 1.48 (0.091–7.47) nmol/L	NR
			52 men	Median, 3.71 (0.146–533) ppb [11.85 (0.47–1702.55)]	Median, 1.09 (0.132–5.78) μmol/L	Median, 3.24 (0.591–68.1) nmol/L	Median, 1.59 (0.091–130) nmol/L	NR
Fustinoni et al. (2005)	Italy, 1999–2000	Filling station attendants	78	Median, 61 (11–478)	NS: Median, 49 (< 10–581) μg/L	Median, 5.8 (0.2–10.9) μg/L	Median, 0.342 (0.042–2.836)	NR
					S: Median,144 (15–321) μg/L	Median, 7.5 (0.2–24.8) μg/L	Median, 1.168 (0.055–5.111)	Section 1.01 NR
		Traffic police	77	Median, 22 (9–316)	NS: Median, 82 (< 10–416) μg/L	NS: Median, 5.3 (0.2–13.8) μg/L	NS: Median, 0.151 (0.025–0.943)	NR
					S: Median, 213 (52–909) μg/L	S: Median, 9.1 (2.4–13.8) μg/L	S: Median, 0.753 (0.054–4.246)	NR
		Office workers	58	Median, 6 (< 6–115)	NS: Median, 33 (< 10–1089) μg/L	NS: Median, 4.1 (0.2–12.5) μg/L	NS: Median, 0.133 (< 0.015–0.409)	NR
					S: Median, 71 (< 10–270) μg/L	S: Median, 8.0 (0.2–13.9) μg/L	S: Median, 0.331 (0.064–4.615)	NR

Table 1.3 (continued)

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (μg/m³)[a, b]	Urinary t,t-MA (μg/g creatinine)[a,c]	Urinary SPMA (μg/g creatinine)[a,c]	Urinary benzene (μg/L)[a,d]	Other biomarkers[a]
Fustinoni et al. (2005) (cont.)		Bus drivers	152	Median, 21 (< 6–92)	NS: Median, 57 (< 10–536) μg/L	NS: Median, 5.6 (0.2–13.3) μg/L	NR	NR
					S: Median, 174 (< 10–695) μg/L	S: Median, 9.3 (0.2–65.9) μg/L	NR	NR
		Researchers	49	Median, 9 (< 6–46)	NS: Median, 51 (< 10–181) μg/L	NS: Median, 9.0 (0.2 – 182.2) μg/L	NR	NR
					S: Median, 195 (< 10–444) μg/L	S: Median, 13.7 (3.0–19.9) μg/L	NR	NR
Chakroun et al. (2002)	Tunisia, NR	Tanker fillers	20	0.16 (0.02–0.42) ppm [510 (63.89–1340)]	350 (80–1110)	NR	NR	NR
		Filling station attendants	10	0.20 (0.09–0.52) ppm [640 (290–1660)]	310 (150–590)	NR	NR	NR
		Controls	20	ND	110 (20–390)	NR	NR	NR
Waidyanatha et al. (2001, 2004)	China, ~1995	Rubber, adhesive, and paint manufacturers	42	14.5 (1.65–30.6) ppm [46 320 (5270–97 740)]	16 200 (1140–77 800) μg/L	712 (050–5890) μg/L	8.42 (0.837–27.9)	NR
				109 (31.5–329) ppm [348 180 (100 620–1 050 920)]	51 300 (7250–133 000) μg/L	9420 (123–27 500) μg/L	50.2 (1.30–284)	NR
		Sewing machine manufacturing workers (controls)	41	0.015 (0.0–0.11) ppm [48 (0.0–350)]	108 (020–338) μg/L	21 (2–79) μg/L	0.145 (0.027–2.06)	NR
Kivistö et al. (1997)	Estonia, 1994	Benzene production (in winter)	25	1.6 (0.06–14.7) ppm [5110 (190–46 960)]	38 (< 0.2–210) μmol/L	99 (< 0.3–1030)	965 (10–6250) nmol/L	Benzene in blood, 174 (8–1160) nmol/L
		Cokery workers (in winter)	27	1.3 (0.09–11.7) ppm [4150 (290–37 370)]	11 (< 0.2–35) μmol/L	73 (< 0.3–1020)	372 (22–1750) nmol/L	Benzene in blood, 160 (18–1690) nmol/L
		Rural controls (in winter)	10	0.009 ppm [28.75]	0.8 (< 0.2–8.1) μmol/L	2.1 (< 0.3–18)	12 (2–45) nmol/L	Benzene in blood, 7 (< 3–22) nmol/L

Table 1.3 (continued)

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (µg/m³)[a, b]	Urinary t,t-MA (µg/g creatinine)[a,c]	Urinary SPMA (µg/g creatinine)[a,c]	Urinary benzene (µg/L)[a,d]	Other biomarkers[a]
Boogaard & van Sittert (1995, 1996)	Several countries including Belgium, Germany, and the Netherlands, 1992–1994	Natural gas production platforms	24	< 100–19 200	< 10–9920 µmol/mol creatinine	< 0.5–378 µmol/mol creatinine	NR	NR
		Chemical manufacturing	130	< 10–100 000	< 10–31 300 µmol/mol creatinine	< 1–1096 µmol/mol creatinine	NR	NR
		Oil refineries with aromatic plants	16	110–3300	8–1200 µmol/mol creatinine	0.9–46.4 µmol/mol creatinine	NR	NR
		Fuel tanker drivers and gasoline attendants	14	NR	9–830 µmol/mol creatinine	0.5–8. µmol/mol creatinine	NR	NR
		Employees without potential benzene exposure	38 NS	NR	29 µmol/mol creatinine	0. 94 µmol/mol creatinine	NR	NR
			14 S	NR	46 µmol/mol creatinine	1.71 µmol/mol creatinine	NR	NR

GM, geometric mean; ND, not detected; NR, not reported; NS, non-smokers; ppb, parts per billion; ppm, parts per million; S, smokers; SPMA, *S*-phenylmercapturic acid; t,t-MA, *trans*,*trans*-muconic acid

[a] Benzene exposure and biomarker concentrations are reported as mean (minimum–maximum), if not indicated otherwise

[b] Exposure concentration and range given in µg/m³ unless indicated otherwise; if published in another unit, the conversion to µg/m³ is given in square brackets

[c] Exposure concentration and range given in µg/g creatinine unless indicated otherwise; if published in another unit, the conversion to µg/g creatinine is given in square brackets

[d] Exposure concentration and range given in µg/L unless indicated otherwise; if published in another unit, the conversion to µg/L is given in square brackets

[e] 5–95th percentile

The separation and processing of crude oil and natural gas into crude oil, condensate, gas, and produced water before transport to shore via pipelines or tank ships takes place in a closed processing equipment and pipeline system. All four petroleum streams contain benzene, however, and the likelihood of exposure to benzene increases whenever the system is opened. The composition of crude oil and gas condensate varies between oil and gas fields and depends upon several factors, such as geological conditions in the reservoirs and the production age of the oil field, but typically lies within the range of < 0.01 and 3.0% by weight (Verma & des Tombe, 1999; Verma et al., 2000; Kirkeleit et al., 2006a), with benzene content in condensate being higher. The full-shift mean exposure in the production of oil and natural gas is usually well below 1 ppm [3.19 mg/m^3] benzene, the 8-hour permissible exposure limit set by the Occupational Safety and Health Administration (OSHA, 2017), during ordinary activity (Glass et al., 2000; Verma et al., 2000; Kirkeleit et al., 2006a; Bråtveit et al., 2007; Steinsvåg et al., 2007) (Table 1.2). However, some specific tasks, such as cleaning and maintenance of tanks and separators, pipeline pigging operations, and storage tank gauging, may cause short-term exposures in excess of this (Runion, 1988; CONCAWE, 2000; Glass et al., 2000; Verma et al., 2000; Kirkeleit et al., 2006a; Esswein et al., 2014).

With technological advances and more efficient reservoir completion techniques, UOGD has grown in the past decades. The only study available for this segment indicates that the potential for exposure is higher than for conventional oil and gas extraction (Esswein et al., 2014; Table 1.2).

(ii) Downstream petroleum industry: refining

The full-shift exposure to benzene during ordinary activity in the refining petroleum industry tends to be higher than for upstream activities, but still with average values well below 1 ppm [3.19 mg/m^3] (Nordlinder & Ramnäs, 1987; Verma et al., 1992, 2001; CONCAWE, 2000, 2002; Glass et al., 2000; Akerstrom et al., 2016; Almerud et al., 2017) (see Table 1.2). However, the range of exposure indicates potential for exceeding 1 ppm [3.19 mg/m^3]; this is particularly true for refinery maintenance, laboratory technicians, and dock workers. Specific tasks such as sampling, opening of vessels for maintenance and cleaning, and loading of petrol may cause high short-term exposure (Runion, 1988; Hakkola & Saarinen, 1996; Vainiotalo & Ruonakangas, 1999; Davenport et al., 2000; Verma et al., 2001; Kreider et al., 2010; Widner et al., 2011). However, while workers before 2000 were likely to have been exposed to higher concentrations of benzene because of a higher content of benzene in reformate stream (Burns et al., 2017), the range of benzene exposures reported in recent studies is considerably reduced (Campagna et al., 2012; Akerstrom et al., 2016; Almerud et al., 2017; Burns et al., 2017). Some of the reported exposure levels are given in Table 1.2.

(iii) Downstream petroleum industry: distribution

In the petroleum transport chain there is a potential for exposure at each point where the products are stored and transferred, and the reported exposures tend to be higher than for production and refinery workers (Halder et al., 1986; Javelaud et al., 1998; CONCAWE, 2000, 2002; Glass et al., 2000). However, because of a lowered content of benzene in petrol (Verma & des Tombe, 2002; Williams & Mani, 2015), as well as the recent introduction of vapour recovery systems in the petroleum distribution chain in at least developed countries, the exposure to benzene for these groups of workers has declined over the years. Some of the reported exposure levels are given in Table 1.2.

Williams et al. (2005) reviewed the available industrial hygiene data describing exposure during the marine transport of products

containing benzene (1975–2000). Although there were differences in sampling strategies and in the benzene content of the liquids being transported, air monitoring data revealed concentrations of 0.2–2.0 ppm [0.64–6.4 mg/m³] during closed-loading and 2–10 ppm [6.4–31.9 mg/m³] during open-loading operations. These estimates are somewhat higher than average values, but in line with the range of exposures reported in other reviews (CONCAWE, 2000, 2002; Verma et al., 2001).

(iv) Oil spill clean-up operations

The petroleum production and distribution scenario for which there is a lack of knowledge on exposure levels is the clean-up of an oil spill. In an oil spill field trial in the North Sea in 2016, full-shift measurements of benzene for personnel closest to the slick yielded a geometric mean exposure of 0.2 ppm benzene [0.64 mg/m³] (Gjesteland et al., 2017). No exposure to benzene was detected in personal samples collected during the Deepwater Horizon spill of light crude oil (Ahrenholz & Sylvain, 2011). In the Prestige and Nakhodka spills of heavy fuel oil, the measured benzene exposure was low because of the low content of volatile organic compounds (Morita et al., 1999; Pérez-Cadahía et al., 2007).

(v) Retail petrol stations

Averaged full-shift exposures of up to 0.65 mg/m³ (McDermott & Vos, 1979; Runion & Scott, 1985; Foo, 1991; Lagorio et al., 1993; CONCAWE, 2000, 2002; Verma et al., 2001; Chakroun et al., 2002; van Wijngaarden & Stewart, 2003; Fustinoni et al., 2005) and 59 µg/m³ [0.059 mg/m³] (Carrieri et al., 2006; Lovreglio et al., 2010, 2014; Campo et al., 2016) have been measured before and after 2000, respectively. Reported benzene exposure levels (Table 1.2 and Table 1.3) suggest that, in higher-income countries, at least, they have decreased with time. The decline is mainly ascribed to a decrease in benzene content in gasoline, as well as the installation of vapour recovery systems at retail gas stations capturing vapours during vehicle fuelling. The information on exposure for petrol station attendants in low- and middle-income countries is scarce, but available studies indicate somewhat higher concentrations of benzene for these workers compared with those reported from more developed countries (Navasumrit et al., 2005; Bahrami et al., 2007).

(b) Exposure from engine exhaust

Benzene from engine exhaust represents a potential exposure for professional drivers and urban workers, including taxi drivers, police, street workers, and others employed at workplaces with exposure to exhaust gases from motor vehicles (Nordlinder & Ramnäs, 1987). Reported exposure concentrations for these workers differ with region (Table 1.2). Reported median exposures for traffic police in Italy (2005) and Thailand (2006) were 6.1 µg/m³ and 38.2 µg/m³, respectively (Manini et al., 2008; Arayasiri et al., 2010). Cloth vendors and grilled-meat vendors in Thailand have been reported to have experienced mean exposures of 22.61 ppb [73 µg/m³] and 28.19 ppb [90 µg/m³], respectively (Navasumrit et al., 2005). Although the data for urban workers in low- and middle-income countries are scarce, the available information on both workers and outdoor air concentrations (see Section 1.4.2) indicates exposure to higher concentrations for these workers relative to the levels typical of higher-income countries.

(c) Automobile repair

Workers employed in automobile repair shops and recycling are potentially exposed to benzene through contact with gasoline vapour and engine products. Measured mean exposures before 2000 are typically less than 1 ppm (Nordlinder & Ramnäs, 1987; Foo, 1991; Hotz et al., 1997; Javelaud et al., 1998; Egeghy et al., 2002) (Table 1.2).

(d) Coke production

Benzene exposure is a potential hazard in the carbonization of coal to form coke used in the manufacture of steel, produced in the refining of the crude coke fractions and the by-products. China is currently the largest coke producer globally; average benzene exposure concentrations of 0.705 and 0.290 mg/m^3 were measured during a survey of two plants, where charging and pushing activities accounted for almost 70% of the exposure at the topside (He et al., 2015). During the period 2005–2010 in Poland, median exposures of 0.09–0.37 mg/m^3 according to job category were reported (Bieniek & Łusiak, 2012). Median exposure at a cokery in a shale oil petrochemical plant in Estonia was reported as 0.4 ppm [1.28 mg/m^3] one decade earlier (Kivistö et al., 1997). Average exposure for coke oven workers in the USA during 1978–1983 was reported as 8.46 ppm [27.02 mg/m^3] (van Wijngaarden & Stewart, 2003), while reported mean levels in the United Kingdom in 1986 ranged from 0.31 ppm [0.99 mg/m^3] in coke oven workers to 1.32 ppm [4.22 mg/m^3] in by-product workers (refining process of benzene) (Drummond et al., 1988).

(e) Rubber manufacturing

Benzene has historically been used in the manufacture of rubber, including the production of tyres and general rubber goods, and the process of retreading. It has also been used as a component in cement, glue, binding agents, and release agents, but has mainly been replaced by other agents (IARC, 2012). Some of the solvents used today still contain low benzene concentrations, however. From a pooled dataset on rubber manufacture workers in China used in a nested case–cohort study within the National Cancer Institute-Chinese Academy of Preventive Medicine (NCI-CAPM) cohort (n = 585), arithmetic and geometric means of 157.3 mg/m^3 and 45.6 mg/m^3 (geometric standard deviation, GSD, 6.4 mg/m^3) were reported from 1949 until after 2000 (Portengen et al., 2016). Averages of 1.42 ppm [4.54 mg/m^3] (n = 179) and 0.34 ppm [1.09 mg/m^3] (n = 4358) for rubber manufacture and production of tyres and inner tubes, respectively, have been reported from the USA and Canada (Runion & Scott, 1985; van Wijngaarden & Stewart, 2003).

The exposure levels in a cohort of workers producing rubberized food-coating materials have been estimated several times (Rinsky et al., 1981, 1987; Paustenbach et al., 1992; Crump, 1994; Utterback & Rinsky, 1995). In the latest retrospective assessment, the highest exposures (involving the jobs of neutralizer, quencher, knifeman, and spreader) were typically 50–90 ppm during 1939–1946 (lower during 1942–1945) and 10–40 ppm during 1947–1976 at the 50th percentile (Williams & Paustenbach, 2003). These estimated exposure levels were two to four times as great as for other jobs in this same cohort.

Kromhout et al. (1994) performed an exposure assessment of solvents in 10 rubber-manufacturing plants in the Netherlands in 1988. The use of particular solvents varied widely, and those selected for the quantitative assessment of exposure were based on the individual solvents, cements, and release and bonding agents used in the plants included in the study. The final assessment was restricted to paraffins, aromatic compounds, chlorinated hydrocarbons, ketones, alcohols, and esters. Benzene was not included, suggesting that the products used in the European rubber industry did not contain benzene from the late 1980s (Kromhout et al., 1994).

(f) Shoemaking

Shoemaking consists of several steps, including: the cutting of the material (leather, rubber, plastic, etc.), fitting of parts, sewing and gluing the various parts together, and finally the trimming and buffing of the shoes (Wang et al., 2006). Benzene is used as a solvent in glues, adhesives, and paint in the shoe-manufacturing process. Dermal exposure to benzene has

been reported as low, and does not significantly contribute to systemic exposure of benzene (Vermeulen et al., 2004). Although no longer as relevant in Europe and North America as in the past, this source of occupational benzene exposure is still of importance in some countries, notably in Asia.

In a recent Chinese study of shoe factory workers, mean exposures of 21.86 ppm [69.83 mg/m^3] and 3.46 ppm [11.05 mg/m^3] were reported for a small and large factory, respectively (Vermeulen et al., 2004). Benzene and toluene exposures were partly determined by the degree of contact with glues, the benzene and toluene content of each glue, air movement, and ventilation patterns. From a pooled dataset on workers in the shoemaking industry in China used in a nested case–cohort study within the NCI-CAPM cohort (n = 635), arithmetic and geometric means of 69.2 mg/m^3 and 8.1 mg/m^3 (GSD, 10.8 mg/m^3) were reported for the period from 1949 to after 2000 (Portengen et al., 2016).

In a benzene exposure assessment in 12 Iranian shoemaking workshops (semiautomated, year not given) mean exposures (standard error) for three consecutive months were 1.10 (0.11) ppm [3.51 (0.35) mg/m^3], 1.37 (0.14) ppm [4.38 (0.45) mg/m^3], and 1.52 (0.18) ppm [4.86 (0.57) mg/m^3] (Azari et al., 2012).

In the shoemaking industry in Spain, where benzene was unintentionally present in the adhesive as a contamination, the mean benzene exposure concentrations for the periods 2002–2003, 2004–2005, and 2006–2007 were 0.05 mg/m^3, 0.07 mg/m^3, and 0.05 mg/m^3, respectively (Estevan et al., 2012).

(g) Firefighting

Because of the incomplete combustion and pyrolysis of organic and synthetic materials, respectively, firefighters are potentially exposed to benzene during firefighting (municipal and wildfire), overhaul, and training. The heterogeneity of types of fires, time spent at fires, and types of structure or material burning, as well as the limited collection of data due to the extreme conditions, have hampered the characterization of exposure to benzene by firefighters and data are scarce. However, the few reported data suggest that the full-shift exposure is much less than 0.5 ppm, and is higher for the knockdown of wildfires compared with structure fires (Reinhardt & Ottmar, 2004); the potential for short-term exposure much higher than 1 ppm [3.19 mg/m^3] has also been reported (Bolstad-Johnson et al., 2000; Austin et al., 2001).

(h) Occupational use of products containing benzene

Benzene was formerly a common solvent and ingredient in a variety of products, including paint, printing inks, and glues, and is a natural component in products derived from petroleum. However, the benzene content in these products has either been replaced or reduced following regulations and other initiatives in the 1980s and 1990s.

(i) Application of paint

Benzene has been largely replaced as a solvent in paint, but is still used in some countries. Although this was a significant source of benzene exposure historically, data are lacking on benzene exposure during the use of paint that contains benzene as a constituent or contamination. In a review of benzene exposure in industries using paint in China, combining all the years during 1956–2005, relevant median exposures were reported for many activities, including: spray painting, 43.9 mg/m^3 (maximum, 3212 mg/m^3); brush painting, 58.2 mg/m^3 (maximum, 3373.5 mg/m^3); mixing, 53.6 mg/m^3 (maximum, 139.4 mg/m^3); immersion, 27.4 mg/m^3 (maximum, 540.0 mg/m^3); and paint manufacturing, 15.08 mg/m^3 (maximum, 344.0 mg/m^3) (Liu et al., 2009). From a pooled dataset on spray painting in China (n = 3754) used in a nested case–cohort study within the NCI-CAPM

cohort, arithmetic and geometric means of 62.5 mg/m^3 and 9.4 (GSD, 8.9) mg/m^3 averaged over the period from 1949 to after 2000 were reported (Portengen et al., 2016). The corresponding exposure concentrations for painting (n = 1099) were 115.3 mg/m^3 and 17.1 (GSD, 10.2) mg/m^3. In a pilot study, eight painters in small car repair shops in Italy were reported to have experienced an arithmetic mean exposure of 9.8 mg/m^3 (range, 0.4–53 mg/m^3) over a period of 236–323 min (Vitali et al., 2006). The authors ascribed the benzene exposure mainly to fuel vapour and gasoline used for degreasing and paint dilution.

(ii) Printing industry

Benzene was withdrawn from its significant use as a solvent of printing inks in Europe in the 1950s, but was used in the USA in the rotogravure processes from the 1930s until the beginning of the 1960s (IARC, 1996). Reported mean exposures from the printing industry are 0.58 ppm [1.85 mg/m^3] in the USA (van Wijngaarden & Stewart, 2003), and 0.017 ppm [0.0543 mg/m^3] in the Republic of Korea (Kang et al., 2005), but it is still a concern in some low- and middle-income countries. From the pooled dataset from the NCI-CAPM cohort (n = 232), arithmetic and geometric means of 94.1 mg/m^3 and 8.2 (GSD, 13.0) mg/m^3 averaged over the period from 1949 until after 2000 were reported (Portengen et al., 2016).

(iii) Use of petroleum-based products containing benzene in small amounts

Benzene is a residual component (< 0.1%) in petroleum-based products such as mineral spirit, jet fuel, degreasing agents, and other solvents. There are insufficient data to draw any conclusions on air concentrations generated when using these products, but estimations and reported exposure after simulations and controlled testing performed in relation to lawsuits can be found in several publications (Fedoruk et al., 2003; Williams et al., 2008; Hollins et al., 2013). There have been some reports on exposure to benzene during handling of various types of jet fuel; although exposure concentrations vary between the studies, work tasks, and circumstances, the reported values indicate a potential for exceeding exposures of 1 ppm [3.19 mg/m^3] (Holm et al., 1987; Egeghy et al., 2003; Smith et al., 2010).

(i) Biological monitoring of occupational exposure to benzene

Although the measurement of benzene in air is the most common method of investigating exposure in occupational settings, biomonitoring is considered the best technique as the characteristics of the individual and the use of protective equipment are taken into account. Moreover, when dermal exposure is a consideration, biological monitoring is the only system that can integrate both exposure routes.

A summary of selected studies on occupational exposure to benzene using biological monitoring is provided in Table 1.3. Investigated occupational settings include: the petrochemical industry (Boogaard & van Sittert, 1995, 1996; Kirkeleit et al., 2006b; Bråtveit et al., 2007; Hoet et al., 2009; Carrieri et al., 2010; Fustinoni et al., 2011; Hopf et al., 2012); cookery (Kivistö et al., 1997); and manufacturing, including chemical manufacturing (Boogaard & van Sittert, 1995, 1996; Kivistö et al., 1997), shoemaking (Kim et al., 2006a; Lv et al., 2014), adhesive production, and rubber and paint manufacturing (Waidyanatha et al., 2001, 2004). Exposure to gasoline vapours encountered by filling station attendants, tanker fillers, and fuel tanker drivers (Boogaard & van Sittert, 1995, 1996; Chakroun et al., 2002; Fustinoni et al., 2005; Bahrami et al., 2007; Lovreglio et al., 2010; Campo et al., 2016) and traffic exhaust exposure, such as that incurred by traffic police, and taxi and bus drivers (Fustinoni et al., 2005; Manini et al., 2006; Bahrami et al., 2007), were also investigated. A few studies have

investigated exposure to benzene encountered by firefighters (Caux et al., 2002; Fent et al., 2014).

Benzene is present in a complex mixture of chemicals in the large majority of these settings, although this percentage can be small in the case of gasoline vapours and traffic exhaust fumes, for example.

In 1995 and 1996, Boogaard and van Sittert investigated 184 workers exposed to benzene in various occupational settings (natural gas production platforms, chemical manufacturing, oil refineries, fuel tank drivers, and gasoline attendants), measuring personal benzene exposure and two minor urinary metabolites (t,t-MA and SPMA) in urine samples collected at the end of shifts. Personal exposure ranged from less than 0.01 to 100 mg/m^3. A group of 52 unexposed employees was also investigated as controls. It was estimated that about 4% and 0.1% of the inhaled dose was excreted in urine as t,t-MA and SPMA, respectively, with half-lives of about 5 hours and 9 hours. The correlation between personal benzene exposure and both biomarkers was very good, demonstrating their utility as biomarkers of exposure. Owing to the presence of background levels of t,t-MA in the urine of workers not exposed to benzene, this biomarker would be of limited use for assessing low benzene concentrations (Boogaard & van Sittert, 1995, 1996).

In later years, other studies in China investigated manufacturing workers exposed to high benzene concentrations in the rubber, adhesive, and paint production industries (up to 329 ppm [1051 mg/m^3]) (Waidyanatha et al., 2004) and in factories manufacturing glue, shoes, and sporting goods (up to 107 ppm [342 mg/m^3]) (Qu et al., 2003). Several benzene metabolites, such as urinary phenol, catechol, hydroquinone, t,t-MA, and SPMA, were investigated and all found to be correlated with personal benzene exposure. SPMA and t,t-MA demonstrated their superior ability as biomarkers of recent exposure, however; they were present in lower background concentrations in workers not exposed to benzene and they revealed a higher sensitivity in correlating with lower concentrations of occupational benzene. Urinary unmetabolized benzene was also measured, and demonstrated a very good correlation with personal benzene exposure and with the other urinary biomarkers (Waidyanatha et al., 2001).

Another study in China in 2000 applied urinary biomarkers to assess exposure in 250 shoemaking workers, using 139 clothes manufacturing workers as controls. Biomarkers were consistently elevated when the median benzene exposure level of the group was at or above 0.2 ppm for t,t-MA and SPMA, 0.5 ppm for phenol and hydroquinone, and 2 ppm for catechol (Kim et al., 2006a).

Much lower occupational exposures in fuel tanker drivers, filling station attendants, taxi and bus drivers, and traffic police were reported in Italy, with levels of up to 1017 µg/m^3 [1.017 mg/m^3] (Fustinoni et al., 2005; Manini et al., 2006; Lovreglio et al., 2010; Campo et al., 2016). Only the most specific biomarkers were measured in these studies, including urinary t,t-MA, SPMA, and unmetabolized benzene. These studies reported on the possibility of correlating very low benzene concentrations with both SPMA and urinary benzene, but not with t,t-MA. Moreover, these studies demonstrated the impact of tobacco smoking on the levels of biomarkers; smokers without occupational exposure to benzene had higher levels of benzene biomarkers than non-smoking filling station attendants (Fustinoni et al., 2005).

[The Working Group noted that, considered together, these studies showed that urinary SPMA and unmetabolized benzene are the most specific and sensitive biomarkers for the investigation of low occupational exposures, such as those found in most work settings. They are short-term biomarkers of exposure, and the best sampling time is at the end of the exposure or shift.]

1.4.2 General population exposure

Benzene is present ubiquitously in the environment, for example as a result of emissions from forest fires and volcanoes. However, the major environmental sources of benzene are anthropogenic. Such sources include industrial emissions, the burning of coal and oil, motor vehicle exhaust, and fuel evaporation. The primary route of environmental exposure to benzene is through inhalation, although exposure from ingestion of water and foods contaminated with benzene can also occur (ATSDR, 2007). Exposure to benzene can occur in microenvironments due to the evaporation of gasoline from parked cars in attached garages, while driving, or while pumping gasoline, or by spending time outdoors in close proximity to heavily trafficked areas or gasoline service stations. Benzene is a component of tobacco smoke; exposure therefore occurs when smoking or inhaling sidestream smoke (environmental tobacco smoke) (IARC, 2004).

(a) Outdoor air levels of benzene

Outdoor air concentrations of benzene vary widely throughout the world (see Table 1.4). In a review of air quality data from 42 European countries in 2014, the European Environment Agency reported no exceedances of the annual limit for benzene (5 μg/m^3) (European Environment Agency, 2016). Earlier, Guerreiro et al. (2014) reported that very few (0.9%) monitoring stations in Europe in 2011 exceeded this annual guideline for benzene. Over the period from mid-2009 to November 2012, mean and median benzene levels in northern Italy (Mestre) averaged 1.8 and 1.1 μg/m^3, respectively (Masiol et al., 2014). For a 5-year period from 2009 to 2013, benzene levels as measured at a single monitoring station in Edmonton, Canada, averaged 0.72 μg/m^3 (Bari & Kindzierski, 2017). In 2013, average benzene levels across 343 monitoring stations in the USA ranged from 0 ppb carbon (equivalent to ppb multiplied by the number of carbon atoms) in Queen Valley, a sparsely populated town in southern Arizona, to 8.27 ppb carbon [~1.38 ppb = 4.41 μg/m^3] in Steubenville, an industrial city in eastern Ohio (ATSDR, 2015). Based on data from seven continuous monitors in Tehran, Islamic Republic of Iran, in 2012 and 2013, annual benzene concentrations of 3.444 μg/m^3 were reported (Miri et al., 2016). The highest reported levels were in China, where benzene levels averaged 6.81 ppb [21.75 μg/m^3] over approximately 20 years, with city-specific averages from 0.73 ppb [2.33 μg/m^3] (Hong Kong Special Administrative Region) to 20.47 ppb [65.39 μg/m^3] (Ji'nan) (Zhang et al., 2017).

There is evidence that benzene outdoor air concentrations have declined significantly over time in Europe (> 70% decline during 2000–2014) (European Environment Agency, 2016) and the USA (66% decline during 1994–2009) (EPA, 2010). In addition to long-term trends, levels may vary seasonally. Jiang et al. (2017) reported average benzene concentrations in outdoor air of 502.5, 116.8, 111.21, and 294.8 parts per trillion [1.61, 0.37, 0.36, and 0.94 μg/m^3] in the spring, summer, autumn, and winter, respectively in Orleans, France. Similarly, outdoor air concentrations of benzene in the United Kingdom were reported to vary over the calendar year, with higher levels in the winter than during the summer (Duarte-Davidson et al., 2001).

Disasters may affect short-term air quality. After the Deepwater Horizon oil spill in the Gulf of Mexico in April 2010, mean benzene concentrations in air over the ensuing 5 months averaged 4.83 μg/m^3 (min., 0.12 μg/m^3; max., 81.89 μg/m^3) and 2.96 μg/m^3 (min., 0.14 μg/m^3; max., 290 μg/m^3) in regional and coastal areas of Louisiana, USA, respectively. These concentrations were higher than those measured from six urban areas in the state over the same period, which averaged 0.86 μg/m^3 (min., 0.51 μg/m^3; max., 2.33 μg/m^3) (Nance et al., 2016).

Table 1.4 Environmental monitoring of benzene

Reference	Country, year	No. of samplings	Sampling matrix	Exposure concentration (mean)[a]	Range[a]	Comments
Bruinen de Bruin et al. (2008)	European Union, from 2003	11 13 8 10 7 9 6 17 11 12 3 8	Personal exposures	5.1 (average overall) 2.0 (Helsinki) 2.3 (Leipzig) 3.2 (Brussels) 3.3 (Arnhem) 3.3 (Budapest) 4.1 (Dublin) 4.2 (Nijmegen) 5.2 (Catania) 7.5 (Athens) 8.0 (Nicosia) 8.5 (Milan) 9.4 (Thessaloniki)	NR	7 d average levels of benzene (μg/m^3) measured in indoor work (12 cities; n = 150; AM, 5.1); indoor home (9 cities; n = 59; AM, 3.2); and outdoor work (12 cities; n = 91; AM, 2.7) environments
Masiol et al. (2014)	Italy, 2000–2013	102 074	Outdoor air	1.8	0–10.2	
Miri et al. (2016)	Islamic Republic of Iran, March 2012–March 2013	NR	Outdoor air	3.444	NR	
Bari & Kindzierski (2017)	Canada, 2009–2013	NR	Outdoor air	0.72	Maximum, 3.31	
Jiang et al. (2017)	France, Oct 2010–Aug 2011	49 30 30 56	Outdoor air	502.50 ppt (spring) [1.61] 116.80 ppt (summer) [0.37] 111.21 ppt (fall) [0.36] 294.80 ppt (winter) [0.94]	16.8–2296 ppt [0.05–7.33] 16.6–674.4 ppt [0.05–2.15] 14.6–431.5 ppt [0.046–1.38] 49.8–1163.3 ppt [0.16–3.72]	2 h samples
McMahon et al. (2017)	USA, 2015–2016	116	Drinking-water wells	NR	< 0.026–0.127 μg/L	Benzene detection frequencies (≥ 0.013 μg/L) were 9.3%, 13.3%, and 2.4% in Eagle Ford (Texas), Fayetteville (Arkansas), and Haynesville (Texas) shale hydrocarbon production areas, respectively

Table 1.4 (continued)

Reference	Country, year	No. of samplings	Sampling matrix	Exposure concentration (mean)[a]	Range[a]	Comments
Zhang et al. (2017)	China, 1990–2014	NR	Outdoor air	4.42 ppb (Beijing) [14.12] 14.16 ppb (Guangzhou) [45.23] 6.95 ppb (Shanghai) [22.2] 3.94 ppb (Jiaxing) [12.59] 3.76 ppb (Nanjing) [12.01] 6.30 ppb (Hangzhou) [20.12] 6.61 ppb (Macau) [21.11] 13.09 ppb (Changchun) [41.81] 20.10 ppb (Changzhou) [64.2] 20.47 ppb (Ji'nan) [65.39] 11.36 ppb (Lianyungang) [36.29] 4.71 ppb (Nanning) [15.05] 6.47 ppb (Zhengzhou) [20.67] 6.45 ppb (Dongguan) [20.6] 0.92 ppb (Tianjin) [2.94] 1.89 ppb (Anshan) [6.04] 1.61 ppb (Shenyang) [5.14] 1.42 ppb (Shaoxing) [4.54] 0.94 ppb (Tai'an) [3.00] 0.73 ppb (Hong Kong SAR) [2.33]	NR	

AM, arithmetic mean; d, day(s); NR, not reported; ppb, parts per billion; ppt, parts per trillion; SAR, Special Administrative Region

[a] Exposure concentration and range given in μg/m^3; if published in another unit, the conversion to μg/m^3 is given in square brackets.

(b) Personal exposures to benzene

A study published in 2008 reported on personal monitoring data for benzene collected in 12 European cities, with the lowest arithmetic mean concentration reported for residents of Helsinki, Finland (2.0 µg/m^3), and the highest for residents of Thessaloniki, Greece (9.4 µg/m^3) (Bruinen de Bruin et al., 2008).

(c) Benzene in drinking-water and food

Benzene exposure can occur due to ingestion of water and food contaminated with benzene (ATSDR, 2007). During 1985–2002, the United States Geological Survey detected benzene in 37 of 1208 (3.1%) domestic water well samples that were collected at sites across the country; all but one sample had concentrations that were less than 1 µg/L (Rowe et al., 2007). In 2015 and 2016, a small proportion of the samples from 116 drinking-water (domestic and public supply) wells in the Eagle Ford (9.3%), Fayetteville (13.3%), and Haynesville (2.4%) shale hydrocarbon production areas in Texas and Arkansas, USA, had detectable levels, and all concentrations were less than 0.15 µg/L (McMahon et al., 2017).

Based on a review of studies published during 1996–2013, relatively low concentrations were reported in carbonated beverages and other foodstuffs (< 1 ppb); the highest levels (18 ppb) were found in organ meats (Salviano Dos Santos et al., 2015). Over a 5-year period (1996–2006), the United States Food and Drug Administration evaluated 70 "table-ready" foods. Benzene was found in all of them except American cheese and vanilla ice cream; levels ranged from 1 ppb (in milk-based infant formula and raw strawberries) to 190 ppb (fully cooked ground beef) (Fleming-Jones & Smith, 2003). Medeiros Vinci et al. (2012) detected benzene in 58% of 455 food samples purchased and analysed from four supermarkets in Belgium in 2010, with the highest mean levels found in smoked (18.90 µg/kg) and canned (7.40 µg/kg) fish, as well as in fatty fish (3.1 µg/kg) and ready-to-eat salads (2.79 µg/kg). Mean levels were much lower in non-fatty (0.52 µg/kg) fish, raw meat (0.31 µg/kg), and eggs (below the limit of detection).

(d) Biomonitoring of benzene exposure

Nationally conducted surveys that include a biomonitoring component have documented benzene exposures in the general population (see Table 1.5). Based on data collected as part of the Canadian Health Measures Survey during 2012–2013 for people aged 12–79 years (n = 2488), geometric mean blood benzene concentrations were 0.036 µg/L (Haines et al., 2017). Based on the United States National Health and Nutrition Examination Survey (NHANES) in 2001–2002, 2003–2004, 2005–2006, and 2007–2008, median benzene blood concentrations for the United States population were 0.03 µg/L (n = 837), 0.027 µg/L (n = 1345), 0.026 µg/L (n = 3091), and less than the limit of detection (n = 2685), respectively (US Department of Health and Human Services, 2018). Using NHANES biomonitoring data, Arnold et al. (2013) reported differences in median blood benzene concentrations between those individuals who had pumped gasoline into a car or motor vehicle during the previous 3 days (0.029 µg/L) and those who had not (0.025 µg/L). Benzene concentrations were also higher for individuals who reported having inhaled diesel exhaust during the previous 3 days (0.039 µg/L) compared with those who had not (0.027 µg/L).

Biomonitoring studies have also documented environmental exposure to benzene by measuring metabolites of benzene in urine. The Korean National Environmental Health Survey, which was conducted among adults aged 19 years and older during 2012–2014 (n = 6376), reported geometric mean levels of urinary t,t-MA of 58.8 µg/L (Choi et al., 2017). Among 336 adults (age, 35–69 years) living in central Italy who had cotinine levels less than 100 µg/g creatinine (the cut-off value above that was used to define a smoker), reported median

Table 1.5 Summary of selected studies with biological monitoring of environmental exposure to benzene[a]

Reference	Country, year	Population	*n*	Biomarker concentration (μg/L)[a, b]					Comments
				Urinary t,t-MA	Urinary SPMA	Urinary benzene	Blood benzene	Breast milk	
Haines et al. (2017)	Canada, 2012–2013	Adults (12–79 yr)	2488	NR	NR	NR	GM, 0.036 (0.020–0.067)[c]	NR	CHMS uses a stratified, multistage household-based sampling strategy; sample size indicated is the number of unweighted participants
US Department of Health and Human Services (2018)	USA, 2001–2002	Adults (≥ 12 yr)	837	NR	NR	NR	Median, 0.030 (0.100–0.190)[d]	NR	NHANES uses a complex multistage probability design; sample size indicated is the number of unweighted participants; 25th percentiles not provided
	2003–2004		1345	NR	NR	NR	Median, 0.027 (0.064–0.170)[d]	NR	
	2005–2006		3091	NR	NR	NR	Median, 0.026 (0.056–0.220)[d]	NR	
	2007–2008		2685	NR	NR	NR	Median, < LOD (0.041–0.198)[d]	NR	
	2011–2012		2466	NR	Median, < LOD (1.07–1.95)[d]	NR	NR	NR	
Choi et al. (2017)	Republic of Korea, 2012–2014	Adults (≥ 19 yr)	6376	GM, 58.8 (30.2–118)[c]	NR	NR	NR	NR	
Schoeters et al. (2017)	Belgium, 2003–2004	Adolescents (14–15 yr)	1586	GM, 99 (92–107)	NR	NR	NR	NR	FLEHS uses a stratified clustered multistage design; geometric mean concentrations are adjusted for age, sex, smoking, and creatinine levels

Table 1.5 (continued)

Reference	Country, year	Population	*n*	Biomarker concentration (µg/L)[a, b]					Comments
				Urinary t,t-MA	Urinary SPMA	Urinary benzene	Blood benzene	Breast milk	
Tranfo et al. (2017)	Italy, NR	Adults (35–69 yr) with cotinine < 100 µg/g creatinine	336	85.48 µg/g creatinine	0.23 µg/g creatinine	NR	NR	NR	
Blount et al. (2010)	USA (Baltimore, Maryland)	Women	12	NR	NR	NR	NR	Median, 0.080	Convenience sample via announcements and word of mouth
Protano et al. (2012)	Italy, NR	Children (5–11 yr)	396	127.59 (13.76–972.918) µg/g creatinine	0.62 (0.06–4.35) µg/g creatinine	NR	NR	NR	
Lovreglio et al. (2011)	Italy (Puglia), 2009	Adult men	137	52.0 (< 20 to 734) µg/g creatinine	< 0.03 (< 0.03–5.22) µg/g creatinine	0.08 (< 0.02 to 11.40)	NR	NR	
Fustinoni et al. (2010)	Italy, 2007–2008	Adults (19–75 yr)	108	NR	NR	0.122 (0.083–0.294)[c]	NR	NR	
Fabietti et al. (2004)	Italy (Rome), NR	Women	23	NR	NR	NR	NR	0.06 (0.01–0.18) µg/kg	

CHMS, Canadian Health Measures Survey; FLEHS, Flemish Environment and Health Study; GM, geometric mean; LOD, limit of detection; NHANES, US National Health and Nutrition Examination Survey; NR, not reported; yr, year(s)

[a] Biomarker concentrations are reported as arithmetic mean levels (minimum–maximum) unless indicated otherwise.

[b] Concentrations are given in µg/L (micrograms/L) unless indicated otherwise.

[c] 25–75th percentile

[d] 75–95th percentile

urinary levels of t,t-MA and SPMA were 85.48 and 0.23 µg/g creatinine, respectively (Tranfo et al., 2017). Fustinoni et al. (2010) reported a urinary benzene level of 0.122 µg/L (median) in 108 Italian men and women.

A few studies have examined the exposure of adolescents and children to benzene using biomonitoring data. Geometric mean concentrations of urinary t,t-MA, adjusted for age, sex, smoking status, and creatinine concentrations in adolescents aged 14 and 15 years, were reported by the Flemish Environment and Health Study of 99 µg/L in 2003–2004 (n = 1586), 94 µg/L in 2007–2008 (n = 206), and 61 µg/L in 2013 (n = 204) (Schoeters et al., 2017). Based on urine samples collected from 396 Italian children (age, 5–11 years), Protano et al. (2012) reported mean levels of 127.59 and 0.62 µg/g creatinine for t,t-MA and SPMA, respectively.

In workers who are not exposed to benzene through their occupation, the combined effects of smoking and environmental tobacco smoke contribute, on average, 85% and 23% to total benzene exposure among smokers and non-smokers, respectively (Weisel, 2010). In a 2009–2011 nationally representative study of exposure to volatile organic compounds in Canada, statistically significant differences in indoor residential concentrations of benzene were detected between homes with and without smokers (difference, 1.12 µg/m^3) (Zhu et al., 2013). Geometric mean benzene concentrations in blood were 0.136 and 0.024 µg/L for smokers and non-smokers, respectively, as assessed using biomonitoring data from the 2003–2004 NHANES survey (Kirman et al., 2012). Similarly, Tranfo et al. (2017) reported urinary levels of t,t-MA and SPMA of 141.32 and 1.83 µg/g creatinine in smokers, compared with 90.68 and 0.20 µg/g creatinine in non-smokers, respectively.

1.5 Regulations and guidelines

The International Labour Organization Benzene Convention (C136) Article 2(1) states: "Whenever harmless or less harmful substitute products are available, they shall be used instead of benzene or products containing benzene." This convention was passed in 1971 and ratified by 38 countries (ILO, 1971). The European Union classified benzene as a category I carcinogen under Directive 67/548/EEC (European Commission, 1967). Benzene is not allowed to be placed on the market with the exception of fuel, or used as a substance or as a constituent of mixtures in concentration greater than 0.1% by weight (EU-OSHA, 2006). The USA withdrew benzene from consumer products in 1978 (IARC, 1982).

1.5.1 Occupational exposure limits

(a) USA

The 8-hour permissible exposure and short-term limits set by the Occupational Safety and Health Administration are 1 ppm [3.19 mg/m^3] and 5 ppm [15.95 mg/m^3], respectively (CFR 1910.1028) (OSHA, 2017) (Table 1.6).

Occupational exposure limit (OEL) recommendations for benzene have been made by the American Conference of Governmental Industrial Hygienists (ACGIH). ACGIH recommends a threshold limit value (TLV) during an 8-hour work shift of 0.5 ppm [1.6 mg/m^3] and a short-term exposure limit (STEL) of 2.5 ppm [~8 mg/m^3]. ACGIH also recommends a biological exposure index (BEI) for t,t-MA in urine of 500 µg/g creatinine and for SPMA in urine of 25 µg/g creatinine (ACGIH, 2012). The United States National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL) for the time-weighted average is 0.1 ppm [0.32 mg/m^3] (NIOSH, 2010) and the short-term limit value is 1 ppm [3.2 mg/m^3].

Table 1.6 International occupational exposure limits for benzene

Country	Limit value – 8 h		Limit value – short-term		Remarks
	ppm	mg/m³	ppm	mg/m³	
Australia	1	3.2			
Austria	1	3.2	4	12.8	TRK value (based on technical feasibility)
Belgium	1	3.25			
Canada – Ontario	0.5		2.5		
Canada – Quebec	1	3	5	15.5	
China		6		10 (1)	(1) 15 min average value
Denmark	0.5	1.6	1.0	3.2	
European Union	1	3.25			
Finland	1 (1)	3.25 (1)			(1) Binding limit value
France	1	3.25			
Germany (AGS)	0.6 (1) 0.06 (2)	1.9 (1) 0.2 (2)	4.8 (1)(3)	15.2 (1)(3)	(1) Workplace exposure concentration corresponding to the proposed tolerable cancer risk (2) Workplace exposure concentration corresponding to the proposed preliminary acceptable cancer risk (3) 15 min average value
Hungary				3	
Ireland	1	3			
Israel	0.5	1.6	2.5 (1)	8 (1)	(1) 15 min average value
Italy	1	3.25			skin
Japan	10				
Japan – JSOH	1 (1)(2) 0.1 (1)(3)				(1) Reference value corresponding to an individual excess lifetime risk of cancer (2) Individual excess lifetime risk of cancer 10^{-3} (3) Individual excess lifetime risk of cancer 10^{-4}
Latvia	1	3.25			
New Zealand	1		2.5		
Poland		1.6			
Romania	1	3.25			
Singapore	1	3.18			
Republic of Korea	1	3	5	16	
Spain	1	3.25			Skin
Sweden	0.5	1.5	3 (1)	9 (1)	(1) 15 min average value
Switzerland	0.5	1.6			
The Netherlands		3.25			
Turkey	1	3.25			

Table 1.6 (continued)

Country	Limit value – 8 h		Limit value – short-term		Remarks
	ppm	mg/m³	ppm	mg/m³	
USA – NIOSH	0.1	0.32	1 (1)	3.2	(1) Ceiling limit value (15 min)
USA – OSHA	1		5		
United Kingdom	1				

Current OELs are reported here but are subject to revisions over time

AGS, German Committee on Hazardous Substances; h, hour(s); JSOH, Japan Society for Occupational Health; min, minute(s); NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OEL, occupational exposure limit; OSHA, Occupational Safety and Health Administration; ppm, parts per million; TRK, technical guidance concentration

From GESTIS (2017)

(b) Europe

The European Union and most European countries have an OEL of 1 ppm, as does the Scientific Committee on Occupational Exposure Limits (SCOEL) (from 1991), but a few countries have opted for lower values (Table 1.6). The biological exposure limits set by the committee are 28 µg of benzene per litre of blood and 46 µg SPMA per gram of creatinine (SCOEL, 2014). The OEL set by the European Chemicals Agency (ECHA) is 1 ppm (3.25 mg/m³) (Annex III of Directive 2004/37/EC, European Commission, 2004).

In Germany, the Committee for Hazardous Substances has proposed a tolerable risk of 4 : 1000 and an acceptable risk of 4 : 10 000 (changing to 4 : 100 000), applicable over a working lifetime of 40 years with continuous exposure every working day. For benzene, the tolerable and acceptable risks correspond to 8-hour concentrations of 1.9 mg/m³ and 0.2 mg/m³ (0.02 mg/m³ by 2018), respectively (Bau, 2013).

1.5.2 Environmental exposure limits

(a) Air

The World Health Organization (WHO) states that there is no safe level of exposure to benzene; for general guidance, the concentrations of airborne benzene associated with excess lifetime risks of leukaemia of 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} are 17, 1.7, and 0.17 µg/m³, respectively (WHO, 2000). The benzene air concentration limit in Europe since 1 January 2010 is 5 µg/m³ averaged over 1 year (European Commission, 2008). The maximum limit value for benzene in petrol (gasoline) is 1.0% v/v limit (Directive 2009/30/EC, European Commission, 2009).

The United States EPA has specified cancer risk levels: 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} risk, corresponding to concentrations of 13–45, 1.3–4.5, and 0.13–0.45 µg/m³, respectively. The EPA reference concentration, the estimated continuous inhalation exposure without risk to health, is 3×10^{-2} mg/m³ (EPA, 2000).

The United States Agency for Toxic Substances and Disease Registry has derived minimal risk levels for acute duration (≤ 14 days) of 0.009 ppm, intermediate duration (15–364 days) of 0.006 ppm, and chronic duration (≥ 365 days) of 0.003 ppm (ATSDR, 2007).

WHO guidelines for indoor air recommend reducing indoor benzene concentrations to the lowest achievable level by eliminating indoor sources of benzene and adjusting ventilation (WHO, 2010).

(b) Water

WHO guidelines for drinking-water recommend a maximum concentration of benzene of 0.01 mg/L (WHO, 2003, 2008). The European Council Directive 98/83/EC on the quality of water intended for human consumption (adopted in 1998) has set the benzene limit to 0.001 mg/L water (European Commission, 1998).

The United States EPA sets regulatory limits for the amount of benzene contaminants in water provided by public water systems: specified cancer risk levels of 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} correspond to drinking-water concentrations of 100–1000, 10–100, and 1–10 µg/L, respectively. The EPA reference dose is 4×10^{-3} mg/kg per day (EPA, 2000).

1.6 Exposure assessment methods in epidemiological studies of cancer

1.6.1 Industry-based studies of occupational exposure

Selected epidemiological studies of cancer and occupational exposure are summarized in Table 1.7. The most common metrics of benzene exposure in these studies are the presumption of occupational exposure by duration (years),

Table 1.7 Exposure assessment method for selected occupational epidemiological studies of exposure to benzene

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert exposure estimation for individual participants' work histories based on measured benzene exposure data	Distribution workers in Canadian petroleum industry; retrospective estimates account for job, site, and era in participants' work histories; base estimates from exposure measurements	Mean intensity 0.0–6.16 ppm [0.0–19.6 mg/m^3]; cumulative exposure 0.0–219.8 ppm-yr [0.0–702.1 (mg/m^3)-yr]; dermal exposure ranking	Work histories and site information well characterized; exposure estimates based on personal measurements collected from 1970 onwards; some task-based hydrocarbon measurements used to validate estimates	Relatively few data points for some base estimates; extrapolation back to as early as 1910 increases uncertainty; potential for other hydrocarbon exposures	Armstrong et al. (1996)	Schnatter et al. (1996)
	Marketing and distribution workers in UK petroleum industry; retrospective estimates of each job or task in the participants' work histories; base estimates developed from exposure measurements adjusted using modifying factors (e.g. job activity, % benzene in fuel, loading technology)	Mean intensity < 0.02 to ≥ 0.4 ppm [< 0.06 to ≥ 1.28 ppm]; cumulative exposure < 0.45 to ≥ 45 ppm-yr [< 1.44 to ≥ 143 (mg/m^3)-yr] Peaks by frequency: daily, weekly, monthly; intensity: 1–3 ppm [3–10 mg/m^3], > 3 ppm [> 10 mg/m^3]; peaks by duration: 1–15 min, 15–60 min Potential for skin exposure (none, low medium, high)	Based on measured exposure data; background exposure assigned for 40% work histories less likely to need extrapolation	Limited job history information for participants pre-1975; extrapolation back to as early as 1910 increases uncertainty; relatively few data points for some base estimates; potential for other hydrocarbon exposures	Lewis et al. (1997)	Rushton & Romaniuk (1997)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert exposure estimation for individual participants' work histories based on measured benzene exposure data (cont.)	Upstream, refinery, and distribution workers in Australian petroleum industry; retrospective estimates account for each job or task in the participants' work histories; base estimates from exposure measurements adjusted with modifying factors (e.g. exposure differences over time or between worksites)	Intensity group range ≤ 0.1 to > 3.2 ppm [≤ 0.32 to > 10.2 mg/m^3]; cumulative exposure mean and range 4.7 (0.01–57.3) ppm-yr [15.0 (0.03–183) (mg/m^3)-yr]; peak as exposure to products with > 70% benzene	Work histories and site information from companies well established; estimates based on measured exposure data; the majority of participants' exposure in 1970s, so less extrapolation needed	Relatively few data points for some estimates; extrapolation back to as early as 1955 increases uncertainty; potential for other hydrocarbon exposures	Glass et al. (2000)	Glass et al. (2003)
	Petroleum industry workers from Canada, UK, and Australia (see Armstrong et al., 1996; Lewis et al., 1997; Glass et al., 2003); exposure assessment by individual study; pooled data compared and adjusted by country	Mean average intensity (SD) 0.22 (0.7) ppm [0.7 (2.24) mg/m^3], mean maximum intensity (SD) 0.41 (1.3) ppm [1.31 (4.2) mg/m^3]; median cumulative exposure (SD) 5.2 (17.0) ppm-yr [16.6 (54.3) (mg/m^3)-yr]; peaks > 3 ppm [10 mg/m^3] for 15–60 min; dermal exposure likelihood; exposure certainty ranking	All studies used measured exposure data; exposure estimation quality scores allowed sensitivity analyses	Relatively few data points for some base estimates; some extrapolation back to pre-1920; different countries, industry sectors, and eras may limit comparability of exposure estimates; potential for other hydrocarbon exposures	Armstrong et al. (1996), Lewis et al. (1997), Glass et al. (2003, 2010, 2017)	Schnatter et al. (2012), Rushton et al. (2014)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert exposure assessment for individual participants' work histories based on measured benzene exposure data, taking account of job title, site, and era	Workers in two USA waterproof cloth manufacturing facilities; JEMs based on air sampling data and detailed work histories to provide individual time-specific exposure estimates; Rhomberg et al. (2016) used Monte Carlo techniques to estimate exposures used in tertile, quartiles, and quintiles	Cumulative exposure 0.001 to > 400 ppm-yr [0.003 to > 1280 (mg/m^3)-yr] (Rinsky et al., 1987), 6.64 ppm [21.2 mg/m^3] platform, 10.46 ppm [33.4 mg/m^3] scrap area (Utterback & Rinsky, 1995)	Occupational hygiene measurements for some sites; exposure estimates adjusted to era; compared exposure estimates with contemporary TLVs; no other exposures	Limited occupational hygiene measurements at some sites; some measurements are spot samples and area samples (not personal); accuracy of detector tube and combustible gas indicator measurements unclear	Rinsky et al. (1981), Paustenbach et al. (1992), Crump (1994), Utterback & Rinsky (1995)	Schnatter et al. (1996), Rinsky et al. (2002), Rhomberg et al. (2016)
Expert assessment using exposure measurements grouped by work characteristic (e.g. job title, work area, industry)	Workers in USA chemical plant; job titles of workers in three areas of a chemical plant assigned to four exposure categories based on measured data	Cumulative exposure groups: 0–3.9, 4.0–24.9, and > 25 ppm-yr [0–12, 13–79, and > 80 (mg/m^3)-yr] (Collins et al., 2015)	Measured exposure data available, adjusted for time period, department, and job	Some estimates based on few personal measurements per job (extent of personal data unclear); most exposures before 1980 estimated without personal exposure data (Collins et al., 2003); limited detail on exposure estimation methods; potential exposure to other known or suspected human carcinogens	Bloemen et al. (2004)	Collins et al. (2003), Bloemen et al. (2004), Collins et al. (2015)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Categoric expert assessment of exposure to form industry JEM by era (1970–1979, 1980–1989, 1990–1999, 2000–2009)	Workers in Norwegian offshore petroleum industry; industry experts coded workers' job histories into 27 job categories in five job sections; exposure burden score created for each job by summing task scores (multiplying categoric scores from intensity, duration, and frequency); job-STEL scores created by summing task-STEL scores (adjusting categoric scores for frequency of task and peak/task)	Intensity 0–0.040 ppm [0–0.130 mg/m^3]; cumulative exposure 0–0.948 ppm-yr [0–3.03 (mg/m^3)-yr] (Stenehjem et al., 2015); STEL probability score	Expert job grouping, some personal measurement data; limited range of tasks and exposures all likely low; extrapolation back to 1970 only	Limited benzene exposure data available (most post-2000); personal measurement data did not cover all jobs and time periods; potential exposure to other known and suspected human carcinogens	Steinsvåg et al. (2007, 2008), Bråtveit et al. (2011, 2012)	Stenehjem et al. (2015)
Expert assessment of job title/area use of JEM to derive exposure estimates	Workers in 672 Chinese facilities in range of industries in 12 cities (712 factories in Yin et al., 1994); estimated using ambient exposure measurements, and production and process information for seven calendar periods for each job title; individual work histories linked to measured exposure data	For leukaemia cases intensity 6.5–487 mg/m^3, cumulative exposure 37.7–5438.4 (mg/m^3)-yr (Yin et al., 1989)	Individualized exposure assessments based on participants' work histories; exposure estimates predictive of benzene poisonings in a validation paper (Dosemeci et al., 1997)	Comparatively few measured data points in pre-1975 period; most measurements based on short-term ambient samples, not personal measurements; overall, 22% of estimates have a high confidence rating	Dosemeci et al. (1994, 1997), Yin et al. (1994), Portengen et al. (2016)	Yin et al. (1996b), Hayes et al. (1997), Linet et al. (2015)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert assessment of job title and allocation of ppm exposure estimates, then grouped into categories	Chemical workers from seven USA plants; jobs classified as continuous, intermittent, or no exposure; exposure in ppm assigned to tasks in jobs; estimates varied by era	Cumulative exposure presented in three exposure categories: < 180, 180–719, ≥ 720 ppm-mo [< 575, 575–2297, ≥ 2300 (mg/m^3)-mo] (Wong, 1987b)	Exposure based on measured data adjusted for production and process changes for five of the seven plants	Exposure data sparse before 1970, requiring extrapolation to pre-1910; proportion of exposure data obtained via personal versus fixed ambient sampling unclear; limited employment records for two of the seven plants; potential for other unspecified chemical exposures	Wong (1987a,b)	Wong (1987a,b)
Expert assessment from job title based on measured hydrocarbon exposure data and extrapolation for era	Distribution workers in USA petroleum industry; 8-h TWA THC exposure (for each task and summed) and annual frequency of peak exposures estimated for eight job categories in case–control study (four for cohort study) for four eras (pre-1950, 1950–1964, 1965–1974, 1975–1985)	Cumulative exposure in ppm-yr, peak of at least 500 ppm [1600 mg/m^3]; THC averaged over 15–90 min	Individualized exposure assessments based on participant work histories using measured personal and ambient sample data	Based on THC, not benzene; few data for certain jobs; measured data from 1975–1980 extrapolated to earlier periods back to pre-1950	Smith et al. (1993)	Wong et al. (1993, 1999)
Expert assessment of workplace to form JEM, then applied to participants' job histories	Workers in Italian shoe factory; questionnaires used to gather data on determinants of exposure (e.g. amount of glue, % benzene, production rate, work process changes over time); modelled exposure estimates used to form JEM	Intensity 0–92 ppm [0–294 mg/m^3], mean cumulative exposure (SD) 58.4 (93.9), range 0–522.4 ppm-yr [0–1670 (mg/m^3)-yr] (Seniori Costantini et al., 2003) calculated; dichotomization at 40 ppm-yr [128 (mg/m^3)-yr] used (Costantini et al., 2009)	Model validated using measured exposure data; historical changes in benzene concentration in glue and work processes characterized	Complete job history data available only for 16% cohort; no measured personal exposure data; potential for other exposures (i.e. solvents in glues) unspecified	Seniori Costantini et al. (2003)	Costantini et al. (2009)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert assessment of job title/area; industry JEM converted to ppm exposure estimates	Gas and electric utility workers in France; TWA estimations (expressed in units of exposure) based on JEM	Cumulative unit-yr (conversion to ppm-yr); range 0 to ≥ 1.98 ppm-yr [0–6.32 (mg/m^3)-yr])	Estimates considered measured occupational data from other studies; exposure estimates accounted for changes in % benzene in petrol	Relative exposures between groups only (e.g. "use of solvents" for cleaning or degreasing and "exposure to gasoline"); no measured data from the population of interest; potential for other exposures (e.g. herbicides, chlorinated solvents, styrene, ionizing radiation)	Guénel et al. (2002)	Guénel et al. (2002)

h, hour(s); JEM, job–exposure matrix; min, minute(s); mo, month(s); ppm, parts per million; SD, standard deviation; STEL, short-term exposure limit; THC, total hydrocarbons; TLV, threshold limit value; TWA, time-weighted average; yr, year(s)

average exposure intensity (ppm or mg/m^3), or cumulative exposure, which is the intensity of exposure multiplied by the number of years exposed (ppm-years or (mg/m^3)-years). These metrics indicate that inhalation is the major route of entry, although some studies have also considered dermal exposure (e.g. Lewis et al., 1997; Schnatter et al., 2012; Rushton et al., 2014). The likelihood of peak exposure (using various definitions of peak) has also been examined in some studies (e.g. Lewis et al., 1997; Schnatter et al., 2012; Stenehjem et al., 2015).

The main sectors where exposure assessment for benzene has been carried out for epidemiological studies are the petroleum industry (e.g. Wong et al., 1993 ; Armstrong et al., 1996 ; Lewis et al., 1997 ; Glass et al., 2000; Steinsvåg et al., 2007, 2008; Bråtveit et al., 2011), the chemical industry (e.g. Wong, 1987a, b), and industries that use benzene in manufacturing processes (e.g. Rinsky et al., 1981; Yin et al., 1994; Utterback & Rinsky, 1995), including shoemaking (Seniori Costantini et al., 2003).

Exposure to benzene is often assessed by experts who group workers by job or facility (where appropriate) and then assign exposure to each person using a job–exposure matrix, which may have a time dimension. The exposure estimates in the job–exposure matrix may be quantitative, that is, based on personal and/or area benzene sampling (Rinsky et al., 1981), or they may be semiquantitative. Relative measures can later be translated into benzene concentration (e.g. ppm or mg/m^3) (Guénel et al., 2002; Bråtveit et al., 2012). Very large studies where multiple experts examine different facilities can increase variability in assessments, but this can be mitigated by standardization across facilities (e.g. Portengen et al., 2016).

Most cohort studies and their nested case–control studies are retrospective, with some including participants from as early as 1910 (Wong, 1987a, b; Armstrong et al., 1996; Lewis et al., 1997). Because exposure data were sparse before 1970, the validity of exposure estimates extrapolated to earlier time periods may be uncertain (e.g. Rinsky et al., 1981; Utterback & Rinsky, 1995; Collins et al., 2015). Even for recent time periods, measured data may not be available or may be inadequate to describe exposures from all jobs. In some studies, data from one facility may be attributed to workers at a similar facility, for example offshore workers on different platforms (Bråtveit et al., 2012; Stenehjem et al., 2015). These differences in data availability may result in varying exposure assessments and outcomes (see Section 2.1.1).

Personal sampling data became more common from the 1970s onwards. Recent studies are therefore more likely to assess exposure using personal measurement data, from which more robust exposure estimates can be derived. It is preferable to assess a high proportion of the participants' time at risk of exposure with contemporary exposure measurement data (Glass et al., 2000; Vlaanderen et al., 2010). When personal measurement data are available, temporal and between-worker exposure variability should be considered (Kromhout et al., 1993).

Changes in facilities over time have been considered in some studies listed in Table 1.7; for example, Dosemeci et al. (1994) and Wong (1987a) took production rate into account. Portengen et al. (2016) used a modelling process to consider several factors affecting exposure. Some studies incorporated factors to account for changes over time and between sites, for example due to changing technology and variations in products handled (Armstrong et al., 1996; Lewis et al., 1997; Glass et al., 2000).

Uncertainty is also introduced when exposure to benzene is based on modelling from total hydrocarbon exposure, as the proportion of benzene may vary with the source of the hydrocarbons (e.g. Smith et al., 1993).

Studies based mainly on grab or area sampling data (e.g. Rinsky et al., 1981; Dosemeci

et al., 1994; Yin et al., 1994) have been used to derive average long-term exposure estimates, which can be less certain than those based on individual-level measurement data collected over longer periods (e.g. full work shifts).

Other exposures may have been incurred by participants in the studies listed in Table 1.7, for example, from other hydrocarbons for petroleum industry workers. Coexposures identified in these studies are listed in the limitations column. Some coexposures, for example styrene, have been associated with an increased risk of leukaemia (e.g. Guénel et al., 2002). Other exposures may not have been described, including low exposure to X-rays for some petroleum industry workers and possibly 1,3-butadiene for some refinery workers (Akerstrom et al., 2016; Almerud et al., 2017).

The application of validation methods can increase confidence in the exposure estimates. Such methods include the use of exposure estimation quality scores (e.g. Schnatter et al., 2012) and the assessment of interrater agreement (e.g. Steinsvåg et al., 2008).

1.6.2 General population studies

(a) Childhood cancer

Epidemiological studies focused on associations between benzene in outdoor air pollution and risks of childhood cancer in Denmark, France, Italy, and the USA. Primary methods to assess exposure to benzene are summarized for selected studies in Table 1.8, which provides a summary of the general approach and metric(s) that were used, along with strengths and limitations. All the studies used a geographical information system (GIS) to manage spatially referenced data from different sources in their benzene exposure assessments.

One investigation (Heck et al., 2014) used routine air monitoring data from 1990 to 2007 (collected every 12 days) from 39 monitors in the state of California (163 696 square miles or 423 970 km^2), USA, and developed exposure estimates by linking maternal residences to the closest outdoor air monitor. However, not all monitors were operating throughout the study period; for example, in 2008 there were only 17 benzene monitors in operation (Cox et al., 2008). In addition, stationary monitors were often sited near heavy industry, busy freeways, or in agriculturally rich areas (Heck et al., 2014).

All other key studies relied on Gaussian dispersion models to predict outdoor benzene concentrations in air, for example: the California Line Source Dispersion model, version 4 (CALINE4) (Vinceti et al., 2012), the Danish Operational Street Pollution Model (Raaschou-Nielsen et al., 2001), or the EPA Assessment System for Population Exposure Nationwide (ASPEN) (Symanski et al., 2016; Janitz et al., 2017). Developed by the Department of Transportation in California, USA, CALINE4 is an air dispersion model for roads (and other linear air pollutant sources) used to estimate outdoor air concentrations of benzene and other contaminants at defined locations in a given area. The National-Scale Air Toxics Assessment (NATA) uses ASPEN, a dispersion model that relies upon a national inventory of emissions data for hazardous air pollutants, as well as other characteristics that affect the fate and transport of pollutants in the environment (e.g. the rate, location, and height of release of pollutants, and wind speed and direction).

The CALINE4 model used in the Italian study by Vinceti et al. (2012) used locally collected traffic flow data for a single year, but relied on vehicular emission factors over a longer period (1990–2007). One drawback in using the ASPEN model is that modelled estimates are only available for selected years (i.e. 1996, 1999, 2002, 2005, and 2011). Symanski et al. (2016) used all available estimates at the time of their study (until 2005) whereas Janitz et al. (2017) relied on data for a single year (2005). Because the NATA model inputs change over time, Symanski et al. (2016)

Table 1.8 Exposure assessment from selected environmental epidemiological studies of benzene exposure

Exposure assessment method	Location	Exposure metrics reported	Strengths[a]	Limitations[a]	Reference
Childhood cancers: routine air monitoring data					
Outdoor air measurements of benzene obtained from stationary (state) regulatory monitors that collected 24-h samples every 12 d; linked to geocoded participant residences (buffer of 2 km for ALL and 6 km for AML)	California, USA	Residential benzene levels (μg/m^3) calculated for each maternal trimester of pregnancy, entire pregnancy period, and child's first year of life	Exposure estimates available for pregnancy and childhood (first year of life) periods	Variable distances between residences and closest monitor; unable to account for residential mobility during pregnancy or the first year of life	Heck et al. (2014)
Childhood cancers: Gaussian dispersion models					
Modelling outdoor air concentrations of benzene from vehicular emissions at geocoded residential addresses using CALINE4 (considers traffic flow, vehicle emissions factors, and meteorological data)	Northern Italy	Quartiles of average annual residential benzene levels < 0.10, ≥ 0.10–0.25, ≥ 0.25–0.50, and ≥ 0.50 μg/m^3; quartiles of maximum hourly residential benzene levels < 2, ≥ 2–4, ≥ 4–6, and ≥ 6 μg/m^3	Validation conducted with air measurements from monitoring stations; considered coexposures to PM_{10}	Uncertainty associated with emissions and traffic data sources, and use of a single calendar year to estimate exposures; limited validation due to small number of air monitoring stations; Pearson's correlation coefficient of 0.43 between modelled and monitored (n = 6 monitors) data; unable to account for residential mobility during childhood (up to 14 yr from birth)	Vinceti et al. (2012)
Modelled annual census tract level estimates of outdoor benzene levels for 1996, 1999, 2002, and 2005 from the US EPA NATA linked to geocoded maternal addresses at birth of infants	Texas, USA	Quartile estimates of outdoor benzene levels (based on the distribution in the controls for each NATA year): low, medium, medium–high, and high	NATA estimates account for point, mobile, and area sources of benzene emissions; considered coexposures to 1,3-butadiene and PAHs	Modelled annual estimates available only at the census tract level for specific year (1996, 1999, 2002, and 2005); unable to account for residential mobility during pregnancy and early childhood (up to 4 yr from birth)	Symanski et al. (2016)
Address at birth linked to the census tract concentration for benzene using the 2005 US EPA NATA database (see Symanski et al., 2016)	Oklahoma, USA	Quartiles of estimated outdoor benzene levels: 0.11 to < 0.39, 0.39 to < 0.67, 0.67 to < 0.91, and 0.91–2.03 μg/m^3	NATA estimates account for point, mobile, and area sources of benzene emissions	Modelled annual estimates of outdoor benzene levels for 1 yr only (2005) to assess exposure at birth; unable to account for residential mobility during pregnancy and childhood (up to 19 yr from birth)	Janitz et al. (2017)

Table 1.8 (continued)

Exposure assessment method	Location	Exposure metrics reported	Strengths[a]	Limitations[a]	Reference
Annual benzene concentrations for grids of area 10 m^2 (Paris), 25 m^2 (inner suburbs), or 50 m^2 (outer suburbs), estimated from a dispersion model linked to air monitoring data; benzene estimates linked to geocoded addresses at the time of diagnosis (cases) or inclusion (controls), and data on proximal roadways using the Navteq database	Paris and surrounding areas, France	Subjects were classified based on whether estimated annual benzene concentration at their residence was < 1.3 μg/m^3 (median exposure for the controls) or ≥ 1.3 μg/m^3; major road length classified as low, medium, and high	Use of an air dispersion model to account for vehicular emissions, meteorology, and information on fate, transport, and transformation of pollutants; inclusion of a term to account for local traffic in the exposure metric	Unable to account for residential mobility before time of diagnosis (up to 14 yr from birth)	Houot et al. (2015)
Modified version of the Operational Street Pollution Model used to estimate average residential exposure to benzene; estimated air concentrations based on measurements made during 1994–1995 at four sites; included other info (e.g. traffic pattern, vehicle emission factors, meteorology); residential history ascertained from the Danish population registry	Denmark	Tertiles of cumulative exposure to benzene (in 1000 ppb-d): < 0.5, 0.5 to < 1.3, and ≥ 1.3	Temporally resolved estimates during pregnancy and during the child's life; accounted for residential mobility; validation conducted with 204 air measurements in urban and rural locations	Did not account for sources of pollution other than traffic; benzene exposure estimated using measurements taken after case diagnosis (1968–1991); validation results indicate poor correlation in rural areas between modelled and monitored results	Raaschou-Nielsen et al. (2001)
Cancer in adults					
Self-reported via completed questionnaires	Rochester, Minnesota, USA	Ever exposed regularly to benzene or derivative (yes/no)	May capture exposure to benzene in work and non-work environments	Little contrast in exposure metric; recall of exposures may not be accurate	Antwi et al. (2015)
Address at baseline was linked to the census tract concentration for benzene using the 2005 US EPA NATA database (see Symanski et al., 2016)	California, USA	Quintile estimates of outdoor benzene levels	NATA estimates account for point, mobile, and area sources of benzene emissions	Use of modelled annual estimates of outdoor benzene levels for 1 yr (2005) to assess exposures at baseline; unable to account for residential mobility during follow-up period; no information about exposure in non-residential environments, exposure to cigarette smoke, and housing characteristics that may influence exposures at home	Garcia et al. (2014)

Table 1.8 (continued)

Exposure assessment method	Location	Exposure metrics reported	Strengths[a]	Limitations[a]	Reference
Reconstructed levels of benzene (from multiple sources of contamination) in the water distribution system using fate and transport models linked to residential histories of navy and marine personnel stationed at the base	US Marine Corps Base, Camp Lejeune, North Carolina	Quartiles of cumulative exposure (μg/L-mo) to benzene: < 2, 2–45, > 45–110, and > 110–601	Rigorous methods used to reconstruct solvent contamination of drinking-water sources	Inaccuracies in residential histories likely; did not account for time spent away from the base for training or deployment	Bove et al. (2014)

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CALINE4, California Line Source Dispersion Model, version 4; d, day(s); h, hour(s); mo, month(s); NATA, National-Scale Air Toxics Assessment; PAHs, polycyclic aromatic hydrocarbons; PM_{10}, particulate matter of diameter < 10 μm; ppb, parts per billion; US EPA, United States Environmental Protection Agency; yr, year(s)

[a] List not exhaustive

conducted a sensitivity analysis by limiting the study population to cases and controls born within 1 year of a NATA release; estimated odds ratios were similar in magnitude, but less precise. Two investigations focused their assessments on exposures due to emissions from vehicular traffic: Raaschou-Nielsen et al. (2001) and Vinceti et al. (2012).

Houot et al. (2015) derived final estimates at geocoded locations using geostatistical methods that combined the dispersion modelled data for a 10 m^2 grid in the city of Paris, a 25 m^2 grid in the inner suburb, and a 50 m^2 grid in the outer suburb with available air monitoring data.

Studies based in the USA (Symanski et al., 2016; Janitz et al., 2017) used NATA estimates that were generated for all census tracts within the continent of North America. Census tract boundaries are drawn based on population size (average population size, 4000 people) and therefore vary by size and shape.

Because the exposure assessments in the reviewed studies relied on a records-based linkage to develop the exposure metrics, there was no response or recall bias in the exposure assessments. The study by Raaschou-Nielsen et al. (2001) offered an advantage over other studies because it addressed residential mobility in estimates of cumulative exposure; residential histories obtained from a national database, from 9 months before birth to the time of diagnosis, were used. All other studies relied on a single residence (either at birth or at the time of diagnosis) upon which to base the exposure assessment. The use of a single residence may have increased uncertainty in the exposure assessments, particularly in studies that included children diagnosed at older ages (e.g. 14–19 years) (Vinceti et al., 2012; Houot et al., 2015; Janitz et al., 2017).

A strength of the studies by Raaschou-Nielsen et al. (2001) and Heck et al. (2014) was their ability to construct temporally resolved estimates of exposure during pregnancy and childhood that allowed for an assessment of exposure at different life stages. However, none of the studies incorporated information on time spent away from the residence for the mother or the child and, by not accounting for exposures in other environments (e.g. maternal exposures at work), uncertainty in the exposure assessments was likely introduced.

Outdoor air includes multiple pollutants from diverse natural and anthropogenic sources; the air pollutant mixture can therefore vary both locally and regionally. Methods for addressing multiple exposures included the application of co-pollutant models (Symanski et al., 2016) and factor analysis (Heck et al., 2014). Information on indoor air sources of benzene (e.g. environmental tobacco smoke) was unavailable in all studies, as was information on housing characteristics (e.g. living in a residence with an attached garage); only one investigation had information about maternal smoking (Symanski et al., 2016).

In most of the studies, the control population (all of the investigations in Table 1.8 used a case–control study design) represented the source population and was therefore unlikely to be affected by exposure-related selection bias. However, some bias may have been introduced in the investigation by Heck et al. (2014) who excluded 2978 cases and 142 188 controls from the parent study because residences were not within defined buffers around a stationary air monitor (2 km for acute lymphoblastic leukaemia and 6 km for acute myeloid leukaemia). Vinceti et al. (2012) also excluded individuals living in mountainous areas (< 10% of the total population in the study area) because the CALINE4 dispersion model was not developed to incorporate rocky terrain in predicting air pollutant concentrations near roadways.

Vinceti et al. (2012) presented results from a validation study and, based on measurements collected at six monitoring stations, reported a modest correlation (Pearson correlation coefficient, 0.43) between the CALINE4 modelled

estimates and outdoor air benzene levels. Raaschou-Nielsen et al. (2001) compared the results from their dispersion model with passive sampler measurements of benzene at various street locations in Copenhagen, Denmark and in rural areas. Pearson correlation coefficients of 0.62–0.68 were reported for urban locations (range in values based on differences in meteorological inputs); correlations were much lower for rural locations (0.15–0.19) where there is little variation in traffic levels. Regarding the NATA data, previous studies reported good agreement between the ASPEN modelled estimates and monitored levels of benzene in ambient air (Symanski et al., 2016).

(b) Cancer in adults

Studies on cancer risks associated with environmental benzene exposure have used a variety of approaches in their exposure assessments (see Table 1.8 for a summary).

In a nested case–control study of 82 cases and 83 controls among lifelong never-smokers of the Shanghai Cohort Study (a prospective cohort of 18 244 Chinese men, aged 45–64 years at enrolment) (Yuan et al., 2014), exposures to benzene were assessed using SPMA based on measured concentrations of stored urine samples collected at baseline. While SPMA is a specific biomarker for benzene exposure, its half-life in the body is relatively short; relying on a single urinary measurement of SPMA is problematic as it is not representative of average exposure.

Two drinking-water systems at the United States Marine Corps Base, Camp Lejeune, North Carolina were contaminated with tetrachloroethylene and other solvents, including benzene, from 1975 until February 1985. Bove et al. (2014) reconstructed monthly contaminant levels in the water distribution system using fate and transport models; these were linked to residential histories of marine and navy personnel living at the base to generate lagged (10-, 15-, and 20-year) and unlagged estimates of cumulative exposure. Exposures may have been misclassified due to errors in the reconstructed levels of benzene in the water distribution system, as well as inaccuracies in identifying units assigned to the base, in determining the location of the barracks or housing for marine/navy personnel with families, or in accounting for time spent away from the base for training or deployment.

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2. CANCER IN HUMANS

The published evidence on the association between benzene exposure and cancers of the lymphatic and haematopoietic system was last reviewed in *IARC Monographs* Volume 100F (IARC, 2012a), when it was concluded that there was *sufficient evidence* in humans for acute myeloid leukaemia (AML)/acute non-lymphocytic leukaemia (ANLL) and *limited evidence* for acute lymphocytic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), multiple myeloma (MM), and non-Hodgkin lymphoma (NHL).

This Working Group reviewed the association between benzene exposure and cancers of the lymphatic and haematopoietic system again, including those studies considered in *IARC Monographs* Volume 100F as well as studies published since that review in 2009. According to the 2017 *WHO Classification of Tumours of Haematopoietic and Lymphatic Tissues* (Swerdlow et al., 2017), the Working Group considered AML and myelodysplastic syndrome (MDS) as well as chronic myeloid leukaemia (CML) and myeloproliferative disorder (MPD) in the broader category of leukaemia; the category of lymphomas was considered to include NHL as well as its various subtypes (e.g. MM, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and hairy cell leukaemia (HCL)), CLL, ALL, and Hodgkin lymphoma (HL). These studies are reviewed in Sections 2.1 and 2.2, respectively.

The Working Group also reviewed all available studies of the association between benzene exposure and other cancers in children and adults published before and after *IARC Monographs* Volume 100F. These reviews are presented in Sections 2.3 and 2.4, respectively.

Studies of adult cancers in occupational cohorts and in the general population are considered separately in Sections 2.1, 2.2, and 2.4, due to differences in approaches to the assessment of benzene exposure and the analysis of data according to the study setting.

Although tobacco smoke is an important source of benzene exposure for the population at large, accounting for half of population exposure to benzene in the USA (American Cancer Society, 2016), the Working Group did not review studies of smoking-related exposures, because tobacco smoke contains numerous correlated components that could confound the effects of benzene. Studies of tobacco smoking and exposure to secondhand tobacco smoke are reviewed in *IARC Monographs* Volume 100E (IARC, 2012b).

2.1 Adult leukaemia

2.1.1 Occupational cohort studies

(a) Introduction

This section reviews epidemiological studies of leukaemia in occupational cohorts, including occupational cohort studies and nested case–control analyses of such studies. Data on adult leukaemia in non-occupational cohort studies

and population-based case–control studies are reviewed in Section 2.1.2.

Benzene was first classified as a human carcinogen with *sufficient evidence* in *IARC Monographs* Supplement 1 and Volume 29 (IARC, 1982). Substantial support for this classification has since come from associations between exposure to benzene and leukaemia, particularly AML/ANLL, in several occupational cohorts described in *IARC Monographs* Supplement 7 (IARC, 1987) and later in *IARC Monographs* Volume 100F, compiled in 2009 (Baan et al., 2009; IARC, 2012a).

Among the studies that were published after the period covered by *IARC Monographs* Volume 100F, the Working Group chose not to consider results for broad aggregations of different cancer types, including "haematopoietic cancers", "leukaemia", or "myelogenous leukaemia" (Richardson, 2009; Merlo et al., 2010; Koh et al., 2011, 2014; Bonneterre et al., 2012); these diagnostic categories are not specific enough or sufficiently informative. Studies of occupational groups where exposure to benzene was not clearly documented and characterized were also excluded (Gudzenko et al., 2015). First, the main features of occupational cohort studies considered in this chapter are described (Table 2.1). Leukaemia risks associated with benzene exposure by histological type are described in the following sections for each of the cohort studies.

(b) Studies published since IARC Monographs Volume 100F

(i) Petroleum distribution workers

Three cohort studies of petroleum distribution workers conducted in Australia (Glass et al., 2003), Canada (Schnatter et al., 1996), and the United Kingdom (Rushton & Romaniuk, 1997) were updated with new cases of cancers of the lymphatic and haematopoietic system diagnosed up until December 2006 (Australia), 1994 (Canada), and 2005 (United Kingdom), and were pooled for reanalysis using a nested case–control study design (Schnatter et al., 2012). Only male cases and matched controls were included in the analysis (370 leukaemia cases and 1587 controls). All leukaemia diagnoses were reviewed by haematopathologists, who reclassified 8 leukaemia cases of the original publications to MDS or MPD. Benzene exposure was reassessed to allow comparability among the three studies, using exposure measurement data and individual work histories obtained from company records in Canada and the United Kingdom, or from trained interviewers in Australia. Six exposure metrics were derived: cumulative exposure (ppm-years), average intensity (ppm), maximum intensity (ppm, i.e. the highest job-specific exposure estimate), duration of employment (years), peak exposure (yes/no, when employed in a particular job for at least 1 year and having experienced > 3 ppm exposure for 15–60 minutes at least weekly), and dermal exposure (no, low, medium, high; defined as the highest job-specific probability of skin contact for at least 1 year). [The strengths of this study included the high quality of the assessment of benzene exposure and of diagnostic classification. The size of the study was relatively large, but small numbers were available in some subgroup analyses. Scarce or no information on potential confounders (e.g. smoking or multiple exposures other than benzene at the workplace) was available.]

(ii) Dow Chemical workers, Midland, Michigan

A retrospective cohort mortality study of 2266 workers exposed to benzene at Dow Chemical plant in Michigan (USA) (Bloemen et al., 2004) (included in *IARC Monographs* Volume 100F, Table 2.1, available at: http://publications.iarc.fr/123) was later updated (Collins et al., 2015). Vital status and cause of death were derived from the company's research database, regularly updated from several sources including the National Death Index. The follow-up, starting in 1940, was extended by

Table 2.1 Occupational cohort studies of exposure to benzene and leukaemia subtypes in adults

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schnatter et al. (2012) Australia, Canada, UK 1981–2006 (Australia), 1964–1994 (Canada), 1950–2005 (UK) Nested case–control	Cases: 370 diagnoses based on incidence and mortality data (hospital records, cancer registries, death certificates) Controls: 1587, 5 age-matched (Australia) or 4 age- and company-matched (Canada and UK) controls selected using incidence density-based sampling Exposure assessment method: quantitative measurements; exposure assessment was conducted at the job/ worksite/era level, based on routinely collected industry exposure measurements; work history was collected from company records (Canada and UK) or through interview and company records (Australia)	Leukaemia (AML)	Cumulative exposure tertiles (ppm-yr)			NR	Exposures are relatively low; MDS (potentially previously reported as AML) may be the more relevant health risk for such low exposure; strongest suggestion of a risk of MPD is for the exposure time window 2–20 yr (reported in Glass et al., 2014); based on limited data, smoking was unlikely to be a confounder Strengths: large study size; review of diagnosis by haematopathologists; re-assessment of exposure across the three studies Limitations: smoking data were incomplete
			≤ 0.348	20	1.00		
			0.348–2.93	19	1.04 (0.50–2.19)		
			> 2.93	21	1.39 (0.68–2.85)		
		Leukaemia (CML)	Cumulative exposure tertiles (ppm-yr)			NR	
			≤ 0.348	4	1.00		
			0.348–2.93	16	5.04 (1.45–17.50)		
			> 2.93	8	2.20 (0.63–7.68)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 workers exposed to benzene at a chemical plant Exposure assessment method: quantitative measurements; job titles were assigned to exposure categories by an industrial hygienist, based on IH measurements (JEM)	Leukaemia (AML): C92.0	Cumulative exposure (ppm-yr) 0–3.9 4.0–24.9 ≥ 25 Trend test *P* value, 0.88	 0 3 2	 0 (0–2.50) 1.87 (0.39–5.47) 1.39 (0.17–5.03)	Age, race, sex	Third update of the Dow Chemical plant retrospective cohort; one death for MDS, which was reported from the high-exposure group (SMR, 25.05; 95% CI, 0.63–139.58) Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: mortality study based on death certificates

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Stenehjem et al. (2015) Norway 1965–1999/1999–2011 Cohort	24 917 male petroleum workers; offshore oil industry workers for at least 20 days during 1965–1999, all men, extracted from a cohort who responded to a survey conducted with postal questionnaires Exposure assessment method: quantitative measurements; a JEM was developed using monitoring data and job-specific information, giving semiquantitative estimates; JEM scores converted into corresponding ppm values	Leukaemia (myeloid): ICD-10 (codes C92, D45–7)	Cumulative exposure tertiles (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	Nested case–cohort study based on an updated cohort of Norwegian offshore workers; evidence of dose-related patterns for cumulative exposure, exposure intensity and peak exposures for AML; weak links with duration; risks are higher for those with first exposure before 1980 Strengths: prospective case–cohort design; data from Cancer Registry of Norway ensure a high degree of completeness; independent exposure estimates developed for this cohort; analyses adjusted for some confounders Limitations: potential recall bias for distant occupations (non-differential); individual differences in exposure within each occupational group could not be taken into account
			T1 (< 0.001–0.037)	5	1.12 (0.31–4.01)		
			T2 (> 0.037–0.123)	4	1.12 (0.30–4.23)		
			T3 (0.124–0.948)	6	2.24 (0.65–7.71)		
			Trend test *P* value, 0.188				
		Leukaemia (AML): ICD-10 (code C92.0)	Cumulative exposure tertiles (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	
			T1 (< 0.001–0.037)	2	1.40 (0.18–11.00)		
			T2 (> 0.037–0.123)	1	0.85 (0.08–9.29)		
			T3 (0.124–0.948)	5	4.85 (0.88–27.00)		
			Trend test *P* value, 0.052				
		NHL (CLL): ICD-10 (codes C83.0, C91.1)	Cumulative exposure tertiles (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	
			T1 (< 0.001–0.037)	4	6.23 (0.71–54.00)		
			T2 (> 0.037–0.123)	2	3.08 (0.28–34.00)		
			T3 (0.124–0.948)	5	6.74 (0.75–60.00)		
			Trend test *P* value, 0.212				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhomberg et al. (2016) USA 1940–1996 Cohort	1696 workers from three rubber manufacturing plants (Pliofilm) for at least 1 d Exposure assessment method: quantitative measurements; updated benzene exposure estimates based on job classifications, reconstructed by additional interviews of former workers	Leukaemia (AML)	Cumulative exposure quintiles (ppm-yr)			NR	One of many re-evaluations of the Pliofilm cohort; evidence of a threshold effect and relevant exposure window (exposure within 10 yr of cancer onset appeared to be most relevant) Strengths: re-evaluated benzene exposure estimates based on quintiles Limitations: mortality-based; no control for potential confounders; low number of cases; no new cases; exposure reassessment for this cohort was based on few additional data and was supported by the chemical industry; elevated estimates increase the likelihood of observing an apparent threshold
			< 1.55	0	0 (0–8.88)		
			1.55–6.33	0	0 (0–8.68)		
			6.34–20.24	0	0 (0–8.57)		
			20.25–80.10	0	0 (0–7.53)		
			> 80.11	6	10.11 (3.71–22.01)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ireland et al. (1997) USA 1940–1977/ through 1991 Cohort	4172 hourly male chemical plant workers who began employment during 1940–1977 Exposure assessment method: expert judgement; benzene-using departments: nitrobenzene, phenol, chlorobenzene, muriatic acid, and alkylbenzene production; most exposures estimated by IH judgement with information on process changes	Leukaemia: all (AML, ALL, CML, CLL) ICD-8 (codes 204–207)	Cumulative exposure (ppm-mo)			Age	Cumulative exposures were low compared with rubber hydrochloride cohort Strengths: examined exposure categories and number of days with peak exposures Limitations: collection of exposure data began in 1980 when only chlorobenzene and muriatic acid departments were still running, so most exposure assignments were estimated by industrial hygienists (including during 1940s–1950s, when exposure data were very sparse); death certificates were the primary ascertainment source; some leukaemias likely missed or misclassified; possibility of exposure to contaminants in coal-tar-derived benzene used at facility; benzene exposures for maintenance workers could not be estimated
			Unexposed	5	1.1 (0.4–2.6)		
			< 12	2	2.5 (0.3–8.9)		
			12–72	0	0 (0–5.4)		
			≥ 72	3	4.6 (0.9–13.4)		
		Leukaemia: acute nonlymphatic	Cumulative exposure (ppm-mo)			Age	
			Unexposed	2	1.4 (0.2–5.0)		
			< 12	1	3.7 (0.1–20.6)		
			12–72	0	0 (0–44.1)		
			≥ 72	1	4.5 (0.1–25.3)		
		NHL (CLL)	Cumulative exposure (ppm-mo)			Age	
			Unexposed	1	1.0 (0–5.5)		
			< 12	1	5.9 (0.1–32.6)		
			12–72	0	0 (0–24.7)		
			≥ 72	1	6.7 (0.2–37.7)		
		Multiple myeloma	Cumulative exposure (ppm-mo)			Age	
			Unexposed	1	0.5 (0–2.8)		
			< 12	0	0 (0–10.1)		
			12–72	2	6.8 (0.8–2.5)		
			≥ 72	1	3.7 (0.1–20.1)		
		Hodgkin lymphoma	Cumulative exposure (ppm-mo)			Age	
			Unexposed	0	0 (0–3.3)		
			< 12	0	0 (0–16.8)		
			12–72	0	0 (0–21.4)		
			≥ 72	0	0 (0–27.4)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Linet et al. (2015) China, 12 cities 1972–1987/1972–1999 Cohort	73 789 benzene-exposed and 35 504 unexposed Chinese workers; spray and brush painting (coatings), rubber, chemical (including pharmaceutical manufacturing), shoemaking, and other (including printing and insulation) industries Exposure assessment method: records; workers dichotomized (benzene-exposed/unexposed) based on job titles and factory records of use of benzene-containing materials	Pharynx (nasopharynx): ICD-8 (code 147)	Exposed	29	1.9 (0.9–4.3)	Sex, attained age, attained calendar year	Update of the NCI-CAPM cohort; supersedes Yin et al. (1996a), Hayes et al. (1996); lag 2 yr for HLD, 10 yr for all other outcomes; no unexposed incident cases available for CLL Strengths: large sample size; follow-up of 28 yr Limitations: exposure dichotomized to exposed/unexposed only (no further classification); wide range of industrial processes included; limited control for confounders
		Stomach/gastric cancer	Exposed	211	1.0 (0.8–1.3)	Sex, attained age, attained calendar year	
		NHL (B-cell lymphoma): ICD-8 (codes 202–202); lymphomas and Hodgkin lymphoma	Exposed	31	4.0 (1.6–13.4)	Sex, attained age, attained calendar year	
		NHL (B-cell lymphoma): ICD-9 (codes 202–202); lymphomas and Hodgkin lymphoma	Exposed	31	3.2 (1.4–9.4)	Sex, attained age, attained calendar year	
		NHL (B-cell lymphoma): ICD-9 (codes 202, 202)	Exposed	30	3.9 (1.5–13.2)	Sex, attained age, attained calendar year	
		Multiple myeloma: ICD-9 (code 20)	Exposed	1	0.12 (0.01–0.96)	Sex, attained age, attained calendar year	
		Leukaemia: ICD-9 (codes 204–208)	Exposed	60	2.5 (1.4–4.9)	Sex, attained age, attained calendar year	
		Leukaemia (lymphoid): ICD-9 (codes 204.0, 204.1, 204.2)	Exposed	10	5.4 (1.0–99.3)	Sex, attained age, attained calendar year	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Linet et al. (2015) (cont.)		Leukaemia (ALL): ICD-9 (code 204.0)	Exposed	18	4.5 (0.8–83.9)	Sex, attained age, attained calendar year	
		Leukaemia (myeloid): ICD-9 (codes 205, 206)	Exposed	39	2.2 (1.1–4.6)	Sex, attained age, attained calendar year	
		Leukaemia (AML): ICD-9 (codes 205.0, 206.0, 207.0, 207.1, 207.2)	Exposed	26	2.1 (0.9–5.2)	Sex, attained age, attained calendar year	
		Leukaemia (CML): ICD-9 (codes 205.1, 205.2)	Exposed	13	2.5 (0.8–10.7)	Sex, attained age, attained calendar year	
		Leukaemia: acute, NOS, ICD-9 (code 208.0)	Exposed	6	3.5 (0.6–66.1)	Sex, attained age, attained calendar year	
		Leukaemia: NOS, ICD-9 (codes 208.8, 208.9)	Exposed	5	2.4 (0.4–44.4)	Sex, attained age, attained calendar year	
		NHL (CLL): ICD-9 (codes 204.1, 204.2)	Exposed	2	NR	Sex, attained age, attained calendar year	
		NHL (CLL): ICD-9 (codes 204.1, 204.2)	Exposed	2	NR	Sex, attained age, attained calendar year	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kirkeleit et al. (2008) Norway 1981–2003 Cohort	27 919 offshore petroleum workers registered to the Norwegian registry of employers and employees, and 366 114 matched controls from the general working population Exposure assessment method: other; location of work and job category	Leukaemia (ALL): ICD-9 (code 204.0)	Exposed in upstream offshore workers	1	2.20 (0.30–16.60)	Sex, age, year of first exposure, education	
		Leukaemia (AML)	Exposed in upstream offshore workers	6	2.89 (1.25–6.67)	Sex, age, year of first exposure, education	
		Leukaemia (CML)	Exposed in upstream offshore workers	1	1.44 (0.19–10.70)	Sex, age, year of first exposure, education	
Guénel et al. (2002) France 1978–1989 Nested case–control	Cases: 72 identified among male workers Controls: 285 controls matched to the cases by year of birth Exposure assessment method: expert judgement; JEM developed from expert judgement	Leukaemia (ALL): ICD-9 (code 204.0)	Exposure (benzene unit-yr)			Age matched	
			Never	9	1.0		
			> 0 to < 5.5	1	0.6 (0.1–5.3)		
			> 5.5	2	3.3 (0.3–43.3)		
			Trend test *P* value, 0.16				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wong et al. (1993) USA 1946–1985 Cohort	18 135 employees with potential exposure to gasoline for at least 1 yr at land-based terminals (n = 9026) or on marine vessels (n = 9109) Exposure assessment method: questionnaire	Leukaemia (ALL)	Land-based employees exposed to gasoline	2	1.3 (0.1–4.5)	NR	
			Marine-based employees exposed to gasoline	1	0.8 (0–4.4)		

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; d, day(s); HLD, haematopoietic, lymphoproliferative, and related disorders; ICD, International Statistical Classification of Diseases and Related Health Problems; IH, industrial hygiene; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; mo, month(s); MPD, myeloproliferative disorder; NCI-CAPM, National Cancer Institute-Chinese Academy of Preventive Medicine; NHL, non-Hodgkin lymphoma;
NOS, not otherwise specified; NR, not reported; ppm, parts per million; SMR, standardized mortality ratio; yr, year(s)

13 years to the end of 2009. Industrial hygiene measurements of benzene were used to estimate job-specific exposures over time. The average exposure duration of cohort members was 4.9 years (range, 30 days–44.7 years), and cumulative exposure of the subjects was divided into three categories (0–3.9, 4.0–24.9, and ≥ 25 ppm-years). [The strengths of this study included its long and complete follow-up and comprehensive exposure assessment. However, it was based on mortality rather than incidence, there was no control for potential confounders, and the number of cases was small.]

(iii) Chinese workers

The incidence of and mortality from cancers of the lymphatic and haematopoietic system were studied in a large cohort of Chinese workers comprising 74 828 workers exposed to benzene and 35 805 unexposed workers (National Cancer Institute-Chinese Academy of Preventive Medicine (NCI-CAPM) cohort). The initial follow-up period of 1972–1987, which had a quantitative assessment for exposure to benzene (Hayes et al., 1997), was extended to 1999 using factory records, hospital records, and death certificates (Linet et al., 2015). Benzene exposure assessment was based on factory and job-specific information on the use of material containing benzene, and was limited to classification as ever (for at least 6 months) versus never exposed, preventing any dose–response evaluation. The study included 60 and 13 incident cases of leukaemia of all types in exposed and unexposed workers, respectively. [The strengths of this study included the large size of the cohort, which included both sexes and covered several different industries, and the long follow-up, with small numbers lost to follow-up. Control for potential confounders was limited to sex, age, and calendar period. The numbers of cases were relatively small in some subgroups, particularly among unexposed workers.]

(iv) Norwegian offshore oil workers

Kirkeleit et al. reported on a prospective cohort study of 27 919 workers listed as having been employed in the offshore oil industry in the Norwegian Registry of Employers and Employees between 1981 and 2003, and followed up for cancer incidence in the Cancer Registry of Norway until the end of December 2003 (Kirkeleit et al., 2008). No quantitative estimates of benzene exposure were derived.

Stenehjem et al. (2015) reported on 24 917 male petroleum workers with at least 20 days employment offshore between 1965 and 1999. The cohort was established by means of a postal questionnaire in 1998, asking participants to report on occupational history and potential confounding factors. About 50% of the offshore workers overlapped with the register-based cohort of male and female offshore workers followed up by Kirkeleit et al. (2008). The follow-up periods of the two studies overlapped by only 5 years out of a total of 31 years of observation; Kirkeleit et al. (2008) covered 1981–2003 and Stenehjem et al. (2015) covered 1999–2011. The overlap is described in (Stenehjem et al., 2014). Incident cancers were identified prospectively by linkage with the Cancer Registry of Norway (Stenehjem et al., 2015). A total of 112 cases of cancers of the lymphatic and haematopoietic system diagnosed during 1999–2011 were identified and compared with a reference subcohort of 1661 workers using a nested case–cohort design (Stenehjem et al., 2015). A job-exposure matrix (JEM) was developed to assess exposure to benzene. The JEM scores were then translated into corresponding ppm values estimated on the basis of industrial benzene measurement data in Norway (Steinsvåg et al., 2007; Bratveit et al., 2011). In all analyses, adjustment was made for benzene exposure from other work (coded as yes or no, depending on the self-reported job titles and/or industry sector where the worker had ever been employed, e.g. shipping, chemical industry,

painting and surface treatment, farming and forestry, or other industry) and smoking status (yes, no, unknown). [The main strengths of this study were the prospective design, the reliability of incidence data, and detailed exposure estimates (Steinsvåg et al., 2007).]

(v) Reassessment of the Pliofilm cohort study

The cohort of workers at three Pliofilm (rubber hydrochloride) manufacturing plants in Ohio (USA) consisted of 1696 workers followed up for mortality between 1940 and 1996 (Wong, 1995; Rinsky et al., 2002) included in *IARC Monographs* Volume 100F, Table 2.1 (available at: http://publications.iarc.fr/123). Methods of exposure assessment differed between investigators, leading to different distributions of benzene exposure in the cohort and different risk values depending on the exposure levels assigned to the cases. In a recent publication, Rhomberg and collaborators reassessed exposure to benzene using a probabilistic approach based on air sampling data and assumptions about how workplace concentrations decreased over time (Rhomberg et al., 2016). The uptake of benzene from dermal exposures was also estimated, and new exposure information was obtained through additional interviews of former workers (Williams & Paustenbach, 2003). Using these new estimates, the authors divided cohort members according to quantiles of benzene exposure distribution; about 20% of the cohort members were found to have cumulative exposures of more than 80.11 ppm-years. Previous investigators (Wong, 1995; Rinsky et al., 2002) had both used fixed cut-offs of 40, 200, and 400 ppm-years. [The Working Group noted that both the outcome categorization (leukaemia subtypes) and the exposure assessment methods and cut-offs were revised from multiple analyses reported from this cohort, and that this had an important impact on different risk estimates reported for the same set of study participants.]

(c) Acute non-lymphocytic leukaemia/acute myeloid leukaemia and myelodysplastic syndrome

Studies of AML and ANLL were reviewed by a previous Working Group in *IARC Monographs* Volume 100F. That review included studies also present in previous evaluations for Volume 29 (IARC, 1982) and Supplement 7 (IARC, 1987). The data reviewed in *IARC Monographs* Volume 100F (IARC, 2012a) were described as follows by that Working Group: "...analyses of cohort studies (e.g. results in Crump (1994) and Wong (1995), based on the cohort study described in Infante et al. (1977) and Rinsky et al. (1981, 1987), which reported an excess risk for combined (mostly acute) myelogenous and monocytic leukaemia) and new cohort studies with quantitative data on benzene exposure have shown evidence of a dose–response relationship between exposure to benzene and risk for ANLL/AML in various industries and in several countries (Hayes et al., 1997; Rushton & Romaniuk, 1997; Divine et al., 1999b; Guénel et al., 2002; Collins et al., 2003; Glass et al., 2003; Bloemen et al., 2004; Gun et al., 2006; Kirkeleit et al., 2008). It was also noted that the NCI-CAPM cohort study [of Chinese workers exposed to benzene] found evidence of an increased risk for the combined category of ANLL and myelodysplastic syndromes (Hayes et al., 1997)".

New results on AML/ANLL and CML published since that time are described in the following and summarized in Table 2.1. Results regarding myelodysplastic syndromes (MDS) are also described in the text (not included in the table), as some cases of MDS can progress to AML and may have been classified in this way in earlier publications.

(i) Petroleum distribution workers

In the pooled analysis of three updated nested case–control studies of petroleum distribution workers from Australia, Canada, and the United Kingdom, 60 cases were classified as AML

(241 matched controls) and 29 as MDS (129 matched controls) (Schnatter et al., 2012).

Conditional logistic odds ratios (ORs) for AML were above unity for most exposure metrics, although none reached statistical significance (highest vs lowest cumulative exposure tertiles OR, 1.39; 95% confidence interval (CI), 0.68–2.85; average exposure intensity OR, 1.90; 95% CI, 0.86–4.18; maximum exposure intensity OR, 1.65; 95% CI, 0.75–3.73; duration of employment OR, 1.70; 95% CI, 0.75–3.87; peak exposure OR, 1.50; 95% CI, 0.82–2.75; dermal exposure OR, 1.15; 95% CI, 0.60–2.22), but no clear dose–response relationship could be demonstrated. In a further analysis of the same AML data, these associations were found to be more consistent in the subgroup of terminal workers who experienced higher exposure levels (Rushton et al., 2014). Finally, MDS showed a consistent monotonic trend for all benzene exposure metrics (e.g. for cumulative exposure, highest vs lowest tertile OR, 4.33; 95% CI, 1.31–14.3; *P* for trend, 0.01; based on 29 cases) (Schnatter et al., 2012).

[Quantitative exposure assessment and ascertainment of leukaemia subtypes were conducted carefully in this pooled analysis. The average exposure to benzene was found to be much lower than in studies of other populations exposed at higher levels, possibly explaining the non-statistically significant associations with AML. A monotonic trend was observed between benzene exposure and MDS. Previous studies relied upon an outcome classification where MDS was typically not identified (e.g. from death certificate). Some cases classified as AML in the original cohort studies were reclassified as MDS in the pooled analysis, leading to a more precise definition of outcomes, and therefore also likely contributing to the lack of associations with AML.]

(ii) Dow Chemical workers, Midland, Michigan

There were five deaths from AML in the cohort of 2266 workers exposed to benzene at a Dow Chemical plant, giving a standardized mortality ratio (SMR) of 1.11 (95% CI, 0.36–2.58) in the total population (*P* for trend, 0.88) (Collins et al., 2015). Standardized mortality ratios were similar when considering the whole ANLL subgroup (five deaths) or taking account of a latency period of more than 30 years (four deaths). No associations with AML were observed by tertiles of cumulative benzene exposure (in ppm-years). There was one MDS death in the group exposed to the highest concentrations of benzene. [This study had important limitations in terms of the small number of leukaemia cases, the use of mortality rather than incidence data, and the absence of an internal reference group.]

(iii) Chinese workers

Previously published results of ANLL incidence in this cohort of Chinese workers revealed statistically significantly elevated relative risks (RRs) for cumulative benzene exposure of 40 ppm-years or more (*P* for trend, 0.06). In analyses of ANLL/MDS, a significant positive trend was also observed (*P* for trend, 0.01) (Hayes et al., 1997). This updated study confirmed previous results, with more precise estimates (Linet et al., 2015). A total of 26 AML cases were ascertained among the subjects exposed to benzene and 7 among the unexposed, resulting in a relative risk of 2.1 (95% CI, 0.9–5.2). In addition, there were 8 MDS cases among the exposed and none among the unexposed group. Relative risks for AML/MDS were lower in 1988–1999 (RR, 1.3; 95% CI, 0.4–5.9) compared with 1972–1987 (RR, 3.7; 95% CI, 1.5–12.8), but the difference was not significant. [The strengths of this study included the large size of the cohort and the long and complete follow-up, with small numbers of subjects lost to follow-up. The main limitation was the lack of analysis of quantitative exposure

to benzene, as workers were simply categorized as exposed or not exposed.]

(iv) Norwegian offshore oil workers

A study based on a Norwegian cohort of offshore oil industry workers (included in *IARC Monographs* Volume 100F, Table 2.1, available at: http://publications.iarc.fr/123) showed an increased risk of AML (RR, 2.89; 95% CI, 1.25–6.67) compared with the general working population (Kirkeleit et al., 2008).

In a later, partially overlapping cohort study analysed using a case–cohort approach (Stenehjem et al., 2015), the hazard ratio (HR) of AML for offshore workers ever exposed versus never exposed to benzene was 2.18 (95% CI, 0.47–10.00). The risk estimate was substantially higher in the highest tertile of cumulative exposure (0.124–0.948 ppm-years) compared with the lowest tertile (< 0.001–0.037 ppm-years), with a hazard ratio of 4.85 (95% CI, 0.88–27.00; *P* for trend, 0.052). Regarding other metrics evaluated, hazard ratios were greatest in the highest tertile of average intensity (HR, 3.21; 95% CI, 0.63–19; *P* for trend, 0.092), cumulative peak (HR, 3.61; 95% CI, 0.59–26.00; *P* for trend, 0.166), and average peak (HR, 4.87; 95% CI, 0.90–26.00; *P* for trend, 0.056). No clear pattern was observed for duration of exposure in years.

[The main strengths of these studies included the prospective design, the reliability of incidence data, and the detailed exposure estimates. Stenehjem et al. (2015) included new cases of AML diagnosed during 1999–2011 but not the cases included in the earlier follow-up; this led to a relatively small number of cases. The narrow distribution of benzene exposure was an important limitation.]

(v) Reassessment of the Pliofilm cohort study

After reassessment of exposure to benzene in the Pliofilm cohort study in Ohio, all six deaths from AML were observed in the highest quintile of benzene exposure (SMR, 10.11; 95% CI, 3.71–22.01), possibly indicating a threshold effect of benzene exposure of more than 80.11 ppm-years (Rhomberg et al., 2016). By contrast, using fixed cut-offs for categories of benzene exposure (based on a balanced distribution of cases), Rinsky et al. (2002) classified four deaths from exposure to benzene at more than 400 ppm-years, giving an unstable standardized mortality ratio of 34.79 (95% CI, 9.48–89.09) in this exposure category. In the analysis using lag times of 0, 5, 10, 15, or 20 years, Rhomberg et al. (2016) found that the highest risk of AML mortality remained in the highest category of exposure, and the observations were consistent with an association with benzene exposure within the past 10 years. [The elevated exposure estimates increased the likelihood of observing an apparent threshold by assigning exposed workers to a higher exposure category; these results were questioned by the Working Group, however, due to the retrospective reassessment of exposure and the use of simulation methods.]

(d) Chronic myeloid leukaemia and myeloproliferative disorder

Studies of CML and occupational exposure to benzene were also reviewed in *IARC Monographs* Volume 100F. Occupational cohort studies available at that time were described as follows: "Several studies in the petroleum industry and in other settings show non-significantly increased risks for CML, whereas other studies show no evidence of an association, including two that had quantitative estimates of exposure to benzene but no dose–response relationship (Rushton & Romaniuk, 1997; Guénel et al., 2002)".

Additional data for CML/MPD in occupational cohorts that have become available since that time are described here and summarized in Table 2.1.

(i) Petroleum distribution workers

The pooled analysis of updated case–control studies nested within three occupational cohorts of petroleum distribution workers from Australia, Canada, and the United Kingdom exposed to low concentrations of benzene included 28 cases of CML (characterized by the presence of the Philadelphia chromosome, a specific genetic abnormality in chromosome 22) and 30 cases of MPD (Schnatter et al., 2012). Matched controls included 122 and 124 men for CML and MPD, respectively. For CML, compared with the lowest tertile, the odds ratio for cumulative exposure was 5.04 (95% CI, 1.45–17.50) in the second tertile (exposure of 0.34–2.93 ppm-years) and 2.20 (95% CI, 0.63–7.68) in the highest tertile (*P* for trend, 0.02). No clear indication of the existence of a monotonic dose–response relationship emerged when incorporating the additional exposure metrics considered in the study (see Section 2.1.1(b)(i)).

For MPD, odds ratios for cumulative exposure were 1.28 (95% CI, 0.47–3.98) in the second tertile and 1.79 (95% CI, 0.68–4.74) in the upper tertile; the trend was not significant (*P* for trend, 0.49). No strong relationship was shown with any other metrics for the whole exposure period. After restricting the exposure window to 2–20 years before diagnosis, statistically or borderline significant dose–response trends were found for cumulative exposure, dermal exposure, maximum intensity, and average intensity. An odds ratio of 3.81 (95% CI, 1.36–10.70) was reported for peak exposure, based on 18 cases ever exposed to more than 3 ppm for 1 year or more (Schnatter et al., 2012; Glass et al., 2014). [This study was the first to examine CML and MPD as separate entities. The Working Group noted that exposure to benzene was relatively low in these cohorts.]

(ii) Chinese workers

The incidence of CML in the NCI-CAPM cohort of Chinese workers was non-significantly elevated in exposed workers compared with non-exposed workers (13 exposed cases; OR, 2.5; 95% CI, 0.8–10.7) (Linet et al., 2015). Results for mortality were almost identical (not reported). [No dose–response relationship was reported, because workers were simply classified as exposed or unexposed to benzene.]

2.1.2 General-population studies

See Table 2.2

General-population studies of leukaemia in adults and exposure to benzene were also reviewed in *IARC Monographs* Volume 100F, reporting the following for ANLL: "In one case–control study an increased risk for childhood ANLL was found for maternal self-reported occupational exposure to benzene (Shu et al., 1988; see Table 2.1, available at: http://publications.iarc.fr/123). One case–control study of childhood cancer in Denmark did not find an association of estimates of environmental benzene exposure from air pollution with an increased risk for ANLL (Raaschou-Nielsen et al., 2001)." Regarding CML, Volume 100F reported: "Case–control studies have shown inconsistent results, with both increased risks (exposure for > 15 years was associated with an OR of 5.0 (1.8–13.9; Adegoke et al., 2003)) and no increase in risk (Björk et al., 2001) reported (see Table 2.6, available at: http://publications.iarc.fr/123)".

For the current evaluation, the Working Group included all general-population cohort studies and case–control studies published in 2009 or later that examined the relationship between benzene exposure (assessed quantitatively or qualitatively) and AML or CML. Studies were excluded if they did not specifically address benzene exposure, but instead used other indicators of traffic-related air pollution (Raaschou-Nielsen et al., 2016) or residential proximity to

Table 2.2 General-population studies of exposure to benzene and leukaemia in adults

Reference, location, follow-up/ enrolment period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kaufman et al. (2009) Bangkok, Thailand 1997–2003 Case–control	Cases: 87 incident cases at Siriraj Hospital, Bangkok Controls: 756 initially age- and sex-matched hospital patients with diagnoses "considered generally unrelated to the exposures of interest" Exposure assessment method: questionnaire	Leukaemia (AML)	Ever exposed Unexposed Exposed	 81 6	 1.0 4.9 (1.4–17.0)	Age, sex, income, use of cellphones, occupational and nonoccupational pesticide exposure, pesticides used near the home, working with powerlines, living near powerlines	Strengths: high response (100%) Limitations: small study; self-reported ever/never exposure; potentially substantial selection and/ or recall bias
Wong et al. (2010a) Shanghai 2003–2007 Case–control	Cases: 722 newly diagnosed AML cases in 29 hospitals; response 94.6% Controls: 1444 patients without malignant diseases and without diseases of the lymphatic and haematopoietic system admitted to the same hospital as the individually matched case (2 controls per case); response 99.0% Exposure assessment method: expert judgement; exposure classification carried out on a job-by-job basis (jobs identified by questionnaire) by an expert committee (blind for case–control status)	Leukaemia (AML)	Benzene exposure (yr) Never (reference) Ever ≤ 10 > 10 to < 20 > 20 Benzene exposure (mg/m³) Group 1: < 1 Group 2: 1–10 Groups 3, 4: >> 10 Period of first exposure 1940–1959 1960–1979 1980–1999 after 2000 Trend test *P* value, 0084 (length of exposed job); 0.01 (maximum exposure)	 644 78 43 21 14 40 20 18 8 22 36 12	 1.00 1.43 (1.05–1.93) 1.99 (1.29–3.07) 1.44 (0.82–2.51) 0.74 (0.39–1.39) 1.18 (0.79–1.76) 1.63 (0.90–2.94) 2.05 (1.05–3.98) 1.33 (0.54–3.26) 0.97 (0.57–1.62) 1.57 (1.00–2.46) 4.18 (1.56–11.15)	Age, sex, hospital	Funding: Benzene Health Effects Consortium Strengths: large study; complete occupational histories with expert assessment Limitations: hospital-based study including potential for selection bias; expert assessment of benzene exposure based on self-reported questionnaire data

Table 2.2 (continued)

Reference, location, follow-up/ enrolment period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Saberi Hosnijeh et al. (2013) 23 centres in 10 European countries 1992–2000 Cohort	241 465 men and women aged 35–70 yr at recruitment, with no prevalent cancer Exposure assessment method: expert judgement; occupational exposures of high-risk occupations estimated by linking them to a general-population JEM originally developed for another study; exposure to benzene classified as "high", "low", and "no exposure" based on job code; 113 cases of AML, but not specified by exposure	Leukaemia (AML)	No exposure	NR	1.00	Sex, smoking status, alcohol intake, age at recruitment, country	Strengths: large cohort with long follow-up; detailed information on confounders Limitations: lack of occupational histories in large number of participants; different procedures to identify cases; exposure classification not very detailed
			Low exposure	NR	1.06 (0.63–1.81)		
			High exposure	NR	1.52 (0.78–2.98)		
			Trend test *P* value, 0.28				
		Leukaemia (CML)	No exposure	NR	1.00	Sex, smoking status, alcohol intake, age at recruitment, country	
			Low exposure	NR	1.00 (0.45–2.22)		
			High exposure	NR	1.97 (0.75–5.19)		
			Trend test *P* value, 0.30				
		NHL (CLL)	No exposure	NR	1.00	Sex, smoking status, alcohol intake, age at recruitment, country	
			Low exposure	NR	1.11 (0.78–1.58)		
			High exposure	NR	0.56 (0.27–1.14)		
			Trend test *P* value, 0.37				
Talibov et al. (2014) Finland, Iceland, Norway, Sweden 1961–2005 Nested case–control	Cases: 15 332 incident cases Controls: 76 660 randomly selected among cohort members who were alive and free from AML on the date of diagnosis of the matched index case (5 controls per case) Exposure assessment method: other; NOCCA JEM based on FINJEM; quantitative assessment (ppm-yr)	Leukaemia (AML)	Cumulative exposure (ppm-yr)			Year of birth, sex, country	The study was funded by Doctoral Programs in Public Health (DPPH)/ Academy of Finland Strengths: very large nested study; selection bias improbable Limitations: exposure classification by JEM relatively unprecise; "cross-sectional" information on jobs held (based on census records); no adjustment for smoking or genetic factors
			50th and 90th percentiles: unexposed	NR	1.00		
			≤ 3.7	430	1.02 (0.84–1.24)		
			3.7–13.6	310	0.88 (0.71–1.11)		
			> 13.6	68	0.80 (0.56–1.15)		
			Trend test *P* value, 0.33				

AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; FINJEM, Finnish job-exposure matrix; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NOCCA, Nordic Occupational Cancer Study; NR, not reported; ppm, parts per million; yr, year(s)

gasoline plume (Talbott et al., 2011), or if they only combined benzene exposure with exposure to other solvents (Poynter et al., 2017), even if the text explicitly referred to "benzene exposure".

Since 2009, one new cohort study in the general population (European Prospective Investigation into Cancer and Nutrition study by Saberi Hosnijeh et al., 2013), one nested case–control study in the Nordic Occupational Cancer Study cohort (Talibov et al., 2014), and two new case–control studies (Kaufman et al., 2009; Wong et al., 2010a) have investigated the relationship between occupational benzene exposure and adult leukaemia.

A large cohort study with 241 465 participants covering 23 centres in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) identified 113 AML cases by either population cancer registries, health insurance records, pathology registries, or active contact with study subjects or next of kin (Saberi Hosnijeh et al., 2013). Occupational exposure to benzene was assessed through a general-population JEM based on self-reported occupations of high risk. Exposure to benzene was classified as either "no exposure", "low exposure", or "high exposure". In the high-exposure category, the hazard ratio for AML was 1.52 (95% CI, 0.78–2.98; *P* for trend, 0.28). The same study reported on CML (46 cases in total) and found an increased hazard ratio of 1.97 in the high-exposure group (95% CI, 0.75–5.19; *P* for trend, 0.37). [The strengths of this study included its large size, its long follow-up, and the detailed information about confounders. The limitations included the lack of complete occupational histories in large numbers of participants, different procedures for case identification, and the lack of specificity in the exposure classification.]

Talibov et al. (2014) conducted a very large case–control study nested within the Nordic Occupational Cancer Study cohort. The study in Finland, Iceland, Norway, and Sweden comprised 15 332 AML cases and 76 660 control subjects. The authors did not find an association between occupational benzene exposure, as assessed by a JEM, and AML. With occupational unexposed workers as a reference, the hazard ratios of those exposed to benzene at 3.7 or less, 3.7–13.6, and more than 13.6 ppm-years was 1.02 (95% CI, 0.84–1.24), 0.88 (95% CI, 0.71–1.11), and 0.80 (95% CI, 0.56–1.15), respectively (*P* for trend, 0.33). [The strengths of this study included its very large size and its nested design, making selection bias improbable. The limitations included incomplete work histories for many participants and the imprecise exposure classification by JEM.]

In a hospital-based case–control study in Shanghai, China, Wong et al. (2010a) compared 722 newly diagnosed AML cases with 1444 control subjects without malignant diseases or diseases of the lymphatic and haematopoietic system. The authors found a monotonic exposure–response relationship between maximum occupational benzene exposure and AML (*P* for trend, 0.01). The odds ratios were 1.18 (95% CI, 0.79–1.76), 1.63 (95% CI, 0.90–2.94), and 2.05 (95% CI, 1.05–3.98) for maximum exposure to benzene at less than 1, 1–10, and more than 10 mg/m^3, respectively. Individuals with a first diagnosis after the year 2000 had a higher risk than individuals with an earlier date of first diagnosis. [The strengths of this study included its large size, as well as complete occupational history with job-specific questions, and expert assessment of exposures. The limitations included the potential for selection bias as a consequence of the hospital-based control selection.]

In a small hospital-based case–control study in Bangkok, Thailand, 87 AML cases were compared with 756 patients of the same hospital (Kaufman et al., 2009). For self-reported occupational benzene exposure, an elevated odds ratio of 4.9 (95% CI, 1.4–17.0) was found. [The high response rate was a strength of this study. Limitations included the potential for selection and recall bias as a consequence of the

hospital-based control selection and the use of self-reported benzene exposure (ever vs never).]

2.2 Adult lymphoma

This section presents the Working Group's review of studies of NHL and HL in adults. Because most of the available studies did not group the entities now included within NHL according to the current WHO classification (Swerdlow et al., 2017), the disease entities presented here are those used in the original publications. For occupational cohort studies, which were more numerous, data are presented for total NHL as defined in the original studies and MM when separate risk data were reported (in the same subsection), for CLL, for ALL, and for HL.

2.2.1 Occupational cohort studies

(a) Non-Hodgkin lymphoma and multiple myeloma

Twenty-one studies on the association between NHL, including MM, and exposure to benzene in occupational cohorts were included in *IARC Monographs* Volume 100F (see Table 2.9, available at: http://publications.iarc.fr/123). The purpose of the current update is to establish whether new studies contribute to the causal assessment of the overall evidence. Several articles on adult lymphomas included in *IARC Monographs* Volume 100F or published later were excluded by the Working Group either because the exposure assessment was considered inadequate to determine whether workers were exposed to benzene (Guberan & Raymond, 1985; Cuzick & De Stavola, 1988; La Vecchia et al., 1989; Blair et al., 1993; Walker et al., 1993; Lagorio et al., 1994; Satin et al., 1996; Lynge et al., 1997; Anttila et al., 1998; Gérin et al., 1998; Lundberg & Milatou-Smith, 1998; Divine et al., 1999b; Persson & Fredrikson, 1999; Mao et al., 2000; Wong et al., 2001a, b; Sorahan et al., 2002; Kauppinen et al., 2003; Xu et al., 2003; Dryver et al., 2004; Huebner et al., 2004; Punjindasup et al., 2015), or because these were either methodological articles or focused on mechanisms (Vineis et al., 2007; Barry et al., 2011; Faisandier et al., 2011).

Studies in occupational cohorts published after the compilation of *IARC Monographs* Volume 100F that are included for evaluation here are those published by Koh et al. (2011, 2014), Collins et al. (2015), Linet et al. (2015), and Stenehjem et al. (2015). These studies are summarized in Table 2.3.

Most studies reported a small number of NHL cases as a result of exposure to benzene, usually less than 20, and generally presented mortality as an outcome, leading to low sensitivity of ascertainment for NHL. The exceptions are the studies by Hayes et al. (1997), Nilsson et al. (1998), Glass et al. (2003), Sorahan et al. (2005), Kirkeleit et al. (2008), Koh et al. (2011, 2014), Linet et al. (2015), and Stenehjem et al. (2015), which identified incident cases. To broadly characterize the available studies, exposure contexts included a variety of manufacturing processes including the petroleum industry, chemical plants, or others, as well as different exposure assessment methods (see Section 1.4.1 on Occupational exposure). Among the studies published before the previous evaluation in *IARC Monographs* Volume 100F, the current Working Group considered those with high-quality exposure assessment, case ascertainment, and follow-up, as well as a large sample size and adjustment for confounders, to be the most informative. None of the studies in the previous Monograph fulfilled all these criteria. All the studies considered in the evaluation are described below (chronologically), but only studies published after *IARC Monographs* Volume 100F are included in Table 2.3.

Wong (1987a) studied male workers from seven chemical plants in the USA, where jobs were classified based on past quantitative measurements. An apparent dose–response

Table 2.3 Occupational cohort studies of exposure to benzene and lymphoma in adults

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 chemical industry workers exposed to benzene as solvent and raw material Exposure assessment method: quantitative measurements; job-specific exposure estimated from measurements taken from 1944 to the late 1970s	NHL: ICD-10 (codes C82–C85.9)	Cumulative exposure (ppm-yr)			Age, race, sex	Third update of the Dow Chemical plant retrospective cohort Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: small cohort; mortality data (based on death certificates) for a period over which diagnosis and classification were uncertain
			> 30 yr latency	12	1.02 (0.53–1.78)		
			0–3.9	6	1.23 (0.45–2.69)		
			4–24.9	6	1.10 (0.41–2.40)		
			≥ 25	3	0.58 (0.12–1.69)		
			Trend test *P* value, 0.26				
		Hodgkin lymphoma: ICD-10 (code C81)	Cumulative exposure (ppm-yr)			Age, race, sex	
			> 30 years latency	1	1.32 (0.03–7.36)		
			0–3.9	0	0 (0–5.28)		
			4–24.9	2	2.63 (0.32–9.51)		
			≥ 25	0	0 (0–4.85)		
			Trend test *P* value, 0.35				
Linet et al. (2015) China 1972–1987/1972–1999 Cohort	35 804 benzene-exposed workers in 672 factories (spray and brush painting, rubber, chemical, shoemaking, and other) Exposure assessment method: records; factory and job title-specific information on the use of benzene-containing materials formed the basis for determining benzene-exposed or unexposed jobs; no quantitative assessment	Multiple myeloma: ICD-9 (code 203)	Exposed/unexposed			Sex, age, calendar year	Strengths: very large cohort; few losses to follow-up; long follow-up (28 yr); very careful ascertainment of haematolymphopoietic malignancies Limitations: no quantitative assessment of exposure; wide range of industrial processes included; coexposures vary and were not addressed in the analyses; very small numbers for CLL (zero unexposed cases)
			Mortality	1	0.10 (0.01–1.00)		
			Incidence	1	0.12 (0.01–0.96)		
		NHL: ICD-9 (codes 200, 202)	Exposed/unexposed			Sex, age, calendar year	
			Mortality	31	4.0 (1.6–13.4)		
			Incidence	30	3.9 (1.5–13.2)		
		NHL (CLL): ICD-9 (codes 204.1, 204.2)	Exposed/unexposed			Sex, age, calendar year	
			Incidence	2	NR		
		Leukaemia (ALL): ICD-9 (code C204.0)	Exposed/unexposed			Sex, age, calendar year	
			Incidence	8	4.5 (0.8–83.9)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koh et al. (2011) Korea 1960–2007 Cohort	8866 male workers in refinery/petrochemical complex in Korea producing benzene or using benzene as a raw material Exposure assessment method: other; job title	NHL	Subgroups of workers (exposure-based) in the petrochemical complex			Age, calendar period	Strengths: incidence data Limitations: small number of cases; exposure assessment based on job title
			Manufacturing workers	2	0.70 (0.08–2.52)		
Koh et al. (2014) Korea 2002–2007 (2002–2005 for incidence) Cohort	14 698 male workers registered in a regional petrochemical plant maintenance workers union Exposure assessment method: none; job title	NHL: ICD-10 (codes C82–C85)	Maintenance workers, incidence	3	1.83 (0.38–5.34)	Age	Limitations: very small number of cases
		NHL: ICD-10 (codes C82–C85)	Maintenance workers, mortality	2	1.24 (0.15–4.47)	Age	
Stenehjem et al. (2015) Norway 1965–1999/1999–2011 Cohort	24 917 male petroleum workers; offshore oil industry workers for at least 20 d during 1965–1999, extracted from a cohort who responded to a survey conducted with postal questionnaires Exposure assessment method: quantitative measurements; a JEM was developed using monitoring data and job-specific information, giving semiquantitative estimates; JEM scores then translated into corresponding ppm values	NHL (B-cell lymphoma): ICD-10 (codes C82–C91)	Exposed/unexposed			Age, benzene exposure from other work, ever daily smoker	Nested case–cohort study based on an updated cohort of Norwegian offshore workers Strengths: prospective case–cohort design; data from Norway cancer registry ensure a high degree of completeness; independent exposure estimates developed for this cohort; analyses adjusted for some confounders Limitations: potential recall bias for distant occupations (non-differential); individual differences in exposure within each occupational group could not be taken into account
			Exposed	61	1.49 (0.90–2.48)		
			Trend test *P* value, 0.245				
		Multiple myeloma: ICD-10 (code C90.0)	Exposed/unexposed			Age, benzene exposure from other work, ever daily smoker	
			Exposed	13	1.64 (0.55–4.89)		
			Trend test *P* value, 0.024				
		NHL (CLL): ICD-10 (codes C83.0, C91.1)	Cumulative exposure tertile (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	
			T1 (< 0.001–0.037)	4	6.23 (0.71–54.00)		
			T2 (> 0.037–0.123)	2	3.08 (0.28–34.00)		
			T3 (0.124–0.948)	5	6.74 (0.75–60.00)		
			Trend test *P* value, 0.212				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 workers exposed to benzene at a chemical plant Exposure assessment method: quantitative measurements; job titles were assigned to exposure categories by an industrial hygienist, based on IH measurements (JEM)	Leukaemia: ICD-10 (codes C91–C95)	Cumulative exposure (ppm-yr)			NR	Third update of the Dow Chemical plant retrospective cohort; one death for MDS, and it was in the high-exposure group (SMR 25.05; 95% CI: 0.63–139.58) Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: mortality study based on death certificates; no evaluation of possible confounders
			0–3.9	3	0.60 (0.12–1.76)		
			4.0–24.9	7	1.23 (0.49–2.53)		
			≥ 25	10	1.72 (0.86–3.17)		
			Trend test *P* value, 0.15				
		Leukaemia (myeloid): ICD-10 (code C92)	Cumulative exposure (ppm-yr)			NR	
			0–3.9	0	0 (0–1.79)		
			4.0–24.9	4	1.78 (0.48–4.54)		
			≥ 25	4	1.93 (0.53–4.94)		
			Trend test *P* value, 0.24				
		Leukaemia (AML): ICD-10 (code C92.0)	Cumulative exposure (ppm-yr)			NR	
			0–3.9	0	0 (0–2.50)		
			4.0–24.9	3	1.87 (0.39–5.47)		
			≥ 25	2	1.39 (0.17–5.03)		
			Trend test *P* value, 0.88				
		Leukaemia (lymphoid): ICD-10 (code C91)	Cumulative exposure (ppm-yr)			NR	
			0–3.9	1	0.78 (0.02–4.36)		
			4.0–24.9	1	0.68 (0.02–3.78)		
			≥ 25	2	1.31 (0.16–4.72)		
			Trend test *P* value, 0.53				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schnatter et al. (2012) Australia, Canada, UK 1981–2006 (Australia), 1964–1994 (Canada), 1950–2005 (UK) Nested case–control	Cases: 370 diagnoses based on incidence and mortality data (hospital records, cancer registries, death certificates) Controls: 1587; 5 age-matched (Australia) or 4 age- and company-matched (Canada and UK) controls selected using incidence density-based sampling Exposure assessment method: quantitative measurements; conducted at the job/ worksite/era level, based on routinely collected industry exposure measurements; work history was collected from company records (Canada and UK) or through interview and company records (Australia)	NHL (CLL)	Cumulative exposure (ppm-yr) ≤ 0.348 0.348–2.93 > 2.93 Trend test *P* value, 0.9	 24 32 24	 1.00 1.49 (0.81–2.76) 1.05 (0.56–1.98)	NR	Exposures are relatively low; based on limited data, smoking was unlikely to be a confounder Strengths: large study size; review of diagnosis by haematopathologists; reassessment of exposure across the three studies Limitations: Smoking data were incomplete

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; ICD, International Statistical Classification of Diseases and Related Health Problems; IH, industrial hygiene; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million;
SMR, standardized mortality ratio; yr, year(s)

relationship between NHL and cumulative exposure to benzene was observed, with a relative risk of 3.7 (*P* value, < 0.04) for continuous or intermittent benzene exposure, and one of 3.8 (*P* value, < 0.04) for continuous benzene exposure compared with no exposure. [The Working Group noted the very small number of subjects in this study and that 95% confidence intervals were not reported.] Wong & Raabe (1997) conducted a nested case–control study on MM in gasoline distribution workers (17 MM deaths among the exposed). Total hydrocarbon concentrations in air were used as a surrogate measure of exposure to benzene. *P* values for trend were 0.06 for exposure duration, 0.77 for cumulative exposure to total, and 0.08 for peak exposure. [The Working Group considered the use of indirect estimates of exposure to benzene and the very small number of MM deaths to be strong limitations of this study.]

Schnatter et al. (1996) and Glass et al. (2003) both report on NHL and MM or NHL/MM, and were later included in a pooled analysis (Schnatter et al., 2012). Schnatter et al. (1996) conducted a nested case–control study of petroleum distribution workers in Canada where benzene exposure was quantitatively assessed and standardized mortality ratios were reported. Eight deaths from NHL were observed, and exposure–response analyses based on these showed no consistent pattern. Similarly, the study found non-significant standardized mortality ratios for MM based on seven deaths only (Schnatter et al., 1996). [The Working Group noted the adjustment for several potential confounders, but the very small sample size of the study.] Glass et al. (2003) conducted a nested case–control study in the petroleum industry in Australia, and did not observe a relationship between cumulative exposure to benzene and NHL/MM.

Consonni et al. (1999) report on a cohort of 1583 male oil refinery workers employed during 1949–1982 and followed up to May 1991. Comparing exposed with non-exposed workers, a standardized mortality ratio of 2.12 (95% CI, 0.68–4.95) was found based on 5 exposed NHL cases. The excess risk was significantly increased among workers with 15 years or more of employment, and 30 years or more since first employment.

Using a JEM based on air sampling data, Rinsky et al. (2002) assessed quantitative exposure to benzene for a cohort of workers manufacturing Pliofilm in the USA; the number of NHL or MM cases was very limited, however (5 for each, based on death certificates). The standardized mortality ratio for white males was 1.00 (95% CI, 0.32–2.33) for NHL and 2.12 (95% CI, 0.69–4.96) for MM (reference group unexposed). [The Working Group noted that exposure–response for NHL was not modelled because of the small numbers of cases.] Using the same Pliofilm cohort reported by Rinsky et al. (2002), Wong (1995) focused specifically on MM. Results were reported in terms of levels of cumulative exposure, and no increased mortality risk was observed. [The Working Group noted that results were based on extremely small numbers; 4 MM cases in total were categorized across four exposure strata.]

Collins et al. (2003) reported on NHL and MM in a small study with long follow-up (from 1940 to 1997) at a single chemical plant that was studied previously by Bond et al. (1986). The study was based on individual exposure measurements, and no increased risks for NHL mortality were observed (25 exposed cases in total). An increased standardized mortality ratio was reported for MM, but with a dose–response relationship that did not reach statistical significance (reference group unexposed). [The Working Group noted the generally low exposure levels ranging from less than 1 ppm-years to 632 ppm-years, but with a median of 3 ppm-years.]

Sorahan et al. (2005) reported on a cohort of workers considered occupationally exposed to benzene based on records of the Factory Inspectorate in the United Kingdom. A total of

15 NHL deaths (SMR, 0.94; 95% CI, 0.53–1.56) and 24 incident NHL cases (standardized incidence ratio (SIR), 1.00; 95% CI, 0.64–1.49) occurred in the exposed workers compared with the unexposed. Additionally, based on 8 incident cases and six deaths, the same study found no increased risk of MM. [The Working Group noted the small numbers for MM and that the exposure assessment was limited, although exposure levels were historically high.]

Kirkeleit et al. (2008) reported on a large cohort of more than 27 000 offshore petroleum workers in Norway (see Section 2.1.1(b)(iv) for further details). Compared with the reference unexposed group, the overall relative risk was 1.01 for incident NHL (95% CI, 0.58–1.75) and 2.49 for incident MM (95% CI, 1.21–5.13). [This study overlaps partially with Stenehjem et al. (2015), described below. No quantitative assessment of benzene exposure was available.]

Several new studies reporting pertinent data for NHL or MM and benzene exposure in occupational settings have been published since the previous *IARC Monographs* Volume 100F (Table 2.3).

Stenehjem et al. (2015) studied 61 NHL incident cases. Overall, compared with a reference unexposed group, there was a slight excess of NHL cases among the exposed subjects (RR, 1.49; 95% CI, 0.9–2.48), and a stronger but not statistically significant association with specific histological type. No *P* trend was observed for NHL (*P* trend of 0.245 based on tertiles of exposure). The incidence of MM (13 exposed cases) was increased among exposed workers (RR, 1.64; 95% CI, 0.55–4.89), and a significant dose–response relationship with exposure tertiles was found (*P* for trend, 0.024). [The Working Group noted the very low levels of exposure in these workers: the upper values of average intensity and cumulative exposure were estimated to be 0.040 ppm and 0.948 ppm-years, respectively.]

Linet et al. (2015) updated the study by Hayes et al. (1997) that was based on 35 804 male and female workers exposed to benzene in 672 factories in China, with a long follow-up (28 years) and good case ascertainment. This study did not assess benzene exposure quantitatively. A total of 31 NHL deaths (RR, 4.0; 95% CI, 1.6–13.4) and 30 incident cases (RR, 3.9; 95% CI, 1.5–13.2) were reported. Only one death and 1 incident case of MM were recorded. [The Working Group considered this a strong study due to the robust case ascertainment and long follow-up.] Data on exposure–response relationships in the same cohort were reported earlier by Hayes et al. (1996) (see *IARC Monographs* Volume 100F, Table 2.9, available at: http://publications.iarc.fr/123). The relative risk of mortality from NHL for those exposed to benzene for more than 10 years, compared with a reference unexposed group, was 4.2 (95% CI, 1.1–15.9) based on 11 exposed cases. A non-monotonic dose–response relationship was observed with average (*P* for trend, 0.04) and cumulative (*P* for trend, 0.02) benzene exposure.

Collins et al. (2015) updated a cohort study previously reported by Bloemen et al. (2004) on mortality among 2266 chemical workers in the USA. Controlled for age, sex, and gender, the standardized mortality ratio for NHL observed in workers with more than 30 years latency (n = 12) was 1.02 (95% CI, 0.53–1.78). [The Working Group noted the robust exposure assessment but very small numbers, particularly in analyses by cumulative exposure.]

(b) Chronic lymphocytic leukaemia

The data on the association between CLL and exposure to benzene that were available at the time (until 2009) were reviewed in *IARC Monographs* Volume 100F and described as follows: "Several cohort studies in the petroleum industry [subsequently included in a pooled analysis by Schnatter et al. (2012)] showed mixed results, with some non-significantly increased risks reported and other studies showing no association (see Table 2.7, available at http://publications.iarc.fr/123). In a nested

case–control study in the Australian petroleum industry an increasing risk for CLL was detected with increasing exposure to benzene over a relatively small range of ppm–years, but the increase was not significant (Glass et al., 2003). Similarly, in a nested case–control study within a cohort of French gas and electrical utility workers, a non-significant increase in risk with increasing years of benzene exposure was detected (Guénel et al., 2002). Some evidence of risk with increasing benzene exposure was also found in a cohort study among petroleum workers in the United Kingdom, but the trends were not clear and interpretation is difficult as white- and blue-collar workers were mixed in the analysis and interactions may have been present (Rushton & Romaniuk, 1997)".

The current Working Group reviewed these studies and determined that several did not meet the criteria established for inclusion (see Section 2.1.1(a)) (McCraw et al., 1985; Satin et al., 1996; Lynge et al., 1997; Divine et al., 1999b; Divine & Hartman, 2000; Wong et al., 2001a; Lewis et al., 2003; Bloemen et al., 2004; Huebner et al., 2004). One study reviewed previously was superseded by later updates (Glass et al., 2003).

Three of the five occupational cohort studies published after *IARC Monographs* Volume 100F and described in Section 2.2.1(b) (petroleum distribution workers in Australia, Canada, and the United Kingdom; Chinese workers; and Norwegian offshore oil workers) presented data on CLL and benzene exposure (Table 2.3), as described in the following sections.

(i) Petroleum distribution workers in Australia, Canada, and the United Kingdom

Exposure to benzene was compared between 80 cases of CLL and 345 matched controls in the pooled analysis of updated case–control studies nested in occupational cohorts of petroleum distribution workers (Schnatter et al., 2012; Rushton et al., 2014). When compared with subjects in the lowest exposure tertile of cumulative exposure (< 0.348 ppm-years), the odds ratio of CLL was more elevated in the intermediate exposure tertile (0.348–2.93 ppm-years; OR, 1.49; 95% CI, 0.81–2.76; 32 cases) than in the highest exposure tertile (> 2.93 ppm-years; OR, 1.05; 95% CI, 0.56–1.98; 24 cases). No clear indication of an association was shown with the other exposure metrics reported in this study (see Section 2.1.1(b)). No dose–response relationship was observed for CLL, except with duration of employment (*P* for spline, < 0.03). Refinery workers (mainly from the Australian study) showed a higher risk of CLL compared with subjects who had never worked as a refinery operator or craftsman (RR, 1.99; 95% CI, 0.87–4.57).

(ii) Chinese workers exposed to benzene

The large NCI-CAPM cohort of Chinese workers included only two CLL cases among the workers exposed to benzene and none among the unexposed; no relative risk could be computed (Linet et al., 2015).

(iii) Norwegian offshore oil workers

In the nested case–cohort study on Norwegian offshore oil industry workers, 12 cases of CLL were compared with 1661 reference workers from the same cohort (Stenehjem et al., 2015). A five-fold hazard ratio of CLL for workers ever versus never exposed to benzene was reported (HR, 5.4; 95% CI, 0.7–41.0). The risk estimates for cumulative exposure were substantially higher in the exposed subjects with respect to the unexposed (HR in the upper tertile, 6.74; 95% CI, 0.75–60.00; 5 cases), but no exposure–response relationship was found (*P* for trend, 0.212). Hazard ratios were consistently elevated when considering all the other metrics reported in the study (see Section 2.1.1(b)), although the highest risks were often in the intermediate tertiles of exposure (e.g. the HR for the middle tertile of average peak exposure was 6.66; 95% CI, 1.32–34.00; 6 exposed cases) and no statistically significant dose–response trend was observed for any of the

metrics. [The Working Group noted that exposure levels in the study were generally low.]

(c) Acute lymphocytic leukaemia

ALL is a rare cancer in adults, and this makes it difficult to study its association with exposure to benzene. The maximum number of exposed cases in the studies included in *IARC Monographs* Volume 100F was 8 (IARC, 2012a; Table 2.3, available at: http://publications.iarc.fr/123). The evidence for the association between ALL in adults and benzene exposure that was available at the time of the previous evaluation was described as follows: "In multiple cohorts there was a non-significantly increased risk for ALL, but the numbers of cases were small (Rushton, 1993; Wong et al., 1993; Satin et al., 1996; Yin et al., 1996a; Divine et al., 1999b; Guénel et al., 2002; Lewis et al., 2003; Gun et al., 2006; Kirkeleit et al., 2008)".

The Working Group reviewed these studies and determined that most (Rushton, 1993; Satin et al., 1996; Divine et al., 1999b; Lewis et al., 2003; Gun et al., 2006) did not meet the criteria for inclusion (see Section 2.1.1(a)) in the current evaluation.

The Working Group identified one earlier study that met the inclusion criteria, but had not been reviewed in *IARC Monographs* Volume 100F: Sorahan et al. (2005). In this study, which was conducted in the United Kingdom, no cases of ALL were observed (0.83 expected) in a cohort of 5514 male and female workers exposed to benzene.

Only one new study with pertinent data for adult ALL and benzene exposure has been published since the previous IARC review. Eight cases of incident ALL were ascertained in the NCI-CAPM cohort of Chinese workers among those who held jobs entailing exposure to benzene, and one among the unexposed (Linet et al., 2015). An elevated relative risk for incidence of 4.5 (95% CI, 0.8–83.9) was found. Formal statistical significance was reached in the "all lymphoid leukaemia" group (9th International Statistical Classification of Diseases and Related Health Problems (ICD-9), code 204), that is, after the addition of 2 cases of CLL among the exposed (RR, 5.4; 95% CI, 1.0–99.3) based on a total of 10 cases. A dose–response evaluation was not conducted, because benzene exposure assessment was limited to a categorization of ever versus never exposed.

Among the included studies (except Sorahan et al., 2005, which had no ALL cases), the risk estimates for ALL as a result of benzene exposure ranged from 0.8 to 4.5, and all the 95% confidence intervals included the null.

(d) Hodgkin lymphoma

The evidence for HL was reviewed in *IARC Monographs* Volume 100F. At that time the Working Group noted that the data on HL in studies of cohorts exposed to benzene were sparse, with most studies having very small numbers of cases and reporting no association (see Table 2.13, available at: http://publications.iarc.fr/123). The evidence from these studies was judged to be *inadequate*.

With the exception of the study by Collins et al. (2015) (described in Section 2.1.1(b) and summarized in Table 2.3), which found no association between HL mortality and cumulative exposure to benzene based on only 2 cases of HL, no additional data have been reported on the association between HL and exposure to benzene.

2.2.2 General-population studies

This review included all published, peer-reviewed epidemiological studies reporting a risk estimate for the association between exposure to benzene and NHL, CLL, DLBCL, follicular lymphoma, HCL, MM, ALL, or HL in study populations enrolled from the population at large, distinct from industry-based cohorts. Relevant studies by Clavel et al. (1996), Orsi et al.

(2010), and Wong et al. (2010b) were not included in *IARC Monographs* Volume 100F but have been added to this chapter, in addition to Bassig et al. (2015) (all summarized in Table 2.4). One study by Jiao et al. (2012) on a gene-environment interaction with a BRCA2 variant was excluded as it did not report overall risks for benzene exposure. All the studies were of a case–control design, with the exception of a cohort study from Shanghai (Bassig et al., 2015). The case–control studies were a mixture of hospital-based and population-based designs. The quality of the population controls varied extensively; studies conducted within the USA were often based on random digit dialling (Wang et al., 2009) or driving licence rosters (Kato et al., 2005), often obtaining low response rates. All studies included newly diagnosed incident cases, usually with a re-examination of diagnoses. Histological reviews were performed in the studies by Scherr et al. (1992), Fritschi et al. (2005), and Miligi et al. (2006) (see *IARC Monographs* Volume 100F, Table 2.10, for details of studies included, available at: http://publications.iarc.fr/123). Exposure contexts for the studies reviewed here mainly included occupational exposure of the subjects. The assessment of benzene exposure ranged from a self-report ascertained by questionnaire [which the Working Group did not deem to be of sufficient quality for assessing exposure to benzene] to expert judgement based on quantitative measurements in factories (Bassig et al., 2015), although most studies used a JEM (see Section 1.3 and Section 1.6 for further information). The studies judged most informative by the Working Group were those that scored high for exposure assessment features, with a large sample size and high-quality design (including a histological review of cases and high response rates). Several studies were excluded due to small sample size (Linet et al., 1987; Kato et al., 2005; Ruckart et al., 2013) or because the exposure assessment was very limited (Micheli et al., 2014).

Clavel et al. (1996) found no association between exposure to benzene and HCL [currently classified as a subtype of NHL]. Exposure assessment was based on a JEM. [The Working Group noted that the response rate was low among controls, at around only 57%.]

The large European multicentre Epilymph study (Cocco et al., 2010), which included population- and/or hospital-based controls depending on the areas and used a JEM to assess benzene exposure, was previously included in *IARC Monographs* Volume 100F. Cocco et al. (2010) found no association between exposure to benzene and NHL, DLBCL, follicular lymphoma, or MM; a positive association between CLL and benzene exposure was observed, but there was no evidence of a dose–response relationship (OR of exposed versus never exposed to benzene isolated from other organic solvents, 1.8; 95% CI, 1.0–3.2; *P* for trend, 0.14). [The Working Group noted that the response rate was low in the population controls, at around only 52%. An earlier study by Seidler et al. (2007), which was already included in the Epilymph analysis, reported no association with NHL.]

Fritschi et al. (2005) found no association between NHL and exposure to benzene in a population-based study in Australia that included 68 exposed cases. Exposure to benzene was assessed by a JEM. [The Working Group noted the relatively low response rate among the population-based controls, at around only 61%.]

Miligi et al. (2006) describe a well-conducted population-based study on more than 1400 NHL cases and 1500 controls from the general population, with high response rates of 79%. Cases were examined by a panel of pathologists. Exposure assessment was based on detailed questionnaires, expert judgement, and a JEM (with assessment of probability and intensity of exposure). Positive associations were found for medium and high benzene exposure versus very low and low benzene exposure (OR, 1.6; 95% CI; 1.0–2.4) based on 58 cases, as well as a non-significant

Table 2.4 Epidemiological studies of exposure to benzene and adult lymphoma in the general population

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bassig et al. (2015) China (Shanghai) 1996–2000/until 2009 Cohort	73 087 women only, aged 40–70 yr; Shanghai Women's Study (population-based), with 92.7% response rate Exposure assessment method: quantitative measurements; JEM that combined benzene measurements in factories (since 1954) plus questionnaire data and other information; probability and intensity of exposure assigned	NHL	Cumulative exposure (mg/m^3)-yr) Unexposed (reference) Ever exposed Tertile 1: ≤ 35.2 Tertile 2: 35.21–102.4 Tertile 3: > 102.4 Trend test *P* value, 0.006 for duration, 0.005 for cumulative exposure	 78 24 3 9 12	 1.00 1.86 (1.17–2.96) 0.92 (0.29–2.94) 2.20 (1.10–4.41) 2.16 (1.17–4.00)	Ever smoking, alcohol intake, BMI, education, age	Strengths: highly representative of the general female population in Shanghai; accurate data on exposure; accurate data from cancer registry on incident cancers (very low losses to follow-up) Limitations: only 24 NHL among the exposed
Clavel et al. (1996) France 1980–1990 Case–control	Cases: 226 hairy cell leukaemia patients recruited in 18 French hospitals; only living cases included (60% of 368 eligible) Controls: 425 hospital-based, matched to cases by sex, birth date, admission date, residence; mainly from orthopaedic and rheumatology departments; response rate, 57% Exposure assessment method: expert judgement; JEM that assessed a score for ppm of exposure to benzene; exposure blindly assigned to cases and controls	NHL (HCL)	Unexposed Cumulative benzene exposure (ppm-yr) < 1 (score) 1–5 (score) ≥ 5	189 15 10 7	1.0 0.7 (0.4–1.3) 0.7 (0.3–1.4) 0.5 (0.2–1.2)	Matching variables age and sex, smoking status, residence, admission date	Strengths: large series of rare tumour; good exposure assessment Limitations: low response rate among controls; only living cases (prevalent) included, meaning potential source of bias

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Orsi et al. (2010) France 2000–2004 Case–control	Cases: 244 NHL, 87 HD, 56 MM; hospital-based, men only (aged 20–75 yr); incident cases; response rate, 95% Controls: 456 mainly from orthopaedic and rheumatology departments, residing in hospital catchment area; matched by age, sex, centre; cancers excluded, as well as diseases related to occupation, alcohol, or smoking; response rate, 91.2% Exposure assessment method: expert judgement; job-specific questionnaires evaluated by chemical engineer; experts derived ppm estimates from previously published intensity measurement campaigns	NHL	All benzene, exposed vs unexposed	94	1.0 (0.7–1.5)	Age, centre, socioeconomic status	Strengths: good exposure assessment by expert; very high response rate Limitations: hospital-based study
			Benzene > 1 ppm-yr	70	1.4 (0.9–2.1)		
			Pure benzene	6	3.0 (0.8–11.2)		
			Pure benzene, definite exposure	5	3.4 (0.8–15.0)		
			Latency 30 yr, pure benzene	5	5.5 (1.0–30.7)		
			High intensity of exposure	4	2.6 (0.6–11.2)		
			Same, diffuse large cell lymphoma	4	7.2 (1.6–33.2)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wong et al. (2010b) Shanghai 2003–2008 Case–control	Cases: 649 hospital-based from 25 hospitals; response rate, 76% Controls: 1298 hospital-based controls matched by age and sex, with exclusion of blood malignancies; response rate, NR Exposure assessment method: expert judgement; exposure assessment conducted by experts by estimating ppm of exposure	NHL	Maximum exposure to benzene			Age, sex, hospital	Limitations: hospital-based study
			Score 1	32	1.14 (0.73–1.78)		
			Score 2	9	0.75 (0.35–1.64)		
			Score 3–4	9	1.21 (0.53–2.76)		
			Trend test *P* value, 0.76 for maximum exposure, 0.80 for duration of exposure				

BMI, body mass index; CI, confidence interval; HCL, hairy cell leukaemia; HD, Hodgkin disease; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million; vs, versus; yr, year(s)

threefold increased risk for exposure duration of more than 15 years (OR, 2.9; 95% CI, 0.9–9.0) based on 14 cases.

In a hospital-based case–control study of men only with very high response rates (83%) conducted in France, Orsi et al. (2010) reported no increased NHL risk with exposure to benzene compared with no exposure (OR, 1.0; 95% CI, 0.7–1.5), and a positive association for those exposed to "pure benzene" (OR, 3.0; 95% CI, 0.8–11.2) based on 6 exposed cases. No associations were observed for HL and MM. [The Working Group noted an unclear choice of controls and the small numbers for the different exposure metrics, for example, "pure benzene".]

A population-based case–control study in the Boston metropolitan area by Scherr et al. (1992), with high response rates of 80% and including a review of pathological material (slides), found no significant association with NHL in ever versus never exposed cases (OR, 1.2; 95% CI, 0.5–2.6). [The Working Group noted the potential for information bias due to the use of self-reported exposure via questionnaires, as well as the small sample size.]

In a population-based case–control study of women aged 21–84 years, Wang et al. (2009) used a JEM (with assessment of probability and intensity of exposure) to report slightly elevated, non-statistically significant risks for NHL in the group exposed to medium to high concentrations of benzene, particularly for specific histological types (*P* for trend, 0.04 for DLBCL, 0.08 for CLL, and 0.18 for follicular lymphoma). [The Working Group noted generally low response rates.]

A hospital-based study by Wong et al. (2010b), including 649 cases and 1298 matched controls, found no association between NHL and benzene exposure assessed by experts based on job questionnaires, and a significant association for follicular lymphoma (based on 7 cases only).

A population-based cohort study conducted after the publication of *IARC Monographs* Volume 100F evaluated the association between benzene exposure and NHL: Bassig et al. (2015) followed up 73 087 women in the Shanghai general population for NHL incidence through the Cancer Registry, and used a quantitative JEM based on actual benzene measurements (with assessment of probability and intensity of exposure to benzene). Response rates were very high (93%). The overall hazard ratio for the ever exposed versus the unexposed group was 1.86 (95% CI, 1.17–2.96) based on 24 exposed cases, and an exposure–response relationship was reported with both duration of exposure to benzene (*P* for trend, < 0.006) as well as cumulative exposure (*P* for trend, < 0.005). A case–control study in Italy showed evidence of a dose–response relationship between exposure to benzene for a duration of more than 15 years and CLL (*P* for trend, 0.05) (Costantini et al., 2008).

In a case–control study in residents in Shanghai (532 cases and 502 controls from the general population), a significant 3.9-fold increased risk for ALL was reported for the group with 15 years or more of self-reported occupational exposure to benzene, based on 5 exposed cases in this category (Adegoke et al., 2003); no association was observed in another study in the USA, with only 3 cases in the highest exposure group (see *IARC Monographs* Volume 100F, Table 2.4, available at: http://publications.iarc.fr/123).

2.3 Childhood cancer

Age-specific incidence rates for several types of childhood cancer peak at ages < 5 years, indicating that risk factors exist in the early life environment or might be inherited. Few risk factors have been identified, with the exception of ionizing radiation and chemotherapy, meaning that the majority of cases are unexplained.

It is known that benzene causes AML/ANLL in adults. Positive associations have also been observed between exposure to benzene and ALL, CLL, MM, and NHL (IARC, 2012a). Leukaemia

is the most common type of childhood cancer, leading to the hypothesis that benzene could also cause leukaemia in children. Benzene often occurs as part of mixed exposures, such as in utero in pregnant women who smoke, or from second-hand smoke and traffic exhaust in ambient air. Studies of childhood cancers have used various indicators of such mixtures, for example, traffic (von Behren et al., 2008; Amigou et al., 2011), petrol stations, and automotive repair garages near the residence (Steffen et al., 2004; Brosselin et al., 2009). Studies of parental occupational exposures and childhood cancer have also used indicators for mixed exposure which may include benzene, such as "solvent use" (van Steensel-Moll et al., 1985; Carlos-Wallace et al., 2016). [The Working Group is aware of these studies but decided to review only those specifically assessing exposure to benzene. The Working Group noted that, even in studies where benzene is specifically assessed, benzene is often one of many correlated air pollutants; confounding from such correlated air pollutants can rarely be excluded.]

Ecological studies have compared incidence rates of childhood leukaemia (Whitworth et al., 2008; Senkayi et al., 2014) and tumours of the central nervous system (CNS) (Danysh et al., 2015) with benzene levels assessed at census tracts or county level. These studies were not reviewed by the current Working Group because of the usual limitations of the ecological design for causal inference.

The Working Group reviewed a series of case–control studies that quantified ambient benzene levels, either assessed at the exact address or as a mean for the area where childhood cancer cases and controls lived. A case–control study that assessed exposure by measuring a benzene metabolite in urine from childhood cases and controls (Jiang et al., 2016) was also reviewed. One additional case–control study (Ruckart et al., 2013) was not reviewed, because the information that could be extracted was limited because the study included only 13 verified childhood cases of cancer of the haematopoietic system (11 leukaemia and 2 NHL) and because exposure to benzene was not quantified. Another case–control study that investigated the distance from the residence to industries emitting benzene was not reviewed because benzene concentrations were not quantified (García-Pérez et al., 2015).

The Working Group also reviewed a series of case–control studies and two cohort studies that compared the occupational exposure to benzene of the parents of childhood cancer cases and controls. Table 2.5 includes only relevant studies of cancer sites with sufficient or limited evidence that were either not included in, or published after, *IARC Monographs* Volume 100F (IARC, 2012a).

2.3.1 Childhood exposure to benzene in outdoor air

Four case–control studies assessed benzene concentrations at the exact address(es) where childhood cancer cases and controls lived (see Table 2.5).

In a population-based study included in *IARC Monographs* Volume 100F, Raaschou-Nielsen et al. (2001) identified 1989 cases (age, 0–14 years) of leukaemia, lymphoma, and tumours of the CNS in the Danish Cancer Registry, and selected 5506 controls at random among the whole Danish childhood population using the Danish Population Registry. Controls were matched to cases by sex, age, and calendar time. The residential history of each child was traced from 9 months before birth to the time of diagnosis. Benzene exposure was calculated from a dispersion model based on traffic and the configuration of the street and buildings at the address. The analyses adjusted for urban development, geographical region, type of residence, low-frequency electromagnetic fields (power lines and transformer stations), mother's age, and birth order. For exposure to benzene during childhood between the 90th and 99th percentile

Table 2.5 Epidemiological studies of exposure to benzene and childhood leukaemia in the general population

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Crosignani et al. (2004) Province of Varese, Italy 1978–1997 Case–control	Cases: 120 from cancer registry Controls: 480 population-based from Health Service Archives Exposure assessment method: other; modelled concentration of benzene outside the residence at time of diagnosis	Leukaemia: ICD-9 (codes 204.0–208.9), lymphoid leukaemia, myeloid leukaemia, monocytic leukaemia, other specified and unspecified leukaemia	Benzene concentration (μg/m³) < 0.1 0.1–10 > 10 Trend test *P* value, 0.005	 88 25 7	 1.0 1.5 (0.9–2.5) 3.9 (1.4–11.3)	Sex, age, SES of municipality	Incidence, ages 0–14 yr Strengths: population-based; exposure model Limitations: small number of cases; only address at diagnosis; non-differential misclassification of exposure

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vinceti et al. (2012) Emilia-Romagna region, northern Italy 1998–2009 Case–control	Cases: 83 from cancer registry Controls: 332 population-based from Health Service Archives Exposure assessment method: other; CALINE4 dispersion model estimating exposure to benzene from road traffic; based on address at diagnosis; exposure categories are approximate quartiles of annual average benzene concentrations	Leukaemia: acute leukaemia	Benzene concentration (μg/m³)			Sex, age, province of residence, PM_{10} concentration	Incidence within previous 0–14 yr; validation of dispersion model reported; linear trends were not statistically significant; stronger and statistically significant associations with AML for children < 5 yr (OR, 5.46; 95% CI, 1.12–26.51; 11 cases) Strengths: population-based, exposure model Limitations: small number of cases; only address at diagnosis; limited confounder adjustment; non-differential misclassification of exposure
			< 0.10	16	1.0		
			0.10 to < 0.25	18	0.8 (0.5–2.6)		
			0.25 to < 0.50	17	1.1 (0.5–2.6)		
			≥ 0.50	32	1.7 (0.8–3.6)		
		Leukaemia (ALL)	OR per 1 μg/m³ increase in average benzene concentration and with a 10 μg/m³ increase in average PM_{10} concentration	64	0.97 (0.49–1.93)	Sex, age, province of residence, PM_{10} concentration	
		Leukaemia (AML)	OR per 1 μg/m³ increase in average benzene concentration and with a 10 μg/m³ increase in average PM_{10} concentration	19	1.92 (0.64–5.78)	Sex, age, province of residence, PM_{10} concentration	

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Houot et al. (2015) Île-de-France, France 2002–2007 Case–control	Cases: 517 from cancer registry Controls: 6147 population-based, from tax databases Exposure assessment method: records; modelled concentration of benzene outside the residence at time of diagnosis	Leukaemia (ALL)	Benzene concentration (μg/m³)			Age	Incidence within previous 0–14 yr Strengths: population-based; exposure model Limitations: small number of AML cases; only address at diagnosis; limited confounder adjustment; non-differential misclassification of exposure
			< 1.3	215	1.0		
			≥ 1.3	210	0.9 (0.7–1.0)		
		Leukaemia (AML)	Benzene concentration (μg/m³)			Age	
			< 1.3	33	1.0		
			≥ 1.3	59	1.6 (1.0–2.4)		
Heck et al. (2014) California, USA 1990–2007 Case–control	Cases: 66 ALL and 41 AML from cancer registry Controls: 2627 for ALL and 17 299 for AML, randomly from population with California birth certificates Exposure assessment method: other; air toxics measured at monitoring station nearest to home address at time of birth (ALL, ≤ 2 km; AML, ≤ 6 km)	Leukaemia (ALL)	OR per IQR increase in benzene concentration (1.2 ppb) during pregnancy			Year of birth, mother's birth place, parity, neighbourhood SES, mother's race/ethnicity	Incidence within previous 0–5 yr; exploratory study of 22 air toxics Strengths: population-based; monitoring-based exposure assessment Limitations: small number of cases; no address history; multiple testing; non-differential misclassification of exposure
			1st trimester	66	0.85 (0.58–1.26)		
			2nd trimester	66	1.16 (0.80–1.67)		
			3rd trimester	66	1.50 (1.08–2.09)		
			Entire pregnancy	66	1.44 (0.84–2.48)		
			1st year of life	61	1.23 (0.62–2.43)		
		Leukaemia (AML)	OR per IQR increase in benzene concentration (1.2 ppb) during pregnancy			See above	
			1st trimester	41	1.13 (0.64–2.01)		
			2nd trimester	41	1.30 (0.74–2.28)		
			3rd trimester	41	1.75 (1.04–2.93)		
			Entire pregnancy	41	1.94 (0.89–4.19)		
			1st year of life	25	2.61 (0.97–6.99)		

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Janitz et al. (2017) Oklahoma, USA 1997–2012 Case–control	Cases: acute leukaemia, 307; ALL, 228; AML, 79; cancer registry Controls: 1013 population with Oklahoma birth certificates Exposure assessment method: other; USEPA National Air Toxics Assessment database modelled benzene 2005 concentration of the census tract where living at time of birth; exposure level cut-points were based on quartiles of benzene concentration (μg/m³) among controls	Leukaemia: ALL and AML combined	Benzene concentration (μg/m³)			Week of birth, race/ ethnicity, age at diagnosis, sex, birth order, electromagnetic fields, urbanization, maternal education, smoking during pregnancy	Incidence within previous 0–19 yr; an alternative exposure categorization showed substantially lower ORs in association with the very highest exposures (above 95th percentile, 1.33–2.03 μg/m³) Strengths: population-based exposure model Limitations: small number of cases; no address history; exposure assessment for only 1 yr, non-differential misclassification of exposure
			0.11 to < 0.39	73	1.00		
			0.39 to < 0.67	71	1.06 (0.71–1.58)		
			0.67 to < 0.91	77	1.21 (0.79–1.87)		
			0.91–2.03	86	1.28 (0.83–1.97)		
		Leukaemia (ALL): childhood ALL	Benzene concentration (μg/m³)			Week of birth, race/ ethnicity, age at diagnosis, sex, birth order, electromagnetic fields, urbanization, maternal education, smoking during pregnancy	
			0.11 to < 0.39	NR	1.00		
			0.39 to < 0.67	NR	0.91 (0.58–1.44)		
			0.67 to < 0.91	NR	1.07 (0.66–1.76)		
			0.91–2.03	NR	1.06 (0.65–1.74)		
		Leukaemia (AML): childhood AML	Benzene concentration (μg/m³)			Week of birth, race/ ethnicity, age at diagnosis, sex, birth order, electromagnetic fields, urbanization, maternal education, smoking during pregnancy	
			0.11 to < 0.39	NR	1.00		
			0.39 to < 0.67	NR	1.60 (0.67–3.82)		
			0.67 to < 0.91	NR	1.92 (0.77–4.74)		
			0.91–2.03	NR	2.42 (0.98–5.96)		

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Spycher et al. (2017) Switzerland 1990 or 2000/ until end of 2008 Cohort	1 664 801 children aged < 16 yr Exposure assessment method: other; census data on parental occupation and JEM (based on ISCO-88) to categorize potential for benzene exposure	Leukaemia (ALL) Leukaemia (AML)	Maternal occupational exposure Ever exposed Maternal occupational exposure Ever exposed	19 3	1.92 (1.18–3.13) 1.05 (0.32–3.48)	Sex; year of birth; census; education; household crowding; neighbourhood SES; background ionizing radiation and electromagnetic fields from radio and TV transmitters; distance to nearest highway, petroleum refinery, petrol station, motor vehicle service station	No associations with AML or ALL and paternal exposure were observed Strengths: population-based study with data on incidence; models adjusted for a range of socioeconomic, perinatal, and environmental factors; accurate and complete outcome ascertainment Limitations: no adjustment for maternal smoking; small number of exposed AML cases

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CALINE4, California Line Source Dispersion model, version 4; CI, confidence interval; ICD, International Statistical Classification of Diseases and Related Health Problems; IQR, interquartile range; ISCO, International Standard Classification of Occupations; JEM, job-exposure matrix; NR, not reported; OR, odds ratio; PM, particulate matter; ppb, parts per billion; SES, socioeconomic status; USEPA, United States Environmental Protection Agency; yr, year(s)

of the distribution of exposure, or above the 99th percentile compared with below the 50th percentile, the respective adjusted relative risks were 0.9 (95% CI, 0.6–1.5) and 0.4 (95% CI, 0.1–1.6) for leukaemia, 1.1 (95% CI, 0.7–1.8) and 0.6 (95% CI, 0.2–1.7) for tumours of the CNS, and 0.8 (95% CI, 0.4–1.8) and 0.4 (95% CI, 0.1–2.0) for lymphomas. The results also indicated no associations between benzene concentrations at the address(es) during pregnancy and cancer risk, although the group exposed to the second-highest levels of benzene was associated with a relative risk for lymphomas of 2.2 (95% CI, 1.2–3.9). This higher risk for lymphomas in association with benzene concentrations during pregnancy was restricted to HL; based on 19 exposed cases, a relative risk of 4.3 (95% CI, 1.5–12.4) was observed in association with exposure above the 90th percentile compared with below the 50th percentile (see *IARC Monographs* Volume 100F, Table 2.14, available at: http://publications.iarc.fr/123). [The strengths of this study included the population-based design, the large sample size, the assessment of cumulative exposure over all addresses during pregnancy and childhood, and the objective, model-based exposure assessment method. However, the study was limited by the non-differential misclassification of exposure.]

A small study of leukaemia incidence was undertaken in Varese, Italy (Crosignani et al., 2004). A total of 120 incident cases were identified from the population-based Lombardy Cancer Registry, and 480 population controls, matched to cases by age and sex, were selected from the population-based Health Service Archives. Benzene concentration at the address at diagnosis was calculated on the basis of traffic density on surrounding roads and distances from the home address to roads with heavy traffic. When comparing children exposed to high concentrations of benzene (estimated annual average, benzene at > 10 μg/m^3) with children not exposed to road traffic emissions (estimated annual average, benzene at < 0.1 μg/m^3), the relative risk was 3.9 (95% CI, 1.4–11.3) based on 7 exposed cases, and 4.3 (95% CI, 1.5–12.6) after adjustment for socioeconomic status of the municipality. There was a trend across the three exposure categories (*P* for trend, < 0.005). [The strengths of the study included the population-based design and the objective, model-based, exposure assessment method. The limitations included the small number of cases, the use of only the address at diagnosis for exposure assessment, and the non-differential misclassification of exposure.]

In the Emilia-Romagna region, northern Italy, Vinceti et al. (2012) identified 83 incident cases of acute leukaemia among children (age, 0–14 years) in the population-based Italian Association of Paediatric Haematology and Oncology cancer registry. A total of 332 population controls, individually matched to cases by sex, year of birth, and province of residence during the diagnosis year, were selected. Traffic-related benzene and PM_{10} (particulate matter of diameter ≤ 10 μm) concentrations were estimated by the California Line Source Dispersion model, version 4 (CALINE4), at the address at time of diagnosis. Modelled benzene concentrations were validated against those measured at fixed-site monitoring stations, showing a correlation coefficient of 0.43 (95% CI, −0.48–0.89) for annual mean values. The odds ratio for acute leukaemia was 1.7 (95% CI, 0.8–3.6) for the highest (≥ 0.50 μg/m^3) compared with the lowest (< 0.10 μg/m^3) category of benzene exposure, after adjustment for PM_{10}. Linear analyses adjusted for PM_{10} showed odds ratios of 0.97 (95% CI, 0.49–1.93; 64 cases) and 1.92 (95% CI, 0.64–5.78; 19 cases) for ALL and AML, respectively, in association with a 1 μg/m^3 increase in average benzene concentration. Restricting the linear analyses to children diagnosed when aged 0–4 years yielded odds ratios of 1.95 (95% CI, 0.58–6.50; 27 cases) and 5.46 (95% CI, 1.12–26.51; 11 cases) for ALL and AML, respectively. [A strength of this study was the population-based design and the objective exposure assessment by

use of a validated exposure model, although the correlation between calculated and measured benzene was relatively low. Limitations included the small sample size resulting in wide confidence intervals, the use of only the address at the time of diagnosis for exposure assessment, and non-differential misclassification of exposure.]

Houot et al. (2015) estimated benzene concentrations at the addresses at the time of diagnosis of 517 incident acute leukaemia cases (age, 0–14 years) and 6147 control children living in the Île-de-France region in France. Cases were identified from the National Registry of Childhood Haematopoietic Malignancies, and control children were selected from a population-based tax database. The benzene modelling took into account contributions from both urban background pollution and local traffic. The subjects were classified on the basis of the benzene exposure estimate at their home being either less than 1.3 μg/m^3 (median exposure for the controls) or 1.3 μg/m^3 or more. When comparing the two groups, the odds ratio for ALL was 0.9 (95% CI, 0.7–1.0; based on 210 exposed cases) and for AML was 1.6 (95% CI, 1.0–2.4; $P < 0.05$, based on 59 exposed cases). The analyses were adjusted for age. [Strengths included the population-based design and the objective, model-based exposure assessment method. Limitations included the limited adjustment for potential confounders, the use of only the address at the time of diagnosis for exposure assessment, the non-differential misclassification of exposure, and the limited contrast in the analysis of exposures to above versus below the median.]

A series of studies from California, USA, studied the association between a wide range of air toxics (including benzene) measured at 39 different sites and different types of childhood cancers. Cases (age, 0–5 years) were identified from the California Cancer Registry, and population controls were selected randomly from the California birth records. Birth addresses of cases and control children were allocated to 1 of the 39 monitoring stations if the address was within a certain distance, and the measured air toxic concentration was averaged over certain time periods; both the distance and the time period differed between the studies. Odds ratios were calculated for one interquartile range (IQR) increase in benzene concentration, corresponding to 1.2 ppb (3.8 μg/m^3). Heck et al. (2013) compared benzene concentrations during pregnancy for 74 neuroblastoma cases and 13 115 control children, and observed an odds ratio of 1.36 (95% CI, 0.82–2.25) per IQR increase of benzene concentration after adjustment for year of birth, maternal race/ethnicity, maternal age, and method of payment for prenatal care. Shrestha et al. (2014) studied 337 cases of Wilms tumour and 96 514 control children, and found an odds ratio of 1.07 (95% CI, 0.84–1.36) per IQR of benzene concentration during pregnancy after adjustment for birth year, maternal age, maternal race/ethnicity, parity, and census-based socioeconomic status. Heck et al. (2014) included 66 cases of ALL and 41 cases of AML, and found odds ratios of 1.44 (95% CI, 0.84–2.48) for ALL and 1.94 (95% CI, 0.89–4.19) for AML per IQR increase of benzene concentration during the entire pregnancy. Results were adjusted for year of birth, maternal race/ethnicity, mother's birthplace, parity, and neighbourhood socioeconomic status. Exposure during the third trimester was associated with statistically significant odds ratios. Odds ratios in association with benzene concentration during the first year of life were similar to those reported for exposure during pregnancy. Heck et al. (2015) included 88 retinoblastoma cases and 25 144 control children, and found a significant odds ratio of 1.67 (95% CI, 1.06–2.64) per IQR increase of benzene concentration during pregnancy after adjustment for year of birth, maternal race and birthplace, paternal age, and method of payment for prenatal care. Von Ehrenstein et al. (2016) investigated the risk for tumours of the CNS based on 168 cases and 27 199 control children; odds

ratios of 2.14 (95% CI, 1.12–4.06; 38 cases) for primitive neuroectodermal tumour, 0.82 (95% CI, 0.36–1.87; 30 cases) for medulloblastoma, and 0.83 (95% CI, 0.53–1.29; 100 cases) for astrocytoma per IQR increase of benzene concentration during pregnancy were reported. Results were adjusted for year of birth, maternal race/ethnicity, maternal age, birthplace, and education. [The strengths of these studies included the population-based design and the objective, monitoring-based exposure assessment method. Limitations included the small sample sizes, the assessment of exposure at only one address, the non-differential misclassification of exposure, and the explorative nature including analyses of many pollutants. Not adjusting for smoking at home, and especially maternal smoking during pregnancy, were other common limitations of many studies. The results for neuroblastoma and retinoblastoma appear to be generating, rather than testing, hypotheses.]

Symanski et al. (2016) identified 1248 cases (age, 0–4 years) of ALL from the Texas Cancer Registry and selected 12 172 population-based control children from birth certificates. The address at birth was used to allocate each child to a census tract. Concentrations of benzene, 1,3-butadiene and polycyclic organic matter at census tract level were extracted from the United States Environmental Protection Agency National-Scale Air Toxics Assessment (NATA), which provided modelled concentrations for years 1996, 1999, 2002, and 2005; each address was allocated to an exposure quartile for the year closest in time. The statistical models adjusted for time of birth, census tract (random effect), maternal age, infant birth weight, sex, and maternal race/ethnicity, and reported odds ratios of 1.19 (95% CI, 1.00–1.41), 1.16 (95% CI, 0.98–1.38), and 1.17 (95% CI, 0.98–1.39) for the second, third, and fourth exposure quartiles compared with the first. Models including both benzene and 1,3-butadiene showed associations between childhood cancer and exposure to 1,3-butadiene, but not to benzene. [The Working Group noted that it is difficult to disentangle the effect of correlated pollutants. The strengths of this study included: the population-based design; the large sample size; the objective, model-based exposure assessment method; and the mutual adjustment for other air pollutants. Limitations included the lack of information about address history and non-differential misclassification of exposure. Further, the transformation of absolute exposure concentrations into quartiles based on 4 different years makes a quantitative interpretation of the results difficult: for example, the third exposure quartile for benzene in 1996 has a lower range of benzene levels than the second exposure quartile in 1999.]

Janitz et al. (2017) studied benzene and acute leukaemia including 228 cases of ALL and 79 of AML (age, 0–19 years) from the Oklahoma Central Cancer Registry, USA. A total of 28% of identified cases were excluded, however, because they could not be linked to birth certificates. Population controls (n = 1013) were selected from birth certificates, matched by week of birth. Address at birth was allocated to the census tract, and benzene concentrations for 2005 for each census tract were extracted from the NATA database. Children were divided into quartiles of exposure and, in a secondary analysis, the cut-off point at the 40th (0.53 μg/m^3), 60th (0.78 μg/m^3), and 95th (1.33 μg/m^3) percentiles were used to form exposure categories. The results indicated no association between benzene and ALL, with an odds ratio for the highest quartile compared with the lowest of 1.06 (95% CI, 0.65–1.74) and that for above the 95th percentile compared with below the 40th percentile being 0.67 (95% CI, 0.28–1.62). In contrast, the two corresponding odds ratios for AML were 2.42 (95% CI, 0.98–5.96) and 1.58 (95% CI, 0.53–4.69), with an indication of an exposure–response relationship over quartiles but not over the alternative exposure categorization. The analyses adjusted for time of birth, race/ethnicity, age at diagnosis, sex, birth order,

exposure to electromagnetic fields, urbanization, and maternal education and smoking during pregnancy. [The strengths included the population-based design and the objective, model-based exposure assessment method. Limitations included the limited sample size, the lack of information about address history, the exposure assessment being based on only 1 year, and the non-differential misclassification of exposure. The Working Group noted the exclusion of cases that could not be linked to a birth certificate as a potential source of selection bias.]

Jiang et al. (2016) measured the benzene metabolite *trans*,*trans*-muconic acid (t,t-MA) in urine samples from 71 cases of ALL identified at the Shenzhen Children's Hospital, China, and from those of 142 control children selected from the orthopaedics section and matched to cases by sex and age. A higher proportion was above the detection limit among cases compared with controls, and higher t,t-MA concentration was associated with an increased risk (OR, 1.09; 95% CI, 1.00–1.19). [The Working Group noted that the related exposure contrast was not reported in the article. Other limitations included: the risk for reverse causation because urine was collected after the ALL diagnosis; a lack of translation between concentrations of t,t-MA in urine (which only reflect exposure during the few hours before urine collection) and exposure to benzene, meaning that the use of t,t-MA in this context did not provide a good context for exposure to benzene and the validity of this exposure assessment method is low (see Section 1.3); and the non-differential misclassification of exposure.]

2.3.2 Parental occupational exposure to benzene

Nine case–control and two cohort studies assessed the occupational exposure to benzene of parents of childhood cancer cases and controls. In 8 of the 11 studies, the exposure assessment was based on information about parental occupation, industry, or exposure collected by interviews with parents after their child received a diagnosis of cancer. Three other studies used information from birth certificates (Shaw et al., 1984) or census data (Feychting et al., 2001; Spycher et al., 2017), which could not be influenced differently by parents of cases and controls. Exposure was assessed in two (yes/no) or three (e.g. no/possible/probable) categories either by parents themselves or by experts or JEM, but was never quantified. [The Working Group noted that the interview-based exposure assessment in 8 of the 11 studies implied a risk for recall and interviewer bias. Differential participation among parents of cases and controls may also have biased the results. Other general limitations were the low numbers of exposed parents, leading to imprecise risk estimates, and the lack of quantification of benzene exposure. For these reasons, the Working Group gave little weight to this group of studies when evaluating benzene as a potential cause of childhood cancer.]

The results from the 11 studies are inconsistent. Shaw et al. (1984) used information about job from birth certificates in a case–control study in California, USA, and reported no difference in childhood leukaemia risk between case and control groups for paternal occupation with potential for benzene exposure. In a cohort study in Sweden, Feychting et al. (2001) reported no association between parental job (2–26 months before the child's birth, obtained from census data) with the potential for benzene exposure (possible and probable exposure combined) and the risk for leukaemia (RR, 1.23; 95% CI, 0.39–3.85; 3 cases) or tumours of the CNS (RR, 0.91; 95% CI, 0.23–3.70; 2 cases) in their children. The risk of leukaemia in children younger than 5 years was 2.0 (95% CI, 0.6–6.3) based on 3 exposed cases [although benzene exposure was too rare for a meaningful analysis].

In a nationwide cohort study in Switzerland, Spycher et al. (2017) used census data about parental occupation and a JEM to categorize

potential for benzene exposure (Table 2.5). In association with maternal exposure to benzene, the study showed a hazard ratio for ALL of 1.92 (95% CI, 1.18–3.13; 19 exposed cases) using the partially adjusted model and 2.63 (95% CI, 1.58–4.38) using the fully adjusted model, which included 14 variables but not maternal smoking. No association between maternal exposure to benzene and risk for AML (3 exposed cases only), lymphoma, NHL, tumours of the CNS, or glioma (Table 2.5) was found. The study also found no association between paternal benzene exposure and risk for ALL or AML, although an increased non-significant risk was observed for AML (HR, 2.66; 95% CI, 0.79–9.00; 3 exposed cases in the upper exposure category). [The Working Group considered this study to be informative because of: the lack of potential for participation, recall, or interviewer bias; the objective assessment of benzene exposure using a JEM; the proper adjustment for potential confounders; and the greater number of cases with exposed parents compared with most other studies. No adjustment was made for parental smoking, however, and there was potential for exposure misclassification.]

Among the eight case–control studies with subjective (interview-based) information about benzene exposure, three found statistically significant associations, two found increased odds ratios without statistical significance, and three found no association.

Shu et al. (1988) (see Table 2.4 in *IARC Monographs* Volume 100F, available at: http://publications.iarc.fr/123) investigated parental exposures in relation to childhood leukaemia in Shanghai and found an association between maternal exposure during pregnancy and risk for ANLL (OR, 4.0; 95% CI, 1.8–9.3; 11 cases) but not for ALL (OR, 1.3; 95% CI, 0.5–3.0; 8 cases).

McKinney et al. (1991), evaluated in *IARC Monographs* Volume 100F, investigated the associations between parental exposure to benzene and risk for leukaemia and NHL (combined) in north England; statistically significant associations with paternal preconceptional exposure (OR, 5.8; 95% CI, 1.7–26.4; 12 cases) and with maternal preconceptional exposure (OR, 4.0; 95% CI, 0.3–118.0; 2 cases) were found.

Castro-Jiménez & Orozco-Vargas (2011) investigated parental benzene exposure and risk for ALL in Columbia, and reported unadjusted odds ratios of 3.0 (95% CI, 1.3–7.1) and 1.6 (95% CI, 0.8–3.1) in association with maternal and paternal exposure, respectively, before conception, and an unadjusted odds ratio of 1.9 (95% CI, 0.8–4.2) in association with maternal exposure during pregnancy. After adjustment for maternal age, parental preconception smoking status, and maternal socioeconomic status during pregnancy, odds ratios of 5.50 (95% CI, 1.38–21.92) and 11.65 (95% CI, 2.98–45.59) were observed for exposure to benzene by mother only and by both parents, respectively.

Feingold et al. (1992) investigated childhood cancer in Denver, Colorado, USA, and reported odds ratios in association with paternal occupational benzene exposure during the year before the child's birth of 0.7 (95% CI, 0.1–3.1) for tumours of the CNS and 1.6 (95% CI, 0.5–5.8) for ALL. Peters et al. (2014) investigated childhood tumours of the brain in Australia, and reported odds ratios of 2.4 (95% CI, 0.2–25.7) and 2.7 (95% CI, 0.9–7.9) in association with maternal exposure in the year before birth and paternal exposure in the year before conception.

Kaletsch et al. (1997) studied childhood leukaemia and lymphoma in Germany, and found no association with parental occupational exposure to benzene.

Shu et al. (1999) investigated parental occupational exposures and risk of ALL in offspring (see Table 2.4 in *IARC Monographs* Volume 100F, available at: http://publications.iarc.fr/123). The authors reported odds ratios of 0.7 (95% CI, 0.3–1.8), 0.5 (95% CI, 0.1–1.6), and 0.6 (95% CI, 0.2–1.6) for maternal exposure to benzene before conception, during pregnancy, and after birth, respectively. Corresponding odds ratios for

paternal occupational exposure to benzene were 1.2 (95% CI, 0.8–1.2), 1.0 (95% CI, 0.6–1.7), and 1.2 (95% CI, 0.7–1.9).

Infante-Rivard et al. (2005) studied childhood ALL in Canada and reported an odds ratio of 0.8 (95% CI, 0.2–3.1) in association with maternal benzene exposure during the 2 years before birth, and one of 1.4 (95% CI, 0.3–6.3) in association with maternal exposure during pregnancy.

[Treating all leukaemias as a single entity is a limitation of several of the preceding studies, given the evidence of etiological heterogeneity.]

2.4 Other cancers

This section describes epidemiological studies on benzene exposure and cancer in adults in sites other than in the lymphohaematopoietic system. The tables (Table 2.6 and Table 2.7) include only relevant studies of cancer sites with sufficient or limited evidence that either were not included in *IARC Monographs* Volume 100F (IARC, 2012a) or were published later.

2.4.1 Occupational cohort studies

Several occupational cohort studies have reported results for multiple solid tumour sites. Some earlier studies were updated for specific outcomes, most often leukaemia or lymphomas, but results for other cancers were not reported in the updates.

The Working Group included occupational cohort studies that reported risk estimates specifically for benzene, based on either individual estimates of exposure or identification of subcohorts exposed to benzene. Studies were excluded if they did not meet these inclusion criteria (Guberan & Raymond, 1985; Dagg et al., 1992; Rushton, 1993; Schnatter et al., 1993; Tsai et al., 1993, 1996, 2003; Walker et al., 1993; Honda et al., 1995; Collingwood et al., 1996; Fu et al., 1996; Satin et al., 1996; Järvholm et al., 1997; Lynge et al., 1997; Lundberg & Milatou-Smith, 1998; Pukkala, 1998; Consonni et al., 1999; Divine et al., 1999a, b; Wong et al., 2001a, b; Sorahan et al., 2002; Kauppinen et al., 2003; Lewis et al., 2003; Huebner et al., 2004; Gun et al., 2006; Bonneterre et al., 2012), were superseded by updates (Ott et al., 1978), presented results only for excessively broad outcome groupings, or had very small study populations (Decouflé et al., 1983).

Studies publicly available at the time were reviewed in *IARC Monographs* Volume 100F, and a new search of the literature for this review identified a few additional studies in occupational cohorts. The following studies available at the time of the previous IARC review are included in the current evaluation: Tsai et al. (1983), Wilcosky et al. (1984), Bond et al. (1986), Wong (1987a, b), Wong et al. (1993), Greenland et al. (1994), Hayes et al. (1996), Bulbulyan et al. (1999), and Sorahan et al. (2005). A brief description of these cohorts is provided in the following section.

(a) Description of occupational cohorts

Bond et al. (1986) studied mortality among 956 workers from a chemicals production plant in Michigan, USA, exposed to benzene (see Section 2.1.1). Industrial hygiene data were used to weight jobs as incurring very low, low, moderate, or high levels of exposure, a representative time-weighted average exposure value was assigned to each level, and cumulative dose indices were calculated for each worker by summing daily time-weighted average values over the work history. Exposure–response analyses were made both including and excluding workers who were exposed to arsenic, asbestos, or high levels of vinyl chloride. Updated results for this cohort were reported by Collins et al. (2015) (see Section 2.1.1(b)), but data were not provided for all cancer sites.

Bulbulyan et al. (1999) reported cancer mortality among women in the Russian printing

Table 2.6 Occupational cohort studies of exposure to benzene and cancer of the lung

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Linet et al. (2015) China, 12 cities 1972–1987/1972–1999 Cohort	74 827 benzene-exposed and 35 504 unexposed Chinese workers; spray and brush painting (coatings), rubber, chemical (including pharmaceutical manufacturing), shoemaking, and other (including printing and insulation) industries Exposure assessment method: records; workers dichotomized (benzene exposed/unexposed) based on job titles and factory records on use of benzene-containing materials	Lung: ICD-9 (code 162)	Exposed	351	1.5 (1.2–1.9)	Sex, attained age, attained calendar year	Supersedes Yin et al. (1996a), Hayes et al. (1996); lag of 2 yr for HLD and 10 yr for all other outcomes; MDS RR mortality, infinity (1.5–infinity), $n = 7$; incidence, infinity (1.9–infinity), $n = 8$ Strengths: large sample size, 28-yr follow-up Limitations: exposure dichotomized to exposed/unexposed only (no further classification); wide range of industrial processes included; coexposures vary and were not addressed in the analyses
Koh et al. (2014) Republic of Korea 2002–2007 Cohort	14 698 male workers registered in a regional petrochemical plant maintenance workers union 2002–2007 Exposure assessment method: none; benzene-exposed workers	Lung: ICD-10 (codes C33–34) Lung: ICD-10 (codes C33–34)	SMR exposed SIR exposed	9 5	0.68 (0.31–1.29) 0.73 (0.24–1.71)	Age Age	Strengths: good coverage of target population Limitations: short follow-up time; no quantitative exposure assessment; occupational histories and specific tasks not available; tobacco exposure history not available
Koh et al. (2011) Republic of Korea 1992–2007 follow-up (16 yr) Cohort	8866 male workers at seven petrochemical plants producing or using benzene Exposure assessment method: none; classified by job	Lung (mortality) Lung (incidence) 	All workers Manufacturing workers Office workers All workers Manufacturing workers Office workers	5 3 2 8 2 6	0.35 (0.11–0.83) 0.31 (0.06–0.91) 0.44 (0.05–1.95) 0.60 (0.26–1.17) 0.22 (0.03–0.78) 1.42 (0.52–3.09)	Age and calendar period Age and calendar period 	Strengths: the first investigation of cancer risk of workers in a refinery/petrochemical complex in the Republic of Korea; data from cancer registry; ICD-10 coding Limitations: no control for smoking; short follow-up; small number of cases; healthy worker effect

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 workers exposed to benzene at a chemical plant Exposure assessment method: quantitative measurements; job titles were assigned to exposure categories by an industrial hygienist, based on IH measurements (JEM)	Lung: ICD-10 (codes C33–C34)	Ever exposed 0–3.9 ppm-yr	146	1.05 (0.89–1.24)	Age, race, sex	Third update of the Dow Chemical plant retrospective cohort Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: mortality study based on death certificates

CI, confidence interval; HLD, haematopoietic, lymphoproliferative, and related disorders; ICD, International Statistical Classification of Diseases and Related Health Problems; IH, industrial hygiene; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; ppm, parts per million; RR, relative risk; SIR, standard incidence ratio; SMR, standardized mortality ratio; yr, year(s)

Table 2.7 Epidemiological studies of exposure to benzene and cancer at other sites in adults in the general population

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bove et al. (2014) USA (California) 1975–1985/1979–2008 Cohort	309 901 marine and naval personnel who began service during 1975–1985 and were stationed anytime during this period in one of two camps, one of them having contaminated drinking-water and the other not Exposure assessment method: records; monthly average estimates of contaminant concentrations in drinking-water, published in peer-reviewed agency record; each individual was assigned estimated exposure based on residence	Lung: trachea, bronchus, and lung; ICD-9 (code 162)	Camp Pendleton (referent, non-contaminated water)	216	0.81 (0.71–0.93)	Age, sex, race, rank, education	Despite the possible healthy veteran effect bias, elevations in SMR were observed in the exposed camp Strengths: large cohorts; low loss to follow-up Limitations: exposure misclassification; for a mortality endpoint a longer follow-up is necessary; 97% of the Camp Lejeune cohort was of age < 55 yr and < 6% had died by the end of the study
			Camp Lejeune (exposure to contaminated drinking-water)	237	0.92 (0.80–1.04)		
Yuan et al. (2014) China, Shanghai 1986–1989 Nested case–control	Cases: 82 men, lifelong non-smokers aged 45–64 yr at enrolment Controls: 83 members of the Shanghai Cohort study without cancer, non-smokers and alive at the time of cancer diagnosis of the case; matched by age at enrolment (±2 yr), year, month of urine sample collection (±1 month), and neighbourhood of residence at recruitment Exposure assessment method: other; levels of urinary PAH and VOCs (SPMA for benzene) prospectively analysed	Lung	Quartiles of SPMA (metabolite of benzene)			Age at baseline, neighbourhood of residence at enrolment, years of sample storage, urinary cotinine level	Strengths: active follow-up with annual in-person interviews; after 22 yr loss of follow-up low (only 5%); urinary cotinine was also quantified to confirm non-smoking status Limitations: relatively small sample size; 26% of cases not histologically confirmed; small number of cases of SCC (n = 16); urinary samples were only collected at baseline
			1st quartile (ref)	17	1.00		
			2nd quartile	18	1.03 (0.39–2.69)		
			3rd quartile	19	1.10 (0.44–2.78)		
			4th quartile	20	1.57 (0.65–3.80)		
			Trend test P value, 0.31				
		Lung (SCC)	Tertiles of SPMA			Age at baseline, neighbourhood of residence at enrolment, years of sample storage, urinary cotinine level	
			1st tertile (ref)	NR	1.00		
			2nd tertile	NR	1.97 (0.31–12.65)		
			3rd tertile	NR	5.76 (1.11–28.96)		
			Trend test P value, 0.023				

Table 2.7 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Villeneuve et al. (2014) Toronto, Canada 1997–2002 Case–control	Cases: 445 incident cases of cancer of the trachea, bronchus, or lung; men and women aged 20–84 yr; non-smokers were oversampled (35%); recruited from four tertiary-care hospitals in Toronto Controls: 948 (425 population and 523 hospital); one control series was population-based (from tax assessment files), the other control series was recruited from a nonspecialized family medicine clinic Exposure assessment method: other; land-use regression models linked to residential addresses; questionnaire on exposures	Lung: trachea, bronchus, and lung; ICD-9 (code 162) Lung: trachea, bronchus, and lung; ICD-9 (code 162)	IQR increase in time-weighted average benzene concentration IQR increase ($0.15\ \mu g/m^3$) Residential benzene exposure, 10 yr before interview IQR increase ($0.15\ \mu g/m^3$)	NR NR	1.84 (1.26–2.68) 1.58 (1.15–2.16)	Controls frequency-matched to cases by ethnicity, age, sex, pack-years of smoking (continuous variable, summed over pipe, cigar, and cigarette use), exposure to second-hand smoke, BMI, family history of cancer, neighbourhood measures of unemployment, median family income	The exposure profile among hospital controls would be expected to be higher than that in population controls (because of residential location of the hospital) Strengths: good exposure assessment including smoking history Limitations: low participation rate of population controls (59%) and lung cancer cases (62%)

BMI, body mass index; CI, confidence interval; ICD, International Statistical Classification of Diseases and Related Health Problems; IQR, interquartile range; NR, not reported; PAH, polycyclic aromatic hydrocarbon; SCC, squamous cell cancer; SMR, standardized mortality ratio; SPMA, *S*-phenylmercapturic acid; VOCs, volatile organic compounds; yr, year(s)

industry; the occupation of bookbinding was known to have used benzene until 1958. [The Working Group noted that many women in the bookbinding group probably did not work during the time when benzene was used.]

Greenland et al. (1994) followed up 1821 male workers at a transformer assembly facility from 1969 to 1984. Interviews with long-term employees were used to develop JEMs for seven types of exposure, including benzene, classified as none, indirect (nearby), or direct. [The notable limitations of this study included work history records not being available for many (34%) of the workers, so the size of the underlying cohort was unknown.]

Hayes et al. (1996) examined mortality among a cohort of Chinese workers from multiple industries, of whom 74 828 were exposed to benzene and 35 805 were unexposed. The study provided relative risk estimates and assessed trends across several cumulative exposure categories (0, < 10, 10–39, 40–99, 100–400, and > 400 ppm-years) for a subset of outcomes.

A total of 5514 workers from 233 facilities in the United Kingdom who were judged by their employers to be exposed to benzene were followed up for cancer incidence (1971–2001) and mortality (1968–2002) by Sorahan et al. (2005). No exposure assessment was included in the analyses because air sampling data were from 1966/1967 and only available from 130 of the facilities. [The study was retained by the Working Group as each cohort member was reported as exposed. Some under-ascertainment in the incidence component of the study was reported.]

Tsai et al. (1983) reported on 454 male benzene workers ever employed at a refinery in Texas, USA. Exposure evaluation used 1973–1982 air sampling data to determine which employees worked in benzene areas and for what duration.

Wilcosky et al. (1984) examined 6678 active and retired rubber industry workers from Akron, Ohio, USA. The study assigned worker exposures based on employment in a process area where a specific solvent was authorized for use, but the authors acknowledged that the solvents may not have been used in some of the areas.

Wong (1987a, b) reported on a study of 4602 workers exposed to benzene and 3074 unexposed workers in seven chemical plants in the USA. Wong (1987a) reported results for exposure characterized as intermittent or continuous. A companion paper presented results for this same population by cumulative exposure category for select outcomes, in which Mantel–Haenszel extension χ^2 trend test results were given (Wong, 1987b).

Wong et al. (1993) reported data for a cohort of gasoline distribution workers in the USA, 9026 of whom were based on land and 9109 who operated on marine vessels between 1946 and 1985, who were followed up for mortality outcomes until 1989. The exposure assessment for this study developed metrics for cumulative and peak exposures to total hydrocarbons as a surrogate for benzene exposure to components of gasoline.

Many of these cohort studies had limitations. In some of the occupational settings, all or subsets of workers had potential exposure to other substances such as asbestos, dust, other solvents, and industrial chemicals. Bond et al. (1986) addressed some of these additional exposure types in the analysis, in this case by removing workers exposed to arsenic, asbestos, or high levels of vinyl chloride. None of these studies adjusted for covariates other than demographic factors. The studies were also inconsistent in addressing latency; some presented results by time since first exposed or employed.

Of the more recent studies included in this evaluation, Stenehjem et al. (2017) prospectively followed up a cohort of 24 917 offshore petroleum workers in Norway (see Section 2.2.1(b)) for 13.5 years for incident cases of cancer, including cancer of the skin (melanoma and squamous cell carcinoma of the forearm and hand). An update of a cohort of Chinese workers from multiple industries (see Section 2.2.1(b)) (Linet et al.,

2015) provided rate ratios comparing outcomes between ever- and never-exposed workers. Koh et al. (2014) followed up 14 698 temporary maintenance workers in a refinery/petrochemical complex in the Republic of Korea for mortality from 2002 to 2007 and for incidence from 2002 to 2005; the workers' exposure to benzene was characterized by Chung et al. (2010). [The Working Group noted the short follow-up time as a limitation of this study.] Koh et al. (2011) also examined cancer mortality during 1992–2007 and incidence during 1997–2005 among 8866 male workers in units of a refinery/petrochemical complex in the Republic of Korea that produced or used benzene. Results were given separately for production, maintenance, laboratory, and office workers, but the authors were not able to quantify exposures because they lacked adequate job history and exposure records.

(b) Cancer sites reviewed in IARC Monographs Volume 100F

(i) Cancer of the lung

The evidence from occupational cohort studies of an association between exposure to benzene and cancer of the lung that was available at the time of publication of *IARC Monographs* Volume 100F was judged to be *inadequate*. Cohort studies available at that time, with information on potential or estimated benzene exposure and cancer of the lung, are described in Table 2.15 of Volume 100F (available at: http://publications.iarc.fr/123).

Bond et al. (1986) reported that among 956 chemical manufacturing plant workers in the USA, who had been employed for 1 month or longer during 1938–1978 and followed up through 1982, overall mortality from cancer of the lung was not increased (SMR, 0.99; 95% CI, 0.59–1.57; 18 deaths). Among cumulative exposure categories, an excess risk (SMR, 2.04; 5 deaths) was observed for the category of 500–599 ppm-months, while no excess risk was observed for the categories of 0–499 ppm-months (SMR, 0.62; 6 deaths) and 1000 ppm-months or more (SMR, 0.49; 2 deaths) [95% CIs were not reported]. In an update of this cohort (see Table 2.6), Collins et al. (2015) reported that among 2266 United States chemical manufacturing plant workers beginning employment during 1940–1982 and followed up through 2009, no excess risk of cancer of the lung (including lung, trachea, and bronchus; ICD-10, codes C33–C34) was observed (SMR, 1.05; 95% CI, 0.89–1.24; 146 deaths).

Collins et al. (2003) reported that among 4417 chemical manufacturing hourly workers in Illinois, USA, who began employment during 1940–1977 and were followed up through 1997, a 60% excess risk of cancer of the lung was observed among those with cumulative exposure to benzene at more than 6 ppm-years (SMR, 1.6; 95% CI, 1.2–2.1; based on 55 deaths). There was a monotonic trend in standardized mortality ratios across cumulative exposure groups exposed to benzene at less than 1 ppm-years (SMR, 1.1; 95% CI, 0.7–1.5), 1–6 ppm-years (SMR, 1.3; 95% CI, 1.0–1.8), and more than 6 ppm-years (SMR, 1.6; 95% CI, 1.2–2.1), although the reference group also presented an elevated risk for cancer of the lung (SMR, 1.3; 95% CI, 1.1–1.5).

Sorahan et al. (2005) reported that among 5514 workers exposed to benzene in 233 factories in the United Kingdom during 1966/1967 or earlier, followed up for mortality during 1968–2002, there was a significant increase in mortality from cancer of the lung (SMR, 1.21; 95% CI, 1.07–1.35; based on 294 deaths) and in incidence of cancer of the lung (SIR, 1.19; 95% CI, 1.06–1.34; based on 293 cases). There was no clear evidence of heterogeneity by type of industry, despite exposure of some of these workers to other carcinogens such as asbestos and polycyclic aromatic amines. [The Working Group noted that some of the deaths coded to cancer of the lung may actually have been due to mesothelioma. Some cancer cases may have been missed or misclassified.]

Wong (1987a, b) reported a standardized mortality ratio of 1.12 (95% CI, 0.90–1.39; *n* = 86), but no exposure–response relation was observed. Among land-based gasoline distribution and marine distribution workers, Wong et al. (1993) reported standardized mortality ratios of 0.66 (95% CI, 0.57–0.77; *n* = 165) and 1.07 (95% CI, 0.94–1.21; *n* = 208), respectively.

The Working Group identified several other pertinent studies that were available at the time, but not included in the previous review (see Table 2.6). Tsai et al. (1983) reported a standardized mortality ratio of 0.52 (95% CI, 0.06–1.86; *n* = 2) in a study of refinery workers. Greenland et al. (1994) observed an odds ratio of 0.58 (95% CI, 0.31–1.07) when directly comparing exposed transformer repair workers with their indirectly exposed or unexposed counterparts. Bulbulyan et al. (1999) reported a standardized mortality ratio of 0.7 (95% CI, 0.1–2.0; *n* = 3) among female bookbinders.

Additional data were available for several more recent studies. Linet et al. (2015) (see Section 2.1.1(b) for details) updated data on cancer among Chinese workers exposed to benzene, studied previously by Hayes et al. (1996). Workers exposed to benzene demonstrated a significant excess of mortality from cancer of the lung (RR, 1.5; 95% CI, 1.2–1.9; *n* = 351). The highest relative risk for mortality from cancer of the lung was for workers in the rubber and coatings industries. Relative risks for death from cancer of the lung were significantly elevated and of the same magnitude in the early (1972–1987) and later (1988–1999) follow-up periods. All analyses were stratified according to sex, attained age, and attained calendar year. [The Working Group noted that there was no control for potential confounding by smoking or other occupational exposures. However, the authors noted that associations were similar in women and men, although the prevalence of smoking is generally much lower among Chinese women.] Hayes et al. (1996) and Yin et al. (1996b) previously reported data for this cohort. Mortality from cancer of the lung (also including trachea and bronchus; ICD-9, code 162) was in excess in the cohort overall (RR, 1.4; 95% CI, 1.0–2.0) due to men (RR, 1.5; 95% CI, 1.0–2.2; 109 cases) but not women (RR, 1.0; 95% CI, 0.4–2.9; 16 cases), and it was increased among workers with greater estimated cumulative benzene exposure (RR, 1.7 for those with ≥ 400 ppm-years exposure vs no exposure; *P* value for trend, 0.01).

Koh et al. (2011) reported on a study of cancer mortality and incidence among petrochemical workers at plants producing or using benzene; no excess in cancer of the lung mortality (SMR, 0.31; 95% CI, 0.06–0.91) or incidence (SIR, 0.22; 95% CI, 0.03–0.78) was reported in manufacturing workers. [The Working Group noted the presence of a healthy worker effect and the short follow-up time.]

Koh et al. (2014) reported on a study of cancer mortality and incidence among temporary maintenance workers at a refinery/petrochemical complex in the Republic of Korea; no excess in cancer of the lung mortality (SMR, 0.68; 95% CI, 0.31–1.29) or incidence (SIR, 0.73; 95% CI, 0.24–1.71) was observed.

(ii) Cancer of the kidney

The evidence available on the association between occupational exposure to benzene and cancer of the kidney was reviewed in *IARC Monographs* Volume 100F, and judged to be *inadequate* at that time; pertinent occupational cohort studies reviewed in Volume 100F are described in Table 2.17 (available at: http://publications.iarc.fr/123). The results of these studies generally do not show a consistent association, although several studies did report elevated but not statistically significant risks for cancer of the kidney (Wong, 1987a, b; Tsai et al., 1993; Sorahan et al., 2005).

Wong et al. (1993) reported on a nested case–control study of United States land-based or marine petroleum distribution workers for

exposure to gasoline containing 2–3% benzene (Wong et al., 1999); several quantitative indices of gasoline exposure (duration of exposure, cumulative exposure, frequency of peak exposure, age at first exposure, and year of first exposure) were used for analysis, but an excess mortality risk was not found for cancer of the kidney. [The Working Group noted potential exposure misclassification and a healthy worker effect.]

Wong (1987a, b) observed a standardized mortality ratio of 0.85 (95% CI, 0.27–1.98; $n = 5$), and did not observe any association between level of exposure and increased risk of cancer of the kidney: no cases were reported in the highest cumulative exposure category.

An odds ratio of 4.29 (95% CI, 1.33–13.8) for death from cancer of the kidney was reported for transformer manufacturing facility workers directly exposed to benzene compared with workers who were unexposed or indirectly exposed (Greenland et al., 1994).

Bulbulyan et al. (1999) reported a standardized mortality ratio of 1.9 (95% CI, 0.4–5.6; $n = 3$) among female bookbinders exposed to benzene.

Collins et al. (2015) reported no excess of death from cancer of the kidney (SMR, 0.78; 95% CI, 0.34–1.55; $n = 8$).

(c) Other cancer sites

Data for other cancer sites were not reviewed in detail in *IARC Monographs* Volume 100F. The key findings of pertinent studies that reported data for several additional types of cancer are reported in the following sections.

(i) Cancers of the nasal cavity, pharynx, larynx, and other respiratory sites

Several studies have reported data on other cancers of the respiratory tract, including the nasal cavity, buccal cavity, pharynx, and larynx (Tsai et al., 1983; Wong, 1987b; Greenland et al., 1994; Hayes et al., 1996; Bulbulyan et al., 1999; Sorahan et al., 2005; Koh et al., 2011, 2014; Linet et al., 2015). Results were based on small numbers of cases or deaths and were generally close to expectation. Two studies reported increased, albeit non-significant, relative risks for cancer of the nasopharynx. Among temporary maintenance workers at a refinery/petrochemical complex in the Republic of Korea, excess mortality (SMR, 5.88; 95% CI, 1.21–17.2; based on 3 deaths) and incidence (SIR, 8.33; 95% CI, 1.72–24.50; based on 3 cases) of cancer of the nasopharynx were reported. The relative risk of death from cancer of the nasopharynx was also elevated (RR, 1.9; 95% CI, 0.9–4.3; $n = 29$) in the Chinese cohort of workers exposed to benzene (Linet et al., 2015), although there was no evidence of a trend with cumulative benzene exposure (Hayes et al., 1996).

(ii) Cancer of the oesophagus

Chinese worker cohorts reported a P value for trend of 0.09 for the association between mortality from cancer of the oesophagus and cumulative exposure to benzene (Hayes et al., 1996). An elevated risk (RR, 1.6; 95% CI, 1.0–2.5; 70 exposed deaths) was reported when comparing workers exposed to benzene with unexposed workers in an updated analysis of the Chinese worker cohort (Linet et al., 2015). Greenland et al. (1994) reported an odds ratio of 1.23 (95% CI, 0.26–5.72) for mortality from cancer of the oesophagus among directly exposed workers compared with indirectly exposed or unexposed workers. Bulbulyan et al. (1999) observed an increase in female bookbinders (SMR, 4.1; 95% CI, 1.0–10.4; $n = 4$). Wong (1987a) observed no excess mortality from cancer of the oesophagus in workers continuously exposed to benzene, and no dose–response relationship was detected for the four deaths observed (Wong, 1987b). Sorahan et al. (2005) reported incidence and mortality results that were not statistically significant and were near or below expectation. Koh et al. (2014) observed a standardized mortality ratio of 0.51 (95% CI, 0.01–2.85; $n = 1$) and no incident cases.

(iii) Cancer of the stomach

The results from most studies of cancer of the stomach are generally at or below expectation. Hayes et al. (1996) reported a *P* value for trend of 0.63 for the association between mortality from cancer of the stomach and cumulative benzene exposure. Greenland et al. (1994) observed an odds ratio of 0.32 (95% CI, 0.04–2.42) for directly exposed workers compared with indirectly exposed or unexposed workers. A cancer of the stomach mortality deficit was reported by Wong (1987a) for workers exposed to benzene (SMR, 0.43; 95% CI, 0.16–0.94; $n = 6$). Tsai et al. (1983) reported a non-significantly elevated standardized mortality ratio of 2.32 for men working 1 year or more in areas exposed to benzene, based on a single case. Sorahan et al. (2005) reported a standardized mortality ratio of 1.06 (95% CI, 0.80–1.37; $n = 57$) and a similar standardized incidence ratio. Bulbulyan et al. (1999) reported a significantly elevated mortality from cancer of the stomach among press operators (SMR, 2.2; 95% CI, 1.0–4.2; $n = 9$) but no elevation among bookbinders (SMR, 1.0; 95% CI, 0.5–1.8; $n = 12$). Wilcosky et al. (1984) reported an odds ratio of 1.3 (based on 12 exposed cases) comparing male workers with potential cumulative benzene exposure of more than 1 year with those with no benzene exposure. Linet et al. (2015) reported no excess risk (RR, 1.0; 95% CI, 0.8–1.3; 211 exposed deaths) for mortality from cancer of the stomach when comparing exposed workers with those not exposed. Koh et al. (2014) reported a standardized mortality ratio of 0.83 (95% CI, 0.41–1.48; $n = 11$) and a standardized incidence ratio of 0.99 (95% CI, 0.56–1.64; $n = 15$). Koh et al. (2011) found significant deficits of mortality from cancer of the stomach in all workers (SMR, 0.24; 95% CI, 0.06–0.60; $n = 4$) and in manufacturing workers (SMR, 0.25; 95% CI, 0.05–0.74; 3 cases), as well as in all-cause mortality and all-cancer mortality among all workers and manufacturing workers.

(iv) Cancers of the colon, rectum, and anus

A study of the Chinese worker cohort reported no evidence (*P* for trend, 0.91) of an exposure–response relationship between exposure to benzene and cancers of the colon and rectum (Hayes et al., 1996). In the update of that study, Linet et al. (2015) observed a relative risk of 1.5 (95% CI, 1.0–2.3; 79 exposed deaths) for cancers of the colon and rectum. Greenland et al. (1994) reported deficits in odds ratios for mortality from cancers of the colon (OR, 0.74; 95% CI, 0.33–1.66) and rectum (OR, 0.85; 95% CI, 0.29–2.47) in directly exposed workers compared with those who were indirectly or not exposed. Wong (1987a) reported a standardized mortality ratio of 1.08 (95% CI, 0.52–1.98; $n = 10$) in continuously exposed workers; no significant exposure–response relationships for cancer of the colon were reported (Wong, 1987b). Sorahan et al. (2005) reported a deficit for mortality (SMR, 0.81; 95% CI, 0.57–1.11; 38 cases) from and incidence (SIR, 0.86; 95% CI, 0.65–1.10; 60 cases) of cancer of the colon. However, for cancer of the rectum increased risks for mortality (SMR, 1.05; 95% CI, 0.71–1.48; $n = 31$) and incidence (SIR, 1.13; 95% CI, 0.86–1.45; $n = 61$) were reported. Among women exposed to benzene while employed as bookbinders, Bulbulyan et al. (1999) reported standardized mortality ratios of 1.3 (95% CI, 0.6–2.6; $n = 8$) for cancer of the colon and 1.3 (95% CI, 0.4–3.1; $n = 5$) for cancer of the rectum. Tsai et al. (1993) reported a standardized mortality ratio for cancer of the colon of 0.94 (95% CI, 0.60–1.40; $n = 24$). For cancers of the intestine and anus, Koh et al. (2014) reported a standardized mortality ratio of 0.33 (95% CI, 0.04–1.20; $n = 2$) and a standardized incidence ratio of 0.91 (95% CI, 0.37–1.88; $n = 7$) among temporary maintenance workers. Koh et al. (2011) reported a standardized mortality ratio of 0.49 (95% CI, 0.06–1.78; $n = 2$) for cancers of the colon and anus among manufacturing workers.

(v) Cancers of the liver and biliary tract

When analysing mortality from cancers of the liver and gall bladder among Chinese workers by cumulative benzene exposure, no notable exposure–response relationships or elevations in exposed versus unexposed workers were observed by Hayes et al. (1996) (*P* for trend, 0.16). Linet et al. (2015) reported a relative risk for cancers of the liver, gallbladder, and bile duct of 1.2 (95% CI, 0.9–1.4; 286 exposed deaths) in the updated study of Chinese benzene workers. Greenland et al. (1994) reported a non-significant odds ratio of 2.76 (95% CI, 0.68–11.20; $n = 9$) for cancers of the liver, gallbladder, and biliary tract combined among workers directly exposed to benzene compared with their unexposed or indirectly exposed counterparts. Elevations in mortality (SMR, 1.54; 95% CI, 0.74–2.84; $n = 10$) and morbidity (SIR, 1.31; 95% CI, 0.57–2.59; $n = 8$) from cancer of the liver were reported by Sorahan et al. (2005); regarding cancer of the gallbladder, the same study reported deficits in mortality (SMR, 0.60; 95% CI, 0.08–2.26; two deaths) and morbidity (SIR, 0.66; 95% CI, 0.14–1.92; three deaths). Bulbulyan et al. (1999) reported 1 observed death from liver cancer, compared with 1.2 cases expected in bookbinders. Wong (1987b) reported no excess risk or exposure–response trends among chemical workers in the USA. Koh et al. (2014) reported a standardized mortality ratio of 0.82 (95% CI, 0.51–1.25; $n = 21$) and a standardized incidence ratio of 1.07 (95% CI, 0.58–1.79; $n = 14$) for cancer of the liver. Koh et al. (2011) reported a standardized mortality ratio of 0.64 (95% CI, 0.34–1.09; $n = 13$) for cancers of the liver and biliary tract among manufacturing workers.

(vi) Cancer of the prostate

Greenland et al. (1994) reported an odds ratio of 1.02 (95% CI, 0.49–2.12) for directly exposed workers compared with indirectly exposed or unexposed workers. No associations were seen by Wong (1987a) (SMR, 0.93; 95% CI, 0.34–2.03; 6 deaths) or Wilcosky et al. (1984) (OR, 0.73; 11 deaths; CI not reported). Sorahan et al. (2005) reported a standardized mortality ratio of 0.94 (95% CI, 0.70–1.24; $n = 50$) and a standardized incidence ratio of 1.10 (95% CI, 0.91–1.32; $n = 121$). Koh et al. (2014) reported a standardized mortality ratio of 2.51 (95% CI, 0.06–14.00; $n = 1$) and a standardized incidence ratio of 1.20 (95% CI, 0.03–6.71; $n = 1$). Koh et al. (2011) observed no deaths from cancer of the prostate in manufacturing workers.

(vii) Cancer of the bladder

No association between exposure to benzene and cancer of the bladder was seen by Greenland et al. (1994) or Wong (1987a). Sorahan et al. (2005) reported a standardized mortality ratio of 1.00 (95% CI, 0.66–1.46; 27 cases) and a standardized incidence ratio of 1.04 (95% CI, 0.81–1.31; 69 cases). Bulbulyan et al. (1999) observed 1 death from cancer of the bladder in bookbinders, where 0.5 was expected. Among press operators, standardized mortality ratio was 12.5 (95% CI, 1.5–45.1; $n = 2$). Linet et al. (2015) reported a relative risk for cancer of the bladder for exposed versus unexposed workers of 0.9 (95% CI, 0.4–2.2; 18 exposed deaths). Koh et al. (2011) observed no deaths from cancer of the bladder.

(viii) Cancer of the skin

Bond et al. (1986) found four deaths from cancer of the skin, all in the lowest exposure category (0–499 ppm-months). The overall standardized mortality ratio for cancer of the skin was 4.41 unlagged and 6.22 with a 15-year lag. Wong (1987a) observed a non-significant deficit in workers continuously exposed to benzene, with the single exposed case (of 3 cases in total) occurring in the lowest exposure category (Wong, 1987b). For all cancers of the skin, Koh et al. (2014) reported a standardized mortality ratio of 5.05 (95% CI, 0.13–28.20; $n = 1$) and no incident cases. No deaths from cancer of the skin were observed by Koh et al. (2011) in manufacturing workers.

For non-melanoma cancer of the skin, Sorahan et al. (2005) reported one observed death (SMR, 0.55; 95% CI, 0.01–3.05). Stenehjem et al. (2017) reported an adjusted odds ratio of 3.51 (95% CI, 0.45–27.00; n = 6) for squamous cell carcinoma of the forearm and hand after adjustment for age, sunburn frequency, and education.

For malignant melanoma, Sorahan et al. (2005) reported a standardized mortality ratio of 0.81 (95% CI, 0.22–2.06; n = 4) and a standardized incidence ratio of 1.21 (95% CI, 0.64–2.07; n = 13). Among women potentially exposed to benzene, Bulbulyan et al. (1999) reported an elevated risk (SMR, 8.7; 95% CI, 1.1–31.3; n = 2) among press operators and no melanoma deaths among bookbinders. In offshore petroleum workers, Stenehjem et al. (2017) reported an odds ratio for benzene exposure of 2.43 (95% CI, 0.30–20.00; n = 5) for melanomas of the forearm and hand after adjustment for age, sunburn frequency, and education. [The Working Group noted that adjustment for sunburn was not a good proxy for adjusting for occupational exposure to ultraviolet radiation. There may also have been potential confounding as a result of other co-exposures.]

(ix) Cancers of the brain and central nervous system

Wong (1987a) reported an increased mortality from cancer of the CNS (ICD-8, code 191–912) for workers continuously exposed to benzene (SMR, 1.54; 95% CI, 0.56–3.35; n = 6), with no linear trend by cumulative exposure (Wong, 1987b) or overall elevation for the three exposure categories.

An odds ratio of 2.11 (95% CI, 0.66–6.73; n = 16) was reported for transformer assembly workers directly exposed to benzene compared with those indirectly exposed or unexposed (Greenland et al., 1994). These results include both malignant and unspecified tumours of the brain.

An exposure–response analysis for the Chinese cohort (Hayes et al., 1996) saw no trend (P for trend, 0.48), but did find an elevation in the highest exposure category (RR, 2.3; five deaths) with deficits in two intermediate exposure categories. In an update of the study, Linet et al. (2015) reported a relative risk of 0.8 (95% CI, 0.4–1.6; 18 exposed deaths) for benign and malignant tumours of the brain for any exposure to benzene.

For cancers of the brain and spine, Koh et al. (2014) reported a standardized mortality ratio of 1.21 (95% CI, 0.15–4.36; n = 2) and a standardized incidence ratio of 2.36 (95% CI, 0.29–8.52; n = 2). Koh et al. (2011) observed no deaths from neurological cancers in manufacturing workers. Collins et al. (2015) reported a standardized mortality ratio of 1.01 (95% CI, 0.48–1.86; 10 deaths) for cancer of the CNS among chemical production workers.

Several older studies provided only summary standardized mortality ratio results for these cancers. Bulbulyan et al. (1999) reported a standardized mortality ratio of 2.6 (95% CI, 0.5–4.6; n = 3) for cancers of the brain and nervous system among women employed as bookbinders. Tsai et al. (1983) included benign neoplasms of the brain and other parts of the nervous system, and neoplasms of an unspecified nature of the eye, brain, and other parts of the nervous system, and reported a standardized mortality ratio of 3.23 (95% CI, 0.04–17.95; n = 1) among men employed as refinery workers. Sorahan et al. (2005) reported non-significant increases in mortality (SMR, 1.05; 95% CI, 0.60–1.70; n = 16) and morbidity (SIR, 1.16; 95% CI, 0.68–1.83; n = 18) for malignant neoplasms of the brain and other parts of the nervous system.

(x) Cancer of the pancreas

A study of male chemical workers observed a standardized mortality ratio below expectation (Wong 1987a) and no exposure–response association between exposure to benzene and cancer

of the pancreas (Wong, 1987b). Greenland et al. (1994) reported an odds ratio of 0.58 (95% CI, 0.18–1.93; *n* = 33) when comparing transformer assembly workers who had been directly exposed to benzene with those indirectly exposed or unexposed. Elevated risks for mortality (SMR, 1.21; 95% CI, 0.85–1.68; *n* = 36) and incidence (SIR, 1.29; 95% CI, 0.90–1.79; *n* = 36) were observed by Sorahan et al. (2005). Bulbulyan et al. (1999) reported a standardized mortality ratio of 1.1 (95% CI, 0.2–3.3; *n* = 3) in female bookbinders and one of 2.0 (95% CI, 0.3–7.4; *n* = 2) among female press operators potentially exposed to benzene. Koh et al. (2014) observed a standardized mortality ratio of 0.57 (95% CI, 0.07–2.07; *n* = 2) and a standardized incidence ratio of 1.41 (95% CI, 0.17–5.09; *n* = 2) in temporary maintenance workers in a refinery/petrochemical complex in the Republic of Korea. For refinery/petrochemical facility manufacturing workers, Koh et al. (2011) reported a standardized mortality ratio of 1.21 (95% CI, 0.25–3.52; *n* = 3) for cancer of the pancreas. Linet et al. (2015) reported a relative risk of 1.7 (95% CI, 1.0–3.1; 45 exposed deaths) for the Chinese benzene worker cohort.

(xi) Additional cancers

Cancers assessed by less than four studies each include the following malignancies: other urinary and genitourinary (Sorahan et al., 2005); other endocrine (Sorahan et al., 2005); small intestine (Sorahan et al., 2005); testis (Sorahan et al., 2005); ovary (Bulbulyan et al., 1999, Sorahan et al., 2005); uterine corpus (Bulbulyan et al., 1999; Sorahan et al., 2005; Linet et al., 2015); uterine cervix (Bulbulyan et al., 1999; Sorahan et al., 2005); thyroid (Sorahan et al., 2005); pleural cancer and mesothelioma (Sorahan et al., 2005); breast (Bulbulyan et al., 1999; Sorahan et al., 2005; Linet et al., 2015); bone (Wong, 1987a, b; Sorahan et al., 2005); lip (Sorahan et al., 2005); and tongue (Sorahan et al., 2005; Koh et al., 2014). Small numbers of cases or deaths were observed for most of these sites. Exceptions included: a standardized mortality ratio of 2.9 (95% CI, 1.5–5.0; *n* = 12) for cancer of the ovary among bookbinders (Bulbulyan et al., 1999); a relative risk of 2.6 (95% CI, 0.9–10.9; *n* = 19) for death from cancer of the uterus; and a relative risk of 1.2 (95% CI, 0.6–2.5; *n* = 32) for death from cancer of the breast among Chinese workers exposed to benzene (Linet et al., 2015).

2.4.2 General-population studies

(a) Cancer of the lung

One cohort study of environmental exposure and three case–control studies examined cancer of the lung in relation to indicators of exposure to benzene (Table 2.7).

Bove et al. (2014) reported on cancer of the lung in the cohort study of United States military personnel exposed to contaminated drinking-water. No quantitative estimate of benzene or other agents was derived. There was an elevated hazard ratio (adjusted for sex, race, rank, and education, but not for smoking) for cancer of the lung among the personnel exposed to drinking-water contaminated with solvents, including benzene (HR, 1.16; 95% CI, 0.96–1.40; 10-year lag time), where the elevation was due entirely to those with higher cumulative exposures. The standardized mortality ratio was 0.92 (95% CI, 0.80–1.04; 237 deaths), and most people in the cohort were younger than 55 years at the end of follow-up.

A case–control study in Montreal reviewed in *IARC Monographs* Volume 100F showed no association between exposure to benzene and overall cancer of the lung (*n* = 857) or for histological subtypes (see Table 2.16, available at: http://publications.iarc.fr/123). Covariates adjusted for in the study included cumulative smoking index, and exposure to arsenic, asbestos, chromium VI, nickel, crystalline silica, beryllium, cadmium, and polycyclic aromatic hydrocarbons (Gérin et al., 1998).

Villeneuve et al. (2014) reported on a case–control study of 445 incident cases of cancer of the lung, trachea, and bronchus, and 948 hospital- and population-based controls in Toronto (1997–2002). Exposure to ambient volatile organic compounds, including benzene, from outdoor air pollution was assessed using land-use regression models and residential history data. The investigators collected information on confounders including tobacco use and exposure to cigarette smoke. An interquartile range increase in estimated time-weighted average benzene exposure across previous residences was associated with cancer of the lung only when using population-based controls (OR, 1.84; 95% CI, 1.26–2.68). Associations were also positive when using exposure 10 years before interview (OR, 1.58; 95% CI, 1.15–2.16) or at the time of interview (OR, 1.51; 95% CI, 1.13–2.01), but smaller in magnitude.

Yuan et al. (2014) reported on a nested case–control study of 82 cases of cancer of the lung and 83 controls among lifelong non-smoking Chinese men in the Shanghai Cohort Study, aged 45–64 years at enrolment. Prospective urine samples were taken and levels of urinary metabolites of polycyclic aromatic hydrocarbons and volatile organic compounds were examined for an association with risk of cancer of the lung. None of the metabolites of volatile organic compounds were associated with overall risk of cancer of the lung. However, elevated urinary *S*-phenylmercapturic acid (SPMA, a metabolite of benzene) was associated with an increased risk of squamous cell carcinoma of the lung (16 cases); odds ratios for the second and third tertiles of SPMA were 1.97 (95% CI, 0.31–12.65) and 5.76 (95% CI, 1.11–28.96), respectively. Overall, there was a monotonic but non-significant trend in odds ratios across quartiles of SPMA (ORs, 1.00, 1.03, 1.10, and 1.57; *P* for trend, 0.31). [The Working Group noted that the study size was small and, although specific to benzene, SPMA was measured at a single point in time and is not a good proxy for occupational benzene exposure (half-life, 9.1 hours). Results also appear to be sensitive to the grouping of the exposure data.]

(b) Cancer of the kidney

In the previous review of the evidence for associations between exposure to benzene and cancer of the kidney, *IARC Monographs* Volume 100F identified two case–control studies in the general population. In the first study, conducted in Germany, an association was found between exposure to benzene and an increased risk for cancer of the kidney (specifically, renal cell carcinoma; Pesch et al., 2000). The study included 935 incident cases and 4298 controls interviewed between 1991 and 1995, with exposure estimated according to occupational history and a JEM. Results indicated that employment durations exceeding the 90th percentile (classified as "very long exposures") in the chemical, rubber, and printing industries were associated with renal cell carcinoma. Substantial exposure to organic solvents was a significant risk factor for both men and women. In the second study in Montreal, Canada, benzene exposure levels were low for most exposed subjects, and there was little evidence of an association between medium and high levels of exposure and risk of cancer of the kidney (OR, 1.3; 95% CI, 0.7–2.4; $n = 12$) (Gérin et al., 1998). The evidence available at the time was judged to be *inadequate*.

Subsequently, Bove et al. (2014) reported on a cohort study of United States marine ($n = 154\,932$) and naval ($n = 154\,969$) personnel who began service during 1975–1985 and were stationed at two United States military bases. Drinking-water systems in Camp Lejeune, North Carolina, were contaminated with solvents, and drinking-water in Camp Pendleton, South Carolina, was uncontaminated. Although the study population was an occupational group, the exposure of interest was environmental; the agents of primary concern were perchloroethylene and trichloroethylene in drinking-water. Benzene was also a contaminant,

with monthly average concentrations above the current United States maximum contaminant levels for 63 months. Personnel in Camp Lejeune had an elevated mortality for cancer of the kidney (HR, 1.35; 95% CI, 0.84–2.16) and, within the cohort, a monotonic categorical cumulative exposure trend was observed for cancer of the kidney and total contaminants. A risk estimate for the association between benzene exposure and mortality from cancer of the kidney was not reported. Less than 6% of the cohort had died, but a risk estimate for the association between benzene exposure and cancer of the kidney was not reported.

(c) Additional cancers

Several studies of associations between various cancers and environmental exposures to benzene have been published recently.

Bove et al. (2014) reported no excess mortality from cancer of the oral cavity or larynx among marine and naval personnel exposed to contaminated drinking-water.

Garcia et al. (2015) reported on a cohort study of 112 378 participants in the California Teachers Study, including 5676 women diagnosed with cancer of the breast. Modelled annual average air concentrations of 24 mammary gland carcinogen pollutants were derived from the NATA database; the mean benzene concentration was 1.40 μg/m^3. There was little evidence for a trend in the hazard of breast cancer overall with estimated benzene concentration (*P* value for trend, 0.38). Analyses restricted to tumours that were both estrogen-receptor and progesterone-receptor negative (704 cases) suggested increased risk of cancer of the breast with exposure to benzene, with a hazard ratio for the highest quintile of benzene concentration of 1.45 (95% CI, 1.15–1.83; *P* for trend, 0.016). Confining the analysis to never smokers did not weaken the association.

Cigarette smoking is the most important environmental factor for cancer of the pancreas. Among smokers, 90% of benzene exposure comes from smoking (National Cancer Institute, 2015). Antwi et al. (2015) reported on a case–control study based at the Mayo Clinic in Minnesota on environmental exposures and risk of cancer of the pancreas; the study included 2092 cases and 2316 hospital-based matched controls from primary care clinics with self-reported exposure in the form of questionnaire responses. Self-reported regular exposure to benzene was associated with cancer of the pancreas, adjusted for age, sex, smoking, diabetes, body mass index, and education (OR, 1.70; 95% CI, 1.23–2.35). [The Working Group noted that significant risks associated with regular exposure to asbestos (OR, 1.54; 95% CI, 1.23–1.92) and chlorinated hydrocarbons (OR, 1.63; 95% CI, 1.32–2.02) were reported. Exposure assessment in the study was limited by self-reported exposure to benzene.]

2.5 Quantitative data

Following the recommendation of an Advisory Group on quantitative risk characterization (IARC, 2014), the Working Group carried out meta-analyses and meta-regression analyses of quantitative associations between occupational benzene exposure and several cancers of the haematopoietic tissues. These analyses update and extend those published earlier by Vlaanderen et al. (2010, 2011, 2012). The association between occupational benzene exposure and the development of AML and CLL was estimated in meta-analyses. Meta-regression analyses investigating the slope and shape of exposure–disease functions were conducted for AML. Studies were selected for inclusion in these analyses using the criteria applied throughout this section; the analytical approach was similar to that previously reported by Vlaanderen et al. (2010, 2011).

2.5.1 Data extraction

Risk ratios, odds ratios, and standardized mortality or incidence ratios were extracted from published studies. The term "relative risk" will be used to refer to these measures of association collectively. Where both mortality and incidence data were reported, the incidence data were used for analysis.

For the meta-regression analyses, only relative risk estimates reported for cumulative exposure to benzene (expressed in ppm-years or ppm-months) were used.

2.5.2 Statistical analysis

(a) Meta-analysis

Random-effects meta-analyses were performed to pool relative risks for AML and CLL. To allow the inclusion of studies without quantitative exposure estimates, only relative risks for "any occupational benzene exposure" versus "background benzene exposure" were used in the meta-analysis. For those studies which included exposure estimates, relative risks for categories of exposure were pooled either by summing observed and expected cases for studies that reported standardized mortality ratios, or by conducting a within-study random-effects meta-analysis of the non-reference exposure groups for studies which reported relative risks or odds ratios.

(b) Meta-regression

The data extracted for use in meta-regression analysis were relative risk estimates for categories of cumulative benzene exposure. Each exposure category relative risk was assigned a specific cumulative exposure value for the purpose of regression analysis, defined as the mid-point of the exposure category. If an open-ended exposure category was reported for the highest exposure group, then the Working Group assigned it an exposure value equal to the reported lower limit for that exposure category plus one half the width of the previous exposure category. The variance of each relative risk was estimated using the reported confidence intervals under the assumption of Wald-type bounds. As risk estimates of a study based on a common internal reference group will be correlated, the covariance between different risk estimates within a study were estimated. For studies that reported standardized mortality ratios, covariance was not estimated.

Meta-regression analyses were performed on the natural logarithm of the reported relative risk estimates, fitting a linear model as well as natural spline models with knots at the 20th, 50th, and 80th percentiles of the benzene exposure distribution. All statistical analyses were performed using the MIXED and IML procedures in SAS 9.4; the meta-analysis was conducted using the "metafor" package in R, version 3.1.2 (Viechtbauer, 2010).

2.5.3 Results

(a) Meta-analysis

Results of the meta-analysis of selected studies on adult AML and CLL are shown in Fig 2.1, stratified by outcome assessment (incidence, mortality, and both combined).

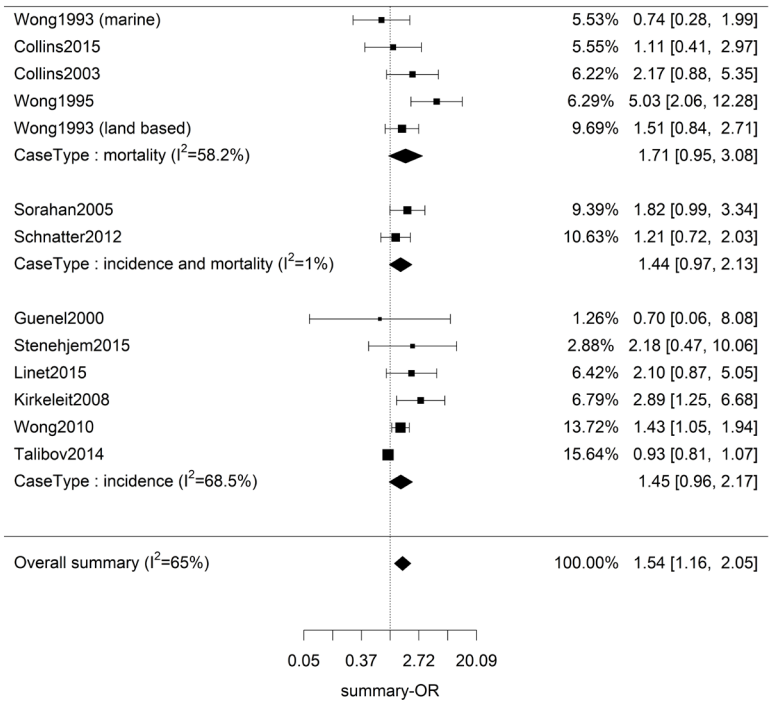
(b) Meta-regression

Visual examination of the natural spline of the cumulative benzene exposure and AML function, including six occupational cohort studies, strongly supported a linear model, as did a statistical comparison of the linear and spline models with respect to goodness of model fit. Subsequent meta-regression analysis focused on results for the linear model, presented in Fig. 2.2.

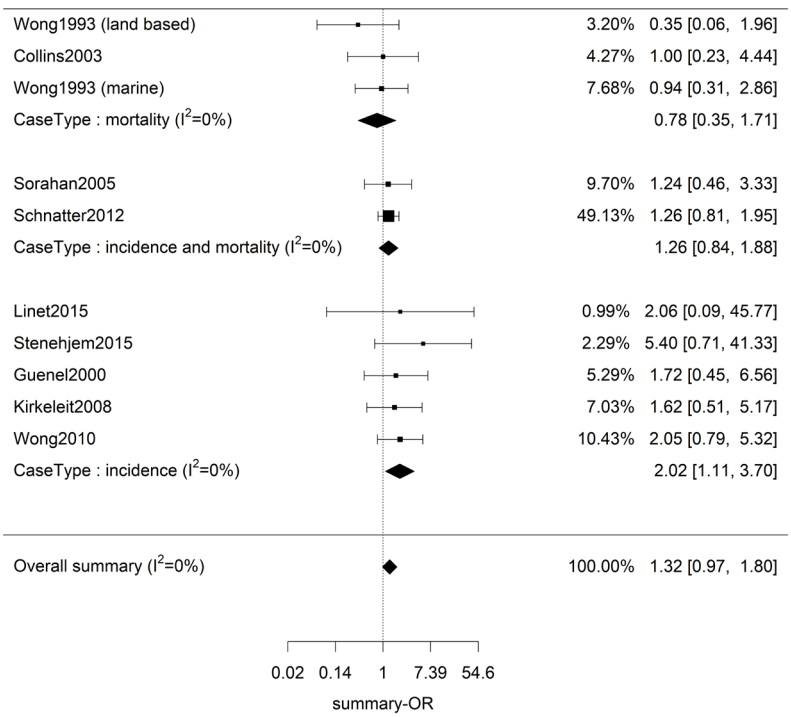
The sensitivity of the exposure–response trend to a single influential study was assessed by refitting the model upon exclusion of one study at a time (Table 2.8). These results indicate that the exposure–response estimate is robust for

Fig. 2.1 Forest plots of (A) AML and (B) CLL stratified by type of outcome (incidence or mortality)

A

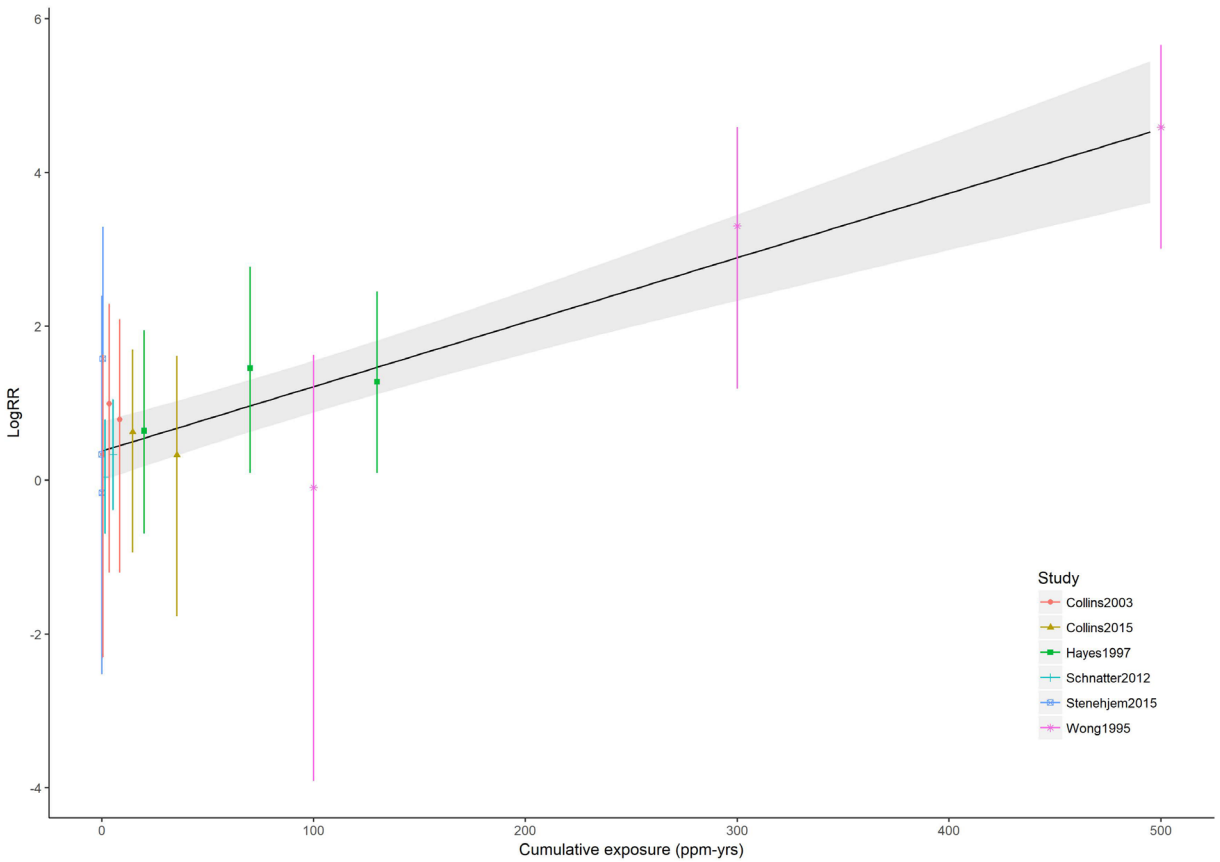


B



Compiled by the Working Group

Fig. 2.2 Meta-regression model of cumulative benzene exposure and AML, including fitted curve and confidence bands



Compiled by the Working Group

Table 2.8 Sensitivity analyses of the linear function of cumulative exposure to benzene (ppm-yr) and AML by sequential exclusion of individual cohort studies

Studies	Intercept	Slope (/100)
All studies	0.38 (0.20)	0.84 (0.11)
Excluding Stenehjem et al. (2015)	0.34 (0.20)	0.85 (0.11)
Excluding Collins et al. (2015)	0.40 (0.22)	0.84 (0.11)
Excluding Schnatter et al. (2012)	0.50 (0.25)	0.81 (0.11)
Excluding Hayes et al. (1997)	0.36 (0.21)	0.86 (0.11)
Excluding Collins et al. (2003)	0.28 (0.23)	0.86 (0.11)
Excluding Wong (1995)	0.45 (0.21)	0.59 (0.40)

AML, acute myeloid leukaemia; ppm, parts per million; yr, year(s)

the exclusion of all individual studies, with the exception of Wong (1995) (reanalysis of Pliofilm manufacturing plants in Ohio), which leads to an overall lower estimated association (0.59 vs 0.85). The Stenehjem et al. (2015) study, which seems to indicate higher risks at low levels of exposure, and the Wong (1995) study were particularly influential on the exposure–response function due to the high exposure estimates. The observed instability in the derivation of the meta-exposure–response association underscores some uncertainty in these results.

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3. CANCER IN EXPERIMENTAL ANIMALS

Benzene has been evaluated by four *IARC Monographs* Working Groups (IARC, 1974, 1982, 1987, 2012). The Working Group in 2012 concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of benzene (IARC, 2012).

3.1 Mouse

3.1.1 Inhalation

See Table 3.1

Numerous studies have been conducted in mice exposed to benzene by inhalation. Even though some of these studies focused on the effects of benzene in transgenic mice, data from these studies reporting the effects of benzene in wildtype mice are included in this section.

C57BL/6 female mice (age, 7–9 weeks) were exposed to clean air (116 mice) or benzene at 300 ppm [purity not reported] (118 mice) for 6 hours per day, 5 days per week, for 16 weeks in whole-body inhalation chambers. The 116 clean-air controls and 118 mice exposed to benzene were reduced to 88 and 90 mice, respectively, by interim killing for assays for haematopoietic stem cells. Preliminary observations were reported 64 weeks after beginning exposure (Cronkite et al., 1984). During this period there was increased mortality in the mice exposed to benzene: one of the clean-air control mice died and 10 mice exposed to benzene died. The dead control mouse did not have lymphoma or leukaemia. Of the 10 mice exposed to benzene that died, 6 had lymphoma of the thymus gland, 2 had unspecified lymphomas, 1 did not have lymphoma or leukaemia, and 1 carcass was lost due to cannibalism and autolysis of tissues. The incidence of lymphoma or leukaemia 64 weeks after beginning exposure was 8/89 (9.0%) in mice exposed to benzene compared with 0/88 in control mice. [These tumour incidences indicated a significant increase in the incidence of lymphoma or leukaemia [$P = 0.007$, Fisher exact test]; however, because 87 of 88 clean-air control mice and 80 of 89 mice exposed to benzene were not comprehensively examined, the actual tumour incidences in the control groups and groups exposed to benzene were unknown. The study was considered inadequate for the evaluation.]

C57BL/6 female mice (age, 8–12 weeks) were exposed to clean air (88 mice) or benzene at a dose of 300 ppm [purity not reported] (89 mice) for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers, and then observed for their lifetimes. The observations reported were made 692 days after beginning exposure (580 days after the end of the exposure period) (Cronkite et al., 1985). During this period, there was increased mortality in the mice exposed to benzene: 23 deaths in the control group and 48 deaths in the group exposed to benzene were observed. There was a significantly higher incidence of tumours in the mice exposed to benzene that had died by day 692 of the experiment: leukaemia (all

Table 3.1 Studies of carcinogenicity in mice exposed to benzene by inhalation

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (F) 7–9 wk 64 wk Cronkite et al. (1984)	Inhalation (whole-body exposure) Benzene, reagent grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 88, 90 87, 80	*Haematopoietic and lymphoid tissues*: lymphoma/leukaemia 0/88, 8/89	See comments	Principal limitations: preliminary data obtained from dead or moribund animals; animals alive at 64 wk were not assessed Surviving mice were not examined, so the tumour incidence in the control and benzene-exposed populations is unknown; the study was considered inadequate
Full carcinogenicity Mouse, C57BL/6 (F) 8–12 wk 692 d Cronkite et al. (1985)	Inhalation (whole-body exposure) Benzene, probably reagent grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 88, 89 65, 41	*Haematopoietic and lymphoid tissues* Thymic lymphoma 1/88, 10/89 Non-thymic lymphoma 2/88, 6/89 Myelogenous leukaemia 3/88, 0/89 Unspecified leukaemia 2/88, 4/89 Leukaemia (all types) 8/88, 20/89 *Zymbal gland*: benign and malignant epidermoid tumours and lymphoepithelioma 1/88, 16/89 *Ovary*: unspecified solid tumours 0/88, 8/89 *Liver*: hepatoma (not specified) 1/88, 1/89 *Other neoplasms*: unspecified solid tumours 2/88, 4/89	See comments See comments See comments See comments See comments See comments See comments See comments See comments	Principal limitations: data obtained from dead or moribund animals; animals alive at 692 d after the commencement of the study were not assessed; the data presented in Table 1 appear to be the continuing observations of the study reported in Cronkite et al. (1984), although this is not specifically stated Surviving mice were not examined, so tumour incidence in the control and in the benzene-exposed populations was therefore unknown; the study was considered inadequate

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, CBA/Ca BNL (M) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 60, 57 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 7/60, 1/57 Myelogenous neoplasms 0/60, 11/57* *Liver*: hepatoma (not specified) 16/60, 6/57 *Other neoplasms*: unspecified solid tumours 13/60, 30/57*	 NS *$P < 0.001$ NS *$P < 0.001$	Principal strengths: lifetime study; studies in male and female mice
Full carcinogenicity Mouse, CBA/Ca BNL (F) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 60, 54 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 5/60, 4/54 Myelogenous neoplasms 1/60, 6/54* *Liver*: hepatoma (not specified) 8/60, 0/54 *Other neoplasms*: unspecified solid tumours 21/60, 43/54*	 NS *$P = 0.040$ (chi-squared test) NS (for an increase) *$P < 0.001$	Principal strengths: lifetime study; studies in male and female mice *P* values for the difference in the incidence of myelogenous neoplasms between control and benzene-exposed females are different when using the one-tail and the two-tail Fisher exact test (as calculated by the Working Group): 0.0420 and 0.0514 (not significant), respectively
Full carcinogenicity Mouse, CBA/Ca BNL (M) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk, 16 wk 70, 85 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 12/70, 7/85 Myelogenous neoplasms 0/70, 2/85 *Liver*: hepatoma (not specified) 27/70, 35/85 *Other neoplasms*: unspecified solid tumours 14/70, 38/85*	 NS NS NS *$P = 0.001$	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, CBA/Ca (M) 10–12 wk 22 mo Farris et al. (1993)	Inhalation (whole-body exposure) Benzene, purity NR but certified HPLC grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 125, 125 75, 25	*Haematopoietic and lymphoid tissues*: malignant lymphoma 2/119, 14/118* *Preputial gland*: squamous cell carcinoma 0/118, 71/118* *Lung*: adenoma 17/119, 42/118* *Zymbal gland*: carcinoma 1/125, 14/125 *Forestomach*: squamous cell carcinoma 0/125, 9/125 *Harderian gland*: adenoma 6/125, 7/125	*$P < 0.01$ (table), $P < 0.002$ (text) *$P < 0.01$ *$P < 0.01$ [$P < 0.01$]; see comments [$P < 0.01$]; see comments NS; see comments	Principal strengths: covers most of the lifespan Benzene decreased survival ($P < 0.01$); the incidence of Zymbal gland carcinoma and of forestomach squamous cell carcinoma was significantly increased by Fisher's exact test: $P < 0.01$ and $P < 0.01$, respectively; Zymbal gland, forestomach, and Harderian gland were examined microscopically only when gross lesions were evident
Full carcinogenicity Mouse, AKR (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk 50, 50 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/50, 0/50	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described
Full carcinogenicity Mouse, AKR (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 80, 80 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/80, 0/80	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57Bl (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/40, 0/40	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described
Full carcinogenicity Mouse, CD-1 (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/40, 2/40	NS; see comments	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described Spontaneous cases of myelogenous leukaemia are rare in CD-1 mice; the authors argue that the two occurrences of myelogenous leukaemia are likely due to benzene inhalation
Full carcinogenicity Mouse, C57BL/6 (wildtype) (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 33, 100, 300 ppm (in air) 6 h/d, 5 d/wk, 26 wk 20, 19, 19, 18 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/20, 0/19, 2/19, 5/18* Non-thymic lymphoma 2/20, 4/19, 1/19, 5/18 Myeloid leukaemia 0/20, 0/19, 0/19, 0/18 Neoplasms of haematopoietic and lymphoid tissues (combined) 2/20, 4/19, 3/19, 10/18* *Other neoplasms*: solid tumours (not specified) excluding lymphomas 3/20, 3/19, 8/19, 2/18	 *$P < 0.05$ [$P = 0.004$] (Cochran–Armitage trend test) NS NS *$P < 0.05$ NS	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C3H/He (wildtype) (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 100, 300 ppm (in air) 6 h/d, 5 d/wk, 26 wk 23, 24, 23 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/23, 4/24, 0/23 Non-thymic lymphoma 2/23, 2/24, 5/23 Myeloid leukaemia 0/23, 0/24, 2/23 Neoplasms of haematopoietic and lymphoid tissues (combined) 2/23, 6/24, 7/23 *Other neoplasms*: solid tumours (not specified) excluding lymphomas 11/23, 5/24, 8/23	 [$P = 0.0031$] (Cochran–Armitage trend test) NS NS NS NS	Principal strengths: lifetime study
Full carcinogenicity Mouse, C57BL/6 (wildtype) (NR) NR Lifetime Li et al. (2006)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 300 ppm 6 h/d, 5 d/wk, 26 wk 8, 10 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/8, 3/10 Non-thymic lymphoma 6/8, 5/10	 [NS] [NS]; see comments	Principal strengths: lifetime study Principal limitations: small numbers of animals were used The time to non-thymic lymphoma was shorter in benzene-exposed mice compared with controls
Full carcinogenicity Mouse, AKR/J (M) 8 wk Lifetime Snyder et al. (1980)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk 50, 50 NR	*Haematopoietic and lymphoid tissues*: malignant lymphoma 24/50, 29/49	 NS	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1980)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues* Haematopoietic neoplasms (combined) 2/40, 8/40* Bone marrow hyperplasia in animals without haematopoietic neoplasm 0/38, 13/32*	 *$P < 0.005$; see comments *$P < 0.001$	Principal strengths: lifetime study Principal limitations: poor data presentation Using the two-tail Fisher exact test to compare the incidence of haematopoietic neoplasms in clean air and benzene-exposed C57BL/6J mice, the Working Group determined that the *P* value was NS (0.0872). On the other hand, using the log-rank (χ^2) test, which compares events and time to event, the difference in malignant lymphoma incidence between control and benzene-exposed mice was found to be significant by the authors ($P < 0.005$). The significance found by the authors therefore depends on tumour induction time
Full carcinogenicity Mouse, CD-1 (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 1200 ppm (in air) 6 h/d, 5 d/wk, 10 wk 80, 80 71 (at risk), 71 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 11/71, 11/71 *Zymbal gland*: carcinoma 0/71, 4/71* *Lung*: adenoma 17/71, 33/71* *All sites* Malignant tumours 22/71, 24/71* Benign tumours 21/71, 35/71* Benign and malignant tumours 36/71, 45/71*	 NS *$P < 0.05$ [NS]; see comments *$P < 0.001$ *$P < 0.05$ [NS]; see comments *$P < 0.001$ *$P < 0.001$ [NS]; see comments	Principal strengths: lifetime study Principal limitations: short duration of exposure; use of the log-rank (χ^2) test is questionable Using the two-tail Fisher exact test, the Working Group determined that only the incidence of lung adenoma and of benign tumours was significantly increased in benzene-exposed mice ($P = 0.0081$ and 0.0252, respectively)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 1200 ppm (in air) 6 h/d, 5 d/wk, 10 wk 80, 80 67 (at risk), 68 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 15/67, 11/68 *Zymbal gland*: carcinoma 0/67, 4/68 *Lung*: adenoma 11/67, 15/68 *All sites* Malignant tumours 19/67, 15/68 Benign tumours 13/67, 16/68 Benign and malignant tumours 28/67, 30/68	 NS NS NS NS NS NS	Principal strengths: lifetime study Principal limitation: short duration of exposure
Full carcinogenicity Mouse, CD-1 (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 1 wk then 2 wk unexposed, for life 60, 60 46 (at risk), 54 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 1/46, 7/54* *Zymbal gland*: carcinoma 0/46, 2/54 *Lung*: adenoma 3/46, 14/54* *All sites* Malignant tumours 1/46, 12/54* Benign tumours 3/46, 15/54* Benign and malignant tumours 4/46, 25/54*	 *[$P < 0.05$] (one-tail Fisher exact test) NS *$P < 0.005$ *$P < 0.005$ *$P < 0.001$ *$P < 0.001$	Principal strengths: lifetime study In the group of exposed mice, the Working Group determined that increases in the incidence of lung adenoma, malignant tumours, benign tumours, and of total tumours were also significant by the two-tail Fisher exact test, with *P* values of 0.015, 0.003, 0.008, and < 0.0001, respectively

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 1 wk then 2 wk unexposed, for life 60, 60 46 (at risk), 54 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 1/46, 3/54 *Zymbal gland*: carcinoma 0/46, 19/54* *Lung*: adenoma 5/46, 3/54 *All sites* Malignant tumours 2/46, 24/54* Benign tumours 6/46, 5/54 Benign and malignant tumours 8/46, 25/54*	 NS *$P < 0.001$ NS *$P < 0.001$ NS *$P < 0.001$	Principal strengths: lifetime study In the group of exposed mice, the Working Group determined that increases in the incidence of Zymbal gland carcinoma, malignant tumours, and of total tumours were also significant by the two-tail Fisher exact test, with P values of < 0.0001, < 0.0001, and 0.003, respectively

d, day(s); F, female; h, hour(s); HPLC, high-performance liquid chromatography; M, male; mo, month(s); NR, not reported; NS, not significant; ppm, parts per million; wk, week(s)

types): control, 8/88 (9.1%) and benzene, 20/89 (22.5%) [$P = 0.0223$, Fisher]; tumours of the Zymbal gland: control, 1/88 (1.1%) and benzene, 16/89 (18.0%) [$P = 0.001$, Fisher]; tumours of the ovary: control, 0/88 and benzene, 8/89 (9.0%) [$P = 0.0066$, Fisher]; hepatoma [not further specified]: control, 1/88 (1.1%) and benzene, 1/89 (1.1%); and other tumours: control, 2/88 (2.3%) and benzene, 4/89 (4.5%). [These incidences indicated a significant increase in carcinogenicity in mice exposed to benzene; however, the animals that were still alive at experimental day 692 (65 control and 41 exposed to benzene) were not comprehensively examined. The actual tumour incidences in the control group and the group exposed to benzene were therefore unknown. The similarities between the mice described in Table 1 of the 1985 article and Table 1 of the 1984 article authored by Cronkite et al. suggested that these were the same groups of experimental animals; this is not specifically stated in the 1985 article, however (Cronkite et al., 1984, 1985). The study was considered inadequate for the evaluation.]

Six groups of CBA/Ca BNL male or female mice (age, 12 weeks) were exposed to clean air or benzene at a dose of 100 or 300 ppm [purity not reported] for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers and then observed for their lifetimes (Cronkite et al., 1989). Groups 1 and 2 consisted of 60 males exposed to clean air and 57 males exposed to benzene at 300 ppm; groups 3 and 4 consisted of 60 females exposed to clean air and 54 females exposed to benzene at 300 ppm; and groups 5 and 6 consisted of 70 males exposed to clean air and 85 males exposed to benzene at 100 ppm. Mice exposed to benzene at 300 pm, but not 100 ppm, had shorter lifespans than controls. Median lifespans of males and females were 1030 and 1100 days (clean air) and 510 and 580 days (300 ppm benzene), respectively, while the median lifespans of males exposed to clean air and 100 ppm benzene were 1020 and 1000 days, respectively. Neither lymphomatous neoplasms nor hepatoma were increased in male or female mice exposed to benzene at 300 ppm; however, the mice exposed to benzene at 300 ppm had increased incidences of myelogenous neoplasms (controls, 0/60 (males) and 1/60 (females); benzene, 11/57 (males) ($P < 0.001$) and 6/54 (females) ($P = 0.040$) [$P = 0.0420$, one-tail Fisher exact test; $P = 0.0514$ (not significant), two-tail Fisher exact test]) and solid tumours other than hepatoma or of the haematopoietic or lymphoid tissues (controls, 13/60 (males) and 21/60 (females); benzene, 30/57 (males) ($P < 0.001$) and 43/54 (females) ($P < 0.001$)). Male mice exposed to benzene at 100 ppm also had an increased incidence of solid tumours other than hepatoma or of the haematopoietic or lymphoid tissues (controls, 14/70; benzene, 38/85 ($P = 0.001$)).

Groups of 125 CBA/Ca male mice (age, 10–12 weeks) were exposed to clean air or benzene [high-performance liquid chromatography grade] at a dose of 300 ppm for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers, and then observed for up to 18 months (Farris et al., 1993). There was a significant decrease in survival in the group exposed to benzene ($P < 0.01$). There was a significant increase in the incidences of malignant lymphoma (control, 2/119; benzene, 14/118; $P < 0.01$), squamous cell carcinoma of the preputial gland (control, 0/118; benzene, 71/118; $P < 0.01$), adenoma of the lung (control, 17/119; benzene, 42/118; $P < 0.01$), carcinoma of the Zymbal gland (control, 1/125; benzene, 14/125 [$P < 0.01$, Fisher]), and squamous cell carcinoma of the forestomach (control, 0/125; benzene, 9/125 [$P < 0.01$, Fisher]).

Groups of 50–80 AKR mice, 40 C57Bl mice, and 40 CD-1 mice [sex and age not reported] were exposed to clean air or benzene at a dose of 100 or 300 ppm [purity not reported] in inhalation chambers for 6 hours per day, 5 days per week, for life (Goldstein et al., 1982). Of the CD-1 mice exposed to benzene at 300 ppm, one developed chronic myelogenous leukaemia

and another developed acute myelogenous leukaemia. [The Working Group noted that only myeloproliferative disorders were assessed and that, while there was no statistical increase in the incidence of myelogenous leukaemia in mice exposed to benzene compared with controls, spontaneous cases of myelogenous leukaemia are rare in CD-1 mice. The authors argued that "the absence of a background incidence of acute and chronic myelogenous leukaemia in CD-1 mice … suggests that the present observations are due to a direct effect of benzene inhalation."] No leukaemias were observed in AKR or C57Bl mice. [The Working Group also noted the very low incidence of neoplasms of the haematopoietic and lymphoid tissues in all three strains of mice tested.]

In lifetime studies, groups of male C57BL/6 wildtype (18–20 animals per group), heterozygous *Trp53*-deficient or homozygous Trp53-deficient mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 33, 100, or 300 ppm [purity not reported, chemical grade] for 6 hours per day, 5 days per week, for 26 weeks in inhalation chambers, and groups of 18–24 male C3H/He wildtype (23–24 animals per group), heterozygous *Trp53*-deficient or homozygous Trp53-deficient mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 100 or 300 ppm for 6 hours per day, 5 days per week, for 26 weeks in inhalation chambers (Kawasaki et al., 2009; see also Section 3.3.1). Wildtype mice of both strains exposed to benzene at 300 ppm had decreased survival rates ($P < 10^{-5}$). Wildtype C57BL/6 mice exposed to benzene at 300 ppm had significant increases in the incidences of lymphoma of the thymus gland (control, 0/20; benzene, 5/18; $P < 0.05$) and total neoplasms of the haematopoietic and lymphoid tissues (control, 2/20; benzene, 10/18; $P < 0.05$). Wildtype C3H/He mice exposed to benzene at 300 ppm had a non-significant increase in the incidence of total neoplasms of the haematopoietic and lymphoid tissues (control, 2/23; benzene, 7/23; not significant), and two of these mice developed myeloid leukaemia (compared with none from the control group). There was also a significant positive trend in the incidence of lymphoma of the thymus gland for both strains of mice. There was no increase in the incidence of solid tumours in any of the groups of wildtype mice exposed to benzene.

In a study using C57BL/6 h-Trx-Tg transgenic mice, Li et al. (2006) exposed 8 wildtype C57BL/6 mice to clean air and 10 wildtype C57BL/6 mice [sex not reported] to benzene at 300 ppm [purity not reported, chemical grade] in inhalation chambers for 6 hours per day, 5 days per week, for 26 weeks, and monitored the mice for their lifetimes. Until the mice reached an age of approximately 2 years, the proportion surviving in wildtype mice exposed to benzene was lower than in wildtype mice exposed to clean air. The cumulative incidence of thymic lymphoma in clean-air controls and wildtype mice exposed to benzene was 0/8 and 3/10 [no significant increase], respectively, and the cumulative incidence of non-thymic lymphoma was 6/8 and 5/10, respectively; however, the time to non-thymic lymphoma was shorter in mice exposed to benzene compared with controls.

Groups of 50 male AKR/J mice and 40 male C57BL/6J mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 100 ppm [purity not reported] (AKR mice) or 300 ppm (C57BL/6J mice) in inhalation chambers for 6 hours per day, 5 days per week, for life (Snyder et al., 1980). In the AKR mice, there was no difference in weight gain or median survival between those exposed to clean air and those exposed to benzene at 100 ppm. In contrast, C57BL/6J mice exposed to benzene at 300 ppm had a decreased weight gain and median survival: median survival of mice exposed to benzene was 41 weeks compared with 75 weeks for the controls. Inhalation of benzene at 100 ppm did not increase the incidence of malignant lymphoma in AKR mice: malignant lymphoma was found in 24/50 mice exposed to clean air and 29/49 mice exposed to benzene.

Haematopoietic neoplasms were found in 2/40 C57BL/6J mice exposed to clean air and 8/40 C57BL/6J mice exposed to benzene at 300 ppm ($P < 0.005$, log-rank (χ^2) test [$P = 0.0872$, two-tail Fisher exact test]), and hyperplasia of the bone marrow without neoplasia was found in 0/38 control mice and 13/32 mice exposed to benzene ($P < 0.001$, log-rank (χ^2) test [$P < 0.001$, Fisher exact test]). [Using the two-tail Fisher exact test, the incidence of haematopoietic neoplasms was not statistically significantly increased in C57BL/6J mice exposed to benzene at 300 ppm. On the other hand, using the log-rank test, which compares events and times to event, the difference in haematopoietic neoplasm incidence between control groups and mice exposed to benzene was found to be significant. The significance found by Snyder et al. (1980) therefore depends on tumour induction time. In support of the authors' conclusion that C57BL/6J mice exposed to benzene at 300 ppm had a significant increase in the incidence of haematopoietic neoplasms, hyperplasia of the bone marrow without neoplasia was significantly increased in mice exposed to benzene.]

Male CD-1 and C57BL/6J mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 300 or 1200 ppm [purity not reported] in inhalation chambers (Snyder et al., 1988). In a first protocol, groups of 80 CD-1 and 80 C57BL/6 mice were exposed to clean air or to benzene at 1200 ppm for 6 hours per day, 5 days per week, for 10 weeks, and then observed for their lifetimes. In a second protocol, groups of 60 CD-1 and 60 C57BL/6 mice were exposed to clean air or to benzene at 300 ppm for 6 hours per day, 5 days per week, for 1 week, followed by non-exposure for 2 weeks; this regimen was repeated for life. Exposure to benzene did not affect the mortality rate of either CD-1 or C57BL/6 mice; however, for clean-air controls, the 50% mortality rate occurred earlier in mice exposed according to the first protocol than in mice exposed according to the second protocol (approximately 460 days vs 600 days for CD-1 mice, and 740 days vs 840 days for C57BL/6 mice). Tumour incidences in CD-1 mice exposed to clean air or benzene at 1200 ppm were: malignant tumours, 22/71 or 24/71; benign tumours, 21/71 or 35/71; total tumours, 36/71 or 45/71; adenoma of the lung, 17/71 or 33/71; leukaemia/lymphoma, 11/71 or 11/71; and carcinoma of the Zymbal gland, 0/71 or 4/71, respectively. [Using the log-rank (χ^2) test, Snyder et al. (1988) reported that the increases in the incidence of malignant tumours, benign tumours, total tumours, adenoma of the lung, and carcinoma of the Zymbal gland were significant; however, using the two-tail Fisher exact test, the Working Group determined that only the incidence of benign tumours and of adenoma of the lung was significantly increased in the exposed mice.] Tumour incidence was not increased in C57BL/6 mice exposed to benzene at 1200 ppm. Tumour incidence in CD-1 mice intermittently exposed to clean air or benzene at 300 ppm was: malignant tumours, 1/46 or 12/54; benign tumours, 3/46 or 15/54; total tumours, 4/46 or 25/54; adenoma of the lung, 3/46 or 14/54; leukaemia/lymphoma, 1/46 or 7/54 [$P < 0.05$, one-tail Fisher exact test]; and carcinoma of the Zymbal gland, 0/46 or 2/54. [Using the two-tail Fisher exact test, the Working Group confirmed the conclusions of Snyder et al. that the incidence of total tumours, malignant tumours, benign tumours, and of adenoma of the lung was significantly increased in mice exposed to benzene.] Tumour incidence in C57BL/6 mice intermittently exposed to clean air or benzene at 300 ppm was: malignant tumours, 2/46 or 24/54; benign tumours, 6/46 or 5/54; total tumours, 8/46 or 25/54; adenoma of the lung, 5/46 or 3/54; leukaemia/lymphoma, 1/46 or 3/54; and carcinoma of the Zymbal gland, 0/46 or 19/54. [Using the two-tail Fisher exact test, the Working Group confirmed the conclusions of Snyder et al. that the incidence of total tumours, malignant tumours, and of carcinoma of the Zymbal gland was significantly increased in mice exposed to benzene. The Working Group noted the short duration of exposure in the first protocol.]

3.1.2 Oral administration

See Table 3.2

Good laboratory practice (GLP) studies of carcinogenicity with benzene (purity, > 99.7%) were conducted in groups of 50 B6C3F$_1$ mice of each sex (age, 6.5–8.5 weeks); four groups were given benzene at a dose of 0 (control), 25, 50, or 100 mg/kg body weight (bw) in corn oil by gavage 5 days per week, for 103 weeks (NTP, 1986; Huff et al., 1989). At the age of 2 years, mean body weights of male and female mice given the high dose were significantly decreased. Survival of male and female mice given the high dose was also significantly decreased (males, 28/50 (control), 23/50, 18/50, 7/50; females, 30/50 (control), 26/50, 24/50, 18/50). Most mice exposed to benzene that died before week 103 had neoplasia. Compound-related non-neoplastic and neoplastic effects were found for the adrenal gland, forestomach, Harderian gland, haematopoietic and lymphoid tissues, liver, lung, mammary gland, ovary, preputial gland, and Zymbal gland.

In males and females, benzene caused a significant increase in the incidence of the following lesions, with a significant positive trend: epithelial hyperplasia and carcinoma of the Zymbal gland; hyperplasia of the bone marrow (haematopoietic system), and lymphoma or leukaemia (combined); alveolar epithelial hyperplasia, bronchioloalveolar adenoma and carcinoma, and bronchioloalveolar adenoma or carcinoma (combined); hyperplasia and adenoma or carcinoma (combined) of the Harderian gland; epithelial hyperplasia, hyperkeratosis, and squamous cell papilloma of the forestomach; and hyperplasia of the adrenal gland. In males only, benzene caused a significant increase in the incidence of adenoma of the Harderian gland and pheochromocytoma of the adrenal gland. In females only, benzene caused a significant increase in the incidence of carcinoma of the Harderian gland, with a significant positive trend.

In male mice, benzene caused significant increases in the incidence, and a significant positive trend in the incidence, of hyperplasia of the preputial gland, squamous cell carcinoma of the preputial gland, carcinoma (not otherwise specified) of the preputial gland, and of carcinoma (all types) of the preputial gland. There was also a small but significant increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined).

In female mice, benzene caused significant increases in the incidence, and a significant positive trend in the incidence, of tubular adenoma of the ovary, granulosa cell tumours [benign] of the ovary, granulosa cell tumours or carcinoma (combined) of the ovary, mixed tumours (benign) of the ovary, carcinoma of the mammary gland, and of carcinosarcoma of the mammary gland, and a significant increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) (NTP, 1986; Huff et al., 1989). [The Working Group noted that this was a well-conducted GLP study with multiple doses, using both males and females, covering most of the lifespan, and with complete histopathology. Mice treated with benzene had lower body weights and survival.]

A group of 40 male and 40 female Swiss mice (age, 7 weeks) was given benzene (purity, > 99.93%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by stomach tube once per day, 4–5 days per week, for 78 weeks. Mice were observed for life. The authors stated that body weights were lower in treated mice, and particularly in males. Survival was comparable among groups. Necropsies were performed on all animals, with histopathological examinations on 29 tissues and organs (several with multiple sections, e.g. gastrointestinal tract), and all lesions. There were significant increases in the incidence of primary tumours (benign and malignant) (male, 15/40 vs 24/40; female, 16/40 vs 32/40), malignant tumours (female, 11/40 vs 28/40), total tumours [mainly adenomas] of the lung (male, 3/40 vs 17/40; female, 4/40 vs 15/40)

Table 3.2 Studies of carcinogenicity in mice exposed to benzene by gavage or intraperitoneal injection

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6.5–8.5 wk 103 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 5 days/wk, 103 wk 50, 50, 50, 50 28, 23, 18, 7	*Zymbal gland*: squamous cell carcinoma 0/43*, 1/34, 4/40**, 21/39***	*P < 0.001 (trend), **P = 0.012, ***P < 0.001 (life-table test)	Principal strengths: 2 yr bioassay; control and three dose groups; complete histopathology; well-conducted GLP study; studies in both male and female mice Principal limitations: much of the lowered body weights and slightly reduced survival could be attributed to tumour-bearing animals; the survival of high-dose groups of male and female mice was significantly lower than respective vehicle control groups; mean body weights of high-dose groups of male and female mice were lower than vehicle controls The incidence of preneoplastic hyperplasia was increased in tumours of Zymbal gland, preputial gland, Harderian gland, lung, haematopoietic system (bone marrow), forestomach, and adrenal gland caused by benzene
		Haematopoietic and lymphoid tissues Lymphoma 4/49*, 9/48, 9/50**, 15/49***	*P < 0.001 (trend), **P = 0.030, ***P < 0.001 (life-table test)	
		Lymphoma or leukaemia (combined) 4/49*, 10/48**, 10/50***, 15/49****	*P < 0.001 (trend), **P = 0.048, ***P = 0.018, ****P < 0.001 (life-table test) Leukaemia in one low-dose and one intermediate-dose group mouse	
		Lung Alveolar/bronchiolar adenoma 6/49*, 6/48, 8/50, 12/49**	*P < 0.001 (trend), **P = 0.005 (life-table test)	
		Alveolar/bronchiolar carcinoma 5/49*, 11/48, 12/50**, 14/49***	*P < 0.001 (trend), **P = 0.017, ***P < 0.001 (life-table test)	
		Alveolar/bronchiolar adenoma or carcinoma (combined) 10/49*, 16/48, 19/50**, 21/49***	*P < 0.001 (trend), **P = 0.007, ***P < 0.001 (life-table test)	
		Adrenal gland: pheochromocytoma 1/47, 1/48, 7/49*, 1/46	*P = 0.010 (life-table test)	
		Preputial gland Squamous cell carcinoma 0/21*, 3/28, 18/29**, 28/35**	*P < 0.001 (trend), **P < 0.001 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6.5–8.5 wk 103 wk NTP (1986) (cont.)		Carcinoma, NOS 0/21*, 2/28, 1/29, 3/35**	*P < 0.019 (trend), **P < 0.043 (life-table test)	
		Carcinoma (all types) 0/21*, 5/28, 19/29**, 31/35**	*P < 0.001 (trend), **P < 0.001 (life-table test)	
		Harderian gland Adenoma 0/49*, 9/46**, 13/49***, 11/48***	*P < 0.001 (trend), **P = 0.001, ***P < 0.001 (life-table test)	
		Adenoma or carcinoma (combined) 1/49*, 10/46**, 13/49***, 14/48***	*P < 0.001 (trend), **P = 0.002, ***P < 0.001 (life-table test)	
		Forestomach Squamous cell papilloma 2/45*, 1/42, 2/44, 5/38**	*P = 0.003 (trend), **P = 0.014 (life-table test)	
		Squamous cell papilloma or carcinoma (combined) 2/45*, 2/42, 3/44, 5/38**	*P = 0.004 (trend), **P = 0.014 (life-table test) One carcinoma in each benzene-treated group; one mouse in the high-dose group had papilloma and carcinoma	
		Liver Hepatocellular carcinoma 9/49, 8/48, 17/50*, 8/47	*P = 0.028 (life-table test)	
		Hepatocellular adenoma or carcinoma (combined) 15/49, 17/48, 22/50*, 11/47	*P = 0.029 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6.5–8.5 wk 103 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 5 d/wk, 103 wk 50, 50, 50, 50 30, 26, 24, 18	*Zymbal gland*: squamous cell carcinoma 0/43*, 0/32, 1/37, 3/31**	*P = 0.007 (trend), **P = 0.045 (life-table test)	Principal strengths: 2 yr bioassay; control and three dose groups; complete histopathology; well-conducted GLP study; studies in both male and female mice Principal limitations: see principal limitations for NTP (1986) male mice study The incidence of preneoplastic hyperplasia was increased in tumours of the Zymbal gland, ovary, Harderian gland, lung, haematopoietic system (bone marrow), forestomach, and adrenal gland Historical incidence of lymphoma at laboratory (mean ± SD): 22/99 (22.2%); historical incidence in NTP studies: 237/1187 (20.0 ± 8.7%). Historical incidence of ovarian tumours at laboratory: 0/100; historical incidence in NTP studies: no more than two ovarian tumours were present in any single control group. Historical incidence of forestomach squamous cell papilloma at laboratory (mean): 0/99; historical incidence in NTP studies: 7/1077 (0.6%)
		Haematopoietic and lymphoid tissues Lymphoma 15/49*, 24/45**, 24/50***, 20/49****	*P = 0.031 (trend), **P = 0.021, ***P = 0.025, ****P = 0.037 (life-table test)	
		Lymphoma or leukaemia (combined) 15/49*, 25/45**, 26/50***, 22/49****	*P = 0.014 (trend), **P = 0.014, ***P = 0.012, ****P = 0.017 (life-table test) Leukaemia in 1 low-dose, 2 intermediate-dose, and 2 high-dose mice	
		Lung Alveolar/bronchiolar adenoma 4/49*, 2/42, 5/50, 9/49**	*P = 0.003 (trend), **P = 0.011 (life-table test)	
		Alveolar/bronchiolar carcinoma 0/49*, 3/42, 6/50**, 6/49***	*P = 0.002 (trend), **P = 0.010, ***P = 0.004 (life-table test)	
		Alveolar/bronchiolar adenoma or carcinoma (combined) 4/49*, 5/42, 10/50**, 13/49***	*P < 0.001 (trend), **P = 0.039, ***P < 0.001 (life-table test)	
		Ovary Tubular adenoma 0/47*, 0/44, 3/49, 3/48**	*P = 0.008 (trend), **P = 0.047 (life-table test)	
		Granulosa cell tumour [benign] 1/47*, 1/44, 6/49**, 7/48***	*P < 0.001 (trend), **P = 0.040, ***P = 0.008 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6.5–8.5 wk 103 wk NTP (1986) (cont.)		Granulosa cell tumour or carcinoma (combined) 1/47*, 1/44, 6/49**, 8/48*** Mixed tumour, benign 0/47*, 1/44, 12/49**, 7/48*** *Mammary gland* Carcinoma 0/49*, 2/45, 5/50**, 10/49*** Carcinosarcoma 0/49*, 0/45, 1/50, 4/49** *Harderian gland* Carcinoma 0/48*, 0/44, 0/50, 4/47** Adenoma or carcinoma (combined) 5/48*, 6/44, 10/50, 10/47** *Forestomach*: squamous cell papilloma 1/42*, 3/40, 6/45**, 5/42*** *Liver* Hepatocellular adenoma 1/49, 8/44*, 5/50, 4/49 Hepatocellular adenoma or carcinoma (combined) 4/49, 12/44*, 13/50**, 7/49	*P < 0.001 (trend), **P = 0.040, ***P = 0.004 (life-table test) One high-dose group mouse had carcinoma *P < 0.001 (trend), **P < 0.001, ***P = 0.001 (life-table test) *P < 0.001 (trend), **P = 0.026, ***P < 0.001 (life-table test) *P < 0.001 (trend), **P = 0.017 (life-table test) *P < 0.001 (trend), **P = 0.020 (life-table test) *P = 0.009 (trend), **P = 0.017 (life-table test) *P = 0.022 (trend), **P = 0.038, ***P = 0.040 (life-table test) *P = 0.008 (life-table test) *P = 0.014, **P = 0.008 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) 7 wk Lifetime Maltoni et al. (1988)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 78 wk 40, 40 NR	*Lung*: all tumours [mainly adenomas] 3/40, 17/40* *All sites* "Benign and malignant tumours" 15/40, 24/40* "Malignant tumours" 9/40, 14/40	*[$P < 0.001$][a] *[$P < 0.04$][b] [NS]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 908; also reported in Maltoni et al. (1989)
Full carcinogenicity Mouse, Swiss (F) 7 wk Lifetime Maltoni et al. (1988)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 78 wk 40, 40 NR	*Mammary gland*: carcinoma 2/40, 19/40* *Lung*: all tumours [adenomas] 4/40, 15/40* *All sites* "Benign and malignant tumours" 16/40, 32/40* "Malignant tumours" 11/40, 28/40*	*[$P < 0.0001$][a] *[$P < 0.01$][a] *[$P \leq 0.0005$][a] *[$P \leq 0.0003$][a]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 908; also reported in Maltoni et al. (1989)
Full carcinogenicity Mouse, RF/J (M) 6 wk Lifetime Maltoni et al. (1989)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 52 wk 45, 45 NR	*Lung*: all tumours [adenomas] 5/45, 23/45* *Haematopoietic and lymphoid tissues*: leukaemia 17/45, 26/45* *All sites* "Benign and malignant tumours" 18/45, 33/45* "Malignant tumours" 19/45, 26/45	*[$P < 0.0001$][a] *[$P < 0.05$][b] *[$P < 0.003$][a] [NS]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 909

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, RF/J (F) 6 wk Lifetime Maltoni et al. (1989)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 52 wk 40, 40 NR	*Mammary gland*: carcinoma 1/40, 9/40* *Haematopoietic and lymphoid tissues*: leukaemia 14/40, 24/40* *Lung*: all tumours [mainly adenomas] 3/40, 18/40* *All sites* "Benign and malignant tumours" 20/40, 34/40* "Malignant tumours" 3/40, 18/40*	*[$P < 0.02$][a] *[$P < 0.05$][a] *[$P < 0.0002$][a] *[$P < 0.002$][a] *[$P \leq 0.0002$][a]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 909
Full carcinogenicity Mouse, A/J (M) 6–8 wk 24 wk Stoner et al. (1986)	Gavage Benzene, purity NR (reagent grade) Tricaprylin 0, 100 mg/kg bw 3×/wk for 8 wk 16, 16 15, 16	*Lung*: adenoma 3/15, 8/16 Tumour multiplicity: 0.27 ± 0.59, 0.63 ± 0.72*	 NR [NS] *$P < 0.05$	Principal limitations: incomplete histopathology reporting; only one dose group Cumulative dose was 2400 mg/kg bw, or 100 mg/kg bw/dose, as animals were gavaged 3×/wk for 8 wk
Full carcinogenicity Mouse, A/J (F) 6–8 wk 24 wk Stoner et al. (1986)	Gavage Benzene, purity NR (reagent grade) Tricaprylin 0, 100 mg/kg bw 3×/wk for 8 wk 16, 16 14, 15	*Lung*: adenoma 2/14, 5/15 Tumour multiplicity: 0.14 ± 0.36, 0.53 ± 0.92	 NR [NS] NS	Principal limitations: incomplete histopathology reporting; only one dose group Cumulative dose was 2400 mg/kg bw or 100 mg/kg bw/dose, as animals were gavaged 3×/wk for 8 wk

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, A/J (M) 6–8 wk 24 wk Stoner et al. (1986)	i.p. Benzene, purity NR (reagent grade) Tricaprylin 0, 20, 50, 100 mg/kg bw 3×/wk for 8 wk 16, 16, 16, 16 16, 15, 16, 16	*Lung*: adenoma 3/16, 5/15, 8/16, 10/16* Tumour multiplicity: 0.25 ± 0.58, 0.53 ± 0.92, 0.63 ± 0.72*, 0.69 ± 0.60*	*[$P < 0.03$] *$P < 0.05$	Principal limitations: incomplete histopathology reporting Cumulative doses were 480, 1200, and 2400 mg/kg bw, or 20, 50, and 100 mg/kg bw, as animals were i.p. injected 3×/wk for 8 wk
Full carcinogenicity Mouse, A/J (F) 6–8 wk 24 wk Stoner et al. (1986)	i.p. Benzene, purity NR (reagent grade) Tricaprylin 0, 20, 50, 100 mg/kg bw 3×/wk for 8 wk 16, 16, 16, 16 16, 16, 16, 15	*Lung*: adenoma 4/16, 4/16, 4/16, 6/15 Tumour multiplicity: 0.31 ± 0.60, 0.44 ± 0.89, 0.25 ± 0.45, 0.47 ± 0.64	NR [NS] NS	Principal limitations: incomplete histopathology reporting Cumulative doses were 480, 1200, and 2400 mg/kg bw, or 20, 50, and 100 mg/kg bw, as animals were i.p. injected 3×/wk for 8 wk
Full carcinogenicity Mouse, CD-1 (M) In utero 12 mo Badham et al. (2010)	i.p. Benzene, purity NR Corn oil 0, 200, 400 mg/kg bw to pregnant dams on gestation days 8, 10, 12, and 14 25, 25, 25 NR	*All sites*: total tumours 6/22, 14/22*, 8/23 *Liver tumours* [primarily adenomas] 3/22, 10/22*, 4/23	*$P = 0.0329$[a] *$P = 0.0452$[a]	Transplacental carcinogenesis The authors also reported an experiment in male C57BP/6N mice with a similar study design, which gave negative results

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) In utero 12 mo Badham et al. (2010)	i.p. Benzene, purity NR Corn oil 0, 200, 400 mg/kg bw to pregnant dams on gestation days 8, 10, 12, and 14 25, 25, 25 NR	*All sites*: total tumours 1/25, 10/24*, 5/22 Lesions of the haematopoietic and lymphoid tissues: hyperplasias, myeloproliferative disorders, and myeloid/lymphoid neoplasias 1/25, 9/24*, 5/22	*P = 0.0019[a] *P = 0.0232[a]	Transplacental carcinogenesis The authors also reported an experiment in female C57BP/6N mice with a similar study design, which gave negative results

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; i.p., intraperitoneal; M, male; mo, month(s); NOS, not otherwise specified; NR, not reported; NS, not significant; NTP, National Toxicology Program; SD, standard deviation; wk, week(s); yr, year(s)

[a] Fisher exact test

[b] One-tail Fisher exact test

in males and/or females, and of carcinoma of the mammary gland in females (2/40 vs 19/40). Carcinoma of the Zymbal gland occurred in four males and one female and dysplasia of the Zymbal gland in three males and four females, compared with none in controls (Maltoni et al., 1988, 1989). [The Working Group noted that this lifetime study did not report on numerical body weight data, statistical analyses, or incidence of benign tumours of the mammary gland.]

A group of 45 male and 40 female RF/J mice (age, 6 weeks) was given benzene (purity, > 99.93%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by stomach tube once per day, 4–5 days per week, for 52 weeks. Mice were observed for life. Necropsies were performed on all animals, with histopathological examinations on 29 tissues and organs (several with multiple sections, e.g. gastrointestinal tract), and all lesions. There were significant increases in the incidence of primary tumours (benign and malignant) (male, 18/45 vs 33/45; female, 20/40 vs 34/40), malignant tumours (female, 3/40 vs 18/40), total tumours [mainly adenomas] of the lung (male, 5/45 vs 23/45; female, 3/40 vs 18/40), and leukaemia (male, 17/45 vs 26/45; female, 14/40 vs 24/40) in males and/or females, and of carcinoma of the mammary gland in females (1/40 vs 9/40) (Maltoni et al., 1989). [The Working Group noted that this lifetime study did not report on body weights, statistical analyses, or incidence of benign tumours of the mammary gland.]

Groups of 16 male and 16 female A/J mice (age, 6–8 weeks) were given benzene (reagent grade) [purity not reported] at a dose of 0 (control) or 100 mg/kg bw [reported as cumulative dose of 2400 mg/kg bw] in 0.1 mL of tricaprylin per mouse, by gavage 3 days per week, for 24 weeks. Of the male mice, 15/16 (control) versus 16/16 (exposed to benzene) survived; for female mice, 14/16 (control) versus 15/16 (exposed to benzene) survived. At necropsy, tumours of the lung (pearly white nodules on the surface of the lungs) were counted under a dissecting microscope. Random samples of lung nodules were taken from control groups and groups treated with benzene for histopathological evaluation and confirmation of adenoma of the lung. The liver, kidneys, spleen, intestines, stomach, thymus, and salivary and endocrine glands were also examined grossly. The incidence of adenoma of the lung was numerically increased, but not significantly (males, 3/15 vs 8/16; females, 2/14 vs 5/15). Lung adenoma multiplicity was increased in exposed males (0.27 ± 0.59 vs 0.63 ± 0.72, $P < 0.05$) but not in exposed females (0.14 ± 0.36 vs 0.53 ± 0.92) (Stoner et al., 1986). [The Working Group noted that benzene purity was not reported, the histopathological report was incomplete, and only one dose was tested.]

3.1.3 *Intraperitoneal injection*

See Table 3.2

Groups of 16 male and 16 female A/J mice (age, 6–8 weeks) were given benzene (reagent grade) [purity not reported] at a dose of 0 (control), 20, 50, and 100 mg/kg bw [reported as cumulative doses of 480, 1200, and 2400 mg/kg bw] in 0.1 mL tricaprylin per mouse, by intraperitoneal injection, 3 days per week, for 24 weeks. One male from the group given the lowest dose and one female from the group given the highest dose died early. At necropsy, tumours of the lung (pearly white nodules on the surface of the lungs) were counted under a dissecting microscope. Random samples of lung nodules were taken from control groups and groups treated for benzene for histopathological evaluation and confirmation of adenoma of the lung. Liver, kidneys, spleen, intestines, stomach, thymus, and salivary and endocrine glands were also examined grossly. The incidence of adenoma of the lung was significantly increased in males given the highest dose (3/16 vs 5/15, 8/16, and 10/16 [$P < 0.03$]), as was multiplicity of adenoma of the lung in males given medium and high doses (0.25 ± 0.58 vs 0.53 ± 0.92, 0.63 ± 0.72 ($P < 0.05$), and 0.69 ± 0.60 ($P < 0.05$)). Neither

lung tumour incidence (4/16 vs 4/16, 4/16, and 6/15) nor multiplicity (0.31 ± 0.60 vs 0.44 ± 0.89, 0.25 ± 0.45, and 0.47 ± 0.64) were significantly increased in treated females (Stoner et al., 1986). [The Working Group noted the incomplete histopathological report.]

CD-1 and C57Bl/6N male and female mice (age, 7–9 weeks) were acclimated for 1 week before use, and given access to rodent chow and tap water. A maximum of 3 females were housed with 1 male overnight, and vaginal plugs the next morning designated day 1 of gestation. Pregnant mice were given corn oil (vehicle) or benzene at a dose of 200 or 400 mg/kg bw [purity not reported] by intraperitoneal injection on days 8, 10, 12, and 14 of gestation (Badham et al., 2010). At an age of 1 year, offspring mice were killed, necropsied, and tissues or organs collected (i.e. heart, intestines, kidneys, liver, lung, spine, spleen, stomach, thymus, and any abnormal tissues). Blinded histopathology was performed under light microscopy.

All tumours observed originated from the lung, liver, or haematopoietic and lymphoid tissues. CD-1 mice exposed in utero to benzene at 200 mg/kg bw had significant increases in the incidence of total tumours (combined), while the group exposed to benzene at 400 mg/kg bw had non-significant numerical increases only: males, 6/22 (27.3%) vs 14/22 (63.6%; $P = 0.0329$, Fisher exact test) and 8/23 (34.8%); females, 1/25 (4.0%) vs 10/24 (41.7%; $P = 0.0019$, Fisher exact test) and 5/22 (22.7%). In C57Bl/6N mice, there was a low incidence of total tumours (combined) only in groups treated with benzene: males, 0/21 vs 1/22 (4.5%) and 1/25 (4.0%); females, 0/19 vs 3/20 (15.0%) and 2/22 (9.1%). CD-1 male mice given the low dose had significant increases in the incidence of tumours of the liver (primarily adenomas, with some focal nodular hyperplasias and carcinomas): 3/22 (13.6%) vs 10/22 (45.5%, $P = 0.0452$) and 4/23 (17.4%). CD-1 female mice given the low dose had significant increases in the incidence of tumours [lesions] of the haematopoietic and lymphoid tissues (hyperplasias, myeloproliferative disorders, or myeloid/lymphoid neoplasias, combined): 1/25 (4.0%) vs 9/24 (37.5%, $P = 0.0232$) and 5/22 (22.7%). In C57Bl/6N mice, there was a low incidence of tumours [lesions] of the haematopoietic and lymphoid tissues only in the groups treated with benzene: males, 0/21 vs 0/22 and 1/25 (4.0%); females, 0/19 vs 3/20 (15.0%) and 2/22 (9.1%) (Badham et al., 2010). [The Working Group noted that dams were not carried to the end of the study for possible tumour occurrence, and that the study did not report benzene purity, injection volumes, beginning and ending body weights, and numbers of surviving mice. The Working Group also noted the short study duration of 12 months, that not all tissues or organs were taken or examined microscopically, and that tumours of the haematopoietic and lymphoid tissues were lesions grouped as hyperplasias, myeloproliferative disorders, and myeloid/lymphoid neoplasias.]

3.2 Rat

See Table 3.3

3.2.1 Oral administration

Groups of 30–35 male and 30–35 female Sprague-Dawley rats (age, 13 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control), 50, or 250 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 52 weeks (Maltoni & Scarnato, 1979; Maltoni et al., 1983; see also Maltoni & Scarnato, 1977; Maltoni et al., 1985, 1989; Mehlman, 2002) [experiment BT901]. The rats were kept under observation for their lifespan. Mortality, correlated with the direct toxic effects and the higher incidence of malignant tumours, was higher in male and female rats treated with benzene. In treated males, a significant increase in the incidence of tumours of the haematopoietic and lymphoid

Table 3.3 Studies of carcinogenicity in rats exposed to benzene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Maltoni & Scarnato (1979), Maltoni et al. (1983)	Gavage Benzene, > 99.9% Olive oil 0, 50, 250 mg/kg bw 1×/d, 4–5 d/wk, 52 wk 30, 30, 35 NR	*Zymbal gland*: carcinoma 0/30, 0/30, 0/35 *Oral cavity*: carcinoma 0/30, 0/30, 0/35 *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia (lymphoma, leukaemia, or histiocytic sarcoma, combined) 0/30*, 0/30, 4/35 (11.4%) *Mammary gland*: carcinoma 0/30, 0/30, 0/35 *All sites*: malignant tumours 2/30 (6.7%)*, 1/30 (3.3%), 8/35 (22.9%)	[NS] [NS] *[$P = 0.033$] (Cochran–Armitage trend test) [NS] *[$P = 0.025$] (Cochran–Armitage trend test)	Principal strengths: lifetime study; studies in male and female mice; multiple-dose study; complete histopathology Principal limitations: mortality was higher in benzene-treated rats Experiment BT901
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Maltoni & Scarnato (1979), Maltoni et al. (1983)	Gavage Benzene, > 99.9% Olive oil 0, 50, 250 mg/kg bw 1×/d, 4–5 d/wk, 52 wk 30, 30, 35 NR	*Zymbal gland*: carcinoma 0/30*, 2/30 (6.7%), 8/35 (22.9%)** *Oral cavity*: carcinoma 0/30, 0/30, 2/35 (5.7%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia (lymphoma, leukaemia, or histiocytic sarcoma, combined) 1/30 (3.3%), 2/30 (6.7%), 1/35 (3%) *Mammary gland*: carcinoma 3/30 (10%), 4/30 (13%), 7/35 (20.0%) *All sites*: malignant tumours 6/30* (20.0%), 10/30 (33.3%), 21/35 (60.0%)	*[$P = 0.005$] (Cochran–Armitage trend test), **[$P = 0.006$] (Fisher exact test) [NS] [NS] [NS]	Principal strengths: lifetime study; studies in male and female mice; multiple-dose study; complete histopathology Principal limitations: mortality was higher in benzene-treated rats Experiment BT901

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 7 wk Lifetime Maltoni et al. (1982a, 1983, 1985)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 50, 40 NR	*Zymbal gland*: carcinoma 1/50 (2.0%), 18/40 (45.0%)*	*[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; studies in male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights Experiment BT902
		Oral cavity: squamous cell carcinoma 0/50, 21/40 (52.5%)*	*[$P < 0.001$] (Fisher exact test)	
		Nasal cavity: carcinoma 0/50, 3/40 (7.5%)	[NS]	
		Forestomach Acanthomas and dysplasias 0/50, 10/40 (25.0%)*	*[$P < 0.001$] (Fisher exact test)	
		Carcinoma in situ 0/50, 0/40	[NS]	
		Liver Hepatoma [hepatocellular carcinoma] 3/50 (6.0%), 3/40 (7.5%)	[NS]	
		Angiosarcoma 0/50, 2/40 (5.0%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 3/50 (6.0%), 1/40 (2.5%)	[NS]	
		Skin: carcinoma 0/50, 9/40 (22.5%)*	*[$P < 0.001$] (Fisher exact test)	
		All sites: malignant tumours 11/50 (22.0%), 36/40 (90.0%)*	*[$P < 0.001$] (Fisher exact test)	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 7 wk Lifetime Maltoni et al. (1982a, 1983, 1985)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 50, 40 NR	*Zymbal gland*: carcinoma 0/50, 16/40 (40.0%)* *Oral cavity*: squamous carcinoma 0/50, 20/40 (50.0%)* *Nasal cavity*: carcinoma 0/50, 1/40 (2.5%) *Forestomach* Acanthomas and dysplasias 0/50, 7/40 (17.5%)* Carcinoma in situ 0/50, 6/40 (15.0%)* *Liver* Hepatoma [hepatocellular carcinoma] 0/50, 1/40 (2.5%) Angiosarcoma 0/50, 3/40 (7.5%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 1/50 (2.0%), 3/40 (7.5%) *Skin*: carcinoma 1/50 (2.0%), 0/40 *All sites*: malignant tumours 10/50 (20.0%), 35/40 (87.5%)*	*[$P < 0.001$] (Fisher exact test) *[$P < 0.001$] (Fisher exact test) [NS] *[$P = 0.002$] (Fisher exact test) *[$P = 0.006$] (Fisher exact test) [NS] [NS] [NS] [NS] *[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; studies in both male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights Experiment BT902

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) 7 wk Lifetime Maltoni et al. (1988, 1989)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 40, 40 NR	*Zymbal gland*: carcinoma 0/40, 7/40 (17.5%)* *Oral cavity*: carcinoma 1/40 (2.5%), 2/40 (5.0%) *Nasal cavity*: carcinoma 0/40, 2/40 (5.0%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 1/40 (2.5%), 2/40 (5.0%) *All sites*: malignant tumours 8/40 (20.0%), 19/40 (47.5%)*	*[$P = 0.012$] (Fisher exact test) [NS] [NS] [NS] *[$P = 0.017$] (Fisher exact test)	Principal strengths: lifetime study; studies in male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Experiment BT907
Full carcinogenicity Rat, Wistar (F) 7 wk Lifetime Maltoni et al. (1988, 1989)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 40, 40 NR	*Zymbal gland*: carcinoma 0/40, 6/40 (15.0%)* *Oral cavity*: carcinoma 0/40, 4/40 (10.0%) *Nasal cavity*: carcinoma 0/40, 1/40 (2.5%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 3/40 (7.5%), 4/40 (10.0%) *All sites*: malignant tumours 10/40 (25.0%), 21/40 (52.5%)*	*[$P = 0.026$] (Fisher exact test) [NS] [NS] [NS] *[$P = 0.021$] (Fisher exact test)	Principal strengths: lifetime study; studies in both male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Experiment BT907

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7–8 wk 104 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 50, 100, 200 mg/kg bw 1×/d, 5 d/wk, 103 wk 50, 50, 50, 50 32, 29, 24, 16	*Zymbal gland*: carcinoma 2/32 (6.3%)*, 6/46 (13.0%), 10/42 (23.8%)**, 17/42 (40.5%)***	*$P < 0.001$[a], $P < 0.001$[c], $P = 0.003$[d] **$P = 0.040$[b], $P = 0.017$[c] ***$P < 0.001$[b], $P < 0.001$[c], $P = 0.024$[d]	Principal strengths: studies in male and female mice; multiple-dose study; covered most of the lifespan; complete histopathology; well-conducted GLP study Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Dose-related lymphocytopenia was observed in benzene-treated rats Statistical tests used: [a] Cochran–Armitage trend test; [b] Fisher exact test; [c] Life-table test; [d] Incidental tumour test
		Oral cavity Squamous cell carcinoma 0/50*, 3/50, 5/50**, 7/50***	*$P = 0.006$[a], $P = 0.001$[c], $P = 0.002$[d] **$P = 0.028$[b], $P = 0.030$[c] ***$P = 0.006$[b], $P = 0.001$[c], $P = 0.001$[d]	
		Squamous cell papilloma 1/50*, 6/50, 11/50**, 13/50***	*$P < 0.001$[a], $P < 0.001$[b], $P < 0.001$[d] **$P = 0.002$[b], $P = 0.001$[c], $P = 0.004$[d] ***$P < 0.001$[b], $P < 0.001$[c], $P = 0.002$[d]	
		Tongue: squamous cell carcinoma 0/50*, 3/50, 4/50, 4/50**	*NS[a], $P = 0.039$[c], $P = 0.049$[d] **$P = 0.028$[c], $P = 0.012$[d]	
		Lip: squamous cell carcinoma 0/50*, 0/50, 0/50, 3/50**	*$P = 0.012$[a], $P = 0.002$[c], $P = 0.008$[d] **$P = 0.035$[c]	
		Skin Squamous cell carcinoma 0/50*, 5/50**, 3/50, 8/50***	*$P = 0.007$[a], $P < 0.001$[c] **$P = 0.028$[b], $P = 0.032$[c] ***$P = 0.003$[b], $P = 0.001$[c], $P = 0.039$[d]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7–8 wk 104 wk NTP (1986) (cont.)		Squamous cell papilloma 0/50*, 2/50, 1/50, 5/50**	*$P = 0.014$[a], $P = 0.001$[c], $P = 0.002$[d] **$P = 0.028$[b], $P = 0.005$[c], $P = 0.009$[d]	
		Palate Squamous cell papilloma 0/50*, 4/50, 4/50, 9/50**	*$P = 0.002$[a], $P < 0.001$[c], $P = 0.005$[d] **$P = 0.001$[b], $P < 0.001$[c], $P = 0.006$[d]	
		Squamous cell carcinoma 0/50, 0/50, 1/50, 0/50	NS	
Full carcinogenicity Rat, F344/N (F) 7–8 wk 104 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 1×/d, 5 d/wk, 103 wk 50, 50, 50, 50 46, 38, 33, 25	*Zymbal gland*: carcinoma 0/45*, 5/40**, 5/44***, 14/46 (30.4%)****	*$P < 0.001$[a], $P < 0.001$[c], $P < 0.001$[d] **$P = 0.020$[b], $P = 0.022$[c], $P = 0.036$[d] ***$P = 0.026$[b], $P = 0.018$[c] ****$P < 0.001$[b], $P < 0.001$[c], $P < 0.001$[d]	Principal strengths: studies in male and female mice; multiple-dose study; covered most of the lifespan; complete histopathology; well-conducted GLP study Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Dose-related lymphocytopenia was observed in benzene-treated rats; historical incidence of endometrial stromal polyps: 22/98 (22.4%) at laboratory; 248/1125 (22.0 ± 7%) (4/49–17/50) overall Statistical tests used: [a] Cochran–Armitage trend test; [b] Fisher exact test; [c] Life-table test; [d] Incidental tumour test
		Oral cavity Squamous cell carcinoma 0/50*, 1/50, 4/50, 5/50**	*$P = 0.011$[a], $P = 0.003$[c] **$P = 0.028$[b], $P = 0.010$[c]	
		Squamous cell papilloma 1/50*, 4/50, 8/50**, 5/50***	*NS[a], $P = 0.017$[c], $P = 0.047$[d] **$P = 0.015$[b], $P = 0.006$[c], $P = 0.022$[d] ***$P = 0.032$[c]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 7–8 wk 104 wk NTP (1986) (cont.)		*Tongue*: squamous cell carcinoma 0/50*, 0/50, 4/50**, 4/50***	*P = 0.014[a], P = 0.004[c] **P = 0.047[c] ***P = 0.024[c]	
		Uterus: endometrial stromal polyp 7/50* (14.0%), 7/50 (14.0%), 7/49 (14.3%), 14/50 (28.0%)**	*P = 0.032[a], P = 0.001[c], P = 0.049[d] **P = 0.003[c], P = 0.049[d]	
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk (breeders) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200–300 ppm 4–7 h/d, 5 d/wk, 104 wk 60, 54 NR	*Zymbal gland*: carcinoma 1/60 (1.7%), 3/54 (5.6%)	[NS]	Principal strengths: lifetime study; complete histopathology Principal limitations: breeders were aged 13 wk at the start of exposure Experiment BT4004, BT4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/60, 2/54 (3.7%)	[NS]	
		Forestomach: carcinoma in situ 0/60, 0/54	[NS]	
		Liver: hepatoma [hepatocellular carcinoma] 0/60, 1/54 (1.9%)	[NS]	
		Mammary gland: malignant tumours 2/60 (3.3%), 6/54 (11.1%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 2/60 (3.3%), 0/54	[NS]	
		All sites: malignant tumours 9/60 (15.0%), 14/54 (25.9%)	[NS]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Embryo (gestation day 12) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200, 200–300 ppm 4–7 h/d, 5 d/wk, 15 wk (200 ppm) or 104 wk (0 ppm or 200–300 ppm) 158, 70, 75 NR	*Zymbal gland*: carcinoma 2/158 (1.3%), 4/70 (5.7%), 6/75 (8.0%)*	*[$P = 0.015$] (Fisher exact test)	Principal strengths: lifetime study; multiple-dose study; studies in male and female mice; in utero exposure; complete histopathology Experiment BT4004, 4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/158, 2/70 (2.9%), 1/75 (1.3%)	[NS]	
		Forestomach: carcinoma in situ 0/158, 0/70, 0/75	[NS]	
		Liver: hepatoma [hepatocellular carcinoma] 1/158 (0.6%), 2/70 (2.9%), 2/75 (2.7%)	[NS]	
		Mammary gland: malignant tumours 3/158 (1.9%), 0/70, 0/75	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 12/158 (7.6%), 4/70 (5.7%), 6/75 (8.0%)	[NS]	
		All sites: malignant tumours 28/158 (17.7%), 20/70 (28.6%), 24/75 (32.0%)**	*[$P = 0.018$] (Fisher exact test)	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) Embryo (gestation day 12) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200, 200–300 ppm 4–7 h/d, 5 d/wk, 15 wk (200 ppm) or 104 wk (0 ppm or 200–300 ppm) 149, 59, 65 NR	*Zymbal gland*: carcinoma 0/149, 1/59 (1.7%), 8/65 (12.3%)*	*[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; multiple-dose study; studies in male and female mice; in utero exposure; complete histopathology Experiment BT4004, 4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/149, 6/59 (10.2%)*, 10/65 (15.4%)**	*[$P < 0.001$] **[$P < 0.001$] (Fisher exact test)	
		Forestomach: carcinoma in situ 0/149, 0/59, 3/65 (4.6%)*	*[$P = 0.027$] (Fisher exact test)	
		Liver: hepatoma [hepatocellular carcinoma] 0/149, 5/59 (8.5%)*, 7/65 (10.8%)**	*[$P = 0.002$] **[$P < 0.001$] (Fisher exact test)	
		Mammary gland: malignant tumours 8/149 (5.4%), 8/59 (13.6%), 9/65 (13.8%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 1/149 (0.7%), 4/59 (6.8%)*, 0/65	*[$P = 0.024$] (Fisher exact test)	
		All sites: malignant tumours 26/149 (17.4%), 26/59 (44.1%)*, 38/65 (58.5%)**	*[$P < 0.001$] **[$P < 0.001$] (Fisher exact test)	

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; h, hour(s); M, male; NR, not reported; NS, not significant; ppm, parts per million; wk, week

tissues (lymphoma, leukaemia, or histiocytic sarcoma, combined) was observed: 0/30, 0/30, and 4/35 (11%) [P = 0.033, Cochran–Armitage trend test]. A significant dose-related increase in the incidence of carcinoma of the Zymbal gland was observed in treated female rats: 0/30, 2/30 (7%), and 8/35 (23%) [P = 0.006, Fisher exact test; P = 0.005, Cochran–Armitage trend test]. The overall incidence of malignant tumours in male rats was 2/30 (7%), 1/30 (3%), and 8/35 (23%) [P = 0.025, Cochran–Armitage trend test]. The overall incidence of malignant tumours in female rats was 6/30 (20%), 10/30 (33%), and 21/35 (66%) [P = 0.003, Cochrane–Armitage trend test]. [The Working Group noted the small number of animals and the start of the treatment from age 13 weeks, slightly later than the usual 7–8 weeks. This might partly explain why certain malignant tumours increased in incidence, in particular carcinoma of the oral cavity and carcinoma of the mammary gland, but these increases were not statistically significant. The Working Group noted that the principal strengths of the study were: lifespan was covered; both male and female rats were studied; multiple doses tested; and complete histopathology. The principal limitation was that mortality was higher in rats treated with benzene.]

Groups of 40–50 male and 40–50 female Sprague-Dawley rats (age, 7 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 104 weeks (Maltoni et al., 1982a, 1983, 1985; see also Maltoni et al., 1989) [experiment BT902]. The rats were kept under observation for their lifespan. Male and female rats treated with benzene had lower body weights and showed lymphocytopenia. A significant increase in the incidence of carcinoma of the Zymbal gland was observed in treated male rats: 1/50 (2.0%) versus 18/40 (45.0%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of carcinoma of the Zymbal gland was also observed in treated female rats: 0/50 versus 16/40 (40.0%) [P < 0.001, Fisher exact test]. A significantly increased incidence of squamous cell carcinoma of the oral cavity was observed in treated male rats: 0/50 versus 21/40 (53%) [P < 0.001, Fisher exact test]. The incidence of squamous cell carcinoma of the oral cavity was also significantly increased in treated female rats: 0/50 versus 20/40 (50.0%) [P < 0.001, Fisher exact test]. In treated female rats, a significant increase in the incidence of carcinoma in situ of the forestomach was reported: 0/50 versus 6/40 (15.0%) [P = 0.006, Fisher exact test]. The incidence of precancerous lesions of the forestomach (acanthomas and dysplasias) was significantly increased in treated male and female rats: 0/50 versus 10/40 (25.0%) [P < 0.001, Fisher exact test] and 0/50 versus 7/40 (17.5%) [P = 0.002, Fisher exact test]. In treated male rats, the incidence of carcinoma of the skin was significantly increased: 0/50 versus 9/40 (22.5%) [P < 0.001, Fisher exact test]. The incidence of a rare tumour (angiosarcoma of the liver) was non-significantly increased in both male and female rats: 0/50 versus 2/40 (5.0%) and 0/50 versus 3/40 (7.5%). The overall incidence of malignant tumours in male rats was 11/50 (22.0%) versus 36/40 (90.0%) [P < 0.001, Fisher exact test]. The overall incidence of malignant tumours in female rats was 10/50 (20.0%) versus 35/40 (87.5%) [P < 0.001, Fisher exact test]. [The Working Group noted that the principal strengths of the study included: lifespan covered; both male and female rats studied; and complete histopathology. The principal limitation was that rats treated with benzene had lower body weights.]

Groups of 40 male and 40 female Wistar rats (age, 7 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 104 weeks (Maltoni et al., 1988, 1989; see also Maltoni et al., 1985) [experiment BT907]. Rats were kept under observation for their lifespan. Mortality was higher and body weights lower in male and female rats treated with benzene.

A significantly increased incidence of carcinoma of the Zymbal gland was reported in treated male and female rats: 0/40 versus 7/40 (17.5%) [$P = 0.012$, Fisher exact test] and 0/40 versus 6/40 (15.0%) [$P = 0.026$, Fisher exact test]. The overall incidence of malignant tumours in male and female rats was 8/40 (20.0%) versus 19/40 (47.5%) [$P = 0.017$, Fisher exact test] and 10/40 (25.0%) versus 21/40 (52.5%) [$P = 0.021$, Fischer exact test]. [The Working Group noted the principal strengths: the study covered the lifespan; both male and female rats were studied; and complete histopathology was reported. The principal limitations were the lower body weights and higher mortality in rats treated with benzene.]

In a GLP study, groups of 50 male F344/N rats (age, 7–8 weeks) were given benzene (purity, > 99.7%) at a dose of 0 (control), 50, 100, or 200 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 103 weeks. Groups of 50 female F344/N rats (age, 7 weeks) were given benzene (purity, > 99.7%) at a dose of 0 (control), 25, 50, or 100 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 103 weeks (NTP, 1986; see also Maronpot, 1987; Huff et al., 1989). The rats were kept under observation for 104 weeks and then killed. Higher mortality, lower body weights, and dose-related lymphocytopenia were all observed in male and female rats treated with benzene. A significant increase in the incidence, and positive trend in the incidence, of carcinoma of the Zymbal gland was observed in treated male and female rats. A significant increase in the incidence, and positive trend in the incidence, of squamous cell carcinoma of the oral cavity was observed in treated male and female rats. A significant increase in the incidence, and positive trend in the incidence, of squamous cell papilloma of the oral cavity was observed in treated male rats. A significant increase in the incidence of squamous cell papilloma of the oral cavity was also reported in treated female rats. There was a significant increase in the incidence, and positive trend in the incidence, of squamous cell carcinoma of the lip in treated male rats. In male rats, a significant increase in the incidence of squamous cell carcinoma of the tongue was reported. In female rats, a significant positive trend in the incidence of squamous cell carcinoma of the tongue was reported. There was a significant increase in the incidence, and positive trend in the incidence, of squamous cell papilloma of the palate in treated male rats. In male rats, significant increases in the incidence, and positive trend in the incidence, of squamous cell carcinoma and of squamous cell papilloma of the skin were reported. In female rats, a significant positive trend in the incidence of stromal polyp of the endometrium was reported. [The Working Group noted that the principal strengths included: well-conducted GLP study; multiple-dose study; most of the lifespan covered; both male and female rats studied; and complete histopathology was reported. The principal limitations were the lower body weights and higher mortality in rats treated with benzene.]

3.2.2 Inhalation

One group of pregnant breeders (54 female Sprague-Dawley rats; age, 13 weeks) was exposed to benzene (purity, > 99.9%) at a concentration of 200 ppm by inhalation from day 12 of gestation for 4 hours per day, 5 days per week, for 7 weeks (Maltoni et al., 1983, 1985, 1989; see also Maltoni et al., 1982b, c) [experiments BT4004, BT4006]. The embryos were exposed transplacentally by inhalation during the prenatal period, and possibly by ingestion (via lactation) during weaning. After weaning, a first group of offspring (70 males and 59 females) was exposed to benzene at 200 ppm by inhalation for 7 hours per day, 5 days per week for 8 weeks (total duration of exposure to benzene, 15 weeks). A second group of offspring (75 males and 65 females) and the breeders were exposed to benzene at 200 ppm by inhalation for 7 hours per day, 5 days per week, for 12 weeks, then exposed to benzene at

300 ppm for 7 hours per day, 5 days per week, for 85 weeks (total duration of exposure to benzene, 104 weeks). All groups of animals were located in inhalation chambers. The breeders control group (60 females) and the offspring control group (158 males and 149 females) were not exposed to benzene (untreated controls exposed to filtered air). The rats were then kept under observation for their lifespan. Mortality was higher in groups of male and female offspring treated with benzene that had lymphocytopenia. No significant increase in tumour incidence was found in the breeders. The overall incidence of malignant tumours in breeders was 9/60 (15.0%, control) versus 14/54 (25.9%). [Breeders were treated starting from age 13 weeks, slightly later than the usual 7–8 weeks. This might partly explain why malignant tumours increased in incidence, particularly carcinoma of the Zymbal gland, carcinoma of the oral cavity, and malignant tumours of the mammary gland, but none of these increases were statistically significant.] In the offspring, a significant increase in the incidence of carcinoma of the Zymbal gland was observed in treated male and female rats: 2/158 (1.3%) versus 4/70 (5.7%) and 6/75 (8.0%) [P = 0.015, Fisher exact test], and 0/149 versus 1/59 (1.7%) and 8/65 (12.3%) [P < 0.001, Fisher exact test], respectively. In female offspring, a significant increase in the incidence of carcinoma of the oral cavity was observed: 0/149 versus 6/59 (10.2%) [P < 0.001, Fisher exact test] and 10/65 (15.4%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of carcinoma in situ of the forestomach was observed in treated female offspring: 0/149 versus 0/59 and 3/65 (4.6%) [P = 0.027, Fisher exact test]. A significant increase in the incidence of liver hepatoma [hepatocellular carcinoma] was observed in treated female offspring: 0/149 versus 5/59 (8.5%) [P = 0.002, Fisher exact test] and 7/65 (10.8%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of tumours of the haematopoietic and lymphoid tissues (haemolymphoreticular neoplasia) was observed in treated female offspring: 1/149 (0.7%) versus 4/59 (6.8%) [P = 0.024, Fisher exact test] and 0/65. The overall incidence of malignant tumours in male and female offspring was: 28/158 (17.7%) versus 20/70 (28.6%) and 24/75 (32.0%) [P = 0.018, Fisher exact test], and 26/149 (17.4%) versus 26/59 (44.1%) [P < 0.001, Fisher exact test] and 38/65 (58.5%) [P < 0.001, Fisher exact test]. [The Working Group noted the principal strengths of the study: multiple-dose study; lifespan was covered; both male and female rats studied; in utero exposure; and complete histopathology. The principal limitation was that breeders were aged 13 weeks at the start of exposure.]

3.3 Genetically modified animals

See Table 3.4

Genetically modified animals have been used for accelerated testing of chemicals for their carcinogenic potential under specific conditions of exposure (Tennant et al., 1995, 2001; French et al., 2001a). These models are focused primarily on heritable mutations in: (1) tumour-suppressor genes with loss of function (e.g. transformation protein 53 or *Trp53*, cyclin-dependent kinase 2a or *Cdnk2a* [splice variants produce either p16Ink4a or p19Arf proteins]) and/or (2) proto-oncogenes (e.g. *Hras* or *vHras*) with gain of function due to acquired mutations associated with cancer in humans and mouse models of human cancer. Susceptibility or predisposition to chemical carcinogenesis is based on acquired or heritable mutations in tumour-suppressor gene and/or proto-oncogene functional pathways, and other modifiers of cancer in genetically modified animal models that are consistent with known hallmarks of human cancer (Hanahan & Weinberg, 2000, 2011). Accelerated tests for carcinogenic potential have shown reliability and potential, but with limitations that must be carefully considered (Pritchard et al., 2003; Eastmond et al., 2013).

Table 3.4 Studies of carcinogenicity in genetically modified animals exposed to benzene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, C57BL/6 or h-Trx-Tg (NR) NR Lifetime Li et al. (2006)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300, 0, 300 ppm 6 h/d, 5 d/wk, 26 wk 8, 10, 8, 13 NR	*Thymus*: lymphoma 0/8, 3/10, 0/8, 0/13	NS	Principal strengths: lifetime study Principal limitations: small numbers of animals used The h-Trx-Tg mouse overexpresses human thioredoxin; survival was higher in control mice than in benzene-treated mice
Carcinogenicity with other modifying factor Mouse, B6.CBA-*Trp53* ^{tm1Sia} (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR Clean air 0, 33, 100, 300 ppm 6 h/d, 5 d/wk 24, 27, 25, 26 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/24, 1/27, 4/25, 19/26* Non-thymic lymphoma 9/24, 10/27, 5/25, 2/26 Acute myelocytic leukaemia 0/24, 0/27, 0/25, 2/26	*$P < 0.05$ (Fisher exact test), [$P < 0.001$] (Cochran–Armitage trend test) NS (for an increase) NS	Principal strengths: lifetime study Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor; heterozygous wildtype and null allele (*Trp53* haploinsufficient) *Trp53* allelotype was used; moribund mice or mice presenting with masses or significant body weight loss were killed for gross and histopathological examination
Carcinogenicity with other modifying factor Mouse, C3.CBA-*Trp53*^{tm1Sia} (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR Clean air 0, 100, 300 ppm 6 h/d, 5 d/wk 24, 24, 24 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 1/24, 12/24*, 6/24* Non-thymic lymphoma 3/24, 6/24, 10/24* Acute myelocytic leukaemia 2/24, 2/24, 9/24*	*$P < 0.05$ (Fisher exact test), [$P = 0.001$] (Cochran–Armitage trend test) *$P < 0.05$ (Fisher exact test) *$P < 0.05$ (Fisher exact test), [$P = 0.016$] Cochran–Armitage trend test)	Principal strengths: lifetime study Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor; heterozygous wildtype and null allele (*Trp53* haploinsufficient) *Trp53* allelotype was used; moribund mice or mice presenting with masses or significant body weight loss were killed for gross and histopathological examination

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (M) 7–10 wk 26 wk French & Saulnier (2000), French et al. (2001b)	Gavage Benzene, purity NR Corn oil 0, 200 mg/kg bw, 5×/wk, 26 wk 20, 40 20, 39	*Subcutis*: sarcoma, NOS 0/20, 16/39* *Thymus*: lymphoma 0/20, 3/39 *Pancreas*: acinar cell carcinoma 0/20, 1/39 *Lung*: tumours 0/20, 0/22	*[$P \leq 0.004$] (Fisher exact test) LOH in 13/16 sarcomas tested NS LOH in 3/3 lymphomas tested NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); study terminated 1 d after exposure period
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (M) NR 26 wk Storer et al. (2001)	Gavage Benzene, purity NR Corn oil 0, 100 mg/kg bw 5–7×/wk 15 + 15, 15 + 15 NR	*Thymus* Lymphoma 0/30, 4/30 Atypical hyperplasia [preneoplastic lesion] 0/30, 7/30* *Subcutis*: sarcoma 1/30, 1/30 *Bone*: osteosarcoma 0/30, 1/30	[NS] *[$P \leq 0.01$] (Fisher exact test) NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); data (combined) extracted from use of benzene at a single dose as a positive control in two studies of another agent
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (F) NR 26 wk Storer et al. (2001)	Gavage Benzene, purity NR Corn oil 0, 100 mg/kg bw, 5–7×/wk 15 + 15, 15 + 15 NR	*Thymus* Lymphoma 1/30, 1/30 Atypical hyperplasia [preneoplastic lesion] 0/30, 2/30 *Subcutis*: sarcoma 1/30, 1/30 *Bone*: osteosarcoma 1/30, 0/30	NS NS NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); data (combined) extracted from use of benzene at a single dose as a positive control in two studies of another agent

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/ or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, B6.129-*Cdkn2a*^{tm1Dep} (M) 7–8 wk 27 wk NTP (2007)	Gavage Benzene, > 99% Corn oil 0, 25, 50, 100, 200 mg/kg bw, 5×/wk 15, 15, 15, 15, 15 15, 15, 15, 15, 14	*Multiple organs*: malignant lymphoma 0/15, 0/15, 0/15, 0/15, 5/15*	*P = 0.021 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	Genetically modified mouse model based on *Cdkn2a* tumour-suppressor gene wildtype and null allele (*Cdkn2a* haploinsufficiency) modifying factor (heterozygous); both *p16Ink4a* and *p19Arf* tumour-suppressor gene functions were haploinsufficient
		Bone marrow: atrophy 0/15, 0/15, 0/15, 10/15*, 12/15*	*P ≤ 0.01 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	
		Mesentery, lymph node: atrophy 1/15, 2/15, 2/14, 13/15*, 13/15*	*P ≤ 0.01 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	
Carcinogenicity with other modifying factor Mouse, B6.129-*Cdkn2a*^{tm1Dep} (F) 7–8 wk 27 wk NTP (2007)	Gavage Benzene, > 99% Corn oil 0, 25, 50, 100, 200 mg/kg bw, 5×/wk 15, 15, 15, 15, 15 15, 15, 15, 15, 15	*Multiple organs*: malignant lymphoma 0/15, 0/15, 0/15, 0/15, 0/15	NS	Genetically modified mouse model based on *Cdkn2a* umour-suppressor gene wildtype and null allele (*Cdkn2a* haploinsufficiency) modifying factor (heterozygous); both *p16Ink4a* and *p19Arf* tumour-suppressor gene functions were haploinsufficient
		Mesentery, lymph node: atrophy 0/15, 2/15, 3/15, 8/15*, 6/15*	*P ≤ 0.01 (Fisher exact test), [P = 0.005] Cochran–Armitage trend test)	
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) 7 wk 20 wk Spalding et al. (1999)	Skin application Benzene, purity NR Acetone, neat 0, 400, 800, 1600 µL/wk 5, 10, 10, 10 4, 8, 8, 8	*Skin*: squamous cell papilloma 3/5, 7/10, 8/10, 10/10*	*P < 0.05 (life-table test)	FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (two copies or homozygous state); to achieve the weekly dosage regimen, benzene was applied 2 d/wk; skin papilloma incidence in control mice due to fighting
		Tumour multiplicity: 1.4 ± 1.7, 7.0 ± 10.3, 10.6 ± 8.5*, 12.6 ± 10.3*	*P < 0.05 (Mann–Whitney U-test)	
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (M) 8 wk 26 wk Holden et al. (1998)	Skin application Benzene, purity NR Acetone, neat 0, 100, 150 µL, 3×/wk for 20 wk 10, 10, 10 9, 8, 10	*Skin*: squamous cell papilloma 0/10, 0/10, 3/10	NS	FVB/N mouse carrying a v-Ha-*Ras* transgene insert (single-copy or hemizygous state); acetone control group treated daily

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/ or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) 8 wk 26 wk Holden et al. (1998)	Skin application Benzene, purity NR Acetone, neat 0, 100, 150 µL, 3×/wk, 20 wk 10, 10, 10 5, 9, 7	*Skin*: squamous cell papilloma 0/10, 1/10, 1/10	NS	FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (single-copy or hemizygous state); acetone control group treated daily
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) NR 32 wk French & Saulnier (2000)	Skin application Benzene, purity NR Acetone, neat 0, 450, 800 µL/wk, 20 wk NR	*Bone marrow*: leukaemia, granulocytic 0/19, 4/14*, 11/15*	*$P < 0.05$ (Fisher exact test)	Principal limitations: limited reporting FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (single copy or hemizygous state); acetone control group treated with 200 µL/d; low-dose group was given 150 µL benzene 3×/wk; high-dose group was given 200 µL benzene 2×/d, 2×/wk

bw, body weight; d, day(s); F, female; h, hour(s); LOH, loss of heterozygosity; M, male; NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s)

3.3.1. Inhalation

Groups of 8 and 10 C57BL/6 wildtype and 8 and 13 h-Trx-Tg mice (overexpressing human thioredoxin) [sex and age at start not reported] were sham exposed (controls) or exposed by whole-body inhalation to benzene at a dose of 300 ppm [purity not reported], 6 hours per day, 5 days per week, for 26 weeks. Mice were maintained for their lifetime (or killed when showing symptoms of advanced haematopoietic neoplasms) and examined histopathologically. Survival of control h-Trx-Tg mice was higher than survival of the three other groups (which was comparable). There was a non-significant numerical increase in the cumulative incidence of lymphoma of the thymus gland (30%, 3/10) in the wildtype group exposed to benzene, while no lymphomas of the thymus gland were observed in the h-Trx-Tg group exposed to benzene (0/13) or in either of the control groups (Li et al., 2006). [The Working Group noted that this was a lifetime study, but the number of animals was small.]

Male B6.CBA-*Trp53*^{tm1Sia} congenic inbred mice (age, 8 weeks) (backcrossed repeatedly to C57BL/6 or B6 to homozygosity) were exposed to benzene at a concentration of 0, 33, 100, or 300 ppm [purity not reported, chemical grade] by whole-body inhalation for 6 hours per day, 5 days per week, for 26 weeks, and observed over their lifetime for tumour development (Kawasaki et al., 2009). Male B6.CBA-*Trp53*^{tm1Sia} mice heterozygous for a null and wildtype *Trp53* allele (haploinsufficient) were observed with a significant increase, with a significant positive trend, in the incidence of lymphoma of the thymus gland. [The Working Group noted that this was a lifetime study.]

Male C3.CBA-*Trp53*^{tm1Sia} congenic mice (age, 8 weeks) (backcrossed repeatedly to C3/He or C3 to homozygosity) were exposed to benzene at a concentration of 0, 100, or 300 ppm [purity not reported, chemical grade] for 6 hours per day, 5 days per week, for 26 weeks, and observed over their lifetime for the development of tumours (Kawasaki et al., 2009). Male C3.CBA-Trp53tm1Sia congenic mice heterozygous for a null and wildtype *Trp53* allele (haploinsufficient) were observed with significant increases in the incidence, with significant positive trends, of thymic lymphoma and of myeloid leukaemia, and a significant increase in the incidence of non-thymic lymphoma. [The Working Group noted that this was a lifetime study.]

3.3.2 Oral administration

Male B6.129-*Trp53*^{tm1Bra} N5 (the fifth C57BL/6 backcross generation, 97% homozygous) congenic mice (age, 7–10 weeks), heterozygous for a null and wildtype *Trp53* allele (Donehower et al., 1992), were given benzene [purity not reported] at a dose of 0 (vehicle only; $n = 20$) or 200 mg/kg bw ($n = 40$) by gavage (vehicle, corn oil) for 5 days per week, for 26 weeks (French & Saulnier, 2000; French et al., 2001b). After 26 weeks of exposure, the *Trp53* haploinsufficient mice were observed with sarcoma (of the subcutis, around the head and neck region or thoracic cavity) (0/20, 16/39 [$P \leq 0.004$, Fisher exact test]), lymphoma of the thymus (0/20, 3/39), and acinar cell carcinoma of the pancreas (0/20, 1/39), but without tumours of the lung (0/20, 0/22). Loss of the residual *Trp53* wildtype allele was observed in tested sarcoma (subcutis) (13/16) and lymphoma of the thymus (3/3).

Benzene [purity not reported] was used as a positive control in the Alternatives to Carcinogenicity Testing project of the International Life Sciences Health and Environmental Sciences Institute in two studies. Male and female B6.129-*Trp53*^{tm1Bra} N5 congenic mice (age at start not reported), heterozygous for *Trp53* wildtype and null allele (Donehower et al., 1992), were given benzene at a dose of 0 or 100 mg/kg bw by gavage, for 5 or 7 days per week, for 26 weeks (Storer et al., 2001). There were 15 males and 15 females per group per study.

Male *Trp53* haploinsufficient mice exposed to benzene showed a non-significant increase in the incidence of lymphoma of the thymus, supported by a significant increase in the incidence of atypical hyperplasia of the thymus [preneoplastic lesion]. The incidence of lymphoma of the thymus was 0/30 and 4/30 (males) and 1/30 and 1/30 (females), and of atypical hyperplasia of the thymus 0/30 and 7/30 [$P \leq 0.01$] (males) and 0/30 and 2/30 (females), for control and exposed groups, respectively. [The Working Group noted the combination of data from two different studies with a similar design.]

The National Toxicology Program tested the B6.129-*Cdkn2a*^{tm1Dep} congenic heterozygous mouse for a null and wildtype *Cdkn2a* allele with reduced expression of both the p16Ink4a and transcript variant p19Arf tumour-suppressor proteins (Serrano et al., 1996). Male and female mice (age, 7–8 weeks) were exposed to benzene (purity, > 99%) at a dose of 0, 25, 50, 100, or 200 mg/kg bw by gavage for 5 days a week, for 27 weeks. Malignant lymphoma was observed in males (0/15, 0/15, 0/15, 0/15, 5/15; $P = 0.021$, Fisher exact test [$P < 0.001$, Cochran–Armitage trend test]), but not in females. In addition, male *Cdkn2a* haploinsufficient mice showed several preneoplastic lesions (including bone marrow, thymus, and lymph node atrophy) associated with dose-related benzene exposure. Significantly increased preneoplastic lesions in female mice were restricted to lymph node (mesenteric) atrophy (NTP, 2007).

3.3.3 Skin application

The application of mutagenic or non-mutagenic chemicals to the shaved dorsal skin of the FVB/N-Tg.AC(v-Ha-*Ras*) mouse (Tg.AC for short) can result in squamous cell papilloma of the skin, which can convert to malignant skin neoplasms due to the abrogation of the first step in the two-step process of initiation–promotion of tumorigenesis (Leder et al., 1990).

A quantity of 200 µL of neat benzene [purity not reported] was applied to the skin of a group of 10–15 female Tg.AC mice twice a week, resulting in the rapid development of papillomas of the skin in 5 weeks. After 20 weeks, 76.9% [10/13] of treated Tg.AC mice had an average of 7.4 papillomas per mouse. A control group of 10–15 mice served as vehicle control [results not reported] (Tennant et al., 1995). [The Working Group noted that no results were given for controls. This study was inadequate for the evaluation of the carcinogenicity of benzene.]

Application of neat benzene [purity not reported] to the shaved dorsal skin of female Tg.AC mice (age, 7 weeks) at a dose of 0 (acetone control group), 400, 800, or 1600 µL per week for 20 weeks resulted in a significant increase in the incidence of squamous cell papilloma of the skin (3/5, 7/10, 8/10, 10/10 ($P < 0.05$)) and a significant increase in tumour multiplicity (1.4 ± 1.7, 7.0 ± 10.3, 10.6 ± 8.5 ($P < 0.05$), 12.6 ± 10.3 ($P < 0.05$)) (Spalding et al., 1999).

Compared with the above skin application studies, at lower benzene [purity not reported] exposure levels of 0 (acetone control group), 100, or 150 µL applied three times a week for 20 weeks in hemizygous Tg.AC mice (age, 8 weeks), the incidences of papilloma of the skin were reduced (males, 0/10, 0/10, 3/10; females, 0/10, 1/10, 1/10) after 26 weeks (Holden et al., 1998).

Blanchard et al. (1998) applied neat benzene [purity not reported] to the shaved dorsal skin of Tg.AC male and female mice (age at start not reported) in both hemizygous (single transgene copy) and homozygous (two copies of transgene) states at a dose of 0 (acetone control group) or 200 µL three times per week, for 20 weeks. A significant difference [$P < 0.05$] in the incidence of papilloma of the skin between hemizygous (males, 3/10; females, 4/10) and homozygous (males, 10/10; females, 9/10) Tg.AC mice was reported. [The Working Group noted that no results were given for controls. This study was inadequate for the evaluation of the carcinogenicity of benzene.]

Benzene [purity not reported] was applied neat to the shaved dorsal skin of homozygous Tg.AC female mice [age at start not reported] at a dose of 0 (acetone control group), 450, or 800 µL once per week, for 20 weeks. After an additional observation period of 12 weeks, the incidences of bone marrow leukaemia (granulocytic) (0/19, 4/14 ($P < 0.05$, Fisher exact test), 15/15 ($P < 0.05$, Fisher exact test)) were significantly increased (French & Saulnier, 2000). [The Working Group noted the limited reporting of the study.]

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4. MECHANISTIC AND OTHER RELEVANT DATA

4.1 Toxicokinetic data

Benzene is extensively metabolized, and the fate of benzene in the body is informed by measurements of various metabolites and of benzene itself. The section on metabolism, metabolic activation, and electrophilicity (Section 4.1.1) therefore precedes the discussion of absorption, distribution, and elimination (Section 4.1.2).

4.1.1 Metabolism, metabolic activation, and electrophilicity

The metabolism of benzene is complex (Ross, 1996; Snyder & Hedli, 1996), as summarized in Fig. 4.1. Qualitatively, the same metabolites are excreted by humans after occupational or environmental exposures and in animals exposed to benzene (Inoue et al., 1988, 1989; Sabourin et al., 1988, 1992; Henderson et al., 1989; Boogaard & van Sittert, 1996). Accordingly, the discussion below integrates findings from humans and from experimental systems in providing a synthetic overview of the metabolism and activation of benzene.

Metabolism of benzene is required for benzene toxicity. Studies in experimental systems using both pharmacological tools and genetically modified animals indicate that benzene requires metabolism to generate reactive electrophilic intermediates and subsequent toxicity (Snyder & Hedli, 1996; Ross, 2000). The first step of benzene metabolism is primarily mediated by cytochrome P4502E1 to form benzene oxide (Johansson & Ingelman-Sundberg, 1988; Koop et al., 1989; Snyder & Hedli, 1996), although other forms of cytochrome P450 may also play a role (Gut et al., 1996a; Powley & Carlson, 2001; Sheets et al., 2004). In CYP2E1-knockout mice, urinary benzene metabolites were reduced by approximately 90% with a concomitant complete lack of benzene-induced genotoxicity and cytotoxicity in bone marrow, blood, and lymphoid tissues (Valentine et al., 1996). Inhibition of CYP2E1-mediated metabolism reduced benzene-induced genotoxicity in mice (Tuo et al., 1996). Co-administration of toluene, a competitive inhibitor of benzene metabolism, reduced both benzene metabolism and benzene toxicity (Andrews et al., 1977). In agreement with rodent data that indicated a critical role for metabolism in toxicity, occupationally exposed individuals were more susceptible to benzene toxicity if they had a phenotype corresponding to rapid CYP2E1 metabolism (Rothman et al., 1997).

The sites of benzene metabolism have also been investigated. Benzene is metabolized in both liver and lung by CYP450 (Chaney & Carlson, 1995; Powley & Carlson, 2000). Partial hepatectomy reduced both benzene metabolism and benzene toxicity in rats (Sammett et al., 1979), indicating that the liver may play a primary role in benzene metabolism. Some metabolites distribute to bone marrow from

hepatic or other sites of generation, and metabolism to electrophiles can also occur in situ in bone marrow (Ross et al., 1996a). In particular, secondary metabolism of phenolic metabolites of benzene via myeloperoxidase (MPO) occurs in bone marrow to generate semiquinone radicals and quinones, providing one potential metabolic mechanism of benzene toxicity (Ross et al., 1996a; Smith, 1999). Metabolism of benzene in situ in rat bone marrow may also occur, and CYP450 has been detected at low levels in bone marrow (Gollmer et al., 1984; Schnier et al., 1989).

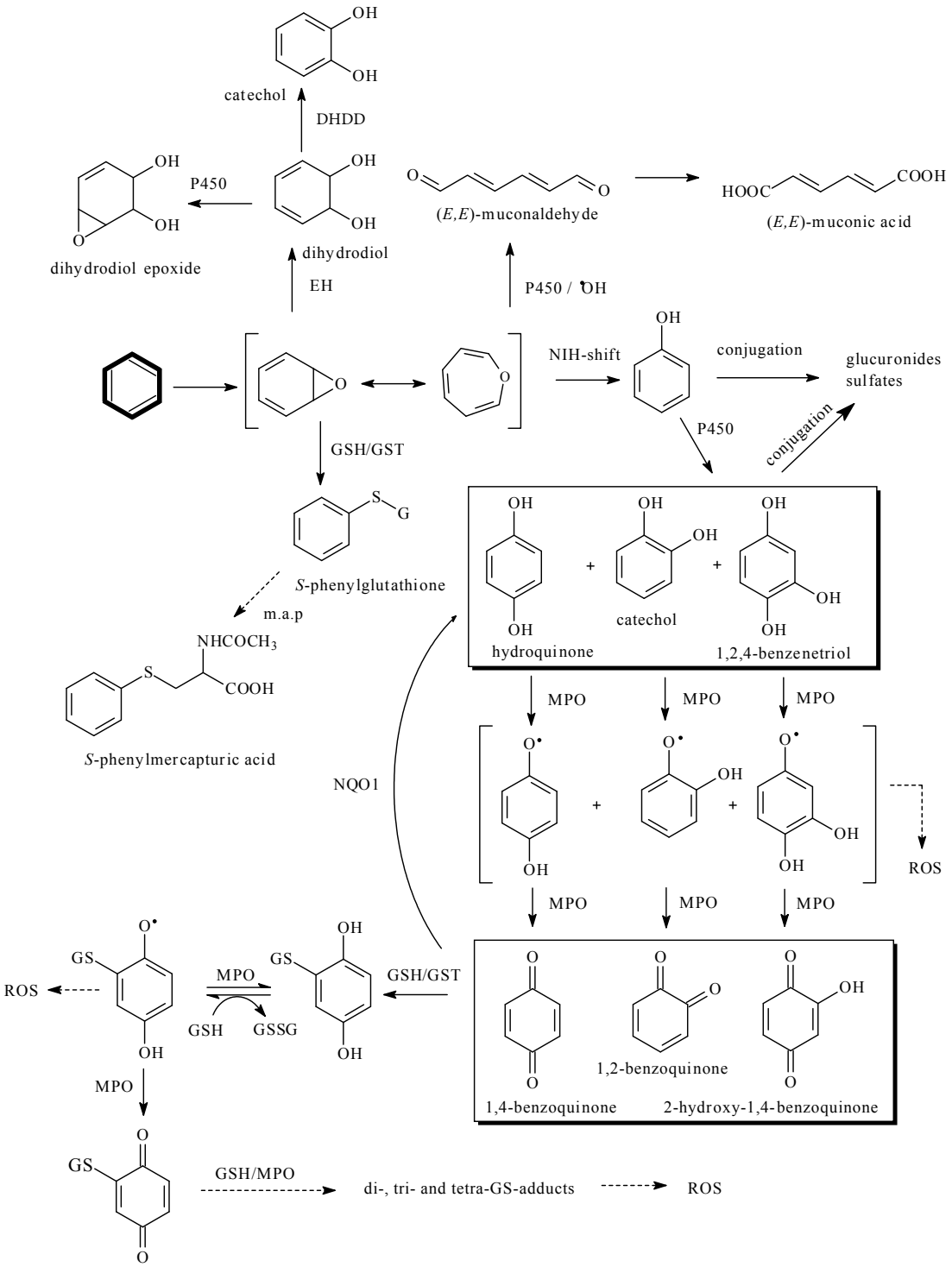
The main urinary metabolites of benzene in humans are phenol, hydroquinone, and catechol (the sum of free plus conjugated), *trans*,*trans*-muconic acid (t,t-MA), and *S*-phenylmercapturic acid (SPMA) (Inoue et al., 1988, 1989; Boogaard & van Sittert, 1996). t,t-MA and SPMA have been commonly used as biomarkers of benzene exposure in occupational and environmental studies in humans (Inoue et al., 1989; Boogaard & van Sittert, 1996). However, several metabolites have only been observed in experimental animals and/or in vitro. Benzene oxide formed by initial metabolic oxidation exists in equilibrium with its tautomer, oxepin. It can rearrange to generate phenol or undergo ring opening mediated by microsomal epoxide hydrolase to form benzene dihydrodiol; benzene dihydrodiol can spontaneously aromatize, releasing a water molecule to give phenol, and can be further oxidized to catechol and/or to a diol epoxide. Phenol can be further oxidized to catechol, hydroquinone, and benzene-1,2,4-triol. Oxidation of these phenols by MPO in bone marrow and bone marrow progenitor cells (Schattenberg et al., 1994) leads to the formation of semiquinone radicals and electrophilic benzoquinones (Gut et al., 1996b; Smith, 1996). Conjugation with glutathione (GSH) also plays an important role in the metabolism of benzene. Reaction of benzene oxide with GSH leads to SPMA.

In parallel, benzene oxide or oxepin and/or benzene dihydrodiol can undergo ring opening reactions to form *trans*,*trans*-muconaldehyde, which is further oxidized to t,t-MA. In studies using both animal and human cells in vitro, quinones generated from polyphenolic metabolites of benzene could be detoxified by NAD(P)H quinone oxidoreductase 1 (NQO1), maintaining them in their hydroquinone forms (Moran et al., 1999). Extensive glucuronidation and sulfation of phenols have been reported in animals and humans (Parke & Williams, 1953a, b; Seaton et al., 1995). GSH is also conjugated with electrophilic quinones (1,2-, 1,4-benzoquinone) leading to the corresponding *S*-(dihydroxyphenyl)glutathione (only one isomer, *S*-(2,5-dihydroxyphenyl) glutathione, is depicted in Fig. 4.1). The multiplicity of electrophilic metabolites formed during benzene metabolism, discussed in the sections that follow, are capable of reaction with GSH, which may occur chemically or be catalysed by glutathione-*S*-transferases (GSTs) (Snyder & Hedli, 1996). Benzene metabolism generates a variety of different reactive electrophiles. Several products of benzene metabolism are electrophilic and can interfere with cellular function.

(a) Epoxides

Benzene oxide is an electrophilic metabolite, identified in vitro when benzene is oxidized by human and mouse microsomes (Lovern et al., 1997) that can spontaneously rearrange to phenol by NIH shift (Jerina & Daly, 1974). It can also form adducts with GSH, cysteine residues in proteins, and DNA (Bechtold et al., 1992a; McDonald et al., 1994; Henderson et al., 2005a; Míčová & Linhart, 2012). The reaction of benzene oxide with soluble thiols such as GSH and N-acetylcysteine, followed by dehydration, leads to the formation of *S*-phenylglutathione and SPMA, respectively, although this reaction is relatively inefficient relative to ring opening in the generation of phenol (Henderson et al., 2005a; Míčová & Linhart, 2012). Benzene oxide is a substrate for GSTs (Zarth et al., 2015) and has sufficient stability in blood (a half-life of

Fig. 4.1 Simplified metabolic scheme of benzene



Compiled by the Working Group from Glatt et al. (1989), Ross (2000), Snyder (2004), Waidyanatha et al. (2005), and Monks et al. (2010).
DHDD, dihydrodiol-dehydrogenase; EH, epoxide hydrolase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione-*S*-transferase; m.a.p., mercapturic acid pathway; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase 1; P450, cytochrome P450; ROS, reactive oxygen species.

~8 minutes) to reach extrahepatic target sites, as indicated by the production of benzene oxide-protein adducts as biomarkers in animals (McDonald et al., 1994). Benzene oxide-protein adducts have been found in the blood and bone marrow of mice and rats exposed to benzene (McDonald et al., 1994; Rappaport et al., 1996), and benzene oxide-haemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Yeowell-O'Connell et al., 1998, 2001; Rappaport et al., 2002; Lin et al., 2007). Benzene oxide can form 7-phenylguanine and other DNA adducts, although its reactivity has been reported to be low relative to its reactions with thiols (Míčová & Linhart, 2012). Recent work failed to detect 7-phenylguanine in DNA from liver, lung, or bone marrow in mice exposed to benzene (Zarth et al., 2014). Benzene dihydrodiol epoxide is another putative electrophilic metabolite. Its half-life at nearly physiological conditions (pH, 7.6) was greater than 5 hours (Waidyanatha & Rappaport, 2005), suggesting it can be distributed to target tissues distal from the initial site of generation.

(b) Muconaldehyde and other ring-opened products

Ring opening of benzene resulting in t,t-MA as a metabolic end-product (Parke & Williams, 1952), commonly used as a biomarker of benzene exposure (Carbonari et al., 2016; Section 1.3.1, occurs in vivo. In vitro or animal studies have identified potential metabolic intermediates in the production of t,t-MA. Latriano et al. (1986) identified muconaldehyde (t,t-MA dialdehyde) in mouse liver microsomes after incubation with benzene. Potential mechanisms of formation include ring opening of benzene oxide or oxepin by cytochrome P450 (CYP) or reactions mediated by oxygen radicals (Zhang et al., 1995; Golding et al., 2010). Muconaldehyde is a bifunctional aldehyde and a reactive electrophilic compound (Latriano et al., 1986; Witz et al., 1996) that reacts with thiols and nucleic acids (Latriano et al., 1989; Bleasdale et al., 1993; Henderson et al., 2005b; Monks et al., 2010; Harris et al., 2011). In studies in vitro, each aldehyde functionality in muconaldehyde can be reduced or oxidized to generate alcohol and/or aldehyde (6-hydroxy-hexa-2,4-dienoic acid) or acid and/or aldehyde (6-oxohexa-2,4-dienoic acid, muconic acid semi-aldehyde) derivatives that retain some of the electrophilicity of the parent dialdehyde but have greater diffusibility (Goon et al., 1992; Witz et al., 1996).

(c) Quinones and semiquinones derived from phenolic metabolites of benzene

The phenolic metabolites, major metabolites of benzene, have been shown to reach the bone marrow of mice and rats in free or conjugated forms (Rickert et al., 1979; Sabourin et al., 1988). In isolated mouse and human bone marrow cells, bi- or triphenolic metabolites could be oxidized to quinones via MPO-mediated reactions to form benzoquinones (Smith et al., 1989; Ross, 1996). Phenol can be metabolized by purified human MPO and horseradish peroxidase to generate 4–4′-diphenoquinone (Eastmond et al., 1986). Quinones are electrophilic compounds that can interact with thiols, proteins, and nucleic acids (Sadler et al., 1988; McDonald et al., 1994; Bodell et al., 1996; Monks et al., 2010). Both 1,2- and 1,4-benzoquinone protein adducts have been found in the blood and bone marrow of mice and rats exposed to benzene (McDonald et al., 1994), and 1,4-benzoquinone protein adducts have been detected in workers exposed to benzene (Rappaport et al., 2002; Lin et al., 2007). The importance of quinones in humans exposed to benzene was suggested by the observation that individuals carrying a homozygous null polymorphism for the quinone-metabolizing enzyme NQO1, and therefore lacking the ability to detoxify electrophilic quinones, were more susceptible to benzene toxicity (Rothman et al., 1997). MPO-catalysed oxidation of bi- or triphenolic metabolites of benzene occurs via

semiquinone radical intermediates, which may disproportionate to generate starting compounds together with their respective benzoquinones, or may react with oxygen to generate reactive oxygen species (ROS) (Sawada et al., 1975; Kalyanaraman et al., 1985, 1988; Sadler et al., 1988; Smith et al., 1989; Smith, 1996; Bolton et al., 2000). Hydroquinone induces chromosomal damage in human lymphocytes in vitro (Eastmond et al., 1994; Stillman et al., 1997). Oxidative damage to DNA in human leukaemia HL-60 cells was induced by hydroquinone, phenol, and benzene triol (Kolachana et al., 1993). Benzene triol was the only phenolic metabolite which resulted in oxidative DNA damage in mice when administered alone; however, combinations of phenol and hydroquinone, phenol and catechol, and hydroquinone and catechol were also effective (Kolachana et al., 1993). In vitro treatment of mouse bone marrow with hydroquinone produced the same DNA adducts as found after treatment of mice with benzene (Bodell et al., 1996).

GSH conjugation of quinones is considered a detoxification reaction, and multiple studies of null polymorphisms in GST-T1 and GST-M1 genes resulting in increased benzene toxicity in exposed human populations suggest that GSTs play an important role in the detoxification of reactive benzene metabolites (Wan et al., 2002). However, GST adducts of 1,4-benzoquinone are haematotoxic and have been demonstrated in the bone marrow of mice after administration of benzene (Bratton et al., 1997). GST conjugation of 1,4-benzoquinone primarily generates 2-(*S*-glutathionyl)hydroquinone, which can undergo sequential oxidation and GST conjugation to produce 2,3,5,6-tetra(*S*-glutathionyl) hydroquinone as the final diphenolic metabolite. This process occurs via the production of both semiquinone and electrophilic quinone derivatives, which can generate oxidative stress and adducts, respectively (Lau et al., 1988, 2010).

Combined exposure to phenol and hydroquinone reproduced the myelotoxicity of benzene in mice (Eastmond et al., 1987; Legathe et al., 1994) and rats (Lau et al., 1988, 2010). Experiments in vitro showed that phenol increased the oxidation of hydroquinone, catalysed by horseradish peroxidase, as well as the binding of radiolabelled hydroquinone to rat liver protein (Eastmond et al., 1987).

In summary, benzene metabolism can generate a multiplicity of metabolites, many of which are electrophilic.

4.1.2 Absorption, distribution, and elimination

(a) Humans

Benzene is well absorbed in humans by inhalation, or by the oral or dermal routes. Inhalation is the major route of human exposure, and is the only route for which extensive human data are available. In experiments on human subjects, values of respiratory uptake (lung retention) of 47–52% were found at exposure levels ranging from 1.6 to 62.0 ppm (Nomiyama & Nomiyama, 1974; Pekari et al., 1992). For absorption of benzene at 32–69 ppm from smoking cigarettes, a higher uptake of 64% was reported (Yu & Weisel, 1996).

Skin absorption of benzene has also been studied experimentally on human subjects. The absorption rate of liquid benzene by the skin (under conditions of complete saturation) was calculated to be approximately 0.4 mg/cm^2 per hour. The absorption rate was determined by the amount of urinary phenol excreted, which was not corrected for urinary phenol not derived from benzene (Hanke et al., 1961). In a series of showering experiments using water contaminated with benzene (367 µg/m^3) it was estimated that the total benzene dose resulting from a 20-minute shower was approximately 281 µg, about 40% of which was a result of inhalation

and the remaining 60% via skin absorption (Lindstrom et al., 1994).

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

After absorption, benzene is rapidly distributed throughout the human body (Winek et al., 1967; Winek & Collom, 1971; Pekari et al., 1992). Concentrations of benzene at 3.8 mg/L in blood, 13.8 mg/kg in the brain, and 2.6 mg/kg in the liver were reported in a young male worker who died suddenly from a short exposure to very high air concentrations of the chemical (Tauber, 1970).

Unmetabolized benzene is primarily excreted in exhaled air, but small amounts were found also in urine (Nomiyama & Nomiyama, 1974). In contrast, the main portion of the absorbed dose is excreted in the form of water-soluble metabolites in the urine. Human exposure to benzene in air at concentrations of 0.1–10.0 ppm results in urinary metabolite profiles of 70–85% phenol (free + conjugated), 5–10% each of hydroquinone (free + conjugated), catechol (free + conjugated), and t,t-MA, and less than 1% of SPMA (Kim et al., 2006a).

The profile of urinary metabolites may change depending on the level of exposure. When comparing workers occupationally exposed to benzene at more than 25 ppm with those exposed to benzene at less than 25 ppm, Rothman et al. (1998) observed that the ratios of phenol and catechol to total metabolites were significantly higher and the ratios of hydroquinone and t,t-MA to total metabolites were significantly lower in workers exposed to higher concentrations. Workers with less than 50 ng/g creatinine phenol in urine, corresponding to a concentration of benzene in air of approximately 10 ppm, were excluded from this analysis because phenol is not specific to exposure to benzene at lower concentrations. The differences in the ratio of each metabolite to total metabolites between the groups of higher and lower exposure were 0.65 versus 0.61 for phenol, 0.11 versus 0.09 for catechol, 0.13 versus 0.16 for hydroquinone, and 0.11 versus 0.15 for muconic acid. A controlled study of 4 cases exposed to isotopically labelled benzene at 40 ppb resulted in a urinary muconic acid concentration of 3.2–45.0% (Weisel et al., 2003).

Multiple studies have analysed and reanalysed data first reported in Kim et al. (2006a) to determine if, at lower levels of benzene exposure, a nonlinear relationship exists between exposure to benzene and urinary excretion of unmetabolized benzene, phenol, hydroquinone, catechol, muconic acid, and phenylmercapturic acid. The original data were from 389 workers in Tianjin, China, 250 of whom were from factories using benzene and 139 from factories not using benzene (Kim et al., 2006a, b, 2007). In their original analysis, Kim et al. (2006a, b) reported nonlinear relationships between benzene exposure and urinary metabolite concentrations, adjusted for background levels based on controls who had been exposed to benzene at less than 3 ppb. Specifically, the ratio of excreted metabolites to benzene exposure increased markedly at exposures to benzene at less than 1 ppm. The presence or absence of nonlinearity for exposures at less than 1 ppm in these data has been the subject of multiple commentaries and reanalyses (Price et al., 2012; Rappaport et al., 2013a, b; Cox et al., 2017; McNally et al., 2017).

[The Working Group noted that, overall, there are some data suggesting increased metabolism at low exposures, but the data are not definitive.]

(b) Experimental systems

Animal data confirm that benzene is efficiently absorbed by inhalation and by oral and dermal routes. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were given benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg body weight (bw) per

day (Sabourin et al., 1987). Low-temperature whole-body autoradiography of ^{14}C-benzene showed that benzene is rapidly distributed in the blood and in well-perfused organs such as the heart muscle, liver, and kidney. A very high level of radioactivity was also observed in the bone marrow, body fat, spinal cord, and white matter of the brain. The radioactivity was rapidly cleared from the central nervous system (Bergman & Appelgren, 1983). The bioavailability of benzene through the oral route was influenced by adsorption on soil. When radiolabelled benzene in soil (clay soil or sandy soil from New Jersey) was administered to rats by gavage, the area under the curve of plasma radioactivity versus time increased compared with that for benzene suspension in water, a difference that was significant with clay soil. The half-life in plasma was not affected by the type of soil used (Turkall et al., 1988). After exposing mice repeatedly to benzene, DNA adducts were detected by ^{32}P-postlabelling in peripheral blood, bone marrow, and liver. The adducts were still detectable in leukocytes 21 days after the last exposure (Li et al., 1996).

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), discussing whether: benzene induces oxidative stress; is genotoxic; alters DNA repair or causes genomic instability; is immunosuppressive; alters cell proliferation, cell death, or nutrient supply; modulates receptor-mediated effects; induces chronic inflammation; and induces epigenetic effects.

In the consideration of mechanistic studies in exposed humans, the Working Group focused on studies in which the following issues were reasonably addressed in their design and/or analysis: definition and comparability of control groups, statistical power, and confounding by relevant covariates or co-exposures. An additional issue was whether bias due to disease (i.e. a diagnosis of benzene poisoning) was avoided.

4.2.1 Oxidative stress

Several potential mechanisms may contribute to benzene-induced oxidative stress. Benzene can produce a multiplicity of electrophilic and pro-oxidant metabolites capable of depleting cellular-reduced GSH (see Section 4.1), a critical defence system against oxygen radicals. Hydroquinones can generally autoxidize in pH-dependent reactions to produce hydrogen peroxide (Song & Buettner, 2010). Polyphenolic metabolites of benzene can generate semiquinone radicals during peroxidase-mediated oxidation (Yamazaki et al., 1960; Yamazaki & Piette, 1963; Kalyanaraman et al., 1991). The primary fate of 1,2- and 1,4-benzosemiquinone radicals is disproportionation to quinone and hydroquinone, although reaction with oxygen can occur in the presence of superoxide dismutase (Sawada et al., 1975; Sadler et al., 1988; Subrahmanyam et al., 1991). The semiquinone derived from 1,2,4-benzenetriol has been shown to react with oxygen (Kalyanaraman et al., 1988), and phenoxy radicals generated during peroxidase-mediated oxidation of phenol can react with GST leading to the generation of thiyl radicals and subsequent production of oxidized GST (Subrahmanyam & O'Brien, 1985; Sadler et al., 1988). Multiglutathione adducts of 1,4-benzoquinone generated by successive cycles of hydroquinone oxidation and glutathione addition retain the capability to generate active oxygen species and oxidative stress (Lau et al., 1988, 2010). Metals such as copper (II) and iron (III) may facilitate the production of reactive and oxidizing species, capable of damaging DNA and inducing lipid peroxidation, from hydroquinone and catechol (Kasai & Nishimura, 1984; Rao & Pandya, 1989; Li et al., 1995).

(a) Humans

Oxidative damage to DNA as indicated by 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels has been commonly used as an indicator of oxidative stress. Examples of studies indicating oxidative damage to DNA in occupationally exposed workers or in environmentally exposed urban populations, where DNA damage was correlated with benzene exposure levels and/or metabolic biomarkers of benzene exposure, are listed in Table 4.1. Four studies are further discussed in Section 4.2.3.

Decreases in GST levels, decreased superoxide dismutase, increased lipid peroxidation, and increased ROS in the blood were detected in 428 gasoline filling station workers compared with 78 unexposed controls (Uzma et al., 2010). The mean benzene exposure over the 12-hour study period in gasoline station attendants was 0.35 ppm (0.12–0.53 ppm). Individual exposure via air sampling was not monitored in controls, but the concentration of benzene in both pre- and post-shift urine and blood in gasoline station attendants was significantly higher than in controls. Both blood and urine benzene concentrations were increased in post-shift samples compared with pre-shift values. Significant correlations were observed between concentrations of benzene in the blood and changes in GST, superoxide dismutase, lipid peroxidation, and ROS (Uzma et al., 2010).

Exposure to benzene in 43 gasoline station attendants significantly increased DNA damage compared with 28 non-exposed individuals, as indicated by comet assay and micronuclei (MN) induction, increased oxidative protein damage, and decreased antioxidant capacity, including decreased GST levels (Moro et al., 2013; see also Table 4.2). Gasoline station attendants were exposed to median benzene values of 76.2 μg/m^3 compared with 42.0 μg/m^3 in controls, and median levels of t,t-MA were increased 4.4-fold in gasoline station attendants compared with the control group (Moro et al., 2013). Personal benzene exposure and urinary muconic acid levels were directly correlated with increases in oxidative protein damage and decreases in antioxidant capacity (Moro et al., 2013).

Two cross-sectional studies evaluated the relationship between benzene exposure and mitochondrial DNA (mtDNA) copy number. Compared with controls without occupational exposure to benzene, one study reported higher mtDNA levels in leukocyte DNA from highly exposed (arithmetic mean, 14 ppm) workers in China (Shen et al., 2008). A second study reported that benzene was associated with increased mtDNA among workers exposed to relatively low levels of benzene in Italy (geometric mean, 21.5 ppb among a group exposed to the highest concentrations) (Carugno et al., 2012).

(b) Human cells in vitro

Benzene metabolites hydroquinone, benzenetriol, and benzoquinone produced ROS in HL-60 cells and enhanced myeloid cell growth (Wiemels & Smith, 1999). A global proteomic analysis in cells after the addition of benzene revealed an enrichment of proteins involved in oxidative stress response (Murugesan et al., 2013). Oxidative DNA damage, indicated by the presence of 8-OHdG, has been detected in several studies of human cells in vitro after treatment with benzene metabolites; these are summarized in Section 4.2.2. Both GST and NQO1, which maintain reactive quinones in their reduced form and can also function as a superoxide reductase (Siegel et al., 2004), are important determinants of hydroquinone-induced toxicity in bone marrow cells (Ross et al., 1996a, b; Trush et al., 1996). The addition of benzene to human myeloid cells led to the production of ROS, an effect suggested to be due to benzene alone (Nishikawa et al., 2011).

Table 4.1 Oxidative damage to DNA in humans exposed to benzene

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
(a) Occupational studies								
8-OHdG Positive Significant correlation $P < 0.01$ between benzene exposure and 8-OHdG (r, 0.34)	65 filling station attendants	Average annual concentration used as a measure of exposure: 0.06–5.85 mg/m^3 (AM ± SD, 0.45 ± 0.96 mg/m^3)	Estimated on the basis of seven repeated personal air samples taken at worksite over 1 yr and questionnaire on personal habits	Not used	Urine	Methyl-benzenes	Increased 8-OHdG with increasing benzene exposure; not related to toluene or xylenes	Lagorio et al. (1994)
8-OHdG Positive $P < 0.05$ in medium- and high-dose groups vs controls; not significant in low-dose group	Three exposed groups and controls: low exposure (35 shoe factory workers); medium exposure (24 paint workers in car factory); high exposure (28 shoe factory workers) Controls: 30 university staff	Mean ± SD: low (n = 35), 2.5 ± 2.4 mg/m^3; medium (n = 24), 103.3 ± 50.3 mg/m^3; high (n = 28), 424.4 ± 181.7 mg/m^3	Personal air sampler	Urinary t,t-MA	Blood lymphocytes	Toluene	8-OHdG significantly correlated with both external and internal measures of benzene; dose–response; higher in women than men exposed to same benzene level; negative correlation of 8-OHdG with toluene levels	Liu et al. (1996)
8-OHdG Positive $P < 0.015$ for coefficient different from 0; adjusted for smoking	Total of 33 men occupationally exposed to gasoline (16 auto mechanics, 14 refinery workers, and 3 gasoline pump repairmen) and 33 male non-occupationally exposed controls	Mean 8-h TWA in workers, 0.13 ppm (range, 0.003–0.6 ppm) Mean exposure of controls 0.002 ppm (range, 0.001–0.003 ppm)	Personal air sampling	Not used	Urine	NR	Late evening/next morning 8-OHdG significant ($P < 0.002$ and $P < 0.02$) relative to pre-shift; no control samples analysed late evening/next morning	Nilsson et al. (1996)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
Urinary markers of nucleic acid oxidation Positive $P < 0.0001$	239 traffic policemen, taxi drivers, and gasoline pump attendants in Parma, Italy		Urine	Urinary t,t-MA, SPMA	Urine	Cotinine	Significant correlation between urinary metabolites of benzene (t,t-MA and SPMA) and DNA and RNA oxidation products	Manini et al. (2010)
8-OHdG Positive $P < 0.001$	31 gasoline service station attendants, 31 petrochemical laboratory workers, and 40 temple workers exposed to incense; controls from an office site with no incense burning in Bangkok, Thailand	Individual benzene exposure (mean ± SE): gasoline service station attendants, 360.9 ± 44.7 μg/m^3; petrochemical laboratory workers, 78.3 ± 18.7 μg/m^3; controls, 4.5 ± 0.5 μg/m^3	Area and personal air sampling	Not used	Leukocytes	1,3-butadiene, PAHs	For occupational exposures, gasoline service station attendants had significantly higher 8-OHdG ($P < 0.001$) than controls; 8-OHdG responding to increasing concentrations of benzene exposure; low to non-detectable 1,3-butadiene and PAHs in gasoline service station attendants and petrochemical laboratory workers	Ruchirawat et al. (2010)
8-OHdG Positive $P < 0.05$	43 gas station attendants, 34 taxi drivers, and 22 controls with no occupational exposure, Rio Grande do Sul, Brazil	NR	NR	Urinary t,t-MA	Urine	Carboxy-haemoglobin	Increased 8-OHdG ($P < 0.05$) in gas station attendants and taxi drivers relative to controls; t,t-MA not measured in taxi drivers	Göethel et al. (2014)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
8-OHdG Negative	18 fuel tanker drivers, 13 filling station attendants, and 20 non-occupationally exposed controls in Bari, Italy	Benzene exposure, mean ± SD: drivers, 279.9 ± 248.6 μg/m³; attendants, 19.9 ± 15.5 μg/m³; controls, 4.7 ± 3.0 μg/m³	Personal air sampling	Urinary t,t-MA, SPMA, benzene	Lymphocytes	NR	No increase in 8-OHdG in benzene-exposed groups relative to controls	Lovreglio et al. (2016)
(b) Exposed urban populations								
8-OHdG Positive (see comments)	40 individuals living and working in Copenhagen	Median (range) benzene exposure, 2.5 (1.9–3.6) μg/m³	Personal air sampling	Urinary t,t-MA, SPMA	Lymphocytes and urine	Toluene, MTBE	Significant correlation between 8-OHdG in lymphocytes (but not urine) and SPMA excretion ($P < 0.04$); no correlation of 8-OHdG with external benzene, toluene or MTBE levels; external benzene was a 5-d cumulative measure while internal markers were measured for only 1 of those days	Sørensen et al. (2003)
8-OHdG Positive (see comments)	Taxi-moto drivers, city residents, and village residents in Cotonou, Benin	Personal benzene exposure, mean ± SD: taxi-moto drivers, 76.0 ± 26.8 μg/m³; village residents, 3.4 ± 3.0 μg/m³	Personal air sampling	Urinary SPMA, benzene	Lymphocytes	Toluene, xylenes	Significantly higher 8-OHdG in taxi-moto drivers than village residents ($P < 0.05$)	Ayi-Fanou et al. (2006)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
8-OHdG Positive (see comments)	109 urban and 62 rural schoolboys in Bangkok, Thailand	Average benzene exposure: 5.50 ± 0.40 ppb in urban vs 2.54 ± 0.23 ppb in rural group	Area and personal air sampling	Urinary t,t-MA, SPMA, benzene	Leukocytes and urine	NR	Level of 8-OHdG in leukocytes was threefold higher in urban vs rural schoolchildren ($P < 0.001$) and was significantly associated with benzene exposure level ($P < 0.05$); urinary 8-OHdG significantly higher in urban vs rural schoolchildren ($P < 0.05$) but no correlation with benzene exposure levels	Buthbumrung et al. (2008)
8-OHdG PAHs were the major contributor to 8-OHdG levels (see comments)	165 city centre and 111 rural schoolchildren in Bangkok, Thailand	Mean ± SE benzene exposure: city, 19.38 ± 1.11 μg/m^3 vs rural, 8.40 ± 0.61 μg/m^3	Area and personal air sampling	Not used	Leukocytes	1,3-butadiene, PAHs	Levels of 8-OHdG were higher in city vs rural schoolchildren ($P < 0.001$); 8-OHdG levels correlated significantly with benzene ($P < 0.001$) and PAH ($P < 0.001$) levels; multivariate analysis identified PAH concentrations as the only factor significantly affecting 8-OHdG levels (r, 0.895; $P < 0.05$)	Ruchirawat et al. (2010)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
8-OHdG, 8-oxoGuo Positive (see comments)	396 children from central Italy districts with different levels of urbanization and air pollution		Urinary t,t-MA, SPMA, benzene	Urinary t,t-MA, SPMA, benzene	Urine	Cotinine	Multiple linear regression ($P < 0.0001$) indicated that benzene exposure (assessed by urinary SPMA and TTMA) was significantly associated with 8-OHdG and 8-oxoGuo	Andreoli et al. (2012)
8-OHdG, 8-oxoGuo, 8-oxoGua Positive (see comments)	155 children living close (< 15 km) to an oil refinery, 58 children living 70 km from the refinery and not close to an industrial hub		Urinary t,t-MA, SPMA, benzene	Urinary t,t-MA, SPMA, benzene	Urine	Cotinine, MTBE	8-OHdG and 8-oxoGuo significantly correlated with markers of benzene exposure ($P < 0.01$)	Andreoli et al. (2015)

8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxoGua, 8-oxo-guanine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; AM, arithmetic mean; d, day(s); h, hour(s); MTBE, methyl tertiary-butyl ether; NR, not reported; PAHs, polycyclic aromatic hydrocarbons; ppm, parts per million; ppb, parts per billion; SD, standard deviation; SE, standard error; SPMA, *S*-phenylmercapturic acid; t,t-MA, *trans*,*trans*-muconic acid; TWA, time-weighted average; vs, versus; yr, year(s)

Table 4.2 DNA strand breaks, adducts and mutations in humans exposed to benzene

End-point Test system	Tissue, cell type	Description of exposed and controls	Results[a]	Agent Concentration (LEC or HIC)	Comments	Reference
DNA damage/strand breaks DNA elution rate through filters	Blood, peripheral lymphocytes	20 female shoemakers, 12 of which had an additional dermal exposure, 20 matched controls	+ $P < 0.001$	Benzene and toluene 4.16 ± 4.15 µg/m³	No evidence for contribution of skin absorption to the effect	Popp et al. (1992)
DNA damage/strand breaks	Blood, the pellet from centrifuged blood	33 men occupationally exposed to gasoline, 3 unexposed controls (smokers and non-smokers)	± $P = 0.2$	Benzene, VOC from gasoline HIC, 0.6 ppm	No increase overall, but in some benzene exposure subgroups	Nilsson et al. (1996)
Mutation/other NN GPA variant cell frequency	Blood, erythrocytes	44 benzene-exposed workers (23 men and 21 women), 44 matched controls	+ $P < 0.05$ (trend)	Benzene 100 ppm-yr		Rothman et al. (1996)
DNA damage/strand breaks Comet assay, tail moment, heavily damaged cell number	Blood, peripheral lymphocytes	12 gasoline station attendants, unspecified matched controls	+ $P = 0.028$	Benzene, gasoline vapours 0.3 ppm (8h TWA)		Andreoli et al. (1997)
DNA damage/strand breaks Comet assay, percentage of damaged cells	Blood, peripheral lymphocytes	83 exposed workers (29 low exposure, 29 high exposure, 25 benzene poisonings), 29 controls	+	Benzene HIC < 300 mg/m³		Wu et al. (1998)
Mutation/oncogene K-ras mutation	Tumour tissue, exocrine cancer of the pancreas, null	107 patients with exocrine cancer of the pancreas, 83 K-ras mutated and 24 K-ras wildtype; among these, 16 cases were previously exposed to benzene	+ $P < 0.05$	Benzene, possibly other solvents, exposure estimated retrospectively (high, low, none)	Significant for men after the only women exposed to benzene were excluded (OR, 7.07; $P < 0.05$)	Alguacil et al. (2002)
DNA damage/strand breaks Alkaline comet assay, comet tail	Blood, peripheral lymphocytes	133 traffic control policemen exposed to traffic emissions, 59 office policemen	–	Benzene, VOC from traffic HIC 9.5 µg/m³, 7-h TWA		Carere et al. (2002)
DNA damage/strand breaks	Blood, peripheral lymphocytes	158 petrochemical workers, 50 matched controls	+ *P* NR	Benzene 1.75 ± 3.6 ppm (inhalation)	NQO1 deficiency correlated with twofold higher SSBs	Garte et al. (2005)

Table 4.2 (continued)

End-point Test system	Tissue, cell type	Description of exposed and controls	Results[a]	Agent Concentration (LEC or HIC)	Comments	Reference
DNA damage/strand breaks Comet tail moment	Blood, peripheral lymphocytes	22 clothes vendors, 21 grilled meat vendors, 29 gasoline station attendants, 23 factory workers, 27 controls Schoolchildren: 41 from Bangkok and 30 provincial	+ $P < 0.05$	Benzene, VOCs 5.5 ± 0.4 ppb	DNA repair capacity was also altered in exposed subjects	Navasumrit et al. (2005)
DNA damage/adducts Comet assay, olive tail moment	Blood, peripheral lymphocytes	41 workers from six plants: printing, shoemaking, production of nitrobenzene, benzene, methylene dianiline and carbomer	+ $P = 0.001$	Benzene, different solvents ND		Sul et al. (2005)
DNA damage/adducts ^{32}P-postlabelling, bulky adducts	Blood, peripheral lymphocytes	34 taxi-moto drivers from Cotonou, 6 controls from a nearby village	+ $P < 0.05$	Complex mixture containing benzene 76.0 ± 26.8 μg/m^3	8-oxo-dG and m^{5}dC levels were also elevated in the exposed group	Ayi Fanou et al. (2006)
DNA damage/adducts Comet assay, olive tail moment	Blood, peripheral lymphocytes	115 schoolchildren from heavy traffic area in Bangkok, 69 controls from a rural area	+ $P < 0.001$	Benzene, PAHs 17.55 ± 1.29 μg/m^3		Ruchirawat et al. (2007)
DNA damage/strand breaks SSB by alkaline elution method	Blood, peripheral lymphocytes	158 petrochemical workers, 50 matched controls	+ P NR	Benzene 1.75 ± 3.6 ppm, long-term	NQO1 deficiency correlated with a twofold higher and GSTT1 gene deletion in a 35–40% higher SSBs	Garte et al. (2008)
DNA damage/strand breaks Comet assay, tail intensity	Blood, peripheral lymphocytes	20 petrol station attendants (11 smokers), 20 matched controls (11 smokers)	+ $P < 0.05$	Benzene and other petrol VOCs 0.65 ± 0.47 mg/m^3		Keretetse et al. (2008)
DNA damage/strand breaks Comet tail length, tail moment	Blood, peripheral lymphocytes	33 petrochemical industry operators, 28 service station attendants, 21 gasoline pump maintenance workers, 51 non-exposed controls	+ $P < 0.008$	Benzene, VOCs 40 μg/m^3		Fracasso et al. (2010)
DNA damage/adducts Total DNA adducts	Blood, peripheral lymphocytes	57 healthy inhabitants of Cotonou (high exposure), 20 suburbans (low exposure), 17 villagers (control)	+ $P < 0.001$	Benzene, PAHs 76 ± 26.8 μg/m^3	Adducts mainly from exposure to PAHs rather than benzene	Ayi-Fanou et al. (2011)

Table 4.2 (continued)

End-point Test system	Tissue, cell type	Description of exposed and controls	Results[a]	Agent Concentration (LEC or HIC)	Comments	Reference
DNA damage/strand breaks Comet assay, mean tail DNA (%) and tail moment	Blood, peripheral lymphocytes	324 USA Air Force personnel maintenance workers (high), service workers (moderate), others (low exposure)	±	Jet fuel JP-8 ND	Positive correlation between both pre-shift benzene concentrations in breath and the mean tail DNA (%) and tail moment ($P < 0.05$)	Krieg et al. (2012)
DNA damage/strand breaks Comet assay, DNA damage index	Blood peripheral lymphocytes	43 gasoline station attendants with 9.1 ± 1.1 yr of exposure, 28 controls matched by age, all non-smoking	+ $P < 0.001$	Benzene, VOC 76.20 (54.34–1285.48) μg/m^3		Moro et al. (2013)
DNA damage/other Comet assay, tail intensity	Blood, peripheral lymphocytes	18 fuel tanker drivers, 13 filling station attendants, 20 controls with no occupational exposure to benzene	–	Benzene, VOCs, fuel HIC, 280 ± 249 μg/m^3	Smokers and non-smokers in all groups	Lovreglio et al. (2014)

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study)

8-oxo-dG, 8-oxo-2′-deoxyguanosine; HIC, highest ineffective concentration; LEC, lowest effective concentration; m^5 dC, methylated deoxycytosine; ND, not determined; NQO1, NAD(P)H:quinone oxidoreductase 1; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; ppb, parts per billion; ppm, parts per million; SSB, single-strand break; TWA, time-weighted average; VOC, volatile organic compounds; yr, year(s)

(c) Experimental systems

In rodents, benzene increased oxygen radical generation and lipid peroxidation (Verma & Rana, 2004). The exposure of mice to benzene via inhalation increased lipid peroxidation and DNA damage in bone marrow (Yu et al., 2014). The activation of bone marrow phagocytes after the administration of benzene to mice led to increased oxidative stress, nitric oxide generation, and protein-bound 3-nitrotyrosine in bone marrow (Laskin et al., 1989; Chen et al., 2005; Melikian et al., 2008). Oxidative stress has also been implicated in several effects induced by benzene metabolites in animal cells, including homologous recombination (Winn, 2003), DNA damage and recombination (Tung et al., 2012), and altered c-Myb transcriptional activity (Wan & Winn, 2007). Attenuation of oxidative stress in mice by transgenic overexpression of thioredoxin reductase decreased clastogenic effects and completely suppressed lymphoma of the thymus gland induced by benzene inhalation (Li et al., 2006).

4.2.2 Genetic and related effects

(a) Oxidative damage to DNA

(i) Humans

Representative studies examining oxidative damage to DNA in either occupationally exposed workers or in environmentally exposed urban populations, as measured by the production of oxidized DNA bases, are described in Table 4.1. Oxidative damage to DNA was detected primarily using the oxidation of guanine residues, and correlated with benzene exposure levels and/or metabolic biomarkers of benzene exposure.

In several occupational studies in Table 4.1 that were the focus of the Working Group's review, discussed in more detail below, significant effects of benzene exposure on oxidative damage to DNA, as indicated by increased 8-OHdG levels or other oxidized DNA bases, were observed. Dose–response relationships between exposure to benzene and 8-OHdG levels were suggested by Lagorio et al. (1994), Liu et al. (1996), and Ruchirawat et al. (2010).

Lagorio et al. (1994) examined urinary 8-OHdG in a random sample of 65 filling station attendants, and both benzene and methylbenzene exposure levels were calculated from seven personal air samples taken over 1 year. No control group was used in this study. A significant correlation was found between urinary 8-OHdG levels and exposure to benzene ($r = 0.34$; $P < 0.01$).

Liu et al. (1996) demonstrated a dose–response increase in oxidative damage to DNA associated with both external and internal measures of benzene exposure. Lymphocyte 8-OHdG was assessed in blood samples of 87 benzene-exposed workers from shoemaking and car-painting factories and 30 controls from a university staff. Workers from different factories were exposed to different concentrations of benzene; median levels in environments considered to have low, medium, and high concentrations of benzene in air were measured as 2.5, 103.3, and 424.4 mg/m^3, respectively.

Manini et al. (2010) measured urinary nucleic acid oxidation in 239 traffic policemen, taxi drivers, and gasoline pump attendants in Parma, Italy. A separate control group was not used. Urinary t,t-MA and SPMA were used as internal markers of benzene exposure. Multiple linear regression analyses showed that benzene exposure was associated with oxidative damage to DNA, particularly RNA as indicated by the production of 8-oxo,7,8-dihydroguanosine (8-oxoGuo). The modulating effects of NQO1 and GST polymorphisms on DNA damage and SPMA excretion, respectively, were reported.

Ruchirawat et al. (2010) performed a study of oxidative damage to DNA in multiple populations in Thailand, either primarily exposed to benzene (31 petrochemical laboratory workers and 31 gasoline service station attendants) or

other pollutants (165 city centre and 111 rural schoolchildren, and 40 temple workers exposed to incense). Individuals from an office building with no incense burning were used as a control population. The group exposed to the highest concentrations of benzene (gas station attendants; mean benzene exposure, 360.9 μg/m^3) had higher leukocyte 8-OHdG than control subjects, and both petrochemical laboratory workers (benzene exposure levels, 78.3 μg/m^3) and gas station attendants had significantly higher single-strand break levels and DNA repair capacity.

(ii) Human cells in vitro

Several studies have demonstrated that phenolic metabolites of benzene can induce oxidative damage to DNA, as indicated by 8-OHdG formation in cellular systems in vitro. Zhang et al. (1993) demonstrated that 1,2,4-benzenetriol induced 8-OHdG and MN formation in the human leukaemia HL-60 cell line. This and other benzene metabolites (phenol, hydroquinone, catechol) were shown to induce 8-OHdG formation in HL-60 cells (Kolachana et al., 1993). Catechol was shown to induce 8-OHdG in HL-60 cells (Oikawa et al., 2001). Hydroquinone induced DNA strand breaks, DNA–protein cross-links, and 8-OHdG formation in human hepatoma HepG2 cells (Luo et al., 2008), and single-strand breaks, chromosomal aberrations (CAs), and 8-OHdG formation in A549 human lung cancer cells (Peng et al., 2013).

(iii) Experimental systems

Increased 8-OHdG formation was also observed in mouse bone marrow 1 hour after administration of benzene or 1,2,4-benzenetriol (Kolachana et al., 1993). Although phenol, catechol, and hydroquinone were without effect when administered separately, binary combinations of phenol, catechol, and hydroquinone induced 8-OHdG formation in mouse bone marrow when administered together (Kolachana et al., 1993).

(b) DNA binding, DNA strand breaks, and gene mutations

In several occupational studies of DNA binding, strand breaks, and mutations that were the focus of the Working Group's review, discussed in more detail below, the observed effects could be reasonably attributed to benzene (Popp et al., 1992; Rothman et al., 1995, 1996; Wu et al., 1998; Table 4.2). The study of Rothman et al. (1995, 1996) showed an apparent dose–response relationship. In this study, glycophorin A gene (*GPA*) loss and subsequent duplication of its NN allele in MN-heterozygous was demonstrated in subjects with long-term occupational exposure to benzene; cumulative exposures ranged from 8 to 3488 ppm-years. The NN *GPA* variant cell frequency was 13.9 ± 1.7 per million cells in workers exposed to benzene versus 7.4 ± 1.1 per million cells in control individuals. In contrast, no significant difference existed between the two groups for the null allele frequency. In this study, benzene produced gene-duplicating mutations, but did not produce gene-inactivating mutations at the *GPA* locus in bone marrow cells of humans.

There are no reports on benzene-specific DNA adducts in exposed humans or in human cells treated with benzene in vitro. However, DNA adducts were detected in human promyelocytic HL-60 cells treated with a mixture of benzene metabolites, hydroquinone and 1,4-benzoquinone, by ^{32}P-postlabelling (Levay et al., 1991). The adduct formed after treatment of the HL-60 cells with either benzoquinone or hydroquinone was later identified as N^2-(4-hydroxyphenyl)-2′-deoxyguanosine-3′-phosphate (Pongracz & Bodell, 1996). Furthermore, two depurinating adducts, namely 7-(3,4-dihydroxyphenyl)-2′-deoxyguanosine and 3-(3,4-dihydroxyphenyl) adenine, were identified in cultured human blood mononuclear cells (Chakravarti et al., 2006).

In human lymphocytes treated in vitro, benzene and its metabolites hydroquinone, catechol, 1,2,4-benzotriol, and 1,4-benzoquinone

(but not t,t-MA) significantly increased DNA damage (single-strand breaks) in the comet assay (Anderson et al., 1995). In HeLa cells treated with hydroquinone, the alkaline comet assay also showed significant DNA damage compared with untreated cells (Galván et al., 2008). In vitro studies on human cells are also listed in Table 4.3.

(c) *Chromosomal damage and cytogenetic effects*

(i) *Humans*

Chromosomal damage

Chromosomal end-points, including sister-chromatid exchanges (SCEs), MN, and CAs, have typically been examined in peripheral blood lymphocytes (PBLs), although some of the benzene studies examined buccal cells, sperm, or other blood cells, such as granulocytes. Two of these end-points, CAs and MN, have been found to be associated with increased cancer risk in humans in large, prospective studies (Liou et al., 1999; Hagmar et al., 2004; Boffetta et al., 2007; Bonassi et al., 2007). The third end-point, SCEs, is an indicator of effects on DNA; however, the relevance of SCEs to cancer risk is uncertain (Norppa et al., 2006), and the data for benzene in exposed humans are inconclusive (see Table 4.4).

Studies that examined structural CAs in humans occupationally exposed to benzene are summarized in Table 4.4. Of this range of occupations, most show increases in CAs associated with jobs involving exposure to benzene, including some large studies (> 100 exposed workers) with significant positive exposure–response relationships (e.g. Kim et al., 2004a; Rekhadevi et al., 2011).

Similarly, although fewer in number, the majority of studies of MN in humans exposed to benzene show increases in MN frequencies associated with jobs involving benzene exposure (see Table 4.4). In addition, several large studies have reported significant positive exposure–response relationships (e.g. Kim et al., 2008; Rekhadevi et al., 2011 in buccal cells; Zhang et al., 2014).

Aneuploidy was reported in other studies of humans occupationally exposed to benzene that examined specific chromosomes, generally in lymphocytes or sperm (e.g. Kim et al., 2004b; Xing et al., 2010 and in studies of human cells in vitro; see Table 4.4, Table 4.5 and Table 4.6). Ji et al. (2012) compared aneuploidy results between lymphocytes and sperm in their study population and across other studies, reporting the induction of aneuploidy in different chromosomes in different cell types.

Over 20 studies of human cells in vitro are available, primarily using PBLs although some have used other lymphohaematopoietic cells or cell lines (see Table 4.5). In vitro studies using benzene without metabolic activation have been inconsistent for chromosomal end-points; however, the few that used S-9 to activate benzene metabolism were uniformly positive. In vitro studies directly assessing the benzene metabolites phenol, hydroquinone, benzoquinone, catechol, and benzenetriol have been consistently positive for the various chromosomal end-points examined, including SCEs (e.g. Morimoto & Wolff, 1980; Yager et al., 1990). In the study of Erexson et al. (1985), catechol exhibited greater potency in inducing SCEs than benzoquinone, hydroquinone, and benzenetriol, which were in turn more potent than phenol, which was in turn more potent than benzene.

Specific cytogenetic effects

Some studies have examined specific cytogenetic changes in humans exposed to benzene to investigate the mechanisms of benzene carcinogenesis (see Table 4.6). The cytogenetic changes include alteration of the number of specific chromosomes, loss of particular regions of certain chromosomes, and acquisition of specific translocations.

Among the variety of cytogenetic changes, significant exposure-related trends for –5, –7,

Table 4.3 DNA damage in human cells in vitro

End-point Test system	Tissue, cell line	Results[a]		Agent	Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation				
DNA adducts ^{32}P-postlabelling	Myeloid leukaemia (HL-60)	+	NT	Benzene metabolites (HQ, 1,4-BQ)	HQ, 50 µM per 8 h; 1,4-BQ, 25 µM per 2 h		Levay et al. (1991)
DNA strand breaks Comet assay	Lymphocytes	+	–	Benzene	12 mM/h		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	+	+	1,2,4-BT	100 µM per 30 min, $P < 0.05$		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	+ NT	– +	HQ CAT	200 µM/h 1 mM per 2 h		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	– NT	– +	1,4-BQ 1,4-BQ	200 µM/h 0.5 mM per 4 h		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	–	–	t,t-MA	800 µM/h		Anderson et al. (1995)
DNA strand breaks Comet assay, tail moment	Non-proliferating peripheral lymphocytes	+		Benzene metabolites (HQ, BQ, BT)	LEC: HQ, 0.5 µg/mL; BQ, 0.3 µg/mL; BT, 5.0 µg/mL		Andreoli et al. (1997)
DNA adducts Tandem mass spectrometry	Blood mononuclear cells	+	NT	Benzene metabolite (1,2-BQ)	75 µM		Chakravarti et al. (2006)
DNA strand breaks Comet assay, tail moment	HeLa cells	+		HQ	150 µM per 12 h	WRN depletion increased DNA damage (SSBs by comet assay)	Galván et al. (2008)

[a] +, positive; –, negative

BQ, benzoquinone; BT, benzenetriol; h, hour(s); HIC, highest ineffective concentration; HL, human leukaemia; HQ, hydroquinone; LEC, lowest effective concentration; NT, not tested; SSB, single-strand break; t,t-MA, *trans*,*trans*-muconic acid

Table 4.4 Chromosomal damage in humans exposed to benzene

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
20 (of 38) workers using benzene as solvent, 5 industrial controls from other areas of plant	1–20	NR (mean, ~25–150 in high-exposure area; Tough et al., 1970)	+	NT	NT	Blood samples 2–3 yr after benzene substituted with toluene	Tough & Brown (1965)
Factory workers (solvent) and onsite controls G1: 20 exposed, 5 controls (from Tough & Brown, 1965) G2: 12 exposed, 6 controls G3: 20 exposed, 5 controls Controls listed above from other areas of plant; 8 general-population controls	G2: 6–25 G3: 2–26	G1: ~25–150 in high-exposure area G2: similar to G1 G3: ~12	G1: see above G2: +/– G3: –	NT	NT	G2: blood samples almost 4 yr after benzene; G3: exposure up to time of study G2: + vs general population; – vs onsite controls; onsite controls also had some exposure, although earlier in time	Tough et al. (1970)
52 workers exposed to benzene, 44 pre-employment control group	0.1–26	2.1 (TWA)	+	NT	NT	+ for chromosome breaks (deletions) and other CAs (rings, dicentrics, translocations, exchanges); – for chromatid breaks	Picciano (1979)
22 benzene production workers, 22 controls from metallurgical factory	11.4 (mean)	0.2–12.4 (8-h TWA)	+	–	NT	+ for chromosome-type CAs; (+) for total CAs; – for chromatid-type	Sarto et al. (1984)
16 non-smoking female benzene-exposed worker, 7 controls	NR	3–50	NT	–	NT		Watanabe & Endo (1984)
66 ethylbenzene production workers, 20 general-population controls of same social position, etc. as exposed	3–18	0.47–11.7 (8-h TWA)	(+)	+	NT		Jablonická et al. (1987)
66 refinery workers, 33 controls SCE: 28 refinery workers, 23 controls	NR	< 1–10 (TWA)	NT	(+)	NT		Yardley-Jones et al. (1988)
33 workers exposed to benzene, 15 general-population controls	10–23	< 31.3	+	NT	NT	+ for CAs	Sasiadek et al. (1989)
36 non-smoking female shoemakers, 11 factory worker controls	5.5 (mean)	3–210 (GM, 54) (one 8-h shift)	NT	–	NT		Seiji et al. (1990)
66 refinery workers, 33 controls CAs: 48 refinery workers, 29 controls	> 5	1–10 (TWA)	+	NT	NT		Yardley-Jones et al. (1990)

Table 4.4 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
20 female shoemakers, 20 general-population controls	3–NR (mean, 18)	0.25–5.0 (mean, 1.3) (one 8-h shift)	NT	+	NT		Popp et al. (1992)
56 workers in plants with other exposures, 20 controls	10–20 (not clearly reported) (average, 6 h/d as solvent)	< 10	+	NT	NT		Sasiadek (1992)
42 benzene distillers at oil refinery, 42 controls	2–NR	0.3–15 (mean, 2.3)	+	+	NT		Major et al. (1994)
49 oil refinery workers, 91 historical controls, 122 industrial controls	0–2 (n = 10) 2–10 (n = 22) > 10 (n = 17)	0.9–21.5 in 1990 (start of study) 0.3–5.8 by 1992	+	+	NT	+ in both chromatid- and chromosome-type CAs and achromatic lesions (gaps); followed from 1990 to 1992, during which exposures decreased along with CAs (excluding gaps) and gaps only, but not SCEs	Tompa et al. (1994)
38 high-exposure and 45 low-exposure female shoemakers, 35 worker controls	High exposure, 2–31 (mean, 13.4) Low exposure, 1–33 (mean, 17.7)	High exposure, 2–15 (mean, 8) Low exposure, 2–13 (mean, 5)	+	+	NT		Karacić et al. (1995)
35 low-benzene (high-toluene) shoeworkers, 24 medium-benzene car painters, 28 high-benzene (low-toluene) shoeworkers, 30 university staff controls	NR	Low, 0.77 (mean) Medium, 32 (mean) High, 133 (mean) (8-h TWA)	NT	NT	+		Liu et al. (1996)
58 shoemakers, 20 general-population controls	5–50	NR	+	NT	NT		Tunca & Egeli (1996)
437 factory workers, 150 controls		0.02–9.2 (mean, 1.4)	NT	NT	+		Zhang (1996)
49 exposed female shoemakers, 27 controls employed in confectionary industry	1–33 (mean, 17)	1.9–14.8 (median, 5.9) (at time of study)	(+)	(+)	NT		Bogadi-Sare et al. (1997)

Table 4.4 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
Lymphocytes: 38 petrochemical workers, benzene plant or coke oven workers, 13 controls (included office workers) Buccal: 18 petrochemical workers, benzene plant or coke oven workers, 15 controls (included office workers)	NR	Benzene, 0.8–1.1 Coke, 0.04–0.30 (8-h TWA)	NT	NT	–	Chromosome 9; buccal cells and lymphocytes	Surrallés et al. (1997)
23 painters, 22 factory controls	NR	0.17–3.06 (TWA, 0.71)	NT	–	NT		Xu et al. (1998)
12 benzene factory workers, 5 coke oven workers, 8 rural village population controls	Benzene, 0.7–19 (GM, 4.1) Coke, 0.5–30.6 (GM, 4.8)	Benzene, 0.03–9.0 (GM, 0.41) Coke, 0. 16–0.53 (GM, 0.31)	+	NT	NT	Chromosomes 1 and 9; blood smear granulocytes and lymphocytes, and stimulated (cultured) lymphocytes; + for breakages in both chromosomes in benzene factory workers in cultured lymphocytes; (+) for chromosome 1 (9 not reported) in benzene factory worker smear cells	Marcon et al. (1999)
44 Chinese workers exposed to benzene, 44 controls 12 Estonian benzene production, 5 coke oven workers, and 8 controls	Estonian benzene production, 6.6 (mean) Estonian coke workers, 11.4 (mean)	Chinese, 31 (median) Estonian benzene production, 4.1 (mean) Estonian coke workers, 1.1 (mean)	–	NT	NT		Eastmond et al. (2001)
178 petroleum refinery workers, 36 office worker controls	10.6 (mean)	0.004–4.52	+	NT	NT		Kim et al. (2004a)
82 coke oven workers, 76 controls	0.75–19.67 (mean, 8)	0.014–0.743 (GM, 0.557) (8-h TWA)	+	NT	NT	Chromosomes 8, 21 were evaluated; + for t(8,21) translocations	Kim et al. (2004b)
39 ethylbenzene production workers, 55 controls	NR	0.13–4.7	+	NT	NT	CAs in high-CA subset decreased 10 mo after improved work conditions	Sram et al. (2004)
10 oil refinery workers, 87 industrial controls, 26 matched controls	12–28 (mean, 22.8)	13.7 (mean) in 1994, reduced to 0.56 (mean) in 1995	+	+	NT	Followed annually from 1990 to 2003; decreased over time as exposures decreased	Tompa et al. (2005)
44 factory workers, 44 factory controls	0.7–16 (mean, 6.3) (Smith et al., 1998)	31 (median) 8-h TWA, based on geometric mean of five 8-h measures	+	NT	NT	Chromosomes 2, 4, 6, 11, 12, 14, 18; same study as Smith et al. (1998) and other Zhang et al. studies with different chromosomes evaluated	Zhang et al. (2007)

Table 4.4 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
108 petroleum refinery workers, 33 controls	10.5 (mean)	0.004–4.52 (shift TWA, 0.51)	+	NT	+		Kim et al. (2008)
30 petroleum refinery workers, 10 office worker controls		0.51 (mean)	NT	NT	+		Kim et al. (2010)
200 filling station workers, 200 general-population controls	10.7 (mean)	0.34–0.47	+	NT	+		Rekhadevi et al. (2011)
30 workers in China who used benzene-containing glues, 11 factory controls	> 1–NR	< LOD–23.6 (GM, 2.8)	+	NT	NT	Chromosome 1 in sperm; + for "structural aberrations", including duplications, deletions, and breaks; (+) for "numerical aberrations" (disomy, nullisomy)	Marchetti et al. (2012)
459 unspecified benzene-exposed workers, 88 controls			NT	NT	+		Zhang et al. (2012b)
385 shoemakers, 197 controls (102 indoor local controls, 95 teachers from Shanghai as external controls)	> 1	0.8–17.8 (median, 2.0)	NT	NT	+		Zhang et al. (2014)
317 shoemakers, 102 office worker controls	> 1	0.80–12.09 (median, 1.60)	NT	NT	+		Zhang et al. (2016)

[a] Benzene exposure level conversion: 1 ppm = 3.19 mg/m^3 = 3190 µg/m^3

[b] +, positive; (+), positive result in a study of limited quality; –, negative

CA, chromosomal aberrations; d, day(s); G1, group 1; G2, group 2; GM, geometric mean; h, hour(s); LOD, limit of detection; MN, micronuclei; mo, month(s); NR, not reported; NT, not tested; ppm, parts per million; SCE, sister-chromatid exchanges; TWA, time-weighted average; vs, versus; yr, year(s)

Table 4.5 Chromosomal end-points in human cells in vitro

Cells	End-point	Benzene and metabolite(s)	Results[a]	Reference
Leukocytes	Combined effects of benzene and radiation on CAs	Benzene	+ for CAs	Morimoto (1976)
Lymphocytes	QM staining	Benzene	– for SCE, structural CAs	Gerner-Smidt & Friedrich (1978)
Lymphocytes	SCEs	Benzene	– for SCE	Morimoto & Wolff (1980)
		Phenol, CAT, HQ	+ for SCE	
Lymphocytes	SCEs	Benzene	+ for SCE with S-9	Morimoto (1983)
		CAT, HQ	+ for SCE	
Lymphocytes	SCEs	Benzene, CAT, HQ, phenol	+ for SCE with S-9	Morimoto et al. (1983)
T-lymphocytes	Stained using a modified fluorescence-plus-Giemsa technique for SCE	Benzene, phenol, CAT, BT, HQ, BQ	+ for SCE	Erexson et al. (1985)
		t,t-MA, 4,4′-biphenol, 4,4′-dipheno-quinone, 2,2′-biphenol	(+)/–	
Lymphocytes	Modified MN assay with anti-kinetochore antibody	HQ, phenol, CAT	+ for MN and K+	Yager et al. (1990)
		BQ	+ for MN	
Lymphocytes and HL-60 cells	MN with anti-kinetochore antibody	BT	+ for MN and K+	Zhang et al. (1993)
Lymphocytes	FISH (chromosomes 1, 7, and 9)	HQ	+ for hyperploidy	Eastmond et al. (1994)
Human lymphoblast cell line (GM09948)	FISH with specific probes for chromosomes 5, 7, and 8	HQ	+ for loss of one hybridization signal for chromosomes 5, 7, and 8	Stillman et al. (1997)
Lymphocytes	FISH with centromeric probes for chromosomes 1, 5, 7 and specific probes for 5q31 and 7q36-qter	HQ, BT	+ for HQ and BT for monosomy 5 and 7 and –5q and –7q	Zhang et al. (1998b)
Human lymphoblast cell line (GM09948)	FISH with specific probes for chromosomes 5, 7, and 8	CAT	– for CAT for loss of one hybridization signal for chromosomes 5, 7, and 8	Stillman et al. (1999)
		HQ	+ CAT/HQ synergy; – for CAT/HQ hyperploidy [(+) for 8]	
Lymphocytes	MN	Benzene	– for MN	Zarani et al. (1999)
CD34+ and CD34– cells from cord blood	FISH with probes for chromosomes 7 and 8	HQ	+ for monosomy and trisomy 7 and 8 in CD34+; + for monosomy 7 only in CD34–; – for tetrasomy 7 and 8 in both cell types	Smith et al. (2000)

Table 4.5 (continued)

Cells	End-point	Benzene and metabolite(s)	Results[a]	Reference
Human CD34+CD19– bone marrow cells	FISH with 5q31, 5p15.2, and centromeric probes specific for human chromosomes 7 and 8	HQ	+ for monosomy 7; + for –5q31; – for monosomy 5; no loss or gain of 8	Stillman et al. (2000)
Lymphocytes	FISH (chromosomes 5, 7, 8, and 21)	BT, HQ, t,t-MA	+ for aneuploidy	Chung & Kim (2002)
Lymphocytes	CBMN, FISH (chromosomes 7 and 8)	BT	+ for MN, aneuploidy	Chung et al. (2002)
Metabolites incubated with human topoisomerase IIα and then DNA	Measuring DNA cleavage	BT HQ BQ CAT, 4,4'-biphenol, 2,2'-biphenol	– (+) + for targeting topoisomerase IIα –	Lindsey et al. (2005)
Whole blood	FISH with probes for chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21; six chromosomes (1, 5, 7, 8, 9, and 21) analysed for BT and high-dose HQ	HQ BT	(+) for HQ for monosomy for all chromosomes except 21; ++ for 5, 7, 9, and 11; highest IRRs for 5, 6, and 12 + for trisomy for 7, 8, 9, and 21; highest IRRs for 7, 8, 12, and 21; + for BT for monosomy for 5, 7, 8, and 9 but not 1 and 21; highest IRRs for 5 and 7; (+) for BT for trisomy for all chromosomes; no selection for trisomy 8	Zhang et al. (2005)
Lymphocytes (interphase and metaphase)	FISH with probes for chromosome 1	Benzene	(+) for MN in interphase cells, aneuploidy in metaphase	Holeckova et al. (2008)
HL-60 cells	Gamma-H2AX	HQ, BQ	+	Ishihama et al. (2008)
TK-6 cells (lympho-blastoid cell line)	Stained with DAPI, chromosomes 11 and 21	HQ	+ for structural CAs; + for translocations of chromosome 21; – for translocations of chromosome 11	Ji et al. (2009)
Peripheral blood mononuclear cells	CBMN	BQ	+ for MN with PMA act of MPO; – for MN without PMA	Westphal et al. (2009)
Lymphocytes	umuC test, CBMN assay	Benzene	– for umuC; (+)/– for MN	Bonnefoy et al. (2012)
Lymphocytes	MN	Benzene with S-9, HQ	+ for MN	Peng et al. (2012)

[a] +, positive; (+), positive result but in a study of limited quality; –, negative

BQ, benzoquinone; BT, benzenetriol; CA, chromosomal aberration; CAT, catechol; CBMN, cytokinesis-blocked micronucleus; DAPI, 4',6-diamidino-2-phenylindole; FISH, fluorescence in situ hybridization; H2AX, histone H2AX; HL, human leukaemia; HQ, hydroquinone; IRR, incidence rate ratio; K+, kinetochore positive; MN, micronuclei; MPO, myeloperoxidase; PMA, phenylmercapturic acid; QM, QM protein (transcription cofactor inhibiting the activity of AP-1 transcription factors and is also a ribosomal protein participating in protein synthesis); SCE, sister-chromatid exchanges; t,t-MA, *trans*,*trans*-muconic acid

Table 4.6 Cytogenetic changes in humans exposed to benzene

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
Woman (age, 38 yr) with benzene-induced leukaemia (case study)			+ for extra chromosomes, mostly in C group (group C trisomy)	All chromosomes	Forni & Moreo (1967)
Woman (age, 37 yr) with benzene-induced acute erythroleukaemia (case study)			+ for cytogenetic changes	All chromosomes	Forni & Moreo (1969)
5 women with benzene haemopathy, diagnosed 5 yr previously, 1 control	NR	NR	+	% aneuploid lymphocytes (40%) decreased from time of diagnosis (70%)	Pollini et al. (1969)
4 women with benzene myelopathy diagnosed 10 yr previously, 1 control	NR	NR	Same subjects as Pollini et al. (1969)	All had lower % aneuploid cells than control	Pollini et al. (1976)
4 women with benzene myelopathy diagnosed 12 yr previously, 1 control	NR	NR	Same subjects as Pollini et al. (1969)	All had lower % aneuploid cells than at previous follow-ups, but closer to control	Pollini & Biscaldi (1977)
33 workers exposed to benzene, 15 general-population controls; all smokers	10–23	< 31.3	+ for structural CAs In exposed workers, chromosomes 2, 4, and 9 almost twice as susceptible to breaks; 1 and 2 almost twice as susceptible to gaps; chromosome 18 underrepresented for CAs In unexposed controls, more random distribution of the breakpoints	All chromosomes	Sasiadek et al. (1989)
56 workers in plants, 20 controls	10–20 (not clearly defined)	< 10	+ for structural CAs (mainly breaks and gaps) and non-random distribution of breakpoints, which accumulated mainly on chromosomes 2, 4, and 7	All chromosomes	Sasiadek (1992)
58 shoemakers, 20 general-population controls	5–50	NR	(+) for polyploidy	All chromosomes	Tunca & Egeli (1996)
18 petrochemical workers (benzene plant or coke oven workers), 15 controls (including some office workers)	NR	Benzene plant, 1.1 (mean) Coke oven, 0.04 (mean) (8-h TWA)	– for chromosome 9 numerical abnormalities	Chromosome 9; buccal cells	Surrallés et al. (1997)

Table 4.6 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
43 Chinese factory workers, 44 other factory controls	0.7–16 (mean, 6.3)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for hypo- and hyperdiploidy of chromosome 8; hyperdiploidy of chromosome 21; t(8,21), t(8;?), t(21,?), and chromosome 8 breaks but not deletions	Chromosomes 8 and 21; same study population as some Zhang et al. studies (2007 and others), different chromosomes evaluated	Smith et al. (1998)
43 Chinese factory workers, 44 factory controls	0.7–16.0 (mean, 6.3)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for monosomy 5 and 7 but not 1; trisomy 1, 5, and 7; tetrasomy 1, 5, and 7; –5q; –7q; total structural CAs in 5 and 7 (+) for chromosome 1 breaks at the centromere	Chromosomes 1, 5, and 7; same study population as for some Zhang et al. studies (2007 and others), different chromosomes evaluated	Zhang et al. (1998a)
12 benzene factory workers, and 5 cokery workers; 17/8 rural village population controls	0.7–19 (GM, 4.1)	0.0–9.0 (GM, 0.41)	(+) for hyperploidy in both chromosomes 1 and 9 in cultured lymphocytes; (+) for chromosome 1 hyperploidy and breakages (9 not reported) in smear cells; + for breakages in chromosomes 1 and 9 in cultured lymphocytes	Chromosomes 1 and 9; blood smear granulocytes and lymphocytes, and stimulated (cultured) lymphocytes	Marcon et al. (1999)
5 cokery workers, 8 rural village population controls	0.5–30.6 (GM, 4.8)	0.16–0.53 (GM, 0.31)	(+) for hyperploidy in both chromosomes 1 and 9 in cultured lymphocytes		
43 Chinese factory workers, 44 factory controls	0.7–16 (mean, 6.3)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for monosomy (one hybridization signal) 7 and 8 in metaphase but not interphase + dr for trisomy (3 hybridization signals) 7 and 8 in metaphase and interphase, but more pronounced in metaphase	Chromosomes 7 and 8; same study population as for other Zhang et al. studies (e.g. 2007), different chromosomes evaluated; compared sensitivity of metaphase and interphase FISH, metaphase more sensitive	Zhang et al. (1999)
44 Chinese workers, 44 controls	NR	1.6–328.5 (median, 31)	(+) dr for hyperploidy in Ch chromosome 1	Chromosomes 1 and 9	Eastmond et al. (2001)
12 Estonian benzene production workers, 5 coke oven workers, 8 controls	Benzene workers, 6.6 (mean) Coke workers, 11.4 (mean)	Benzene workers, 4.1 (mean) Coke workers, 1.1 (mean)			

Table 4.6 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
82 coke oven workers, 76 controls	0.75–19.67 (mean, 8)	0.014–0.743 (GM, 0.557) (8-h TWA)	+ for both monosomy and trisomy of both 8 and 21; + for t(8,21) translocations	Chromosomes 8 and 21	Kim et al. (2004b)
43 factory workers, 44 factory controls	0.7–16 (mean, 6.3) (from Smith et al., 1998)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for monosomy and trisomy of all 7 chromosomes; + for exposed vs non-exposed for tetrasomy of all 7 chromosomes; some selectivity at lower exposures: only monosomy 6 and trisomy 4, 6, and 11 were + in the < 31 ppm group; + for −6q and t(14;18); – for t(4,11) and t(6,11)	Chromosomes 2, 4, 6, 11, 12, 14, and 18; same study population as for Smith et al. (1998) and other Zhang et al. studies, different chromosomes evaluated	Zhang et al. (2007)
57 Chinese factory workers exposed to benzene (20 low-dose and 37 high-dose), 31 unexposed factory workers (not clearly reported) Subset: 37 benzene-exposed workers, 20 unexposed factory workers (not clearly reported)	NR	Low, 1.8 (mean) High, 21.9 (mean) Subset, 22.6 (mean)	t(14,18) signif ↓d no t(8,21) observed 2 t(15,17), but 1 in unexposed, 1 in exposed	t(15;17) and t(8,21) t(14,18) in subset	McHale et al. (2008)
649 MDS cases, 80 with benzene exposure (13.2%), 29 highly exposed > 21 ppm	High, > 0.5 (mean, 12)	> 21	– *	−5/5q−, −7/7q−, +8, del(20q) and 11q23/*MLL* * – for benzene-exposed vs non-exposed MDS cases	Irons et al. (2010)
30 petroleum refinery workers, 10 office worker controls		0.51 (mean)	+ for chromosomes 7, 9 for aneuploidy	Chromosomes 7 and 9	Kim et al. (2010)
33 Chinese male factory workers using benzene-containing glues, 33 factory worker controls	> 1	< 0.2–23.6 (median, 2.9) (8-h TWA)	+ for disomy X and disomy Y; – for disomy 21	Chromosomes 21, X, and Y in sperm; same population as Ji et al. (2012) (PBL results)	Xing et al. (2010)
47 shoemakers (22 exposed to benzene at < 10 ppm, 25 at ≥ 10 ppm), 27 clothing factory controls	NR	Low, 4.95 (mean) High, 28.33 (mean) (Based on multiple samples over 3 mo)	+ selectivity; + dr for monosomy 5, 6, 7, 10, 16, and 19; + dr for trisomy 5, 6, 7, 8, 10, 14, 16, 21, and 22	All chromosomes; OctoChrome FISH: chromosome-wide aneuploidy study (CWAS)	Zhang et al. (2011)

Table 4.6 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
33 Chinese male factory workers, 33 control factory workers	> 1	< 0.2–23.6 (median, 2.9) (8-h TWA)	(+) for trisomy 21; – for gain X and gain Y	Chromosomes 21, X, and Y; same population as Xing et al. (2010) (sperm results)	Ji et al. (2012)
30 workers in China who used benzene-containing glues, 11 factory worker controls	> 1–NR	< LOD–23.6 (GM, 2.8)	(+) for disomy 1	Chromosome 1 in sperm	Marchetti et al. (2012)
Man (age, 43 yr) with MDS and AML (case study)	16 (HQ)		+ CAs in chromosomes 5 and 7		Regev et al. (2012)
28 shoemakers (18 exposed to benzene at < 10 ppm, 10 at ≥ 10 ppm), 14 clothing factory controls	NR	Low, 2.64 (mean) High, 24.19 (mean) (Based on multiple samples over 3 mo)	+ dr for monosomy 7 and 8; no trisomy effects	Chromosomes 7 and 8; in interphase CFU-GM cells	Zhang et al. (2012a)
722 AML cases, 78 with benzene exposure (10.8%), 38 > 0.31 ppm			– *	–5/5q–, –7/7q–, +8, del(20q) and 11q23/*MLL*; t(8,21), t(15,17) * – for benzene-exposed vs non-exposed AML cases	Irons et al. (2013)

[a] Benzene exposure level conversion: 1 ppm = 3.19 mg/m^3 = 3190 ug/m^3

[b] +, positive; (+), positive but in a study of limited quality; –, negative

AML, acute myeloid leukaemia; CA, chromosomal aberration; CFU-GM, colony-forming-unit granulocyte-macrophage; CWAS, chromosome-wide aneuploidy study; dr, dose–response relationship; FISH, fluorescence in situ hybridization; GM, geometric mean; h, hour(s); HQ, hydroquinone; LOD, limit of detection; MDS, myelodysplastic syndrome; *MLL*, mixed lineage leukaemia gene; mo, month(s); NR, not reported; PBL, peripheral blood lymphocyte; ppm, parts per million; TWA, time-weighted average; vs, versus

–5q, and –7q, as well as for +8 in PBLs, have been observed (Zhang et al., 1998a, 1999, 2011). Zhang et al. (2012a) also observed a significant exposure-related trend for –7, but not +7 or +8, in circulating interphase colony-forming-unit (CFU) granulocyte-macrophage (GM) cells, myeloid progenitor cells that are probable targets for the induction of myeloid leukaemia. In independent studies with PBLs, the benzene-associated aneuploidy of chromosome 7 (Kim et al., 2010) and +8 (Kim et al., 2004b) were confirmed. Zhang et al. (2007) also observed a significant exposure-related trend for trisomy 12.

In studies on several translocations implicated in some cancers of the lymphoid and haematopoietic tissues, Smith et al. (1998) observed a significant exposure-related trend using fluorescence in situ hybridization testing for t(8,21). This association was independently confirmed by Kim et al. (2004b), but not replicated using polymerase chain reaction analysis (McHale et al., 2008; IARC, 2012). Similarly, using fluorescence in situ hybridization Zhang et al. (2007) observed a significant exposure-related increase in t(14,18) in the group exposed to the highest concentration of benzene, but this was not replicated in the polymerase chain reaction analyses of McHale et al. (2008). In addition, Zhang et al. (2007) did not observe significant trends for some common translocations involving the *MLL* (mixed lineage leukaemia) gene on chromosome 11q23; however, a significant exposure-related trend was observed for –6q. McHale et al. (2008) did not observe an exposure-related effect on t(15,17).

In vitro studies similarly report certain cytogenetic changes with exposure to benzene or its metabolites. For example, Chung & Kim (2002) reported significant concentration-related trends for –5 and –7, as well as inductions of +8 without significant trends, in human lymphocytes treated with the benzene metabolites hydroquinone, benzenetriol, or t,t-MA, although no t(8,21) translocations were observed. Similarly, Zhang et al. (1998b) reported significant increases in –5 and –7, as well as in –5q and –7q, in human lymphocytes treated with hydroquinone or benzenetriol. Smith et al. (2000) reported that hydroquinone also induced significant positive dose–response relationships in +8 in CD34+ cells and in –7 in both CD34+ and CD34– cells from human cord blood, CD34+ cells being haematopoietic progenitor cells. Stillman et al. (1997, 1999) observed increased –5 and –7 in a human lymphoblastoid cell line from exposure to hydroquinone, but not to catechol. Stillman et al. (1999) further found that catechol acted synergistically with hydroquinone to induce significant positive dose–response relationships in –5 and –7, as well as –5q, which was not observed for hydroquinone alone. A concentration-related trend for +8 was also observed, although it was reportedly not statistically significant. In addition, Stillman et al. (2000) treated human CD34+CD19– bone marrow cells with hydroquinone and reported significant concentration-related trends for –7 and –5q but not –5 or +8; further, a greater susceptibility to hydroquinone- induced –5q and –7 was seen in the bone marrow cells than in the lymphoblastoid cell line. Zhang et al. (2005) exposed lymphocytes in whole blood to hydroquinone or benzenetriol, and reported that chromosomes 5 and 7 were selectively more susceptible to loss induced by those benzene metabolites than several other chromosomes that were examined; further, chromosome 8 was one of a few chromosomes that were more susceptible to gain. Also of note, similar to the findings of Zhang et al. (2007) in workers exposed to benzene, no increases in translocations involving 11q23 were observed in a lymphoblastoid cell line (TK6) treated with hydroquinone (Ji et al., 2009).

In addition to the investigation of chromosomal end-points in healthy people exposed to benzene, some studies have examined cytogenetic changes in cases of acute myeloid leukaemia (AML) or myelodysplastic syndromes

(MDS) in people who have likely been exposed to benzene. Zhang et al. (2002) reviewed 18 cases of AML attributed to benzene exposure from case reports with cytogenetic analyses. Only 1 of the 18 cases had a normal karyotype (Zhang et al., 2002), in contrast to some cytogenetic studies which reported over 40% of de novo cases of AML with a normal karyotype (Schoch et al., 2004; Sanderson et al., 2006). Zhang et al. (2002) also reviewed over 30 abnormal karyotypes from leukaemia patients with likely prior benzene exposure from several large-scale leukaemia studies, and noted that several cases exhibited the same translocation (e.g. t(8,21) for AML and t(9,22) for chronic myeloid leukaemia). Overall, Zhang et al. (2002) found that there were insufficient data from which to discern a specific pattern of clonal chromosomal changes in patients with leukaemia associated with benzene, indicating that benzene produces a variety of cytogenetic changes that may induce or contribute to leukaemogenesis.

Irons et al. (2013) investigated 722 AML cases identified in Shanghai, China and determined that 78 cases had likely benzene exposure. Irons et al. (2013) compared the cytogenetic findings in the 78 cases exposed to benzene first with those from the 644 unexposed cases and then with those from several studies of therapy-related AML. In a subsequent study of 710 of these AML cases, 75 of which were determined to have likely been exposed to benzene, Kerzic & Irons (2017) assessed chromosome breakpoints across 441 identifiable regions. Likewise, Irons et al. (2010) studied 649 MDS cases in Shanghai, China, and determined that 80 cases had likely been exposed to benzene, 29 of which had likely been exposed to high concentrations (> 21 ppm) of benzene. Irons et al. (2010) first compared the cytogenetic findings in the cases exposed to benzene with those from all of the MDS cases. A case–case analysis was then conducted, in which each of the 29 highly exposed cases was matched by age and sex to two cases with no suspected benzene exposure, and levels of various abnormalities characteristic of t-MDS in the highly exposed cases were compared with levels in the unexposed cases. [The Working Group noted that the implications of the reported comparisons between cases exposed to benzene and unexposed cases are uncertain, given that only a portion of the MDS cases in those exposed to benzene were actually attributable to benzene, which can have a diluting effect.]

(ii) Experimental systems

Benzene induced CAs, MN, and SCEs in bone marrow cells of mice, CAs in bone marrow cells of rats, rabbits, and Chinese hamsters, and sperm-head anomalies in mice treated in vivo. Most of the induced aberrations were breaks or deletions. Chromosome-type aberrations also occurred however, particularly after prolonged exposure when toxicity, manifested by a drop in the peripheral blood leukocyte count, appeared. Benzene did not induce SCE in rodent cells in vitro, but it did induce aneuploidy and CAs in cultured Syrian hamster embryo cells. Benzene induced mutation and DNA damage in some studies in rodent cells in vitro. In *Drosophila*, benzene was reported to be weakly positive in assays for somatic mutation and for crossing-over in spermatogonia; in single studies, it did not induce sex-linked recessive lethal mutations or translocations. It induced aneuploidy, mutation, and gene conversion in fungi. Benzene was not mutagenic to bacteria (IARC, 1982, 1987, 2012). In agreement with a possible role of combinations of multiple metabolites of benzene in genotoxicity, Barale et al. (1990) demonstrated that combinations of phenol and hydroquinone were highly genotoxic to mouse bone marrow as indicated by the formation of MN.

In utero exposure to benzene increased the frequency of MN and SCEs in haematopoietic tissue of fetal and postnatal mice (Ning et al., 1991; Xing et al., 1992). French et al. (2015) observed a dose-dependent increase in benzene-induced

chromosomal damage and estimated a benchmark concentration limit of 0.205 ppm benzene using Diversity Outbred mice. This estimate is an order of magnitude below the value estimated using B6C3F1 mice.

After exposure of mice to benzene, DNA adducts were detected by ^{32}P-postlabelling in both the bone marrow and leukocytes (Bodell et al., 1996; Lévay et al., 1996). Mild but statistically significant mutagenic responses were found in transgenic mice carrying the *lacI* reporter gene exposed to benzene (Mullin et al., 1995; Provost et al., 1996). The clastogenic potential of benzene is partly due to its metabolites. Specifically, benzene oxide, benzoquinones, muconaldehydes, and benzene dihydrodiol epoxides are electrophiles that readily react with peptides, proteins, and DNA (Bechtold et al., 1992b; McDonald et al., 1993; Bodell et al., 1996; Gaskell et al., 2005; Henderson et al., 2005a; Waidyanatha & Rappaport, 2005), and can thereby interfere with cellular function (Smith, 1996).

The importance of CYP2E1 (see Section 4.1) in inducing benzene toxicity was shown in studies of *Cyp2e1*$^{-/-}$ mice, in which no benzene-induced cytotoxicity or genotoxicity were observed (Valentine et al., 1996). Similar studies showed the importance of NQO1, which detoxifies benzoquinones, proposed toxic metabolites of benzene. Compared with *NQO1*$^{+/+}$ mice, *NQO1*$^{-/-}$ mice exhibited more severe benzene-induced haematotoxicity and were more sensitive to benzene-induced MN formation in peripheral blood cells. These results indicate that NQO1 deficiency results in substantially greater benzene-induced toxicity. However, the specific patterns of toxicity differed between the male and female mice (Bauer et al., 2003). In fact, male mice were more sensitive than females to the induction of MN by benzene administered either orally or intraperitoneally (Meyne & Legator, 1980; Siou et al., 1981). This may be due, at least in part, to a function of greater oxidative metabolism in male mice (Kenyon et al., 1996). Castration of males reduces their sensitivity to that of females (Siou et al., 1981).

4.2.3 Altered DNA repair or genomic instability

Several DNA reactive metabolites are formed during benzene metabolism, and the type and the frequency of lesions, the respective DNA repair systems involved in their removal, and the repair capacity of the target organ are influenced by the different metabolites (Winn, 2003; Pandey et al., 2009; Au et al., 2010; Hartwig, 2010). Table 4.7 reports examples of in vivo and in vitro studies indicating altered DNA repair or epigenetic alterations related to benzene exposure or its metabolites.

Benzene exposure at occupational and environmental concentrations influences DNA repair systems in human studies in vivo, as reviewed by Ravegnini et al. (2015). In subjects who worked at a spray-painting plant, the exposure to benzene had significantly altered mRNA expression of some critical cell regulatory and DNA repair genes such as *Xpc*, *Xpa*, and *Apel* (Wang et al., 2012). Exposure to a time-weighted average concentration of benzene in a workplace of up to 1.8 mg/m^3 may cause chromosomal damage in workers; in particular, the *XRCC1* rs25487 and rs1799782 polymorphisms may be associated with an increase in MN frequency (Huang et al., 2016). Frequencies of MN and CAs in 108 petroleum refinery workers exposed to 0.51 ppm of benzene (full-shift time-weighted average) were higher than in 33 office workers, and the frequencies were influenced by the polymorphism of the *XRCC1* gene (Kim et al., 2008).

In human cell systems and in exposed mice, chemically reactive benzene metabolites, particularly 1,4-benzoquinone and hydroquinone, directly inhibited isolated topoisomerase II (Frantz et al., 1996; Hutt & Kalf, 1996; Eastmond et al., 2001, 2005; Ji et al., 2009). Possible mechanisms of this inhibition include covalent binding

Table 4.7 Studies of benzene or metabolites indicating altered DNA repair, genomic instability, or epigenetic alterations

Description of exposed and controls Study type Tissue	Benzene exposure (range or median) or its metabolites	Comments	Reference
DNA repair or genomic instability			
In vitro Isolated topoisomerase IIα	BQ, 10 μM; HQ 10 mM	Metabolites catalytically inhibited topoisomerase II	Baker et al. (2001)
In vitro Isolated topoisomerase IIα; human CEM leukaemia	BQ, 0–100 μM BQ, 10 μM	BQ strongly inhibited topoisomerase II BQ underwent covalent binding with topoisomerase IIα	Lindsey et al. (2004)
108 exposed petroleum refinery workers, 33 controls Occupational exposure	0.004–4.52 ppm	Both the CA and MN frequencies were significantly higher in exposed compared with unexposed workers and influenced by polymorphism of XCCR1 gene	Kim et al. (2008)
Spray painters: A, 46 direct exposed; B, 26 indirect exposed; C, 29 controls Occupational exposure	A, 0.21 ± 0.19 mg/m^3; B, 0.06 ± 0.12 mg/m^3; C, ND	The mRNA expression levels of *Rad51*, *Bcl-2*, *Bax*, *Apel*, *Xpa*, and *Xpc* in groups A and B were downregulated significantly compared with group C	Wang et al. (2012)
Haematopoietic stem and progenitor cells, human CD34+ cells In vitro	0.5–1 mg/mL	DNA breakage	Thys et al. (2015)
CD-1 mouse fetal liver cells In vitro	BQ, 5, 15, and 25 μM	Benzoquinone exposure significantly decreased the transcript levels of 8-oxo-guanine glycosylase	Philbrook & Winn (2016)
Epigenetic alterations			
DNA methylation			
78 gas station attendants, 77 urban traffic officers, and 57 controls Occupational exposure	0.040–0.132, 0.09–0.031, and < 0.006–0.014 mg/m^3	Airborne benzene was associated with hypomethylation of *Line-1* and *AluI*	Bollati et al. (2007)
In vitro Human lymphoblastoid TK6 cells	Benzene: 1, 10, and 100 μM; HQ, 0.005, 0.05, and 0.5 μM	Benzene and its metabolite HQ exposure induced global DNA hypomethylation in TK6 cells	Tabish et al. (2012)
In vitro Human hepatic L02 cells	Benzene, HQ, and BQ: 5, 10, 25, and 50 μM	HQ and 1,4-BQ, but not benzene, induced global DNA hypomethylation	Hu et al. (2014)
Histone modifications			
In vitro Human myeloid leukaemia HL-60 cells	HQ Single treatment: 1, 5, 15, and 25 μM Repeated treatment: 1, 5, and 15 μM four times every 48 h Long-term treatment with 1μM: five times a week for 5 wk	Epigenetic modifications (instauration in *LINE-1* sequences) after in vitro treatment with HQ were transitory and reversible	Mancini et al. (2017)

BQ, benzoquinone; CA, chromosomal aberration; CEM, human acute lymphoblastic leukaemia cells; h, hour(s); HQ, hydroquinone; *LINE-1*, long interspersed nuclear element-1; MN, micronuclei; ND, not detectable; ppm, parts per million; wk, week(s)

or catalytic action (Baker et al., 2001; Lindsey et al., 2004, 2005; Chen et al., 2016a).

Benzoquinone exposure significantly decreased the transcript levels of the critical base excision repair gene, 8-oxo-guanine glycosylase, in CD-1 mouse fetal liver cells in vitro (Philbrook & Winn, 2016); it was also able to rapidly increase ROS production, followed by a statistically significant increase in both c-H2A.X foci and DNA recombination in fetal haematopoietic cells (Tung et al., 2012).

4.2.4 Immunosuppression

This section focuses on the studies that directly or indirectly inform immune response outcomes, and is divided into haematotoxicity (inclusive of all such data in Section 4), genes related to immune function, and immunoproteins.

(a) Humans

(i) Haematotoxicity

Acute exposure to benzene has been associated with diseases and symptoms in the blood-forming system such as aplastic anaemia, specific cytopenias, and pancytopenia (Aksoy et al., 1971; Yin et al., 1987; IARC, 2012). These diseases are associated with a functional reduction in immune competence by virtue of the reduced number of immunocompetent cells resulting from impaired haematopoiesis (IARC, 2012; McHale et al., 2012). In addition, several studies have found that various levels of severity of benzene-associated haematotoxicity have been associated with a future risk of developing a haematological malignancy or related disorder (Aksoy & Erdem, 1978; Yin et al., 1987; Rothman et al., 1997).

Many studies investigating the association between benzene exposure and altered blood cell counts reported haematological changes in exposed humans, especially at relatively high levels of exposure (e.g. > 10 ppm) (Rothman et al., 1996; Ward et al., 1996; Qu et al., 2002; Lan et al., 2004); some studies have demonstrated that haematological alterations can also occur at lower levels of exposure (< 10 ppm) (Ward et al., 1996; Zhang, 1996; Qu et al., 2002; Lan et al., 2004; Miao & Fu, 2004; Uzma et al., 2008; Robert Schnatter et al., 2010; Chen et al., 2012; Wang et al., 2012; Zhang et al., 2016). In particular, leukocyte counts were consistently reduced in an exposure-related manner (Rothman et al., 1996; Ward et al., 1996; Qu et al., 2002; Lan et al., 2004; Robert Schnatter et al., 2010). Reductions in leukocyte counts were observed with median benzene air concentrations of 1.2 ppm in Lan et al. (2004) and 3.8 ppm (4-week average) in Qu et al. (2002), with lowered counts in subgroups of workers exposed to less than 1 ppm. Decreased neutrophil counts were associated with benzene exposure down to a level of about 7.8–8.2 ppm (Robert Schnatter et al., 2010). However, numbers of band neutrophils, which are precursors and later mature into granulocytes, were increased as well as mean corpuscular volume (Bogadi-Sare et al., 2003).

A few studies reported no statistically significant differences in blood cell counts (Hancock et al., 1984; Kipen et al., 1989; Biró et al., 2002). Additionally, several studies reported no or minimal changes in haematological parameters in workers with occupational exposures of less than 5 ppm, in particular, less than 1 ppm. Several of these studies used historical haematological data collected as part of routine surveillance (Collins et al., 1991, 1997; Tsai et al., 2004; Swaen et al., 2010). [The Working Group noted that the timing of collection of blood samples relative to the most recent benzene exposure was not reported.]

Total lymphocyte counts were reduced in humans exposed to benzene (Rothman et al., 1996). Numbers of circulating CD19+ B-lymphocytes were consistently reduced in several studies (Rothman et al., 1996; Bogadi-Sare et al., 2000, 2003; Lan et al., 2004). CD4+ T-lymphocytes were consistently decreased in

multiple studies (Luan, 1992; Lan et al., 2004; Kirkeleit et al., 2006; Uzma et al., 2008; Chen et al., 2007, 2012; Wang et al., 2012); however, CD8+ T-lymphocyte populations were increased (Chen et al., 2012). In a study of paint factory workers exposed to benzene, a continual increase in the percentage of CD8+ T-cells measured every 4 months for a year was observed (Chen et al., 2012). No significant change in absolute number of CD8+ cells was observed in other studies (e.g., Chen et al., 2007). The decreased CD4+ and increased CD8+ T-cells resulted in a lowering of the CD4+/CD8+ ratio (Luan, 1992; Lan et al., 2004; Chen et al., 2007, 2012; Wang et al., 2012). Increased CD3+ lymphocytes were additionally noted (Chen et al., 2012).

Benzene exposure also reduced T-cell receptor excision circles (TRECs), a marker of T-cell maturity. Decreased TRECs in peripheral blood mononuclear cells (PBMCs) of patients with benzene poisoning were found in two separate studies, suggesting impaired T-cell immune function (Li et al., 2005, 2009a). Decreased TRECs were also found in the peripheral blood mononuclear cells in 62 workers exposed to benzene at a concentration in air of 1.72–37.8 mg/m^3 compared with 11 healthy controls (Han et al., 2004). However, Lan et al. (2005a) reported no significant difference in TREC levels in shoe factory workers exposed to benzene at a mean concentration in air of 15.8 ppm.

Relatively low levels of benzene (i.e. < 5 ppm) could result in haematological suppression after continuous exposure with no observed threshold for a response (Ward et al., 1996). Most types of blood cells, with the exception of leukocytes, from complete blood count levels were decreased in workers exposed to benzene, correlated with length of employment (Khuder et al., 1999).

Overall, decreased red blood cell counts (Rothman et al., 1996; Khuder et al., 1999; Qu et al., 2002; Miao & Fu, 2004; Koh et al., 2015), platelets (Rothman et al., 1996; Qu et al., 2002; Uzma et al., 2008; Ye et al., 2008; Chen et al., 2012; Wang et al., 2012), and haemoglobin content (Bogadi-Sare et al., 2003; Wang et al., 2012; D'Andrea & Reddy, 2016) were consistently reported. Benzene exposure increased haemoglobulin content and platelets. Haemoglobin content and red blood cell counts in workers who had been exposed to benzene for longer periods were significantly increased compared with controls (Uzma et al., 2008). Chemical and rubber factory workers who had been exposed to benzene at 0.07–872.0 mg/m^3 (median level, 7.4 mg/m^3) had reduced red blood cell count and mean platelet volume, the most affected haematological peripheral blood parameters (Robert Schnatter et al., 2010).

Finally, several studies examined circulating haematopoietic stem and/or progenitor cells, which may also be affected in individuals exposed to benzene. In a cross-sectional study of 17 petroleum refinery workers exposed to very low levels of benzene (0.28–0.41 ppm), increased burst-forming-unit erythroid and CFU-GM colonies without any growth stimulation were observed compared with 20 unexposed controls; this effect was not observed after the addition of growth factors, either erythropoietin (EPO) or granulocyte colony-stimulating factor (Quitt et al., 2004). However, another study of 10 subjects with occupational exposure to more than 10 ppm benzene (mean, 24.2 ppm), 19 subjects with exposure to less than 10 ppm benzene (mean, 2.6 ppm), and 24 controls with no occupational exposure to benzene reported an inverse monotonic exposure–response relationship with haematopoietic progenitor cell colony formation in cultured peripheral blood, including: CFU granulocyte, erythroid, macrophage, and megakaryocyte (with EPO stimulation); CFU-GM (with and without EPO stimulation); and burst-forming-unit erythroid cells (with EPO stimulation) (Lan et al., 2004).

(ii) Genes related to immune function

Several studies have investigated human susceptibility to benzene exposure and its relationship with single-nucleotide polymorphisms in genes that encode immune-related proteins. For instance, the tumor necrosis factor alpha (TNF-α) single-nucleotide polymorphism was associated specifically with an increased risk of persistent benzene-induced dysplasia in workers (Lv et al., 2007), and a significantly higher frequency of TNF-α was observed in benzene-poisoned patients (Lv et al., 2005).

Corresponding to altered leukocyte counts in benzene-exposed shoe factory workers, changes were reported in the expression of various genes, including the vascular cell adhesion molecule *VCAM1*, interleukin (*IL*)*-1A*, *IL-4*, *IL-10*, *IL-12A*, *CSF3*, *MPO*, and *CRP* (Lan et al., 2005b; Shen et al., 2011). Gene expression related to T-cells was also altered. The distributions of the T-cell receptor variable (TCRV) family TCRVα, TCRV*β*, and TCRVγ gene repertoires in individuals exposed to benzene were significantly lower compared with the reference group (Chen et al., 2006; Li et al., 2007, 2008, 2009b). PBMC gene expression levels of *CD3δ*, *CD3ε*, and *CD3ζ* were increased in workers exposed to benzene versus controls; in workers diagnosed with benzene poisoning, however, some regions were decreased in severe cases and other regions were unchanged (i.e. *CD3γ* and *CD3ε*) in mild cases (Li et al., 2012). *CXCL16*, a gene responsible for encoding a chemokine that activates T-cells and natural killer cells, was found to be consistently upregulated in workers exposed to benzene (Forrest et al., 2005; McHale et al., 2009).

(iii) Immunoproteins

Regarding B-cell effects, immunoglobulin (Ig) G production was positively correlated with air benzene levels (Bogadi-Sare et al., 2000). Increased IgG was also reportedly correlated with benzene urinary metabolite t,t-MA measured in petrochemical workers exposed to benzene (Dimitrova et al., 2005). IgM and IgA were reduced in cargo tank workers exposed to benzene (Kirkeleit et al., 2006). Furthermore, one study reported that plasma concentrations of soluble CD27 and CD30, two immune markers indicative of B-cell activation, were decreased by 17% for sCD27 but non-significantly reduced for sCD30 in the group exposed to the highest concentration of benzene (≥ 10 ppm) compared with control workers, after adjusting for age and sex (Bassig et al., 2016). [The Working Group noted that several prospective cohorts, although not specifically related to benzene, found that higher levels of sCD27 were associated with increased risk of non-Hodgkin lymphoma (Purdue et al., 2011; De Roos et al., 2012; Bassig et al., 2015a; Hosnijeh et al., 2016; Späth et al., 2017).]

Similar to effects in cytokine gene expression, cytokine serum concentrations were also modified. TNF production was significantly reduced in paint factory workers exposed to benzene vapours (Haro-García et al., 2012). IL-10 serum concentrations were positively correlated with the number of working years in those exposed to benzene (Spatari et al., 2015).

(b) Human cells in vitro

Alterations in cytokine production were also observed in studies of human cells in vitro. Both IL-1α and IL-1β were decreased in human blood monocytes after exposure to hydroquinone (Carbonnelle et al., 1995). Catechol, hydroquinone, 1,2,4-benzenetriol, and *p*-benzoquinone were also found to stimulate the production of T-helper cell (Th2) cytokines IL-4 and IL-5 (Gillis et al., 2007).

Supporting the haematotoxicity observed in humans, phenol, hydroquinone, and 1,2,4-benzenetriol decreased haemoglobin synthesis in K562 cells in a concentration-dependent manner (Wu et al., 2011). CD34+ haematopoietic progenitor cells treated with hydroquinone inhibited erythroid differentiation

in an exposure-related response, and miRNA-451a and miRNA-486–5p were upregulated during erythroid differentiation (Liang et al., 2017).

(c) Experimental systems

(i) Mouse

Several murine studies demonstrated consistent immunosuppressive effects on assays for humoral and cell-mediated immune function after oral and inhalation exposure. The only animal study to evaluate the effect of benzene exposure on the ability of T-cells to respond to a tumour challenge was conducted by Rosenthal & Snyder (1987), who exposed C57Bl/6 male mice to three concentrations (10, 30, and 100 ppm) of benzene for 100 days before tumour challenge. Inhalational exposure to 100 ppm increased lethal tumour incidence, which suggests reduced tumour surveillance (Rosenthal & Snyder, 1987). Further examination demonstrated that the same benzene concentration reduced T-cell cytolytic activity after 20 days of exposure, and reduced proliferative responses in the spleen in the mixed lymphocyte reaction (Rosenthal & Snyder, 1987). These reductions in cytotoxic and proliferative activity all occurred without any corresponding changes in the total number of T-cell or lymphocyte subpopulations in the spleen (Rosenthal & Snyder, 1987). In addition, Rosenthal & Snyder (1985) also demonstrated that 9 days of continuous inhalation exposure to benzene reduced cell-mediated immunity to bacterial infection. Exposure to benzene at several concentrations (30–300 ppm) increased bacterial load after a 4-day infection by the intracellular pathogen *Listeria monocytogenes*. Accompanying this effect, total lymphocytes and T- and B-cell populations were all reduced in the spleen for up to 7 days post-infection under the same benzene exposure concentrations (Rosenthal & Snyder, 1985).

Changes in assays for humoral immune function were first observed in BALB/c male mice after inhalation exposure to benzene at concentrations of 50 or 200 ppm for 14 days (Aoyama, 1986). Seven days after immunization with sheep red blood cells (SRBC), both benzene concentrations reduced IgM SRBC-specific plaque-forming cells (PFC) by up to 87% relative to controls, and IgG PFCs were reduced by approximately 94% (Aoyama, 1986). IgG PFCs remained suppressed 10 days after immunization, and IgM PFCs were not significantly different from controls. Reductions in total lymphocytes and B- and T-cells in the blood were also observed at the same concentration.

The oral exposure database of assays for immune function is less robust than that for inhalation; only one study has reported reduced humoral immunity. Male CD-1 mice were exposed to benzene in drinking-water at a concentration of 166 mg/L (the only tested dose) for 28 days, resulting in reduced IgM SRBC-specific PFCs as well as anti-SRBC antibody titres (Hsieh et al., 1990). Reductions were also observed in the mixed lymphocyte response and T-cell proliferation in response to concanavalin A and phytohaemagglutinin stimulation, and in the B-cell proliferative responses to pokeweed mitogen and lipopolysaccharide stimulation. In addition, benzene exposure increased serum corticosterone, which is known to suppress immune function (Hsieh et al., 1991). Different results were observed in a study of BALB/c male mice exposed to benzene by oral gavage at a concentration of 150 mg/kg bw per day (8 hours per day/5 days a week, for 2 weeks); no changes in total serum antibody titres were reported, but reduced counts of leukocytes, total lymphocytes, monocytes, and neutrophils in the blood were observed (Wen et al., 2016). In C57BL/6 mice exposed orally to benzene at 27 mg/kg bw per day for 28 days, briefly increased splenic natural killer cell activity was observed by day 21 and

splenic production of IL-2 was reduced by day 28 (Fan, 1992).

The haematotoxic effects of benzene exposure are well established in experimental animals (Cronkite et al., 1985; Farris et al., 1997a). Rats and mice of both sexes exhibited leukocytopenia and anaemia after subchronic inhalation exposure, but only mice demonstrated evidence of severe femoral hypoplasia (Ward et al., 1985). Nucleated bone marrow cells were significantly reduced in $B6C3F_1$ mice after inhalation exposure at a concentration of 100 ppm benzene for 8 weeks (Farris et al., 1997a). From analysis of the differentiation and maturation of haematopoietic precursor cells, exposure to benzene at 200 ppm for 8 weeks resulted in a sustained reduction of the primitive precursor CFU high proliferative progenitor cells, downstream progenitor CFU-GM cells, bone marrow granulocytes, and leukocytes in the blood (Farris et al., 1997a). In C57BL/6 mice exposed intraperitoneally to hydroquinone at 50 mg/kg bw per day or to benzene at 600 mg/kg bw per day for 2 days, or in DBA/2J mice exposed to benzene at 10 ppm via inhalation for 5 days, CFU-GM proliferation was not significantly affected but differentiation was significantly increased (Dempster & Snyder, 1991; Hazel et al., 1996). When Swiss Webster mice of both sexes were exposed to benzene in utero at 10 ppm and re-exposed to benzene at 10 ppm at age 10 weeks, greater reductions in splenic CFU-GM were observed compared with mice that were not exposed to benzene in utero (Keller & Snyder, 1986). Intraperitoneal exposure to benzene increased the production of nitric oxide in bone marrow cells, which may contribute to the reduced proliferation (Punjabi et al., 1994). Benzene exposure was also found to suppress the progenitor cell cycle of CFU-GM in the bone marrow of C57BL/6 mice by overexpressing the cyclin-dependent kinase inhibitor p21 (Yoon et al., 2001).

(ii) Rat

Two rat studies examined the effect of benzene exposure on assays for immune function: one oral and one inhalation. In male Wistar rats exposed to a single dose of benzene in drinking-water at 0.6 mL/kg of drinking water per day for 90 days, reductions in the total number of SRBC-specific antibody-forming cells in the spleen by 40%, and in the total anti-SRBC serum titres by 64% after immunization with SRBC (immunization protocol not specified), were observed (Karaulov et al., 2017). Cell-mediated immunity, assessed by the delayed-type hypersensitivity response, was also reduced by 52% compared with controls. Karaulov et al. (2017) additionally examined the effect of benzene exposure after 45, 90, and 135 days by stimulating splenocytes ex vivo with the concanavalin A; increased cytokine production of IL-4 and IL-6 and a reduced number of CD4+ T-cells were reported for all time periods.

In male Sprague-Dawley rats exposed to benzene via inhalation at a range of concentrations (30–400 ppm) for 2 or 4 weeks, no changes in anti-SRBC serum antibodies were induced (Robinson et al., 1997). However, the highest tested concentration reduced the numbers of splenic B-cells after 2 and 4 weeks of exposure and of CD4+/CD5+ T-helper cells after 4 weeks of exposure.

4.2.5 Altered cell proliferation, cell death, and nutrient supply

(a) Humans

Representative studies were included if the biological end-point was considered relevant for this key characteristic (studies of peripheral blood cell counts, benzene poisoning, cultured haematological progenitor cells, and genetic susceptibility to these events are presented in Section 4.2.4(a)). The Working Group focused on studies in which presence of benzene in the study population was documented, the presence

of co-exposures was evaluated and addressed, the control group was comparable to the exposed study population, and the study had adequate statistical power.

A case series report in China of 23 subjects with a history of benzene poisoning, with quantitative data for 17 subjects indicating very high exposure to benzene before diagnosis (i.e. concentration in air at 50–300 ppm), described a distinct pattern of bone marrow dysplasia including marked dyserythropoiesis, eosinophilic dysplasia, and abnormal cytoplasmic granulation of neutrophilic precursors. In addition, clonal and oligoclonal proliferation in bone marrow T-lymphocytes, including clonal rearrangements in T-cell receptor gene segments, was present in 14 out of 23 cases (Irons et al., 2005).

Several cross-sectional studies of workers with occupational exposure to benzene and unexposed controls measured miRNA in peripheral leukocytes or plasma and mRNA in leukocytes, and found altered levels of these end-points for genes that play a role in apoptosis; these studies provided indirect evidence of the possible influence of benzene on apoptosis in healthy subjects (Forrest et al., 2005; Sun et al., 2009; McHale et al., 2011; Wang et al., 2012; Li et al., 2014; Chen et al., 2016b, 2017; Hu et al., 2016; Liu et al., 2016).

In studies in vitro, benzene or its metabolites induced apoptosis in CD34+ human bone marrow progenitor cells, PBLs, PBMCs, bone marrow mesenchymal stem cells, and HL-60 human promyelocytic leukaemia cells (Moran et al., 1996; Ross et al., 1996a; Wiemels & Smith, 1999; Bratton et al., 2000; Nishikawa et al., 2011; Hu et al., 2012; Lee et al., 2012; Peng et al., 2012; Zolghadr et al., 2012). Inhibition of nuclear-factor kappa-light-chain-enhancer of activated B-cells (NF-κB) by hydroquinone sensitizes human bone marrow progenitor cells to TNF-α-induced apoptosis (Kerzic et al., 2003). Inhibition of DNA-dependent protein kinase, catalytic subunit, potentiated the apoptotic and growth inhibitory effects of hydroquinone in proerythroid leukaemia K562 cells (You et al., 2013). Apoptosis was prevented when NQO1 was induced by hydroquinone in KG-1a human promyeloblastic leukaemia cells. Induction of NQO1 by hydroquinone in human bone marrow cells depends on its genotype (Moran et al., 1999); in cells with a T/T genotype, NQO1 activity and protein were not detected (Ross et al., 1996b; Traver et al., 1997). This finding is consistent with the observation that the NQO1 null genotype increases the risk of benzene poisoning (i.e. haematotoxicity) (Rothman et al., 1997).

The benzene metabolite orthoquinone stimulated hyperproliferation of human mononuclear cells cultured with T- and B-cell mitogens (Chakravarti et al., 2006).

(b) Experimental systems

Benzene is reported as a bone marrow depressant as it decreases cell counts in circulating blood, bone marrow, and haematopoietic progenitor cells of animals treated with benzene (IARC, 1982). The cycling fraction of bone marrow or progenitor cells is also suppressed during exposure to benzene, although this suppression is rapidly reversed when exposure to benzene ceases (Moeschlin & Speck, 1967; Irons et al., 1979; Cronkite et al., 1982; Lee & Garner, 1991; Farris et al., 1997a). Suppression of the number of progenitor cells as well as of their cycling fraction is induced by a p53-mediated checkpoint for damaged cells (Kastan et al., 1991; el-Deiry et al., 1994), as evidenced by the lack of suppression of either parameter in the Trp53 knockout mouse (Yoon et al., 2001). On and off regulation of Trp53 therefore results not only in the direct suppression of haemopoiesis but also in a dynamic recovery proliferation after suppression of haemopoiesis during and after benzene exposure in wildtype mice. These dynamic changes may be responsible for the oscillatory proliferation of bone marrow cells to counter any additional epigenetic haematopoietic

neoplastic impacts (Yoon et al., 2001). Indeed, the studies of Snyder and co-workers (Snyder et al., 1981; Dempster & Snyder, 1990) demonstrated that exposing mice to benzene by inhalation for varying periods of time resulted in a growth advantage for granulopoietic cells and proliferation of myeloblasts and/or promyelocytes.

Benzene has been shown to induce apoptosis in murine haematopoietic cells in vitro (Martínez-Velázquez et al., 2006; Gao et al., 2011), as well as spleen cells, femoral B-lymphocytes, and thymic T-lymphocytes in vivo (Farris et al., 1997b; Wen et al., 2016). In mice exposed to benzene by inhalation at 100 ppm for 6 hours per day, 5 days per week for 2 weeks, no change in the level of apoptosis in bone marrow as measured by flow cytometric analysis using Annexin V staining (Faiola et al., 2004) was observed. However, genes involved in apoptosis (Trp53-mediated *caspase 11*, *bax*, and *ccng*) were upregulated in the bone marrow cells of mice exposed to benzene by inhalation at 300 ppm for 6 hours per day, 5 days per week for 2 weeks (Yoon et al., 2003). In mice exposed to benzene by inhalation at 300 ppm for 6 hours per day, 5 days per week, a higher ratio of apoptosis (i.e. Annexin V staining) in bone marrow cells was observed on day 60 after the start of the experiment when compared with control mice (Das et al., 2012). Trp53-mediated gene expression alterations were also observed in the bone marrow cells of mice exposed to benzene by inhalation at 100 ppm for 6 hours per day, 5 days per week for 15 weeks (Boley et al., 2002). Simultaneously, in mice exposed to benzene a reduction of immune function (phagocytic capacity and cytotoxic efficacy) of cells derived from bone marrow, a reduced generation of adherent stromal cells, and a decreased expression of the adhesion molecule (CXCR4) in bone marrow cells were observed, which might be responsible for inducing myelodysplasia (Das et al., 2012).

Inhalation of benzene at 300 ppm for 23 hours per day for 7 consecutive days induced apoptotic changes in the parenchymal components of the lung of Sprague-Dawley rats. An assay for terminal deoxynucleotidyl transferase dUTP nick end-labelling (TUNEL) and electrophoretic analysis of internucleosomal DNA fragmentation of benzene-exposed lung tissue exhibited 180–200 base pairs of laddering subunits, indicative of genomic DNA degradation (Weaver et al., 2007).

4.2.6 Receptor-mediated effects

Although no data on aryl hydrocarbon receptor (AhR) were available in exposed humans or in human cells, several experimental studies in vitro and in vivo have examined the potential role of AhR in benzene carcinogenicity. This transcription factor appears to be involved in the regulation of immature haematopoietic stem or progenitor cell populations, and AhR dysregulation may result in changes to the bone marrow microenvironment that can lead to excessive or unnecessary proliferation (Singh et al., 2009, 2014). Yoon et al. (2002) reported that AhR-knockout ($AhR^{-/-}$) mice do not show any haematotoxicity after exposure to benzene (Yoon et al., 2002). Follow-up studies reported that mice in which the bone was ablated by irradiation and repopulated with marrow cells from AhR-knockout mice did not display any sign of benzene-induced haematotoxicity (Hirabayashi et al., 2008; Hirabayashi & Inoue, 2010). Benzene and its metabolites hydroquinone and benzoquinone did not activate AhR in mouse hepatoma cells in vitro, suggesting that direct interaction with AhR is not involved in these haematotoxic effects (Badham & Winn, 2007).

4.2.7 Chronic inflammation

(a) Humans

TNF-α (an important mediator of inflammation), IL-6 (a pro-inflammatory cytokine), and IL-8 (a chemokine) were studied in 196

rural Indian women who used benzene-contaminated biomass to cook, and compared with 149 age-matched women who cooked with the cleaner fuel of liquefied petroleum gas (Dutta et al., 2013). This study analysed sputum samples and revealed markedly elevated levels of TNF-α (6.9-fold) as well as significantly higher IL-6 and IL-8 levels in the exposed women, suggesting airway inflammation and trafficking of inflammatory cells from circulation to the airways, compared with control women who cooked with the cleaner fuel (Dutta et al., 2013). [The Working Group noted that the women using biomass were not only exposed to benzene, but also to particulate matter of diameter less than 10 μm (PM_{10}) and other toxic chemicals such as formaldehyde, which may also induce inflammation.]

Several studies in human cells in vitro indicate that several benzene metabolites (t,t-MA, hydroquinone, catechol, benzoquinone, and 1,2,4-benzenetriol) may play important roles in the mechanisms of benzene toxicity and inflammation. Hydroquinone (1–10 M) inhibited TNF-α-induced activation of NF-κB in primary human CD4+ T-lymphocytes and in primary human CD19+ B-lymphocytes (Pyatt et al., 1998, 2000). Gillis et al. (2007) showed that benzene metabolites (catechol, hydroquinone, 1,2,4-benzenetriol, and benzoquinone) increased production of pro-inflammatory cytokines (TNF-α and IL-6) in PBMCs. TNF-α production was increased in a dose-dependent manner. Concurrently, suppression of anti-inflammatory cytokine IL-10 expression was also observed in the activated PBMCs treated with higher concentrations of hydroquinone and catechol (Gillis et al., 2007).

(b) Experimental systems

(i) Mouse

Exposure to benzene for 14 days has been demonstrated to affect inflammation in mouse models in two studies. Aoyama (1986) reported that exposure to benzene by inhalation at 200 ppm for 14 days increased ear swelling in BALB/c mice immunized with the contact sensitizer picryl chloride. In mice given benzene by oral gavage at 150 mg/kg bw per day (8 hours per day/5 days a week, for 2 weeks), slight, but not significant, paw swelling was observed in the delayed-type hypersensitivity test (Wen et al., 2016).

Benzene metabolites were also shown to directly induce inflammatory responses in mice. In C57BL/6 female mice given a single subcutaneous injection (100 nmol/mouse), benzoquinone and (to a lesser extent) hydroquinone, but not benzene itself, was observed to increase popliteal lymph node cell count indices 6 days later as determined by popliteal lymph-node assay (Ewens et al., 1999). Bando et al. (2017) also demonstrated the direct inflammatory capacity of hydroquinone. The ears of BALB/c and C57BL/6 mice were observed to swell within 24 hours of dermal application of hydroquinone at concentrations of as low as 1% and 8%, respectively. Further examination revealed accumulation of Th2 cytokines such as IL-4, decreased Th1 cytokines, and increased accumulation of T-, B-, and natural killer cells, total serum IgE, hydroquinone-specific IgE, macrophages, neutrophils, and eosinophils (Bando et al., 2017). In female BALB/c mice, hydroquinone induced IL-4 and IgE and increased total and keyhole limpet haemocyanin-specific IgE (Lee et al., 2002).

(ii) Rat

In a single rat study that examined the effect of 1 hour of dermal exposure to benzene of hairless male rats, occlusive and unocclusive dermal exposure increased erythema at the site of application. Blood IL-1 and skin concentrations of TNF-α increased by 2.4-fold and 3.7-fold, respectively (Chatterjee et al., 2005).

4.2.8 Epigenetic alterations

Epigenetic alterations related to benzene exposure were observed in studies in vivo and in vitro, as reviewed by Zhang et al. (2010), Chappell et al. (2016), and Salemi et al. (2017).

Epigenetic alterations, including DNA methylation and non-coding RNA, were correlated with benzene exposure (Fenga et al., 2016). Occupational or environmental exposure to benzene can produce epigenomic changes. More recently, the effect of benzene exposure on miRNA expression has been reported in occupationally exposed workers (Liu et al., 2016). Downregulation of miR-133a was observed in 50 workers exposed to benzene at 3.50 ± 1.6 mg/m^3 compared with 50 controls exposed to benzene at 0.06 ± 0.01 mg/m^3 (Chen et al., 2016b). Overexpression of miR-221 was observed in PBLs of 97 petrol station attendants exposed to benzene at 0.073 ± 0.02 mg/m^3 compared with 103 controls exposed to benzene at 0.008 ± 0.001 mg/m^3 (Hu et al., 2016).

The results of in vitro studies of benzene-induced changes in DNA methylation are influenced by cell line type and substance used for the specific experiment, that is, benzene or its metabolite. A global DNA hypomethylation was observed in human lymphoblastoid TK6 cells after exposure to benzene at concentrations of 1, 10, and 100 μM (Tabish et al., 2012), and after exposure to hydroquinone at concentrations of 2.5, 5, 10, 15, and 20 μM in a dose-dependent manner (Ji et al., 2010). In human normal hepatic L02 cells a global DNA methylation change was observed only after exposure to hydroquinone and 1,4-benzoquinone, but not to benzene itself or other metabolites (Hu et al., 2014). A reversible poised state of chromatin, identified by the simultaneous presence of histone modifications associated with both gene activation and repression in long interspersed nuclear element-1 (*LINE-1*) sequences, was observed after an in vitro long-term treatment of human myeloid leukaemia HL-60 cell line with a low-concentration dose (1μM (correspond to 110 ng/mL)) of hydroquinone (Mancini et al., 2017). In human leukaemia U937 cells exposed to 1,4-benzoquinone, Chen et al. (2016b) observed dose-dependent alterations in miR-133a expression.

4.2.9 Other mechanisms

Other effects of benzene primarily concern telomere length and transformation. Bassig et al. (2014) reported that workers who had been exposed to high concentrations of benzene (> 31 ppm) had a mean telomere length that was increased by about 10% compared with matched unexposed workers. A study of human lung cells in vitro reported that exposure to benzene (0.01 and 1 μM) increased telomerase activity in the fibroblast-like human lung LL24 cell line, but not in the human adenocarcinoma A549 cell line at higher concentrations (10 and 1000 μM) (Giuliano et al., 2009).

Two studies in vitro examined indicators of transformation. Tsutsui et al. (1997) reported increases in transformed colonies of Syrian hamster embryo cells after treatment with benzene and its metabolites phenol, catechol, or hydroquinone (1–100 μM), and Ibuki & Goto (2004) described anchorage-independent growth in soft agar after treatment of NIH3T3 cells with benzoquinone and hydroquinone.

4.3 Data relevant to comparisons across agents and end-points

This section analyses the responses and/or activity of benzene, its metabolites (Fig. 4.1), and its agents, as evaluated by IARC in a diverse set of in vitro assays performed as part of the United States Environmental Protection Agency Toxicity Forecaster (ToxCast) (Kavlock et al., 2012) and Toxicology in the 21st Century (Tox21) (Tice et al., 2013) initiatives. The inclusion of analyses from high-throughput in vitro assays in the

evaluation of the carcinogenicity of agents has been identified as a priority by IARC (Straif et al., 2014). Consequently, analyses involving these assays have been part of recent *Monographs* that have evaluated the carcinogenicity of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4,4′-dichlorodiphenyltrichloroethane (DDT) (Loomis et al., 2015).

Benzene has not been tested as part of ToxCast and Tox21. The benzene metabolites that have been evaluated are phenol (IARC Group 3), hydroquinone (IARC Group 3), catechol (IARC Group 2B), and 1,4-benzoquinone (IARC Group 3).

Exposure to agents could potentially lead to human cancer through a diverse set of mechanisms. Each individual agent has a specific pathway or a set of pathways leading to a particular kind of cancer. Despite this heterogeneity in the possible mechanisms, Smith et al. (2016) have identified 10 common characteristics of carcinogens by examining all agents classified as Group 1 carcinogens by IARC. The *IARC Monographs* Volume 113 Working Group (Loomis et al., 2015; IARC, 2017) systemically evaluated the assays performed as part of ToxCast and Tox21 and assigned an estimation of activity in each assay for an agent as an indication of 1 of the 10 key characteristics of the carcinogens. In this *Monograph*, we use these same assignments of activities (i.e. mapping of assays) to the key characteristics. Assays were assigned to 6 out of the 10 key characteristics of carcinogens, namely: is electrophilic or can undergo metabolic activation (31 end-points); induces epigenetic alterations (11 end-points); induces oxidative stress (18 end-points); induces chronic inflammation (45 end-points); modulates receptor-mediated effects (92 end-points); and alters cell proliferation, cell death, or nutrient supply (68 end-points).

The 10 key characteristics are listed in full as follows.

(1) *Is electrophilic or can be metabolically activated*: 31 assay end-points consisting of CYP biochemical activity assays and aromatase, which regulates conversion of androgens to estrogens. [The Working Group noted that these assays largely indicate inhibition of CYP activity, and do not directly measure metabolic activation or electrophilicity.]

(2) *Is genotoxic*: 0 assay end-points.

(3) Alters DNA repair or causes genomic instability: 0 assay end-points.

(4) *Induces epigenetic alterations*: 11 assay end-points including 4 DNA-binding assays in HepG2 liver cell lines, biochemical assays targeting histone deacetylases, and other enzymes modifying chromatin, as well as cellular transcription factor assays involved in epigenetic regulation. [The Working Group noted these end-points have not been extensively validated with reference compounds for epigenetic alterations.]

(5) *Induces oxidative stress*: 18 assay end-points, all cellular assays, targeting nuclear erythroid-related factor-2, antioxidant response element, and other stress-related transcription factors, as well as protein upregulation in response to ROS.

(6) *Induces chronic inflammation*: 45 assay end-points, mostly using primary human cells, measuring protein expression levels indicative of inflammatory responses, including cytokines, cell adhesion molecules, and NF-κB. [The Working Group noted these in vitro end-points are short-term assays and therefore not directly indicative of chronic inflammation.]

(7) *Is immunosuppressive*: 0 assay end-points.

(8) *Modulates receptor-mediated effects*: 92 assay end-points targeting nuclear receptors (e.g. AhR, androgen receptor (AR), estrogen receptor (ER), farnesoid X receptor, peroxisome proliferator-activated receptor

(PPAR), pregnane X receptor (PXR), and retinoic acid receptor, among others) in cellular assays for transactivation, receptor dimerization, and nuclear translocation, as well as biochemical radioligand binding assays and coregulatory recruitment assays.

(9) *Causes immortalization*: 0 assay end-points.

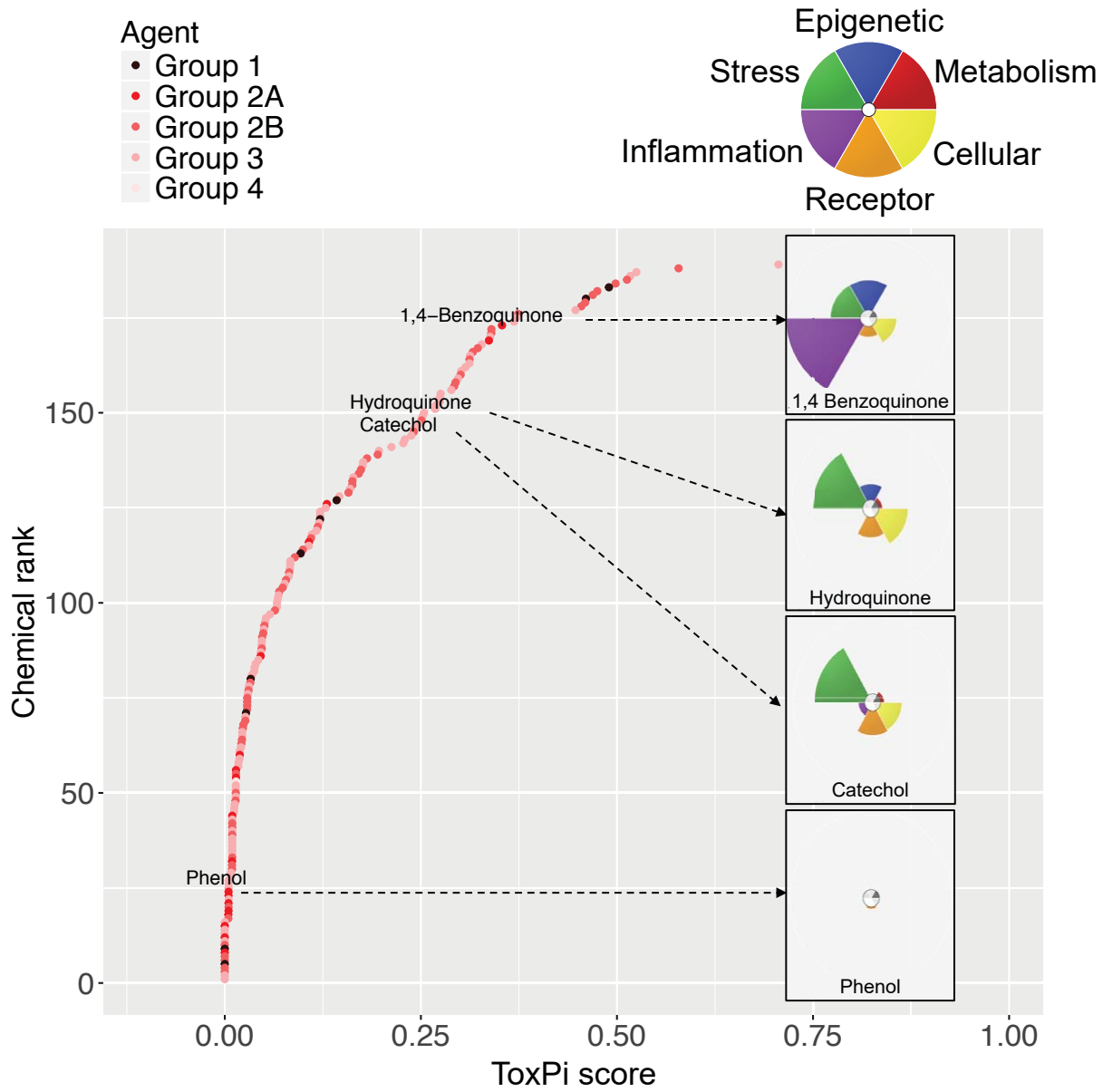
(10) *Alters cell proliferation, cell death, or nutrient supply*: 68 assay end-points measuring cell cycle markers, proliferation, cytotoxicity, and mitochondrial toxicity by a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

The activity of an agent on a given assay is determined by the statistical significance of the association between all tested concentrations of the agent with assay response (Sipes et al., 2013). The activity of each agent in each assay and across groups of assays was summarized using the Toxicological Prioritization Index (ToxPi) approach (Reif et al., 2010). In the Working Group's analysis, each agent–assay pair was summarized as "active" (1) or "inactive" (0). Within each key characteristic slice for a given agent, the distance from the origin represents the relative agent-elicited activity of the component assays (i.e. slices extending further from the origin were associated with "active" calls on more assays). The overall score of an agent is the aggregation of all slice-wise scores (Fig. 4.2, inset) and provides an activity ranking relative to the 189 agents screened in ToxCast/Tox21 that have been evaluated in the *IARC Monographs* (Fig. 4.3, rank chart).

A tabular and graphical summary of the results is given in the supplementary material (Annex 1), and a summary for each relevant compound follows. Note that the activity calls across these assays represent exposure to each of the four individual metabolites, and may not necessarily be indicative of exposure to benzene or its other metabolites.

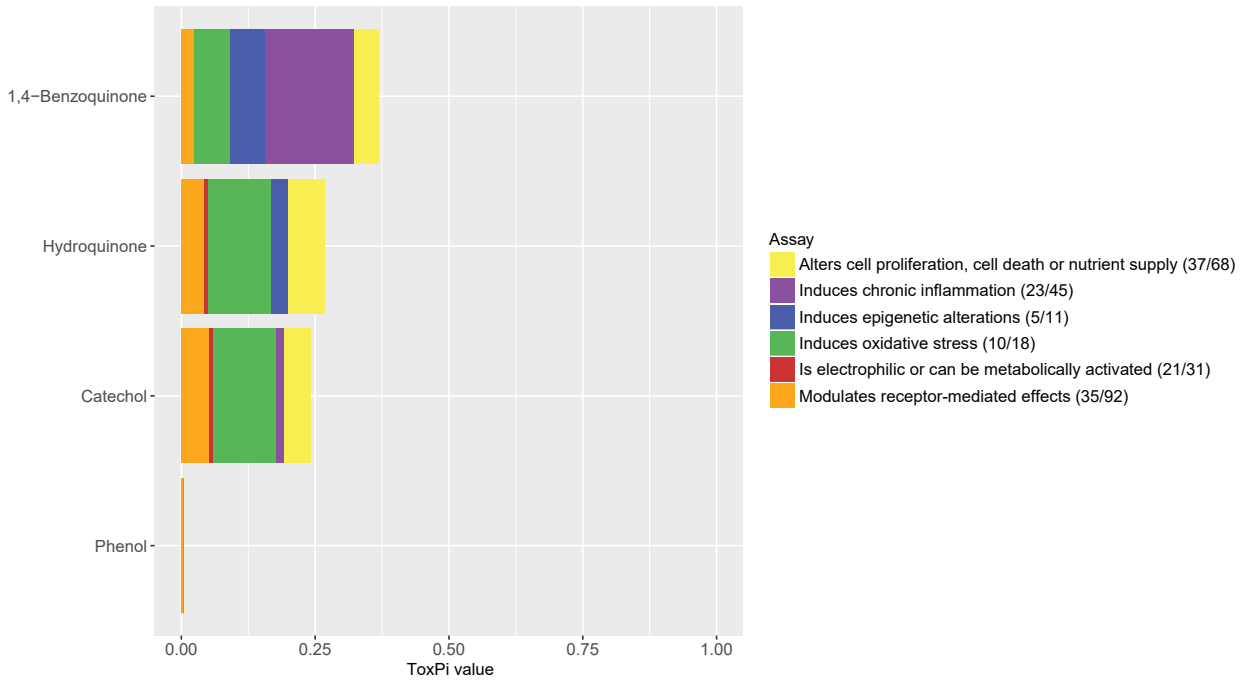
1,4-Benzoquinone (Chemical Abstracts Service, CAS, Registration No. 106-51-4) has the highest ToxPi value among the benzene metabolites evaluated, and has the 16th highest value among the 189 agents (16/189) evaluated by IARC (see Fig. 4.2). The largest contribution to this ToxPi value is from active hits to assays mapped to the "*Induces chronic inflammation*" category (Fig. 4.2). This represents the maximum number of hits to this category among the 189 evaluated IARC agents. Exposure results in upregulation of cell adhesion proteins E-selectin, P-selectin, and vascular cell adhesion molecule 1 (VCAM1), and in the upregulation of chemokines and cytokines such as CXCL9, CXC10, CCL2, IL-1a, IL-8, TNFα, CD38, CD40, and CD69 in multiple human cell cultures and co-cell cultures. NF-κb is also upregulated in the HepG2 cell line. The second-largest contributor to the ToxPi value derives from active hits mapped to the "*Induces epigenetic alterations*" category, with two hits out of four assays mapping to DNA binding (the seven assays associated with measuring chromatin alterations were not performed). Assays indicating upregulation of matrix metalloproteinase 1 in two cells cultures are linked to the "*Induces oxidative stress*" category, and the tissue inhibitor of metalloproteinases 2 is also upregulated in a co-culture involving one of the former lines. Assays suggesting upregulation of hypoxia-inducible factor-a and metal regulatory transcription factor-1 in HepG2 liver cell lines are also linked to 1,4-benzoquinone exposure. Hits associated with upregulation of six genes, including *TGFβ1*, *MYC*, and vascular endothelial growth factor *VEGFRII*, which are markers of cell-cycle across multiple platforms, downregulation of cellular proliferation across multiple cell-cultures as assayed by the sulforhodamine B colorimetric assay, and upregulation of two markers of cell proliferation were mapped to the "*Alters cell proliferation, cell death, or nutrient supply*" category. Upregulation of the gene expression of six receptors, including AhR, AR,

Fig. 4.2 ToxPi ranking for benzene metabolites phenol, catechol, hydroquinone, and 1,4-benzoquinone and all agents evaluated by IARC with available data using ToxCast and Tox21 assay end-points mapped to six key characteristics of carcinogens



Inset are the ToxPi diagrams for these metabolites. ToxPi diagram colour coding is provided in the legend. Each agent in the rank chart is plotted in a colour according to its IARC classification

Fig. 4.3 Stacked bar plots of ToxPi values by assay category for benzene metabolites phenol, catechol, hydroquinone, and 1,4-benzoquinone using ToxCast and Tox21 assay end-points mapped to six key characteristics of carcinogens



The numbers in the parentheses next to each category represent the ratio of the maximum number of hits to assays assigned to category over 189 IARC agents to the total number of assays assigned to the category

glucocorticoid receptor, and retinoid X receptor, all on the Attagene platform performed on HepG2 liver cell line, were linked to the "*Modulates receptor-mediated effects*" component of the ToxPi value.

Hydroquinone (CAS 123-31-9) has the second-highest ToxPi value of the benzene metabolites and the 39th highest ToxPi value among the 189 agents (39/189) evaluated by IARC (see Fig. 4.2). The largest contributor to this value came from active hits linked to the "*Induces oxidative stress*" category. This represents the ninth-highest number of hits to this category among the 189 evaluated IARC agents. This category contains assays mapped to regulation of matrix metalloproteinase 1 in one of the three cell cultures, regulation of oxidative stress and stress kinase after 72 hours of exposure, and four (hypoxia-inducible factor-a, metal regulatory transcription factor-1, nuclear erythroid-related factor-2, and antioxidant response element) out of six markers of oxidative stress measured in three separate assay platforms (Apredica, Attagene, and Tox21). The second-largest contributor came from hits linked to the "*Alters cell proliferation, cell death, or nutrient supply*" category, with indications of upregulation of cell cycle, downregulation of proliferation, and upregulation of mitochondrial toxicity. For assays assigned to "*Modulates receptor-mediated effects*" there are active hits for AhR, AR, glucocorticoid receptor, PPAR-response element, PXR-vitamin D response element, ER, and PPARγ. One out of the four assays associated with DNA binding (from the "*Induces epigenetic*

alterations" category) is active for hydroquinone exposure.

Catechol (CAS 120-80-9) has the third-highest ToxPi value of the benzene metabolites and the 45th highest ToxPi value among the 189 agents (45/189) evaluated by IARC (see Fig. 4.2). The largest contributor to this value came from the exact same active hits as for hydroquinone in assays linked to the "*Induces oxidative stress*" category. For assays assigned to "*Modulates receptor-mediated effects*" there are active hits for AhR, AR, ER, and PPAR. The assays linked to "*Alters cell proliferation, cell death, or nutrient supply*" category suggested upregulation of cell cycle and downregulation of proliferation across multiple cell cultures.

Phenol (CAS 108-95-2) was only active on a biochemical assay for ER, with no activity hits on any other assays assigned to the six categories of carcinogenicity.

In conclusion, 1,4-benzoquinone is the benzene metabolite most strongly associated with assays mapped to the six key characteristics of carcinogens, and with the "*Induces chronic inflammation*" category in particular. Hydroquinone and catechol showed a moderate number of hits. Phenol showed activity on only one assay. These four benzene metabolites were tested in different phases of the ToxCast or Tox21 programmes, resulting in different percentages of missing data (i.e. "not-tested" in a given assay). Relative to the full assay set, 1,4-benzoquinone was tested in 46% of all assays and catechol, hydroquinone, and phenol were tested in 88% of all assays. [The Working Group noted that a reanalysis of data, in which only assays with data for most of the full list of IARC chemicals tested were considered, resulted in similar ToxPi scores.]

4.4 Observed exposure–response relationships in mechanistic studies

Based on the *Report of the IARC Advisory Group to Recommend on Quantitative Risk Characterization* (IARC, 2014), a more detailed review of the availability of exposure–response information for mechanistic and other data from studies in exposed humans was performed. The purpose of this review was to explore the observed exposure–response relationships by summarizing information across studies on the magnitudes of response (e.g. relative percentage change) and the corresponding levels of exposure.

First, the representative studies of human benzene exposure that were the focus of Sections 4.1 and Section 4.2 relating to the key characteristics of carcinogens were further reviewed for availability of information relating level of exposure to degree of response. Only studies relating to key characteristics of carcinogens for which there was strong evidence in exposed humans were considered (see Section 5.4). Additional considerations for selecting studies included the availability of multiple exposure categories with associated measurements of benzene concentrations in air, adequate sample size, consideration of potentially confounding co-exposures, and/or completeness of reporting. Candidate studies were evaluated for their adequacy in terms of exposure assessment.

Based on these considerations, it was determined that exposure–response information was available for the key characteristics of carcinogens of "is genotoxic" and "is immunosuppressive", the second of which includes measures of haematotoxicity. Specifically, exposure–response information was available for the end-points for which there was strong evidence in exposed humans (see Section 5.4): (i) genotoxicity (oxidative DNA damage, indicated by 8-OHdG, and

chromosomal effects, indicated by MN and CAs); and (ii) immunosuppression and haematotoxicity (peripheral pluripotent stem cell and leukocyte counts).

Representative studies, independent of the presence or direction of a statistically significant effect, were then selected (see Section 4.2), and numbers in the exposed and reference groups, duration of exposure, and level of exposure among those exposed were examined. Further, for each end-point the measure of response, the evidence of an exposure–response gradient, the central tendency and measure of variance of end-point in the reference group, and the measure of exposure were all considered. Finally, considerations of each exposure category included: the central tendency and measure of variance of exposure; the central tendency of difference in response from the reference group as a percentage change (i.e. (mean of category – mean of the reference group)/mean of the reference group); and a test for significance of difference from the reference group (e.g. *t*-test) and *P* value.

[The Working Group noted that, because representative studies were selected, the existence of additional mechanistic studies with exposure–response information cannot be excluded. The possibility of publication bias on mechanistic end-points also cannot be excluded.]

4.4.1 Genotoxicity

Two studies with exposure–response information for oxidative DNA damage (8-OHdG) were selected (Lagorio et al., 1994; Liu et al., 1996). Both had statistically significant exposure–response trends, but the population in Lagorio et al. (1994) were exposed to benzene at much lower concentrations (mean, 0.45 mg/m^3 or 0.14 ppm) compared with those in Liu et al. (1996) (mean, 166.1 mg/m^3 or 51 ppm). Of the two studies, only the study conducted by Liu et al. (1996) divided exposed populations into categories; the group exposed to low concentrations (mean, 2.46 mg/m^3 or 0.76 ppm) was not statistically different, with an effect size of 25%, and the group exposed to medium concentrations (mean, 103.3 mg/m^3 or 31 ppm) was statistically significantly increased, with an effect size of 600%.

Four studies with exposure–response information for MN were selected (Liu et al., 1996; Rekhadevi et al., 2011; Zhang et al., 2014, 2016). [The Working Group noted that the study by Rekhadevi et al. (2011) was not informative for exposure–response because it had a narrow range of benzene exposures (1.1–1.5 mg/m^3 or 0.34–0.46 ppm) that was further divided into three categories.] All studies except for that of Rekhadevi et al. (2011) tested for exposure–response trends, all of which were statistically significant. Exposures in the Rekhadevi et al. (2011) (mean, 1.32 mg/m^3 or 0.41 ppm), Zhang et al. (2016) (median, 1.6 ppm), and Zhang et al. (2014) (median, 6.4 mg/m^3 or 2 ppm) studies were lower than those of the Liu et al. (1996) study (mean, 166.1 mg/m^3 or 51 ppm). Three of these studies divided exposed populations into categories that were compared with the reference group and, in each case, the group exposed to the lowest concentrations demonstrated a statistically significant increase in MN compared with the reference group, with effect sizes of 45–55% (Liu et al., 1996; Zhang et al., 2014, 2016).

Numerous publications with exposure–response information for CAs were selected (Bogadi-Sare et al., 1997; Zhang et al., 1998b, 2007, 2011, 2012b; Kim et al., 2004b; Xing et al., 2010; Rekhadevi et al., 2011; Marchetti et al., 2012), although many were of the same study population. All had statistically significant exposure–response trends with the exception of Rekhadevi et al. (2011) (see Working Group comment, above) and Bogadi-Sare et al. (1997). Exposure concentrations considered by Rekhadevi et al. (2011) (mean, 1.322 mg/m^3 or 0.41 ppm), Kim et al. (2004b) (geometric mean, 0.56 ppm), Marchetti et al. (2012) (mean, 2.8 ppm), Xing et al. (2010) (median, 2.9 ppm), and Bogadi-Sare et al. (1997)

(median, 5.9 ppm) were lower than those in the Zhang et al. studies (median, 10–31 ppm). Among the studies of exposure to lower concentrations, two divided exposed populations into categories that were compared with the reference group: Xing et al. (2010) and Marchetti et al. (2012). The response of the group exposed to the lowest concentration (median, 1.2 ppm) in the study by Marchetti et al. (2012) was statistically significantly different from the reference group, with an effect size of 33%. In the case of Xing et al. (2010), the group exposed to the lowest concentration (median, 1.0 ppm) was not statistically different with an effect size of 50%; the group exposed to the higher concentration (median, 7.7 ppm) was statistically significantly increased, with an effect size of 70%.

[The Working Group noted that, in the majority of studies examined, an exposure–response gradient between benzene exposure and both MN and CAs was reported.]

4.4.2 Immunosuppression and haematotoxicity

Numerous studies with exposure–response information for leukocyte counts were selected (Liu et al., 1996; Rothman et al., 1996; Ward et al., 1996; Qu et al., 2002; Lan et al., 2004; Robert Schnatter et al., 2010; Swaen et al., 2010; Zhang et al., 2016). All had statistically significant exposure–response trends with the exception of the study by Swaen et al. (2010), which was not statistically significant, and by Liu et al. (1996), in which no trend test was performed. Exposures in the studies by Swaen et al. (2010) (mean, 0.22 ppm), Zhang et al. (2016) (median, 1.6 ppm), Robert Schnatter et al. (2010) (median, 2.3 ppm), Qu et al. (2002) (mean, 3.8 ppm), and Lan et al. (2004) (mean, 5.1 ppm) were lower than in the studies by Rothman et al. (1996) (median, 31 ppm) and Liu et al. (1996) (mean, 166.1 mg/m^3 or 51 ppm). Ward et al. (1996) only reported the maximum exposure level (34 ppm).

Six studies divided exposed populations into categories (Liu et al., 1996; Rothman et al., 1996; Qu et al., 2002; Lan et al., 2004; Swaen et al., 2010; Zhang et al., 2016). The groups exposed to the lowest concentrations in these studies had effect sizes ranging from a 0.8% increase to a 14.5% decrease in leukocytes, with the effects observed in Lan et al. (2004) (14.5% decrease at a mean exposure of 0.57 ppm) and Qu et al. (2002) (4.3% decrease at a mean exposure of 3.07 ppm) being statistically significant. In the case of Swaen et al. (2010), none of the groups (< 0.5 ppm, 0.5–1.0 ppm, > 1.0 ppm) demonstrated statistically significant changes (effects ranging from 1% increase to 1% decrease). In Zhang et al. (2016), the groups exposed to the two lowest concentrations (3.55 ppm-yr and 6.51 ppm-yr) had non-significant decreases of 5.3%, whereas the third exposure group (10.72 ppm-yr) had a significant decrease of 11.2%. In Liu et al. (1996), the lowest exposure category (mean, 2.46 mg/m^3 or 0.76 ppm) demonstrated a 0% change, but the middle exposure category (mean, 103.3 mg/m^3 or 31 ppm) showed a 17% decrease (no statistical tests were performed). In Rothman et al. (1996), the group exposed to the lower concentration (median, 13.6 ppm) demonstrated a non-significant decrease of 5.8%, whereas the group exposed to the higher concentration (median, 91.9 ppm) had a significant decrease of 17.6%.

[The Working Group noted that, in the majority of studies examined, an exposure–response gradient between exposure to benzene and leukocyte count was reported. Some other studies discussed in Section 4.2.4(a), which evaluated populations exposed to relatively low levels of benzene, reported no effects on leukocytes; these studies are not included here, however, because they were not informative for exposure–response analyses (e.g. they only compared all levels of exposure with the reference group).]

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5. SUMMARY OF DATA REPORTED

5.1 Exposure data

Benzene is the simplest aromatic hydrocarbon. It is a volatile and ubiquitous air pollutant, mainly arising from anthropogenic sources such as combustion processes. It is found in crude oil and hence in petroleum products. Historically, benzene was used as a solvent, for example in glues and paints, and also in the rubber and chemical industries. At the present time, benzene is a high production volume chemical, despite being banned in consumer products in many countries. Its primary use is in the synthesis of ethylbenzene, used in plastics manufacturing.

Benzene exposure may occur in several industries and occupations, including the petroleum, chemical, and manufacturing industries, and coke making. In low- and middle-income countries, exposure may still occur in several industrial sectors, including shoemaking, painting, printing, and rubber product manufacturing. It is a component of gasoline, vehicular exhaust, industrial emissions, and tobacco smoke, all sources of environmental exposure.

Exposure to benzene mainly occurs via inhalation, but skin absorption is also possible. Benzene can be measured in both workplace and community settings using air monitoring and personal monitoring as well as biomonitoring, capturing all routes of exposure. Urinary *S*-phenylmercapturic acid (SPMA) and unmetabolized benzene in blood and urine are specific biomarkers, whereas *trans*,*trans*-muconic acid is not. The current preferred method for biomonitoring is to measure urinary benzene and SPMA; these measurements are non-invasive and reflect daily benzene exposure.

Occupational exposure to benzene is regulated in many countries, and there are also some environmental guidelines for benzene in air and drinking-water.

Full-shift occupational exposures in high-income countries are usually less than 1 ppm (3.19 mg/m^3) for most industries and occupational groups, including the upstream and downstream petroleum industry and automobile repair, and for diverse workers exposed to vehicle exhausts. However, workers in most of these industries may conduct short-term tasks that possibly result in exposure to high concentrations of benzene, such as maintenance activities where pipelines are open, tank cleaning, or top filling of road tankers with gasoline. Data from low-and middle-income countries are sparse; however, exposures considerably higher than those described above have been reported from China.

Environmental air levels, as determined at fixed monitoring sites, are generally orders of magnitude lower than occupational exposures. There is evidence that outdoor air levels have declined significantly over time in both Europe and the USA, where annual average concentrations are currently less than 5 μg/m^3; however, higher concentrations are measured in some cities in other regions of the world.

For occupational cancer epidemiology, high-quality exposure assessments use benzene measurements to derive individual exposure estimates. In studies of occupational exposures, these estimates represent long-term exposure in the workplace. Participants should have complete and detailed job histories for which the measured data are applied. For environmental (air pollution) epidemiology, exposure assessment typically relies on measurements of benzene in outdoor air collected from routine monitoring stations, or from modelled ambient concentrations for geographically defined gridded areas. These spatially referenced data for temporally relevant critical windows are linked to geocoded residences of study participants and used to generate individual-level estimates of benzene exposure.

5.2 Human carcinogenicity data

5.2.1 Acute myeloid leukaemia

The classification of benzene as a Group 1 carcinogen in previous *IARC Monographs* was based on sufficient evidence of an association between benzene exposure and risk of acute myeloid leukaemia (AML) and/or acute non-lymphocytic leukaemia (ANLL). This conclusion was supported by several occupational cohort studies that collected quantitative exposure data, revealing exposure–response trends between benzene exposure and AML and/or ANLL. According to the recent WHO classification of AML, related neoplasms are included in this category as AML not otherwise specified (e.g. pure erythroid leukaemia, acute megakaryoblastic leukaemia, and acute monocytic leukaemia). The following discussion referring to AML therefore includes ANLL.

Occupational and general-population studies published since the previous *IARC Monographs* on benzene, including two studies in occupational cohorts with careful assessment of benzene exposure, confirm the association between AML and exposure to benzene, and also demonstrate an exposure–response trend with quantitative exposure metrics.

5.2.2 Chronic myeloid leukaemia

Several cohort studies in the petroleum industry and other settings demonstrated increased risks for chronic myeloid leukaemia (CML). Other studies showed no evidence of an association, including two studies that were previously included in *IARC Monographs* volume 100F with quantitative estimates of exposure to benzene but did not report any exposure–response relationship.

An elevated risk of CML was reported in two new publications of occupational cohort studies with extended follow-ups, and a significant exposure–response trend was seen in the study that evaluated exposure–response. Among the four studies judged to be the most informative by the Working Group, the point estimates were above the null for all; however, only three studies included 6 or more exposed cases. The Working Group further noted a lack of clear evidence of an exposure–response gradient in the four available studies. Other co-exposures were present, but the potential for confounding could not be assessed.

5.2.3 Non-Hodgkin lymphoma

The broad category of non-Hodgkin lymphoma (NHL) includes chronic lymphocytic leukaemia (CLL), multiple myeloma (MM), and acute lymphocytic leukaemia (ALL), as well as follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and hairy cell leukaemia. In considering the data available at the time, the Working Group of *IARC Monographs* Volume 100F concluded that there was *limited evidence* in humans that benzene causes NHL. The current Working Group

examined all of the pertinent studies published before and after Volume 100F. In doing so, the Working Group assessed the quality of the old and new studies and noted that several high-quality cohort studies provided data for NHL in occupational settings and in the general population. These studies showed elevated relative risks for NHL as categorized in the studies, which were statistically significant in two studies. Two of these studies were conducted in China, and with relatively high levels of exposure to benzene in one occupational cohort. The studies that reported on NHL used different classifications of lymphoma, which varied over time and between studies. The Working Group therefore noted that associations were observed between benzene exposure and a heterogeneous classification of NHL.

CLL is currently included as a subgroup of NHL, but in the past it was generally considered as a separate entity (not always reported as such in papers). As noted in *IARC Monographs* Volume 100F, CLL can be an indolent disease of the elderly; this raises questions about cohorts that are not followed up until the study population is relatively old, and about studies that use mortality instead of incidence data. The diagnostic accuracy of CLL has also improved over time. Because of these concerns, the Working Group accorded the greatest weight to recent studies and those that reported incidence data; these included four studies (two occupational cohorts, and case–control studies in Italy and China) that used current classifications for lymphomas, including CLL. Three of these studies (three occupational cohorts) found positive associations between CLL and exposure to benzene, but the 95% confidence intervals included the null. Confounding from other occupational exposures was judged to be unlikely in these studies.

MM was considered separately in one case–control study and in nine occupational cohort studies. The numbers of exposed cases were generally small. Elevated relative risks were observed in four studies. The remaining studies did not find robust positive associations, but some showed elevated risks in the exposure category of highest concentration.

In *IARC Monographs* Volume 100F, the evidence of an association between benzene and ALL in adults was regarded as limited, based on a few occupational cohorts that included very small numbers of exposed cases and reported increased risks that were not statistically significant. Data for adult ALL and benzene exposure remain sparse: only one occupational cohort study has reported on ALL after the publication of the previous review. That study reported a non-significantly elevated risk based on a few incident cases, and did not provide exposure–response results. Among all of the included studies of adult ALL, the magnitude of the risk ratio estimates ranged from 0.8 to 4.5, and all confidence intervals included the null.

Other specific subtypes of NHL were reported in a few studies, including outcomes such as DLBCL, follicular lymphoma, and mantle B-cell lymphoma, but results were inconsistent.

Overall, the Working Group concluded that the evidence of carcinogenicity for NHL is *limited*; however, a small minority of the Working Group concluded that the evidence of carcinogenicity is *sufficient* for NHL.[1]

[1] The meta-analysis of five incidence studies revealed a statistically significant association between benzene exposure and CLL (pooled relative risk estimate, 1.53; 95% CI, 1.04–2.25). The relative risk estimates of four of these five studies were above 1.6; only one study found a slightly lower risk estimate of 1.40 (95% CI, 0.90–2.19). Therefore, the CLL risk is comparable to the AML risk (pooled risk estimate, 1.45; 95% CI, 0.96–2.17 when combining the six incidence studies). Chance and confounding can be ruled out with reasonable confidence. *(continued on next page)*

5.2.4 Childhood cancer

Information about a possible association between environmental benzene exposure and childhood cancer derives mainly from case–control studies. Seven studies investigated leukaemia, two studies reported on tumours of the central nervous system, and there were single studies published for each of lymphoma, Wilms tumour, retinoblastoma, and neuroblastoma. The seven studies reporting on leukaemia were inconsistent in the definition of leukaemia (including a variation over all leukaemias, acute leukaemia, ALL, and AML) and reported heterogeneous results for association with benzene exposure. The Working Group noted that all four studies reporting separate results for ALL and AML showed associations between benzene and AML in children (although most associations were not statistically significant), but no or weaker associations with ALL. Parental exposures to benzene before or during pregnancy were also considered in several studies of both cohort and case–control format. The Working Group noted a consistent association between exposure to benzene and AML for children, and coherence with findings for adult AML and benzene exposure, but could not rule out chance, bias, and confounding as alternative explanations.

5.2.5 Cancer of the lung

Several epidemiological studies of workers exposed to benzene have examined cancer of the lung. The most informative studies, which include those with larger cohort sizes, longer follow-up times, and either larger numbers of workers exposed to high concentrations or better-quality exposure assessments, have all reported statistically significant excesses of cancer of the lung among workers exposed to benzene. Positive trends between cumulative exposure to benzene and cancer of the lung were reported in two of these studies. However, none of these studies controlled for potential confounding by smoking or by occupational exposure to other lung carcinogens. The Working Group noted that smoking is a strong risk factor for cancer of the lung, and an important potential confounder of this association; in addition, the workers in these cohorts were potentially exposed to other occupational lung carcinogens.

Overall, the Working Group concluded that the evidence of carcinogenicity for the lung is *limited*; however, a small minority of the Working Group concluded that the evidence of carcinogenicity is *inadequate* for the lung.

5.2.6 Other cancers

Occupational cohort studies also reported data for several other cancer types and tumour sites, including cancer of the: nasal cavity, pharynx, larynx, and related sites; oesophagus;

(continued from previous page) Most studies show a positive association between benzene exposure and NHL incidence. Among the incidence studies, there are three high-quality cohort studies, which reveal clearly increased NHL risks: one found a relative risk of 3.90 (95% CI, 1.31–11.57) for ever versus never exposure to benzene (not including CLL and MM), another reported a hazard ratio of 1.86 (95% CI, 1.17–2.96) including MM but not CLL, and a third reported a hazard ratio of 1.49 (95% CI, 0.90–2.47) for the whole NHL group (including MM and CLL).
Most older studies, as well as some newer studies, did not include MM and CLL in the NHL group. However, the inclusion of CLL and MM in the NHL entity in these studies should not have changed the finding of a clear association between benzene and NHL, for the following two reasons. First, studies allowing for a direct comparison between MM and the (remaining or total) NHL group give no overall indication for lower MM risks compared with NHL risks as a whole. Second, as noted above, CLL risks are also clearly elevated.

stomach; colon, rectum, and anus; pancreas; kidney; liver and biliary tract; prostate; bladder, brain, and central nervous system; and skin. Each of these cancers was addressed in a small number of studies. For each cancer site, results were inconsistent across studies, exposure–response data were generally lacking, and potential confounding from other occupational exposures and behavioural factors was typically not controlled.

5.2.7 Quantitative data

Meta-regression analysis of data from six occupational cohort studies strongly supported a linear exposure–response relationship for AML and cumulative exposure to benzene.

5.3 Animal carcinogenicity data

There were 17 studies that reported on the effects of benzene inhalation in male and female mice. Several studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in mice exposed to benzene.

In one study in male and female mice, benzene caused significant increases in the incidence of myelogenous neoplasms (myeloid leukaemia) and solid tumours (other than of the liver or lymphomas) in males and females. In a second study in male mice, there was a significant increase in the incidence of solid tumours (other than of the liver or lymphomas). In a third study in male mice, there was a significant increase in the incidence of malignant lymphoma, squamous cell carcinoma of the preputial gland, carcinoma of the Zymbal gland, squamous cell carcinoma of the forestomach, and adenoma of the lung. In a fourth study in male mice, there was a significant increase in the incidence of lymphoma of the thymus gland (with a significant positive trend), and neoplasms of the haematopoietic and lymphoid tissues. In a fifth study in male mice, there was a significant positive trend in the incidence of lymphoma of the thymus gland. In a sixth study in male mice, there was a significant increase in the incidence of neoplasms of the haematopoietic tissues. In a seventh study in male mice, with a short duration of exposure, there was a significant increase in the incidence of adenoma of the lung. In an eighth study in male mice, there was a significant increase in the incidence of leukaemia or lymphoma (combined) and of adenoma of the lung. In a ninth study in male mice, there was a significant increase in the incidence of carcinoma of the Zymbal gland. Seven other studies were negative. One study was considered inadequate for the evaluation.

There were four oral administration (gavage) and two intraperitoneal studies of benzene in male and female mice. Some studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in mice exposed to benzene.

In a first study in which benzene was administered by gavage, benzene caused a significant increase in the incidence of the following lesions in males and females: bronchioloalveolar adenoma and carcinoma, hepatocellular adenoma or carcinoma (combined), squamous cell carcinoma of the Zymbal gland, adenoma or carcinoma (combined) of the Harderian gland, lymphoma or leukaemia (combined), and squamous cell papilloma of the forestomach. In males only, benzene caused a significant increase in the incidence of hepatocellular carcinoma, adenoma of the Harderian gland, carcinoma of the preputial gland, squamous cell papilloma or carcinoma (combined) of the forestomach, and pheochromocytoma of the adrenal gland. In females only, benzene induced hepatocellular adenoma, carcinoma of the Harderian gland, tubular adenoma of the ovary, mixed tumours (benign) of the ovary, tumour (benign or malignant) of the granulosa cell, and carcinoma and carcinosarcoma of the mammary gland.

In a second gavage study, benzene caused a significant increase in the incidence of tumours of the lung in males and females, and of carcinoma of the mammary gland in females. In a third gavage study, there was a significant increase in the incidence of tumours of the lung and leukaemia in males and females, and of carcinoma of the mammary gland in females. In a fourth gavage study in male and female mice, strain A/J, benzene induced a significant increase in the multiplicity of adenoma of the lung in males.

In a first study in which benzene was administered by intraperitoneal injection, there was a significant increase in the incidence and multiplicity of adenoma of the lung in male A/J mice but not in females. In a second (transplacental) study there was a significant increase in the incidence of tumours of the liver in the male offspring, and of lesions of the haematopoietic and lymphoid tissues (hyperplasia, myeloproliferative disorders, and myeloid/lymphoid neoplasia, combined) in the female offspring of pregnant mice given benzene intraperitoneally.

There were five studies of the carcinogenicity of benzene in rats: four oral administration studies (by gavage of males and females of different strains, i.e. Sprague-Dawley, Wistar, and F344) and one inhalation study in Sprague-Dawley rats (in pregnant females and their male and female offspring). All studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in rats exposed to benzene.

Benzene significantly increased the incidence of carcinoma of the Zymbal gland in male and/or female rats in four gavage studies, and in male and female offspring in a study of transplacental exposure followed by inhalation. It also significantly increased the incidence of squamous cell carcinoma of the oral cavity (including lip and tongue) in males and females in two gavage studies, and in the female offspring in the study of transplacental exposure followed by inhalation. Exposure to benzene significantly increased the incidence of carcinoma in situ of the forestomach in females and of acanthoma or squamous cell dysplasia (combined) of the forestomach in males and females in one gavage study, and carcinoma in situ of the forestomach in the female offspring in the study of transplacental exposure followed by inhalation. A significantly increased incidence of hepatocellular carcinoma was observed in the female offspring in the study of transplacental exposure followed by inhalation. Benzene caused a significant positive trend in the incidence of tumours of the haematopoietic and lymphoid tissues in males in one of the gavage studies, and a significant increased incidence of those same tumours in female offspring in the study of transplacental exposure followed by inhalation. There were also significant increases in the incidence of carcinoma of the skin in males in two gavage studies and of stromal polyps of the endometrium in females in one gavage study.

There were 12 studies that reported on neoplasms and preneoplastic effects induced by benzene (three whole-body inhalation, three oral administration (gavage), and six skin application studies) in one or both sexes of four different genetically modified mouse models of different genetic backgrounds. It was demonstrated that benzene induced cancer in different tissues (including tumours of the haematopoietic and lymphoid tissues) of genetically modified mice, depending upon the route of exposure.

In inhalation studies, B6.CBA-*Trp53*^{tm1Sia} haploinsufficient congenic inbred mice showed significant exposure-related increases in the incidence of lymphoma of the thymus gland in one study; C3.CBA-*Trp53*^{tm1Sia} congenic mice demonstrated a significant exposure-related increase in the incidence of lymphoma of the thymus gland, non-thymic lymphoma, and myeloid leukaemia in another study. One inhalation study in C57BL/6 h-Trx-Tg mice was negative.

In studies of B6.129-*Trp53*^{tm1Bra} N5 haploinsufficient mice exposed to benzene by gavage, increases in the incidence of sarcomas of the subcutis were observed in one study and atypical hyperplasia of the thymus gland in another. In another model of a haploinsufficient mouse with tumour-suppressor gene (the B6.129-*Cdkn2a*^{tm1Dep} congenic), oral exposure to benzene by gavage was associated with a significant dose-related increase in malignant lymphoma in males, but not in females.

Benzene application to the skin of female v-Ha-*Ras* mice resulted in a significant and rapid development of exposure-related squamous cell papillomas of the skin in one study, and of a significant increase in the incidence of granulocytic leukaemia in another; all other skin application studies were negative or inadequate for the evaluation.

5.4 Mechanistic and other relevant data

Benzene is well absorbed via inhalation as well as by oral and dermal exposure in all species studied, including humans and rodents. Benzene is widely distributed in the body by blood circulation; unchanged benzene is largely excreted by exhaled breath, with small amounts appearing in urine. The initial step of metabolism is oxidation to benzene oxide by cytochrome P450. Subsequent metabolism is complex, and includes the creation of a multiplicity of reactive electrophiles via multiple metabolic pathways in multiple tissues, including bone marrow. Major urinary metabolites detected in exposed humans include phenol, hydroquinone, catechol, (*E,E*)-muconic acid, and SPMA. There are some data suggesting increased metabolism at exposure to low concentrations, but these data are not definitive. Electrophiles are generated during benzene metabolism, as indicated by metabolite profiles and the production of epoxide- and benzoquinone-protein adducts in individuals exposed to benzene. There is *strong* evidence, including in exposed humans, that benzene is metabolically activated to electrophilic metabolites. There is *strong* evidence, including in exposed humans, that benzene induces oxidative stress and associated oxidative DNA damage. Several studies in exposed humans reported that exposure to benzene is associated with markers of oxidative stress, such as decreased serum glutathione levels, increased lipid peroxidation, increased reactive oxygen species, oxidative protein damage, and/or decreased antioxidant capacity. In addition, multiple studies in exposed humans reported oxidative DNA damage in the form of 8-hydroxy-2′-deoxyguanosine. Benzene or its metabolites induced oxidative stress in human and other mammalian cells in vitro, and in various tissues, including bone marrow, in mice.

There is *strong* evidence, including in exposed humans, that benzene is genotoxic, inducing DNA damage and chromosomal changes. Benzene induces DNA strand breaks and gene mutations in occupationally exposed humans, and DNA damage in human cells in vitro. In experimental animals exposed in vivo, benzene induced DNA adducts in bone marrow and leukocytes. Benzene metabolites induced benzene-derived DNA adducts in several studies in human haematopoietic cells. The multitude of studies of chromosomal end-points in humans exposed to benzene is largely consistent with respect to the induction of chromosomal aberrations and micronuclei. Specific cytogenetic changes have also been observed in exposed humans, including aneuploidy, translocations, and various other structural chromosome changes. Furthermore, in human cells in vitro, benzene with metabolic activation and benzene metabolites consistently induce chromosomal alterations.

The evidence is *strong* that benzene alters DNA repair or causes genomic instability, inhibiting topoisomerase II, which is involved in DNA

replication. No data on topoisomerase II were available in exposed humans. Benzene metabolites, particularly 1,4-benzoquinone and hydroquinone, directly inhibited topoisomerase II in human cell systems and in exposed mice.

The evidence is *strong* that benzene is immunosuppressive, including in exposed humans. Although no studies in humans were available that directly examined changes in immune function, many studies in exposed humans have demonstrated haematotoxicity, from decreased leukocyte counts at lower exposures to aplastic anaemia and pancytopenia at higher exposures. Specifically, reduced numbers and/or maturity of B-lymphocytes and CD4+ T-lymphocytes have been reported in multiple studies in exposed humans. Multiple experimental animal studies have demonstrated consistent immunosuppressive effects on assays for humoral and cell-mediated immune function, in addition to haematotoxicity, consistent with studies in exposed humans. In addition, several studies have found that haematotoxicity induced by benzene, at various levels of severity, has been associated with a future risk of developing a haematological malignancy or related disorder.

Haematotoxicity observed in exposed humans and experimental animals provides indirect evidence that benzene exposure leads to alterations of cell proliferation and cell death. In human cells in vitro, benzene or its metabolites induced apoptosis consistently across multiple haematopoietic cell types, which could be prevented by induction of the detoxifying enzyme NAD(P)H quinone oxidoreductase 1. In addition, in mice, benzene depressed the cycling fraction of bone marrow cells/progenitor cells mediated by Trp53, and induced apoptosis in various mouse haematopoietic cells in vivo and in vitro. After cessation of benzene exposure, dynamic recovery proliferation of bone marrow cells/progenitor cells was observed. Overall, the evidence is *strong* that benzene alters cell proliferation, cell death, or nutrient supply, specifically with respect to induction of apoptosis.

The evidence is *strong* that benzene modulates receptor-mediated effects, specifically with respect to aryl hydrocarbon receptor (AhR). No data on AhR were available in exposed humans or in human cells. Benzene does not induce haematotoxicity in AhR-knockout ($AhR^{-/-}$) mice, or in wildtype mice whose marrow cells were repopulated with cells from $AhR^{-/-}$ mice after irradiation. Benzene and its metabolites hydroquinone and *p*-benzoquinone did not directly activate AhR in vitro in mouse hepatoma cells.

There are few data on the remainder of the 10 key characteristics of carcinogens (induces chronic inflammation, induces epigenetic alterations, or causes immortalization).

In the ToxCast/Tox21 high-throughput testing programmes of the United States government, four metabolites of benzene (phenol, catechol, hydroquinone, and 1,4-benzoquinone) were individually tested in several assays in vitro that have been mapped to the key characteristics of carcinogens. Few of these assays demonstrated metabolic capacity. Phenol was largely inactive, while the activity of the other three metabolites for oxidative stress and AhR corroborated other mechanistic data on these key characteristics. 1,4-Benzoquinone was also active in many assays mapped to inflammation.

Studies in exposed humans examining exposure–response gradients were available for the end-points of micronucleus formation, chromosomal aberrations, and leukocyte counts. In the majority of studies examined, an exposure–response gradient was reported.

6. EVALUATION AND RATIONALE

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of benzene. Benzene causes acute myeloid leukaemia in adults.

Positive associations have been observed for non-Hodgkin lymphoma, chronic lymphoid leukaemia, multiple myeloma, chronic myeloid leukaemia, acute myeloid leukaemia in children, and cancer of the lung.

A small minority of the Working Group considered that benzene also causes non-Hodgkin lymphoma. A separate small minority considered that a positive association was not observed for cancer of the lung.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of benzene.

6.3 Overall evaluation

Benzene is *carcinogenic to humans (Group 1).*

6.4 Rationale

Support for Group 1 from mechanistic data

A Group 1 evaluation was supported by mechanistic data demonstrating that benzene exhibits many of the key characteristics of carcinogens. In particular, there is *strong* evidence, including in exposed humans, that benzene: is metabolically activated to electrophilic metabolites; induces oxidative stress and associated oxidative DNA damage; is genotoxic, inducing DNA damage and chromosomal changes; is immunosuppressive; and causes haematotoxicity.

LIST OF ABBREVIATIONS

8-OHdG	8-hydroxy-2′-deoxyguanosine
AhR	aryl hydrocarbon receptor
ALL	acute lymphocytic leukaemia
AML	acute myeloid leukaemia
ANLL	acute non-lymphocytic leukaemia
AR	androgen receptor
ASPEN	EPA Assessment System for Population Exposure Nationwide
bw	body weight
CA	chromosomal aberration
CALINE	California Line Source Dispersion model
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CI	confidence interval
CLL	chronic lymphocytic leukaemia
CML	chronic myeloid leukaemia
CNS	central nervous system
DLBCL	diffuse large B-cell lymphoma
EPA	United States Environmental Protection Agency
EPO	erythropoietin
ER	estrogen receptor
GLP	good laboratory practice
GM	granulocyte-macrophage
GSD	geometric standard deviation
GSH	glutathione
GST	glutathione-*S*-transferase
HCL	hairy cell leukaemia
HL	Hodgkin lymphoma
HR	hazard ratio
IARC	International Agency for Research on Cancer
ICD	International Statistical Classification of Diseases and Related Health Problems
Ig	immunoglobulin
IL	interleukin
IQR	interquartile range

JEM	job-exposure matrix
MDS	myelodysplastic syndrome
MLL	mixed lineage leukaemia
MM	multiple myeloma
MN	micronuclei
MPD	myeloproliferative disorder
MPO	myeloperoxidase
mtDNA	mitochondrial DNA
NATA	National-Scale Air Toxics Assessment
NCI-CAPM	National Cancer Institute-Chinese Academy of Preventive Medicine
NF-κB	nuclear-factor kappa-light-chain-enhancer of activated B-cells
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NQO1	NAD(P)H quinone oxidoreductase 1
OEL	occupational exposure limit
OR	odds ratio
PBL	peripheral blood lymphocyte
PBMC	peripheral blood mononuclear cell
PFC	plaque-forming cell
PM_{10}	particulate matter of diameter < 10 μm
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion
ppm	parts per million
PXR	pregnane X receptor
ROS	reactive oxygen species
RR	relative risk
SCE	sister-chromatid exchange
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SPMA	*S*-phenylmercapturic acid
SRBC	sheep red blood cell
TCRV	T-cell receptor variable
Tg.AC	FVB/N-Tg.AC(v-Ha-*Ras*)
Th	T-helper cell
TNF-α	tumour necrosis factor alpha
Tox21	Toxicity in the 21st Century
ToxCast	Toxicity Forecaster
ToxPi	toxicological prioritization index
TREC	T-cell receptor excision circle
t,t-MA	*trans*,*trans*-muconic acid
UOGD	unconventional oil and gas development
vs	versus
WHO	World Health Organization

ANNEX 1. SUPPLEMENTAL MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online at: http://publications.iarc.fr/576) contains a spreadsheet (.xlsx) and a zip folder containing several ToxPi data files (.csv) and associated figures (.pdf) analysed by the Working Group for Volume 120 of the *IARC Monographs*. The spreadsheet lists the ToxCast/Tox21 assay end-points, the associated target and/or model system (e.g. cell type, species, detection technology, etc.), their mapping to 6 of the 10 "key characteristics" of known human carcinogens, and whether each chemical was "active" or "inactive" (EPA, 2015). The ToxPi files integrate the results by "key characteristic" and can be accessed using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

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IARC MONOGRAPHS

This volume presents an evaluation of the carcinogenicity of benzene, updating with new data the most recent evaluation provided in Volume 100F of the *IARC Monographs*. Benzene, a simple aromatic hydrocarbon, occurs naturally and as a result of human activity, notably as a result of combustion, and it is a high-volume chemical now used mostly as a chemical intermediate. Human exposure to benzene is widespread through the air, in consumer products, and in industry.

An *IARC Monographs* Working Group reviewed epidemiological studies, animal cancer bioassays, and mechanistic data to assess the carcinogenicity of benzene and conducted quantitative analyses of data on genotoxicity and human cancer risks.

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I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 71–43–2
CAS No.	71–43–2
EINECS Name	benzene
EINECS No.	200–753–7
Molecular Formula	C6H6

Dataset created by: EUROPEAN COMMISSION – European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non–confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

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Cedex: 2539

Name: POLIOLI S.P.A.
Street: Emilia, 85
Town: 20093 Cologno Monzese (MI)
Country: Italy
Phone: 02-25191
Telefax: 02-251940
Telex: 311434

Name: PRAOIL S.R.L.
Street: Milanofiori, Strada 2 Palazzo F7
Town: 20094 ASSAGO MI
Country: Italy
Phone: +39-2-5201
Telefax: +39-2-52026986
Telex: 314215

Name: REPSOL PETROLEO,S.A.
Street: PASEO DE LA CASTELLANA, 278-280
Town: 28046 MADRID
Country: Spain
Phone: 91-3488000
Telefax: 3489494/3142821
Telex: 49840

Name: Rhone-Poulenc Chemicals Limited
Town: WD1 1QH Watford, Hertforshire
Country: United Kingdom

Name: Ruetgers-VfT n.v.
Street: Vredekaai 18
Town: B-9060 Zelzate
Country: Belgium
Phone: +32/(0)9/3458831
Telefax: +32/(0)9/3458533
Telex: 11480 zelohm b

Name: Ruetgerswerke AG
Street: Varziner Str. 49
Town: 47138 Duisburg
Country: Germany

Name: Ruhr Oel GmbH
Street: Alexander-von-Humboldt-Straße
Town: 45896 Gelsenkirchen-Hassel
Country: Germany
Phone: 0209 366-1
Telefax: 0209 366-7820
Telex: 824 881-0 vo d

Name: SHELL FRANCE
Street: 89 bld Franklin Roosevelt
Town: 92564 Rueil Malmaison
Country: France
Phone: 33 1 47.14.71.00
Telefax: 33 1 47.14.82.99
Telex: SHELL 615013F

Name: Shell Nederland Chemie B.V.
Street: P.O. Box 3030
Town: 3190 GH Hoogvliet–Rotterdam
Country: Netherlands
Phone: +31–10–2317005
Telefax: +31–10–2317125

Name: Shell UK Limited
Street: Shell–Mex House, Strand
Town: WC2R 0DX London
Country: United Kingdom
Phone: +44–171–257–3000
Telefax: +44–171–257–3920

Name: Shell UK Limited,
Street: Shell–Mex House, Stand,
Town: WC2R 0DX London.
Country: United Kingdom
Phone: –44–71–257–3000
Telefax: –44–71–257–3212
Telex: 22585 SHELL G

Name: SSAB Tunnplåt AB
Town: 971 88 Luleå
Country: Sweden
Phone: 046 0920/92000

Name: Sächsische Olefinwerke GmbH
Street: Werkstraße
Town: 04564 Böhlen
Country: Germany
Phone: (034206)30
Telefax: (034206) 3 6488
Telex: 312 097 sow d

Name: TOTAL
Street: 24, cours Michelet
Town: 92069 PARIS LA DEFENSE
Country: France
Phone: 33 41 35 40 00
Cedex: 47

Name: Wintershall AG
Street: Friedrich–Ebert –Str. 160
Town: 34119 Kassel
Country: Germany
Phone: (0561) 3011–059
Telefax: (0561) 3011–702

Name: ÖMV – Chemie Linz GMBH
Street: St. Peterstrasse 25
Town: 4021 Linz
Country: Austria
Phone: *43/(0)732/5916–2386
Telefax: *43(0)732/5916–3738
Telex: 221324

Name: ÖMV Deutschland GmbH
Street: Haiminger Str.1
Town: 84489 Burghausen
Country: Germany
Phone: 08677–81587
Telefax: 08677–81259

1.0.2 Location of Production Site

–

1.0.3 Identity of Recipients

–

1.1 General Substance Information

Substance type: organic
Physical status: liquid

Substance type: petroleum product
Physical status: liquid

Substance type:
Physical status:

1.1.1 Spectra

–

1.2 Synonyms

Annulene
Source: Exxon Chemical Holland BV Botlek
Ruetgers–VfT n.v. Zelzate
ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

annulene
Source: TOTAL PARIS LA DEFENSE

Benzen
Source: Sächsische Olefinwerke GmbH Böhlen

Benzene
Source: Exxon Chemical Holland BV Botlek
Bayer Antwerpen N.V. Antwerpen
ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Bayer AG Leverkusen
Deutsche Shell Chemie GmbH Eschborn
Brenntag AG Muehlheim a. d. Ruhr

Benzene (8CI, 9CI)
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

Benzene, Annulene, Benzol, Benzolo, Cyclohexantriene, Phene, Phenylhydride, Pyrobenzol, Pyrobenzole
Source: Aral Aktiengesellschaft Bochum

Benzol
Source: Exxon Chemical Holland BV Botlek
Dow Benelux N. V. Terneuzen
Bayer Antwerpen N.V. Antwerpen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
FINA-BOREALIS Antwerpen
Ruetgers-VfT n.v. Zelzate
REPSOL PETROLEO,S.A. MADRID
ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG
ÖMV – Chemie Linz GMBH Linz
ICI Chemicals & Polymers Limited Runcorn, Cheshire
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
SSAB Tunnplåt AB Luleå
Bayer AG Leverkusen
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn
EC Erdölchemie GmbH Köln
ÖMV Deutschland GmbH Burghausen

benzol
Source: TOTAL PARIS LA DEFENSE

BENZOL
Source: ACNA C.O. Cengio (SV)
Shell UK Limited, London.
Shell UK Limited London

BENZOL(E) PHENYL HYDRIDE
Source: Rhone-Poulenc Chemicals Limited Watford, Hertforshire

Benzol, Phenylwasserstoff
Source: DEA Mineraloel AG Hamburg

Benzol; Cyclohexatriene; Annulene; Benzolo; Coal naphta; Phene; Phenyl hydride; Pyrobenzol; Pyrobenzole
Source: Atochem Paris la Defense

BENZOL; PHENYL HYDRIDE; MINERAL NAPHTHA; COAL NAPHTHA;
Source: Enichem Synthesis Milan

benzol;cyclohexatriene;phenyl hydride;annulene;coal naphtha;pyrobenzol
Source: Enichem S.p.A. Milan

Benzole
Source: Dow Benelux N. V. Terneuzen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
REPSOL PETROLEO,S.A. MADRID
ICI Chemicals & Polymers Limited Runcorn, Cheshire

Benzolene
Source: Dow Benelux N. V. Terneuzen
REPSOL PETROLEO,S.A. MADRID

Benzolo
Source: Exxon Chemical Holland BV Botlek
Ruetgers-VfT n.v. Zelzate
ALUSUISSE Italia SpA - FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

benzolo
Source: TOTAL PARIS LA DEFENSE

BENZOLO
Source: Agip Petroli SpA ROMA
PRAOIL S.R.L. ASSAGO MI
POLIOLI S.P.A. Cologno Monzese (MI)

Carbon oil
Source: Dow Benelux N. V. Terneuzen
REPSOL PETROLEO,S.A. MADRID

CICLOESATRIENE
Source: POLIOLI S.P.A. Cologno Monzese (MI)

Coal naphta
Source: REPSOL PETROLEO,S.A. MADRID

Coal naphtha
Source: Exxon Chemical Holland BV Botlek
Dow Benelux N. V. Terneuzen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
Ruetgers-VfT n.v. Zelzate
ALUSUISSE Italia SpA - FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.

Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

coal naphtha
Source: TOTAL PARIS LA DEFENSE

Cyclohexatrien
Source: Sächsische Olefinwerke GmbH Böhlen

Cyclohexatriene
Source: Exxon Chemical Holland BV Botlek
Dow Benelux N. V. Terneuzen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
Ruetgers-VfT n.v. Zelzate
REPSOL PETROLEO,S.A. MADRID
ALUSUISSE Italia SpA - FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

cyclohexatriene
Source: TOTAL PARIS LA DEFENSE

CYCLOHEXATRIENE
Source: ACNA C.O. Cengio (SV)
Rhone-Poulenc Chemicals Limited Watford, Hertforshire
Shell UK Limited, London.
Shell UK Limited London

Cyclohexatriene; benzol; phenyl hydride
Source: Shell Nederland Chemie B.V. Hoogvliet-Rotterdam
SHELL FRANCE Rueil Malmaison
Deutsche Shell Chemie GmbH Eschborn

FENILIDRIDE
Source: POLIOLI S.P.A. Cologno Monzese (MI)

Phene
Source: Exxon Chemical Holland BV Botlek
Dow Benelux N. V. Terneuzen
Ruetgers-VfT n.v. Zelzate
ALUSUISSE Italia SpA - FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

phene
Source: TOTAL PARIS LA DEFENSE

Phenyl hydride
Source: Exxon Chemical Holland BV Botlek
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
Ruetgers-VfT n.v. Zelzate
ALUSUISSE Italia SpA - FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

phenyl hydride
Source: TOTAL PARIS LA DEFENSE

PHENYL HYDRIDE
Source: ACNA C.O. Cengio (SV)
Shell UK Limited, London.
Shell UK Limited London

Phenylhydride
Source: Dow Benelux N. V. Terneuzen

Phenylwasserstoff
Source: Sächsische Olefinwerke GmbH Böhlen

PIROBENZOLO
Source: POLIOLI S.P.A. Cologno Monzese (MI)

Pyrobenzol
Source: Exxon Chemical Holland BV Botlek
Dow Benelux N. V. Terneuzen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
Ruetgers-VfT n.v. Zelzate
ALUSUISSE Italia SpA - FTALITAL Factory Scanzorosciate BG
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

pyrobenzol
Source: TOTAL PARIS LA DEFENSE

Pyrobenzole
Source: Dow Benelux N. V. Terneuzen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
Ruetgers-VfT n.v. Zelzate
BP Chemicals Ltd. London
Bitmac Limited Scunthorpe.
Ruetgerswerke AG Duisburg
Deutsche Shell Chemie GmbH Eschborn

pyrobenzole
Source: TOTAL PARIS LA DEFENSE

Q9111
Source: Shell UK Limited, London.

Synonym a: Benzol
Source: PCK AG Schwedt Schwedt/Oder

Synonym a: Reinbenzol
Source: PCK AG Schwedt Schwedt/Oder

Source: Bayer Antwerpen N.V. Antwerpen
Brenntag AG Muehlheim a. d. Ruhr

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

Quantity more than 1 000 000 tonnes

1.6.1 Labelling

Labelling: as in Directive 67/548/EEC
Symbols: F
T
Nota: E
other RM: S
Specific limits: no data
R-Phrases: (45) May cause cancer
(11) Highly flammable
(48/23/24/25) Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
S-Phrases: (53) Avoid exposure - obtain special instructions before use
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

1.6.2 Classification

Classification: as in Directive 67/548/EEC
Class of danger: carcinogenic, category 1
R-Phrases: (45) May cause cancer

Classification: as in Directive 67/548/EEC
Class of danger: highly flammable
R-Phrases: (11) Highly flammable

Classification: as in Directive 67/548/EEC
Class of danger: toxic
R–Phrases: (48/23/24/25) Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed

1.7 Use Pattern

Type: type
Category: Non dispersive use

Type: type
Category: Use in closed system

Type: type
Category: Wide dispersive use

Type: industrial
Category: Basic industry: basic chemicals

Type: industrial
Category: Chemical industry: used in synthesis

Type: industrial
Category: Fuel industry

Type: industrial
Category: Paints, lacquers and varnishes industry

Type: industrial
Category: Polymers industry

Type: use
Category: Fuel additives

Type: use
Category: Intermediates

Type: use
Category: Laboratory chemicals

Type: use
Category: Lubricants and additives

Type: use
Category: Pharmaceuticals

Type: use
Category: Solvents

Type: use
Category: other: in ACNA C.O., il benzene, veniva utilizzato come materia prima nella sintesi del benzene, nitro– (n°CAS 98–95–3 e n°EINECS 2027160)

Type: use
Category: other: sintesi di Anidride Maleica

Type: use
Category: other

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

Type of limit: MAC (NL)
Limit value: 10 ml/m3
Remark: Proposal : 1 ppm
With the annotation "H" (huid=skin)
Source: Exxon Chemical Holland BV Botlek

(1)

Type of limit: MAC (NL)
Limit value: 30 mg/m3
Remark: Skin notation
Listed as carcinogen
Source: Shell Nederland Chemie B.V. Hoogvliet-Rotterdam

(2)

Type of limit: MAC (NL)
Limit value: 30 mg/m3
Remark: 8 hr.
Source: DSM Hydrocarbons B.V. Sittard

Type of limit: MAK (DE)
Limit value: mg/m3
Country: Germany
Remark: Benzene is in the carcinogenic group IIIA1, i.e. it is capable of inducing malignant tumors as shown by experience with humans and has a skin notification (H= Danger of cutaneous absorption).
Source: Dow Benelux N. V. Terneuzen

(3)

Type of limit: MAK (DE)
Limit value: 2.5 ml/m3
Remark: Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus), Tankfeld in der Mineraloelindustrie, Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(4)

Type of limit: MAK (DE)
Limit value: 8 mg/m3
Remark: Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus), Tankfeld in der Mineraloelindustrie, Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(4)

Type of limit: MAK (DE)
Limit value: 1 ml/m3
Remark: gilt fuer Benzol im uebrigen
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(4)

Type of limit: MAK (DE)
Limit value: 3.2 mg/m3
Remark: gilt fuer Benzol im uebrigen
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(4)

Type of limit: MAK (DE)
Limit value:
Remark: hautresorptiv, krebserzeugend
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(4)

Type of limit: MAK (DE)
Limit value:
Remark: krebserzeugend, EG-Kategorie 1
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(5)

Type of limit: MAK (DE)
Limit value:
Remark: krebserzeugend, EG-Kategorie 1
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(5)

Type of limit: MAK (DE)
Limit value:
Country: Germany
Remark: Benzene(71–43–2)is assigned to Category A, and as such has no concentration value listed since no values have been established for a safe concentration range. Special protective and surveillance measures are required.
Source: Bitmac Limited Scunthorpe.

(6)

Type of limit: MAK (DE)
Limit value:
Remark: krebserzeugend, EG–Kategorie 1
Source: BASF AG Ludwigshafen

(5)

Type of limit: MAK (DE)
Limit value:
Remark: krebserzeugend, EG–Kategorie 1
Source: BASF AG Ludwigshafen

(5)

Type of limit: MEL (UK)
Limit value: 5 ml/m3
Source: Exxon Chemical Holland BV Botlek

(7)

Type of limit: MEL (UK)
Limit value: 16 mg/m3
Country: United Kingdom
Remark: Benzene is defined as carcinogen for the purpose of the COSHH Regulation as at 1 January 1993 and to which the special provisions for carcinogen in the COSHH Regulations apply.
Source: Dow Benelux N. V. Terneuzen

(8)

Type of limit: MEL (UK)
Limit value: 30 mg/m3
Source: Enichem Synthesis Milan

Type of limit: MEL (UK)
Limit value: 5 ml/m3
Source: ICI Chemicals & Polymers Limited Runcorn, Cheshire

Type of limit: MEL (UK)
Limit value: 16 mg/m3
Source: Rhone–Poulenc Chemicals Limited Watford, Hertforshire

Type of limit: MEL (UK)
Limit value: 16 mg/m3
Source: Bitmac Limited Scunthorpe.

(9)

Type of limit: MEL (UK)
Limit value: 16 mg/m3
Country: United Kingdom
Source: Shell UK Limited, London.

(10)

Type of limit: MEL (UK)
Limit value: 16 mg/m3
Country: United Kingdom
Source: Shell UK Limited London

(11)

Type of limit: OES (UK)
Limit value: 2 mg/m3
Source: BP Chemicals Ltd. London

(12)

Type of limit: OES (UK)
Limit value: 2 mg/m3
Remark: Reference not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn

(12)

Type of limit: TLV (US)
Limit value: 10 ml/m3
Remark: Benzene has been indicated as category A2 carcinogen: "Suspected Human Carcinogen."
The USA ACGIH has proposed a TLV for Benzene of 0.3 ppm.
Source: Exxon Chemical Holland BV Botlek

(13)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Country: USA
Remark: For the exposure value and the classification as A2 carcinogen ('suspected human carcinogen') following changes are intended: TWA = 0.3 mg/m3, A1 carcinogen ('confirmed human carcinogen')
Source: Dow Benelux N. V. Terneuzen

(14)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: Listed as suspected human carcinogen
Source: Shell Nederland Chemie B.V. Hoogvliet-Rotterdam

(15)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: confirmed human carcinogen
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(16)

Type of limit: TLV (US)
Limit value:
Remark: limit value: 10 ppm. Confirmed human carcinogen.
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(16)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: confirmed human carcinogen
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(16)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Source: Atochem Paris la Defense

(17)

Type of limit: TLV (US)
Limit value: .3 mg/m3
Source: TOTAL PARIS LA DEFENSE

Type of limit: TLV (US)
Limit value: 1.6 mg/m3
Short term expos.
Limit value: 8 mg/m3
Schedule: 15 minute(s)
Frequency: 4 times
Source: REPSOL PETROLEO,S.A. MADRID

Type of limit: TLV (US)
Limit value: 1.6 mg/m3
Source: Compañia Española de Petroleos CEPSA Madrid

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: A2 (suspected human carcinogen).A 1992–1993 ACGIH Notice of Intended Changes to lower the benzene TWA to 0.1ppm with a skin notation and A1 (confirmed human carcinogen)
Source: PETROLEOS DE PORTUGAL – PETROGAL,S.A. LISBOA

Type of limit: TLV (US)
Limit value: 1.6 mg/m3
Short term expos.
Limit value: 8 mg/m3
Schedule: 15 minute(s)
Frequency: 4 times
Remark: LIMITI ACGIH:
TLV/TWA: 0,5 ppm A1
1,6 mg/m3 A1
TLV/STEL: 2,5 ppm A1
8 mg/m3 A1
(Skin)
Source: ACNA C.O. Cengio (SV)

(18)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Source: ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG

(19)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: TLV–TWA = 10 ppm = 32 mg/m3
Source: Enichem Synthesis Milan

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: TLV–TWA: 32, A2 mg/m3

Intended change:
TLV–TWA: 0.3, A1 mg/m3
Notes:skin

A1= Confirmed Human Carcinogens
A2= Suspected Human Carcinogens
Source: Enichem Augusta Industriale Milan

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: A2:Suspected Human Carcinogen:The agent is carcinogenic in experimental animals at dose levels,by route(s) of administration,at site(s),of histologic type(s),or by mechanism(s) that are considered relevant to worker exposure.Avalable epidemiologic studies are conflicting or insufficient to confirm an increased risk of cancer in exposed humans.
Source: Enichem S.p.A. Milan

(20)

Type of limit: TLV (US)
Limit value: .3 mg/m3
Remark: A1:Confirmed Human Carcinogen:The agent is carcinogenic to humans based on the weight of evidence from epidemiologic studies of,or convincing clinical evidence in,exposed humans.
Notice of Intended Changes (1993–1994)
Source: Enichem S.p.A. Milan

(20)

Type of limit: TLV (US)
Limit value: .5 other: ppm
Short term expos.
Limit value: 2.5 other: ppm
Schedule: 15 minute(s)
Frequency: 4 times
Source: ACGIH, 1998
Agip Petroli SpA ROMA

Type of limit: TLV (US)
Limit value: 10 other: ppm
Source: PRAOIL S.R.L. ASSAGO MI

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: PROTEZIONE PERSONALE (IN DIPENDENZA DEL TIPO ED ENTITA' DELLA POSSIBILE ESPOSIZIONE):
MASCHERA CON FILTRO PER VAPORI ORGANICI
AUTORESPIRATORE
GUANTI IN PVC A MANICA LUNGA
OCCHIALI A TENUTA
SCHERMO FACCIALE
INDUMENTI E STIVALI IMPERMEABILI AL BENZENE
Source: POLIOLI S.P.A. Cologno Monzese (MI)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: A 1992-1993 Notice of Intended Changes has also been published by US ACGIH to lower the benzene TWA to 0.1 ppm (0.3 mg/m3) with a skin notation and A1 (confirmed human carcinogen).
A2 (suspected human carcinogen) notation given.
Source: BP Chemicals Ltd. London

(21)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Country: United States of America
Remark: Benzene(71-43-2) is assigned to Appendix A2- a Suspected Human Carcinogen. The TLV List of 1993-1994 includes Benzene as being under Notice of Intended Change with 0.3 mg/m3 as the value and reassignment to Appendix A1- a Known Human Carcinogen with an additional Skin notation.
Source: Bitmac Limited Scunthorpe.

(22)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: confirmed human carcinogen
Source: BASF AG Ludwigshafen

(16)

Type of limit: TLV (US)
Limit value: 32 mg/m3
Remark: confirmed human carcinogen
Source: BASF AG Ludwigshafen

(16)

Type of limit: TLV (US)
Limit value: 1 mg/m3
Remark: = 0.3 ppm; 8h TWA
SK (skin) and A1 (confirmed human carcinogen) notation proposed
Source: Deutsche Shell Chemie GmbH Eschborn

(23)

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Remark: With derogations
Source: Exxon Chemical Holland BV Botlek

Type of limit: TRK (DE)
Limit value: 8 mg/m3
Country: Germany
Remark: Benzene (Benzol)

Verwendung	TRK (Technische Richtkonzentration)
Kokereien (Dickteerschneider, Kondensation, Gassaugerhaus)	8 mg/m3
Tankfeld in der Mineraloelindustrie	8 mg/m3
Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen	8 mg/m3
im uebrigen	3.2 mg/m3

Source: Dow Benelux N. V. Terneuzen

(24)

Type of limit: TRK (DE)
Limit value: 16 mg/m3
Source: Atochem Paris la Defense

(17)

Type of limit: TRK (DE)
Limit value: 16 mg/m3
Source: Enichem Synthesis Milan

Type of limit: TRK (DE)
Limit value: 16 mg/m3
Source: ÖMV - Chemie Linz GMBH Linz

Type of limit: TRK (DE)
Limit value: 3.2 mg/m3
Source: Ruetgerswerke AG Duisburg

(25)

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Short term expos.
Limit value: 12.5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: TRK value valid for coke refineries, area of storage tanks in the mineral oil industry and repair and maintenance of fuel or benzene containing parts.
Source: Deutsche Shell Chemie GmbH Eschborn

(26)

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Short term expos.
Limit value: 5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: Valid for all other applications except those mentioned above.
Source: Deutsche Shell Chemie GmbH Eschborn

(27)

Type of limit: TRK (DE)
Limit value: 16 mg/m3
Country: Federal Republic of Germany
Remark: group III A1 (confirmed human carcinogen)
Source: Deutsche Shell Chemie GmbH Eschborn

(28)

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Remark: Wert für : übrige (Anwendungen)
Source: Brenntag AG Muehlheim a. d. Ruhr

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Source: Ruhr Oel GmbH Gelsenkirchen-Hassel
Sächsische Olefinwerke GmbH Böhlen

Type of limit: TRK (DE)
Limit value:
Remark: TRGS 900 (1993): Technische Richtkonzentrationen (TRK–Wert)
– Kokereien (Dickteerschneider, Kondensation, Gassaugerhaus) = 2,5 ppm / 8 mg/m3

– Tankfeld in der Mineralölindustrie = 2,5 ppm / 8 mg/m3

– Reparatur und Wartung von Ottokraftstoff bzw. benzol–führenden Teilen = 2,5 / 8 mg/m3

– im übrigen = 1,0 ppm / 3,2 mg/m3
Source: Aral Aktiengesellschaft Bochum

Type of limit: TRK (DE)
Limit value: 8 mg/m3
Source: Sächsische Olefinwerke GmbH Böhlen

(29)

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Short term expos.
Limit value: 5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Source: PCK AG Schwedt Schwedt/Oder

Type of limit: TRK (DE)
Limit value: 3.2 mg/m3
Short term expos.
Limit value: 16 mg/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: Zum Kurzzeitwert: Der zeitliche Abstand von einer Expositionsspitze zur anderen darf 1h nicht unterschreiten.
Source: PCK AG Schwedt Schwedt/Oder

(30) (31)

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Remark: bei Tankfeld in der Mineralölindustrie
Source: DEA Mineraloel AG Hamburg

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Remark: im übrigen
Source: DEA Mineraloel AG Hamburg

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Remark: – für Kokereien
– Tankfeld in der Mineralölindustrie
– Reparatur und Wartung von Ottokraftstoff bzw. Benzol führenden Teilen
Source: EC Erdölchemie GmbH Köln

(32)

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Remark: – im übrigen (alle weiteren Anwendungen)
Source: EC Erdölchemie GmbH Köln

(33)

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Short term expos.
Limit value: 12.5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: –für Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus)
Source: ÖMV Deutschland GmbH Burghausen

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Short term expos.
Limit value: 12.5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: –Tankfeld in der Mineralölindustrie
Source: ÖMV Deutschland GmbH Burghausen

Type of limit: TRK (DE)
Limit value: 2.5 ml/m3
Short term expos.
Limit value: 12.5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: –Reparatur und Wartung von Ottokraftstoff–bzw.benzenführenden Teilen
Source: ÖMV Deutschland GmbH Burghausen

Type of limit: TRK (DE)
Limit value: 1 ml/m3
Short term expos.
Limit value: 5 ml/m3
Schedule: 15 minute(s)
Frequency: 5 times
Remark: -im übrigen
Source: ÖMV Deutschland GmbH Burghausen

(34) (35)

Type of limit: other
Limit value: 10 mg/m3
Country: Belgium
Remark: 3 ppm
Source: Bayer Antwerpen N.V. Antwerpen

Type of limit: other
Limit value: 16 mg/m3
Country: France
Remark: Type of Limit: VME
Source: Atochem Paris la Defense

(36)

Type of limit: other
Limit value: 32 mg/m3
Source: TOTAL PARIS LA DEFENSE

Type of limit: other
Limit value:
Short term expos.
Limit value: 80 mg/m3
Source: TOTAL PARIS LA DEFENSE

Type of limit: other
Limit value:
Short term expos.
Limit value: 16 mg/m3
Source: TOTAL PARIS LA DEFENSE

Type of limit: other
Limit value: 30 mg/m3
Remark: NP 1796 (1988) Portuguese Standard
Source: PETROLEOS DE PORTUGAL - PETROGAL,S.A. LISBOA

Type of limit: other
Limit value: 15 mg/m3
Remark: MAC (ex URSS), 1980-1987
Value for vapour (for gas 5 mg/m3).
Source: Enichem Synthesis Milan

Type of limit: other
Limit value: 32 mg/m3
Country: Belgium
Remark: C2 (suspected human carcinogen) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value: 16 mg/m3
Country: Denmark, Finland, Switzerland
Remark: SK (skin) and C or, for Finland, C3 (suspected carcinogen) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value: 16 mg/m3
Country: France
Remark: C (suspected carcinogen) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value: 3 mg/m3
Country: Sweden
Remark: SK (skin) and C3 (suspected carcinogenic potential) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value: 32 mg/m3
Country: Former USSR
Remark: SK (skin) and C (suspected carcinogen) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value: 16 mg/m3
Country: Australia
Remark: Carcinogen category 1 (confirmed human carcinogen).
Source: BP Chemicals Ltd. London

(38)

Type of limit: other
Limit value:
Short term expos.
Limit value: 30 mg/m3
Country: Finland
Remark: SK (skin) and C3 (suspected carcinogenic potential) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value:
Short term expos.
Limit value: 16 mg/m3
Country: Sweden
Remark: SK (skin) and C3 (suspected carcinogenic potential) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value:
Short term expos.
Limit value: 80 mg/m3
Country: Former USSR
Remark: SK (skin) and C (suspected carcinogen) notation given.
Source: BP Chemicals Ltd. London

(37)

Type of limit: other
Limit value:
Country: United Kingdom
Remark: Internal Working Limit

3 mg/m3 8 hour TWA
Source: Shell UK Limited, London.
Shell UK Limited London
Test substance: Benzene

Type of limit: other
Limit value: 32 mg/m3
Country: Belgium
Remark: C2 (suspected human carcinogen) notation given.
Reference not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value: 16 mg/m3
Country: Denmark, Finland, Switzerland
Remark: Reference not yet available (12/10/94)
SK (skin) and C or, for Finland, C3 (suspected carcinogen) notation given.
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value: 16 mg/m3
Country: France
Remark: C (suspected carcinogen) notation given.
Reference not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value: 3 mg/m3
Country: Sweden
Remark: Reference not yet available (12/10/94)
SK (skin) and C3 (suspected carcinogenic potential) notation given.
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value: 32 mg/m3
Country: Former USSR
Remark: Reference not yet available (12/10/94)
SK (skin) and C (suspected carcinogen) notation given.
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value: 16 mg/m3
Country: Australia
Remark: Carcinogen category 1 (confirmed human carcinogen).
Reference not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn

(38)

Type of limit: other
Limit value:
Short term expos.
Limit value: 30 mg/m3
Country: Finland
Remark: Reference not yet available (12/10/94)
SK (skin) and C3 (suspected carcinogenic potential) notation given.
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value:
Short term expos.
Limit value: 16 mg/m3
Country: Sweden
Remark: Reference not yet available (12/10/94)
SK (skin) and C3 (suspected carcinogenic potential) notation given.
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other
Limit value:
Short term expos.
Limit value: 80 mg/m3
Country: Former USSR
Remark: Reference not yet available (12/10/94)
SK (skin) and C (suspected carcinogen) notation given.
Source: Deutsche Shell Chemie GmbH Eschborn

(37)

Type of limit: other: CANCS (B)
Limit value: 32 mg/m3
Remark: C2 (suspected human carcinogen) (ILO 1991)
Source: Ruetgers-VfT n.v. Zelzate

(39)

Type of limit: other: Ireland
Limit value: 5 ml/m3
Source: Exxon Chemical Holland BV Botlek

Type of limit: other: Italy
Limit value: 10 ml/m3
Source: Exxon Chemical Holland BV Botlek

Type of limit: other: Norway
Limit value: 1 ml/m3
Source: Exxon Chemical Holland BV Botlek

Type of limit: other: PEL(OSHA): Permitted Exposure Limit
Limit value: 3 mg/m3
Short term expos.
Limit value: 15 mg/m3
Source: REPSOL PETROLEO,S.A. MADRID

(40)

Type of limit: other: Sweden
Limit value: .5 ml/m3
Source: Exxon Chemical Holland BV Botlek

Type of limit: other: TLV
Limit value: 3 other: ppm
Source: Direttiva 97/42/CE
Agip Petroli SpA ROMA

Type of limit: other: TLV (Spanish Ministry of Labour)
Limit value:
Short term expos.
Limit value: 25 ml/m3
Source: REPSOL PETROLEO,S.A. MADRID

Type of limit: other: TRGS 900
Limit value:
Remark: TRK-Wert: 1 ppm = 2,3 mg/m3 Spitzenbegrenzung Kategorie: 4
Gefahr der Hautresorption
Source: Bayer AG Leverkusen

Type of limit: other: TWA (Belgium)
Limit value: 10 ml/m3
Remark: Benzene has been indicated as a C2 "suspected human carcinogen".
Proposal : 3 ppm
Source: Exxon Chemical Holland BV Botlek

(41)

Type of limit: other: VME
Limit value: 16 mg/m3
Short term expos.
Limit value: 3 mg/m3
Remark: Use local exhaust ventilation.
Hand protection : nitrile rubber gloves.
Eye protection : safety monogoggles.
Body protection : standard issue work clothes.
chemicals resistant shoes or boots.
if splashes are likely to occur,
wear : PVC apron.
Source: SHELL FRANCE Rueil Malmaison

Type of limit: other: VME (France)
Limit value: 5 ml/m3
Remark: VME = Valeur Moyenne d'Exposition
Source: Exxon Chemical Holland BV Botlek

(42)

Type of limit:
Limit value:
Source: Exxon Chemical Holland BV Botlek

(43)

Type of limit:
Limit value:
Remark: Gefahr der Hautresorption
Source: EC Erdölchemie GmbH Köln

(32)

1.9 Source of Exposure

Remark: Air Quality Standards:
For :
United Kingdom: 500 ppb
U.S.A.: Louisiana 3.75 ppb ; Massachusetts : 0.0375 ppb
Germany: 4.7 ppb as from July '95 ; 3.1 ppb as from July '96.
Netherlands : 3.1 ppb as from Feb. '93
Benzene as reported here is produced by means of an extraction and fractionation process of benzene from a benzene rich gasoline stream in a petrochemical plant.
EXXON CHEMICAL HOLLAND BV operates one manufacturing site for the production of benzene.

The production of benzene takes place in a completely closed system. The system will only be opened for maintenance, when empty ,after measurement of exposure concentrations and following strict control and working procedures.
Because of the carcinogenic properties of benzene,personel potentially exposed to benzene concentrations is continously monitored and guided.
Consumer Exposure Literature:
Note:
Following literature references have been included for sake of completeness. References report the potential sources of benzene exposure additional to sources as reported by our company for the benzene production – and end–use description in this HEDSET.

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Note:
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Source: Exxon Chemical Holland BV Botlek

Remark: Benzene is used in closed systems.
The occupational hazard is negligible versus the proposed TLV of 0.3 TWA, i.e. below/around 0.1 ppm (*).

(*)Peakexposures may occur during shutdowns but also here, due to surveillance and the taken precautions (protective equipment/cleaning procedures), the exposure will be minimal. The proof of good working practise is the low levels of metabolite (phenol) found in the urine.

Source: Dow Benelux N. V. Terneuzen

(44)

Remark: Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.

Source: Shell Nederland Chemie B.V. Hoogvliet–Rotterdam

(45)

Remark: Benzene is released into the UK environment as a result of its production and of oil refining processes in general, its use as a chemical intermediate (production of ethylbenzene, cyclohexane, phenol, etc), its presence in gasoline and the combustion of fossil fuels.
Studies in UK, Canada, Germany and USE estimate the relative contributions of these sources to be:

Vehicle emissions	79–85%
Petrol distribution	2– 6%
Chemical industry	4–14%
Petroleum refineries	1%
Fossil fuel burning	2– 5%

Source: Shell Nederland Chemie B.V. Hoogvliet–Rotterdam

(46)

Remark: Benzene production facility
Source: DSM Hydrocarbons B.V. Sittard

Remark: Benzene is produced from crude benzene by distillation.
Source: Ruetgers–VfT n.v. Zelzate

Remark: Processes:
– Extraction by solvent (e.g. N–Methylpyrolidone) of a cut predominantly C6 issued from steam cracking
– Hydrodealkylation (thermic catalytic cracking)
– Hydrodealkylation of benzols (issued from coal)
Source: Atochem Paris la Defense

Remark: Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general to benzene.
Benzene is released into environment as :
–vehicle emissions (79–85 %)
–petrol distribution (2–6 %)
–chemical industry (4–14 %)
–fossil fuel burning (2–5 %)
Inhalation or skin contact when loading, unloading or using the product.
In case of accidental release, product may contaminate the environment.
Source: SHELL FRANCE Rueil Malmaison
(47) (48) (49)

Remark: Emissions from petroleum refining, styrene manufacture.
Source: TOTAL PARIS LA DEFENSE

Source: REPSOL PETROLEO,S.A. MADRID
(50) (51) (52)

Remark: The product is only use by industrial profissionals jointly with a Material safety data sheet.¿
Source: PETROLEOS DE PORTUGAL – PETROGAL,S.A. LISBOA

Remark: Nel sito produttivo di Scanzorosciate il Benzene (Bz) viene impiegato come materia prima per la produzione di Anidride Maleica (MA).
Il Bz, stoccato in un serbatoio cilindrico verticale a tetto galleggiante della capacità pari a 6000 mc, viene inviato al relativo impianto di produzione in cui viene trasformato in MA mediante ossidazione catalitica.
Nel sito produttivo sono in esercizio due unità di reazione sostanzialmente identiche denominate AM3 ed AM4 costituite da reattori a letto fisso.
Il Bz non convertito viene inviato ad un impianto di abbattimento costituito da cinque adsorbitori a carbone attivo, in uscita dai quali (a 30 metri dal suolo) la concentrazione in Bz nell’ emissione è pari a 30 mg/mc (portata 81000 Nmc/h).
Source: ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG

Remark: Benzene enters the environment from production, storage, transport, venting, and combustion of gasoline; and from production, storage, and transport of benzene itself. Other sources result from its use as an intermediate in the production of other chemicals, and as a solvent.. from its indirect production in coke, from nonferrous metal manufacture, ore mining, wood processing, coal mining and textile manufacture, from cigarette smoke..
manufacturing methods:
process from coal tar distillation
manufacturing methods:
Process high temperature carbonisation of coal to coke
Manufacturing methods: process petroleum–derived by catalytic reforming or by cracking of parafines
Source: Enichem Synthesis Milan

(53)

Country: Italy
Remark: Benzene is used in three sites in Italy in alkylation processes to produce linear alkylbenzene in close system.
Source: Enichem Augusta Industriale Milan

Remark: Utilizzato in sistemi chiusi: esposizione possibile durante il campionamento.
Source: Agip Petroli SpA ROMA

Remark: Utilizzato in sistemi chiusi: esposizione possibile durante il campionamento.
Source: PRAOIL S.R.L. ASSAGO MI

Remark: Principal raw materials : crude benzole hydrogen.

Separation by distillation giving benzene.
Source: Rhone–Poulenc Chemicals Limited Watford, Hertforshire

Country: United Kingdom
Remark: Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food beverages, and petroleum refining operations play only a minimal role in the total exposure of the general populationto benzene.
Benzene is released into the UK environment as a result of its production and of oil refining processes in general; itsuse as a chemical intermediate (production of ethylbenzene, cyclohexane, phenol, etc.); its presence in gasoline; and combustion of fossil fuels.

Studies in the UK, Canada, Germany and the USA estimate the relative contributions of thesee sources to be :

Vehicle emissions	: 79–85%
Petrol distribution	: 2– 6%
Chemical industry	: 4–14%
Petroleum refineries	: 1%
Fossil fuel burning	: 2– 5%

Source: Shell UK Limited, London.

(54) (55)

Country: United Kingdom
Remark: Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.
Benzene is released into the UK environment as a result of its production and of oil refining processes in general; its use as a chemical intermediate (production of ethylbenzene, cyclohexane, phenol, etc.); its presence in gasoline; and combustion of fossil fuels.

Studies in the UK, Canada, Germany and the USA estimate the relative contributions of thesee sources to be :

Vehicle emissions	: 79-85%
Petrol distribution	: 2- 6%
Chemical industry	: 4-14%
Petroleum refineries	: 1%
Fossil fuel burning	: 2- 5%

Source: Shell UK Limited London

(54) (55)

Source: Ruetgerswerke AG Duisburg

Remark: Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.
Benzene is released into the UK environment as a result of its production and of oil refining processes in general, its use as a chemical intermediate (production of ethyl benzene, cyclohexane, phenol etc.), its presence in gasoline and the combustion of fossil fuels.
Studies in UK, Canada, Germany and USA estimate the relative contributions of these sources to be:

Vehicle emissions	79-85 %
Petrol distribution	2-6 %
Chemical industry	4-14 %
Petroleum refineries	1 %
Fossil fuel burning	2-5 %

Germany: In 1986, an extensive investigation programme on aromatic emissions from gasoline-powered cars was conducted by TUEV-Rheinland as part of a research project from DGMK/FVV/UBA. These measurements provide a good basis for more accurate estimates of car exhaust gas emission in the FRG. Using these results UBA estimated the total benzene emission from combustion engine driven vehicles, including diesel vehicles and motorcycles to be 42,000 t/a.
Source: Deutsche Shell Chemie GmbH Eschborn

(56) (57) (58)

Remark: Benzene may be released to the environment during manufacturing and processing by the chemical industry. Major processing products are ethylbenzene (styrene, polystyrene plastics and synthetic rubber), cyclohexane (nylon), cumene/phenol (phenolic resins), nitrobenzene, chlorobenzenes and alkylbenzenes (detergents). To a significant amount benzene is further emitted into the atmosphere during combustion processes, mainly of fossile fuels (oil and coal), but also from wood burning and volcanoic activities. Important sources of airborne benzene regarding the exposure of individuals are automobile exhaust and cigarette smoke (see also section 3.2 below).
Source: Deutsche Shell Chemie GmbH Eschborn
(59) (60) (61) (62) (63) (64)

Remark: Aus C6– bzw. C7–Schnitt der Aromatendestillation des Aromizers und C7–Aromatenrücklauf der p–Xylol–Anlage werden in einem Mixer–Settler–Extraktor mittels Lösungsmittelgemisch (N–Methyl–Pyrrolidon/Glykol) im Gegenstrom Benzol und Toluol extrahiert, die Raffinatphase verläßt nach einer Wasserwäsche die Anlage. Im Lösungsmittelstripper wird unter Vakuum das Lösungsmittel von den Aromaten abgetrennt. Nach einer Bleicherdebehandlung wird Reinbenzol destillativ gewonnen. Lizenzgeber für das Verfahren ist die Lurgi Deutschland. Benzol wird im Ottokraftstofflager in Festdachtanks mit Schwimmdecke eingelagert. Pumpen, Kolonnen, Behälter und sonstige Apparate sind in Freibauweise errichtet und in Tassen aufgestellt. Sicherheitseinrichtungen wie z.B. Sicherheitsventile sind in ein Fackelsystem eingebunden. Die Verladung von Benzol erfolgt mit Abdichtkissen auf dem Dom des Kesselwagens, um Expositionen der Verlader gegenüber Benzol zu vermeiden.¿
Source: PCK AG Schwedt Schwedt/Oder

Remark: Expositionsgefahr bei Herstellung, Lagerung, Umschlag, Transport.
Source: DEA Mineraloel AG Hamburg

1.10.1 Recommendations/Precautionary Measures

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1.10.2 Emergency Measures

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1.11 Packaging

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1.12 Possib. of Rendering Subst. Harmless

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1.13 Statements Concerning Waste

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1.14.1 Water Pollution

Classified by: KBwS (DE)
Labelled by: KBwS (DE)
Class of danger: 3 (strongly water polluting)
Source: Bayer Antwerpen N.V. Antwerpen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

Classified by: KBwS (DE)
Labelled by:
Class of danger: 3 (strongly water polluting)
Source: Bayer AG Leverkusen

1.14.2 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: yes
Remark: Stoerfall–Stoff–Nr. 39
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(65)

Legislation: Stoerfallverordnung (DE)
Substance listed: yes
Remark: Anhang II, Nr. 39
Source: Bayer AG Leverkusen

Legislation: other: 92/86/EG
Substance listed: yes
Remark: Annex, Part 2, 2
Source: Bayer Antwerpen N.V. Antwerpen

1.14.3 Air Pollution

Classified by: TA–Luft (DE)
Labelled by: TA–Luft (DE)
Number: 2.3 (carcinogenic substances)
Class of danger: III
Source: Bayer Antwerpen N.V. Antwerpen
BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

Classified by: TA–Luft (DE)
Labelled by:
Number: 2.3 (carcinogenic substances)
Class of danger: III
Source: Bayer AG Leverkusen

1.15 Additional Remarks

Remark: Benzene as reported here is only transported by ship or vessel.
Routes for disposal: benzene may be disposed of by controled incineration, re–used as a blending component for fuel or can be recovered by distillation.
Transport information:
By land: (railroad/road, such as RID/ADR):
ADR/RID Class :3,3b ; Danger Number: 33 ; Danger Label :3
Substance ID number: 1114
By inland waterways:
ADN/R Class: IIIa,1.a ; ADN/R Category :K1n
By Sea (IMDG):
UN NUmber:1114 ; IMO Class: 3.2 ; IMDG Code : 3185 ; MFAG Number : 312 ; Packing group: II ; Risk Label : 3
Source: Exxon Chemical Holland BV Botlek

Remark: no additional remarks
Source: Dow Benelux N. V. Terneuzen

Remark: DISPOSAL OPTIONS

Recover or recycle if possible. Otherwise :incineration.

TRANSPORT INFORMATION

UN Number: 1114
Class: 3
Packing group: II
Proper Shipping Name: Benzene

Sea (IMO)
Class: 3.2
Packing Group: II
Symbol: Flammable liquid
Marine Pollutant (Y/N): No

Rail/Road (RID/ADR)
Class: 3
Item: 3(b)
Symbol: Flammable liquid
Kemler Plate: 33/1114

Air (IATA/ICAO)
Class: 3
Packing Group: II
Symbol: Flammable liquid
Source: Shell Nederland Chemie B.V. Hoogvliet–Rotterdam

Remark: By barge (sometimes by roadtanker)
Source: DSM Hydrocarbons B.V. Sittard

Remark: Disposal options : Recover or recycle if possible. Otherwise : incineration.

Avoid electrostatic discharge generation.
Earth all equipment.
Avoid splash filling.
Do not use compressed air or oxygen for filling, discharging or handling.
Use a vapour recovery system.
If positive displacement pumps are used, these must be fitted with a non–integral pressure relief valve.
Restrict line velocity during the pumping in order to avoid generation of electrostatic discharge.
Do not smoke. Avoid naked flames. Remove ignition sources.
Avoid sparks.
Transport Information

UN number : 1114
Class : 3
Packing Group : II
Proper Shipping Group : Benzene

Sea (IMO)
Class/Packing group : 3.2/II
Marine Pollutant (Y/N) : No
Symbol : Flammable liquid

Rail/Road (RID/ADR)
Class : 3
Item : 3(b)
Symbol : Flammable liquid
Kemler Plate : 33/1114

Air (IATA/IACO)
Class : 3
Symbol : Flammable liquid
Source: SHELL FRANCE Rueil Malmaison

Remark: INFORMATIONS RELATIVES AU TRANSPORT
Réglementations internationales:

par voies terrestres: classe 3, énumération: 3°b), étiquette: 3, code danger: 33, code matière: 1114

par voie maritime: classe: 3.2, page: 3185, étiquette:3
Source: TOTAL PARIS LA DEFENSE

Remark: DISPOSAL METHODS :
Small spills : take up with sand or other noncombustible adsorbent material and place into containers for later disposal.
Large spills : avoid dispersion of liquid with barriers or mechanicals containment.
Surplus : disposal methods are combustion or incineration.
HANDLING : Wear self contained breathing apparatus to avoid vapour inhalation. Safety goggles, gloves and impervious suits are recommended when there is risk of contact with the material. Exposure monitoring at work. Do not smoke and eliminate all sources of ignition fron areas where the material is stored, handled or used. Never exposed stored material in containers to open air. Product transfer must be done in earthed, airtight conducts. Used antisparking equipment and tools.
STORED : Protect containers against physical damage or fire. Containers properly labelled and sealed, placed in cool and well ventilated areas.
TRANSPORT :
UN No. : 1114
Hazard Identification No. : 33
ADR/RID : 3, Item 3B
IMDG Class : 3.2
IATA-DGR : 3
Packing group : I
Source: REPSOL PETROLEO,S.A. MADRID

Remark: Transport information :

UN number 1114

RPE 3, 3º b) ; 33 ; 3

IMO 3.2

¿¿¿
Source: PETROLEOS DE PORTUGAL – PETROGAL,S.A. LISBOA

Remark: Smaltimento.
Il Benzene (Bz) può essere inviato all' incenerimento in inceneritori autorizzati.
Il Bz, altamente infiammabile e volatile, deve essere trattato con estrema cautela nella fase di accensione.
Source: ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG

(66)

Remark: Trasporto.
Il Benzene (Bz) arriva al sito produttivo di Scanzorosciate mediante autobotti e vagoni cisterna ferroviari.
Attualmente sono previsti complessivamente da 5 a 6 arrivi giornalieri.
Il Bz è soggetto alla regolamentazione nazionale ed internazionale relativa al trasporto di merci pericolose.

Ai fini del trasporto stradale e ferroviario è classificato nella categoria 3 relativa ai materiali molto infiammabili all' ordianle 3b.

Source: ALUSUISSE Italia SpA – FTALITAL Factory Scanzorosciate BG

Remark: The primary routes of exposure are inhalation of contaminated air, especially in areas with high traffic, and in the vicinity of gasoline service stations and consumption of contaminated drinking water.

For late model cars it has been estimated that over 90% of automotive benzene comes from exhaust and less than 10% from fuel evaporation.. (Re: Verschueren, Handbook of environm. data of organic compound, 2Ed. NY, Van Nostrand Reinhold Co, 1983, 238).

In 1976, an estimated 1.3 billion pounds of benzene were released into the atmosphere from 132 million stationery and mobile sources...(in USA). (Re: DHHS/NTP, Fourth Annual Report on Carcinog., p.35, 1985).

Source: Enichem Synthesis Milan

Remark: La produzione della sostanza è terminata nel 1993

Source: Kuwait Raffinazione e Chimica Napoli

Remark: Benzene delivered in up to 20 tonnes stainless steel road tankers. Offtake by pump.Benzene in181 kg drums delivered ona flatbed lorry (+ 205 l. packages).

UK Road :

Hazchem code : 3 WE
Packing group II – ID No 1114.

RID – ADR –class 3 Hem 3 (b)

IMO : IMDG P 3185

ICAO : Passenger 305 (5 litres). Cargo : 307 (60 litres).

– Water pollution :
  ––––––––––––––
  UK legislation : Environmental pollution Act 1990 monitored by HMIP.
  Categorie de danger WGK : O

– Major accidents hazards :
  UK : complies to (CIMAH) Control of Industrial Major Accidents Hazards Regulations.

– Air pollution : Environmental pollution Act 1990 (part. I) monitored by HMIP.

Source: Rhone–Poulenc Chemicals Limited Watford, Hertforshire

Remark: Disposal: reprocess if feasable, may be incinerated.
Transport:by road, rail and sea. UN No 1114 Hazchem 3WE
Source: CONOCO Limited. Warwick

Remark: Disposal Options

Recover or recycle if possible. Otherwise incinerate.
Transport Conventions

Proper Shipping Name : Benzene
UN No. : 1114

ADR/RID

Class : 3
Item : 3(b)
Hazard ID No. : 33

IMO/IMDG

Class : 3.2
Packing Group : II
Marine Pollutant : No

ICAO/IATA

Class : 3
Packing Group : II
Transport Information

Transported by : Sea
Frequency : 4/5 times per month
Source: Shell UK Limited, London.

Remark: Disposal Options

Recover or recycle if possible. Otherwise incinerate.
Transport Conventions

IMO/IMDG

Class : 3.2
UN No. : 1114
Packing Group : II
Page No. : 3185
Marine Pollutant : No
Transport Information

Transported by : Sea
Frequency : 4/5 times per month
Source: Shell UK Limited London

Remark: Disposal options:
Recover or recycle if possible. Otherwise: incineration.

Transport classification:
UN number: 1114
IMO class/Packing group: 3.2/II
Marine pollutant: No
IMO symbol: Flammable liquid
IMO proper shipping name: benzene
ADR/RID class/item: 3/3 b
ADR/RID symbol: Flammable liquid
ADR/RID Kemler number: 33
ADR/RID packing group: 2
ADR/RID proper shipping name: benzene
ICAO class: 3
ICAO packing group: II
ICAO proper shipping name: benzene

TA-Luft (1986):
Max. emission of 5 mg/m³ (carcinogenic substances class III)

Stoerfall-Verordnung (1991):
Substance number 39

Verordnung ueber brennbare Fluessigkeiten (1990):
A I

Source: Deutsche Shell Chemie GmbH Eschborn

Remark: Analytical methods for the determination of benzene in environmental samples can be summarized as follows:
Air: preconcentration by passing through a solid sorbent (Tenax resin, silica gel or activated carbon), thermal desorption or desorption by a solvent (e.g. CS2), gaschromatographic detction with a flame-ionization detector(FID) or a mass-selective detector (MSD). Detection limits are usually in the low ppb (ug/m3) range.
Water, soil, sediment, food: isolation by purge-and-trap methods, purging of the sample by an inert gas (e.g. N2), gaschromatographic detection (detectors see above). Detection limits are usually in the low to sub ug/l range (water) and as low as 1 ng/kg (soil, sediment, food), respectively. In the latter case, recoveries and precision seem to be frequently low.

Source: Deutsche Shell Chemie GmbH Eschborn

(63)

Remark: Wasserschadstoff:
Wassergefährdungsklasse 3
KBwS-Einstufung

Luftschadstoff:
Benzol ist im Anhang II der Störfallverordnung unter Nr.39 sowieunter Ziffer 2.3 der TA-Luft mit Klasse III zu finden.

Source: Sächsische Olefinwerke GmbH Böhlen

Remark: Die Abgabe an Dritte darf nur erfolgen bei Beachtung der – GGVO
– Verordnung über Verbote und Beschränkungen des Inverkehrbringens gefährlicher Stoffe, Zubereitungen und Erzeugnisse nach dem Chemikaliengesetz (Chemikalienverbotsverordnung–ChemVerbotsV) vom 14.Oktober 1993 BGBl. I S.1720
Source: PCK AG Schwedt Schwedt/Oder

Remark: Entsorgung: Rückgewinnung durch Destillation. Transportvorschriften: Kl.3; Ziff.3b); Kemmlerzahl 33; UN–Nr.1114.
Source: DEA Mineraloel AG Hamburg

Remark: Beförderung in Eisenbahnkesselwagen
Hinweise zur Entsorgung:
Empfehlung:Durch Destillation zurückgewinnen, in geeigneter Verbrennungsanlage vernichten oder als Sondermüll entsorgen.
Source: ÖMV Deutschland GmbH Burghausen

(67)

1.16 Last Literature Search

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1.17 Reviews

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1.18 Listings e.g. Chemical Inventories

–

2.1 Melting Point

Value: = 5 degree C
Decomposition: no
Sublimation: yes
Source: REPSOL PETROLEO,S.A. MADRID
Test substance: It sublimes at -30 to 5 deg C

Value: = 5.5 degree C
Decomposition: no
Method: other
GLP: no data
Remark: No further details were available.
Source: BP Chemicals Ltd. London
(68) (69) (70) (71) (72) (73)

Value: = 5.5 degree C
Source: BASF AG Ludwigshafen
(74)

Value: 5.5 degree C
Decomposition: no
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(75) (76) (77) (78) (79) (80)

2.2 Boiling Point

Value: = 80 degree C
Source: REPSOL PETROLEO,S.A. MADRID

Value: = 80 degree C
Source: BASF AG Ludwigshafen
(74)

Value: = 80.1 degree C at 1013.25 hPa
Decomposition: no
Method: other
GLP: no data
Remark: No further details were available.
Source: BP Chemicals Ltd. London
(68) (69) (70) (71) (72) (73)

Value: 80.1 degree C at 1013.25 hPa
Decomposition: no
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(75) (76) (77) (79) (80)

2.3 Density

Type: relative density
Value: = .8787 g/cm3 at 15 degree C
Source: REPSOL PETROLEO,S.A. MADRID

Type: relative density
Value: = .879 at 15 degree C
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: relative to water at 4 degree C
(76)

Type: relative density
Value: = .8765 at 20 degree C
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: relative to water at 4 degree C
(77) (79)

Type: density
Value: = .8788 g/cm3 at 20 degree C
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(75)

Type: relative density
Value: = .879 at 20 degree C
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: relative to water at 4 degree C
(80)

Type: density
Value: = .879 g/cm3 at 20 degree C
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Type: density
Value: = .879 g/cm3
Method: other
GLP: no data
Remark: Test conducted at 15-20°C. No further details were available.
Source: BP Chemicals Ltd. London
(68) (70) (72) (73)

Type: density
Value: = .88 g/cm3
Source: BASF AG Ludwigshafen
(74)

2.3.1 Granulometry

-

2.4 Vapour Pressure

Value: = 10 hPa at 10 degree C
Remark: Vapour pressure: 92 mmHg at 20 C
Source: REPSOL PETROLEO,S.A. MADRID

Value: 99.7 hPa at 20 degree C
Method: other (calculated)
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data

temperature (degree C)	vapour pressure (hPa)
0	34.66
10	59.65
30	157.8
40	241.9

Source: Deutsche Shell Chemie GmbH Eschborn
(75) (77)

Value: = 100 hPa at 20 degree C
Source: BASF AG Ludwigshafen
(74)

Value: = 126.9 hPa at 25 degree C
GLP: no data
Remark: Value given converted from cited value of 95.18 Torr. No further details available. Other vapour pressures of 100 mmHg at 26.1°C (converts to 133.32 hPa; HSDB, 1993) and 40 mmHg at 7.6°C (converts to 53.33 hPa; OHMTADS, 1987) have been reported.
Source: BP Chemicals Ltd. London
(81) (70) (71) (73)

Value: = 200 hPa at 38 degree C
Source: BASF AG Ludwigshafen
(74)

2.5 Partition Coefficient

log Pow: = 1.56 - 2.15 at 25 degree C
Method: other (calculated)
Year:
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Compilation of literature data.
Value recommended by the author: log Pow = 2.13 at 25 degree C
Source: Deutsche Shell Chemie GmbH Eschborn
(82)

log Pow: 1.83
Method: other (measured)
Year:
GLP: no data
Remark: Reliability: 2 (valid with restrictions)
Comparable to guideline study with acceptable restrictions
Test procedure not described in detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: room temperature; substance concentration: ca. 1 g/l; reversed phase analytical column; mobile phase: methanol/water; UV detection
Test substance: commercial product (purity not given)
(83)

log Pow: 2.11
Method: other (measured)
Year:
GLP: no data
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Room temperature
Test substance: commercial product (spectrograde, purity not given)
(84)

log Pow: = 2.13
Method:
Year:
Source: REPSOL PETROLEO,S.A. MADRID

log Pow: = 2.13
Method:
Year:
GLP: no data
Remark: The log Pow (octanol-water partition coefficient) was reported as above. No further details on test method were available. Other log Pow values have been reported to be 2.15 (Hansch C. & Leo A.J. Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley & Sons, New York, 1979 cited in Hodson P.V. et al. Envir. Toxic. Chem. 7, 443-454, 1988; Jow P. & Hansch C. Unpublished results cited in ISHOW. Information System for

Hazardous Organics in Water Databank. Chemical Information System, Inc., Baltimore, Maryland 21212, 1993; Veith G.D. & Morris R.T. A Rapid Method for Estimating Log P for Organic Chemicals. Report EPA 600/3–78–049. US Environmental Protection Agency, Duluth, Minnesota, 1978 cited in Swann R.L. et al. Residue Rev. 85, 17–28, 1983) and 2.19 (Van Leeuwen C.J. & Van der Zandt P.T.J. Envir. Toxic. Chem. 11, 267–282, 1992).

Source: BP Chemicals Ltd. London

(85) (86) (70) (87) (88) (89) (90)

log Pow: = 2.14
Method:
Year:
Source: BASF AG Ludwigshafen

(74)

log Pow: 2.19
Method: other (measured)
Year:
GLP: no data
Remark: Reference not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn

(91)

2.6.1 Water Solubility

Value: = 1.8 g/l at 20 degree C
Remark: Mischbar mit vielen organischen Lösemitteln.
Source: BASF AG Ludwigshafen

(74)

Value: ca. 1.88 g/l at 23.5 degree C
Method: other
GLP: no data
Remark: cited value: 0.188 % (w/w)
Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn

(76)

Value: = 1.8 mg/l at 25 degree C
Method: other
GLP: no data
Remark: Value given converted from cited value of: 0.18 g/100 g. No further details were available on this study. Other reports of the water solubility have been 1.71 g/l (BUA. Benzene. BUA Substance Report 24. Advisory Committee on Existing Chemicals of Environmental Relevance (BUA), German Chemists' Association. VCH, Weinheim, Germany, 1988) and 1.78 g/l (Mackay D. & Leinomen P.J. Envir. Sci. Technol. 9, 1178–1180, 1975 cited in ATSDR. Draft Toxicological Profile for Benzene. Agency for Toxic Substances and Disease Registry, Public Health Service, US DHHS, 1991), both at 25°C.
Source: BP Chemicals Ltd. London

(71) (92)

Value: 1.8 g/l at 25 degree C
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(78)

Value: = .06 vol%
Source: REPSOL PETROLEO,S.A. MADRID

2.6.2 Surface Tension

-

2.7 Flash Point

Value: -11.1 degree C
Type:
Method: other
Year:
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(78)

Value: = -11 degree C
Type: closed cup
Method: other: see reference
Year: 1983
GLP: no data
Source: REPSOL PETROLEO,S.A. MADRID
(93)

Value: = -11 degree C
Type: closed cup
Method: other
Year:
GLP: no data
Remark: No further details were available.
Source: BP Chemicals Ltd. London
(68) (94) (72)

Value: = -11 degree C
Type:
Method:
Year:
Source: BASF AG Ludwigshafen
(74)

Value: -11 degree C
Type: closed cup
Method: other
Year:
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(75) (76) (77) (80)

2.8 Auto Flammability

Value: = 562 degree C
Method: other
GLP: no data
Remark: No further details on this study were available. Other auto flammability values of 580°C (OHMTADS. Oil and Hazardous Materials Technical Assistance Data System. US Environmental Protection Agency, 1987. SilverPlatter, Chem-Bank. August 1993) and 591°C (CHRIS. Chemical Hazard Response Information System. March 1991 Edition. US Dept of Transportation. SilverPlatter, Chem-Bank. August 1993) have been reported.
Source: BP Chemicals Ltd. London
(94) (70) (95)

Value: = 562 degree C at 1013.25 hPa
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data / reference not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn
(95) (80)

Value: = 590 degree C
Method: other: see reference
Year: 1986
GLP: no data
Source: REPSOL PETROLEO,S.A. MADRID
(96)

Value: 595 degree C at 1013.25 hPa
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Value:
Remark: Zuendtemperatur: 555 Grad C
Source: BASF AG Ludwigshafen
(74)

2.9 Flammability

Result: flammable
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
see section 2.7 above
Source: Deutsche Shell Chemie GmbH Eschborn
(77) (80)

Result: highly flammable
Method: other: Dir. 67/548/EEC
Year: 1967
GLP: no data
Remark: Lower Flammability Limit : 1.3%
Upper Flammability Limit : 7.1
Source: REPSOL PETROLEO,S.A. MADRID
(96)

Result: highly flammable
Method: other
GLP: no data
Remark: See section 2.7 above.
Source: BP Chemicals Ltd. London
(68) (94) (72)

Result: highly flammable
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
see section 2.7 above
Source: Deutsche Shell Chemie GmbH Eschborn
(76)

2.10 Explosive Properties

Result: explosive under influence of a flame
Method: other
GLP: no data
Remark: The vapour may explode if ignited in an enclosed area. It may form an explosive mixture with air.
Source: BP Chemicals Ltd. London
(68) (73)

Result: not explosive
Method: other
GLP: no data
Remark: No further data were available.
Source: BP Chemicals Ltd. London
(97)

Result: other
Method: other
GLP: no data
Remark: The lower and upper explosive limits have been reported to be 1.4 and 8.0 % respectively.
Source: BP Chemicals Ltd. London
(98) (99)

Result: other
Method: other
GLP: no data
Remark: study not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn
(100)

Result: other
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(101) (102)

Result: other
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(80)

Result: other
Method: other
GLP: no data
Remark: Reliability: 2 (valid with restriction)
Handbook data
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Result:
Remark: Lower Explosive Limits : 1.4%
Upper Explosive Limits : 8
Source: REPSOL PETROLEO,S.A. MADRID
(103)

Result:
Remark: Explosionsgrenzen in Luft: 1.2-8.0 Vol.%
Source: BASF AG Ludwigshafen
(74)

2.11 Oxidizing Properties

Result: no oxidizing properties
Method: other
GLP: no data
Remark: No further details were available.
Source: BP Chemicals Ltd. London
(97)

Result: no oxidizing properties
Method: other
GLP: no data
Remark: study not yet available (12/10/94)
Source: Deutsche Shell Chemie GmbH Eschborn
(100)

2.12 Additional Remarks

Remark: STABILITY : Flammable and combustible.
CONDITIONS TO AVOID : Exposure to heat, sparks, static electricity or flames.
INCOMPATIBILITIES : Strong Oxidants.
HAZARDOUS DECOMPOSITION/COMBUSTION PRODUCTS : CO and toxic vapours in case of incomplete combustion.
EXTINGUISHING AGENTS : Foams, dry chemicals, CO2. Water may be ineffective but water should be used to keep fire-exposed containers cool.
SPECIAL HAZARDS : Vapours may travel to a source of ignition and flash back. RUNOFF TO DRAINS OR SEWER MAY CREATE FIRE AND EXPLOSION HAZARD
Source: REPSOL PETROLEO,S.A. MADRID

Remark: Benzene floats on water.
Source: BP Chemicals Ltd. London
(104)

Remark: Liquid surface tension: 28.9 dynes/cm at 20 degree C (0.0289 N/m).
Source: BP Chemicals Ltd. London
(104) (70)

Remark: Liquid-water interfacial tension: 35 dynes/cm at 20 degree C (0.035 N/m).
Source: BP Chemicals Ltd. London
(104)

Remark: Latent heat of vaporisation: 169 Btu/lb (94.1 cal/g; 3.94 x 10+5 J/kg).
Source: BP Chemicals Ltd. London
(104) (70)

Remark: Heat of combustion: -17460 Btu/lb (-9698 cal/g; -406 x 10+5 J/kg).
Source: BP Chemicals Ltd. London
(104) (70)

Remark: Heat of fusion: 30.45 cal/g (127.4 J/g; 9951 J/mol).
Source: BP Chemicals Ltd. London
(104) (70)

Remark: Heat capacity: 135.6 J/mol-degree K at 1 atm (liquid); 81.6 J/mol-degree K at 1 atm (gas).
Source: BP Chemicals Ltd. London
(70)

Remark: Viscosity: 0.6468 mPa's at 20 degree C.
Source: BP Chemicals Ltd. London
(70)

Remark: % in saturated air: 13.15 at 26 degree C and 1 atm.
Source: BP Chemicals Ltd. London
(70)

Remark: Miscible with alcohol, chloroform, ether, carbon disulphide, acetone, oils, carbon tetrachloride, glacial acetic acid.
Source: BP Chemicals Ltd. London
(94) (70)

Remark: Gefaehrliche Reaktion mit: Saeuren, Oxidationsmitteln, Brom, Chlor, Jod, Schwefel (fluessig)
Viskositaet: 0.652 mPa.s bei 20 Grad C
Source: BASF AG Ludwigshafen
(74)

Remark: undissolved benzene floats on water
Source: Deutsche Shell Chemie GmbH Eschborn
(78)

Remark: liquid surface tension (temperature not given): 29 dyn/cm (29 mN/m)
Source: Deutsche Shell Chemie GmbH Eschborn
(80)

Remark: heat of vaporisation: 435.0 J/g at 20 degree C
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Remark: heat of combustion: -3725.3 kJ/mol (-47760 J/g)
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Remark: heat of fusion: 125.9 J/g
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Remark: viscosity: 0.654 mPa x s at 20 degree C
Source: Deutsche Shell Chemie GmbH Eschborn
(77)

Remark: miscible with ethanol, diethylether, carbondisulfide, acetone, tetrachlormethane, acetic acid
Source: Deutsche Shell Chemie GmbH Eschborn
(79) (80)

Remark: Henry's Law Constant: 2.67E–3 atm x m3/mol at 10 degree C (270.5 Pa x m3/mol)
5.5E–3 atm x m3/mol at 25 degree C (557.3 Pa x m3/mol)
Source: Deutsche Shell Chemie GmbH Eschborn
(105)

Remark: Henry's Law Constant: 533–570 Pa x m3/mol at 25 degree C (experimental value: 562 Pa x m3/mol; recommended value: 550Pa x m3/mol; compilation of literature data)
Source: Deutsche Shell Chemie GmbH Eschborn
(106)

Remark: odour threshold (water): 0.17 mg/l
odour threshold (air): 39 mg/m3 (12 ppm)
Source: Deutsche Shell Chemie GmbH Eschborn
(107)

Remark: odour threshold (air): 4.68 ppm (15.2 mg/m3)
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: highest purity commercially available product from large scale production
(108)

Remark: saturation vapour concentration: 320 g/m3 at 20 degree C
489 g/m3 at 30 degree C
Source: Deutsche Shell Chemie GmbH Eschborn
(75)

3.1.1 Photodegradation

Type: air
Light source: other
Conc. of subst.: at 24 degree C
INDIRECT PHOTOLYSIS
Sensitizer: O3
Conc. of sens.: 3000000000000 molecule/cm3
Rate constant: = .0000000000000000000001 cm3/(molecule * sec)
Degradation: = 50 % after 105 year
Method: other (calculated)
Year: **GLP:** no data
Test substance: no data
Result: In an urban atmosphere, the half–life for the reaction of benzene with ozone was calculated to be 105 years. In a rural atmosphere, the half–life was found to be 327 years, using an atmospheric concentration for ozone of 9.6 x 10+11 molecules/cm3. Consideration of the reaction with ozone in isolation from other sensitizer species is not a realistic scenario for benzene atmospheric fate.
Source: BP Chemicals Ltd. London
Test condition: The light source was chemiluminescence. The rate constant used was that of Pate C.T. et al. J. envir. Sci. Hlth A11, 1–10, 1976 and the sensitizer concentration that of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. McGraw–Hill Book Company, New York, 1982.

(109)

Type: air
Conc. of subst.: .013 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: O3
Conc. of sens.: .001 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: Very little aerosol was formed when benzene reacted with ozone in the dark.
Source: BP Chemicals Ltd. London
Test condition: A light scattering photometer was used. No further details were available.

(110) (111)

Type: air
Light source: other
INDIRECT PHOTOLYSIS
Sensitizer: O3
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: Quantum yield (254 nm): 0.05 +/- 0.02; from this result the authors conclude that chain reaction steps are not important for ozone-sensitized benzene photooxidation.
Main degradation products: phenol, biphenyl, benzoic aldehyde, maleic acid anhydride, p-benzoquinone, toluene. Phenol seemed to be the by far abundant component.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: room temperature; substance concentrations: 0.17 and 1.3 hPa; ozone concentration: <= 150 ppm; irradiation time: 3-8 h; product accumulation in a cold trap; GC-MS analysis

(112)

Type: air
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000
Rate constant: = .0000000000012 cm3/(molecule * sec)
Degradation: 50 % after 13.4 day
Method: other (calculated)
Year: 1977 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(113)

Type: air
Light source: other
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000
Rate constant: = .0000000000012 cm3/(molecule * sec)
Degradation: = 50 % after 13.4 day
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: The half-life for the reaction with OH radicals (concentration = 5 x 10+5 radicals/cm3) produced by flash photolysis and using a resonance fluorescence method was 13.4 days.
Source: BP Chemicals Ltd. London

(110) (114) (115)

Type: air
Light source: other
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 100000000000
Rate constant: .0000000000012 cm3/(molecule * sec)
Degradation: day
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 1 (valid)
Comparable to guideline study
Result: Taking a OH radical concentration of 5E+5 radicals/cm3 a half-life of 13.4 days can be calculated.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Room temperature; flow-through system; initial substance concentration: saturated vapour at -22 - + 22 degree C; total pressure: 133 +/- 3 hPa (argon diluent)

(116)

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 7500000 molecule/cm3
Rate constant: = .0000000000013 cm3/(molecule * sec)
Degradation: = 50 % after 19 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: The half-life was found to be 19 hours and the residence time 28 hours.
Source: BP Chemicals Ltd. London
Test condition: This study examined the reaction of benzene with OH radicals under photochemical smog conditions in South East England at summer day temperatures. Presumably sunlight was the light source.

(117) (118)

Type: air
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1100000 molecule/cm3
Rate constant: = .0000000000013 cm3/(molecule * sec)
Degradation: = 50 % after 5.6 day
Method: other (calculated)
Year: 1982 **GLP:** no data
Test substance: no data
Result: A half-life for the reaction with OH radicals in ambient air was 5.6 days and the residence time was 8.1 days. In polluted air, with an OH radical concentration of 1 x 10+8 molecules/cm3, the half-life was 1.5 hours and the residence time was 2.1 hours.
Source: BP Chemicals Ltd. London
Test condition: The half-life was calculated using the method of Lyman W.J.

et al. Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. McGraw-Hill Book Company, New York, 1982.

(110) (119) (118)

Type: air
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Degradation: = 50 % after
Method: other (calculated)
Year: **GLP:** no data
Test substance: no data
Result: A half-life of 2.4-24 hours was estimated from the reaction rates reported by Davis D.D. et al. J. Phys. Chem. 79, 293-294, 1975 (k = 1.59 x 10-12 cm3/molecule-second) and Hansen D.A. et al. ibid. 79, 1763-1766, 1975 (k = 1.24 x 10-12 cm3/molecule-second). Presumably sunlight was the light source and no indication of sensitizer concentration was given.
Source: BP Chemicals Ltd. London

(120) (118)

Type: air
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1200000 molecule/cm3
Degradation: = 50 % after 5.3 day
Method: other (calculated)
Year: **GLP:** no data
Test substance: no data
Result: A half-life for the reaction between benzene and OH radicals of 5.3 days was calculated using the average Dutch OH radical abundance of 1.2 x 10+6 molecules/cm3 and the rate constants reported by Davis D.D. et al. J. Phys. Chem. 79, 293-294, 1975 (k = 1.59 x 10-12 cm3/molecule-second), Hansen D.A. et al. ibid. 79, 1763-1766, 1975 (k = 1.24 x 10-12 cm3/molecule-second) and Perry R.A. et al. ibid. 81, 296-304, 1977 (k = 1.2 x 10-12 cm3/molecule-second), measured at 25°C, and those of Tully F.P. et al. ibid. 85, 2262, 1981, Wahner A. & Zetsch C. In: Physico-chemical Behaviour of Atmospheric Pollutants, 138. Proceedings of the 2nd European Symposium, 1981 and Witte F. & Zetsch C. In: Physico-chemical Behaviour of Atmospheric Pollutants, 168. Proceedings of the 3rd European Symposium, Varese. Edited by B. Versino and G.G. Angeletti. D. Reidel Publishing Co., Dordrecht/Boston/Lancaster, 1984 (no rate constant values reported). (All these references were cited in Nielsen et al. 1991). Presumably sunlight was the light source.
Source: BP Chemicals Ltd. London

(118) (121)

Type: air
Light source: other
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Absolute rate constant measurements
Reliability: 1 (valid)
Comparable to guideline study
Result:

Method	t (degree C)	p (hPa)	k (cm3/(molecule x s))
A	21	533	(12.2+/-2)E-13
A	22	667	(10.2+/-0.4)E-13
B	22	112	(1.1+/-0.2)E-12
B	25	42	(1.2+/-0.2)E-12
C	23	1030	(9.7+/-0.5)E-13

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: A: flash photolysis/resonance fluorescence; light source: spark discharge in N2 at atmospheric pressure; production of OH radicals by pulsed vacuum UV photolysis of H2O (initial OH radical concentration: 5E10molecules/cm3); Ar and He as diluent gas; temperature: 21 and 23 degree C; pressure: 533 and 667 hPa
B: laser photolysis/resonance fluorescence; light source: excimer laser; production of OH radicals from HNO3 (initial OH radical concentration: 4E11 molecules/cm3); Ar as diluent gas; temperature: 22 and 25 degree C; pressure: 42 and 112 hPa
C: laser photolysis/laser absorption; light source: excimer laser; production of OH radicals from photolysis of H20 at 193 nm or from photolysis of H2O2 at 248 nm (initial OH radical concentration: 1E9 molecules/cm3); N2 or air as diluent gases; temperature: 23 degree C; pressure: 1030 hPa
(122)

Type: air
Light source: other
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Relative rate constant measurements
Reliability: 1 (valid)
Comparable to guideline study
Result:

Method	t (degree C)	p (hPa)	k (cm3/(molecule x s))
A	23	267, 933	1.1E-12 (single measurements)
A	27	1000	(1.2+/-0.1)E-12
B	23-32	1050	1.2E-12
C	27	1004	(1.04+/-0.13)E-12

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: A: chamber volume: 420 and 40 l respectively; light source: photolysis lamps; generation of OH radicals by (a) thermal decomposition of HO2NO2 in the presence of NO and (b) by photolysis of CH3ONO; reference substance: n-butane; synthetic air and Ar as diluent gases

B: chamber volume: 450 l; light source: UV–A lamps producingphotochemical smog in the chamber; NO, NO2, O3 concentrations and relative humidity are monitored; initial concentrations of smog–forming compounds (NO, ethene): ca. 1ppm; initial substance concentrations: ca. 100 ppb; reference substance: toluene; synthetic air as diluent gas
C: chamber volume: 520 l; light source: metal halogenide lamp; generation of OH radicals by photolysis of NOx; relative humidity: 70 –90%; NOx concentration: 300–1000 ppb (with an initial NO/NO2 ration of 10:1); initial substance concentration: 10–50 ppb; reference substance: ethene; synthetic air as diluent gas

(122)

Type: air
Light source: other
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restrictions)
compilation of literature data
Result: At a temperature of 25 degree C the measured rate constants for the reaction of benzene with OH radicals were between 1.15E–12 and 1.59E–12 cm3/(molecule x s).
Source: Deutsche Shell Chemie GmbH Eschborn

(123)

Type: air
Light source: other
Light spect.: > 160 nm
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 0 molecule/cm3
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Comparable to guideline study
Result: (a) The reaction rate of the addition of OH radicals to benzene was measured in dependance of the pressure. The rate constants were between 3E–13 and 12.2E–13 cm3s–1 at a temperature of 21 degree C.
(b) The reaction rate of the addition of OH radicals to benzene was measured in dependance of the temperature. The rate constants were between 1.11E–12 and 1.33E–12 cm3s–1 at a pressure of 200 hPa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: OH radicals were generated by pulsed vacuum–UV photolysis ofH2O and monitored by resonance fluorescence technique. The test substance was at gas saturation concentration (Ar diluent gas). (a) pressure: 1.3–533 hPa; (b) –34–81 degree C
Test substance: The test substance contained 0.15% toluene.

(124)

Type: air
Light source: other
Light spect.: > 105 nm
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Comparable to guideline study
Result: The pressure and temperature dependent measurements of the reaction rate of benzene with OH radicals gave following results: k = (0.91–1.02)E–12 cm3s–1. For room temperature at a pressure of 133 hPa an average rate constant of (1.02 +/–0.2)E–12 cm3s–1 is given.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: OH radicals were generated by vacuum–UV flash photolysis of H2O and monitored by a resonance fluorescence method (radical concentration not given. Benzene was used at gas saturation concentration (He as diluent gas). Pressure range: 33–666 hPa; temperature range: –11––37 degree C.
Test substance: The test substance contained 0.04% toluene.

(125)

Type: air
Light source: other
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Comparable to guideline study
Result: Rate constants k for the reaction of benzene with OH radicals were (1.04–2.20)E–12 cm3molecule–1s–1 in the temperature range between –23 and 744 degree C. At 25 degree C a rate constant k of 1.24E–12 cm3molecule–1s–1 was found. In the range between 33 and 267 hPa almost no dependance of the rate constant of the pressure was detected.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Temperature range: –23 – 744 degree C; pressure range: 33–267 hPa; concentration of test substance about 100 times higher than concentration of sensitizer; concentration of sensitizer: 2E–10 – 1E–11 molecules/cm3. The OH radicals were generated by flash photolysis of H2O.

(126)

Type: air
Light source: other
Light spect.: > 290 nm
Conc. of subst.: .32 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: No evidence of benzene degradation was found. It is noted that ambient air is rarely clean or dry.
Source: BP Chemicals Ltd. London
Test condition: Benzene exposed in a 20 l smog chamber filled with clean, dry air. Irradiation with light at a wavelength corresponding to tropospheric sunlight occurred for 6 days. No further details were available.
(110) (127) (118)

Type: air
Light source: other
Light spect.: > 230 nm
Conc. of subst.: .32 mg/l at 25 degree C
DIRECT PHOTOLYSIS
Degradation: = 31.5 % after 2 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: Cited value of 100 ppm converted to 0.32 mg/l. 68 ppm carbon dioxide and 121 ppm carbon monoxide (convert to 0.12 and 0.14 mg/l respectively) were detected. The half–life was not reported.
Source: BP Chemicals Ltd. London
Test condition: Benzene exposed in a 4.4 l reactor for 2 hours. The light source was a mercury vapour lamp with a water–cooled quartz filter. Carbon dioxide and carbon monoxide degradation products measured by infrared–gas chromatography in the case of carbon dioxide or gas chromatography with a molecular sieve column ultrasound detector in the case of carbon monoxide.
(128)

Type: air
Light source: other
Light spect.: > 290 nm
Conc. of subst.: .32 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
Result: No evidence of benzene degradation was found. After the addition of chemicals which produce active species (no further specification) benzene half–lives were between 4 and 5 h. Using light with a higher intensity (wavelengths > 230 nm) a half–life of 6.5 h was detected.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Initial substance concentration: 100 ppm (=0.32 mg/l); benzene exposed in a 20 l smog chamber filled with clean, dry air. Irradiation time: 6 d; no further details.

(129)

Type: air
Light source: other
Light spect.: > 230 nm
Conc. of subst.: .32 mg/l at 25 degree C
DIRECT PHOTOLYSIS
Degradation: = 31.5 % after 2 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Reference not yet available (12/10/94)
Result: 68 ppm carbon dioxide and 121 ppm carbon monoxide (convert to 0.12 and 0.14 mg/l respectively) were detected. The half–life was not reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Initial substance concentration: 100 ppm (=0.32 mg/l); benzene exposed in a 4.4 l reactor for 2 hours. Carbon dioxide and carbon monoxide degradation products were measured by infrared–gas chromatography in the case of carbon dioxide or gas chromatography with a molecular sieve column ultrasound detector in the case of carbon monoxide.

(130)

Type: air
Light source: other
Light spect.: 254 nm
Quantum yield: .05
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: standard deviation: +/– 0.02
quantum yield (185 nm): 0.01 +/– 0.005
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: wavelength 254 nm: substance concentrations 0.13, 1.3 and 8 hPa; NiSO4–filter to eliminate ca. 99% of the 185 nm radiation from the lamp
wavelength 185 nm: substance concentrations 0.1 and 1.3 hPa;O3–filter to eliminate > 99% of the 254 nm radiation from the lamp and of the ethene photolysis as an actinometer reaction

(112)

Type: air
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Conc. of sens.: 72000 molecule/cm3
Rate constant: = .000000000000028 cm3/(molecule * sec)
Degradation: = 50 % after 10.9 year
Method: other (calculated)
Year: 1982 **GLP:** no data
Test substance: no data
Result: The half-life for the reaction between benzene and atomic oxygen presumably in sunlight was calculated to be 10.9 years. The formation of phenol has been reported as a result of such a reaction (Altshuller A.P. & Bufalini J.J. Envir. Sci. Technol. 5, 39, 1971; ATSDR, 1987). This is not reported in the 1991 ATSDR draft report (ATSDR. Draft Toxicological Profile for Benzene. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services, 1991).
Source: BP Chemicals Ltd. London
Test condition: The half-life for the reaction between benzene and atomic oxygen, presumably in sunlight, was calculated using the method of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. McGraw-Hill Book Company, New York, 1982 and the reaction rate constant of Gaffney J.S. & Levine S.Z. Int. J. Chem. Kinet. 11, 1197-1209, 1979 (cited in Envirofate, 1993; Nielsen et al. 1991).

(131) (110) (118)

Type: air
Light source: Sun light
Conc. of subst.: .016 mg/l at 37 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Conc. of sens.: .004 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance:
Remark: A second study conducted in a smog chamber with 1-10 ppm benzene (0.003-0.032 mg/l) and 0.1-3 ppm NO (0.1 x 10-3 - 3.7 x 10-3 mg/l) reported a low reactivity with the maximum NO2 formation occurring after 290 minutes (Levy A. Adv. Chem. Ser. 124, 70-94. Am. Chem. Soc. Washington D.C., 1973 (cited in Envirofate, 1993).
Result: The time for the NO2 formation to reach a maximum concentration was 210 minutes in the FEP bag. No details of the Mylar bag were given.
Source: BP Chemicals Ltd. London
Test condition: A Mylar or FEP bag smog chamber was used. The light source was artificial sunlight (warm white, sun and blacklight fluorescent lamps) and the sensitizer was NO. The FEP bag passes 30-40% more radiation in the 330-400 nm region and much more less than 330 nm than the Mylar bag. The colorim method of analysis was used.

(132) (110)

Type: air
Light source: other
Conc. of subst.: 6.32 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: other
Conc. of sens.: 1.2 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: Degradation to 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2,6-dinitrophenol was found but no levels of these products were given.
Source: BP Chemicals Ltd. London
Test condition: Benzene was tested in an air-NO system in a 1 l quartz reaction vessel. [Concentration of substance converted from cited 2 ml in 1 litre reaction vessel implies concentration was 0.2% v/v = 6.32 mg/l of benzene] [Concentration of sensitizer converted from cited 1 ml in 1 litre reaction vessel implies concentration was 0.1% v/v = 1.2 mg/l of NO] Irradiation with sunlight or xenon lamp was conducted for 5 hours at 25-30°C and degradation followed by thin-layer chromatography, gas chromatography and gas chromatography-mass spectrometry.

(110) (133)

Type: air
Light source: other
Conc. of subst.: .003 mg/l at 26 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: In a second study, benzene was tested at 0.003 mg/l in a smog chamber in the presence of 0.45 ppm NO and 0.05 ppm NO2 (converts to 0.6 x 10-3 and 0.9 x 10-4 mg/l). The rate of photolysis was found to be 1.4 ppb/min (converts to 4.5 µg/l/min) (Dimitriades B. et al. Development and Utility of Reactivity Scales from Smog Chamber Data. RI 8023. US Bureau of Mines, Pittsburgh, Philadelphia, 1975 (cited in Envirofate, 1993).
Result: The rate of photolysis was 0.33 ppb/minute (converts to 1.05 ng/l/min). The rate constant (radical), k(NO2), was 0.29 l/min.
Source: BP Chemicals Ltd. London
Test condition: Concentration of substance 0.003 mg/l converted from cited 1 ppm. Concentration of sensitizer (NO) 0.5 x 10-3 mg/l converted from cited 0.38 ppm. Concentration of sensitizer (NO2) 0.4 x 10-4 mg/l converted from cited 0.02 ppm. The test was conducted in a simulated atmosphere using black fluorescent lamps. The sensitizer was NO and NO2.

(110) (134)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: other
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: The degradation products were found to be phenol and nitrobenzene.
Source: BP Chemicals Ltd. London
Test condition: Benzene was photolysed in air in the presence of NO, NO2 and HNO2. No further details were available.
(110) (135)

Type: air
Light source: other
Light spect.: 345 – 355 nm
Conc. of subst.: .006 mg/l at 30 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Degradation: = 16.5 % after 5 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: In another study, benzene was reported to be unreactive in air, based on the maximum ozone concentration produced (average 3.1% degradation/hour) when 4 ppm benzene (0.013 mg/l) was irradiated in a pyrex glass chamber with 22 black lights and 7 sunlamps in the presence of 0.2 ppm NOx (Dimitriades B. & Joshi S.-B. In: EPA-600/3-77-001B. Inter. Conf. on Photochemical Oxidant Pollution and its Control. p.705-711. Edited by B. Dimitriades. US Environmental Protection Agency, Research Triangle Park, North Carolina, 1977). A similar study in which a benzene-NOx-air system was irradiated in a pyrex flask, showed 2% of the degradation reaction occurring in the first hour and carbon dioxide, carbon monoxide, formic acid, peroxyacetyl nitrate and an unspecified aldehyde to be formed as the degradation products (Kopczynski S.L. Int. J. Air Wat. Pollut. 8, 107-120, 1964). Finally, when 100 ppm benzene (0.32 mg/l) was irradiated, in the presence of 10-110 ppm NOx, with light of wavelength >290 nm, photodegradation occurred with a half-life of 4-5 hours. Approximately 2 days were required for 50% of the benzene to degrade to carbon dioxide (Korte F. & Klein W. Ecotoxic. envir. Saf. 6, 311-327, 1982) (all cited in Envirofate, 1993).
Result: The intensity of irradiation corresponded to K1 = 0.33/min of nitrogen dioxide (NO2) photo-dissociation rate, and 16.5% depletion of benzene was seen in 5 hours. The depletion rate for nitric oxide was found to be 2.2 ppb/min and a maximum concentration of 0.34 ppm NO2 was seen after 300 minutes. No ozone formation was recorded and, after 2 and 4 hours, formaldehyde was measured at 0.02 and 0.03 ppm respectively. NOx was decreased to 0.3 ppm after 5 hours.
Source: BP Chemicals Ltd. London
Test condition: The experiment was conducted with the Mechanical Engineering Laboratory Smog Chamber. The light source was 300 UV fluorescent lamps of "Black light" of relative intensity 700

µW/cm2. Nitrogen oxides were used as the sensitizer at a concentration of 1 ppm (conversion impossible). Substance concentration cited as 2 ppm (converts to 0.006 mg/l). The relative humidity was controlled at 55%. Irradiation was continued for 5 hours. NO2 was measured using the Saltzman method or UV spectro–photometer and nitric oxide was measured by the chemiluminescence method. NOx was reduced to NO by a converter, for measurement. Ozone was measured by the chemiluminescence of the reaction with ethylene and formaldehyde by the para–rozani–line method. Benzene was analysed with a gas chromatograph.

(110) (136)

Type: air
Light source: Sun light
Light spect.: > 290 nm
Conc. of subst.: .32 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: other
Degradation: = 50 % after 6 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: Photodegradation seen, with approximately 2 days required for 50% degradation to carbon dioxide. Half–life found to be 6 hours.
Source: BP Chemicals Ltd. London
Test condition: No further information available on method. The sensitizer was sulphur dioxide at a concentration cited as 10–110 ppm (converts to 0.026–0.288 mg/l). The light source was light at a wavelength corresponding to tropospheric sunlight.

(110) (137)

Type: air
Light source: Sun light
Light spect.: > 290 nm
Conc. of subst.: .32 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: other
Conc. of sens.: .012 mg/l
Degradation: = 50 % after 16 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: Photodegradation seen, with approximately 2 days required for 50% degradation to carbon dioxide. Half–life found to be 16 hours.
Source: BP Chemicals Ltd. London
Test condition: No further information available on method. The sensitizer was isobutane at a concentration cited as 5 ppm (converts to 0.012 mg/l). The light source was light at a wavelength corresponding to tropospheric sunlight.

(110) (138)

Type: air
Light source: Xenon lamp
INDIRECT PHOTOLYSIS
Sensitizer: other
Conc. of sens.: 1.2 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail.
Levels of degradation products not given
Result: Degradation to nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2,6-dinitrophenol was found. During the photochemical reaction the initial substance concentration decreased by about 10 %. About 16 % of the decreased benzene was converted into nitrobenzene (5.5%), nitrophenols (7.7%) and dinitrophenols (2.4 %).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Benzene (6.32 mg/l) was tested in an air-NO system in a 1 l quartz reaction vessel (concentration of substance convertedfrom cited 2 ml in 1 litre reaction vessel implies concentration was 0.2% v/v = 6.32 mg/l of benzene; concentration of sensitizer converted from cited 1 ml in 1 litre reaction vessel implies concentration was 0.1% v/v = 1.2 mg/l of NO). Irradiation was conducted for 5 h at 25-30 degree C and degradation followed by thin-layer chromatography, gas chromatography and gas chromatography-mass spectrometry.

(139)

Type: air
Light source: other
Light spect.: 345 - 355 nm
Conc. of subst.: .006 mg/l at 30 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Degradation: = 16.5 % after 5 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: In another study, benzene was reported to be unreactive in air, based on the maximum ozone concentration produced (average 3.1% degradation/hour) when 4 ppm benzene (0.013 mg/l) was irradiated in a pyrex glass chamber with 22 black lights and 7 sunlamps in the presence of 0.2 ppm NOx (Dimitriades B. & Joshi S.-B., In: EPA-600/3-77-001B. Inter. Conf. on Photochemical Oxidant Pollution and its Control. p.705-711. Edited by B. Dimitriades. US Environmental Protection Agency, Research Triangle Park, North Carolina, 1977, cited in Envirofate, 1993). A similar study in which abenzene-NOx-air system was irradiated in a pyrex flask, showed 2% of the degradation reaction occurring in the first hour. Carbon dioxide, carbon monoxide, formic acid, peroxyacetyl nitrate and an unspecified aldehyde were formed as the degradation products (Kopczynski S.L., Int. J.

Air Wat. Pollut. 8, 107–120, 1964, cited in Envirofate, 1993). Finally, when 100 ppm benzene (0.32 mg/l) was irradiated, in the presence of 10–110 ppm NOx, with light of a wavelength >290 nm, photodegradation occurred with a half–life of 4–5 hours.
Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail.

Result: The depletion rate for NO was found to be 2.2 ppb/min. A maximum concentration of 0.34 ppm NO2 was seen after 300 min. No ozone formation was recorded. After 2 and 4 h formaldehyde was measured at 0.02 and 0.03 ppm respectively. NOx was decreased to 0.3 ppm after 5 hours.

Source: Deutsche Shell Chemie GmbH Eschborn

Test condition: Relative intensity of the light source: 700 uW/cm2. NOx was used as the sensitizer at a concentration of 1 ppm (conversion impossible). Substance concentration cited as 2 ppm. The relative humidity was controlled at 55%. Irradiation was continued for 5 to 6 h. NO2 was measured using the Saltzman method or UV spectro–photometer and nitric oxide was measured by the chemiluminescence method. NOx was reduced to NO by a converter, for measurement. Ozone was measured by the chemiluminescence of the reaction with ethylene and formaldehyde by the para–rozani–line method. Benzene was analysed with a gas chromatograph.

(140)

Type: air
Light source: other
Light spect.: 300 – 430 nm
Conc. of subst.: .139 mg/l at 22.5 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Very high concentrations of test substance

Result: Main photoproducts of the irradiation of a benzene/NO2 mixture were phenol (ca. 0.16 ppm), nitrobenzene (ca. 0.8 ppm), glyoxal (ca. 0.05 ppm), formaldehyde (ca. 0.03 ppm), formic acid and maleic anhydride (ca. 0.01 ppm each) (concentrations given after an irradiation time of 180 min). It is suggested that phenol and nitrobenzene are both generated from the OH–benzene adduct while glyoxal is a ringcleavage product.

Source: Deutsche Shell Chemie GmbH Eschborn

Test condition: Substance concentration: 42.65 ppm; concentration of sensitizer: 1.50 ppm; pressure: 1013 hPa; the chemical structure and the concentration of the photoproducts was determined by FTIR.

(141)

Type: water
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Degradation: = 50 % after 80 day
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: A half–life of 80 days has been reported for the reaction taking place just below the water surface between benzene and OH radicals. The half–life will tend to increase with depth as a result of both decreased light intensity and OH radical concentration.
Source: BP Chemicals Ltd. London
Test condition: No further details on test method or conditions available. (118) (142)

Type: water
Light source: Sun light
DIRECT PHOTOLYSIS
Halflife t1/2: 8.8 – 673 day
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: The authors state that the broad range of values obtained may have been due to loss due to high volatility, varying intensity of sunlight or unsuitable methods of analysis. In any case the relevance of these findings and methodology must be limited by the predominance of volatilization as a removal process for benzene in aquatic systems.
Result: Degradation was reported but no indication of extent was given.
Source: BP Chemicals Ltd. London
Test condition: The method of Leifer A. & Stern A.M. Test guideline: Photochemical transformation in water, EPA, Washington, date unspecified, was used. Experiments were carried out in five different laboratories. No further details were available. (143)

Type: water
Light source: Sun light
DIRECT PHOTOLYSIS
Halflife t1/2: 8.8 – 673 day
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Experiments were carried out in five different laboratories. The authors state that the broad range of values obtained may have been due to loss due to high volatility, varying intensity of sunlight or unsuitable methods of analysis. In any case the relevance of these findings and methodology must be limited by the predominance of volatilization as a removal process for benzene in aquatic systems.
Reliability: 3 (not valid)
Significant methodological deficiences
Suitable only as screening test
Result: Degradation was reported but no indication of extent was

given.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Benzene at a concentration of < 900 mg/l was exposed to sunlight in a closed vessel of duran glas. The substance loss was determined gaschromatographically in the water phase.

(144)

Type: water
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: other
Method:
Year: **GLP:** no data
Test substance: no data
Result: Reaction with singlet oxygen is negligible due to low reactivity, the rate constant having been reported at <<360/M-hour. This is similarly true in the presence of alkyl peroxy radicals (RO2), with the rate constant being reported as <<1/M-hour. In any case the relevance of these findings and methodology must be limited by the predominance of volatilization as a removal process for benzene in aquatic systems.
Source: BP Chemicals Ltd. London

(145) (118)

Type: water
Light source: Sun light
Conc. of subst.: 900 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: other
Degradation: = 50 % after 16.9 day
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: The relevance of these findings are limited by the predominance of volatilization as a removal process for benzene in aquatic systems.
Result: The rate constant (radical) for the reaction was 0.041/day.
Source: BP Chemicals Ltd. London
Test condition: The photolysis of benzene at a concentration of <900 mg/l dissolved in deionized water saturated with air and exposed to sunlight was measured. Benzene concentrations were measured by gas chromatography with flame-ionization detection.

(143)

Type: other
Light source: other
Light spect.: > 230 nm
Conc. of subst.: .32 mg/l
DIRECT PHOTOLYSIS
Halflife t1/2: = 6.5 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Benzene was tested adsorbed on silica gel. No further details were available and the extent of degradation was not reported.

(110) (127)

Type: other
Light spect.: > 290 nm
Conc. of subst.: .32 mg/l
DIRECT PHOTOLYSIS
Degradation: = 5 % after 17 hour(s)
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Result: After 17 hours adsorbed on silica gel and irradiated, 5% photomineralized to carbon dioxide.
Source: BP Chemicals Ltd. London
Test condition: Benzene was tested adsorbed on silica gel. No further details were available and the half–life was not reported.

(110) (127)

Type: other
Light source: other
Method: other (measured)
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
Result:

Test medium	Irradiation time (d)	Light source	Photomineralization (% CO2)
silica gel	6 d	> 290 nm	12–73
natural sand	30 d	sunlight	< 1

Formation of CO2 was dependent on water content of the solid. There was an initial buildup of a high amount of intermediates with significantly delayed CO2 formation.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: No further details were available.

(129)

3.1.2 Stability in Water

Type: abiotic
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Hydrolysis is not a significant process for benzene and it will not be expected to significantly adsorb to sediment.
Source: BP Chemicals Ltd. London

(70)

Type: abiotic
Method: other
Year: **GLP:**
Test substance:
Remark: Reliability: 2 (valid with restriction)
Handbook data
Result: Benzenes appear to be generally resistant to hydrolysis.
Source: Deutsche Shell Chemie GmbH Eschborn

(146)

3.1.3 Stability in Soil

Type: laboratory **Radiolabel:** yes
Concentration: 20 ppm
Soil humidity: 7 g water/100g soil dry weight
Organ. carbon: = 1.3 %
pH: = 7.1
Cation exch. capac.
Microbial biomass:
Method: other
Year: **GLP:** no data
Test substance: no data
Result: The levels of carbon dioxide released were found to be 7.5, 24, 37, 44 and 47% after 3 days and 1, 2, 5 and 10 weeks respectively, showing degradation.
Source: BP Chemicals Ltd. London
Test condition: A base–rich, para–brownish, loess (clay) soil from Flachstöckheim near Braunschweig (100 g; 1.26% carbon; 0.12% nitrogen) was mixed with 2 mg 14C–labelled benzene dissolved in 0.1 ml acetone, and moistened to 70% of the water capacity. [(10 ml water). 70% of water capacity (10 ml water in 100 g soil implies 7% w/w).] 14CO2 production was measured over a period of 10 weeks.

(147)

Type: laboratory **Radiolabel:** no
Concentration: 39.055 ppm
Soil temp.: 30 degree C
Soil humidity: 60 g water/100g soil dry weight
pH: = 7
Cation exch. capac.
Microbial biomass:
Method: other
Year: **GLP:** no data
Test substance: no data
Result: The indigenous microflora needed about 30 hours for adaptation to degrade benzene and 100 hours for the complete removal of benzene.
Source: BP Chemicals Ltd. London
Test condition: Unsterilized soil slurries were prepared from garden soil (clay) in MMY–medium. After addition of 500 µM benzene, the soil was stored for 2 months at 4°C to allow adsorption to take place. A gas–tight system was used for incubation, to prevent volatilization. The flasks were rotated during incubation to ensure mixing. Degradation was measured by gas chromatography.
Test substance: Test substance was laboratory reagent grade.

(148)

Type: laboratory **Radiolabel:** yes
Concentration: 20 mg/kg
Soil humidity: 7 g water/100g soil dry weight
Organ. carbon: 1.3 %
pH: 7.1
Cation exch. capac.
Microbial biomass:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
description of soil parameters not complete
Result: The levels of carbon dioxide released were found to be 7.5, 24, 37, 44 and 47% after 3 days and 1, 2, 5 and 10 weeks respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: A base–rich, para–brownish, loess (clay) soil from Flachstoeckheim near Braunschweig/FRG (100 g; 0.12% nitrogen) was mixed with 2 mg 14C–labelled benzene dissolved in 0.1 ml acetone, and moistened to 70% of the water capacity. [10 ml water; 70% of water capacity (10 ml water in 100 g soil implies 7% w/w)]. 14CO2 production was measured over a period of 10 weeks.

(149)

Type: other **Radiolabel:**
Concentration:
Organ. carbon: = 1.3 %
Cation exch. capac.
Microbial biomass:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: If released to soil, benzene will be subject to rapid volatilization near the surface. That which does not evaporate will be highly to very highly mobile in soil and may leach to groundwater. The effective half–lives for volatilization from soil, without water evaporation, of benzene uniformly distributed to 1 and 10 cm in soil with an organic carbon content of 1.25%, were 7.2 and 38.4 days respectively.
Source: BP Chemicals Ltd. London

(110) (150)

3.2 Monitoring Data (Environment)

Type of measurement: background concentration
Medium: air
Remark: In the United Kingdom, urban atmospheric levels have been reported to range from 0.01 ppb (approximately 32 ng/m3) in a poorly ventilated traffic tunnel in London, to 576 µg/m3 measured during a 1973 study of various sites around London. The majority of the benzene concentrations reported are below 100 µg/m3.
Source: BP Chemicals Ltd. London

(70) (118) (151) (152)

Type of measurement: background concentration
Medium: air
Remark: In the United Kingdom, rural atmospheric concentrations have been reported to range from 1.3 µg/m3 (arithmetic mean hourly concentration measured in 1983 in Silwood Park about 1 km for the nearest main road) to 292 µg/m3 (mean daily concentration from at least seven 24–hour samples taken in Heston, Middlesex under the Heathrow airport flight–path). The majority of the concentrations reported are below 20 µg/m3.
Source: BP Chemicals Ltd. London

(153) (118) (154)

Type of measurement: background concentration
Medium: air
Remark: In the United Kingdom, atmospheric benzene concentrations at sites near motorways have been reported to range from 0.2 µg/m3 (arithmetic mean hourly concentrations measured at Toddington, about 15 metres from the M1 Motorway in 1983) to 489 µg/m3 (mean daily concentrations from at least seven 24–hour samples taken from a garden in Luton about 150 yards from the motorway). All such reported benzene concentrations, apart from the latter, are below 45 µg/m3.
Source: BP Chemicals Ltd. London
(153) (118) (154)

Type of measurement: background concentration
Medium: air
Remark: In April 1986–1987, several sites around Hamburg, West Germany, were assessed for their atmospheric benzene concentrations. In a populated urban site with dense traffic, benzene was present at 15.3 µg/m3. In the residential areas tested, benzene ranged from 7.6 µg/m3 (the area was near a metal working industry) to 19.3 µg/m3 (the area had dense traffic). Suburban and rural levels were reported to be 7.0–7.7 µg/m3 and, at three industrial sites, benzene ranged from 6.9–10.6 µg/m3. All these values were reported to be the annual arithmetic means from short sampling times (order of seconds). 24–Hour sampling periods in 1988–1989 showed the benzene concentrations in the Hamburg Elbtunnel to range from 80.5–95.3 µg/m3.
Source: BP Chemicals Ltd. London
(155) (156) (118)

Type of measurement: background concentration
Medium: air
Remark: Urban atmospheric benzene concentrations were measured in The Netherlands in 1974 (2–hour sampling periods) and in 1982–1983 (annual averages from 80 2–hour samples collected/site). The average levels ranged from 1.6–41.8 µg/m3 (with more than half of those measured being below 3 µg/m3) and the corresponding maxima ranged from 5.3–90.9 µg/m3 (with half the values being below 11 µg/m3). The Maastunnel (traffic) was investigated in 1974 and the average benzene concentration was 79.8 µg/m3, with the maximum recorded level being 105.9 µg/m3. A further study in The Netherlands reported average and maximal concentrations from 1–hour samples taken in 1980 from rural (Island of Terschelling), urban (Delft) and heavily industrialised (near Rotterdam) sites. The average, over all three sites, ranged from 0.8–4.8 µg/m3 and the maxima from 5.4–35.1 µg/m3.
Source: BP Chemicals Ltd. London
(157) (158) (118) (159)

Type of measurement: background concentration
Medium: air
Remark: In Stockholm, Sweden, average benzene atmospheric concentrations in the city centre were measured in 1982 and 1983 from eight 1-hour samples collected from each site over a 3-12 day period. In busy streets, the averages ranged from 44.3-147.7 µg/m3 (minimun-maximum range 4.2-609.3 µg/m3); in light traffic the average was 14.6 µg/m3 (range 4.6-48.2 µg/m3 and in a quiet street benzene averaged 7.7 µg/m3 (range 3.2-20.9 µg/m3). At a recreation ground 12 km WNW of the city centre, benzene was detected at an average of 3.0 µg/m3 (range 0.3-13.7 µg/m3). Two studies were conducted in 1981 of the atmospheres downwind of two car assembly plants. Benzene was detected at 5.1 and 3.1 µg/m3 respectively.
Source: BP Chemicals Ltd. London
(160) (118) (161)

Type of measurement: background concentration
Medium: air
Remark: In Zurich, Switzerland, benzene was detected, in 1971, at an average concentration of 172.3 µg/m3 from samples taken at a distance of 300 m from the main road.
Source: BP Chemicals Ltd. London
(162) (118)

Type of measurement: background concentration
Medium: air
Remark: A large number of cities in the USA has been studied for their atmospheric benzene concentrations. In general, the averages ranged from 0.4 µg/m3 in an industrial area of North Carolina where there is no chemical industry (1982 measurements; 12-hour outdoor samples collected) to 47.85 µg/m3 in Los Angeles in 1966. The majority of these readings were below 20 µg/m3. The lowest level was 0.2 µg/m3 measured between June and July 1979 in Oakland, California by 24-hour, 7 days/week continuous sampling schedules. The highest level was reported to be 431.33 µg/m3 measured in Rutherford, an industrial area in New Jersey, from six 20-minute samples collected on 3 consecutive days and repeated over 4-9 weeks.
Source: BP Chemicals Ltd. London
(163) (164) (165) (70) (118) (166) (167) (168)

Type of measurement: background concentration
Medium: air
Remark: In a source dominated study, ambient air samples from 44 sites in 39 US urban areas were collected in electropolished, stainless steel canisters on week days from 6-9 am, between June and September 1984, 1985 and 1986. The samples were analysed by capillary gas chromatography with flame ionization detection to determine the C2-12 volatile hydrocarbon compositions. Benzene was detected in every sample at an overall average concentration of 40.2 µg/m3.

When each site and year was considered individually the average benzene level ranged from 15.3–111.7 µg/m3.
Source: BP Chemicals Ltd. London
(169) (70)

Type of measurement: background concentration
Medium: air
Remark: In source dominated studies in the USA, concentrations of benzene in the atmosphere near chemical factories where benzene is used, ranged from 1.9–108.8 µg/m3 and that at service stations ranged from 1 µg/m3–10.2 mg/m3.
Source: BP Chemicals Ltd. London
(170) (171) (172) (173) (174) (175)

Type of measurement: background concentration
Medium: air
Remark: The rural background average concentration is reported to range from 0.32–54.33 µg/m3. Levels ranging from 0.03–1.85 µg/m3 have been reported in rural areas of the Atlantic and Pacific Oceans, the Colorado Rockies, Norwegian Arctic, Pullman and Cape Meares. In five remote tropical sites, benzene ranged from undetectable to 5.74 µg/m3, with the range of averages between 0.22–2.07 µg/m3.
Source: BP Chemicals Ltd. London
(176) (70) (172) (177) (178)

Type of measurement: background concentration
Medium: surface water
Remark: A survey of 80 surface water bodies (rivers and estuaries) was conducted across the UK between December 1988 and February 1989. The average level found in the freshwater samples which contained benzene at concentrations greater than the 0.1 µg/l detection limit, was 7.05 µg/l. Over half the samples collected (93 out of 154) contained less than the detection limit of benzene. A maximum level of 89.4 µg benzene/l was found in one sample.
Source: BP Chemicals Ltd. London
(118) (179)

Type of measurement: background concentration
Medium: surface water
Remark: Estuarine water samples taken from the River Tees in the UK, near to industrial effluent outfalls contained 0–5 µg benzene/l. The levels of benzene along the East coast of the UK were the subject of a survey in 1984. Samples were taken at a depth of 1 m from estuaries and benzene levels were reported to be 2–12 µg/l in the Humber Estuary, <1–18 µg/l in the Tees Estuary, 1–3 µg/l in the Tyne Estuary and <1–9 µg/l in the Thames Estuary.
Source: BP Chemicals Ltd. London
(180) (118) (181)

Type of measurement: background concentration
Medium: surface water
Remark: In 1976, benzene levels were measured in the River Rhine. The average levels at Basle, Cologne and Duisburg were 0.2, about 0.2 and 0.8 µg/l respectively. Samples collected from the surface of Lake Zurich, Switzerland in October 1973 were reported to contain 0.028 µg benzene/l. In the same study, levels of 0.022 µg/l were detected in samples taken at a depth of 30 m and, in a sample of fresh spring water collected in the Zurich area in 1973, 0.018 µg benzene/l was detected.
Source: BP Chemicals Ltd. London
(182) (183) (118)

Type of measurement: background concentration
Medium: surface water
Remark: In 1975–1976, 14 heavily industrialized areas in the USA, with water basins, were investigated. It was found that 20% of the river samples contained between 1 and 7 µg/l. In the Brazos River, Texas, samples taken downriver of the outfalls from a chemical plant showed benzene levels of 0.004–0.910 µg/l.
Source: BP Chemicals Ltd. London
(184) (70) (185) (118)

Type of measurement: background concentration
Medium: surface water
Remark: In 1975–1976, benzene levels in Lake Erie and Lake Michigan were reported to range from 0–1 µg/l (one of two sites being positive) and 0–7 µg/l (five of seven sites being positive) respectively. Levels in the Potomac River were found to be less than the 2 µg/l detection limit. In 1975, an average concentration of 5.4 µg/l was reported from samples taken from 700 random sites in the USA. The US EPA STORET database shows that benzene was detected in 15% of 1,271 samples measured and the median concentration was 5 µg/l.
Source: BP Chemicals Ltd. London
(186) (70) (187) (188) (189)

Type of measurement: background concentration
Medium: surface water
Remark: A survey of the concentration of pollutants in urban stormwater runoff from 19 US cities was conducted. Benzene was not found frequently enough for its mean level in urban runoff to be calculated. Benzene was detected in 5% of the 86 samples taken and the range of detected concentrations was 3.5–13 µg/l.
Source: BP Chemicals Ltd. London
(190) (191)

Type of measurement: background concentration
Medium: ground water
Remark: Of the groundwaters of 32 public and private supply boreholes in the UK sampled in summer 1983, eleven were found to contain benzene. The mean level for these eleven was 0.027 µg/l, the average for all 32 samples was 0.009 µg/l and the maximum level was 0.07 µg/l. A further 59 industrial supply boreholes in the Birmingham aquifer which were not used for public supply, were assessed in 1986-1988. The entire sample area was urbanised and the boreholes were all contained within industrial premises. In general, the levels of non-chlorinated organic compounds were low; the maximum benzene level found being 0.6 µg/l.
Source: BP Chemicals Ltd. London
(192) (191) (193)

Type of measurement: background concentration
Medium: ground water
Remark: Benzene levels in European groundwater have been reported as 0.045 µg/l in Zurich, Switzerland and 0.005-0.030 µg/l in unpolluted areas in The Netherlands.
Source: BP Chemicals Ltd. London
(194) (191) (121)

Type of measurement: background concentration
Medium: ground water
Remark: A survey was conducted of the groundwater from 466 randomly selected and 479 US state agency selected sampling sites. Of those sites serving fewer than 10,000 people, 0.4 and 1.6% contained benzene respectively; the median levels for the positive sites being 0.61 and 1.6 µg/l respectively and the maximum levels being 0.61 and 12 µg/l respectively. Of those sites serving >10,000 people, 1.1 and 1.9% contained benzene respectively; the median levels for the positive sites being 9.0 and 2.7 µg/l respectively and the maximum levels being 15 and 12 µg/l respectively. Groundwater sampled from eight monitoring wells in South Ontario, Canada where bituminous and petroliferous sedimentary rocks are expected to influence the aquifer resource, was found to contain levels of up to 100 µg benzene/l. Benzene was reported in 3.2% of 63 private supply wells in Nebraska in 1982 at a median level of 1.6 µg/l and a maximum of 1.8 µg/l. Levels of 30-330 µg/l were reported in groundwater samples from Connecticut, New Jersey and New York and a maximum of 0.1 µg/l was found in sewage-contaminated groundwater from a site at Otis Air Base, Boston, Massachusetts.
Source: BP Chemicals Ltd. London
(195) (196) (118) (197) (198) (199)

Type of measurement: background concentration
Medium: sediment
Remark: Surface sediment taken from two sites in Walvis Bay, near Capetown, South Africa, contained 0.2–20.4 µg/kg (dry weight). Estuarine sediments in the Tees Estuary, UK, were found to contain benzene at levels of 1.3–3.9 µg/kg. In May and June 1980, sediment was collected from Lake Pontchartrain, Louisiana, USA. Benzene levels of 8–21 µg/kg (wet weight) were found. The US EPA STORET database reports 9% of 355 samples to contain benzene at a median concentration of <5 ppb.
Source: BP Chemicals Ltd. London
(200) (70) (118) (201) (202) (181)

Type of measurement: background concentration
Medium: food
Remark: Benzene has been detected in fruits, nuts, vegetables, dairy products, meat, fish, poultry, eggs and beverages. It has been reported to be present in the aroma from cooked fresh ground beef and fresh and frozen cod fillets. Actual levels of 2 µg/kg in heat treated/canned beef, 120 µg/kg in Jamaican rum, 500–2100 µg/kg in eggs and 19 µg/kg in irradiated beef have been reported. Banzene was detected in all eight samples of mother’s milk from four US urban areas.
Source: BP Chemicals Ltd. London
(203) (204) (205) (206) (207) (118) (208) (209)

Type of measurement: background concentration
Medium: drinking water
Remark: There is one report of a benzene concentration of 0.1 µg/l in Czechoslovakian drinking water and, in the USA, levels of 0.1–0.3 µg/l have been reported. In five US cities, tested in 1974–1975, levels were found to range from 0–0.3 µg/l and of 113 public supplies in 1976, seven contained benzene, the average concentration being <0.2 µg/l. Three surveys of community water were conducted. In the first, none of the 111 samples contained benzene; seven of the 113 in the second were positive (mean 4 µg/l) and four of the 16 in the third were positive (0.95 µg/l maximum). The US Groundwater Supply Survey in 1982 looked at 466 samples of finished drinking water taken randomly from the 1000 samples in the survey. Benzene was detected in 0.6%; the median level being 3 µg/l and maximum being 15 µg/l. Of 1174 community and 617 private drinking water wells in Wisconsin tested in June 1984, benzene was detected in 0.34 and 2.9% respectively.
Source: BP Chemicals Ltd. London
(210) (211) (212) (213) (214) (215) (216) (217)

Type of measurement: background concentration
Medium: biota
Remark: Measurements were made of oysters and clams collected in May and June 1980 from Lake Pontchartrain, Louisiana. In one sample of oysters (Crassostrea virginica) benzene was found at a level of 220 µg/kg (wet weight) and in clams (Rangia cuneata) at 260 µg/kg (wet weight). In a second sample of clams collected from the same area, benzene could not be detected however, the detection limits were not reported.
Source: BP Chemicals Ltd. London

(218) (118)

Type of measurement: background concentration
Medium: biota
Remark: Two species of macroalgae, Ulva lactuca and Hypnea musciformis (seaweeds) were grown in tanks using circulating seawater. Benzene was detected in the algae at 20 µg/kg (dry weight).
Source: BP Chemicals Ltd. London

(219)

Type of measurement: background concentration
Medium: biota
Remark: Marine fish and invertebrate samples were collected 6 km from the outfall from the Los Angeles County wastewater treatment plant. Benzene was present at levels ranging from <1 µg/kg (wet weight) in the liver of Pacific sanddab and in the muscle of ridgeback prawn, up to 52 µg/kg (wet weight) in the liver of Dover sole.
Source: BP Chemicals Ltd. London

(220)

Type of measurement: background concentration
Medium: air
Result: From January to March 1994, airborne benzene levels (24-h averages) were measured at three different sites in the cityof Munich/Germany giving following results: 0.72-1.55 ug/m3 (average: 0.96 ug/m3; outskirts with very low traffic density, some 100 m away from roads), 15.63-24.89 ug/m3 (average: 20.44 ug/m3; street with heavy traffic, about 26000 vehicles/d), 2-9 ug/m3 (average: 5 ug/m3; suburban residential area) (TUEV Umwelttechnik, 1994; DEKRA Umwelt, 1994).
In 1992 and 1993, the benzene concentrations in seven German cities ranged between 6 and 45 ug/m3 (monthly averages) (LUEN/UBA, 1993). In the state of Baden-Wuerttemberg monthlyaverage values of 15-28 ug/m3 with an annual average of 20 ug/m3 were found in 1992/93 (UMEG, 1994).
In April 1986-1987, 12 different sites in and around Hamburg, West Germany, were assessed for their atmospheric benzene concentrations. In a downtown residential area near a street with dense traffic a maximum value of 19.3 ug/m3 was found. The minimum value was 7.0 ug/m3 in a suburban

area in rural surroundings. In two purely industrial regions the benzene levels were 6.9 and 10.6 ug/m3 respectively. In three residential areas industrial reagionsin the neighbourhood, benzene concentrations between 7.6 and17.7 ug/m3 were determined. All these values were reported to be the annual arithmetic means from short sampling times (order of seconds) (Bruckmann et al., 1988). 24-Hour sampling periods (date not given) showed the benzene concentrations in the Hamburg Elbtunnel to range from 80.5-95.3 ug/m3 (Dannecker et al., 1990).

Source: Deutsche Shell Chemie GmbH Eschborn (221) (222) (223) (224) (225) (226)

Type of measurement: background concentration
Medium: air
Remark: Compilation of literature data
Result: The Netherlands:
The average levels of airborne benzenes in cities in the early 80s ranged from 1.6-9.0 ug/m3 and the corresponding maxima which mainly occurred in winter ranged from 5.3-59.4 ug/m3. The annual largescale concentration level is estimated to be ca. 2 ug/m3. For cities, the concentrations are by a factor of 2 to 10 higher.
Source: Deutsche Shell Chemie GmbH Eschborn (64)

Type of measurement: background concentration
Medium: surface water
Remark: Compilation of literature data
Result: United Kingdom:
(a) Surface water:
In 1988/89, a survey of 80 surface water bodies (rivers and estuaries) gave an average benzene level found in the freshwater samples which contained benzene at concentrations greater than the 0.1 ug/l detection limit of 7.05 ug/l. About 60% of the samples collected contained less than the detection limit of benzene. A maximum level of89.4 ug benzene/l was found in one sample.
(b) Sea water:
The benzene levels measured in a depth of 1 m along the Eastcoast were 1984 in the range of < 1 - 18 ug/l (Humber, Tees,Tyne and Thames estuaries)
Source: Deutsche Shell Chemie GmbH Eschborn (227)

Type of measurement: background concentration
Medium: ground water
Remark: Compilation of literature data
Result: The Netherlands:
In the early 80s benzene concentrations of 0.005-0.030 ug/l were found in unpolluted areas.
Source: Deutsche Shell Chemie GmbH Eschborn (64)

Type of measurement: background concentration
Medium: biota
Result: Four species of macroalgae, Ulva lactuca, Ascophyllum nodosum, Gracilaria tikvahiae and Hypnea musciformis (seaweeds) were grown in tanks using circulating seawater. Benzene was detected in two species (Ulva lactuca, Hypnea musciformis) at 20 ug/kg (dry weight). The authors suggest that the seaweeds are a possible source of the volatile organic compounds identified in recent marine sediments.
Source: Deutsche Shell Chemie GmbH Eschborn

(228)

Type of measurement: background concentration
Medium: biota
Remark: Reference not yet available (12/10/94)
Result: Marine fish and invertebrate samples were collected 6 km from the outfall from the Los Angeles County wastewater treatment plant. Benzene was present at levels ranging from <1 ug/kg (wet weight) in the liver of Pacific sanddab and in the muscle of ridgeback prawn, up to 52 ug/kg (wet weight) in the liver of Dover sole.
Source: Deutsche Shell Chemie GmbH Eschborn

(229)

Type of measurement: background concentration
Medium: ground water
Remark: Compilation of literature data
Result: United Kingdom:
In the 80s an average benzene concentration of 0.009 ug/l was found in a survey of public and private supply boreholes. The maximum level was 0.07 ug/l.
Source: Deutsche Shell Chemie GmbH Eschborn

(227)

Type of measurement: background concentration
Medium: air
Result: From 1986–1990, three month running mean concentrations of benzene at a rural site in the UK (Harwell, Oxfordshire) were between about 0.4 ppb (1.3 ug/m3) and 1.7 (5.5 ug/m3). The minimum values were always detected during the months June – September, the maximal concentrations were found during November – February.
From 1991–1992, monthly mean concentrations of benzene in Central London (Exhibition Road) were between about 1 ppb (3.25 ug/m3) and 14 ppb (45.5 ug/m3; smog episode in winter)with an annual average of about 4 ppb (13 ug/m3).
Source: Deutsche Shell Chemie GmbH Eschborn

(230)

Type of measurement: background concentration
Medium: other
Remark: Reference not yet available (12/10/94) (Krause et al., 1987)
Result: A comparison of indoor/outdoor benzene levels in 52 private homes in the city of Munich, FRG gave following results:

concentration (ug/m3)	min	max	mean
indoor	0.41	17.40	2.74
outdoor	0.27	22.26	2.68

(detection limit: 0.005 ug/m3; sampling period not given)
From comparison with indoor and outdoor NOx concentrations the authors conclude that the main source is the infiltration of polluted outdoor air (Gebefuegi et al., 1994).
A survey of 479 German households in 1985/86 gave following indoor air benzene levels:
range: < 1–90 ug/m3
non–smokers: 6.5 ug/m3
smokers: 11 ug/m3 (Krause et al., 1987)
Source: Deutsche Shell Chemie GmbH Eschborn
(231) (232)

Type of measurement: background concentration
Medium: surface water
Result: From 1990–1992, benzene was not detected in the river Rhine (1990: sampling sites not given; 1991/92: sampling site: Lobith, The Netherlands) (detection limit 0.1 ug/l).
Source: Deutsche Shell Chemie GmbH Eschborn
(233) (234) (235)

Type of measurement: background concentration
Medium: air
Result: From 1992–1993, following annual average benzene levels were found at different sampling sites in Austria:

two rural areas	1.0/1.8 ug/m3
Vienna/low traffic density	2.0/3.2 ug/m3
Vienna/high traffic density	8.8–15.5 ug/m3
	16.0 ug/m3 (service station)
Lobau/petrol depot	12.7–17.0 ug/m3 (dependent on distance)

(detection limit 0.8 ug/m3)
Measurements of airborne benzene levels in Vienna/Austria and its surroundings from October 1986 – February 1987 and in May 1987 respectively gave following results (arithmetic means each):
locations with heavy traffic: 15.6–179 ppbC (5 sampling sites)
residential areas: 13.1 ppbC (2 sampling sites)
wood (about 10 km from Vienna): 6.9 ppbC (1 sampling site)
(detection limit: 0.1–0.2 ppbC from a 1l air sample)
Source: Deutsche Shell Chemie GmbH Eschborn
(236) (237)

Type of measurement: background concentration
Medium: air
Result: In Italy the benzene level in air of three large cities (Rome, Milan, Taranto), one suburban site (Montelibretti near Rome) and one forest site (Monti Cimini, pine forest) was determined during day-time (9-11 a.m.) in mild seasons (sampling period not given). The sampling lasted at least 15 d. Following average values were found: 39.0-43.9 ug/m3 (cities), 1.84 ug/m3 (suburban site), 4.39 ug/m3 (forest).
Source: Deutsche Shell Chemie GmbH Eschborn

(238)

Type of measurement: background concentration
Medium: air
Result: Airborne benzene concentrations were detected 1991 and 1992 in two wooden areas of Europe (near Storkow/former GDR southeast of Berlin in a pine forest and 25 km west of Rome/Italyin an area with mediterranean macchia) and at a height of 5050 m at the foot of the Mount Everest/Nepal. The concentrations in Europe were 0.54 ug/m3 (former GDR) and 3.08 ug/m3 (Italy) respectively (one measurement each). In Nepal, levels between 0.27 and 1.99 ug/m3 were found. The authors concluded from their results that the airborne benzene in the Himalaya region is due to long-range transport from polluted areas.
Source: Deutsche Shell Chemie GmbH Eschborn

(239)

Type of measurement: background concentration
Medium: other
Result: From 1979-1988, the personal exposures for benzene in 5 US cities were between 4 and 18 ug/m3 (median 24-h averages). The arithmetic mean values were larger, ranging from 6-30 ug/m3 with a grand mean of about 15 ug/m3. These personal exposure exceeded the outdoor concentrations (range of median values: 2-16 ug/m3; arithmetic means: 2-19 ug/m3 witha grand mean of ca. 6 ug/m3). The measured maximum concentrations differ even more. For indoor 24-h personal exposures 30-250 ug/m3 were measured compared with 6-35 ug/m3 for outdoor maxima. Breath measurements identified smoking as the most important single source of benzene exposure. Smokers had mean breath concentrations of 15 ug/m3whereas in the breath of non-smokers benzene concentrations of 1.5-2 ug/m3 were found.
Source: Deutsche Shell Chemie GmbH Eschborn

(240)

Type of measurement: background concentration
Medium: food
Result: A survey of 50 US American foods and beverages (sampling period 1991–1992) gave following results:
(a) foods with no added benzoates:
benzene not detected: strained apple juice, cranberry juice concentrate, raspberry drink, cola soda, brewed instant coffee, strawberry preserves, grape jelly, liquid beef bouillon, raw and baked potato, ground nutmeg, fried egg, fresh tomato
benzene level < 1 ug/kg: apple juice, strained apple–cherry juice, cranberry juice cocktail (2 different brands), fresh cranberries, grape drink, fruit punch, orange soda, hard–boiled egg, smoked fish (chub), roasted peanuts
benzene level >= 1 ug/kg: cranberry juice cocktail (2 different brands; 1 ug/kg), red raspberry preserves (1 ug/kg), black currant preserves (2 ug/kg), liquid smoke (2 different brands; 21 and 121 ug/kg)
(b) foods with added benzoates:
benzene not detected: diet orange soda (1 brand), soy sauce (2 different brands), iced tea
benzene level < 1 ug/kg: fruit punch, diet cola, diet orange (1 brand), diet white grape soda, pickled vegetables, citrus salad, diet raspberry soda, lite strawberry preserves, sweet relish, salad peppers, lite syrup product
benzene level >= 1 ug/kg: Bloody Mary mix (3 ug/kg), imitation grape jelly (5 ug/kg), different sauces (1–22 ug/kg), Margarita mix (2 ug/kg), diet cherry berry soda (2 ug/kg), diet grapefruit soda (1 ug/kg), imitation strawberry preserves (38 ug/kg), lite orange marmalade (1 ug/kg), litegrape jelly (1 ug/kg),
Source: Deutsche Shell Chemie GmbH Eschborn

(241)

Type of measurement: background concentration
Medium: air
Result: In summer 1991 and winter 1992 benzene levels in a Swedish autotunnel (Tingstad tunnel, Goeteborg) were determined. The summer values were 61 and 450 ug/m3 the second one being caused by slow traffic in both directions of the tunnel tube. In winter, 104–301 ug benzene/m3 were found. The proportion of benzene was about 7% of the total non–methane volatile hydrocarbons.
Source: Deutsche Shell Chemie GmbH Eschborn

(242)

Type of measurement: background concentration
Medium: food
Result: Benzene was detected in US American chicken meat, ham and pork samples by irradiation in concentrations between 5 and 12 ug/kg.
Source: Deutsche Shell Chemie GmbH Eschborn

(243)

Type of measurement: background concentration
Medium: other
Result: Benzene was found in the air of new and recently renovated houses in Switzerland in a maximum concentration of 20 ug/m3. 10% of the measured levels were < 5 ug/m3, 50% were max. 7 ug/m3 and 90% were max. 14 ug/m3. As the substance was detected in all of the examined houses and the ratio to outdoor air is almost 1 the authors concluded that it is mainly infiltrated from outdoor air.
Source: Deutsche Shell Chemie GmbH Eschborn

(244)

Type of measurement: background concentration
Medium: other
Result: In 1991, the benzene content of the blood of 55 year old women who were not occupationally exposed to benzene and 6 year old children living either in a large city or in a rural area was determined in the state of Nordrhein–Westfalen/FRG. The geometric mean values were as follows:

	large city	rural area
women	0.072 ug/l	0.048 ug/l
children	0.143/0.133 ug/l*	0.128 ug/l

* 2 different cities

Source: Deutsche Shell Chemie GmbH Eschborn

(245)

Type of measurement: background concentration
Medium: surface water
Remark: Compilation of literature data
Result: The Netherlands:
(a) surface water: in the 80s, the benzene concentrations of surface waters (rivers and lakes) generally were < 0.1 ug/l. In the Rhine a median concentration of 0.026 ug/l were found.
(b) sea water: in the 80s, < 0.005–0.02 ug/l benzene were detected in the coastal parts of the North Sea. In the central part of the North Sea average concentrations of about 0.005 ug/l were found.
Source: Deutsche Shell Chemie GmbH Eschborn

(64)

Type of measurement: background concentration
Medium: air
Remark: Compilation of literature data
Result: United Kingdom:
In the 80s, the airborne benzene levels measured mainly in or near London ranged between 0.2 and 181.8 ug/m3 with average values between 5.2 and 28.1 ug/m3.
Source: Deutsche Shell Chemie GmbH Eschborn

(227)

Type of measurement: background concentration
Medium: air
Remark: Compilation of literature data
Result: USA:
In the 80s, airborne benzene levels ranging from 0.3–210 ug/m3 were found in the large cities (average values: 4.6–19ug/m3). In a rural area the concentrations were 0.07–2.8 ug/m3.
Source: Deutsche Shell Chemie GmbH Eschborn
(59)

Type of measurement: background concentration
Medium: air
Remark: Compilation of literature data
Result: Former FRG:
In the 80s, the airborne benzene levels in cities ranged between 0.4 and 171.8 ug/m3 with average values of 7.5–51.3 ug/m3. In rural areas concentrations of about 1 ug/m3 were found.
Source: Deutsche Shell Chemie GmbH Eschborn
(60)

Type of measurement: background concentration
Medium: surface water
Remark: Compilation of literature data
Result: Former FRG:
In the 80s, benzene concentrations between 0.01 and 0.40 ug/l were found in rivers and lakes.
Source: Deutsche Shell Chemie GmbH Eschborn

Type of measurement: concentration at contaminated site
Medium: ground water
Remark: Samples from a chalk aquifer near to a petrol storage facility in East Anglia, UK were analysed for benzene. At distances of 210, 120 and 10 m from the storage facility, benzene levels were found to be 1–10 µg/l, >250 µg/l and 1250 µg/l respectively.
Source: BP Chemicals Ltd. London
(70) (191) (246)

Type of measurement: concentration at contaminated site
Medium: ground water
Remark: The benzene levels in groundwater sampleed at a site on a solvent recovery plant in Wisconsin were reported to be 12,000 µg/l at the water–table and <10 µg/l (below the detection limit) at depth. At a sampling site 80 m downgradient from the recovery plant, levels of <10 µg/l (below the detection limit) and 20 µg/l were recorded respectively.
Source: BP Chemicals Ltd. London
(247) (248)

Type of measurement: concentration at contaminated site
Medium: soil
Remark: A study of soil near US factories where benzene was either used or produced found levels of benzene in the soil of <2-191 µg/kg.
Source: BP Chemicals Ltd. London

(171) (215)

Type of measurement: concentration at contaminated site
Medium: other
Result: Monthly sampling from November 1983 to May 1984 in eight service stations and two petrol depots in the former FRG gave following average benzene levels:
4 service stations with car-wash: 1.4-5.5 ug/l
1 sevice station with garage: 70.65 ug/l
2 petrol depots: 0.05/1267 ug/l.
Source: Deutsche Shell Chemie GmbH Eschborn

(249)

Type of measurement: concentration at contaminated site
Medium: other
Result: Benzene in leachate from the sanitary landfill site in Hamburg-Georgswerder/former FRG was detected 1981-1982 at 4 sampling sites in a concentration range from not detectable (limit of detection not given) to 96 ug/l.
Source: Deutsche Shell Chemie GmbH Eschborn

(250)

Type of measurement: concentration at contaminated site
Medium: air
Result: Measurements of VOC concentrations in the emissions of three different coal-fired power stations in France showed benzene levels between 3.1 and 20 ug/m3.
Source: Deutsche Shell Chemie GmbH Eschborn

(251)

Type of measurement: concentration at contaminated site
Medium: air
Result: In moped exhaust benzene had a proportion of 5.1% (w/w) related to the non-methane hydrocarbons (for comparison: benzene content of the fuel 4.2% (w/w)) during a test distance of 700-800 m on plain ground at the maximum speed of 30 km/h if conventional unleaded reformate-based grade fuel was used. With alkylate-based petrol the benzene content of the exhaust was reduced to 0.10% (w/w) (benzene content of the fuel 0.013% (w/w)).
In exhaust from a lawn mower operated with conventional unleaded reformate-based grade fuel the benzene content was 7% (w/w). Using alkylate-based petrol the benzene level was reduced to 0.5% (w/w) (= 6 ug/m3).
Source: Deutsche Shell Chemie GmbH Eschborn

(252)

Type of measurement: concentration at contaminated site
Medium: air
Result: In 1987, benzene was found in stack gases of four US American municipal wastewater sludge incinerators in amounts between 0.107 g/h (plant with afterburning device) and 7.87g/h under steady state conditions.
Source: Deutsche Shell Chemie GmbH Eschborn
(253)

Type of measurement: concentration at contaminated site
Medium: other
Result: From 1989-1991 benzene levels in the influent and the effluent of the municipal wastewater treatment plant of Goeteborg/Sweden were determined. The influent range was found to be 0.1-5 ug/l with average concentrations of 3.9 ug/l (1989), 3.5 ug/l (1990) and 0.3 ug/l (1991). The effluent concentrations were generally < 0.5 ug/l.
Source: Deutsche Shell Chemie GmbH Eschborn
(254)

Type of measurement: concentration at contaminated site
Medium: soil
Result: Benzene was found in the soil of 2 gas works stations in the Netherlands in concentrations of 1 and 2 mg/kg respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
(255)

Type of measurement: concentration at contaminated site
Medium: air
Remark: Reference not yet available (12/10/94)
Result: In winter 1985 and summer 1986, airborne benzene concentrations at 2 petrol stations in Munich/Germany were between 462 and 27170 ug/m3.
Source: Deutsche Shell Chemie GmbH Eschborn
(256)

Type of measurement: concentration at contaminated site
Medium: other
Remark: Reference not yet available (12/10/94) (Muecke et al., 1984)
Result: In the inner rooms of automobiles average benzene concentrations of 40-60 ug/m3 were derived from literature values (Eikmann, 1991). Under unfavourable conditions (parking in the sun, closed windows) maximum levels up to 2700 ug/m3 were found (Muecke et al., 1984).
Source: Deutsche Shell Chemie GmbH Eschborn
(257) (258)

Type of measurement: other
Medium: other
Result: In tests with a smoking machine in a chamber to which only the sidestream smoke was lead while the mainstream smoke was vented outside an amount of 500 ug benzene/cigarette was measured. For comparison the indoor air benzene levels in anUS American tavern which are probably mainly caused by smoking were determined. They showed values of 21 and 27 ug/m3 respectively while outdoor concentrations of 8 and 6 ug/m3 were measured simultaneously.
Source: Deutsche Shell Chemie GmbH Eschborn
(259)

Type of measurement: other
Medium: other
Result: In 1989, benzene was found among the volatile compounds of 8 from 11 different microwave susceptors packaged with 10 different US American food products in concentrations between < 1.6 and 34 ng/cm2 susceptor test strip.
Source: Deutsche Shell Chemie GmbH Eschborn
(260)

3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: soil – air
Method: other
Year:
Remark: A study of soil samples near a benzene–using facility showed higher benzene concentrations in the soil than in the air, indicating a potential for benzene adsorption on soil, if the soil is continuously exposed to the chemical.
Source: BP Chemicals Ltd. London
(261) (262)

Type: adsorption
Media: soil – air
Method: other
Year:
Remark: Reference not yet available (12/10/94)
Result: A study of soil samples near a benzene–using facility showed higher benzene concentrations in the soil than in the air, indicating a potential for benzene adsorption on soil, if the soil is continuously exposed to the chemical.
Source: Deutsche Shell Chemie GmbH Eschborn
(263)

Type: adsorption
Media: soil – air
Method: other
Year:
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Result: At a temperature of 30 degree C a sorption coefficient K for benzene at the soil–air interface of 3.01 ml/g was determined. The sorption of the test substance from the gas phase decreased with increasing water content of the soil.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Soil columns were directly tested in a gas chromatograph. The test substances were injected after the dilution of saturated vapour with ambient laboratory air. Soil sorption characteristics: clay and silt fraction of an US American soil (1.2% organic matter content; 52% relative humidity (= 1.68% (w/w) water content)); temperature range: 30–50 degree C.

(264)

Type: adsorption
Media: water – soil
Method: other: see reference
Year: 1983
Result: Benzene will be expected to exhibit very high to high mobility in soil and therefore may leach to groundwater. Koc: woodburn silt loam= 31.7–143
Source: REPSOL PETROLEO,S.A. MADRID

(265)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Using equations developed by Kenaga E.E. & Goring C.A.I. In: Aquatic Toxicology ASTM STP707. Edited by J.C. Eaton et al. American Society for Testing and Materials, 1980, soil sorption coefficients were predicted for compounds whose water solubilities were known. The soil adsorption coefficient (Koc) was calculated for benzene to be 71, indicating that benzene is moderately to highly mobile. An experimental soil adsorption coefficient value of 83 was reported by Kenaga & Goring, 1980.
Source: BP Chemicals Ltd. London

(266)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: The sorptive characteristics of benzene to groundwater aquifer solids were studied. Benzene showed a tendency to adsorb to aquifer solids with an increase in adsorption being seen with increasing organic matter content.
Source: BP Chemicals Ltd. London

(267) (268)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Adsorption to bottom sediments of rivers or lakes is not expected to occur to any significant degree because its volatility favours release to the atmosphere.
Source: BP Chemicals Ltd. London

(269)

Type: adsorption
Media: water – soil
Method: other: berechnet
Year: 1994
Remark: log Koc = 2.22
Computer–Program: PCKOC, PC Software to Estimate Soil Sorption Coefficients, Version 1.22, PH.Howard, W.Meylan (Mai 1993), Syracuse Research Corporation, New York.
Source: BASF AG Ludwigshafen

(270)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 2 (valid with restrictions)
Accepted calculation method
Result: Using equations developed by Kenaga E.E. & Goring C.A.I., In: Eaton, J.C. et al. (eds.), Aquatic Toxicology ASTM STP707. American Society for Testing and Materials (1980) soil sorption coefficients Koc were predicted for compounds whose water solubilities were known. The soil adsorption coefficient was calculated for benzene to be 71 from a givenwater solubility of 1.78 mg/l, indicating that benzene is moderately to highly mobile.
Source: Deutsche Shell Chemie GmbH Eschborn

(271)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: The sorptive characteristics of benzene to two groundwater aquifer solids were studied. Log Koc values were between 2.09 and 3.01 (Koc = 123–1023) being slightly dependent of the adsorbent mass.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Experiments were carried out in capped glass centrifuge tubes with two US American groundwater aquifer materials of following characteristics:

sand	90.0%	70.4%
silt	8.0%	24.0%
clay	2.0%	5.6%
organic matter	4.4%	2.2%
pH	3.8	5.5

3 different adsorbent masses (1.0, 5.0, 10.0 g) were tested with 3 concentrations of test substance (0.078, 0.777, 7.77 mg/l) at a temperature of 20 degree C. The substance concentration was determined in the water phase by LSC.
Test substance: 14C labelled commercial product

(272)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Result: For benzene, a sorption coefficient Koc related to the organic matter content of the sediment of 83 was found.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The adsorption isotherms were taken from batch tests in capped centrifuge tubes at 25 +/– 1 degree C with substance concentrations of 10–50% of the water solubility. Equilibrium conditions were achieved after 4–8 h of shaking. Analysis was carried out in the water phase. Sorbed substance concentrations were computed by difference based on the total substance amount added. Substance concentrations were determined gaschromatographically. Description of the two sediments: coarse silt fraction with an organic matter content of 2.78 and 3.27% respectively.
Test substance: commercial product (spectrograde, purity not given)

(84)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Combination of theoretical considerations with measured parameters
Result: For benzene, a sorption coefficient Koc of 60 was determined related to the organic matter content of a variety of US American soils.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Soil sorption characteristics (e.g. organic matter content, cation exchange capacity) of a variety of US American soils and measured n–octanol/water partition coefficient were combined to calculate Koc values.

(273)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: For benzene, a soil sorption coefficient Koc related to the organic matter content of the soil of 18.2 (log Koc = 1.26) was measured.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The soil–solution mixtures were equilibrated for 24 h at 20 degree C in capped centrifuge tubes. The determination of substance concentration was carried out gaschromatographically in the water phase. Losses by volatilization were avoided by sampling through the septum of the caps. The substance amounts were corrected by the airspace of the tubes under consideration of air volume and Henryos Law Constant. Soil characteristics: 1.9% organic matter, 68% silt, 21% clay, 9% sand, cation–exchange capacity: 14 meqv/100 g soil.
Test substance: commercial product (purity not given)

(274)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 2 (valid with restrictions)
Accepted calculation method
Calculation on the basis of first–order molecular connectivity
Result: For benzene, a soil sorption coefficient Koc related to the oragnic matter content of soils of 123 (log Koc = 2.09) was calculated.
Source: Deutsche Shell Chemie GmbH Eschborn

(275)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Koc values were calculated from distribution coefficients assuming a linear relationship with the n-octanol/water partition coefficient
Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: Dependent on the organic matter content of the aquifer material (given in brackets) following Koc values were calculated for benzene: 759 (0.007 %), 30 (0.025%) and 42 (0.015%). The sorption of benzene seemed to increase drastically with decreasing content of organic matter.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Distribution coefficients were determined in a stainless steel flow-through system with three different aquifer materials described as follows:

coarse sand	84%	90%	63%
fine sand	15%	8%	34%
silt	0%	0%	2%
clay	1%	2%	1%
organic matter	0.007%	0.025%	0.015%

The test substance was applied to the test columns after solution in groundwater from a Danish drinking water plant at an average influent concentration of 39 ug/l. Effluent concentrations were measured gaschromatographically. Evaporative losses were avoided by saturating the gas phase with test substance vapour. The temperature was 10 degree C.
Test substance: commercial product (analytical grade, purity not given)

(276)

Type: adsorption
Media: water – soil
Method: other
Year:
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: The distribution coefficients Kd for benzene measured at 20 different Danish aquifer materials were between 0.04 and 0.23 ml/g dependent on the content of organic matter. By dividing through the organic matter content of the aquifer material Koc values in a range between about 100 and 900 were calculated.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 14C-labelled benzene in concentrations of 100, 200 and 400 ug/l solved in groundwater from a Danish drinking water plant was applied to 20 different Danish aquifer materials in capped tubes. The samples were equilibrated for 96 h at room temperature and the water phase then analysed by scintillation counting. Aquifer material characteristics: 2-94% coarse sand, 3-94% fine sand, 0-4% silt, 0-4% clay, 0.006-0.213% organic matter.
Test substance: commercial product (purity not given)

(277)

Type: volatility
Media: soil – air
Method: other
Year:
Remark: The half–lives of volatilization, without water evaporation, of benzene uniformly distributed at a rate of 1 kg/ha to 1 and 10 cm in soil with an organic carbon content of 1.25% were 7.2 and 38.4 days respectively.
Source: BP Chemicals Ltd. London
(70) (278)

Type: volatility
Media: soil – air
Method: other
Year:
Remark: A model was developed to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank. It was estimated that most (67%) of the benzene in the gasoline would volatilize from the soil within 17 months. Of the remaining benzene, 29% would be expected to leach to groundwater and 3% would remain in the soil.
Source: BP Chemicals Ltd. London
(267) (279)

Type: volatility
Media: soil – air
Method: other
Year:
Remark: Based on benzene’s vapour pressure of 95.2 mmHg at 25°C (Riddick J.A. et al. Organic Solvents: Physical Properties and Methods of Purification. Techniques of Chemistry. 4th Edition. Wiley–Interscience, 1986 cited in HSDB, 1993), volatilization from soil surfaces and other surfaces is expected to be rapid. Benzene which does not evaporate will be highly–very highly mobile in soil and may leach to groundwater.
Source: BP Chemicals Ltd. London
(70) (280)

Type: volatility
Media: soil – air
Method: other
Year:
Remark: Reference not yet available (12/10/94)
Result: The half–lives of volatilization, without water evaporation, of benzene uniformly distributed at a rate of 1 kg/ha to 1 and 10 cm in soil with an organic carbon content of 1.25% were 7.2 and 38.4 days respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
(281)

Type: volatility
Media: soil – air
Method: other
Year:
Remark: Reference not yet available (12/10/94)
Result: A model was developed to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank. It was estimated that most (67%) of the benzene in the gasoline would volatilize from the soil within 17 months. Of the remaining benzene, 29% would be expected to leach to groundwater and 3% would remain in the soil.
Source: Deutsche Shell Chemie GmbH Eschborn

(282)

Type: volatility
Media: water – air
Method: other: see reference
Year: 1982
Result: The estimated half–life for volatilization of benzene from a model river is 2.7 hr at 20 deg C based on a reported Henry's Law Constant of 5.3E–3 atm–cu m/mol
Source: REPSOL PETROLEO,S.A. MADRID

(283)

Type: volatility
Media: water – air
Method: other
Year:
Result: Evaporation was found to be the primary loss mechanism for benzene in the winter, with the half–life being 13 days. In spring and summer, the half–lives were 23 and 3.1 days respectively.
Source: BP Chemicals Ltd. London
Test condition: The half–life for the evaporation of benzene from seawater was investigated in a mesocosm experiment which simulated Narragansett Bay, Rhode Island and contained the associated planktonic and microbial communities.

(70) (118) (284) (285)

Type: volatility
Media: water – air
Method: other
Year:
Remark: The half–life for evaporation of benzene from a 1 m thick still water column was 4.81 hours at 25°C and 5.03 hours at 10°C by thermodynamic calculations. The residence half–time for well–mixed water was 37 minutes.
Source: BP Chemicals Ltd. London

(286) (118)

Type: volatility
Media: water – air
Method: other
Year:
Remark: Based on a reported Henry's Law constant of 5.3 x 10–3 atm–m3/mole (Hine J. & Mookerjee P.K. J. org. Chem. 40, 292–298, 1975), the half–life for volatilization from a model river 1 m deep, flowing at 1 m/second and with a wind velocity of 3 m/second was 2.7 hours at 20°C. The equations of Lyman W.J. et al. were used (Lyman W.J. et al. Handbook of Chemical Property Estimation Methods. p. 15–9 to 15–31. McGraw–Hill, New York, 1982).
Source: BP Chemicals Ltd. London

(70) (280)

Type: volatility
Media: water – air
Method: other
Year:
Remark: The half–life for volatilization in a wind–wave tank with a wind speed of 7.09 m/second was 5.23 hours.
Source: BP Chemicals Ltd. London

(70) (287)

Type: volatility
Media: water – air
Method: other
Year:
Remark: Reliability: 2 (valid with restrictions)
Accepted calculation method
Steady–state conditions
Result: The half–life for evaporation of benzene from a 1 m thick still water column was 4.81 hours at 25 degree C and 5.03 hours at 10 degree C.
Source: Deutsche Shell Chemie GmbH Eschborn

(105)

Type: volatility
Media: water – air
Method: other
Year:
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: The mass transfer coefficients of benzene at the water–air interface were 11.4–34 cm/h dependent of the wind velocity. The volatilization is of first–order kinetics. For a wind speed of 7.09 m/s a half–life of 5.2 h can be calculated.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The wind–wave tank was 6 m long, 0.61 m deep and 0.60 m wide with wind velocities of 5.96–13.2 m/s at a temperature of 20.7 degree C. The testing period was > 50 h so that an approximate 10fold change of solute concentration would occur. The solute concentration was monitored with gas chromatography.

(288)

Type: other
Media: other
Method: other
Year:
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: Benzene was found in concentrations between 0.15 and 0.30 ug/g leaf dry wt. in leaves of Cotoneaster dammeri and in concentrations <= 0.01 ug/g leaf dry wt. in leaves of the other six species.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Uptake of benzene by plants was tested with samples from Cotoneaster dammeri, Pinus mugo Turra, Pinus nigra Arnold, Pseudotsuga menziesii (Mirb.) Franco, Juniperus media Van Melle, Juniperus chinensis L. and Chamaecaparis lawsonania (Murr.) Parrl growing within a distance of about 1000 m along the same road with very dense traffic in the city of Gent/Belgium. Sampling was carried out in a height of about 1 m in March 1992. Benzene concentrations were determined in the air and in the plant leaves by GC-MS.

(289)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: other (calculation)
Year:
Method: An environmental modelling exercise was conducted to estimate benzene's distribution in the UK environment. The computer model used by the Building Research Establishment, developed from Mackay's original model (Mackay D. Envir. Sci. Technol. 13, 1218-1223, 1979; Mackay D. & Paterson S. Envir. Sci. Technol. 15, 1006-1014, 1981) was used. For the model, it was assumed that 38,000 tonnes benzene are released in the UK per year. Other factors required for the model were molecular weight (78.11), water solubility (1780 mg/l at 20°C or 22.8 mg/m3), vapour pressure (76 mmHg at 20°C or 1.01 x 10+2 hPa), octanol-water partition coefficient (2.13, log; 135, non-log) and the rate constants for photo-oxidation in air (0.03/hour, t½=24 hr in polluted air and 4.8 x 10-3/hour,t½=6 days in ambient air), photo-oxidation in water (3.6 x 10-4/hour, t½=80 days) and biodegradation in water (1.8 x 10-3/hour, t½=16 days).
Result: The distribution of benzene in the environment was estimated to be 0.08-0.49 µg/m3 in air, 0.42-2.64 ng/l in water, 1.17-7.30 ng/kg in soil, 2.80-17.5 ng/kg in sediment, 2.80-17.5 ng/kg in suspended sediment and 2.73-17.1 ng/kg in biota.
Source: BP Chemicals Ltd. London

(118)

Media: air – biota – sediment(s) – soil – water
Method: other (calculation)
Year:
Method: An environmental modelling exercise was conducted to estimate benzene's distribution in the UK environment. The computer model used by the Building Research Establishment, developed from Mackay's original model (Mackay D., Envir. Sci. Technol. 13, 1218–1223, 1979; Mackay D. & Paterson S., Envir. Sci. Technol. 15, 1006–1014, 1981) was used. For the model, it was assumed that 38,000 tonnes benzene are released in the UK per year. Other factors required for the model were molecular weight (78.11), water solubility (1780 mg/l at 20 degree C or 22.8 mg/m3), vapour pressure (1.01E2 hPa at 20 degree C), octanol–water partition coefficient (2.13, log; 135, non–log) and the rate constants for photo–oxidation in air (0.03/hour, t1/2 = 24 hr in polluted air and 4.8E–3/hour,t1/2 = 6 days in ambient air), photo–oxidation in water (3.6E–4/hour, t1/2 = 80 days) and biodegradation in water (1.8E–3/hour, t1/2=16 days).
Remark: Reliability: 2 (valid with restrictions)
Accepted calculation method
Result: The distribution of benzene in the environment was estimated to be 0.08–0.49 ug/m3 in air, 0.42–2.64 ng/l in water, 1.17–7.30 ng/kg in soil, 2.80–17.5 ng/kg in sediment, 2.80–17.5 ng/kg in suspended sediment and 2.73–17.1 ng/kg in biota.
Source: Deutsche Shell Chemie GmbH Eschborn

(227)

Media: air – biota – sediment(s) – soil – water
Method: Calculation according Mackay, Level III
Year:
Method: The method is described in detail in Mackay, D. et al., Chemosphere 14, 335–374, 1985. Ideally mixed compartments and photolysis (water and air) and biodegradation (water) are as important degradation mechanisms are assumed.
Remark: Reliability: 2 (valid with restrictions)
Accepted calculation method
Result: For the Netherlands, the model calculation gave following results:

	air	water (fresh)	soil	sediment
concentration	2.1 ug/m3	58 ng/l	12 ng/kg	130 ng/kg
distribution	99.0%	0.9%	0.1%	< 0.1%
removal by reaction	4.6%	0.2%	n.c.*	n.c.
removal by advection	95.2%	< 0.1%	n.c.	n.c.

* n.c. = not calculated
Transportation through the air seemed to be the most important removal mechanism.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Model parameters: 41000 km2 total surface (88% soil/12% water), 1000 m air column, 2.5 m water column, 0.03 m sediment column, 0.15 m soil column, 2.3 d residence time inair, 55% sea air with a benzene level of 0.1 ug/m3 and 45% land air (mainly from south east direction, i.d. from

Belgium and Germany) with a benzene level of 1.8 ug/m3.
(64)

Media: water – air
Method: other (measurement)
Year:
Method: Benzene mixed with other volatile organic compounds was applied to a tank containing sea water from the US American Narragansett Bay (with associated planktonic and microbial communities, without sediment). Mixing was carried through four times a day to simulate tidal currents. Different seasons were simulated: spring, summer, winter.
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Result: Benzene was mainly removed from the system by volatilization. The half–lives were 23 d (spring conditions), 3.1 d (summer conditions) and 13 d (winter conditions).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Benzene concentrations: 1.4–3.0 ug/l; tank volume: 13 m3; spring conditions: temperature 8–16 degree C, summer conditions: temperature 20–22 degree C, winter conditions: temperature 3–7 degree C. Test substance concentrations were monitored by closed–loop stripping and HRGC after adsorption onto charcoal.
Test substance: no data
(290)

Media: water – soil
Method: other (calculation)
Year: 1980
Method: Using the equations of Kenaga E.E. & Goring C.A.I. In: Aquatic Toxicology ASTM STP707. Edited by J.C. Eaton et al. American Society for Testing and Materials, 1980, a soil adsorption coefficient (Koc) for benzene was calculated.
Remark: According to a report in HSDB, 1993, this study was conducted in woodburn silt loam. Other studies have reported Koc values in this type of soil of 31 (Chiou C.T. et al. Envir. Sci. Technol. 17, 227–231, 1983 cited in HSDB, 1993) and 31.7–143 (Sabljic A. J. Agric. Fd Chem. 32, 243–246, 1984 cited in HSDB, 1993).
Result: The soil adsorption coefficient (Koc) was found to be 71, indicating moderate to high mobility. An experimental soil adsorption coefficient of 83 was also reported.
Source: BP Chemicals Ltd. London
(70) (291)

Media: water – soil
Method: other (calculation)
Year: 1982
Remark: Based on a reported octanol/water partition coefficient (log Kow) of 2.13 (Hansch C. & Leo A.J. Medchem Project Issue No.26. Pomona College, Claremont, California, 1985 cited in HSDB, 1993), a Koc of 98 was estimated using the equations of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods. p.4-9. McGraw-Hill, New York, 1982. This value indicates that benzene will be expected to exhibit very high to high mobility in soil (Swann R.L. et al. Res. Rev. 85, 17-28, 1983) and may leach to groundwater.
Source: BP Chemicals Ltd. London

(70) (292)

3.4 Mode of Degradation in Actual Use

Remark: Soil : volatilization and water-soil adsorption.
Water : volatilization.
Atmosphere : indirect photolysis
Source: REPSOL PETROLEO,S.A. MADRID

Remark: In the atmosphere, benzene is mainly degraded by indirect phototransformation, reaction with hydroxy radicals being the most important.
Source: BP Chemicals Ltd. London

(267) (293) (118)

Remark: Due to its high volatility from aqueous solution and its low sorption tendency to soils (see section 3.3.1 above) it is to be expected that benzene released from different sources (see section 1.9 above) at last reaches the atmosphere. There, the substance is mainly degraded by indirect phototransformation, reaction with hydroxy radicals being the most important.
Source: Deutsche Shell Chemie GmbH Eschborn

(59) (60) (227) (63) (64)

3.5 Biodegradation

Type: aerobic
Inoculum: other
Concentration: 100 mg/l related to Test substance
Result: readily biodegradable
Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year: 1981 **GLP:** no data
Test substance: no data
Remark: Inoculum added: 30 mg/l; aniline as reference substance; BOD measurement.
Result: Complete degradation after 2 weeks of incubation.
Reliability: 1 (valid)
Test procedure according to OECD-Guideline.
Source: Deutsche Shell Chemie GmbH Eschborn

(294)

Type: aerobic
Inoculum: activated sludge
Degradation: = 2 % after 5 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 2% theoretical oxidation was recorded after 5 days, indicating that benzene is biodegradable.
Source: BP Chemicals Ltd. London
Test condition: Bench-scale activated sludge (fill and draw operation) was used as the inoculum in the dilution technique to measure BOD. The seed material was acclimated for at least 14 days. The test was conducted at 20°C.

(295) (296)

Type: aerobic
Inoculum: activated sludge
Degradation: = 3 % after .3 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 3% theoretical oxidation was recorded after 0.25 days indicating that benzene is biodegradable.
Source: BP Chemicals Ltd. London
Test condition: Bench-scale activated sludge (fill and draw operation) was used as the inoculum in the Warburg respirometer at 20°C. At least 14 days acclimation was used together with 50-200 mg feed/l.

(295) (296)

Type: aerobic
Inoculum: activated sludge
Concentration: 50 µg/l related to Test substance
Degradation: = 29 % after 5 day
Result: readily biodegradable
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: After 5 days, benzene was degraded (29%) to carbon dioxide.
Source: BP Chemicals Ltd. London
Test condition: The microbial inoculum was based on activated sludge from microbial sewage treatment. 14C–labelled benzene was tested in a closed system at 25°C. 14CO2 evolution was followed.
(110) (297) (118)

Type: aerobic
Inoculum: activated sludge
Concentration: 50 µg/l related to Test substance
Degradation: = 29 % after 5 day
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Method: static test in closed system with 14C–labelled test compound; 14C–analysis; microbial inoculum based on activated sludge from microbial sewage treatment.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Only removal of test substance determined; no information about origin and possible pre–acclimation of inoculum.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 25 degree C.
(298)

Type: aerobic
Inoculum: activated sludge
Concentration: 135 mg/l related to Test substance
Degradation: = 99.9 % after 60 day
Result: readily biodegradable
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The BOD5, TOC and COD values were found to be 0.55, 0.92 and 3.08 mg/mg benzene and 15% benzene was found to be stripped from biological systems.
Source: BP Chemicals Ltd. London
Test condition: Complete–mix, bench–scale, continuous–flow activated sludge reactors, with retention times of 8 hours, were used to treat synthetic wastewater with added benzene. The synthetic wastewater contained ethylene glycol, ethyl alcohol, glucose, glutamic acid, acetic acid, phenol, ammonium sulphate, phosphoric acid and salts. The biological activated sludge for initial seeding was obtained from a municipal activated sludge treatment plant, and acclimated to the benzene–containing synthetic wastewater. The activated sludge system was operated at mean cell residence

times of 2, 4 and 6 days. Following acclimation, samples were collected over a 60-day testing period and monitored for BOD5, total organic carbon (TOC), COD and benzene. A non-biological control was used to assess stripping.

(299)

Type: aerobic
Inoculum: activated sludge, adapted
Concentration: 153 mg/l related to Test substance
Degradation: = 99.9 % after 60 day
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Method: Complete-mix, bench-scale, continuous-flow activated sludge reactors, with retention times of 8 hours, were used to treat synthetic wastewater with added benzene. The synthetic wastewater contained ethylene glycol, ethyl alcohol, glucose, glutamic acid, acetic acid, phenol, ammonium sulphate, phosphoric acid and salts. The biological activated sludge for initial seeding was obtained from a municipal activated sludge treatment plant, and acclimated to the benzene-containing synthetic wastewater. The activated sludge system was operated at mean cell residence times of 2, 4 and 6 days. Following acclimation, samples were collected over a 60-day testing period and monitored for BOD5, total organic carbon (TOC), COD and benzene (GC analysis). A non-biological control was used to assess stripping.
Results: BOD5, TOC and COD values were 0.55, 0.92 and 3.08 mg/mg benzene resp. and 15% benzene was found to be stripped from the test system.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Test compound not sole source of carbon.
Source: Deutsche Shell Chemie GmbH Eschborn

(299)

Type: aerobic
Inoculum: activated sludge, domestic, adapted
Concentration: 1000 mg/l related to Test substance
Degradation: = 3.3 % after 6 hour(s)
Method: other
Year: 1946 **GLP:**
Test substance:
Remark: Degradation result given as % BOD of ThOD; respirometric test in Warburg apparatus according to APHA (1946)
Reliability: 2 (valid with restriction)
Standard test procedure; no description of method or degradation kinetics.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 20 degree C

(300)

Type: aerobic
Inoculum: Arthrobacter sp. (Bacteria)
Concentration: 20 mg/l related to Test substance
Degradation: < .02 % after 7 day
Result: other
Method: other
Year: **GLP:** no data
Test substance:
Remark: Benzene utilizing Arthrobacter sp. (B5; B7) were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on glucose. After removing remaining substrate and suspending in mineral salts medium 14C–labelled benzene was added and the degradation determined by measuring 14CO2 release.
14CO2 release after 7 d incubation was < 0.02% for both strains.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Source: Deutsche Shell Chemie GmbH Eschborn

(147)

Type: aerobic
Inoculum: Nocardia sp. (Bacteria)
Concentration: 20 mg/l related to Test substance
Degradation: = 85 % after 7 day
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene utilizing Nocardia sp. BR1 were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on the test compound. After removing remaining substrate and suspending in mineral salts medium 14C–labelled benzene was added and the degradation determined by measuring 14CO2 release.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Mineral salts medium.

(147)

Type: aerobic
Inoculum: Pseudomonas putida (Bacteria)
Concentration: 20 mg/l related to Test substance
Degradation: = 43 % after 7 day
Method: other
Year: **GLP:** no data
Test substance:
Remark: Benzene utilizing Pseudomonas putida were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on glucose. After removing remaining substrate and suspending in mineral salts medium 14C–labelled benzene was added and the degradation determined by measuring 14CO2 release.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Mineral salts medium.

(147)

Type: aerobic
Inoculum: Pseudomonas sp. (Bacteria)
Concentration: 20 mg/l related to Test substance
Degradation: = 45 – 90 % after 7 day
Result: other
Method: other
Year: **GLP:** no data
Test substance:
Remark: Benzene utilizing Pseudomonas sp. (B6/8; Bw9; B4/3) were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on the test compound or on glucose. After removing remaining substrate and suspending in mineral salts medium 14C–labelled benzene was added and the degradation determined by measuring 14CO2 release.
14CO2 release after 7 d incubation:
Pseudomonas B6/8 (precultured on benzene) 69%
Pseudomonas B6/8 (precultured on glucose) 68%
Pseudomonas Bw9 (precultured on benzene) 90%
Pseudomonas B4/3 (precultured on benzene) 45%
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Mineral salts medium.

(147)

Type: aerobic
Inoculum: other
Concentration: 20 mg/l related to Test substance
Degradation: 0 – 90 % after 7 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: After 1 week incubation of the cultures with benzene, 85, 45–90, 0 and 43% CO2 production was measured respectively from the four species.
Further studies were conducted using 100 g of a base–rich, para–brownish, loess (clay) soil from Flachstöckheim near Braunschweig instead of the pure cultures. The soil (pH 7.1, 1.26% carbon and 0.12% nitrogen) was mixed with 2 mg 14C–labelled benzene dissolved in 0.1 ml acetone, and moistened to 70% of the water capacity. 14CO2 production was again measured, after 3 days and 1, 2, 5 and 10 weeks. The levels of carbon dioxide released were found to be 7.5, 24, 37, 44 and 47% after 3 days and 1, 2, 5 and 10 weeks respectively.
Source: BP Chemicals Ltd. London
Test condition: Microbes were isolated from soil samples of different origin (field, grassland and forest soils) and cultured on benzene or p–dihydroxybenzoic acid. Radio (14C)–labelled benzene was added and the degradation rate determined by measuring the 14CO2 release. Nocardia BR1 and Pseudomonas species (B6/8, BW9 and B/3) were cultured in benzene while Arthrobacter species (B5 and B7) and Pseudomonas putida were cultured in p–hydroxybenzoic acid.

(147)

Type: aerobic
Inoculum: other
Concentration: 3 mg/l related to Test substance
Degradation: = 24 % after 5 day
Result: other
Method: other
Year: 1971 **GLP:** no data
Test substance: no data
Remark: 24, 27, 24 and 29% bio–oxidation was recorded after 5, 10, 15 and 20 days respectively indicating that benzene is biodegradable.
Source: BP Chemicals Ltd. London
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 13th Edition. American Public Health Association, New York, 1971 was followed. Wastewater from domestic sewage was used as inoculum. Settled domestic wastewater was filtered through glass wool and added as seed material. Aerated dilution water (freshwater) containing minerals and buffer was added to the BOD bottles followed by small aliquots of benzene, at concentrations of 3, 7 and 10 mg/l, taken from 0.1% stock solutions. At least two of the concentrations were tested in duplicate. Dissolved oxygen was monitored periodically. Samples were analysed routinely for ammonia, nitrogen and organic nitrogen which may result in an oxygen demand.

(301)

Type: aerobic
Inoculum: other
Concentration: 5 mg/l related to Test substance
Degradation: = 49 % after 7 day
Result: readily biodegradable
Method: other
Year: 1979 **GLP:** no data
Test substance: no data
Remark: Benzene demonstrated significant biodegradation with rapid acclimation, 49 and 37% being biodegraded when tested at concentrations of 5 and 10 mg/l respectively after 7 days. It was completely biodegraded at both concentrations after the second week of incubation.
Source: BP Chemicals Ltd. London
Test condition: The method of Bunch R.L. & Chambers C.W. J. Wat. Pollut. Control Fed. 39, 181, 1967 modified by Tabak H.H. & Hannah S. Static Procedure for Biodegradability Determination. Internal EPA Report, Mun. envir. Res. Lab., Cincinnati, Ohio, 1979 was followed. Wastewater from domestic sewage was used as inoculum. The static-culture flask-screening procedure was used. Benzene was prepared in deionized distilled water as a 10% solution to give concentrations of 5 and 10 mg benzene/l. The solutions were incubated with prechilled dilution water containing 5 mg yeast extract/l, and also with settled domestic wastewater inoculum. The flasks were then incubated at 25°C in the dark for 7 days and three weekly subcultures taken (totalling 28 days of incubation). Duplicate samples at the beginning of each incubation period and triplicate samples at the end of each 7-day period were subjected to GC, total organic carbon (TOC) and dissolved organic carbon (DOC) analyses. The minimum sensitivity of the GC procedures used was about 0.1 mg/l.

(302)

Type: aerobic
Inoculum: other
Concentration: 2.81 mg/l related to Test substance
Degradation: = 50 % after 16 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: In die-away tests, the biodegradation of <=2.81 mg benzene/l in samples of groundwater and Lester River water followed first-order rate kinetics. The rate constants were 0.025/day for groundwater and 0.044/day for river water and the half-lives for the reaction were 28 and 16 days respectively.
Source: BP Chemicals Ltd. London

(303) (304) (305)

Type: aerobic
Inoculum: other
Concentration: 480 µg/l related to Test substance
Degradation: = 54.6 % after 48 hour(s)
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The benzene concentration was seen to drop slowly from 480 µg/l to 218 µg/l in 48 hours. In samples amended with nitrogen (as ammonium nitrate), degradation was much more rapid, with the benzene concentration dropping to 35 µg/l after 20 hours (equivalent to 92.7% degradation). Further incubation of this system did not lead to further reductions in the benzene concentration. It was thought that this was due to 35 µg/l being an insufficient carbon source to sustain the microbial population. In samples amended with an inoculum of microflora and with or without nitrogen, degradation was again seen to be rapid, the rates of benzene removal ranging from 5.7 µg/l/hour in the absence of nitrogen to 25.4 µg/l/hour in its presence.
Source: BP Chemicals Ltd. London
Test condition: The biodegradation of benzene in contaminated groundwater collected from a test well in Los Angeles was studied. A mixed bacterial culture, containing petroleum degrading bacteria from groundwater and soil bacteria capable of utilising gasoline as sole carbon source, was incubated with the benzene-contaminated groundwater under closed, agitated conditions.

(306) (304)

Type: aerobic
Inoculum: other
Concentration: 480 µg/l related to Test substance
Degradation: = 54.6 % after 48 hour(s)
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Elimination determined, amount of stripped or adsorbed test substance not measured.
Removal of benzene in gasoline-contaminated groundwater collected from a monitoring well in Los Angeles by the autochthonic, petroleum degrading microflora was studied. Influence of additional nitrogen and from contaminated soil enriched microbial inocula to the test culture was investigated.
Results: Closed batch flask culture with groundwater containing 6.2 mg/l hydrocarbons (a.o. 477 ug/l benzene, 561 ug/l toluene and 153 ug/l xylenes) were incubated with the autochthonic microflora (710 CFU/ml bacteria; 3430 CFU/ml total heterotrophic population):
(I) sterile control (II) unamended, (III) amended with

100 mg/l NH4NO3-N and (IV) amended with nitrogen and 1 ml inoculum of a soil enrichment culture with bacteria capable of utilising gasoline as sole carbon source; removal of test substance was followed by GC-analysis.
Results: (I) and (II): <50% benzene removal occurs slowly in 48 hours. (III) and (IV): Rapid removal (>90%) of benzene within 20 hours of incubation; longer incubation did not result in further elimination, probably due to 35 ug/l benzene being an insufficient carbon source to sustain the microbial population.

Source: Deutsche Shell Chemie GmbH Eschborn

Test condition: Water characteristic: pH 7.93; TOC 23 mg/l; NH4-N 0.22 mg/l; NO3-N 0.21 mg/l.
Incubation at approx. 23 degree C, shaken.

(307)

Type: aerobic
Inoculum: other
Degradation: = 58 % after 5 day
Result: readily biodegradable
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 58, 67, 76 and 80% bio-oxidation was recorded after 5, 10, 15 and 20 days respectively.

Source: BP Chemicals Ltd. London

Test condition: A mixture of two biologically treated petrochemical effluents, settled domestic wastewater, Kanawha River water taken from the Winfield Locks at Winfield, West Virginia and soil was acclimated for 45-60 days, and the supernatant used as seed material. Freshwater was used as the dilution water. It is unclear from the paper if the method, conditions and benzene concentrations used in the unacclimated test were used in this case.

(301)

Type: aerobic
Inoculum: other
Concentration: 1 mg/l related to Test substance
Degradation: > 99 % after 16 day
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Removal determined.
Removal of benzene in groundwater samples from Gainesville, Florida (drawn from a 9 m deep shallow).
Method: Batch culture experiments according to Bouwer et al., Environ. Sci. Technol. 15, 596-599. Elimination of test substance monitored by GC-MS. Sterile control.
Results: Acclimatization period of about 8 days, followed by slow elimination; 75% of initial concentration removed between day 12-16. No change in benzene concentration in sterile control indicates biological transformation of TS.

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Incubation at 20 degree C in the dark; pH 5.3
(308)

Type: aerobic
Inoculum: other
Result: other
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Phenol was identified at concentrations of 1–5% in samples treated with Penicillium chrysogenum, Cunninghamella blakesleeana, Gliocladium deliquescens, Streptomyces species and Cunninghamella bainieri. Two unknown phenols were produced (1–5%) from reaction with P. chrysogenum. No metabolites were detected in systems using Aspergillus niger, Aspergillus ochraceous, Rhizopus stolonifer, Curvularia lunata, Streptomyces rimosus and Helicostylum piriforme. Catechol and cis–1,2–dihydroxy–1,2–dihydrobenzene have also been reported as metabolites (Gibson D.T. et al. Biochemistry 7, 2653–2662, 1968 cited in HSDB, 1993).
Source: BP Chemicals Ltd. London
Test condition: Microorganisms suspended in a soybean meal–glucose medium were used to inoculate the soybean meal–glucose medium in Erlenmeyer flasks. These were then incubated at 27°C on a rotary shaker for 48–72 hours. After incubation, cultures were transferred to fresh medium and incubated for 24 hours. Benzene (12.5 mg), dissolved in dimethyl formamide, was then added and incubation continued for a further 24–72 hours. Samples were withdrawn and subjected to thin–layer chromatography.
Test substance: Test substance was laboratory reagent grade.
(309) (310)

Type: aerobic
Inoculum: other
Result: readily biodegradable
Method: other
Year: 1979 **GLP:** no data
Test substance: no data
Remark: Method: The static–culture flask–screening procedure of Bunch R.L. & Chambers C.W. J. Wat. Pollut. Control Fed. 39, 181 (1967) modified by Tabak H.H. & Hannah S. Static Procedure for Biodegradability Determination. Internal EPA Report, Mun. envir. Res. Lab., Cincinnati, Ohio (1979) was followed. Wastewater from domestic sewage was used as inoculum. Benzene was prepared in deionized distilled water as a 10% solution to give concentrations of 5 and 10 mg benzene/l. The solutions were incubated with prechilled dilution water containing 5 mg yeast extract/l, and also with settled domestic wastewater inoculum. The flasks were then incubated for 7 days in glass stoppered bottles (to minimize volatilization) and three weekly subcultures were taken (totalling 28 days of incubation). Duplicate samples at the beginning of each incubation period and triplicate samples at the end of each 7–day period were subjected to GC, total organic carbon (TOC) and dissolved organic carbon

(DOC) analyses. The minimum sensitivity of the GC procedures used was about 0.1 mg/l.
Results: Benzene demonstrated significant biodegradation with rapid acclimation, 49 and 37% being biodegraded when tested at concentrations of 5 and 10 mg/l respectively after 7 days. Test substance was completely biodegraded at both concentrations in the first and the following subcultures (7 days incubation each).
Reliability: 1 (valid)
Modification of standard test procedure; method in accordance with generally accepted standards and described in sufficient detail.

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 25 degree C; dark

(302)

Type: aerobic
Inoculum: other
Result: other
Method: other
Year: 1980 **GLP:** no data
Test substance: no data
Remark: Biodegradation of test substance (0, 0.8, 1.6 and 3.2 ul/l) was determined in samples from groundwater, Lester River water and lake water (Lake Superior) by BOD technique according to APHA (1980) Standard methods for the examination of water and wastewater, Washington.
DO concentrations were determined at 0, 5, 10, 14 or 15, and 20 days of incubation.
Results: After 20 days of incubation approx. 53% of test substance in river water, 25% in Superior harbor water and 5% in ground water were degraded (BOD of ThOD); degradation followed first-order rate kinetics. The rate constants were 0.025/day for groundwater and 0.044/day for river water (lake water not calculated); half-lives for the reaction were 28 and 16 days respectively.
Reliability: 2 (valid with restriction)
Standard test procedure; unclear if degradation results were corrected for oxygen consumption by nitrification

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Physicochemical water characteristic:

	ground water	Lester river	Superior harbor
pH	8.23	7.76	7.56
hardness (as CaCO3)	121.35	58.59	50.10
Ammonia-N	0.07	0.59	1.03
total solids	170.3	93.2	131.7
dissolved solids	156.7	80.7	109.0
suspended solids	<1.2	<1.2	5.6
bacterial concentration (cells/ml)	55	420	310

Incubation at 21+/-3 degree C

(311)

Type: aerobic
Inoculum: other: groundwater
Degradation: = 50 % after 28 day
Result: readily biodegradable
Method: other: see reference
Year: 1987 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID
Test substance: Concentration : 3.2 ug/

(312)

Type: anaerobic
Inoculum: other
Concentration: 50 mg/l related to DOC (Dissolved Organic Carbon)
Degradation: = 0 % after 2.8 month
Result: under test conditions no biodegradation observed
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: For benzene, NGP was -10±8.9% of the theoretical, indicating a lack of degradation.
Source: BP Chemicals Ltd. London
Test condition: The inoculum was primary digesting sludge collected from Reading Sewage Works (Berkshire, England) which receives a mixture of domestic and industrial wastewaters. Diluted sludge (2-3 g dry solids/l of medium) was incubated with benzene under a headspace of 90% N2-10% CO2. Sterile controls contained autoclaved sludge and sterile test chemical. Incubation was conducted at 35°C for at least 60 days. Total gas production was measured weekly. Net gas production (NGP) was expressed as a percentage of the theoretical gas production calculated from the stoichiometry of test chemical mineralization to methane and carbon dioxide.
Test substance: The test substance was laboratory reagent grade.

(313)

Type: anaerobic
Inoculum: other
Degradation: = 0 % after 6 month
Result: under test conditions no biodegradation observed
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A second study reported the complete removal of benzene after 6 months following the in situ anoxic biological treatment of a hydrocarbon contaminated aquifer under anaerobic conditions (Battermann G. In: Contaminated Soil. TNO International Conference. p.711-722. Edited by J.W. Assink & W.J. Van den Brink. Nijhoff, Dordrecht, The Netherlands, 1986 (cited in Nielsen et al. 1991).
In the uncontaminated microcosm, benzene was not degraded after 56 days but it did inhibit the basal rate of denitrification of the microcosm. No significant degradation was seen in the contaminated microcosm after 6 months but the denitrification processes were unaffected.
Source: BP Chemicals Ltd. London
Test condition: Microcosms were prepared from both uncontaminated and contaminated aquifer materials from an anaerobic site contaminated by jet fuel. The microcosms were amended with nitrate, nutrients and benzene and incubated under a nitrogen atmosphere at 12°C.

(314) (118)

Type: anaerobic
Inoculum: other
Concentration: 50 mg/l related to DOC (Dissolved Organic Carbon)
Degradation: = 0 % after 2.8 month
Result: under test conditions no biodegradation observed
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
The inoculum was primary digesting sludge collected from Reading Sewage Works (Berkshire, England) which receives a mixture of domestic and industrial wastewaters. Diluted sludge (2-3 g dry solids/l of medium) was incubated with benzene under a headspace of 90% N2-10% CO2. Sterile controls contained autoclaved sludge and sterile test chemical. Incubation was conducted for at least 60 days. Total gas production was measured weekly. Net gas production (NGP) was expressed as percentage of the theoretical gas production calculated from the stoichiometry of test chemical mineralization to methane and carbon dioxide.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 35 degree C
Test substance: The test substance was laboratory reagent grade.

(313)

Type: anaerobic
Inoculum: other
Degradation: = 0 % after 12 month
Result: under test conditions no biodegradation observed
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Removal determined.
Removal of benzene (initial concentration 5-6 mg/l) in microcosms filled with aquifer core samples (a) from an uncontaminated site and (b) from the saturated zone below a jet fuel contaminated interval under denitrifying conditions.
Method: The microcosms were amended with NH4-N, PO4-P and one or more of the following compounds: benzene, toluene, phenol, catechol, nitrate and/or biocides. Removal of test substance was determined by GC.
Results: Within an incubation time of 1 year benzene was neither removed in uncontaminated nor in contaminated samples to a significant degree regardless if it was present as sole source substrate or with other metabolizable compounds.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Aquifer characteristics: pH 9.2; 99.7% sand; 0.3% silt and clay; TOC 0.04%; NO3-N <0.5; NH4-N <0.5;
incubation under nitrogen atmosphere
Test substance: Test substance was reagent grade.

(315)

Type: anaerobic
Inoculum: other
Concentration: 1 mg/l related to Test substance
Degradation: = 0 % after 96 day
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Removal determined.
Removal of benzene in groundwater samples from Gainesville, Florida (raw water intake of a water treatment plant).
Method: Batch culture experiments according to Bouwer et al., Environ. Sci. Technol. 15, 596-599. Elimination of test substance monitored by GC-MS. Sterile control.
Results: No removal during incubation; test terminated after 96 days.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Incubation at 20 degree C in the dark; pH 5.3

(308)

Type: anaerobic
Inoculum: other
Concentration: 1 µg/l related to Test substance
Degradation: = 5 % after 7 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: After incubation at 20°C for 7 days, 5% of the benzene was degraded to 14C-carbon dioxide. Further incubation did not produce further carbon dioxide. Benzene, however, continued to disappear and it was thought that sorption to the test vessel walls accounted for this. Higher benzene concentrations of 100 µg/l followed this same pattern of degradation.
Source: BP Chemicals Ltd. London
Test condition: The degradation of 14C-labelled benzene was followed in a methanogenic sediment microcosm originating from a Ducth river.

(316) (317)

Type: anaerobic
Inoculum: other
Concentration: 1 µg/l related to Test substance
Degradation: = 5 % after 7 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
The mineralization of 14C-labelled benzene was followed in a methanogenic sediment microcosm originating from a Dutch river; measurement of 14CO2.
Results: Total removal of test substance after 7 days was 57-63% Incubation for 63 days did not result in higher removal or further mineralization. Sorption to stoppers or the sediment or formation of intermediates were thought to account for most of the removal. Tests with an initial benzene concentration of 100 ug/l had similar results.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Incubation at 20 degree C;
Sediment characteristics: pH 7.5; 5% organic carbon; 0.42% nitrogen; 0.21% phosphorus; 11% CACO3.

(318)

Type: anaerobic
Inoculum: other
Concentration: 1.17 g/l related to Test substance
Degradation: = 34 % after 4 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: After 4 days, the benzene concentration was reduced to 66% and complete disappearance occurred after 34 days. Phenol (1.88 mg/l) was identified as the major aromatic intermediate with cyclohexanone and propanoic acid also being identified. Carbon dioxide and methane were the final products.
Source: BP Chemicals Ltd. London
Test condition: The mixed methanogenic inoculum was derived from stable methanogenic consortia that degrade ferulic acid to carbon dioxide and methane and that had been enriched from sewage sludge. Acclimation was obtained by adding unlabelled benzene dissolved in 1 ml of methanol to the inoculum, at concentrations of 15 mM. The cultures were refed every 2 months for 9 months and incubated in a 30% CO2-70% N2 gas atmosphere at 35°C in the dark. Following acclimation, the subcultures were again fed 15 mM benzene and incubated, as before, for 2 months. Sterile chemical controls without inocula and autoclaved biological controls were used. Seven samples were withdrawn during the incubation period and assessed by GC-MS for the transformation intermediates.
Test substance: The test substance was laboratory reagent grade; 99.3% pure.
(319)

Type: anaerobic
Inoculum: other
Concentration: 1.17 g/l related to Test substance
Degradation: = 34 % after 4 day
Result: other
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
No standard test procedure but detailed description of method and test conditions; test procedure in accordance with generally accepted scientific standards.
Removal of benzene by a mixed methanogenic inoculum from a stable methanogenic consortia enriched from sewage sludge that had been grown on ferulic acid (as sole carbon and energy source) for 5 years.
Method: Acclimation to test substance for 9 month (1.17 g/l benzene, dissolved in 1 ml of methanol; cultures re-fed every 2 month). Following acclimation, the subcultures were again fed 1.17 g/l benzene (as only organic carbon source) and incubated, as before, for 2 months. Two parallel cultures, sterile chemical controls without inocula and autoclaved biological controls were used. Seven samples were withdrawn during the incubation period and assessed by GC-MS for the test substance and transformation intermediates.
Results: After 4 days, the benzene concentration was reduced

to 66+/–2% of initial concentration and complete disappearance occurred after 34 days. Phenol (1.88 mg/l) was identified as the major aromatic intermediate with cyclohexanone and propanoic acid also being identified. 39+/–8% of test substance was dissolved into rubber stoppers.

Source: Deutsche Shell Chemie GmbH Eschborn

Test condition: Incubation in a 30% CO2–70% N2 gas atmosphere at 35 degree C in the dark; mineral salts medium with reducing agents, ammonium phosphate as nitrogen source and vitamins.

Test substance: Purity: 99.3%.

(319)

Type: anaerobic
Inoculum: other
Degradation: = 72 % after 10 month
Result: other
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: After 20 weeks of incubation at 17°C in the dark, benzene did not show signs of significant degradation. After 40 weeks, the benzene levels were reduced to 28% (i.e. 72% removal) of the originals and after 120 weeks <1% remained (i.e. >99% removal). No evidence of degradation was seen in the autoclaved controls after 40 weeks.
Source: BP Chemicals Ltd. London
Test condition: Benzene was studied in aquifer material from Oklahoma, USA. The living microcosms tested consisted of anaerobic subsurface materials from aquifers known to support methanogenesis. Autoclaved samples were used as controls.

(118) (320)

Type: anaerobic
Inoculum: other
Concentration: .27 mg/l related to Test substance
Degradation: = 72 % after 10 month
Result: other
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.
Removal determined; test substance not sole substrate.
Removal of 0.61 mg/l benzene mixed with 0.55 mg/l toluene, 0.27 mg/l ethylbenzene and 0.26 mg/l o–xylene in methanogenic aquifer material. Aquifer samples were collected below a contaminated landfill, which is located on the bank of a river in Oklahoma, USA. GC–analysis after 0, 6, 12, 20, 40 and 120 weeks of incubation. Autoclaved samples were used as controls.
Results: After 20 weeks incubation benzene did not show signs of significant removal. After 40 weeks, elimination of test substance was 72% and after 120 weeks >99%. No evidence of elimination was seen in the autoclaved controls after 40

weeks.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Incubation at 17 degree C in the dark.
Characteristic of aquifer water: pH 7.3; 0.7 mg/l nitrate; 218 mg/l ammonia; 344 mg/l TOC.
Test substance: High purity

(321)

Type: anaerobic
Inoculum: other
Concentration: .12 g/l related to Test substance
Result: other
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: At the end of the study, 68 and 64% of the 14C activity was found in the liquid and gas phases and 35 and 31% in the rubber stoppers at 1.5 and 3 mM benzene respectively. The initial carbon dioxide contained 0.002 and 0.85% of the 14C activity in the 1.5 and 3 mM benzene cultures respectively and this increased, at the end of the study, to 5.8 and 5.4% respectively. For the tests conducted in the presence of methanol, the corresponding 14C activity levels in the 1.5 and 3 mM benzene cultures were 53 and 56% in the liquid and gas phases, 41 and 46% in the rubber stoppers, 0.25 and 0.05% in the initial carbon dioxide and 2.6 and 2.0% in the final carbon dioxide respectively.
Source: BP Chemicals Ltd. London
Test condition: The mixed methanogenic inoculum was derived and acclimated as described previously. Following acclimation, the subcultures were fed mixtures of umlabelled and 14C-labelled benzene at concentrations of 1.5 and 3 mM (0.12 and 0.23 g/l). Benzene was used either as the sole carbon source or in combination with methanol. Sterile chemical controls without inoculum and sterile controls with autoclaved inoculum were used. The cultures were then incubated at 35 °C in the dark for 60 days. Gas production was measured, gas composition determined and 14C activity in both the liquid and headspace assessed.
Test substance: Purity=99.4%

(319)

Type: anaerobic
Inoculum: other
Concentration: .12 g/l related to Test substance
Result: other
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
No standard test procedure but detailed description of method and test conditions; test procedure in accordance with generally accepted scientific standards.
Removal of benzene by a mixed methanogenic inoculum from a stable methanogenic consortia enriched from sewage sludge that had been grown on ferulic acid (as sole carbon and energy source) for 5 years.
Method: Acclimation to test substance for 9 month (1.17 g/l benzene, dissolved in 1 ml of methanol; cultures re–fed every 2 month). Following acclimation, the subcultures were fed mixtures of umlabelled and 14C–labelled benzene in separate test sets at concentrations of 0.12 and 0.23 g/l (as sole source of carbon). Individual series of 3 cultures for each concentration, sterile chemical controls without inoculum and sterile controls with autoclaved inoculum were used. During incubation gas production was measured, gas composition determined and 14C activity in both the liquid and headspace assessed.
Results:

Benzene concentration	[% liquid and gas phase	mean 14C activity +/– SD] initial CO_2	final CO_2
0.12 g/l			
culture	68+/–1.2	0.002+/–0.0002	5.8 +/–0.36
control	68+/–2.3	0.25 +/–0.25	0.1 +/–0.1
0.23 g/l			
culture	64+/–3.0	0.85 +/–0.044	5.4 +/–0.28
control	57+/–5.2	0.39 +/–0.22	0.17+/–0.17

Amounts of >30% of applied radioactivity were found in the rubber stoppers from the test bottles.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Incubation in a 30% CO_2–70% N_2 gas atmosphere at 35 degree C in the dark; mineral salts medium with reducing agents, ammonium phosphate as nitrogen source and vitamins.
Test substance: Purity = 99.3%; ring–labelled test test substance.

(319)

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Method: other
Year: **GLP:** no data
BOD5: = 0 mgO2/l
Method: other
Year: **GLP:** no data

R A T I O B O D 5 / C O D

BOD5/COD: = 0

Result: The COD was reported to be 0.25 (no units given).
Source: BP Chemicals Ltd. London
Test condition: The standard dilution technique was used with normal sewage as the seed material. No further details on the method of determining the BOD or COD values were available in the citing referencce.

(322) (323)

Method: other
Year: 1971 **GLP:** no data
Concentration: mg/l related to Test substance

C O D

Method: other
Year: 1971 **GLP:** no data
COD: = 1400 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = .53

Result: The BOD5 was reported as 24% ThOD (converts to 0.744 mg/mg or 744 mg/g).
Source: BP Chemicals Ltd. London
Test condition: The methods of the APHA. Standard Method for the Examination of Water and Wastewater. 13th Edition. American Public Health Association, New York (BOD Procedure and chromic acid reflux procedure), 1971 were followed. A settled domestic wastewater was filtered through glass wool and added as seed material. Aerated dilution water (freshwater) containing the specified minerals and buffer was added to the BOD bottles followed by benzene at concentrations of 3, 7 or 10 mg/l. At least two of the concentrations were tested in duplicate. Dissolved oxygen was monitored periodically. Samples were analysed routinely for ammonia, nitrogen and organic nitrogen which may result in an oxygen demand.

(301)

Method: other
Year: **GLP:** no data

C O D

Method: other
Year: 1971 **GLP:** no data
COD: = 1400 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = 1.28

Result: The BOD5 was reported as 58% ThOD (converts to 1.80 mg/mg or 1800 mg/g).
Source: BP Chemicals Ltd. London
Test condition: For the BOD test, a mixture of two biologically treated petrochemical effluents, settled domestic wastewater, Kanawha River water taken from the Winfield Locks at Winfield, West Virginia and soil was acclimated for 45-60 days, and the supernatant used as the seed material. Freshwater was used as the dilution water. It is unclear from the paper if the method, conditions and concentrations used in the unacclimated test were used in this case. COD was measured using the method of the APHA. Standard Method for the Examination of Water and Wastewater. 13th Edition. American Public Health Association, New York (chromic acid reflux procedure), 1971.

(301)

Method: other
Year: 1971 **GLP:** no data

C O D

Method: other
Year: 1974 **GLP:** no data
COD: = 2150 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = 1.01

Result: The BOD5 was reported as 2.18 g/g (converts to 2180 mg/g).
Source: BP Chemicals Ltd. London
Test condition: The methods of the APHA. Standard Method for the Examination of Water and Wastewater. No.219. American Public Health Association Inc., New York, (BOD Procedure), 1971 and the ASTM. Annual Book of ASTM Standards. No. D 1252-67. American Society for Testing and Materials, Philadelphia, 1974 were followed. The standard dilution method for determining BOD was conducted at 20 ± 1°C. Allyl thiourea (0.5 mg/l) was added to prevent nitrification. Filtered effluent from a biological sanitary waste treatment plant was used as seed. The benzene/seed mixture (500 ml, conversion not possible as no data on strength of test solution) was stirred throughout the study. COD was determined by the standard potassium

dichromate method.

(324)

Method: other
Year: **GLP:** no data
Method: other
Year: **GLP:** no data

R A T I O B O D 5 / C O D

BOD5/COD: = .53

Result: The BOD5 was reported to be 10% of ThOD and the COD was reported to be 19% of ThOD.
Source: BP Chemicals Ltd. London
Test condition: The AFNOR T90/103 test was used. The inoculum was taken from three polluted surface waters. No details on COD measurement were available in the citing reference.

(325) (110) (326)

Method: other
Year: 1971 **GLP:** no data
Concentration: mg/l related to Test substance

C O D

Method: other
Year: 1971 **GLP:** no data
COD: = 1400 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = .53

Remark: Reliability: 1 (valid)
Test procedure in accordance with national standard methods. The methods of the APHA. Standard Method for the Examination of Water and Wastewater. 13th Edition. American Public Health Association, New York (BOD Procedure and chromic acid reflux procedure), 1971 were followed. A settled domestic wastewater was filtered through glass wool and added as seed material. Aerated dilution water (freshwater) containing the specified minerals and buffer was added to the BOD bottles followed by benzene at concentrations of 3, 7 or 10 mg/l. At least two of the concentrations were tested in duplicate. Dissolved oxygen was monitored periodically. Samples were analysed routinely for ammonia, nitrogen and organic nitrogen which may result in an oxygen demand and degradation values corrected.
Result: The BOD5 was reported as 24% of ThOD (converts to 0.744 mg/mg or 744 mg/g); BOD after 20 d was 29% of ThOD.
Source: Deutsche Shell Chemie GmbH Eschborn

(301)

Method: other
Year: 1971 **GLP:** no data

C O D

Method: other
Year: 1971 **GLP:** no data
COD: = 1400 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = 1.28

Remark: For the BOD test, a mixture of two biologically treated petrochemical effluents, settled domestic wastewater, Kanawha River water taken from the Winfield Locks at Winfield, West Virginia and soil was acclimated for 45-60 days to a concentration of 40 mg/l benzene (re-fed at approx. 3 day-intervals, and the supernatant used as the seed material. Freshwater was used as the dilution water. It is unclear from the paper if the method, conditions and concentrations used in the unacclimated test were used in this case. COD was measured using the method of the APHA. Standard Method for the Examination of Water and Wastewater. 13th Edition. American Public Health Association, New York (chromic acid reflux procedure), 1971.
Reliability: 4 (validity cannot be judged)
Probably standard test procedure but not clearly stated.
Result: The BOD5 was reported as 58% of ThOD (converts to 1.80 mg/mg or 1800 mg/g); BOD after 20 d was 80 % of ThOD.
Source: Deutsche Shell Chemie GmbH Eschborn
(301)

Method: other
Year: 1971 **GLP:** no data

C O D

Method: other
Year: 1974 **GLP:** no data
COD: = 2150 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = 1.01

Remark: Reliability: 1 (valid)
Test procedure in accordance to national standard methods. The methods of the APHA. Standard Method for the Examination of Water and Wastewater. No.219. American Public Health Association Inc., New York, (BOD Procedure), 1971 and the ASTM. Annual Book of ASTM Standards. No. D 1252-67. American Society for Testing and Materials, Philadelphia, 1974 were followed. The standard dilution method for determining BOD was conducted at 20 +/- 1 degree C. Allyl thiourea (0.5 mg/l) was added to prevent nitrification. Filtered effluent from a biological sanitary waste treatment plant was used as

inoculum. The benzene/seed mixture (500 ml) was stirred throughout the study. COD was determined by the standard potassium dichromate method.
Result: BOD5 = 71% of ThOD.
Source: Deutsche Shell Chemie GmbH Eschborn
(324)

Method: other
Year: 1969 **GLP:** no data
Method: other
Year: 1971 **GLP:** no data

R A T I O B O D 5 / C O D

BOD5/COD: = .53

Remark: Methods: BOD5 = AFNOR T90/103 test (January 1969),
COD = AFNOR T90/101 test (September 1971),
TOC = Method according to Davis, L.F., Eng. Bull. Purdue Univ., Eng. Ext. Ser. 132, 989-999 (1968) and Gallion, F., Agence Financiere de Bassin Seine-Normandie (1971).
The inoculum was taken from three polluted surface waters.
Reliability: 2 (valid with restriction)
Test procedures (BOD5 and COD) in accordance with national standard methods and described in sufficient detail.
Result: The BOD5 was reported to be 10% of ThOD and the COD was reported to be 19% of ThOD; TOC-removal was 40%.
Source: Deutsche Shell Chemie GmbH Eschborn
(327)

Method: other
Year: 1946 **GLP:**
Concentration: 1000 mg/l related to Test substance

Remark: BOD5 = 1.9% of ThOD; Standard Dilution B.O.D. technique according to APHA (1946);
Inoculum: domestic sewage, non adapted
Reliability: 2 (valid with restriction)
Standard test procedure; no description of method or degradation kinetics.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 20 degree C
(300)

Method: other
Year: 1946 **GLP:**
Concentration: 1000 mg/l related to Test substance

Remark: BOD5 = 2.4 % of ThOD; Standard Dilution B.O.D. technique according to APHA (1946);
Inoculum: activated sludge, adapted for 16 d +/– 48 h
Reliability: 2 (valid with restriction)
Standard test procedure; no description of method or degradation kinetics.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 20 degree C

(300)

3.7 Bioaccumulation

Species: Anguilla japonica
Exposure period: 10 day at 20 degree C
Concentration: 50 mg/l
BCF: = 3.5
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The test was conducted in seawater and the bioconcentration factor measured in the flesh.

(110) (70) (328)

Species: Anguilla japonica
Exposure period: 10 day at 20 degree C
Concentration: 50 mg/l
BCF: = 3.5
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Fish (130–180 g) were treated in 130 l water with 60.5 g crude oil (containing 50 mg/l benzene). Benzene was measured in oil suspension and in flesh of the eels (pooled samples from 5 eels) by GC–FID. No further details reported.
Results:
A concentration factor of 3.5 was observed after rearing eels in oil–seawater after 10 days. Benzene was no longer detectable in eel flesh after 3 days depuration in clean seawater.
Reliability: 3 (not valid)
Documentation insufficient for assessment
No information about test procedure and test conditions; fish exposed to a mixture of hydrocarbons (crude oil).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Incubation in seawater.

(329)

Species: Anguilla japonica
Exposure period:
Concentration:
BCF: = 3.5
Elimination: no data
Method: other: see reference
Year: 1978 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(330)

Species: Carassius auratus (Fish, fresh water)
Exposure period:
Concentration: 1 mg/l
BCF: = 4.3
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The log bioconcentration factor was found to be 0.63.
Source: BP Chemicals Ltd. London
Test condition: Goldfish were reared in water containing benzene. The concentration of benzene in the fish was measured by gas chromatography after 5 minutes incubation at 80°C of the fish tissue and air. A sample of the tank water was similarly treated and measured.

(110) (70) (328) (89)

Species: Carassius auratus (Fish, fresh water)
Exposure period:
Concentration: 1 mg/l
BCF: = 4.3
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
No information about test procedure or test conditions.
The log bioconcentration factor was found to be 0.63.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Goldfish were reared in water containing benzene. The concentration of benzene in water as monitored by GC ranged from 0.017 to 0.8 mg/l. No further details reported.

(89)

Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 48 hour(s)
Concentration: 88 µg/l
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The bioconcentration factors measured in the gall bladder, intestine, pyloric caecum of the digestive tract, gills, brain, liver, muscle, kidney and reproductive organs were 31, 6, 6, 7, 6, 5, 4, 4 and 2 respectively.
Source: BP Chemicals Ltd. London
Test condition: Clupea harengus pallasi (Pacific herring) were exposed in static water with a salinity of 24 ppt at a temperature of 9–11°C.

(331) (110) (332)

Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 48 hour(s)
Concentration: 88 µg/l
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
The bioconcentration factors measured in the gall bladder, intestine, pyloric caecum of the digestive tract, gills, brain, liver, muscle, kidney and reproductive organs were 31, 6, 6, 7, 6, 5, 4, 4 and 2 respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Clupea harengus pallasi (Pacific herring) were exposed in static water with a salinity of 24 ppt at a temperature of 9–11 degree C.

(331) (110) (332)

Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Concentration:
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Non–feeding Pacific herring larvae (no. of animals not reported), obtained from naturally fertilized eggs and incubated in the laboratory, were exposed five days after hatching, when yolk was totally consumed, to 14C–labelled benzene in static filtered seawater. Actual initial concentrations of benzene in were determined by GC. Benzene concentrations in water decreased to ca. 37% of initial conc. within 24 h; no 14C was detected after 48 h. No analysis for 14C–labelled metabolites was performed.
Results (after 6 to 12 h exposure):
Initial conc. of 14C–label in larvae

benzene in water [mg/l]	(calculated as benzene) [mg/l]
0.127	0.202
1.05	2.82
1.85	3.60

After 12 h, 14C-label gradually leveled off or near initial exposure concentrations.
Reliability: 3 (not valid)
Documentation insufficient for assessment
Purity/radiochemical purity of test substance, no. of exposed animals not reported; no statistical analysis; since only 14C-uptake was measured, no discrimination between benzene and its metabolites possible.

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Filtered seawater; salinity 23 g/l; oxygen near saturation level; temperature 12.2-13.5 degree C.

(333)

Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Concentration:
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Eggs, yolk-sac and postyolk-sac of Pacific herring larvae (79-144 indiv./sample) were exposed to 14C-labelled benzene in static filtered seawater. Actual initial concentrations of benzene in were determined by GC. Benzene concentrations in water decreased to ca. 23-45% of initial conc. within 24 h; no 14C was detected after 48 h. No analysis for 14C-labelled metabolites was performed.
Results (after 6 h exposure):

Life stage	Initial conc. [mg/l]	14C-label in animals [ug/g]
embryo	0.055	0.51
embryo	0.49	5.36
yolk-sac	0.032	0.22
yolk-sac	0.40	2.11
postyolk-sac	0.13	0.19
postyolk-sac	1.05	2.82
postyolk-sac	1.85	3.60

Reliability: 3 (not valid)
Documentation insufficient for assessment
Purity/radiochemical purity of test substance, no. of exposed animals not reported; no statistical analysis; since only 14C-uptake was measured, no discrimination between benzene and its metabolites possible.

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Filtered seawater; salinity 23 g/l; oxygen near saturation level; temperature 12.2-13.5 degree C.

(334)

Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Concentration:
BCF: = 4.4
Elimination: no data
Method: other: see reference
Year: 1977 **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(335)

Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 3 day
Concentration: .05 mg/l
BCF: < 10
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The methods of Freitag D. Ecotoxic. envir. Saf. 6, 60–81, 1982 and Korte F. et al. Chemosphere 7, 79–102, 1977 were followed. Fish were exposed to 14C–benzene and the concentration of the compound in the fish compared with the medium concentration in the water.
Test substance: Purity: >98%.

(336)

Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 3 day
Concentration: .05 mg/l
BCF: < 10
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: 5 fish (2–5 g) were exposed to 14C–labeled benzene in a closed system and concentrations determined by following radioactivity in fish and water; BCF–values related to wet weight.
Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Determination of radioactivity includes possible metabolized and/or incorporated intermediates.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Temperature 23+/–3 degree C; pH 7; hardness 100 mg CaO/l
Test substance: Purity: >98%.

(336) (337)

Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 2 day
Concentration:
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The half–life (t½) was found to be <1 day, indicating a moderate tendency for benzene accumulation.
Source: BP Chemicals Ltd. London
Test condition: Fish were exposed to benzene in water and the concentration of benzene in the muscle tissue was followed over a 7–day clearance period.

(118) (338)

Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 2 day
Concentration:
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
The half–life (t1/2) was found to be <1 day, indicating a moderate tendency for benzene accumulation.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Fish were exposed to benzene in water and the concentration of benzene in the muscle tissue was followed over a 7–day clearance period.

(118) (338)

Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 6 hour(s)
Concentration: .077 mg/l
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The bioconcentration factors measured in the fat and intestine were 1.14 and 5.45 respectively.
Source: BP Chemicals Ltd. London
Test condition: Fish were exposed under static, saltwater conditions.

(339) (340)

Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 6 hour(s)
Concentration: .077 mg/l
BCF:
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
The bioconcentration factors measured in the fat and intestine were 1.14 and 5.45 respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Fish were exposed under static, saltwater conditions.
(339) (340)

Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period:
Concentration:
BCF: = 19
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The log bioconcentration factor in rainbow trout muscle was calculated to be 1.28.
Source: BP Chemicals Ltd. London
Test condition: Salmo gairdneri/Oncorhynchus mykiss were tested. No further details were available.
(341) (110)

Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period:
Concentration:
BCF: = 19
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
The log bioconcentration factor in rainbow trout muscle was calculated to be 1.28.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Salmo gairdneri/Oncorhynchus mykiss were tested. No further details were available.
(341) (110)

Species: other
Exposure period: 5 day at 25 degree C
Concentration: .05 mg/l
BCF: = 1700
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Activated sludge from a municipal sewage treatment plant (1 g dry weight/l) was exposed to 14C-labeled benzene in a closed system and radioactivity measured after incubation; nutrient solution and sludge replenished once a week; BCF related to dry weight.
Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Determination of radioactivity includes possible metabolized and/or incorporated intermediates.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Synthetic nutrient solution; constant stirring.
Test substance: Purity: >98%.

(336) (337)

Species: other
Exposure period: 8 day
Concentration:
BCF: = 0
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No measurable levels of benzene were detected in the clam tissue at the end of exposure.
Source: BP Chemicals Ltd. London
Test condition: Tapes semidecussata (clam) were exposed to the water-soluble fraction of crude oil in a flow system under natural environmental conditions.

(342) (343)

Species: other
Exposure period: 8 day
Concentration:
BCF: = 0
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 70 Manila clams (size class ca. 20 ml in volume, collected from the sea) were exposed to the water-soluble fraction of crude oil (total 3.1 mg/l) which contained a mixture of 6 monoaromatics (toluene, ethylbenzene, o-, p-, m-xylene and 0.9-2.2 mg/l benzene). Exposure in a continuous-flow system under natural environmental conditions. The amount of aromatics in water was measured three times a day, tissue content every 48 h in pooled tissues of 10 clams by GC-FID.
Results:

Benzene concentration in the exposed clams was below the detection limit (0.6 mg/l) during the whole exposure period.
Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Exposure to benzene in a mixture of hydrcarbons.

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Filtered sterilized seawater; salinity 30 g/l; temperature 14+/-1 degree C; light intensity, wavelenght and photoperiod approximating natural conditions.

(344)

Species: other
Exposure period: 24 hour(s)
Concentration: .05 mg/l
BCF: = 30
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Chlorella fusca (green algae) were tested. No further details were available from the citing reference.

(345) (118)

Species: other
Exposure period: 24 hour(s)
Concentration:
BCF: = 30
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Chlorella fusca var: vacuolata (green algae) were exposed to 14C-benzene according to the methods of Freitag D. Ecotoxic. envir. Saf. 6, 60-81, 1982 and Korte F. et al. Chemosphere 7, 79-102, 1977 and the concentration of the chemical in the algae compared with that remaining in the water.
Test substance: Purity: >98%.

(336)

Species: other
Exposure period: 24 hour(s)
Concentration: .05 mg/l
BCF: = 30
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Algae (20 mg dry weight, ca. 100 mg fresh weight) were exposed to 14C-labeled benzene in a closed system and concentrations determined by following radioactivity in algae and water; BCF-values related to wet weight.
Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Determination of radioactivity includes possible metabolized and/or incorporated intermediates.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Temperature 20-25 degree C; illumination 16 h/day.
Test substance: Purity: >98%.

(336) (346) (337)

Species: other
Exposure period: 24 hour(s)
Concentration: .097 mg/l
BCF: = 4360
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: In the liver, a bioconcentration factor of 45.1 was reported following a similar exposure to 3.3 mg/l. IT IS NOTED THAT THE BCF FROM THIS STUDY IS UNUSUAL IN BEING THE ONLY VERY HIGH VALUE NOTED.
Source: BP Chemicals Ltd. London
Test condition: The bioconcentration factor was measured in the gall bladder of Engraulis mordax (Northern anchovies) exposed under static, saltwater conditions.

(339) (340)

Species: other
Exposure period: 24 hour(s)
Concentration: .097 mg/l
BCF: = 4360
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: In the liver, a bioconcentration factor of 45.1 was reported following a similar exposure to 3.3 mg/l. IT IS NOTED THAT THE BCF FROM THIS STUDY IS UNUSUAL IN BEING THE ONLY VERY HIGH VALUE NOTED.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The bioconcentration factor was measured in the gall bladder

exposed under static, saltwater conditions.
(339) (340)

Species: other
Exposure period: 24 hour(s)
Concentration:
BCF:
Elimination: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Daphnids were exposed in to ca. 10 ug/l 14C–benzene in lake water alone or algae (Ankistrodesmus falcatus, preloaded by incubation with 50 ug/l benzene, 100 cells/ml of test solution with 1.5 pg benzene/cell) or both (water and algae). Exposition in sealed 250 ml flasks with sufficient headspace to accomodate in excess the oxygen demand. Accumulation was measured after incubation of 20 daphnids per set followed by determination of clearance in uncontaminated lake water; UV–spectrofluorometry. Accumulation factors and clearance:
(1) after exposure in water 225; 88% clearance after 72 h,
(2) after feeding with preloaded algae 203;
(3) after incubation in medium and algae loaded 153;
83% clearance in water with unloaded algae.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Not mentioned if BCF–values refer to dry weight or fresh weight of exposed daphnids.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Filtered lake water; pH 7.5; temperature 13–17 degree C.
(347)

Species: other
Exposure period:
Concentration:
BCF: 6 – 24
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Based on reported log Kow of 2.13 (Hansch C. & Leo A.J. Medchem Project Issue No. 26. Pomona College, Claremont, California, 1985 cited in HSDB, 1993), a bioconcentration factor of 24 was estimated using the methods of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods. p.5–5. McGraw–Hill, New York, 1982 (cited in HSDB, 1993). Using this same log Kow value and Mackay's empirical relation (Mackay D. Envir. Sci. Technol. 16, 274–278, 1982 cited in BUA, 1988), the bioconcentration factor was estimated to be about 6, signifying a low bioaccumulation potential.
Source: BP Chemicals Ltd. London
(331) (70) (280)

Species: other
Exposure period:
Concentration:
BCF: = 225
Elimination: no data
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The maximum bioconcentration factor for Daphnia pulex was found to be 225. No further details were available from the citing reference.
Source: BP Chemicals Ltd. London

(118) (121)

3.8 Additional Remarks

Remark: No relevant data identified from the literature searched.
Source: BP Chemicals Ltd. London

Remark: The determination of rate constants for the reaction of the adduct benzene–OH which is the first intermediate after the reaction with OH radicals gave following results: under tropospheric conditions the reaction of the adduct with O2 will be predominant compared to the analogous reaction with NO2. The reaction of NO with the adduct is expected to be negligible, even under extreme pollution conditions.
Source: Deutsche Shell Chemie GmbH Eschborn

(348)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 6 day
Unit: mg/l **Analytical monitoring:** no data
LC : = .7
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A decrease in the survival of ovarian eggs, embryo and larvae was noted.
Source: BP Chemicals Ltd. London
Test condition: Adult males and females (Clupea harengus pallasi) were exposed in saltwater with a dissolved oxygen concentration of 6-10.5 mg/l, a salinity of 23-24 ppt and a temperature of 10-11.5°C.

(349) (350)

Type: flow through
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 6 day
Unit: mg/l **Analytical monitoring:** no data
LC : = .7
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A decrease in the survival of ovarian eggs, embryo and larvae was noted.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Adult males and females (Clupea harengus pallasi) were exposed in saltwater with a dissolved oxygen concentration of 6-10.5 mg/l, a salinity of 23-24 g/l and a temperature of 10-11.5 degree C.

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Type: flow through
Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 9.58
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The LC50 after a 72-hour exposure, under the same conditions, was 9.58 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Juvenile fish were exposed in saltwater at 16.9-17.9°C, alkalinity 110-120 mg/l as CaCO3, dissolved oxygen 7.2-8.2 mg/l and pH 7.6-7.8.

(349) (351)

Type: flow through
Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 9.58
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 1 (valid)
Comparable to guideline study
Saltwater fish species used, no GLP, no LC0 and LC100 data reported.
The LC50 after a 72-hour exposure, under the same conditions, was 9.58 mg/l.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile fish (1-2g) were exposed in filtered and aerated natural saltwater (salinity: 28-30 g/l) at 16.9-17.9 degree C, alkalinity 110-120 mg/l as CaCO3, dissolved oxygen 7.2-8.2 mg/l and pH 7.6-7.8.
Test substance: Reagent grade benzene was used.

(352)

Type: flow through
Species: Mugil curema (Fish, estuary, marine)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC : = 10
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Oxygen consumption was increased in a concentration-dependent manner to ca. 20-fold of the control value at 10 mg/l benzene.
At the same concentration, the excretion of ammonia was reduced to ca. 60% of control.
Reliability: 3 (not valid)
Significant methodological deficiencies
No analytical monitoring, no EC0 value, no confidence limits reported. According to the authors, benzene solutions were "emulsified".
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile white mullet (0.5-1.0 g) were captured from the wild and kept at 18-22 degree C in filtered, well-aerated seawater (salinity: 35 g/l) for 10 days prior to the assay. Fish were fed ad libitum.
Oxygen consumption rates of individual fish (7 replicates) were recorded for 24 h during exposure to 1.0, 5.0, and 10.0 mg/l benzene, respectively.
No further details were available.
In parallel, ammonia excretion rates were monitored in a static system.

(353)

Type: flow through
Species: Oncorhynchus kisutch (Fish, fresh water, marine)
Exposure period: 1 hour(s)
Unit: mg/l **Analytical monitoring:** yes
EC50 : 1.75
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: At 1.9 mg/l, about half the expected number of animals was found in the test arm with treated water. Nearly all fish avoided water with 2.8 mg/l benzene.
Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Only 2 concentrations tested.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Presmolt coho salmon (6-10 cm length), kept in freshwater, were treated at 6.7-9.9 degree C with three different concentrations of benzene in a flow-through system.
Avoidance reaction was tested in a Y-maze chamber with one side continuously perfused with freshwater, the other side with freshwater containing benzene which was added from a reservoir by a syringe pump.
20-60 fish were placed in the holding region of the Y-maze for 15 min before injection of benzene to the water of one arm was started. After 50-70 min, the number of animals in each of the two upstream arms of the Y-maze was counted.
Analytical monitoring showed that measured concentrations of test substance well agreed with calculated concentrations.
The calculated EC50 value represents the concentration of benzene at which half the expected number of fish would be found in the test arm with treated water.

(354)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 7 day
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 10.2
LC50: = 14.01
LOEC : = 17.2
Method: other
Year: 1989 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 12.37-15.86 mg/l. The NOEC, LOEC and subchronic values were found to be 10.2, 17.2 and 13.2 mg/l respectively when examining both survival and growth.
Source: BP Chemicals Ltd. London
Test condition: The method of the U.S. Environmental Protection Agency. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freahwater Organisms. 2nd Edition. EPA 600/4-89-001 and Supplement EPA 600/4-89-001A. Cincinnati, Ohio, 1989 was followed. Chemical analysis was conducted by flame-ionisation chromatography. Groups of 40 fish aged less than 24 hours were exposed to benzene in filtered, aerated and heated Lake Superior water. The

temperature was measured to be 25°C, pH 7.65, dissolved oxygen 7.3 mg/l, alkalinity 39 mg/l as CaCO3 and hardness 45.5 mg/l as CaCO3.

Test substance: Supplied by Burdick & Jackson, Muskegon, Midland; >99.5% pure.

(355)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 7 day
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 10.2
LC50: = 14.01
Method: other
Year: 1989 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 12.37-15.86 mg/l.
The NOEC and LOEC values were found to be 10.2 and 17.2 mg/l, respectively, when examining both survival and growth. However, since NOEC and LOEC values, calculated from hypothesis test statistics, were found to correspond to concentrations causing considerable mortality, the authors conclude "that NOEC and LOEC can be unsuitable to satisfactorily represent thresholds for biological effects". In the same study, a 96h LC50 of 15.59 mg/l (conf. lim. 14.04-17.3 mg/l) was also reported.
Reliability: 1 (valid)
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the U.S. EPA (Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freahwater Organisms. 2nd Edition. EPA 600/4-89-001 and Supplement EPA 600/4-89-001A, Cincinnati, Ohio, 1989) was followed. Chemical analysis was conducted by GC-FID. Groups of 40 fish aged less than 24 hours were exposed to benzene in filtered, aerated and heated Lake Superior water. The temperature was measured to be 24-26 degree C, pH 7.1-8.3, dissolved oxygen 6.3-8.3 mg/l, alkalinity 38-40 mg/l as CaCO3 and hardness 44.5-46.5 mg/l as CaCO3. The photoperiod was 16 h light/ 8 h dark. Fish were fed three times a day with brine shrimp larvae.
The benzene concentrations in the tanks were measured before starting tests and at least twice during the test.
Test substance: Supplied by Burdick & Jackson, Muskegon, Midland; >99.5% pure.

(355)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC30 : 15.1
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
Several parameters were not reported in the study (purity of test substance, EC0, EC100 values).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 14th Edition. American Public Health Association, New York, 1975 was followed. Groups of 20 juvenile fathead minnows (mean weight 0.58 g) were exposed to benzene in freshwater at 13-17 degree C. Dissolved oxygen concentration was about 7 mg/l, pH was 7.9-8, alkalinity 147-165 mg/l as CaCO3 and water hardness 535-596 mg/l as CaCO3. The fish were not fed during treatment. The photoperiod was 16 h light/ 8 h dark. Benzene concentrations in the test tanks were monitored daily by HPLC.

(356)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 5.3
Method: other
Year: 1975 **GLP:** no data
Test substance: no data
Remark: Other investigators exposed Oncorhynchus mykiss, in a flow through system, to benzene for 96 hours. The temperature was 14-16.5°C, dissolved oxygen 5.6-9.4 mg/l and pH 7.6-8.19. The LC50 was found to be 277 µmol/l (converts to 21.6 mg/l) (Hodson P.V. et al. Envir. Toxic. Chem. 3, 243-254, 1984 cited in AQUIRE, 1993).
Source: BP Chemicals Ltd. London
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 14th Edition. American Public Health Association, New York, 1975 was followed. Groups of 20 juvenile fish (Salmo gairdneri/Oncorhynchus mykiss) were exposed to benzene in freshwater at 15°C. Dissolved oxygen concentration was about 7 mg/l, pH was 7.9-8, alkalinity 147-165 mg/l as CaCO3 and water hardness 535-596 mg/l as CaCO3.

(349) (357)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 5.3
Method: other
Year: 1975 **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
Several parameters were not reported in the study (purity of test substance, EC0, EC100 values).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 14th Edition. American Public Health Association, New York, 1975 was followed. Groups of 20 juvenile fish (Salmo gairdneri/Oncorhynchus mykiss, mean weight 13.9 g) were exposed to benzene in freshwater at 13-17 degree C. Dissolved oxygen concentration was about 7 mg/l, pH was 7.9-8, alkalinity 147-165 mg/l as CaCO3 and water hardness 535-596 mg/l as CaCO3. The fish were not fed during treatment. The photoperod was 16 h light/ 8 h dark. Benzene concentrations in the test tanks were monitored daily by HPLC.

(356)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 21.6
Method: other
Year: **GLP:**
Test substance: no data
Remark: In the same study, under similar conditions the toxicity of benzene to rainbow trout was also investigated after i. p. injection (solution in cod liver oil) and after oral administration (intubation of substance in gelatine capsules).
i.p. injection (ten fish weighing 9-30 g each): LD50 value 25.8 mmol/kg (=2.0 g/kg fish).
oral administration (ten fish weighing 9-30 g each): LD50 value 32.3 mmol/kg (=2.5 g/kg fish).
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
No analytical monitoring data presented, no EC0, EC100 values.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish (1-4g weight/fish), preadapted for at least one week, were exposed to benzene in a continuous-flow test system. Each bioassay tank contained 14 l of water. The tanks were not aerated during exposure. Water temperature was 14.1-16.5 degree C. Water was dechlorinated by charcoal filtration and addition of 1 mg/l sodium sulfite. Alkalinity averaged 86 mg/l (as CaCO3), conductivity 340 umhos/cm2, pH 7.6-8.2, oxygen content 5.6-9.4 mg/l.

Benzene concentrations were measured daily by UV–spectrophotometry.

(358)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
LC50: = 8.25
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Fish were exposed to benzene in freshwater at a temperature of 20°C, an oxygen concentration of 7.5 mg/l and pH of 7.7 from fertilisation to 4 days post–hatch.

(359) (360)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
LC50: = 8.25
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Fish were exposed to benzene in freshwater at a temperature of 20 degree C, an oxygen concentration of 7.5 mg/l and pH of 7.7 from fertilisation to 4 days post–hatch.

(359) (360)

Type: other: no specified
Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LD50 : = 46
Method: other: modified ASTM D–1345
Year: **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Source: REPSOL PETROLEO,S.A. MADRID

Type: static
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: < 32
LC50: 70.7
Method: other: nach UBA–Richtlinie vom Maerz 1979 und Ergaenzungen vom 06.02.1980
Year: **GLP:** no
Test substance: as prescribed by 1.1 – 1.4
Source: BASF AG Ludwigshafen
Reliability: (1) valid without restriction
nationale Richtlinien

(361)

Type: static
Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 34.42
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: A second set of investigators tested benzene in accordance with the 1971 edition of these guidelines and found a 24–hour LC50 of 46 mg/l (Bridie A.L. et al. Water Res. 13, 623–626, 1979).
LC50 confidence limits = 26.10–42.83 mg/l. Further tests gave 48– and 96–hour LC50 values of 34.42 mg/l (confidence limits 26.10–42.83 mg/l.
Source: BP Chemicals Ltd. London
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25°C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).
Test substance: Reagent grade benzene tested.

(362)

Type: static
Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 46
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No EC0, EC100 values reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater, Method No. 231, 1971, was followed. Groups of six fish (2.3–4.3 g) were exposed to benzene at 19–21 degree C in 25 l tap–water (100 mg/l Ca2+, alkali as Na+: 30 mg/l, pH 7.8, no aeration during exposure to reduce evaporation of benzene, dissolved oxygen during test at least 4mg/l).
The concentration of the test chemical was determined before and after each test.
Test substance: Purity: no data presented.

(363)

Type: static
Species: Carassius auratus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 34.42
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 26.10–42.83 mg/l.
Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No analytical monitoring.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25 degree C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).
Test substance: Reagent grade benzene tested.

(362)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 7 day
Unit: µg/l **Analytical monitoring:** no data
EC : = 50
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A significant decrease in oxygen consumption was recorded at the lowest concentation tested, 50 µg/l, while the highest concentration, 490 µg/l, caused a significant increase in oxygen consumption. No effects on anabolic energy, tissue weight or growth were found.
Source: BP Chemicals Ltd. London
Test condition: Embryo (Clupea harengus pallasi) were exposed, at hatching, to benzene in saltwater with a salinity of 23 ppt and a temperature of 13.5°C.

(364) (365)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 7 day
Unit: µg/l **Analytical monitoring:** no data
EC : = 88
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A significant decrease in oxygen consumption was recorded at the lower concentation tested, 88 ug/l, while the higher concentration, 880 ug/l, caused a significant increase in oxygen consumption. No effects on anabolic energy, tissue weight or growth were found.
Reliability: 3 (not valid)
Documentation insufficient for assessment
Concentration of test substance not exactly defined.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Embryo (Clupea harengus pallasi) were exposed, at hatching, to benzene in saltwater with a salinity of 23 g/l and a temperature of 13.5 degree C.

(366)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 17 day
Unit: µg/l **Analytical monitoring:** no data
NOEC: = 490
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No effect on oxygen consumption was noted at 490 µg/l, the highest concentration tested.
Source: BP Chemicals Ltd. London
Test condition: Yolk sac and post-yolk sac larvae (Clupea harengus pallasi) were exposed in saltwater with a salinity of 23 ppt and a temperature of 12.2-12.5°C.

(364) (365)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 17 day
Unit: µg/l **Analytical monitoring:** no data
NOEC: = 880
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No effect on oxygen consumption was noted at 880 ug/l, the highest concentration tested.
Reliability: 3 (not valid)
Documentation insufficient for assessment
Concentration of test substance not exactly defined.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Yolk sac and post–yolk sac larvae (Clupea harengus pallasi) were exposed in saltwater with a salinity of 23 g/l and a temperature of 12.2 to 12.5 degree C.

(366)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Unit: µg/l **Analytical monitoring:** no data
LC0: = 490
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No effects on survival or yolk consumption were seen at any of the concentrations tested, 490 µg/l being the highest. A significant decrease in tissue weight and significant delayed growth were seen in those exposed to 50–490 µg/l.
Source: BP Chemicals Ltd. London
Test condition: Embryo (Clupea harengus pallasi) were exposed for 6–7 days, starting on the day of fertilisation, in saltwater at 13.5°C. The salinity was reported to be 23 ppt.

(349) (365)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Unit: µg/l **Analytical monitoring:** no data
LC0: = 460
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No effects on survival, yolk consumption or catabolic energy were seen at 460 µg/l, the highest concentration tested. A significant energy deficit, however, was reported at this concentration when the larvae were tested 24 hours after hatching. In post–yolk sac larvae similarly exposed for 5 days at 12.2°C, a significant increase in growth and caloric assimilation was noted at 1840 µg benzene/l, the highest concentration tested.
Source: BP Chemicals Ltd. London
Test condition: Yolk sac larvae (Clupea harengus pallasi) were exposed for 5–8 days to benzene in saltwater at 12.5°C and a salinity of 23 ppt.

(364) (365)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Unit: µg/l **Analytical monitoring:** no data
LC0: = 880
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No effects on survival or yolk consumption were seen at bothconcentrations tested, 880 ug/l being the higher. A significant decrease in tissue weight and significant delayed growth were seen both in those exposed to 88 ug/l and to 880 ug/l.
Reliability: 3 (not valid)
Documentation insufficient for assessment
Concentration of test substance not exactly defined.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Embryo (Clupea harengus pallasi) were exposed for 6–7 days, starting on the day of fertilisation, in saltwater at 13.5 degree C. The salinity was reported to be 23 g/l.

(367)

Type: static
Species: Clupea harengus (Fish, estuary, marine)
Exposure period:
Unit: µg/l **Analytical monitoring:** no data
LC0: = 880
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No effects on survival, yolk consumption or catabolic energy were seen at 880 ug/l, the highest concentration tested. A significant energy deficit, however, was reported at this concentration when the larvae were tested 24 hours after hatching. In post-yolk sac larvae similarly exposed for 5 days at 12.2 degree C, a significant increase in growth and caloric assimilation was noted at 88 ug benzene/l, the lower concentration tested.
Reliability: 3 (not valid)
Documentation insufficient for assessment
Concentration of test substance not exactly defined.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Yolk sac larvae (Clupea harengus pallasi) were exposed for 5-8 days to benzene in saltwater at 12.5 degree C and a salinity of 23 g/l.

(366)

Type: static
Species: Gasterosteus aculeatus (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 21.8
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 19-25 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Three-year-old adults were exposed in freshwater at 8°C and dissolved oxygen content >85% saturation.

(364) (368)

Type: static
Species: Gasterosteus aculeatus (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 21.8
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 95% confidence limits = 19–25 mg/l.
Most deaths occurred during the first 12 h of exposure.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Three–year–old adult three spine sticklebacks (length ca. 55 mm, 12 fish/concentration) were exposed in freshwater at 8 degree C. Solutions were aerated during the final 48 h to ensure dissolved oxygen content >85% saturation. Concentrations of benzene as monitored by UV–spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: purity not specified

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Type: static
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 425
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 357–505 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Fish exposed in freshwater at 22°C to benzene. Water hardness was 40–50 mg/l as CaCO3, alkalinity was 30–35 mg/l as CaCO3 and pH was 7.2–7.5

(364) (370)

Type: static
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 425
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 357–505 mg/l.
Further 96h LC50 values (confidence limit) given:
rainbow trout (appr. 2.4g), 12 degree C: 9.2 mg/l (7.2–11.7)
Bluegill sunfish (appr. 0.2 g), 22 degree C: 100 mg/l (70–142).
Reliability: 4 (validity cannot be judged)
Secondary literature
Data from handbook with compilation of results
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Fish (approx. 0.1 g) exposed in freshwater aerated prior to use at 22 degree C to benzene. Water hardness was 40–50 mg/l as CaCO3, alkalinity was 30–35 mg/l as CaCO3 and pH was 7.2–7.5.

(371)

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 20
LC100: = 34
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A 48 h LC50 of 20 mg/l was also reported in this study.
At 60 mg/l, all fish died within two hours.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No monitoring of concentration during the test, purity of test substance not reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten bluegill sunfish (length: 5–11 cm, weight ca. 5 g) were exposed for 48 h to benzene in 15 l freshwater (alkalinity as CaCO3: 33–81 mg/l, pH 6.9–7.5, total hardness as CaCO3: 84–163 mg/l, dissolved oxygen at least 5 mg/l). Benzene was added from a saturated solution in water. The concentration in this solution was determined by UV–spectrophotometry.

(372)

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 22.49
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: In static tests conducted by other investigators, 24- and 48-hour LC50 values of 20 mg/l have been reported (Turnbull H. et al. Ind. Engng Chem. 46, 324-333, 1954 cited in AQUIRE, 1993) and following exposure for 96 hours, LC50 values have ranged from 100 to 370 mg/l (Johnson W.W. & Finley M.T. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Resour. Publ. 137. Fish Wildl. Serv., USDI, Washington DC, 1980 cited in AQUIRE, 1993; Mayer F.L. & Ellersieck M.R. Manual for Acute Toxicity: Interpretation and Database for 410 Chemicals and 66 Species of Freshwater Animals. Resour. Publ. 160, 50. Fish Wildl. Serv., USDI, Washington DC, 1986 cited in Nielsen et al. 1991).
LC50 confidence limits = 17.50-28.37 mg/l. Further tests gave 48- and 96-hour LC50 values of 22.49 mg/l (confidence limits 17.50-28.37 mg/l).
Source: BP Chemicals Ltd. London
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25°C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).
Test substance: Reagent grade benzene tested.

(349) (373) (362)

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 22.49
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 17.50-28.37 mg/l.
Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No analytical monitoring.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25 degree C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).
Test substance: Reagent grade benzene tested.

(362)

Type: static
Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
LC50: = 6.1
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Similar exposure for 96 hours gave an LC50 value of 5800 µl/l (converts to 5.1 g/l).
Source: BP Chemicals Ltd. London
Test condition: Juveniles were exposed at 16°C in saltwater.
Test substance: >99% pure.

(349) (374)

Type: static
Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 6.1
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Similar exposure for 96 hours gave an LC50 value of 5.8 ppm (converts to 5.1 mg/l).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juveniles (mean weight: 6.0 g) were exposed at 16 degree C in saltwater (salinity: 25 g/l).
Benzene concentration was assayed by gas chromatography at 0, 24, 48, 72, and 96 hours.
Test substance: >99% pure.

(375)

Type: static
Species: Mugil curema (Fish, estuary, marine)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 22
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Significant methodological deficiencies
No analytical monitoring, no EC0 value, no confidence limits reported, no. of animals not reported. According to the authors, benzene solutions were "emulsified".
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile white mullet (0.5-1.0 g) were captured from the wild and kept at 18-22 degree C in filtered, well-aerated seawater (salinity: 35 g/l) for 10 days prior to the assay. Fish were fed ad libitum.
48 h LC50 bioassays were conducted in 2.5 l glass bottles with water aerated before benzene was added from stock solution (1000 mg/l).

(353)

Type: static
Species: Oncorhynchus gorbuscha (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 15
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 12.6-17.4 mg/l. A second study on fry, exposed in saltwater, gave an LC50 value of 7.4 mg/l (confidence limits 6.9-8 mg/l). When examining eggs and emergent fry in freshwater, LC50 values of 298 and 4.6 mg/l were obtained respectively.
Source: BP Chemicals Ltd. London
Test condition: Fry were exposed at 4°C to benzene in freshwater containing >85% dissolved oxygen saturation.

(349) (368)

Type: static
Species: Oncorhynchus gorbuscha (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 15
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 12.6-17.4 mg/l.
Most deaths occurred during the first 12 h of exposure.
Fry acclimated to saltwater (salinity 28-30 g/l) similarly exposed to benzene were more sensitive: LC50 value 7.4 mg/l (confidence limits 6.9-8 mg/l).
When examining eggs and emergent fry in freshwater, LC50 values of 298 and 4.6 mg/l were obtained, respectively.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Outmigrant Pink salmon fry were exposed at 4 degree C to benzene in freshwater. Solutions were aerated during the final 48 h to ensure >85% dissolved oxygen saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: purity not specified

(376)

Type: static
Species: Oncorhynchus kisutch (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 12.4
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 8.7-16.1 mg/l. This test was repeated using eggs and emergent fry. The 96-hour LC50s, under such conditions, were 476 and 8.6 mg/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Fish of length 40-75 mm were exposed to benzene at 9°C and in freshwater containing >85% dissolved oxygen saturation.
(349) (368)

Type: static
Species: Oncorhynchus kisutch (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 12.4
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 8.7-16.1 mg/l.
Most deaths occurred during the first 12 h of exposure.
This test was repeated using eggs and emergent fry. The 96-hour LC50s were 476 and 8.6 mg/l respectively.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Coho salmon (length 5-50 mm) were exposed to benzene at 9 degree C in freshwater. Solutions were aerated during the final 48 h to ensure >85% dissolved oxygen saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: Purity not specified
(376)

Type: static
Species: Oncorhynchus nerka (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 4.9
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 2.5–7.2 mg/l. A similar study conducted in freshwater gave an LC50 value of 9.5 mg/l (confidence limits 8.3–10.6 mg/l).
Source: BP Chemicals Ltd. London
Test condition: Two–year–old smolts were exposed at 6°C to benzene in saltwater containing >85% dissolved oxygen saturation.
(349) (368)

Type: static
Species: Oncorhynchus nerka (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 9.4
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 8.3–10.6 mg/l.
Most deaths occurred during the first 12 h of exposure.
Smolts acclimated to saltwater (salinity 28–30 g/l) similarly exposed to benzene were more sensitive: LC50 value 4.9 mg/l (confidence limits 2.5–7.2 mg/l).
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Sockeye salmon smolts were exposed at 6 degree C to benzene in freshwater. Solutions were aerated during the final 48 h to ensure >85% dissolved oxygen saturation.
Concentration of benzene as monitored by UV–spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: Purity not specified.
(369)

Type: static
Species: Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 10
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 condifence limits = 7.4–12 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Juvenile fish were exposed at 9°C to benzene in freshwater with >85% dissolved oxygen saturation content.

(349) (368)

Type: static
Species: Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 10.3
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 7.4–12 mg/l.
Most deaths occurred during the first 12 h of exposure.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile chinook salmon (length 75 mm) were exposed (12 fish/concentration) at 9 degree C to benzene in freshwater. Solutions were aerated during the final 48 h to ensure dissolved oxygen content >85% saturation.
Concentrations of benzene as monitored by UV–spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: Purity not specified

(369)

Type: static
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 54 – 74
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: At 10, 20 and 30°C, the 24-hour LC50s were 74, 70 and 54 mg/l respectively. Following exposure for 48 hours at these temperatures, the LC50 values were found to be 74, 70 and 54 mg/l respectively.
Other investigators reported a 48-hour LC50 of 250 mg/l in fish exposed at 4-5 weeks of age to benzene (>98% pure) at 24°C (Slooff W. et al. Aquat. Toxic. 4, 113-128, 1983, cited in AQUIRE, 1993).
Source: BP Chemicals Ltd. London
Test condition: Fish were exposed to benzene in freshwater at various temperatures to examine the effect of temperature on the LC50 values.

(349) (377)

Type: static
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 54 – 74
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: At 10, 20 and 30 degree C, the 24 h as well as the 48 h LC50s were 74, 70 and 54 mg/l respectively.
Reliability: 4 (validity cannot be judged)
Original reference in Japanese
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Killifish were exposed to benzene in freshwater at various temperatures to examine the effect of temperature on the LC50 values.

(378)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 34.42 – 35.56
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 25.81–45.83 mg/l.
Source: BP Chemicals Ltd. London
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene in either soft or hard freshwater at 25°C. The hardness was measured as 20 and 360 mg/l (EDTA) respectively, alkalinity 18 and 300 mg/l respectively, pH 7.5–8.2 and dissolved oxygen 7.8 mg/l.
Test substance: Reagent grade benzene tested.

(362)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: 34.42 – 35.56
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 25.81–45.83 mg/l.
Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No analytical monitoring.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene in either soft or hard freshwater at 25 degree C. The hardness was measured as 20 and 360 mg/l (EDTA) respectively, alkalinity 18 and 300 mg/l respectively, pH 7.5–8.2 and dissolved oxygen 7.8 mg/l.
Test substance: Reagent grade benzene tested.

(362)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 32 – 35.08
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: A further group of workers reported an LC50 value of 84 mg/l in fish exposed at 3–4 weeks of age to benzene (>98% pure) for 48 hours (Slooff W. et al. Aquat. Toxic. 4, 113–128, 1983 cited in AQUIRE, 1993).
LC50 confidence limits = 22.33–43.67 mg/l.
Source: BP Chemicals Ltd. London
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene in either soft or hard freshwater at 25°C. The hardness was measured to be 20 and 360 mg/l (EDTA) respectively, alkalinity 18 and 300 mg/l respectively, pH 7.5–8.2 and dissolved oxygen 7.8 mg/l.
Test substance: Reagent grade benzene tested.

(349) (362)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: 32 – 35.08
Method: other
Year: 1960 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 22.33–43.67 mg/l.
Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No analytical monitoring.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene in either soft or hard freshwater at 25 degree C. The hardness was measured to be 20 and 360 mg/l (EDTA) respectively, alkalinity 18 and 300 mg/l respectively, pH 7.5–8.2 and dissolved oxygen 7.8 mg/l.
Test substance: Reagent grade benzene tested.

(362)

Type: static
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 28.6
Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Other investigators conducted tests on groups of ten Lebistes reticulata in accordance with APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 and obtained 24-, 48- and 96-hour LC50 values of 36.60 mg/l (confidence limits 28.63-54.33 mg/l).
Source: BP Chemicals Ltd. London
Test condition: Groups of ten fish were exposed, in freshwater, to benzene. The water temperature was 21°C. The lethal effect was observed at 3, 6, 24, 48, 72 and 96 hours from the beginning of the experiment.
Test substance: Supplied by Carlo Erba; purity not specified.

(379)

Type: static
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 28.6
Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Other investigators conducted tests on groups of ten Lebistes reticulata in accordance with APHA, Standard Methods for the Examination of Water and Wastewater, 11th Edition. American Public Health Association, New York, 1960 and obtained 24-, 48- and 96-hour LC50 values of 36.60 mg/l (confidence limits 28.63-54.33 mg/l).
Reliability: 2 (valid with restiction)
Guideline study without detailed documentation
Several experimental details not reported (age of fish, measured benzene concentrations, applied concentrations).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish were exposed, in freshwater, to benzene (11 l closed bottles, 10 animals/10 l, renewal of test solution after 48 h to avoid decline of oxygen saturation below 80%).
The water temperature was 21 degree C. The lethal effect was observed at 3, 6, 24, 48, 72 and 96 hours from the beginning of the experiment.
Quantitative analyses of benzene were carried out by HPLC at the beginning and the end of the experiment, and at the renewal of the test solution after 48 h.
Test substance: purity: Carlo Erba GC standards.

(379)

Type: static
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 5.9
Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Other investigators examined Oncorhynchus mykiss exposed, for 96 hours, under static conditions, to benzene at 12°C, a water hardness of 40-50 mg/l at CaCO3, an alkalinity of 30-35 mg/l as CaCO3 and a pH of 7.2-7.5. The LC50 was found to be 9.2 mg/l (confidence limits 7.2-11.7 mg/l) (Johnson W.W. & Finley M.T. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Resour. Publ. 137. Fish Wildl. Serv., USDI, Washington DC, 1980 cited in AQUIRE, 1993; Mayer F.L. & Ellersieck M.R. Manual for Acute Toxicity: Interpretation and Database for 410 Chemicals and 66 Species of Freshwater Animals. Resour. Publ. 160, 50. Fish Wildl. Serv., USDI, Washington DC, 1986 cited in Nielsen et al. 1991).
Source: BP Chemicals Ltd. London
Test condition: Groups of ten fish were exposed to benzene in freshwater. The water temperature was 12°C. The lethal effect was observed at 3, 6, 24, 48, 72 and 96 hours from the beginning of the experiment.
Test substance: Supplied by Carlo Erba, purity not specified.

(349) (379) (118)

Type: static
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 5.9
Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Guideline study without detailed documentation
Several experimental details not reported (age of fish, measured benzene concentrations, applied concentrations).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish were exposed to benzene in freshwater. The water temperature was 12 degree C. The lethal effect was observed at 3, 6, 24, 48, 72 and 96 hours from the beginning of the experiment.
Test substance: purity: Carlo Erba GC standard

(379)

Type: static
Species: Salmo trutta (Fish, fresh water, marine)
Exposure period: 1 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 12
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The method employed in this study developed, with certain modifications, into the standard method to be used in conditions of consent for effluents, by the Ministry of Housing and Local Government. Fish Toxicity Tests. Report of the Technical Committee. HMSO, London, 1969. Groups of ten yearling fish were exposed to benzene at 10°C in freshwater.

(380)

Type: static
Species: Salmo trutta (Fish, fresh water, marine)
Exposure period: 1 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 12
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method employed in this study developed, with certain modifications, into the standard method to be used in conditions of consent for effluents, by the Ministry of Housing and Local Government. Fish Toxicity Tests. Report of the Technical Committee. HMSO, London, 1969. Groups of ten yearling fish were exposed to benzene at 10 degree C in freshwater.

(380)

Type: static
Species: Salmo trutta (Fish, fresh water, marine)
Exposure period: 1 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 12
Method: other: see reference
Year: 1983 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(381)

Type: static
Species: Salvelinus malma (Fish, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 10.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LD50 confidence limits = 7.9-13 mg/l. When the test was conducted in saltwater, the LC50 value was 5.5 mg/l (confidence limits 4-7 mg/l) and, using 40-75 mm fish in freshwater, a value of 10.5 mg/l (confidence limits 7.8-13.2 mg/l) was obtained.
Source: BP Chemicals Ltd. London
Test condition: Three-year-old smolts were exposed in freshwater at 8°C. Dissolved oxygen concentration was >85% of the saturation level.

(349) (368)

Type: static
Species: Salvelinus malma (Fish, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 10.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LD50 confidence limits = 7.9-13 mg/l.
Most deaths occurred during the first 12 h of exposure. Smolts acclimated to saltwater (salinity 28-30 g/l) were more sensitive to benzene (LC50 value 5.5 mg/l, 95% confidence limits 4-7 mg/l) than smolts in freshwater (LC50 value 10.5 mg/l, confidence limits 7.8-13.2 mg/l).
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Dolly varden were exposed to benzene (12 fish/concentration) in freshwater at 8 degree C. Solutions were aerated during the final 48 h to ensure dissolved oxygen content at >85% of the saturation level.
Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96h, respectively.
Test substance: Purity not specified

(376)

Type: static
Species: other
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 12.9
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 9.4-16.5 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Thymallus arcticus (Arctic grayling) yearlings were exposed in freshwater at 9°C to benzene.

(349) (368)

Type: static
Species: other
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 12.9
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 9.4-16.5 mg/l.
Most deaths occurred during the first 12 h of exposure.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Thymallus arcticus (Arctic grayling) yearlings (length 55 mm) were exposed in freshwater at 9 degree C to benzene. Solutions were aerated during the final 48 h to ensure dissolved oxygen content at >85% saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: Purity not specified.

(376)

Type: static
Species: other
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 13.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 12.2-15 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Cottus cognatus (slimy sculpin) yearlings were exposed in freshwater at 9°C. Dissolved oxygen was present at >85% of the saturation concentration.

(364) (368)

Type: static
Species: other
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 13.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: LC50 confidence limits = 12.2–15 mg/l.
Most deaths occurred during the first 12 h of exposure.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 reported, purity of test substance not specified.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Cottus cognatus (slimy sculpin) yearlings (length 50 mm) were exposed in freshwater at 9 degree C. Solutions were aerated during the final 48 h to ensure dissolved oxygen levels at >85% of saturation concentration.
Concentrations of benzene as monitored by UV–spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.
Test substance: Purity not specified.

(376)

Type: other
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 20 – 25
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A second study was conducted in which eggs were exposed in saltwater for 96 hours at 10–17.5°C. The LC50 was 40–45 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Two–day–old larvae (Clupea harengus pallasi) were exposed in a renewal type assay in saltwater to benzene at 11.5–14.0°C. The water pH was 7.4–8.1 and dissolved oxygen content was 4.7–8.0 mg/l. The larvae were observed for 7 days after exposure.

(349) (382)

Type: other
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 20 – 25
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A second study was conducted in which eggs were exposed in saltwater for 96 hours at 10–17.5 degree C. The LC50 was 40–45 mg/l.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Two-day-old larvae (Clupea harengus pallasi) were exposed in a renewal type assay in saltwater to benzene at 11.5–14.0 degree C. The water pH was 7.4–8.1 and dissolved oxygen content was 4.7–8.0 mg/l. The larvae were observed for 7 days after exposure.
(349) (383)

Type: other
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 7 day
Unit: mg/l **Analytical monitoring:** no data
LC0: = 1500
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Fish were exposed in freshwater at 17–18°C and pH 7.1–8.1. No further details were available from the citing reference.
(349) (384)

Type: other
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 7 day
Unit: mg/l **Analytical monitoring:** no data
LC0: = 1500
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Fish were exposed in freshwater at 17–18 degree C and pH 7.1–8.1.
(349) (384)

Type: other
Species: Gambusia affinis (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 395
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: After exposure for 48 and 96 hours, the LC50 values were found to be 395 and 386 mg/l respectively. At 300 mg/l and less, the fish were apparently normal and at 1000 mg/l all were dead within 16 min.
Source: BP Chemicals Ltd. London
Test condition: Groups of ten fish were exposed to benzene in pond water which had a long history of high turbidity. The alkalinity was low (10 ppm methyl orange alkalinity), pH ranged from 8.1-8.4 and temperature from 20-22°C.
Test substance: Chemically pure benzene tested.

(385)

Type: other
Species: Gambusia affinis (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 395
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: After exposure for 48 and 96 hours, the LC50 values were found to be 395 and 386 mg/l respectively. At 300 mg/l and less, the fish were apparently normal and at 1000 mg/l all were dead within 16 min.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish were exposed to benzene in pond water which had a long history of high turbidity. The alkalinity was low (10 ppm methyl orange alkalinity), pH ranged from 8.1-8.4 and temperature from 20-22 degree C.
Test substance: Chemically pure benzene tested.

(385)

Type: other
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 33 - 62
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: No further details available.

(331) (386)

Type: other
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: = 126
LC50: = 250
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Several parameters not reported (No EC100 values, no confidence limits), no analytical monitoring.
The study was conducted to compare the sensitivity of several aquatic species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish (4-5 weeks of age) were exposed to benzene in a total volume of 1 l water (Dutch Standard Water) at 24 degree C.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(387)

Type: other
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: = 54
LC50: = 84
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Several parameters not reported (No EC100 values, no confidence limits), no analytical monitoring.
The study was conducted to compare the different sensitivity of several aquatic species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish (3-4 weeks of age) were exposed to benzene in a total volume of 1 l water (Dutch Standard Water) at 20 degree C.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(388)

Type: other
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 12.6
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 10.7-14.7 mg/l. Affected fish lost schooling behaviour, swam in a corkscrew motion, were underreactive to external stimuli and had increased respiration and convulsions. They were also darkly coloured and were haemorrhaging. Loss of equilibrium was seen prior to death.
Other investigators have reported 96-hour LC50 values ranging from 15-33 mg/l (DeGraeve G.M. et al. Archs envir. Contam. Toxicol. 11, 487-490, 1982; Geiger D.L. et al. 1990; Marchini S. et al. Envir. Toxic. Chem. 11, 187-195, 1992; Pickering Q.H. & Henderson C. J. Wat Pollut. Control Fed. 38, 1419-1429, 1966).
Source: BP Chemicals Ltd. London
Test condition: Chemical analysis was conducted by GLC. Groups of ten fish were exposed at 30 days of age to benzene in freshwater at 22.3°C, 7.3 mg dissolved oxygen/l, 50.7 mg/l as CaCO3 (hardness), 42.5 mg/l as CaCO3 (alkalinity) and pH 7.0.
Test substance: Supplied by J.T. Baker Chemical Co.; >=99.9% pure.

(389)

Type: other
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 12.6
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 10.7-14.7 mg/l. Affected fish lost schooling behaviour, swam in a corkscrew motion, were underreactive to external stimuli and had increased respiration and convulsions. They were also darkly coloured and were haemorrhaging. Loss of equilibrium was seen prior to death.
Other investigators have reported 96-hour LC50 values ranging from 15-33 mg/l (DeGraeve G.M. et al. Archs envir. Contam. Toxicol. 11, 487-490, 1982; Geiger D.L. et al. 1990; Marchini S. et al. Envir. Toxic. Chem. 11, 187-195, 1992; Pickering Q.H. & Henderson C. J. Wat Pollut. Control Fed. 38, 1419-1429, 1966).
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Chemical analysis was conducted by GLC. Groups of ten fish were exposed at 30 days of age to benzene in freshwater at 22.3 degree C, 7.3 mg dissolved oxygen/l, 50.7 mg/l as CaCO3 (hardness), 42.5 mg/l as CaCO3 (alkalinity) and pH 7.0.
Test substance: Supplied by J.T. Baker Chemical Co.; >=99.9% pure.

(389)

Type: other
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 14 day
Unit: mg/l **Analytical monitoring:** no data
LC50: = 63.5
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Groups of eight fish aged 2-3 months were exposed to benzene in freshwater. Water hardness was 25 mg/l as CaCO3, oxygen content remained about 5 mg/l and temperature was 22°C.

(87)

Type: other
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 14 day
Unit: mg/l **Analytical monitoring:** no data
LC50: = 63.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Several parameters not reported (no EC0, EC100 values given; purity of test substance not reported).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of eight fish aged 2-3 months were exposed to benzene in 1 l of standard freshwater in 1.5 l vessels covered with glass. Water hardness was 25 mg/l as CaCO3, oxygen content remained about 5 mg/l and temperature was 21-23 degree C. The test solution was renewed daily.
Benzene was added as stock solution in acetone or isopropanol.

(390)

Type: other
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: = 40
LC50: = 56
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Groups of ten fish (Salmo gairdneri/Oncorhynchus mykiss) were exposed, in tap water, at 5-8 weeks of age to benzene at 15°C and pH 7.8.
Test substance: >98% pure.

(388)

Type: other
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: = 40
LC50: = 56
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Method not described in detail, several parameters not reported (No EC100 values, no confidence limits), no analytical monitoring.
The study was conducted to compare the sensitivity of several aquatic species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten fish (Salmo gairdneri=Oncorhynchus mykiss, 5-8 weeks of age) were exposed to benzene in a total volume of 10 l tap water (hardness 5.5 degree d.H. corresponding to 98 mg CaCO3) at 15 degree C and pH 7.8.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(388)

Type: other
Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 20 - 25
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Engraulis mordax (Northern anchovy) fish eggs were exposed in a renewal type assay in saltwater at 16.8-18.4°C, pH 7.5-8.1 and dissolved oxygen concentration 6.9-7.6 mg/l. Observations were made on days 3 and 6.

(364) (391)

Type: other
Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: 20 - 25
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Engraulis mordax (Northern anchovy) fish eggs were exposed in a renewal type assay in saltwater at 16.8-18.4 degree C, pH 7.5-8.1 and dissolved oxygen concentration 6.9-7.6 mg/l. Observations were made on days 3 and 6.

(364) (391)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Artemia salina (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 66
Method: other
Year: 1969 **GLP:** no data
Test substance: no data
Remark: The 48-hour LC50 was also determined and found to be 21 mg/l. Other investigators exposed Artemia salina, at the larval stage, to ¿97% pure benzene for 24 hours in a static seawater system at 20°C. The LC50 was found to be 1.63 mg/l (Abernethy S. et al. Aquat. Toxic. 8, 163-174, 1986 cited in AQUIRE, 1993).
Source: BP Chemicals Ltd. London
Test condition: The method of Tarzwell C.M. Standard Methods for Determination of Relative Toxicity of Oil Dispersants and Mixtures of Dispersants and Various Oils to Aquatic Organisms. Proc. Joint Conf. on Prevention and Control of Oil Spills, sponsored by API and EPA, December 15-17, 1969 was followed. A preliminary study was conducted to determine the range in which benzene was toxic to Artemia salina. Newly hatched shrimp were exposed in seawater for 24 hours at 24.5°C. At the end of exposure, the number of live and dead shrimp were noted. Benzene was found to be lethal at less than 100 mg/l. The median tolerance limit was then examined at doses established from the preliminary screen. (349) (301)

Species: Artemia salina (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 66
Method: other
Year: 1969 **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
According to the authors, a modified standard procedure was used. No EC0 values reported, no monitoring of actual test concentrations.
The 48-hour LC50 was also determined and found to be 21 mg/l.
Other investigators exposed Artemia salina, at the larval stage, to 97% pure benzene for 24 hours in a static seawater system at 20 degree C. The LC50 was found to be 1.63 mg/l (Abernethy S. et al. Aquat. Toxic. 8, 163-174, 1986 cited in AQUIRE, 1993).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of Tarzwell C.M. (Standard Methods for Determination of Relative Toxicity of Oil Dispersants and Mixtures of Dispersants and Various Oils to Aquatic Organisms, Proc. Joint Conf. on Prevention and Control of Oil Spills, sponsored by API and EPA, December 15-17, 1969) was followed. A preliminary study was conducted to determine

the range in which benzene was toxic to Artemia salina. 30–50 newly hatched brine shrimps were exposed in 100 ml artificial seawater for 24 hours at 24.5 degree C in loosely capped bottles. At the end of exposure, the number of live and dead shrimp were noted. Benzene was found to be lethal at less than 100 mg/l. The median tolerance limit was then examined at doses established from the preliminary screen.

(301)

Species: Artemia sp. (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: 127.3
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: In the study, the EC50 value is listed as 1630 mmol/m3.
Validity: 3 (not valid)
Significant methodological deficiencies
No analytical monitoring, concentrations calculated from solubility data.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Artemia were exposed to benzene at 19–21 degree C in test chambers sealed in glass vials with teflon–lined screw caps. Benzene was added as saturated solution in water (lower phase of water/benzene mixture in a seperatory funnel). The concentration of the stock solution was calculated from solubility data and it was assumed that loss of benzene due to volatilisation was neglible.
No analytical monitoring was performed.
No further details are reported in this study.
Test substance: Purity: at least 97% (no further specification)

(392)

Species: Asellus sp. (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 120
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: A static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113–128, 1982) system at 20°C was used. Asellus aquaticus (sowbugs) were tested in groups of at least five.
Test substance: >98% pure.

(393)

Species: Asellus sp. (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 120
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 Asellus aquaticus (Isopoda) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113–128, 1982) to various concentrations of benzene for 48 h at 19–21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: Daphnia cucullata (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 356 – 390
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Daphnids were exposed at 11 days of age in static freshwater.

(349) (394)

Species: Daphnia cucullata (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 356 – 390
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
Test followed the concept rules of the Dutch Standardization Institute (1978), but no experimental details are reported in this study (no. of animals, temperature, open or closed system).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Daphnids were exposed at 10–12 days of age in static freshwater. The actual concentrations of the test compound during the experiments were not measured and LC50 values were calculated from the quantity added at the start.

(395)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 18
Method: OECD Guide–line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The determination of the immobilization concentration for 50% of the organisms was performed in closed bottles.
Test substance: Supplied by Carlo Erba; purity unspecified.

(379)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 18
Method: OECD Guide–line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Guideline study without detailed documentation
Several experimental details not reported (no. of animals, measured and used benzene concentrations.)
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The determination of the immobilization concentration for 50% of the organisms was performed in closed bottles (150 ml), completely filled with test solution.
Test substance: purity: Carlo Erba GC standard.

(379)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 10
Method: OECD Guide–line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year: **GLP:** no data
Test substance: other TS
Remark: According to the authors, test procedure followed OECD guideline 202, part 1, but details not reported in the study.
After 24 h of exposure, the EC50 was also 10 mg/l.
Reliability: 2 (valid with restriction)
Guideline study without detailed documentation
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Purity: reagent grade

(396)

Species: Daphnia magna (Crustacea)
Exposure period: 1 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 6.3
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: According to the authors, results of this test correlate well with tests done in parallel following OECD–guideline criteria.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The test is based on the ability of the alive animals to enzymatically hydrolyse 4–methylumbelliferyl–beta–D–galactoside (MUF–G) with the formation of fluorescent 4–methylumbelliferon inside the animal.
Toxicity tests were run as follows:
five benzene concentrations plus control,
six 10 ml glass test tubes with five 12–48 h old Daphnia magna per treatment,
incubation for 1 h at 20 degree C,
addition of 0.1 ml of MUF–G stock solution (5 g/l), corresponding to 50 mg/l,
counting of fluorescent animals 15 min later under UV light.
Tests were considered valid if at least 90% of control animals showed fluorescence.
Test substance: Purity: Reagent grade

(396)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 250
Method: other
Year: 1975 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 200-310 mg/l. The no discernible effect concentration was <13 mg/l. A second 24-hour test gave an LC50 value of 1130 mg/l, calculated from an LC0 of 490 mg/l and an LC100 of 1400 mg/l (Bringmann G. & Kuhn R. Z. Wasser Abwasser Forsch. 10, 161-166, 1977).
Source: BP Chemicals Ltd. London
Test condition: The method of the US EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009), 1975 was used. Groups of 15 daphnids were exposed to benzene in reconstituted well water having a total hardness of 173 mg/l as CaCO3, pH of 7.4-9.4, dissolved oxygen concentration of 6.5-9.1 mg/l and a temperature of 22°C. Dilution water was used for the negative control.
Test substance: >= 80% pure.

(397)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 250
Method: other
Year: 1975 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 200-310 mg/l. The no discernible effect concentration was <13 mg/l.
A second 24-hour test gave an LC50 value of 1130 mg/l, calculated from an LC0 of 490 mg/l and an LC100 of 1400 mg/l (Bringmann G. & Kuhn R. Z. Wasser Abwasser Forsch. 10, 161-166, 1977).
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the US EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009), 1975 was used. Groups of 15 daphnids were exposed to benzene in reconstituted well water having a total hardness of 173 mg/l as CaCO3, pH of 7.4-9.4, dissolved oxygen concentration of 6.5-9.1 mg/l and a temperature of 22 degree C. Dilution water was used for the negative control.
Test substance: >= 80% pure.

(397)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: 31.2
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: 95% confidence interval: 22.2-43.3 mg/l.
In the study, values are presented as mmol/m3.
Validity: 3 (not valid)
Significant methodological deficiencies
No analytical monitoring, applied concentrations of test substance calculated from solubility data.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Daphnia (4-6 days old) were exposed to benzene in distilled water as follows:
10 animals in 24 ml glass vials with Teflon-lined screw caps completely filled,
5 concentrations of benzene and control,
temperature: 21-25 degree C,
pH ca. 7 at start and ca. 6 at end of exposure
dissolved oxygen ca. 9 mg/l at start and 5 mg/l at end of exposure.
Benzene was added as saturated stock solution in water (lower phase of water/benzene mixture in a separatory funnel). The concentration of the stock solution was calculated from solubility data and it was assumed that loss of benzene due to volatilisation was neglible.
No analytical monitoring was performed.
Test substance: Purity: at least 97% (no further specification)

(398)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 200
Method: other
Year: 1975 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 140-320 mg/l. The no discernible effect concentration was <13 mg/l. A second 48-hour study reported an LC50 of 682 mg/l (confidence limits 608-752 mg/l) (Eastmond D.A. et al. Archs envir. Contam. Toxicol. 13, 105-111, 1984 cited in AQUIRE, 1993).
Source: BP Chemicals Ltd. London
Test condition: The method of the US EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009), 1975 was followed.
Groups of 15 daphnids were exposed to benzene in reconstituted well water having a total hardness of 173 mg/l as CaCO3, pH 7.4-9.4, dissolved oxygen concentration of 6.5-9.1 mg/l and a temperature of 22°C. Dilution water was used for the negative control.
Test substance: >= 80% pure.

(349) (397)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 200
Method: other
Year: 1975 **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 140–320 mg/l. The no discernible effect concentration was <13 mg/l. A second 48–hour study reported an LC50 of 682 mg/l (confidence limits 608–752 mg/l) (Eastmond D.A. et al. Archs Envir. Contam. Toxicol. 13, 105–111, 1984 cited in AQUIRE, 1993).
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The method of the US EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA–660/3–75–009), 1975 was followed. Groups of 15 daphnids were exposed to benzene in reconstituted well water having a total hardness of 173 mg/l as CaCO3, pH 7.4–9.4, dissolved oxygen concentration of 6.5–9.1 mg/l and a temperature of 22 degree C. Dilution water was used for the negative control.
Test substance: >= 80% pure.

(349) (399)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 356 – 620
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Daphnia magna were exposed at <1 day of age in a freshwater, static system.

(349) (394)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 356 – 620
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
According to the authors, test followed the concept rules of the Dutch Standardization Institute (1978), but no experimental details are reported in this study (no. of animals, temperature, open or closed system).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Daphnids were exposed at <1 day of age in static freshwater. The actual concentrations of the test compound during the experiments were not measured and LC50 values were calculated from the quantity added at the start.

(395)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 682
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: 95% confidence limit: 608–752 mg/l.
Reliability: 3 (not valid)
No. of animals not exactly reported, no ECO, EC100 values, no analytical monitoring, incubation conditions not precisely described (open or closed system).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 5 or 10 animals (no further information) were treated in Petri dishes (no information, if open or closed) with various concentrations of benzene in filtered, autoclaved river water (alalinity as CaCO3: 92 mg/l, hardness as CaCO3: 134, pH 7.4–7.8, oxygen: 3.8–4.0, temperature: 17.5–20.5 degree C). Acetone was used as carrier (0.5 ml/l) and Triton–X100 as a surfactant (0.5 g/l) in all tests including controls.
Test substance: Benzene from a commercial supplier was used without further purification (No additional data given).

(400)

Species: Daphnia pulex (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 265 – 345
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Daphnids were exposed when <1 day–old in freshwater under static conditions.

(349) (394)

Species: Daphnia pulex (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 265 – 345
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
According to the authors, test followed the concept rules of the Dutch Standardization Institute (1978), but no experimental details are reported in this study (no. of animals, temperature, open or closed system).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Daphnids were exposed at <1 day of age in static freshwater. The actual concentrations of the test compound during the experiments were not measured and LC50 values were calculated from the quantity added at the start.

(395)

Species: Daphnia pulex (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 15
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Daphnids were exposed in lake water at 15°C and pH 7.5. Benzene was tested at concentrations ranging from 0 to 20 mg/l. Groups of 20 daphnids were exposed.

(401)

Species: Daphnia pulex (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 15
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
Purity of test substance not reported, no EC0, no EC100 values given.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 20 daphnids per concentration were exposed in filtered lake water at 15 degree C (13–17 degree C) and pH 7.5. Sealed 250 ml Erlenmeyer flasks with sufficient head space to accomodate in excess the oxygen demand were used. Benzene was tested at concentrations ranging from 0 to 20 mg/l. Benzene concentrations were determined by UV–spectrofluorometry.
Test substance: purity: no data

(401)

Species: Daphnia pulex (Crustacea)
Exposure period: 1 day
Unit: mg/l **Analytical monitoring:**
EC : = 50
Method:
Year: **GLP:** no data
Test substance: no data
Remark: In a field study in static freshwater, with a dissolved oxygen concentration of 1.1–1.6 mg/l, pH of 6.2, a temperature of 12°C and a hardness of 2.86 mmol/l, the lowest concentration tested, 50 mg/l, was lethal to Daphnia pulex exposed for <1 day. Benzene loss and oxygen fluctuations were reported.
Source: BP Chemicals Ltd. London

(349) (402)

Species: Daphnia pulex (Crustacea)
Exposure period: 1 day
Unit: mg/l **Analytical monitoring:**
EC : = 50
Method:
Year: **GLP:** no data
Test substance: no data
Remark: In a field study in static freshwater, with a dissolved oxygen concentration of 1.1–1.6 mg/l, pH of 6.2, a temperature of 12 degree C and a hardness of 2.86 mmol/l, the lower concentration tested, 50 mg/l, was lethal to Daphnia pulex exposed for <1 day. Benzene loss and oxygen fluctuations were reported.
Reliability: 3 (not valid)
Significant methodological deficiencies
Near 100% lethality at lower dose tested, no concentration–dependent effects on lethality investigated.
Source: Deutsche Shell Chemie GmbH Eschborn

(403)

Species: Gammarus pulex (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 42
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: A static system using Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113–128, 1982) at 20°C was used. Groups of at least five scud were tested.
Test substance: >98% pure.

(393)

Species: Gammarus pulex (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 42
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 Gammarus pulex (Amphipoda) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113–128, 1982) to various concentrations of benzene for 48 h at 19–21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: Nitocra spinipes (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 82
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: No further details were available in the citing source.

(331) (404)

Species: Nitocra spinipes (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 82
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 95% confidence limit: 73–91 mg/l.
At increased salinity (25 g/l), the EC50 value was 111.5 mg/l (conf. lim. 61–157 mg/l).
Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No monitoring data reported, no EC0, no EC100 values reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: According to the author, the bioassay followed methods outlined in Standard Methods (APHA, 1965).
10–20 Nitocra spinipes (Harpacticoidea, Copepodae, lab culture from wild captures), preadapted without food to test

conditions for 12 h, were exposed to benzene under the following conditions:
150 ml fingerling bowls, salinity: 15 g/l, oxygen concentration: at least >=5.8 ppm at end of experiment, temperature: 20 degree C.
Effects of benzene on animals were recorded by microscopical observation.
Benzene concentrations were measured by UV-spectrophotometry (at 250 nm).

Test substance: Purity: ACS standard, redistilled.

(404)

Species: Palaemonetes pugio (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 33.5
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: At different temperatures and salinities, the following results were obtained:

Stage	Temp. (degree C)	Salin. (g/l)	EC50 (mg/l)	95% confidence limit (mg/l)
Adult				
	20	15	38.0	36.6-40.2
	20	25	33.5	31.0-36.0
	10	15	40.2	33.5-48.2
	10	25	40.8	38.6-43.3
Larval				
	20	15	90.8	61.1-113.5
	20	25	74.4	66.8-81.8

Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
No monitoring data, no EC0, no EC100 values reported.
According to the author, the bioassay followed methods outlined in Standard Methods (APHA, 1965).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Bioassays were conducted with adult and larval brine shrimp, respectively.
13-25 adult shrimp (wild captures acclimated at least 10 days) were exposed in 10 l seawater to benzene at different temperatures and salinities (see below). Oxygen content was at least >/= 5.8 ppm at the end of the experiment.
Approx. 30 larval shrimp (capture bred) were exposed in 350 ml glass bowls.
Benzene concentrations were measured by UV-spectrophotometry (at 250 nm).
Effects of benzene on animals were recorded by microscopical observation.

Test substance: Purity: ACS standard, redistilled.

(405)

Species: Palaemonetes pugio (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 43.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Another study investigated the adult and larval stages of Palaemonetes pugio and recorded 24-hour LC50 values of 31 and 74 mg/l respectively (Potera G.T. The Effects of Benzene, Toluene and Ethylbenzene on Several Important Members of the Estuarine Ecosystem. PhD Thesis, Leigh University, 1975 (cited in BUA, 1988).
When the same test was conducted over 48 or 96 hours, LC50 values of 35 and 27 mg/l were obtained respectively.
Source: BP Chemicals Ltd. London
Test condition: The shrimp were exposed under static conditions in saltwater which had a pH of 8.1, dissolved oxygen concentration of >5 mg/l, salinity of 15 ppt and a temperature of 21°C.
(349) (331) (406)

Species: Palaemonetes pugio (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 43.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
When the same test was conducted over 48 or 96 hours, LC50 values of 35 and 27 mg/l were obtained respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The shrimp were exposed under static conditions in saltwater which had a pH of 8.1, dissolved oxygen concentration of >5 mg/l, salinity of 15 g/l and a temperature of 21 degree C.
(349) (331) (406)

Species: Palaemonetes pugio (Crustacea)
Exposure period: 96 hour(s)
Unit: µg/l **Analytical monitoring:** no data
LC50 : = 27
Method: other: see reference
Year: 1983 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID
(381)

Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 120
EC50: = 230
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of ten Lymnaea stagnalis were exposed at 3-4 weeks of age in Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982) at 20°C.
Test substance: >98% pure.

(393) (388)

Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 120
EC50: = 230
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Several parameters not reported (No EC100 values, no confidence limits), no analytical monitoring.
The study was conducted to compare the sensitivity of several aquatic species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten Lymnaea stagnalis were exposed at 3-4 weeks of age in 1 l of Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982) at 20 degree C.
Test substance: Purity: highest grade available (at least >98% pure, no further information given).

(388)

Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 377
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: ED50 value given refers to median ecological mortality (100 - Perc. Ecological Survival, which is the no. of surviving larvae in the culture minus the no. of abnormal larvae in culture).
When tested at the embryo stage, an LC50 of 920 mg/l was found, following 96 hours exposure (EPA. Ambient Water Quality Criteria for Benzene. NTIS PB81-117293. US Dept of Commerce, US Environmental Protection Agency, Washington DC, 1980 cited in BUA, 1988).
Reliabilty: 3 (not valid)
Documentation insufficient for assessment
No information on several parameters (pH, monitoring of test substance, oxygen content).

Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Crassostrea gigas (Pacific oyster) fertilised eggs (ca. 30000/test container) were exposed in static saltwater at 20-21.5 degree C and at a salinity of 25.3-30.8 g/l.
(331) (407)

Species: other aquatic worm
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 24
EC50: = 34
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: A static Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982) system at 17-20°C was used. Budless Hydra oligactis were exposed in groups of ten.
Test substance: >98% pure.
(393) (388)

Species: other aquatic worm
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 74
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of at least five Dugesia lugubris (turbellarian, flatworm) were exposed in static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113-128, 1982) at 20°C.
Test substance: >98% pure.
(393)

Species: other aquatic worm
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 74
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 Dugesia cf. lugubris (triclad flatworm) were exposed in closed vessels with Dutch Standard Water (Canton

J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other aquatic worm
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 320
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of at least five Tubificidae (Oligochaete family) were exposed under static Dutch Standard Water (Canton J.H.& Slooff W. Ecotoxic. envir. Saf. 6, 113-128, 1982) conditions at 20°C.
Test substance: >98% pure.

(393)

Species: other aquatic worm
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 320
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The exposure system consisted of static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113-128, 1982) at 20°C. Groups of at least five Erpobdella octoculata (leeches) were exposed.
Test substance: >98% pure.

(393)

Species: other aquatic worm
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 320
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals were obtained from a commercial source.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 Tubificidae were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of

benzene for 48 h at 19-21 degree C.
Test substance: purest grade commercially available (>98% pure).
(393)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 5.61
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Similar tests were conducted for 48 and 72 hours and gave LC50 values of 4.41 and 3.29 g/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Scylla serrata (crabs) were exposed in static saltwater during the breeding season.
(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 5.61
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.
Similar tests were conducted for 48 and 72 hours and gave LC50 values of 4.41 and 3.29 g/l respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Scylla serrata (crabs) were exposed in static saltwater during the breeding season.
(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 7.14
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 5.79, 4.62 and 3.67 g/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Immature Scylla serrata (crabs) were exposed in static saltwater during the summer season.
(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 7.14
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Similar tests were conducted for 48 and 72 hours and gave LC50 values of 5.79 and 4.62 g/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Scylla serrata (crabs) were exposed in static saltwater during the non–breeding season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 7.14
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
It is noted that the EC50–values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.
Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 5.79, 4.62 and 3.67 g/l respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Immature Scylla serrata (crabs) were exposed in static saltwater during the summer season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 7.14
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
It is noted that the EC50–values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.
Similar tests were conducted for 48 and 72 hours and gave LC50 values of 5.79 and 4.62 g/l respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Scylla serrata (crabs) were exposed in static saltwater during the non–breeding season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 9.11
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 8.04, 7.33 and 6.08 g/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Immature Scylla serrata (crabs) were exposed in static saltwater during the monsoon season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 9.11
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.
Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 8.04, 7.33 and 6.08 g/l respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Immature Scylla serrata (crabs) were exposed in static saltwater during the monsoon season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 11.29
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 10, 8.93 and 7.63 g/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Immature Scylla serrata (crabs) were exposed in static saltwater during the winter season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:** no data
EC50: = 11.29
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.
Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 10, 8.93 and 7.63 g/l respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Immature Scylla serrata (crabs) were exposed in static saltwater during the winter season.

(349) (408)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 19.3
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: LC50 confidence limits = 17.6-21.1 mg/l. When conducted over 48 hours, the LC50 was found to be 17.6 mg/l (confidence limits 16.7-19.3 mg/l).
Source: BP Chemicals Ltd. London
Test condition: Mature Crangon franciscorum (bay shrimp) were exposed in static, saltwater at 16°C. The salinity was 25 ppt.
Test substance: >99% pure.

(349) (409)

Species: other aquatic crustacea
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 22
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: LC50 95% confidence limits = 20-24 mg/l. When conducted over 96 hours, the LC50 was found to be 20 mg/l (confidence limit 19-22 mg/l).
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 10 mature Crago franciscorum (bay shrimp) were exposed in saltwater at 16 degree C. The salinity was 25 g/l.
Test substance: >99% pure.

(410)

Species: other aquatic crustacea
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 347
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A 96-hour LC50 of 108 mg/l was also reported.
Source: BP Chemicals Ltd. London
Test condition: First instar zoeal crabs (Cancer magister) were exposed in static saltwater at 10.5-14.2°C and a salinity of 29-34 ppt.
(349) (411)

Species: other aquatic crustacea
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 347
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Full details of the method not reported (open/closed system, oxygen content, pH).
The concentration of benzene as measured by UV-spectrophotometry (at 255 nm) was found to decline by about 25-50% within the first 24 h of exposure.
A 96-hour LC50 of 108 mg/l was also reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 50 first instar zoea larvae of the dungeness crab (Cancer magister) were exposed in static saltwater at 10.5-14.2 degree C and a salinity of 29-34 g/l.
(412)

Species: other aquatic crustacea
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 377
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: When tested at the embryo stage, an LC50 of 920 mg/l was found, following 96 hours exposure (EPA. Ambient Water Quality Criteria for Benzene. NTIS PB81-117293. US Dept of Commerce, US Environmental Protection Agency, Washington DC, 1980 cited in BUA, 1988).
Source: BP Chemicals Ltd. London
Test condition: Crassostrea gigas (Pacific oyster) eggs were exposed in static saltwater at 20-21.5°C and at a salinity of 25.3-30.8 ppt.
(349) (331) (413)

Species: other aquatic crustacea
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 710
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: A static freshwater system at 28°C was used to test Diaptomus forbesi (calanoid copepod).
(349) (414)

Species: other aquatic crustacea
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 710
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: A static freshwater system at 28 degree C was used to test Diaptomus forbesi (calanoid copepod).
(349) (414)

Species: other
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 59
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Aedes aegypti (mosquito) larvae were exposed in a static system at 24–26°C.
(415) (416) (417)

Species: other
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: 12.9
EC50: = 59
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 25 fourth–instar larvae of Aedes aegypti (mosquito) (Rockefeller strain) were exposed in a static system at 24–26 degree C to variousconcentrations of benzene in 200 ml solution in 300 ml glass bowls (4 bowls/trial, at least 3 trials).
Test substance: purity: no data

(418)

Species: other
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 1000
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Brachionus calyciflorus (rotifer) neonates were exposed at 25°C in moderately hard, synthetic freshwater.

(419)

Species: other
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 1000
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Brachionus calyciflorus (rotifer) neonates were exposed at 25 degree C in moderately hard, synthetic freshwater.

(419)

Species: other
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 1000
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: In the same study, marine rotifers (Brachyonus plicatilis) were also used and the LC50 value was also >1000 mg/l.
Reliability: 3 (not valid)
Significant methodological deficiencies
No analytical monitoring (possible adsorption of benzene to polystyrene culture wells), no information if culture plates were sealed to reduce evaporation of test compound.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Animals were treated with benzene (plus control) in sterile, 24–well polystyrene tissue culture plates as follows:
5 concentrations of benzene (plus control), added from stock solution in ethanol,
10 animals/ml incubate, 3 replicates/ conc.
water: standard synthetic freshwater (EPA), temperature: 25 degree C, pH 7.4–7.8, hardness 80–100 mg/l as CaCO3, alkalinity 60–70 mg/l,
The number of dead animals was counted after 24 h.
Test substance: Purity: 86%.

(420)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The life stage of Ischnura elegans (dragonfly) (tested in groups of at least five) at the time of study was not given. Exposure was at 20°C in static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113–128, 1982).
Test substance: >98% pure.

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Within the 12 species tested, Ischnura elegans was most sensitive.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 larvae of Ischnura elegans (blue–tailed damselfly) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113–128, 1982) to various concentrations of benzene for 48 h at 19–21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 34
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Exposure of Cloeon dipterum (mayfly) was conducted under static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113–128, 1982) conditions at 20°C.
The age of the mayfly (tested in groups of at least five) was not given.
Test substance: >98% pure.

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 34
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
The study was conducted to compare the sensitivity of several invertebrates from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 larvae of Cloeon dipterum (mayfly) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113–128, 1982) to various concentrations of benzene for 48 h at 19–21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 24
EC50: = 34
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten Hydra oligactis were exposed at 17 degree C in 50 ml Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891–907, 1982).
The actual concentrations of benzene during the experiment were not measured, and the values presented are based on the quantity added at the beginning of the experiment.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(388)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 48
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Exposure was in static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113-128, 1982) at 20°C. No indication of age of the Corixa punctatae (water boatman), at the time of the study, was given. Insects were tested in groups of at least five.
Test substance: >98% pure.

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 48
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 Corixa punctata (water boatman) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 40
EC50: = 71
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of ten third instar Culex pipiens (mosquito) larvae were exposed at 26°C in Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982).
Test substance: >98% pure.

(388)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 40
EC50: = 71
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Several parameters not reported (No EC100 values, no confidence limits), no analytical monitoring.
The study was conducted to compare the sensitivity of several aquatic species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten third instar Culex pipiens (mosquito) larvae were exposed at 26 degree C in 50 ml Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891–907, 1982).
The actual concentrations of benzene during the experiment were not measured, and the values presented are based on the quantitiy added at the beginning of the experiment.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(388)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 100
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of at least five Chironomus thummi (midge) (age not specified) were exposed to benzene in static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113–128, 1982) at 20°C.
Test substance: >98% pure.

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 100
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were obtained from commercial source.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 larvae of Chironomus gr. thummi were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 130
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The age of Nemoura cinerea (stonefly) (tested in groups of at least five) at the time of the study was not given.
Exposure was in static Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. envir. Saf. 6, 113-128, 1982) at 20°C.
Test substance: >98% pure.

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 130
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 larvae of Nemoura cinerea (stonefly) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 170
EC50: = 200
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of ten third instar Aedes aegypti (mosquito) larvae were exposed at 26°C in Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982).
Test substance: >98% pure.

(388)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 170
EC50: = 200
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Several parameters not reported (No EC100 values, no confidence limits), no analytical monitoring.
The study was conducted to compare the sensitivity of several species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten third instar Aedes aegypti (mosquito) larvae were exposed at 26 degree C in 50 ml Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891–907, 1982). The actual concentrations of benzene during the experiment were not measured, and the values presented are based on the quantity added at the beginning of the experiment.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(388)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 320
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restrictions)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No EC0, EC100 values, no confidence limits reported.
The animals (age not specified) were collected from the river Rhine.
In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: At least 5 Erpobdella octoculata were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113–128, 1982) to various concentrations of benzene for 48 h at 19–21 degree C.
Test substance: purest grade commercially available (>98% pure).

(393)

Species: other
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC : .078
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: In the same study, sea urchin sperm cells were treated for 5-90 min with 10 uM benzene (0.78 mg/l) prior to insemination of untreated eggs and subsequent development of embryo to the pluteus larval stage.

Results:
No spermiotoxicity was observed.
The no. of developmental defects in larvae was increased:
Control: 15.9+/-3%, 10 uM benzene: 65.9+/-15.3%.
Reliability: 3 (not valid)
Significant methodological deficiencies
Only one concentration used.
The following effects of benzene were noted:
significant increase in developmental defects:
control: 11.8+/-6.7% (S.D.), 1ug/l benzene: 29.5+/-14.3%, p<0.05

elevated metaphase/anaphase ratio:
control: 2.2+/-0.8, benzene: 3.5+/-0.5, p<0.001

No effect on the no. of mitoses/embryo, % of interphase embryos, % total mitotic aberrations and % anaphase aberrations was noted.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Mediterranean sea urchin (Paracentrotus lividus) embryos were incubated for 48 h, from the zygote to the pluteus larval stage, with 1 um Benzene (0.078 mg/l) in natural filtered seawater (salinity 37.6-37.8 g/l, pH 8.2-8.4, temperature 20 degree C).
No further details of the methods are reported in this study.
Test substance: over 99% pure (Merck, FRG).

(421)

Species: other: Crangon franciscorum
Exposure period: 96 hour(s)
Unit: µg/l **Analytical monitoring:** no data
LC50 : = 20
Method: other: see reference
Year: 1983 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(381)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Ankistrodesmus falcatus (Algae)
Endpoint: biomass
Exposure period: 4 hour(s)
Unit: **Analytical monitoring:** no data
EC50: = 310
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The EC50 was measured in mmoles and was 3.97 mmoles (310 mg).
Source: BP Chemicals Ltd. London
Test condition: Algae were exposed in the log phase of growth, under static conditions at 20°C. The effect noted was on photosynthesis which may indicated a biomass effect but no details were given.

(422) (423)

Species: Ankistrodesmus falcatus (Algae)
Endpoint: other
Exposure period: 4 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 310
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Purity of test substance not reported, no measurement of actual concentrations.
The EC50 was reported in mmoles/l (3.97 mmoles/ corresp. to 310 mg/l).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Algae (400000–700000 cells) were exposed in the log phase of growth, under static conditions at 20 degree C for 4 h under illumination with 5000 Lux. Test were carried in 15 ml medium in capped 25 ml Erlenmeyer flasks.
Benzene was added from stock solution in acetone. Controls were incubated with acetone only and additional controls were incubated in the dark.
Inhibition of uptake of 14C–carbonate was taken as a measure for the toxic effect of benzene.
Test substance: Purity of test substance and of 14C–labelled carbonate not reported.

(424)

Species: Chlamydomonas sp. (Algae)
Endpoint: biomass
Exposure period: 3 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 461
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Exponential phase Chlamydomonas angulasa (green algae) cells (5 x 10+4 cells/ml) were exposed under static conditions at 19°C and pH 6.5. Radiolabelled carbon dioxide uptake was measured as a sign of effects on photosynthesis. This may indicate a biomass effect but no details were given.

(425)

Species: Chlamydomonas sp. (Algae)
Endpoint: other
Exposure period: 3 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 461
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Exponential phase Chlamydomonas angulosa (green algae, 680 Indiana collection) cells (5 x 10+4 cells/ml), grown axenic in Bolds Basal Medium at a 12 h dark/12 h light cyclus, were exposed in glass stoppered flasks under static conditions at 19 degree C and pH 6.5. A dark set of controls was also run. Benzene was added from saturated stock solutions in medium. Radiolabelled carbon dioxide uptake (1.25 uCi/100 ml) from NaHCO3 (purity not specified) was measured as a sign of effects on photosynthesis. This may indicate a biomass effect but no details were given.
Test substance: No information on the purity of the test substance was given.

(425)

Species: Chlorella vulgaris (Algae)
Endpoint: biomass
Exposure period: 3 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 312.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A 35% inhibition of photosynthesis was noted at 55 mg/l (Potera G.T. The Effects of Benzene, Toluene and Ethylbenzene on Several Important Members of the Estuarine Ecosystem. PhD Thesis, Leigh University, 1975 cited in BUA, 1988).
Source: BP Chemicals Ltd. London
Test condition: Exponential phase cells (20 x 10+4 cells/ml) were exposed under static conditions at 19°C and pH 6.5. Radiolabelled carbon dioxide uptake was measured as a sign of effects on photosynthesis. This may indicate a biomass effect but no details were given.

(426) (425)

Species: Chlorella vulgaris (Algae)
Endpoint: biomass
Exposure period: 3 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 312.5
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Short-term incubation, purity of benzene not specified, no determination of actual benzene concentration in test system.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Exponential phase Chlorella vulgaris (green algae, 260 Indiana collection) cells (20 x 10+4 cells/ml), grown axenic in Bolds Basal Medium at a 12 h dark/12 h light cyclus, were exposed in glass stoppered flasks under static conditions at 19 degree C and pH 6.5. A dark set of controls was also run. Benzene was added from saturated stock solution in medium.
Radiolabelled carbon dioxide uptake (0.5 uCi/100 ml) from NaHCO3 (purity not specified) was measured as a sign of effects on photosynthesis. This may indicate a biomass effect but no details were given.
Test substance: No information on the purity of the test substance was given.

(425)

Species: Chlorella vulgaris (Algae)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 525
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A second study exposed 3-10 x 10+4 cells/ml under static conditions and pH 6.5 to benzene at 20°C for 24 hours. A decrease in growth was noted at 25 mg/l (Kauss P. et al. Proc. Joint Conf. Prevent. Control Oil Spills. p.703-714. Washington DC, 1973 (cited in AQUIRE, 1993).
Source: BP Chemicals Ltd. London
Test condition: Algae were exposed in static freshwater at 20°C.

(422) (427)

Species: Chlorella vulgaris (Algae)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 525
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A second study exposed 30000-100000 cells/ml under static conditions and pH 6.5 to benzene at 20 degree C for 24 hours. A decrease in growth was noted at 25 mg/l (Kauss P. et al. Proc. Joint Conf. Prevent. Control Oil Spills. p.703-714. Washington DC, 1973 (cited in AQUIRE, 1993).
In the study, the effects of benzene on growth of the unicellular algae Chlorella vulgaris (culture no. 29, Indiana University Culture Collection of Algae) were studied.
Reliability: 3 (not valid)
Significant methodological deficiencies
The concentration of benzene in the culture medium at 20 degree C was calculated from reported solubility data of a saturated benzene solution in pure water at 25 degree C. Actual benzene concentrations were not measured.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 30000-100000 cells were incubated with various concentrations of benzene in 50 ml Bolds Basal Medium at 18-22 degree C in cotton-plugged 125 ml Erlenmeyer flasks under a 12 h light/12 h dark cycle. Growth was recorded by determination of cell number.
Effective concentrations refer to nominal concentrations at the beginning of the experiment.
Results: 525 mg/l benzene led to a 50% reduction in cell number after one day. At 25 mg/l to 500 mg/l, a lag-phase in growth proportional to the initial nominal benzene concentration was observed. Subsequently, growth of benzene-treated and control cultures did not differ significantly. This effect could be related to rapid volatilisation of benzene from the cultures, but no measurement of actual concentrations was performed.
Test substance: Purity not specified.

(428)

Species: Dunaliella tertiolecta (Algae)
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
EC : = 100
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Growth was inhibited when algae were exposed at 18°C in static saltwater for 4-72 hours. No further details were available.
Source: BP Chemicals Ltd. London
Test substance: Spectro-grade benzene was tested.

(429) (430)

Species: Dunaliella tertiolecta (Algae)
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** yes
EC0: > 100
EC :
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Growth was not inhibited up to 100 mg/l, the highest concentration tested.
Instead, growth was stimulated to ca. 150% of control at 1 mg/l, the lowest concentration tested.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Axenic cultures of Dunaliella (Clone, Dun, WHOI) were incubated under the following conditions:
12h dark/ 12h white light (ca. 4000 uW/cm2 at 380-700 nm)
125 ml flasks with rubber stopper, 3 flasks/concentration.
50 ml enriched filtered seawater
temperature: 18 degree C.
Cell density not specified, but such that growth was not limited due to CO2-shortage.
Benzene concentration was monitored by gas chromatography.
Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.
Test substance: Spectro-grade benzene was tested.

(431)

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint: biomass
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
EC0: > 1400
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No detectable effect on concentration and total biomass was noted.
Source: BP Chemicals Ltd. London

(432)

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint: biomass
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
EC0: > 1400
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.
No detectable effect on concentration and total biomass was noted.
Reliability: 3 (not valid)
3f-c
No data regarding actual concentrations during exposure period presented, possible evaporation of test substance during exposure period cannot be ruled out.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 10 ml test solution (algae in defined medium with appropriate concentration of test substance) is incubated at 27 degree C in capped Kapsenberg culture vials for 8 days under static conditions.

(433) (434)

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
Toxicity threshold :
> 1400
Method: other: see reference
Year: 1983 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(381)

Species: Phaeodactylum tricornutum (Algae)
Endpoint: other
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LOEC: = 50
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: After 24 h of exposure, 50 mg/l, the lowest concentration tested, reduced net photosynthesis to about 80% of controls. At 100, 200, and 300 mg/l, rates were reduced to 68%, 26% and 3% of control, respectively.
After 2 h of treatment, 50 mg/l had no effect, but 100 mg/l reduced photosynthesis to 83% of control. At 200, 300, and 400 mg/l,photosynthesis was reduced to 61%, 37%, and 0% of control, respectively.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
No analytical monitoring, purity of test substance not reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The marine diatom algae was exposed to benzene in glass stoppered bottles as follows:
salinity of growth medium: ca. 30g/l
pH: 7.5
temperature: 15 degree C
illumination: 7W/m2
cell density: 2-3 million cells/ ml
Benzene was added as stock solution in ethanol (50 ul stock sol./ 20 ml culture), control received ethanol.
Oxygen exchange was measured with an oxygen electrode in a closed chamber.

(435)

Species: Scenedesmus quadricauda (Algae)
Endpoint: growth rate
Exposure period: 7 day
Unit: mg/l **Analytical monitoring:** no data
EC0: > 1400
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The toxicity threshold for the cell multiplication inhibition test was measured using a method developed by Bringmann G.

(436) (437)

Species: Scenedesmus quadricauda (Algae)
Endpoint: growth rate
Exposure period: 7 day
Unit: mg/l **Analytical monitoring:** no data
EC0: > 1400
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.
No detectable effect on concentration and total biomass was noted.
Reliability: 3 (not valid)
Unsuitable test system
No data regarding actual concentrations during exposure period presented, possible evaporation of test substance during exposure period cannot be ruled out.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 10 ml test solution (algae in defined medium with appropriate concentration of test substance) is incubated at 27 degree C in capped Kapsenberg culture vials for 7 days under static conditions.

(434) (438)

Species: Scenedesmus sp. (Algae)
Endpoint: growth rate
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 1360
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Scenedesmus abundans (green algae) (10 x 10+4 cells/ml) were exposed in static freshwater at 22°C.
Test substance: 99.7% pure

(429) (439)

Species: Scenedesmus sp. (Algae)
Endpoint: growth rate
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC10: > 1360
EC50: > 1360
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 3 (not valid)
Unsuitable test system
The test was performed in compliance with the test guideline of the Federal Environmental Agency, FRG. However, according to the authors, the method is not suitable for testing volatile substances such as benzene.
The test was performed in accordance with the test guideline of the Federal Environmental Agency (Umweltbundesamt), FRG (Algal Growth Inhibition Test CHODAT EC10, EC50 96h, Berlin, May 1982).
According to the authors, this "test is not suitable for volatile substances such as benzene...".
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Scenedesmus subspicatus (green algae) (ca. 10+4 cells/ml) were exposed to benzene under static conditions (300 ml Erlenmeyer flasks with Kapsenberg caps) in nutrient medium at 20-24 degree C for 96 h. Cells were continuously illuminated with 120 uE/m2 x sec. Cell growth was determined by photometric determination of cell density.
Test substance: 99.7% pure.

(440)

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 4 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC : = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: At the concentrations tested, 14C fixation was found to be decreased by 5, 16 and 95% respectively when compared with the control values. This same investigator also reported an EC20 of 144 mg/l when tested under these same conditions (Giddings J.M. Four-hour Algal Bioassays for Assessing the Toxicity of Coal-Derived Materials. Govt Report Announce. Index Issue 18. US NTIS Conf.-800223-2, 1980 cited in AQUIRE, 1993).
Source: BP Chemicals Ltd. London
Test condition: Benzene was tested at 10, 100 and 1000 mg/l.
Rapidly-growing culture (1.25 x 10+7 cells/ml) were added to the benzene solutions. Radiolabelled sodium hydrogen carbonate (14C) was added after 2 hours incubation at 24°C and after a further 2 hours, the cells were arrested by the addition of formalin. Photosynthesis was determined by measurement of fixed 14C.
Test substance: Reagent grade benzene was used.

(429) (441)

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 4 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC : = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: At the concentrations tested, 14C fixation was found to be decreased by 5, 16 and 95% respectively when compared with the control values. This same investigator also reported an EC20 of 144 mg/l when tested under these same conditions (Giddings J.M. Four-hour Algal Bioassays for Assessing the Toxicity of Coal-Derived Materials. Govt Report Announce. Index Issue 18. US NTIS Conf.-800223-2, 1980 cited in AQUIRE, 1993).
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Benzene was tested at 10, 100 and 1000 mg/l.
Rapidly-growing culture (1.25 x 10+7 cells/ml) were added to the benzene solutions. Radiolabelled sodium hydrogen carbonate (14C) was added after 2 hours incubation at 24 degree C and after a further 2 hours, the cells were arrested by the addition of formalin. Photosynthesis was determined by measurement of fixed 14C.
Test substance: Reagent grade benzene was used.

(429) (441)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 29
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The OECD Test Guide-line was modified to allow for the testing of volatile compounds. The concentration causing 50% growth inhibition was measured.
Test substance: Supplied by Carlo Erba; purity not specified.

(442)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 29
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restiction)
Guideline study without detailed documentation
Several experimental details not reported (measured benzene concentrations, used concentrations).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The OECD Test Guideline was modified to allow for the testing of volatile compounds. The concentration causing 50% growth inhibition was measured.
Test substance: Purity: Carlo Erba GC standards.

(443)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
EC50: = 41
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: The bioassay was performed using Bristol medium with added sodium bicarbonate to supplement the carbon supply and allow algal growth under conditions of restricted gas exchange. The pH was adjusted to pH 7.0 and the medium was inoculated with 1 x 10+5 cells/ml in the exponential growth phase. Neat benzene was injected into the exposure vials through the valve. At the end of exposure, the cell number in each vial was determined. Four separate measurements of algal biomass were made after 4, 6 and 8 days. These were cell number, chlorophyll content, dry weight and absorbance and were compared to ascertain which parameter would be the most sensitive indicator of algal growth inhibition.
Test substance: Supplied by J.T. Baker, Phillipsburg, New Jersey, "photrex" grade.

(444)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
EC50: = 41
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Analysis of benzene in both liquid and headspace of incubation vials was performed by GC. Concentrations were determined in vials containing a hydrocarbon mixture with benzene over a period of 12 days. No significant changes in the concentrations were observed.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
No GLP, no EC0 values reported, temperature during incubation not reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The bioassay was performed using Bristol medium with added sodium bicarbonate (0.4%, w/v) to supplement the carbon supply and allow algal growth under conditions of restricted gas exchange.
The pH was adjusted to pH 7.0 and the medium was inoculated with 1 x 10+5 cells/ml in the exponential growth phase.
Bioassays were performed using 4 replicate hypo–vials (125 ml size containing 50 ml solution) to at least 5 concentrations of benzene. Neat benzene was injected into the exposure vials through the valve. Cultures were shaken at 100 rpm and exposed to benzene for 8 days under a 16h light:8h dark cycle (illumination 32 uEinstein/m2xsec). At the end of exposure, the cell number in each vial was determined by direct counting using a haemocytometer.
Additionally, chlorophyll content, dry weight and absorbance were also measured after 4, 6, and 8 days and compared to ascertain which parameter would be the most sensitive indicator of algal growth inhibition.
Test substance: Supplied by J.T. Baker, Phillipsburg, New Jersey, "photrex" grade.

(444)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 14 day
Unit: mg/l **Analytical monitoring:** no data
EC50: = 292
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Algae were exposed under static conditions.

(429) (445)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 14 day
Unit: mg/l **Analytical monitoring:** no data
EC10: = 155
EC50: = 292
EC90 : = 552
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
Details of the method not reported in this study, but in an earlier publication.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Algae were exposed for 14 days under static conditions as described in an earlier publication (Gaur J.P. & Kumar H.D. Environ. Pollut. Ser. A25, 77-85, 1981).

(446)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: = 600
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Log phase cells (5 x 10+4 cells/ml) were exposed in freshwater at 26°C and the effect on population growth was noted.
Test substance: >98% pure

(447)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 600
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
Several parameters not reported (open or closed system, no confidence limits), no analytical monitoring.
The study was conducted to compare the sensitivity of several aquatic species from different taxa.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Log phase cells were exposed (50000 cells/ml) in 150 ml freshwater at 26 degree C for 96 h and the effect on population growth was noted.
Test substance: Purity: purest grade available (at least >98% pure, no further information given).

(388)

Species: Skeletonema costatum (Algae)
Endpoint: biomass
Exposure period: 7 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 200
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: When exposed for 8 and 12 hours, the EC50 values for photosynthesis effects were 300 and 80 mg/l respectively.
Source: BP Chemicals Ltd. London
Test condition: Algae were exposed in saltwater and effects on photosynthesis noted.

(429) (448)

Species: Skeletonema costatum (Algae)
Endpoint: biomass
Exposure period: 7 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 200
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
When exposed for 8 and 12 hours, the EC50 values for photosynthesis effects were 300 and 80 mg/l respectively.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Algae were exposed in saltwater and effects on photosynthesis noted.

(449) (448)

Species: Skeletonema costatum (Algae)
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** yes
EC50: > 100
EC :
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Growth was not inhibited up to 10 mg/l and was slightly inhibited (ca. 20% inhibition) at 100 mg/l, the highest concentration tested.
In further experiments, 10 mg/l benzene caused a depression of growth (to 17% of control) for the first 160 h of culture, followed by a stimulation of growth afterwards. At 20 mg/l benzene, almost no growth was observed within 160 h.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Axenic cultures of the diatom Skeletonema costatum (Clone, Skelly, WHOI Culture collection) were incubated under the following conditions:
12h dark/ 12h white light (ca. 4000 uW/cm2 at 380-700 nm)

125 ml flasks with rubber stopper, 3 flasks/concentration.
50 ml enriched filtered seawater
temperature: 18 degree C.
Cell density not specified, but such that growth was not limited due to CO2-shortage.
Benzene concentration was monitored by gas chromatography.
Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.

Test substance: Spectro-grade benzene was tested.

(450) (431)

Species: Skeletonema costatum (Algae)
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
EC : = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Population growth was inhibited at 10 mg/l.
Source: BP Chemicals Ltd. London
Test condition: Algae were exposed in static saltwater at 18°C for 4-72 hours.
Test substance: Spectro-grade purity.

(429) (451)

Species: other algae
Endpoint: biomass
Exposure period: 2 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: < 350
EC :
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Photosynthetic oxygen production was inhibited to 67%, 36 %, and 5% of control by 175 mg/l, 350 mg/l, and 525 mg/l, respectively.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Acrosiphonia sonderi (green algae) were exposed in a respiratory chamber in saltwater at 5 degree C for 2 h, and the effect on photosynthesis, which may reflect a biomass effect, was noted by determination of oxygen development by means of a Clark electrode.
Benzene was added as a stock solution in ethanol. Control samples were not affected by addition of ethanol (50 ul/20 ml medium).
Test substance: Purity: Analytical grade

(452)

Species: other algae
Endpoint: biomass
Exposure period: 2 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC : 175 – 525
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Acrosiphonia sonderi (green algae)were exposed under static conditions in saltwater at 5°C and the effect on photosynthesis, which may reflect a biomass effect, was noted.

(429) (453)

Species: other algae
Endpoint: growth rate
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: = 439
Method: other
Year: 1980 **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The toxicity threshold for the cell multiplication inhibition test was measured in Chilomonas paramecium (cryptomonad) using a method developed by Bringmann G.

(454)

Species: other algae
Endpoint: growth rate
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: = 439
Method: other
Year: 1980 **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The toxicity threshold for the cell multiplication inhibition test was measured in Chilomonas paramecium (cryptomonad) using a method developed by Bringmann G.

(455)

Species: other algae
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: > 700
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The toxicity threshold for the cell multiplication inhibition test was measured in Entosiphon sulcatum (flagellate euglenoid) using a method developed by Bringmann G.

(456)

Species: other algae
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: > 700
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.
No detectable effect on concentration and total biomass was noted.
Reliability: 3 (not valid)
Unsuitable test system
No measurement of actual concentrations; no data about purity of test substance.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: 20 ml test solution (Entosiphon in defined medium with appropriate concentration of test substance) is incubated at pH 6.9 and 25 degree C in metal–cap stoppered 300 ml Erlenmeyer flasks for 72 h under static conditions.
The number of cells is determined by means of a cell counter.

(438)

Species: other algae
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** yes
EC50: > 50
EC : = 1
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Growth was inhibited to 60% of control at both 1 mg/l, the lowest concentration tested, and at 50 mg/l.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Axenic cultures of Amphidinium carterae (Clone, Amphi 137, WHOI) were incubated under the following conditions:
12h dark/ 12h white light (ca. 4000 uW/cm2 at 380-700 nm)
125 ml flasks with rubber stopper, 3 flasks/concentration.
50 ml enriched filtered seawater
temperature: 18 degree C.
Cell density not specified, but such that growth was not limited due to CO2-shortage.
Benzene concentration was monitored by gas chromatography.
Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.
Test substance: Spectro-grade benzene was tested.

(431)

Species: other algae
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
EC50: > 100
EC : > 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Growth was not inhibited up to 10 mg/l. At 100 mg/l, the highest concentration tested, benzene inhibited growth to about 70% of controls.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Axenic cultures of Cricosphaera carterae (Clone, 156, Plymouth) were incubated under the following conditions:
12h dark/ 12h white light (ca. 4000 uW/cm2 at 380-700 nm)
125 ml flasks with rubber stopper, 3 flasks/concentration.
50 ml enriched filtered seawater
temperature: 18 degree C.

Cell density not specified, but such that growth was not limited due to CO2-shortage.
Benzene concentration was monitored by gas chromatography. Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.

Test substance: Spectro-grade benzene was tested.

(431)

Species: other algae
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
EC : = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Inhibition of growth was noted.
Source: BP Chemicals Ltd. London
Test condition: Amphidinium carterae (dinoflagellate) exposed in static saltwater at 18°C for 4-72 hours. No further details were available.
Test substance: spectro-grade benzene was tested.

(429) (457)

Species: other algae
Endpoint: growth rate
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
EC : = 10
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Inhibition of growth was noted.
Source: BP Chemicals Ltd. London
Test condition: Cricosphaera carterae (coccolithophorid) were exposed in static saltwater at 18°C for 4-72 hours. No further details were available.
Test substance: Spectro-grade benzene was tested.

(429) (457)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 8 day
Unit: mg/l **Analytical monitoring:** no data
EC0: = 92
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: No detectable effect on concentration and total biomass was noted.
Source: BP Chemicals Ltd. London

(458)

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: = 92
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Established cultures of Pseudomonas putida were incubated with various concentrations of benzene in a total volume of 100 ml in 300 ml Erlenmeyer flasks with cotton lined plastic cups for 16 h at 25 degree C.
The number of cells, which served as a measure to determine cell multiplication inhibition, was determined turbidimetrically.
No analytical monitoring of the actual benzene concentrations was performed.
Test substance: Purity: no data

(459)

Type: aquatic
Species: Tetrahymena pyriformis (Protozoa)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC0: 391
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Value reported is the acute toxicity threshold level, i.e. the intermediate concentration between the lowest and highest concentration causing cell death and permitting replication, respectively.
In the study, the value is given as 5010 mmol/m3.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The ciliate T. elliotti (=T. pyriformis; strain L1630/1C) was used. Ca. 500 cells were treated with different concentrations of benzene in a 10 ml sterile, gas tight syringe to avoid volatilisation of benzene. After 24 h, cell death was recorded using cessation of ciliary movement as criterion.
Benzene solutions were prepared from a saturated stock solution, which concentration was determined by GC analysis.
Test substance: Purity: 99%

(460)

Type: other: see reference
Species: Pseudomonas putida (Bacteria)
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 92
Method: other: cell multiplication inhibition test
Year: 1983 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(381)

Type: other
Species: activated sludge, industrial
Exposure period: 15 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 520
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The test was conducted in a sealed bottle to prevent loss by volatilization. The concentration that inhibited the bacterium culture by 50%, at 35°C, was investigated. Uninhibited controls were used. The seed bacteria were aerobic heterotrophs cultured from the mixed liquor of an activated sludge wastewater treatment plant. Inhibition of oxygen uptake was used as the criterion for toxic inhibition. The method used was not a standard assay but was designed to mimic the standard anaerobic toxicity assay. The cell concentration was typical of the AFNOR and ETAD standard assays (King E.F. & Dutka B.J. In: Toxicity Testing Using Microorganisms. Vol. 1. Chpt. 5. Edited by G. Bitton & B.J. Dutka, CRC Press, Inc., Boca Raton, 1986). The culture was shaken during exposure to allow for aerobic conditions. The results were noted after 27, 38 and 49 hours and the 15-hour IC50 interpreted from these.

(461)

Type: other
Species: activated sludge, industrial
Exposure period: 15 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 520
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The seed bacteria were aerobic heterotrophs obtained from the mixed liquor of an activated sludge wastewater treatment plant. Tests were run with 25 ml liquid volume at a pH of 7 in an atmosphere with a N2/O2 ratio of 1:1 at a temperature of 35 degree C. The test was conducted in 125 ml serum bottles sealed to prevent by volatilisation.
Carbon dioxide was absorbed with NaOH-solution.
Inhibition of oxygen uptake was used as criterion for toxic effects.
The test procedure is not a standard assay but was designed to mimic the standard ATA. The cell concentration was typical of the AFNOR and ETAD standard assays (King E.F. & Dutka B.J. In: Toxicity Testing Using Microorganisms. Vol. 1. Chpt. 5. Edited by G. Bitton & B.J. Dutka, CRC Press, Inc., Boca Raton, 1986).
The culture was shaken during exposure to allow for aerobic conditions. The results were noted after 27, 38 and 49

hours and the 15-hour IC50 interpreted from these.
Using Henrys law constant, the partitioning of benzene between gas and liquid was calculated and the equilibrium concentration in the liquid phase was used as the IC50, i.e the concentration necessary to inhibit oxygen consumption to 50% of control.

(462)

Type: other
Species: activated sludge, industrial
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 13
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The test was conducted in a sealed bottle to prevent loss by volatilization. The concentration that inhibited the bacterium culture by 50%, at 25°C, was investigated. Uninhibited controls were used. The seed bacteria were Nitrosomonas obtained from the mixed liquor of an activated sludge plant treating meat-packing, rendering and hide-curing wastewater. The inhibition of ammonia consumption was used as the criterion for toxic inhibition. The culture was shaken during exposure to allow for aerobic conditions.

(461)

Type: other
Species: activated sludge, industrial
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 13
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The test was conducted in sealed 125 ml bottles to prevent loss by volatilization. 50 ml liquid, pH 6.5-8.0, was incubated at 25 degree C with constant shaking for 24 h under an atmosphere with a N2/O2 ration of 1.6:1.
The seed bacteria were Nitrosomonas obtained from the mixed liquor of an activated sludge plant treating meat-packing, rendering and hide-curing wastewater.
The inhibition of ammonia consumption was used as the criterion for toxic inhibition. Ammonia was measured using an ammonia sensitive electrode.
Using Henrys law constant, the partitioning of benzene between gas and liquid was calculated and the equilibrium concentration in the liquid phase was used as the IC50, i.e. the concentration necessary to inhibit ammonia consumption by 50%.

(462)

Type: other
Species: Anacystis aeruginosa (Bacteria)
Exposure period: 4 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC20 : = 50
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The effect on photosynthesis was examined at 24°C. No further details were available.

(429) (463)

Type: other
Species: Anacystis aeruginosa (Bacteria)
Exposure period: 4 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC20 : = 50
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The effect on photosynthesis was examined at 24 degree C.
No further etails were available.

(429) (464)

Type: other
Species: other bacteria
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 1200
Method: other
Year: 1979 **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: The test was conducted on methanogens according to the method of Owen, 1979. Bacteria were exposed to benzene in a sealed bottle to prevent loss of benzene by volatilization. The concentration that inhibited the bacterium culture by 50%, at 35°C, was investigated. Uninhibited controls were used. The anaerobic toxicity assay used measures the inhibition of gas production as the criterion for toxicity. The results were noted after 24, 72 and 96 hours and the 48-hour IC50 interpreted from these.

(461) (465)

Type: other
Species: other bacteria
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
IC50 : = 1200
Method: other
Year: 1979 **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The test was conducted on methanogens according to the method of Owen et al., 1979. Bacteria from an enriched 10-year established laboratory culture were used.
Bacteria were exposed to benzene in sealed 125 ml serum bottles to prevent loss of benzene by volatilization.
The concentration that inhibited the bacterium culture by 50%, at 35 degree C, was investigated. The anaerobic toxicity assay used measures the inhibition of gas production as the criterion for toxicity.
The results were noted after 24, 72 and 96 hours and the 48-hour IC50 interpreted from these.
Using Henrys law constant, the amount of benzene exiting with the vented gas produced was calculated and the new equilibrium concentration in the liquid was computed.

(462) (466)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species: Morone saxatilis (Fish, estuary, marine)
Endpoint: weight of young fish
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 3.1
EC : = 5.3
Method: other
Year: **GLP:** no data
Test substance: no data
Result: No effects on the wet or dry weight of the exposed fish were seen. However, at a benzene concentration of 5.3 mg/l, a decrease in wet and dry weight was noted.
Source: BP Chemicals Ltd. London
Test condition: Juvenile fish were exposed in a saltwater, flow-through, laboratory system at 15.2-16.4°C. The water contained 7.5-7.9 mg/l dissolved oxygen and <0.5 mg/l ammonia. The salinity was 25-26 ppt and it had a pH of 7.7-7.8.

(429) (467)

Species: Morone saxatilis (Fish, estuary, marine)
Endpoint: weight of young fish
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 5.3
Method: other
Year: **GLP:** no data
Test substance: no data
Result: No effect was seen on the caloric content (ash–free dry weight) of the exposed fish.
Source: BP Chemicals Ltd. London
Test condition: Juvenile fish were exposed in saltwater, in a flow–through laboratory system. The water temperature was 15.2–16.4°C, dissolved oxygen content was 7.5–7.9 mg/l, pH was 7.7–7.8, salinity was 25–26 ppt and ammonia content was <0.5 mg/l.
(429) (467)

Species: Morone saxatilis (Fish, estuary, marine)
Endpoint: weight of young fish
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 3.1
EC : = 5.3
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Result: Decrease in wet and dry weight of exposed fish at a benzene concentration of 5.3 mg/l.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile fish were exposed in a saltwater, flow–through, laboratory system at 15.2–16.4 degree C. The water contained 7.5–7.9 mg/l dissolved oxygen and <0.5 mg/l ammonia. The salinity was 25–26 g/l and it had a pH of 7.7–7.8.
(449) (467)

Species: Morone saxatilis (Fish, estuary, marine)
Endpoint: weight of young fish
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 5.3
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Result: No effect was seen on the caloric content (ash–free dry weight) of the exposed fish.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile fish were exposed in saltwater, in a flow–through laboratory system. The water temperature was 15.2–16.4 degree C, dissolved oxygen content was 7.5–7.9 mg/l, pH was 7.7–7.8, salinity was 25–26 g/l and ammonia content was <0.5 mg/l.

(429) (467)

Species: Morone saxatilis (Fish, estuary, marine)
Endpoint: other
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
LOEC: = 3.1
Method: other
Year: **GLP:** no data
Test substance: no data
Result: A decrease in the percentage fat content of the exposed fish was noted at all concentrations tested, 3.1 mg/l being the lowest test concentration.
Source: BP Chemicals Ltd. London
Test condition: Juvenile fish were exposed in a flow–through laboratory system to benzene in saltwater at 15.2–16.4°C and pH of 7.7–7.8. The dissolved oxygen concentration was 7.5–7.9 mg/l and ammonia was present at <0.5 mg/l. The salinity was 25–26 ppt.

(429) (468)

Species: Morone saxatilis (Fish, estuary, marine)
Endpoint: other
Exposure period: 28 day
Unit: mg/l **Analytical monitoring:** no data
LOEC: = 3.1
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Result: A decrease in the percentage fat content of the exposed fish was noted at all concentrations tested, 3.1 mg/l being the lowest test concentration.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile fish were exposed in a flow-through laboratory system to benzene in saltwater at 15.2-16.4 degree C and pH of 7.7-7.8. The dissolved oxygen concentration was 7.5-7.9 mg/l and ammonia was present at <0.5 mg/l. The salinity was 25-26 g/l.

(429) (468)

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: other aquatic crustacea
Endpoint: mortality
Exposure period: 20 day
Unit: mg/l **Analytical monitoring:** no data
NOEC: ca. .17
LOEC: ca. 1.1
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Full details of the method not reported (open/closed system, pH), analytical monitoring performed, but data not presented.
The concentration of benzene in the test solutions was measured by UV-spectrophotometry (at 255 nm).
Result: Benzene had little effect on the duration of the larval stages and had no effect on the size of surviving larvae. At the lowest concentration used, benzene had no effect on survival. At the other two concentrations used, benzene led to an accelerated mortality rate compared to untreated controls. After 10 days of exposure to the highest concentration of benzene (6.5-7 mg/l), most larvae died. With 1.1-1.2 mg/l, mortality rate was less but most larvae died before day 20 of exposure.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Larval stages of the Dungeness crab (Cancer magister), were continuously exposed after hatching in flowing water laboratory culture systems to benzene for up to 60 days as follows:
Filtered sterile seawater,saturated with air, salinity:

29-34 g/l, temperature: 10.5-14.2 degree C, photoperiod: 11h dark/13h light, 3 benzene concentrations (0.17-0.18 mg/l, 1.1-1.2 mg/l, and 6.5-7.0 mg/l) and control.
In each test, 25 larvae/culture container were used, a total of 300 in controls and 100 in each exposure treatment.
Larvae were fed brine shrimp during exposure and were transferred to clean culture containers three times a week.
Effects on survival, duration of larval development and size were employed as indicators of toxic effects.

(412)

Species: other aquatic crustacea
Endpoint: mortality
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
EC : 1
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Only one concentration used.
Result: A significantly greater number of benzene treated animals died before molting (55.8% vs. 23%).
The mean time required to molt was significantly increased for animals exposed to benzene (control: 33 days, treated: 50 days).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Juvenile C. sapidus (blue crab, carapace widths 1-8 cm), collected from the wild, were kept in 750 ml artificial sea water (osmolality 280-380 osmol, temperature 23 degree C, 12h:12h dark/light cycle) for at least 2 weeks before the experiment was started. Crabs were induced to autotomize the 3rd appendage. One group was exposed to 1 mg/l benzene (nominal concentration, see below) (added as stock solution in ethanol, water with benzene was changed daily), the other received ethanol only (water was changed twice a week).
To assess the concentration of benzene in the water, the actual concentrations were monitored in a control experiment by GC analysis. Concentrations fell linearly within 7 h to about 20% of initial level. It was concluded that the animals were exposed to 0.26-3.0 mg/l benzene for 18 h a day.

(469)

Species: Daphnia magna (Crustacea)
Endpoint: other
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 98
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Daphnia magna were exposed for partial lifetime or lifetime. No further details were available in the citing reference. (426) (470)

Species: Daphnia magna (Crustacea)
Endpoint: other
Exposure period:
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 98
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Daphnia magna were exposed for partial lifetime or lifetime. No further details were available in the citing reference. (426) (470)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

Type: filter paper
Species: Eisenia foetida (Worm (Annelida), soil dwelling)
Endpoint: mortality
Exposure period: 48 hour(s)
Unit: mg/cm2 filter paper
LC50: .1 - 1
Method: Directive 87/302/EEC, part C, p. 95 "Toxicity for earthworms: Artificial soil test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Benzene was classified as moderately toxic as its LC50 value fell within the above range.
Source: BP Chemicals Ltd. London
Test condition: The test protocol used was based on that proposed by the EEC (Edwards C.A. Personal communications relative to his coordination of developing the methodology and protocol for an earthworm testing procedure as directed by the European Economic Commission. Rothamsted Experimental Station, Harpenden, Herts, England, 1981). Benzene was dissolved in acetone and pipetted on to filter paper in the exposure vial. Controls were treated with acetone alone. The solvent was evaporated off the filter paper before the earthworms were exposed. Groups of ten worms were exposed at each test concentration.

Test substance: Technical- or analytical-grade benzene was tested.

(471)

Type: filter paper
Species: Eisenia foetida (Worm (Annelida), soil dwelling)
Endpoint: mortality
Exposure period: 48 hour(s)
Unit: mg/cm2 filter paper
LC50: .1 - 1
Method: Directive 87/302/EEC, part C, p. 95 "Toxicity for earthworms: Artificial soil test"
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Benzene was classified as "moderately toxic" as its LC50 value fell within the the limits of this predefined toxicity class.
Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
According to the authors, experiments were based on the protocol of the EC-directive. However, no EC50 value but only a range is given in this study. Several experimental details were not reported (temperature, nominal or actual benzene concentrations used, purity of test compound, concentration dependent effects on mortality rates).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: The test protocol used was based on that proposed by the EEC (Edwards C.A. Personal communications relative to his coordination of developing the methodology and protocol for an earthworm testing procedure as directed by the European Economic Commission. Rothamsted Experimental Station, Harpenden, Herts, England, 1981). Benzene was added directly to moist filter paper. Groups of ten worms were exposed at each test concentration in capped glass vials. Death was recorded if the worm did not respond to gentle agitation of its anterior end. Severely morbid worms having lost its posterior end when considered alive when still responding to agitation.
Test substance: Technical- or analytical-grade benzene was tested (no details reported).

(472)

Type: filter paper
Species: Eisenia foetida (Worm (Annelida), soil dwelling)
Endpoint: mortality
Exposure period:
Unit: mg/cm2 filter paper
LC50: = .098
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Filter paper was impregnated with benzene and the earthworms were allowed to absorb this into their bodies for an unspecified period.

(473) (474)

Type: filter paper
Species: Eisenia foetida (Worm (Annelida), soil dwelling)
Endpoint: mortality
Exposure period:
Unit: mg/cm2 filter paper
LC50: = .098
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Filter paper was impregnated with benzene and the earthworms were allowed to absorb this into their bodies for an unspecified period.

(473) (474)

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant
Endpoint: growth
Expos. period:
Unit: g/l
EC : .02 - .2
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Low concentrations of benzene in the water used for watering, stimulate plant growth and root formation. In contrast, higher concentrations of 0.9-1.3 g/l (near the saturated solution of 1.8 g/l) have a growth inhibiting effect. No more details were available.
Source: BP Chemicals Ltd. London

(426) (475)

Species: other terrestrial plant
Endpoint: growth
Expos. period:
Unit: mg/l
LC : > .05
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: High concentrations of benzene in air have a lethal effect on plants. No more details were available.
Source: BP Chemicals Ltd. London

(426) (476)

Species: other terrestrial plant
Endpoint: growth
Expos. period:
Unit: g/l
EC : .02 – .2
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Low concentrations of benzene in the water used for watering, stimulate plant growth and root formation. In contrast, higher concentrations of 0.9–1.3 g/l (near the saturated solution of 1.8 g/l) have a growth inhibiting effect. No more details were available.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
(426) (475)

Species: other terrestrial plant
Endpoint: growth
Expos. period:
Unit: mg/l
LC : > .05
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: High concentrations of benzene in air have a lethal effect on plants. No more details were available.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Source: Deutsche Shell Chemie GmbH Eschborn
(426) (476)

4.6.3 Toxicity to other Non–Mamm. Terrestrial Species

Species: other
Endpoint: mortality
Expos. period: 48 hour(s)
Unit: other
LC0: = 105
LC50: = 190
Method: other
Year: **GLP:** no data
Test substance: other TS
Source: BP Chemicals Ltd. London
Test condition: Groups of ten, 3–4 week old Xenopus laevis (clawed toad) larvae were exposed in covered, all–glass aquaria at 20°C. Results are reported in mg/l.
Test substance: Analytical grade benzene was tested.
(429) (426) (477) (478) (479)

Species: other
Endpoint: mortality
Expos. period: 48 hour(s)
Unit: other
LC0: = 120
LC50: = 370
Method: other
Year: **GLP:** no data
Test substance: no data
Source: BP Chemicals Ltd. London
Test condition: Ambystoma mexicanum (Mexican axolotl; Salamander) larvae (3-4 weeks old) were exposed in groups of ten in static freshwater at 20°C. The results are reported in mg/l.
(429) (426) (480) (479)

Species: other
Endpoint: mortality
Expos. period: 48 hour(s)
Unit: other
LC0: = 105
LC50: = 190
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
Several details not reported (oxygen concentration during test, no monitoring of concentrations).
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Groups of ten, 3-4 week old Xenopus laevis (clawed toad) larvae were exposed in 1 l Dutch Standard water in covered, all-glass aquaria at 19-21 degree C.
Test substance: Analytical grade benzene was tested.
(481) (482) (479)

Species: other
Endpoint: mortality
Expos. period: 48 hour(s)
Unit: other
LC0: = 120
LC50: = 370
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
Several details not reported (use of solvent vehicle?, purity of test compound, no monitoring of benzene concentrations, oxygen concentration during treatment)
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Ambystoma mexicanum (Mexican axolotl; Salamander) larvae (3-4 weeks old) were exposed in groups of ten in static freshwater (1 l of Dutch Standard water in covered glass basins) at 19-21 degree C. No further experimental details were reported in this study.
(482) (479)

4.7 Biological Effects Monitoring

Remark: No relevant data identified from the literature searched.
Source: BP Chemicals Ltd. London
Deutsche Shell Chemie GmbH Eschborn

4.8 Biotransformation and Kinetics

Type: animal
Method: Between November 1980 and August 1981, quarterly, five replicate, one week composites of the final effluent from the Los Angeles County wastewater treatment plant were collected for analysis of extractable organics (EOs) and two grab samples of surficial sediments were collected for analysis of volatile organics (VOs). The sampling site was 6 km NW of the discharge zone at Pulos Verdes and a modified Van Veen sampling device was used. During June 1981, biological samples were collected at this same site. These included Citharichthys xanthostigma (Pacific sanddab), Microstomus pacificus (Dover sole), Scorpaena guttata (scorpionfish), Genyonemus lineatus (white croaker), Sicyonia ingentis (ridgeback prawn) and a sample of small invertebrates collected from just above the bottom sediments (containing 74% (by weight) mysids and 23% decapod shrimp). All EOs and VOs were separated from the samples using the standard EPA protocol for analysis of priority pollutants (EPA. Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. Environmental Monitoring and Support Laboratory, US Environmental Protection Agency, Cincinnati, Ohio, 1977) or by using previously reported methods (Young D.R. et al. J. Wat. Pollut. Control Fed. 48, 1919–1928, 1976). VOs from sediments and tissues were separated using EPA protocol method 601. Analysis was done using gas chromatography/mass spectrometry.
Result: Benzene was detected at a level of 220 µg/l of effluent and <1 µg/kg dry sediment. In the biological samples, benzene was present at concentrations of <1, 52, 16, 15 (liver samples), <1 (muscle sample) and 8 (whole invertebrate) µg/kg wet weight respectively.
Source: BP Chemicals Ltd. London

(483)

Type: animal
Method: Oysters and clams were collected from Lake Pontchartrain, Louisiana, USA in May and June 1980 and analysed for the presence of benzene.
Result: One sample of oysters (Crassostrea virginica) was found to contain 220 µg/kg wet weight and one sample of clams (Rangia cuneata) contained 260 µg/kg wet weight. A second sample of clams taken from the same area had no detectable benzene (detection limits not stated).
Source: BP Chemicals Ltd. London

(484)

Type: animal
Method: Two species of macroalgae, Ulva lactuca and Hypnea musciformis (seaweeds) were grown in tanks using circulating seawater. The algae samples were analysed by a modified headspace technique (Whelan J.K. et al. Geochim. Cosmochim. Acta 44, 1767-1785, 1980) using gas chromatography and mass spectrometry. Degassed distilled water and the tank water in which the algae were grown were used as blanks.
Result: Benzene was not detected in either of the blanks but was present in the algae at levels of 20 ng/g dry weight of algae.
Source: BP Chemicals Ltd. London

(485)

Type: animal
Method: The effects of benzene on various mixed-function oxydases and on cytochrome P450 was investigated.
Remark: Reliability: 3 (not valid)
Significant methodological deficiencies
No controls treated with vehicle (corn oil) only were reported to be used.
Result: Cytochrome P450 content was decreased after benzene treatment to ca. 78% of control. Among the monooxygenase dependent enzymes studied, the activity of the 7-ethoxy resorufin-O-deethylase (EROD) was decreased (73% of control). The activity of the aniline 4-hydroxylase and ethylmorphine N-demethylase was not affected.
Source: Deutsche Shell Chemie GmbH Eschborn
Test condition: Gilthead seabream (Sparus aurata. weight 225-275 g) were kept in aerated, filtered seawater at 16.5-17.5 degree C and fed daily. Benzene was administered once daily for 4 days by s.c injection (0.3 ml of benzene/corn oil 1:1).
Microsomes were prepared from the liver and the cytochrome P450 content and the activity of several monooxygenases were determined by standard methods.
Controls receiving corn oil are not reported.

(486)

4.9 Additional Remarks

Remark: No relevant data identified from the literature searched.
Source: BP Chemicals Ltd. London

Remark: In a test developed in part by the EPA, short-term effects of benzene on sexual reproduction of the marine red macroalga Champia parvula was investigated.
Unialgal stock cultures of both male and female gametophytes were grown for 3 weeks in sterile, filtered seawater with nutrients added. Thereafter, branches of female plants and one branch of a male plant producing spermatia were treated with benzene for 2 days (400 ml test solution in 500 ml screw-capped Erlenmeyer flasks, 22-24 degree C, salinity 7-7.5 g/l, 16:8 h light/dark cycle), followed by a 5-7 day recovery period for the females.
Subsequently, the number of cystokarps per female were counted.

Concentrations of benzene showing the following effects are nominal concentrations, no analytical monitoring to determine actualconcentrations was preformed.
No sexual reproduction was observed at a benzene concentration of 73.6 mg/l. The EC50 value, i.e. the concentration decreasing the no. of cystokarps to 50% of control, was reported to be lower than 34.3 mg/l.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

Source: Deutsche Shell Chemie GmbH Eschborn

(487)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 930 mg/kg bw
Method: other: see reference
Year: 1965 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(488)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: 690 – 1230 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: At high doses of 1870 mg/kg bw, animals developed tremors and convulsions and many died within 20 min. At 88 mg/kg bw, slight CNS depression occurred but no deaths. Guidelines followed not specified; groups of ten non–fasted or 24–hr fasted animals.
Source: BP Chemicals Ltd. London
Test substance: Test substance was laboratory reagent grade.

(489)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: 1758 – 6856 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of six to twelve newborn (24–48–hr–old) and immature (14–day–old) rats and groups of six young adult and older adult rats; stomach tube; undiluted; observed for 7 days. The LD50 values for the 14–day–old, young adult and older adult groups were 1758–5010, 2549–4219 and 3516–6856 mg/kg bw respectively. A rough approximation of the acute LD50 in the newborn rats found volumes of about 1 ml/kg bw [about 880 mg/kg bw] to be fatal.
Source: BP Chemicals Ltd. London
Test substance: Test substance was analytical grade laboratory reagent meeting ACS specifications.

(490)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: ca. 5600 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Guidelines followed not specified; 25 male animals; stomach tube; benzene given either undiluted or as an olive–oil or corn–oil solution emulsified with a 5–10% aqueous solution of gum arabic; observed for 14 days.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: 5080 – 7000 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Guidelines followed not specified; groups of ten or 20 animals; stomach tube; observed for 7 days.
The LD50 value was reported as 5960 mg/kg bw (range 5080–7000 mg/kg bw). Hind–limb paralysis, petechial bleeding of the urinary tract, eyes and nose, mild acute gastritis and stripping of the epithelial lining of the glandular portion of the stomach were seen.
Source: BP Chemicals Ltd. London

(492)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: 7380 – 14855 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of five non–fasted males; stomach tube; observed for 14 days.
The LD50 value was reported as 10,020 mg/kg bw (range 7,380–14,855 mg/kg bw).
Source: BP Chemicals Ltd. London

(493)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 810 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: method: groups of ten fasted male Sprague-Dawley rats; geometrically graded doses.
Reliability: 3 (not valid)
Documentation insufficient for assessment
one sex; no pathology; no data about number of doses, post exposure observation period, mortality rate, time to death after dosing
results: 95% confidence limits 690-950; at high doses of 1870 mg/kg bw, animals developed tremors and convulsions and many died within 20 min.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Test substance was laboratory reagent grade.

(489)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 3340 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: methods: 6 male young adult Sprague-Dawley rats per group; additional experiments with a) 6 male older adult rats/group, b) 6-12 male and female newborn rats (24-48-h-old) per group and c) 6-12 14-days-old rats of both sexes per group; stomach tube; undiluted; observed for 7 days.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
one sex; short observation period; no data about clinical examination, mortality rate, time of death after dosing and pathology
results: 95% confidence limits 2550-4220 mg/kg; LD50 values in a) 4920 (3520-6860) mg/kg and in b) 2990 (1760-5010) mg/kg; c) rough approximation of the acute LD50 in the newborn rats found volumes of about 1 ml/kg bw [about 880 mg/kg bw] to be fatal.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Test substance was analytical grade laboratory reagent meeting ACS specifications.

(490)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: ca. 5600 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: methods: guidelines followed not specified; 25 male animals; stomach tube; benzene given either undiluted or as an olive–oil or corn–oil solution emulsified with a 5–10% aqueous solution of gum arabic; observed for 14 days.
Reliability: 3 (not valid)
Documentation insufficient for assessment
no data about number of rats per group, clinical and pathological examination, assessement of toxicity in the other sex, doses
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.98% pure.

(491)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 5960 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Guidelines followed not specified; groups of 10 (pilot study) or 20 Spreague–Dawley Cobb rats; 5 doses tested; stomach tube; observed for 7 days.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about the test substance or time of death after dosing; short observation period after dosing; one sex
The LD50 95% confidence limits were reported as 5080–7000 mg/kg bw. Hind–limb paralysis, petechial bleeding of the urinary tract, eyes and nose, mild acute gastritis and stripping of the epithelial lining of the glandular portion of the stomach were seen.
Source: Deutsche Shell Chemie GmbH Eschborn

(492)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 10020 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 95% confidence limits 7,380-14,855 mg/kg.
Groups of five non-fasted male Carworth-Wistar rats; stomach tube; observed for 14 days;
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions one sex; ; no data about clinical examination, time of death after dosing, mortality rate and pathology;
Source: Deutsche Shell Chemie GmbH Eschborn

(493)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: 8438 mg/kg bw
Method: other
Year: **GLP:** no
Test substance: as prescribed by 1.1 - 1.4
Remark: Zur Bestimmung der akuten Toxizitaet wurden nur weibl. Tiereverwendet.
Source: BASF AG Ludwigshafen
Reliability: (2) valid with restrictions
Rahmendaten liegen vor

(494)

Type: LDLo
Species: human
Sex:
Number of Animals:
Vehicle:
Value: = 50 mg/kg bw
Method: other: see reference
Year: 1980 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(495)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time: 7 hour(s)
Value: = 10000 ppm
Method: other: see ref.
Year: 1960 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(496)

Type: LC50
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time: 4 hour(s)
Value: 13050 – 14380 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Method approximates to OECD Test Guide-line, 403 but females only were tested. Groups of ten female animals; observed for 2 wk; animals dying during exposure and those killed at end of study subjected to necropsy.
The LC50 value was reported as 13,700 ppm (converts to 44.7 mg/l) with a range of 13,050-14,380 ppm (converts to 42.5-46.9 mg/l). Death appeared to be caused by a depression of the CNS. These animals had increased lung and liver weights, lung and liver congestion and an increased number of vacuolated hepatocytes in the liver.
Source: BP Chemicals Ltd. London
Test substance: Reagent grade thiophene-free benzene containing no contaminants above a concentration of 0.05% was tested.

(497)

Type: LC50
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value: > 40000
Method:
Year: **GLP:** no data
Test substance: no data
Remark: Groups of eight male animals; removed from chamber when convulsions or respiratory arrest seen and returned as soon as they recovered; necropsy performed on CNS after 24 hr. Three of the eight animals died following five 25-35 min

exposures to 40,000 ppm (130.4 mg/l). Local irritation, depression of CNS and quivering or twitching were noted. Necropsy revealed no gross lesions other than those attributable to local irritation of the respiratory tract and no specific damage to the CNS.
Source: BP Chemicals Ltd. London

(498)

Type: LC50
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time: 4 hour(s)
Value: = 13700 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Method approximates to OECD Test Guide-line, 403 but females only were tested. Groups of ten female animals; observed for 2 wk; animals dying during exposure and those killed at end of study subjected to necropsy. Chamber concentration monitored.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions one sex; no data about concentrations tested, mortality rate The LC50 value was reported as 13,700 ppm (converts to 44.7 mg/l) with 95% confidence limits of 13,050-14,380 ppm (converts to 42.5-46.9 mg/l). Death appeared to be caused by a depression of the CNS. These animals had increased lung and liver weights, lung and liver congestion and an increased number of vacuolated hepatocytes in the liver.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Reagent grade thiophene-free benzene containing no contaminants above a concentration of 0.05% was tested.

(497)

Type: other
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time: 4 hour(s)
Value: = 16000 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Four of the six animals died following exposure to 16000 ppm (52.2 mg/l).
Groups of six males or females; observed for 14 days.
Source: BP Chemicals Ltd. London

(493)

Type: other
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time: 4 hour(s)
Value: = 16000 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Four of the six animals died following exposure to 16000 ppm (52.2 mg/l).
Reliability: 3 (not valid)
Unsuitable test system
no LC50 determined
Six males or females albino rats; observed for 14 days.
Source: Deutsche Shell Chemie GmbH Eschborn
(493)

Type: other
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value: > 40000 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of eight male animals; removed from chamber when convulsions or respiratory arrest seen and returned as soon as they recovered; necropsy performed on CNS after 24 hr.
Reliability: 3 (not valid)
Unsuitable test system
no LC50 determination
Three of the eight animals died following five 25-35 min exposures to 40,000 ppm (130.4 mg/l). Local irritation, depression of CNS and quivering or twitching were noted. Necropsy revealed no gross lesions other than those attributable to local irritation of the respiratory tract and no specific damage to the CNS.
Source: Deutsche Shell Chemie GmbH Eschborn
(498)

Type: LC50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Exposure time: 7 hour(s)
Value: = 9980 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: method: male and female Swiss mice, concentration analysed by interferometric determination, 16-20 mice per group, 10 concentrations tested, post exposure observation period up to 4 weeks but deaths occurred mostly within 8 h after start of exposure.
results: LC50 after 1 h post exposure observation period, converted to 31.79 mg/l; 95% confidence limits 9180-10860 ppm; symptoms: restlessness, tremor, muscular twitching, S-shaped tail, changes in respiration, incoordination, narcosis; histopathology: congestion of lungs and kidneys, nuclear fragments in spleen follicles.
Reliability: 1
Comparable to guideline study
LC50 not determined for each sex
Source: Deutsche Shell Chemie GmbH Eschborn

(499)

Type: LCLo
Species: human
Sex:
Number of Animals:
Vehicle:
Exposure time: 5 minute(s)
Value: = 2000 ppm
Method: other: see ref.
Year: 1980 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(495)

Type: other
Species:
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: Meerschweinchen, Kaninchen und Katzen wurden gegenueber Benzoldaempfen exponiert. (Die Dosierungen lagen zwischen 14 und 146 mg.) Die Vergiftungssymptome waren bei allen Spezies gleich. Die Benzoldaempfe fuehrten ohne

nennenswertelokale Reizungen unter Kraempfen (Zuckungen) zu Narkose und bei laengerdauernder Exposition zu Atemlaehmung und Tod.
Wurde waehrend der Narkose die Exposition abgesetzt erholtensich die Tiere rasch und vollstaendig.

Source: BASF AG Ludwigshafen
Reliability: (3) invalid
entspricht nicht heutigen Kriterien

(500)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: > 9400 mg/kg bw
Method: other: see ref.
Year: 1965 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(501)

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: > 8260 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: method: 4 albino rabbits of both sexes per group; at least 3 doses tested; abraded skin.
Reliability: 2 (valid with restriction)
Test procedure in accordance with national standard methods with acceptable restrictions
4 animals per group; used guidelines not available, but LD50 value >2000 mg/kg which is recommended in the limit test OECD 402
Source: Deutsche Shell Chemie GmbH Eschborn

(502)

Type: LD50
Species: guinea pig
Sex:
Number of Animals:
Vehicle:
Value: > 8260 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: method: 4 male Hartley derived guinea pigs per group, 3 doses tested, benzene applied undiluted on a 1 inch square cellulose pad fixed to the clipped, intact or abraded abdominal or dorsal skin.
results: no differences between abraded and intact skin.
Reliability: 3 (not valid)
Documentation insufficient for assessment
one sex, exposure and observation period not specified, 4 instead of 5 animals/dose, no data about mortality (time to death after dosing), no clinical examination and no pathology reported
Source: Deutsche Shell Chemie GmbH Eschborn

(502)

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: No relevant data identified from the literature searched.
Source: BP Chemicals Ltd. London

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 2890 other: ug/Kg
Method: other: see ref.
Year: 1977 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(503)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: 2450 – 3530 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of eight female rats; 50–50 (v/v) solution in mineral oil; necropsy on all animals dying during study and on all survivors killed after 14 days.
The LD50 value was reported as 2940 mg/kg bw (range 2450–3530 mg/kg bw). Animals died from CNS depression. No significant histologic damage observed in the lungs of any animal.
Source: BP Chemicals Ltd. London
Test substance: Reagent grade thiophene–free benzene containing no contaminants above a concentration of 0.05% was tested.

(497)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 2940 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: 95% confidence limits 2450–3530 mg/kg bw. Animals died from CNS depression. No significant histologic damage observed in the lungs of any animal.
Groups of eight female rats; 50–50 (v/v) solution in mineral oil; necropsy on all animals dying during study and on all survivors killed after 14 days.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Reagent grade thiophene–free benzene containing no contaminants above a concentration of 0.05% was tested.

(497)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 340 mg/kg bw
Method: other : no data
Year: 1975 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(504)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: 245 – 370 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The LD50 value was reported as 300 mg/kg bw (range 245–370 mg/kg bw). No further details were available.
Source: BP Chemicals Ltd. London

(505)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 298 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: 95% confidence limits 245–370 mg/kg; LD50 of benzene dissolved in DMSO 81 mg/kg; no further details were available.
Source: Deutsche Shell Chemie GmbH Eschborn

(505)

Type: other
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: s.c.
Value: 88 – 2200 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of males administered 88, 440 or 2200 mg/kg bw; 50% (v/v) benzene – olive oil solution; uptake of radioiron (59Fe), given as ferrous citrate, into the circulating erythrocytes followed.
No effect on iron uptake after 72 hr was seen with the lowest dose level or with the mid– and high–doses when administered 1 and 12 hr before iron administration. When 440 or 2200 mg/kg bw were given 24 hr before the iron, the amount of iron incorporated into the circulating erythrocytes after 72 hr was dose–dependently reduced. When determined over a 24–hr period, iron incorporation was reduced dose–dependently when the mid– and high–benzene doses were given 24 and 48 hr before the iron dose but not at 1, 12 and 72 hr before, showing complete recovery of benzene's bone marrow suppression effect.
Source: BP Chemicals Ltd. London
Deutsche Shell Chemie GmbH Eschborn

(506)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result: slightly irritating
EC classificat.: irritating
Method: other: see reference
Year: 1962 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(507)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result: moderately irritating
EC classificat.: irritating
Method: other: see ref.
Year: 1986 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(508)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result: irritating
EC classificat.:
Method: other
Year: 1941 **GLP:** no data
Test substance: other TS
Remark: Slight-moderate irritation (i.e. perceptible-definite erythema) and moderate necrosis (i.e. development of oedema and superficial necrosis resulting in a chapped appearance and exfoliation of large patches of skin). Blistering was seen, particularly when the material was confined.
The method of Adams E.M. et al. Indust. Med. 2, 1, 1941 was followed. Neat material applied 10-20 times to the ear and abdomen (bandaged; type not specified) of an unspecified number of animals over a 2-4 wk period. Animals were observed daily and weighed weekly.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result: irritating
EC classificat.:
Method: other
Year: 1941 **GLP:** no data
Test substance: other TS
Remark: methods: The method of Adams E.M. et al. Indust. Med. 2, 1,

1941 was followed. Neat material applied 10-20 times to the ear and abdomen (bandaged; type not specified) of an unspecified number of animals over a 2-4 wk period. Animals were observed daily and weighed weekly.
Reliability: 3 (not valid)
Significant methodological deficiencies
No data about number of animals tested, type of patch, dose level, observation period; single exposure (4 h) recommended
results: slight-moderate irritation (i.e. perceptible-definite erythema) and moderate necrosis (i.e. development of oedema and superficial necrosis resulting in a chapped appearance and exfoliation of large patches of skin). Blistering was seen, particularly when the material was confined. No abnormal behavior or body weight loss.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.98% pure.

(491)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result: irritating
EC classificat.: irritating
Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1981 **GLP:** no data
Test substance: no data
Remark: method: 6 shaved New Zealand White rabbits, 4 h occlusive application under exposure chamber of 6 cm2; scoring criteria according to Draize, J.H. et al., JPET 82, 377-390 (1944); classification in compliance with 79/831/EEC, Annex VI, part IID.
results: mean score of erythema 1, 24, 48, 72, 144 h after end of application 1.0, 2.0, 2.2, 2.4, 3.0, respectively; mean score of edema 2.2 1 h after end of application (other observation periods = 0)
Reliability: 1 (valid)
Guideline study
Source: Deutsche Shell Chemie GmbH Eschborn

(509)

5.2.2 Eye Irritation

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: highly irritating
EC classificat.: risk of serious damage to eyes
Method: other: see ref.
Year: 1986 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(507)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: moderately irritating
EC classificat.: irritating
Method: other: see ref.
Year: 1956 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(510)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: moderately irritating
EC classificat.:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Method approximates to OECD Test Guide–line, 405. Two drops of liquid [presumably neat and approximately 100 ml] placed onto right eyeball [exact area not specified] and visual observations of irritation and internal and external corneal injury [no further details available] were made 3 min, 1 hr and 1, 2 and 7 days after treatment.
Moderate conjunctival irritation (i.e. inflammation and slight swelling of the eyelids) and very slight transient corneal injury (i.e. questionable or just perceptible superficial necrosis in very small areas of the cornea) were recorded.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: moderately irritating
EC classificat.:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: method: two drops of liquid [presumably neat and approximately 100 ul] placed onto right eyeball [exact area not specified] and visual observations of irritation and internal and external corneal injury [no further details available] were made 3 min, 1 hr and 1, 2 and 7 days after treatment.
Moderate conjunctival irritation (i.e. inflammation and slight swelling of the eyelids) and very slight transient corneal injury (i.e. questionable or just perceptible superficial necrosis in very small areas of the cornea) were recorded.
Reliability: 4 (validity cannot be judged)
Documentation insufficient for assessment
no data about number of animals, effects at designated observation time, effects on iris;
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.98% pure.

(491)

5.3 Sensitization

Type:
Species:
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: **GLP:**
Test substance:
Remark: No relevant data identified from the literature searched.
Source: BP Chemicals Ltd. London
Deutsche Shell Chemie GmbH Eschborn

5.4 Repeated Dose Toxicity

Species: rat **Sex:** female
Strain: Wistar
Route of admin.: inhalation
Exposure period: 7 or 14 days
Frequency of treatment: 8 hours/day
Post. obs. period: none
Doses: 20, 50, 100, 300, 1000, 3000 ppm (converts to 0.065, 0.16, 0.33, 0.98, 3.26, 9.78 mg/l); 5-7 rats/dose
Control Group: yes, concurrent no treatment
NOAEL: = 50 ppm
LOAEL: = 100 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Blood sampled from all animals.
Result: After 7 days exposure, the body weight gain and mean leukocyte count were dose-dependently decreased at 0.98 mg/l or more. Leukocyte alkaline phosphatase activity was increased at 0.33 and 0.98 mg/l with no additional elevation seen at the higher doses after 7 or 14 days exposure. Serum alkaline phosphatase activity tended to be lower than in the controls but this was not significant.
Source: BP Chemicals Ltd. London
Test substance: >=99% pure.

(511)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose
Control Group: yes, concurrent no treatment
NOAEL: = 10 ppm
LOAEL: = 30 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were examined twice daily for mortality and moribundity throughout the study. Observations for signs of toxicity were made weekly. On days 7, 14, 28, 56 and 91, blood samples were obtained at necropsy from ten randomly selected rats/sex/group. Complete necropsies were performed on all of these sacrificed animals and on all animals found dead or moribund.
Result: No exposure-related mortality, clinical observations or mean body weight changes were seen. In the high-dosed animals, a significant decrease in the white blood cell count and, from day 14, the percentage of lymphocytes was noted. No

exposure-related gross pathologic observations were reported. Females exposed to 0.098 mg/l had significantly increased mean thyroid weights and, on histopathological examination of the high-dosed animals, lower femoral bone marrow cellularity was noted on days 7, 28, 56 and 91. No results are reported for the lower dosed animals.

Source: BP Chemicals Ltd. London
Test substance: >99.9% pure.

(512)

Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: inhalation
Exposure period: up to 212 days
Frequency of treatment: 7 hours per day/5 days per week
Post. obs. period: 18-22 hours
Doses: 0.28, 7, 14, 21, 30 mg/l; 10-25 rats/sex/dose
Control Group: yes, concurrent no treatment
NOAEL: < .28 mg/l
LOAEL: = .28 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.
Result: Groups of ten to 25 male and female animals were used. Increased spleen weight and unspecified changes in blood histopathology including leucopenia were recorded at the lowest dose level with, in addition, narcosis, growth depression and unspecified spleen and bone marrow histopathology at 7 mg/l or more. Mortality rate increased at 14 mg/l and increased testis weight and unspecified liver histopathology occurred at 21 mg/l.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(513)

Species: rat **Sex:** female
Strain: Wistar
Route of admin.: inhalation
Exposure period: 7 or 14 days
Frequency of treatment: 8 hours/day
Post. obs. period: none
Doses: 20, 50, 100, 300, 1000, 3000 ppm (converts to 0.065, 0.16, 0.33, 0.98, 3.26, 9.78 mg/l); 5-7 rats/dose
Control Group: other
NOAEL: = 50 ppm
LOAEL: = 100 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Blood sampled from all animals. TS analysed by GC.
Reliability: 3 (not valid)
Significant methodological deficiencies
no clinical biochemistry, no further clinical examination, limited hematology, no pathology, one sex, short exposure period
Result: After 7 days exposure, the body weight gain and mean leukocyte count were dose-dependently decreased at 0.98 mg/l or more. Leukocyte alkaline phosphatase activity was increased at 0.33 and 0.98 mg/l with no additional elevation seen at the higher doses after 7 or 14 days exposure. Serum alkaline phosphatase activity tended to be lower than in the controls but this was not significant.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >=99% pure.

(511)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: = 10 ppm
LOAEL: = 30 ppm
Method: other
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: All animals were examined twice daily for mortality and moribundity throughout the study. Observations for signs of toxicity were made weekly. On days 7, 14, 28, 56 and 91, blood samples were obtained for hematology and clinical biochemistry at necropsy from ten randomly selected rats/sex/group. Complete necropsies organ weight determination and histopathology were performed on all of these sacrificed animals and on all animals found dead or

moribund.
Reliability: 1 (valid)

Result: No exposure-related mortality, clinical observations or mean body weight changes were seen. In the high-dosed animals, a significant decrease in the white blood cell count and, from day 14, the percentage of lymphocytes was noted. No exposure-related gross pathologic observations were reported. Females exposed to 0.098 mg/l had significantly increased mean thyroid weights and, on histopathological examination of the high-dosed animals, lower femoral bone marrow cellularity was noted on days 7, 28, 56 and 91. No further effects reported.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: >99.9% pure.

(512)

Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: inhalation
Exposure period: up to 212 days
Frequency of treatment: 7 hours per day/5 days per week
Post. obs. period: 18-22 hours
Doses: 0.28, 7, 14, 21, 30 mg/l; 10-25 rats/sex/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS

Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.
TS concentration was analysed (no data about method) and adjusted, variation of concentration 10%. Males and females tested only at the low dose, other groups only males.
Reliability: 3 (not valid)
Significant methodological deficiencies
low number of animals, one sex (other than low dose), no clinical biochemistry, limited hematology, limited pathology, no statistical evaluation, no further data about results of enforced investigations

Result: Increased spleen weight and unspecified changes in blood histopathology including leucopenia were recorded at the lowest dose level with, in addition, narcosis, growth depression and unspecified spleen and bone marrow histopathology at 7 mg/l (exposure period 221 d) or more (up to 93 d). Mortality rate increased at >= 14 mg/l and increased testis weight and unspecified liver histopathology occurred at 21 mg/l (exposure period 93 d).

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.98% pure.

(513)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: gavage
Exposure period: 17 weeks
Frequency of treatment: 5 days/week
Post. obs. period: none
Doses: 25, 50, 100, 200, 400, 600 mg/kg bw/day; 10 or 15 rats/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: < 25 mg/kg bw
LOAEL: = 25 mg/kg bw
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn oil. Animals were checked twice daily and weights recorded weekly. Five animals of each sex from the control, 200 and 600 mg/kg bw groups were killed on day 60. Survivors from all groups killed at end of the study period and haematologic analyses were performed on five animals from each group. At necropsy, a large number of organs from animals from the control, the interim-kill and the top-dose groups were removed and examined histopathologically; spleens were examined in all dose groups.
Result: No compound-related deaths. Mean body weight gain was dose-dependently decreased in both the males and females at 200 mg/kg bw or more. White blood cell and, in particular, lymphocyte counts were decreased significantly ($p < 0.05$) on day 60 in both the male and female groups given 200 and 600 mg/kg bw and on day 120 in all the female treated groups and the top two dosed male groups. A significant increase ($p < 0.05$) in white blood cell count was recorded on day 120 in the 25 mg/kg bw male group.
Source: BP Chemicals Ltd. London
Test substance: >99.7% pure.

(514)

Species: rat **Sex:** female
Strain: Wistar
Route of admin.: gavage
Exposure period: 6 months
Frequency of treatment: 5 days/week
Post. obs. period: 18-22 hours
Doses: 1, 10, 50, 100 mg/kg bw/day; 10 rats/dose
Control Group: yes, concurrent vehicle
NOAEL: = 1 mg/kg bw
LOAEL: = 10 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene given as an olive-oil solution. Haematological examinations made on selected animals of each group at varying intervals. Animals were weighed at regular intervals and frequent observations were made of their general appearance and behaviour. Records were kept of food consumption and mortality. Gross appearance of lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Blood was obtained from selected animals of each group at time of autopsy. Histopathological examination of above organs and adrenals, pancreas and femoral bone marrow made.
Result: Very slight leucopenia was seen at 10 mg/kg bw/day, with leucopenia and erythrocytopenia occurring at the top two doses. No further results were reported.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 days/week
Post. obs. period: 1 week
Doses: 50, 100, 200 mg/kg bw/day for males, 25, 50, 100 mg/kg bw/day for females; 60 rats/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: < 25 - 50 mg/kg bw
LOAEL: = 25 - 50 mg/kg bw
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn-oil. Blood was drawn from ten randomly preselected animals from each sex and dose group at 0, 3, 6, 9, 12, 15, 18 and 21 months and from all animals at the terminal kill at 24 months. Ten animals of each sex and group were killed after 51 weeks and necropsies were performed. Animals were observed twice daily and weighed weekly for 13 wk and monthly thereafter.
Result: A dose-related decrease in weight gain was recorded in the

mid- and high-dosed groups and no other clinical signs related to benzene exposure were seen. The high-dosed male and mid- and high-dosed female groups had significantly decreased survival rates when compared to the control groups. The female control group, however, apparently had exceptionally good survival when compared with historic control data for the same strain and sex of animal. Various non-neoplastic lesions were found in the zymbal gland, adrenal gland, spleen, thymus, cardiac stomach and pituitary gland. A decrease in white blood cell counts was reported in males in first 18 months and in females in first 12 months. The female relation to benzene exposure is questionable due to ambiguous results. See section 5.8 for carcinogenic effects.

Source: BP Chemicals Ltd. London
Test substance: >99.7% pure.

(514)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: gavage
Exposure period: 17 weeks
Frequency of treatment: once daily, 5 days/week
Post. obs. period: none
Doses: 25, 50, 100, 200, 400, 600 mg/kg bw/day; 10 or 15 rats/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: < 25 mg/kg bw
LOAEL: = 25 mg/kg bw
Method: other
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Benzene administered in corn oil. Animals were checked twice daily and weights recorded weekly. Five animals of each sex from the control, 200 and 600 mg/kg bw groups were killed on day 60. Survivors from all groups killed at end of the study period and haematologic analyses were performed on five animals from each group. At necropsy, a large number of organs from animals from the control, the interim-kill and the top-dose groups were removed and examined histopathologically; spleens were examined in all dose groups.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no clinical biochemistry, no ophtalmological examination, limited hematology
Result: No compound-related deaths. Mean body weight gain was dose-dependently decreased (14-22%) in both the males and females at 200 mg/kg bw or more. White blood cell and, in particular, lymphocyte counts were decreased significantly ($p < 0.05$) on day 60 in both the male and female groups given 200 and 600 mg/kg bw and on day 120 in all the female treated groups and the top two dosed male groups. A significant increase ($p<0.05$) in white blood cell count was recorded on day 120 in the 25 mg/kg bw male group.

Histopathology: lymphoid depletion in the B-cell of the spleen in male and female rats sacrified on day 60 and dosed with 200 or 600 mg/kg as well as in the high dose groups (male and female) at termination; in the last 2 groups benzene treatment caused also increased extramedullary hematopoiesis.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: >99.7% pure.

(514)

Species: rat **Sex:** female
Strain: Wistar
Route of admin.: gavage
Exposure period: 6 months
Frequency of treatment: 5 days/week
Post. obs. period: 18-22 hours
Doses: 1, 10, 50, 100 mg/kg bw/day; 10 rats/dose
Control Group: yes, concurrent vehicle
NOAEL: = 1 mg/kg bw
LOAEL: = 10 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene given as an olive-oil solution, controls (n=20) received only olive oil emulsified with 5-10% aqueous solution of arabic gum. Haematological examinations made on selected animals of each group at varying intervals. Animals were weighed at regular intervals and frequent observations were made of their general appearance and behaviour. Records were kept of food consumption and mortality. Gross appearance of lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Blood was obtained from selected animals of each group at time of autopsy. Histopathological examination of above organs and adrenals, pancreas and femoral bone marrow made.
Reliability: 3 (not valid)
Documentation insufficient for assessment
low number of animals, one sex, no clinical biochemistry, limited hematology, limited pathology, no further data given about results of enforced investigations, no statistical evaluation

Result: Very slight leucopenia was seen at 10 mg/kg bw/day, with leucopenia and erythrocytopenia occurring at the top two doses. No further results were reported.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.98% pure.

(491)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: once daily, 5 days/week
Post. obs. period: 1 week
Doses: 50, 100, 200 mg/kg bw/day for males, 25, 50, 100 mg/kg bw/day for females; 60 rats/sex/dose
Control Group: yes, concurrent vehicle
Method: other
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Benzene administered in corn–oil. Blood was drawn from ten randomly preselected animals from each sex and dose group at 0, 3, 6, 9, 12, 15, 18 and 21 months and from all animals at the terminal kill at 24 months. Ten animals of each sex and group were killed after 51 weeks and necropsies were performed. Animals were observed twice daily and weighed weekly for 13 wk and monthly thereafter.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no clinical biochemistry, no urinanalysis, limited hematology
Result: A dose–related decrease in weight gain was recorded in the mid– and high–dosed groups and no other clinical signs related to benzene exposure were seen. The high–dosed male and mid– and high–dosed female groups had significantly decreased survival rates when compared to the control groups. The female control group, however, apparently had exceptionally good survival when compared with historic control data for the same strain and sex of animal. Various non–neoplastic lesions were found in the zymbal gland, adrenal gland, spleen, thymus, cardiac stomach and pituitary gland. A decrease in white blood cell counts was reported in males in first 18 months and in females in first 12 months. The female relation to benzene exposure is questionable due to ambiguous results. See section 5.7 for carcinogenic effects. LOEL for noncarcinogenic effects 25 mg/kg for females and 50 mg/kg for males.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99.7% pure.

(514)

Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: for life
Frequency of treatment: 6 hr/day, 5 days/week
Post. obs. period:
Doses: 300 ppm
Control Group: yes, concurrent no treatment
Method: other: see ref.
Year: 1982 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(515)

Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 150 mice/sex/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were examined twice daily for mortality and moribundity throughout the study. Observations for signs of toxicity were made weekly. On days 7, 14, 28, 56 and 91, blood samples were obtained at necropsy from 30 randomly selected mice/sex/group. Complete necropsies were performed on 20 of these sacrificed animals/sex and on all animals found dead or moribund.
Result: Groups of 150 mice/sex were used. No exposure-related mortality, clinical observations or mean body weight changes were seen. In the high-dosed animals, changes in blood composition, relating to decreased red blood cells, white blood cells and platelets, and an increased incidence and severity of various red cell morphologic changes, which was more pronounced in the males, were noted. In the top dosed males, slight increases were noted in the incidence of small thymuses on days 56 and 91 and spleens on day 56. The authors felt that this was of doubtful biological significance. A statistically significant decrease in mean testis weight and increase in mean female liver/body weight ratio occurred at 0.98 mg/l. Histopathological changes were reported, at the high-dose level, in the thymus, femoral marrow, spleen, mesenteric lymph nodes, mandibular lymph nodes and the liver, the severity increasing with time. Histomorphologic changes in testes and ovaries were recorded at 91 days. In a few instances, the lesions seen at the high-dose level were detected at the lower levels but these were not reported in detail. Male mice were reported to be

much more susceptible than the females.
Source: BP Chemicals Ltd. London
Test substance: >99.9% pure.

(512)

Species: mouse **Sex:** male/female
Strain: C57BL
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: 16 weeks
Doses: 10, 25, 100, 300, 400 ppm (converts to 0.03, 0.08, 0.33, 0.98, 1.3 mg/l); group numbers unspecified
Control Group: yes, concurrent no treatment
NOAEL: = 10 ppm
LOAEL: = 25 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group [numbers unspecified] were exposed to conditioned air. At various times during and after the exposure period, five to ten mice were removed from both the treated and control groups and the blood, bone marrow and spleens removed and examined.
Result: The lowest benzene concentration caused a slight decrease in the fraction of haemopoietic stem cells in DNA synthesis after 2 wk exposure. This, however, was only determined once at this level and no such effect was seen at 0.08 mg/l. A dose–related effect on dimunition of lymphocyte concentration in the peripheral blood was apparent at 0.08 mg/l or more after 10 exposures in 12 days. Exposures for this same time to concentrations of 0.33 mg/l and above significantly depressed the femur bone marrow cellularity, the CFU levels in the femur and the haematocrit and caused a slight increase in fraction of haemopoietic stem cells in DNA synthesis. This last finding is of questionable significance. The depressed CFU and blood lymphocyte levels seen following exposure to 0.98 mg/l for 2, 4, 8 and 16 weeks had almost completely recovered 16 weeks after ceasing exposure. Early mortality and an increase in mortality rate were noted after 16 weeks exposure to 0.98 mg/l.
Source: BP Chemicals Ltd. London

(516)

Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 178 days
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 10 ppm (converts to 0.032 mg/l); group numbers unspecified
Control Group: yes, concurrent no treatment
NOAEL: < 10 ppm
LOAEL: = 10 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Chamber atmospheres were analysed at half–hour intervals during the daily 6–hour exposures. Control mice received filtered conditioned air. After 32, 66 and 178 days, mice from each group were bled and the peripheral blood cell counts determined. Five animals from each group were selected for red progenitor cell and nucleated red cell determinations.
Result: Exposure group sizes were not reported. Progenitor cells showed markedly reduced abilities to form colonies compared with the controls. The numbers of marrow colony–forming unit–erythroid (CFU–E) colonies progressively declined during the exposure period. Marrow burst–forming unit–erythroid (BFU–E) colonies were significantly depressed at day 66 but had returned to normal by day 178. Splenic CFU–E colony formation was depressed at day 178 but no effect was seen at any monitoring period on splenic BFU–E colony growth. Changes also occurred in the numbers of differentiated haematopoietic cells and the numbers of circulating red blood cells and lymphocytes were significantly depressed. At 178 days, depressions in splenic nucleated cellularity and splenic nucleated red cell numbers were seen.
Source: BP Chemicals Ltd. London

(517)

Species: mouse **Sex:** male/female
Strain: CD–1
Route of admin.: inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 100 mice/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: = 1 ppm
Method: other
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: All animals were examined twice daily for mortality and moribundity throughout the study. Observations for signs of toxicity were made weekly. On days 7, 14, 28, 56 and 91, blood samples were obtained at necropsy from 20 randomly selected mice/sex/group for clinical pathology (hematology and clinical biochemistry). Complete necropsies, organ weight analysis and histopathology were performed on 20 of these sacrificed animals/sex and on all animals found dead or moribund.
Reliability: 1 (valid)
Result: No exposure–related mortality, clinical observations or mean body weight changes were seen. In the high–dosed animals, changes in blood composition, relating to decreased red blood cells, white blood cells and platelets, and an increased incidence and severity of various red cell morphologic changes, which was more pronounced in the males, were noted. Both sexes showed sign. increased glycerol lysis time. In the top dosed males, slight increases were noted in the incidence of small thymuses on days 56 and 91 and spleens on day 56. The authors felt that this was of doubtful biological significance. A statistically significant decrease in mean testis weight and increase in mean female liver/body weight ratio occurred at 0.98 mg/l. Histopathological changes were reported, at the high–dose level, in the thymus, femoral marrow, spleen, mesenteric lymph nodes, mandibular lymph nodes and the liver, the severity increasing with time. Histomorphologic changes in testes and ovaries were recorded at 91 days. In a few instances, the lesions seen at the high–dose level were detected at 0.033 and 0.098 mg/l (thymic atrophy, increased extramedullary hematopoiesis in the spleen, plasma cell infiltration in mandibular lymph nodes, sinus histiocytosis in mesenteric lymph nodes). Male mice were reported to be much more susceptible than the females.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99.9% pure.

(512)

Species: mouse **Sex:** male/female
Strain: other
Route of admin.: inhalation
Exposure period: 2-16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: up to 16 weeks
Doses: 10, 25, 100, 300, 400 ppm (converts to 0.03, 0.08, 0.33, 0.98, 1.3 mg/l); group numbers unspecified
Control Group: yes, concurrent vehicle
LOAEL: = 10 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group [numbers unspecified] were exposed to conditioned air. At various times during and after the exposure period, five to ten mice were removed from both the treated and control groups and the blood, bone marrow and spleens removed and examined.
Reliability: 3 (not valid)
Significant methodological deficiencies
results not separated by sex, no data about the test substance and TS concentration analysis, no clinical biochemistry, no pathology, limited hematology
Result: The lowest benzene concentration caused a slight decrease in the fraction of haemopoietic stem cells in DNA synthesis after 2 wk exposure. This, however, was only determined once at this level and no such effect was seen at 0.08 mg/l. A dose-related effect on dimunition of lymphocyte concentration in the peripheral blood was apparent at 0.08 mg/l or more after 10 exposures in 12 days. Exposures for this same time to concentrations of 0.33 mg/l and above significantly depressed the femur bone marrow cellularity, the CFU levels in the femur and the haematocrit and caused a slight increase in fraction of haemopoietic stem cells in DNA synthesis. This last finding is of questionable significance. The depressed CFU and blood lymphocyte levels seen following exposure to 0.98 mg/l for 2, 4, 8 and 16 weeks had almost completely recovered 16 weeks after ceasing exposure. Early mortality and an increase in mortality rate were noted after 16 weeks exposure to 0.98 mg/l.
Source: Deutsche Shell Chemie GmbH Eschborn

(516)

Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: up to 178 days
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 10 ppm (converts to 0.032 mg/l); group numbers unspecified
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Chamber atmospheres were analysed at half-hour intervals during the daily 6-hour exposures. Control mice received filtered conditioned air. After 32, 66 and 178 days, mice from each group were bled and the peripheral blood cell counts determined. Five animals from each group were selected for red progenitor cell and nucleated red cell determinations.
Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, one dose, no data about test substance or number of animals used, only erythropoietic (hematological) effects studied
Result: Exposure group sizes were not reported. Progenitor cells showed markedly reduced abilities to form colonies compared with the controls. The numbers of marrow colony-forming unit-erythroid (CFU-E) colonies progressively declined during the exposure period. Marrow burst-forming unit-erythroid (BFU-E) colonies were significantly depressed at day 66 but had returned to normal by day 178. Splenic CFU-E colony formation was depressed at day 178 but no effect was seen at any monitoring period on splenic BFU-E colony growth. Changes also occurred in the numbers of differentiated haematopoietic cells and the numbers of circulating red blood cells and lymphocytes were significantly depressed. At 178 days, depressions in splenic nucleated cellularity and splenic nucleated red cell numbers were seen.
Source: Deutsche Shell Chemie GmbH Eschborn

(517)

Species: mouse **Sex:** male
Strain: CD–1
Route of admin.: drinking water
Exposure period: 28 days
Frequency of treatment: continuous
Post. obs. period: none
Doses: 31, 166, 790 mg/l (estimated average doses of 8, 40, 180 mg/kg bw/day); numbers of animals unspecified
Control Group: yes, concurrent no treatment
NOAEL: < 31 mg/l
LOAEL: = 31 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: No overt clinical symptoms of toxicity, no change in food and water consumption, no gross lesions on any of the organs, and no apparent alteration in body weight gain were seen, although the mid– and high–dose groups had slight non–significant increases in growth rate. Spleen weight was dose–dependently decreased (the high–dose group being significantly different from the controls), kidney weight dose–dependently increased (again the highest dosed animals being significant) and thymus weight was lower in all the treated groups, but this was not statistically significant. Dose–dependent, statistically significant decreases were seen in erythrocyte, leukocyte and lymphocyte counts and haematocrit, the latter being statistically significant only in the mid– and high–dose levels, while mean corpuscular volumes increased significantly as the dose increased.
Result: Gross and pathological examinations performed on all mice; major organs were removed and weighed; blood examined. Actual concentrations of benzene in the drinking water were determined by gas chromatographic analysis on several days and were found to range from 28 to 36, 147 to 178 and 734 to 855 mg/l for the three levels respectively. Based on the observed average intakes, the daily dose of benzene in each animal was estimated to be 8, 40 and 180 mg/kg bw respectively.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent; 99.9% pure.

(518)

Species: mouse **Sex:** male
Strain: CD–1
Route of admin.: drinking water
Exposure period: 28 days
Frequency of treatment: continuous
Post. obs. period: none
Doses: 31, 166, 790 mg/l (estimated average doses of 8, 40, 180 mg/kg bw/day); 5 animals/dose
Control Group: yes, concurrent vehicle
LOAEL: = 31 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Gross pathological examinations performed on all mice; major organs were removed and weighed; blood examined. Actual concentrations of benzene in the drinking water were determined by gas chromatographic analysis on several days and were found to range from 28 to 36, 147 to 178 and 734 to 855 mg/l for the three levels respectively. Based on the observed average intakes, the daily dose of benzene in each animal was estimated to be 8, 40 and 180 mg/kg bw respectively.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no clinical biochemistry, one sex, limited hematology, no histopathology
Result: No overt clinical symptoms of toxicity, no change in food and water consumption, no gross lesions on any of the organs, and no apparent alteration in body weight gain were seen, although the mid– and high–dose groups had slight non–significant increases in growth rate. Spleen weight was dose–dependently decreased (the high–dose group being significantly different from the controls), kidney weight dose–dependently increased (again the highest dosed animals being significant) and thymus weight was lower in all the treated groups, but this was not statistically significant. Dose–dependent, statistically significant decreases were seen in erythrocyte, leukocyte and lymphocyte counts and haematocrit, the latter being statistically significant only in the mid– and high–dose levels, while mean corpuscular volumes increased significantly as the dose increased.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent; 99.9% pure.

(518)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 17 weeks
Frequency of treatment: 5 days/week
Post. obs. period: none
Doses: 25, 50, 100, 200, 400, 600 mg/kg bw/day; 10 or 15 mice/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: = 25 mg/kg bw
LOAEL: = 50 mg/kg bw
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn oil. Animals were checked twice daily and weights recorded weekly. Five animals of each sex from the control, 200 and 600 mg/kg bw groups were killed on day 60. Survivors from all groups killed at end of the study period and haematologic analyses were performed on five animals from each group. At necropsy, a large number of organs from animals from the control, the interim–kill and the top–dose groups were removed and examined histopathologically; spleens were examined in all dose groups.
Result: Groups of ten or 15 animals of each sex were used. No compound–related deaths. Mean body weight gain was dose–dependently decreased in both the males and females at 100 mg/kg bw or more. Tremors were observed intermittently in the 400 and 600 mg/kg bw groups, those during the last 3 weeks of the study being more pronounced in the males than females. At day 60, no changes in the white blood cell or lymphocyte counts were seen in either the male or female animals given 200 or 600 mg/kg bw. Both counts were dose–dependently and significantly ($p < 0.05$) decreased at the end of the study in the males at 50 mg/kg bw or more and in the females at 600 mg/kg bw in the case of the white blood cells and at 400 mg/kg bw or more in the case of the lymphocytes. No compound–related histopathological changes were observed.
Source: BP Chemicals Ltd. London
Test substance: >99.7% pure.

(514)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 days/week
Post. obs. period: 1 week
Doses: 25, 50, 100 mg/kg bw/day; 60 mice/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: < 25 mg/kg bw
LOAEL: = 25 mg/kg bw
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn-oil. Blood was drawn from ten randomly preselected animals from each sex and dose group at 0, 3, 6, 9, 12, 15, 18 and 21 months and from all animals at the terminal kill at 24 months. Ten animals of each sex and group were killed after 51 weeks and necropsies were performed. Animals were observed twice daily and weighed weekly for 13 wk and monthly thereafter.
Result: A decrease in weight gain was noted in the high-dosed males and females after 47 and 87 weeks respectively. No other clinical signs related to benzene exposure were seen. The survival rates were significantly decreased in the high-dosed male and female animals. Various non-neoplastic lesions were found in the zymbal gland, preputial gland, harderian gland, adrenal gland, ovary, lung, spleen, bone marrow and forestomach of the treated animals. Decreased white blood cell counts, which were variable in the female animals, were recorded throughout the study. See section 5.8 for carcinogenic effects.
Source: BP Chemicals Ltd. London
Test substance: >99.7% pure.

(519)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 17 weeks
Frequency of treatment: once daily, 5 days/week
Post. obs. period: none
Doses: 25, 50, 100, 200, 400, 600 mg/kg bw/day; 10 or 15 mice/sex/dose
Control Group: yes, concurrent vehicle
NOAEL: = 25 mg/kg bw
LOAEL: = 50 mg/kg bw
Method: other
Year: 1981 **GLP:** no data
Test substance: other TS
Remark: Benzene administered in corn oil. Animals were checked twice daily and weights recorded weekly. Five animals of each sex from the control, 200 and 600 mg/kg bw groups were killed on day 60. Survivors from all groups killed at end

of the study period and haematologic analyses were performed on five animals from each group. At necropsy, a large number of organs from animals from the control, the interim-kill and the top-dose groups were removed and examined histopathologically; spleens were examined in all dose groups.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no clinical biochemistry, no ophtalmological examination, limited hematology

Result: Groups of ten or 15 animals of each sex were used. No compound-related deaths. Mean body weight gain was dose-dependently decreased in both the males and females at 100 mg/kg bw or more. Tremors were observed intermittently in the 400 and 600 mg/kg bw groups, those during the last 3 weeks of the study being more pronounced in the males than females. At day 60, no changes in the white blood cell or lymphocyte counts were seen in either the male or female animals given 200 or 600 mg/kg bw. Both counts were dose-dependently and significantly ($p < 0.05$) decreased at the end of the study in the males at 50 mg/kg bw or more and in the females at 600 mg/kg bw in the case of the white blood cells and at 400 mg/kg bw or more in the case of the lymphocytes. No compound-related histopathological changes were observed.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: >99.7% pure.

(514)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: once daily, 5 days/week
Post. obs. period: 1 week
Doses: 25, 50, 100 mg/kg bw/day; 60 mice/sex/dose
Control Group: yes, concurrent vehicle
LOAEL: = 25 mg/kg bw
Method: other
Year: 1981 **GLP:** no data
Test substance: other TS

Remark: Benzene administered in corn-oil. Blood was drawn from ten randomly preselected animals from each sex and dose group at 0, 3, 6, 9, 12, 15, 18 and 21 months and from all animals at the terminal kill at 24 months. Ten animals of each sex and group were killed after 51 weeks and necropsies were performed. Animals were observed twice daily and weighed weekly for 13 wk and monthly thereafter.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no clinical biochemistry, no urinanalysis, limited hematology

Result: A decrease in weight gain was noted in the high-dosed males and females after 47 and 87 weeks respectively. No other clinical signs related to benzene exposure were seen. The

survival rates were significantly decreased in the high-dosed male and female animals. Various non-neoplastic lesions were found in the zymbal gland, preputial gland, harderian gland, adrenal gland, ovary, lung, spleen, bone marrow and forestomach of the treated animals. Decreased white blood cell counts, which were variable in the female animals, were recorded throughout the study. See section 5.7 for carcinogenic effects. LOEL related to noncarcinogenic effects.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99.7% pure.

(519)

Species: rabbit **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: 243 days
Frequency of treatment: 7 hours per day/5 days per week
Post. obs. period: 18-22 hours
Doses: 0.26 mg/l; 1 or 2 rabbits tested
Control Group: yes, concurrent no treatment
NOAEL: < .26 mg/l
LOAEL: = .26 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: The animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.
Result: Unspecified histopathological changes in the kidney, testes and blood (including leucopenia) were reported.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Species: rabbit **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: 243 days
Frequency of treatment: 7 hours per day, 5 days per week
Post. obs. period: 18–22 hours
Doses: 0.26 mg/l; 1 or 2 rabbits tested
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Reliability: 3 (not valid)
Significant methodological deficiencies
one dose, low number of animals, one sex, no clinical biochemistry, limited hematology, limited pathology, no further data about results of enforced investigations
The animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy. TS concentration analysed (no further data) and adjusted, 10% variation.
Result: Unspecified histopathological changes in the kidney, testes and blood (including leucopenia) were reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.98% pure.

(491)

Species: rabbit **Sex:** female
Strain: New Zealand white
Route of admin.: s.c.
Exposure period: 10 days
Frequency of treatment: daily
Post. obs. period: 30 days
Doses: 0.25, 0.5 ml/kg bw/day (converts to 219.5, 439 mg/kg bw/day); 8 or 9 rabbits/dose
Control Group: yes, concurrent vehicle
NOAEL: < 219.5 mg/kg bw
LOAEL: = 219.5 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene administered as a 50% (v/v) solution in corn oil.
Blood was collected from the middle ear artery.
Result: A dose– and time–dependent decrease in circulating lymphocytes which partially recovered 30 days after treatment, was noted. Circulating monocytes were significantly decreased in the top–dosed animals.
Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade benzene tested.

(520)

Species: rabbit **Sex:** female
Strain: New Zealand white
Route of admin.: s.c.
Exposure period: 10 days
Frequency of treatment: once daily
Post. obs. period: up to 40 days
Doses: 0, 0.25, 0.5 ml/kg bw/day (converts to 0, 219.5, 439 mg/kg bw/day); 6, 8 and 9 rabbits/dose, respectively
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene administered as a 50% (v/v) solution in corn oil. Blood was collected from the middle ear artery at different intervals (day 0 and 1-40).
Reliability: 3 (not valid)
Unsuitable test system
only effects on lymphocytes studied, no other toxicological effects reported
Result: A dose- and time-dependent decrease in circulating lymphocytes (80% reduction in the high dose group,35% at 0.25 ml/kg) which partially recovered 30 days after treatment, was noted. Circulating monocytes were significantly decreased in the top-dosed animals at day 10. Immunoglogin surface receptor-negative cells (presumably T-lymphocytes) were relatively unaffected.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade benzene, thiophene-free, checked by GCMS

(521)

Species: guinea pig **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: up to 269 days
Frequency of treatment: 7 hours per day/5 days per week
Post. obs. period: 18-22 hours
Doses: 0.28 mg/l; 5 or 10 guinea pigs tested
Control Group: yes, concurrent no treatment
NOAEL: < .28 mg/l
LOAEL: = .28 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of

these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.

Result: A group of five or ten animals used. Increased kidney, spleen and testes weights, growth depression and unspecified histopathological changes in the bone marrow and blood (including leucopenia) were noted.

Source: BP Chemicals Ltd. London

Test substance: 99.98% pure.

(491)

Species: guinea pig **Sex:** male

Strain: no data

Route of admin.: inhalation

Exposure period: 32 or 269 days

Frequency of treatment: 7 hours per day, 5 days per week

Post. obs. period: 18-22 hours

Doses: 0.28 mg/l; 5 to 10 guinea pigs tested

Control Group: yes, concurrent vehicle

Method: other

Year: **GLP:** no data

Test substance: other TS

Remark: Reliability: 3 (not valid)
Significant methodological deficiencies
one dose, low number of animals, one sex, no clinical biochemistry, limited hematology, limited pathology, no further data about results of enforced investigations, no statistical evaluation
TS concentration analysed (no further data) and adjusted, variation was 10% of desired concentration.
All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.

Result: After 269 d increased spleen and testes weights, growth depression and unspecified histopathological changes in the bone marrow and blood (including leucopenia) were noted. After 32 d increased kidney weight and unspecified histopathological changes in blood (including leucopenia).

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.98% pure.

(491)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium strains TA97, 98, 100, 1535
Concentration: 0, 0.01, 0.033, 0.1, 0.333, 1.0 mg/plate
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay"
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Metabolic activation system was Aroclor-1254-induced S9 from male Sprague-Dawley rats and Syrian hamsters. Benzene tested at a minimum of five doses up to a toxic dose or the limit of solubility, to a maximum dose of 10 mg/plate. Triplicate plates were used. The concentration of S9 in the S9 mix was 10 or 30%. The positive controls were 9-aminoacridine, 2-aminoanthracene, 4-nitro-O-phenylenediamine and sodium azide.
Several other investigators have also reported negative findings in S. typhimurium reverse mutation assays using several tester strains. Some of these include Cotruvo J.A. et al. Ann. N.Y. Acad. Sci. 298, 124-140, 1977; Florin I. et al. Toxicology 15, 219-232, 1980; Matsushima T. et al. Prog. Mutation Res. 5, 181-186, 1985; Shimizu M. et al. Mutation Res. 116, 217-238, 1983.
Source: BP Chemicals Ltd. London
Test substance: 99% pure.

(522)

Type: Ames test
System of testing: Salmonella typhimurium strains TA97, 98, 100, 1535
Concentration: 0, 0.01, 0.033, 0.1, 0.333, 1.0 mg/plate
Metabolic activation: with and without
Result: negative
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Metabolic activation system was Aroclor-1254-induced S9 from male Sprague-Dawley rats and Syrian hamsters. Benzene tested at a minimum of five doses up to a toxic dose or the limit of solubility, to a maximum dose of 10 mg/plate. Triplicate plates were used. The concentration of S9 in the S9 mix was 10 or 30%. The positive controls were 9-aminoacridine, 2-aminoanthracene, 4-nitro-O-phenylenediamine and sodium azide.
Reliability: 1 (valid)
Comparable to guideline study
TA 97 instead of TA 1537 used
results: Benzene showed no evidence of mutagenicity in any of the strains when tested in the absence of metabolic activation or in the presence of 10 or 30% hamster or rat liver S9. Several other investigators have also reported

negative findings in S. typhimurium reverse mutation assays using several tester strains. Some of these include Cotruvo J.A. et al. Ann. N.Y. Acad. Sci. 298, 124-140, 1977; Florin I. et al. Toxicology 15, 219-232, 1980; Matsushima T. et al. Prog. Mutation Res. 5, 181-186, 1985; Shimizu M. et al. Mutation Res. 116, 217-238, 1983.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99% pure.

(522)

Type: Cytogenetic assay

System of testing: Balb/c-3T3 mouse embryo cells

Concentration: 0.5, 1.67, 2.0, 3.33, 4.0, 5.0 mg/ml

Metabolic activation: with and without

Result: positive

Method: Directive 87/302/EEC, part B, p. 73 "Mutagenicity: - In vitro mammalian cell transformation tests"

Year: 1988 **GLP:** no data

Test substance: other TS

Remark: A cytotoxicity study was initially carried out to determine the benzene doses to be tested in the cell transformation assay. Doses causing 10-100% survival of the cells were used. Cells were treated with benzene for 72 hours, washed and incubated for a further 24 days. Transformed foci were scored microscopically. Primary rat-liver cells were prepared from Fischer-344 rats and lethally X-irradiated. This made up the activation system and was added to the mouse embryo cells for 3 hours before treatment with benzene for 48 hours. The cultures were then treated with 12-O-tetra-decenoylphorbol-13-acetate biweekly for 3 weeks, starting 1-2 days after benzene treatment.
Dimethylnitrosamine and 3-methylcholanthrene were used as the positive controls in the tests conducted with and without the addition of the rat liver cells respectively. Untreated cells were the negative controls. The tests were conducted in closed tissue culture flasks.
In the cytotoxicity test, 59% survival was seen at doses of 3.0 and 3.33 mg/ml in the absence and presence of activation respectively. All cells were killed at 6.0 mg/ml in the absence of activation and 0.3% survival was found at 5.0 mg/ml with activation. In the transformation assay, doses of 0.5, 2.0 and 4.0 mg/ml and 1.67, 3.33 and 5.0 mg/ml were tested without and with activation respectively. In the absence of activation, no transformation activity was found. In the presence of activation, statistically significant effects were noted at all dose levels, the increases in the numbers of transformed cell foci being dose-related. A second set of investigators also reported a lack of transforming activity in this cell line when benzene was tested in the absence of activation. The test was not conducted, in this case, with added activation (Rundell J.O. et al. Envir. Sci. Res. 27, 309-324, 1983).

Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade; purity unspecified.

(523)

Type: Cytogenetic assay
System of testing: C3H/10T½ mouse embryonic fibroblast cells
Concentration: 50, 80, 100, 160, 200, 320 µg/ml
Metabolic activation: with and without
Result: negative
Method: Directive 87/302/EEC, part B, p. 73 "Mutagenicity: – In vitro mammalian cell transformation tests"
Year: 1988 **GLP:** no data
Test substance: other TS
Remark: Based on the preliminary toxicity tests, benzene was tested at concentrations of 50, 100 and 200 µg/ml in the absence and 80, 160 and 320 µg/ml in the presence of S9. No transformed foci were identified at any benzene concentration in the presence or absence of S9. A similar negative result in this cell line in the absence of activation was reported by Nesnow S. et al. Prog. Mutation Res. 5, 659–664, 1985.
Benzene was dissolved and diluted in dimethylsulphoxide before use. The metabolic activation system (S9) was obtained from the liver of Aroclor–1254–treated male rats. A dose–ranging study was conducted to determine the doses to be used in a preliminary cytotoxicity test. The latter was conducted to establish the range of concentrations to be used in the cell transformation assay. In the transformation assay, the cells were exposed to benzene for 24 hours in the presence or absence of S9 mix. One of the treated cultures/dose was used to assess cell survival in the study. In the remaining cultures, the medium was changed twice weekly until the cells reached confluence and weekly thereafter. After an 8–week incubation period, the cultures were stained and assessed for cell transformation. Dimethylsulphoxide was used as the negative control chemical and cyclophosphamide as the positive control in the presence of S9. In the absence of the activation mix, 3–methylcholanthrene acted as the positive control.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(524)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 200, 600, 1800 µg/ml
Metabolic activation: with and without
Result: positive
Method: OECD Guide–line 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: In cultures fixed after 3 hours, increases in the frequency of chromosomal aberrations were seen in the absence and presence of S9 at 1800 and 600 µg/ml respectively. No such effects were found after 12 hours and after 18 hours, increases were observed at 200 and 600 µg/ml when in the

presence of S9. A second study conducted on a Chinese hamster lung fibroblast cell line also showed benzene-induced chromosome aberrations when tested in the presence but not the absence of S9 (Ishidate M. Jr & Sofuni T. Prog. Mutation Res. 5, 427-432, 1985).
The metabolic activation system (S9 mix) was prepared from the livers of phenobarbital- and ß-naphthoflavone-induced male Sprague-Dawley/CD rats. Benzene was dissolved in dimethylsulphoxide to achieve the required concentration. The negative control consisted of both untreated and solvent treated cultures. Cyclophosphamide and aflatoxin B1 were used as the positive controls. Benzene was tested at concentrations of 200, 600 and 1800 µg/ml, the top dose giving approximately 50% inhibition of the mitotic index. Cells were treated for 3 hours, the medium aspirated and cultures washed. Some cultures were fixed while others were resuspended in fresh medium and incubated for 6 or 12 hours. Colchicine was added to these cultures and 3 hours later they were fixed.

Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(525)

Type: Cytogenetic assay
System of testing: human lymphocytes
Concentration: 9, 44, 88 µg/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1982 **GLP:** no data
Test substance: other TS
Remark: In the cytotoxicity study, a 50-80% reduction in mitosis was seen in cultures treated with 88 µg/ml when compared with the negative control. The mutagenicity test was conducted at benzene concentrations of 9, 44 and 88 µg/ml. A dose-related increase in chromosomal aberrations was noted in both the male and female donated lymphocytes treated in the absence and presence of S9. The incidence of all aberrations, excluding chromatid gaps, was statistically significantly increased over the negative controls, with all treatment regimes; this being more pronounced in the males than females. A second set of investigators reported chromosomal aberrations in human lymphocytes treated in the absence of metabolic activation (Morimoto K. Jap. J. ind. Hlth 18, 23-34, 1976), however, negative results in the absence of activation have also been reported (Gerner-Smidt P. & Friedrich U. Mutation Res. 58, 313-316, 1978).
The method of Richardson C.R. & Wildgoose J. Mutation Res. 100, 287-293, 1982 was followed. Benzene was dissolved in dimethylsulphoxide before use and dimethyl-sulphoxide was used to treat the negative control cultures. Metabolic activation system was derived from the liver of Aroclor-1254-induced rats. Blood was withdrawn from a donor of each sex who had a previously established low incidence of chromosomal damage. Preliminary cytotoxicity tests were

performed in the absence and presence of S9. In the chromosome aberration test, the lymphocyte cultures were treated with benzene, 44 hours after initiation for 3 hours. When activation was used, benzene and the relevant controls were incubated for 30 min with S9 before being added to the blood cultures. The cultures exposed to benzene were agitated throughout the exposure period to maintain homogenicity of the chemical through the culture. Mitomycin C was used as the positive control substance in the absence of S9 and cyclophosphamide in the presence of S9. Lymphocytes were treated with colchicine before analysis.

Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade; 99% pure.

(526)

Type: Cytogenetic assay

System of testing: Chinese hamster ovary cells

Concentration:

Metabolic activation: with and without

Result: negative

Method: other

Year: 1985 **GLP:** no data

Test substance: other TS

Remark: Benzene failed to induce chromosome aberrations when in either the absence or presence of S9. Similar negative findings have also been reported in this same cell line by Natarajan A.T. et al. Prog. Mutation Res. 5, 433-437, 1985; in a Chinese hamster fibroblast cell line in the absence of a metabolic activation system by Danford N. ibid. 5, 397 411, 1985; and in Wistar rat liver cells in the absence of metabolic activation by Priston R.A.J. & Dean B.J. ibid. 5, 387-395, 1985.
The method of Galloway S.M. et al. Envir. Mutagen. 7, 1-51, 1985 was followed. Benzene was dissolved in dimethylsulphoxide to the required test concentration. The metabolic activation system (S9) was prepared from Aroclor-1254-induced male Sprague-Dawley rats. The dose levels used in the study were selected based on the toxicity of benzene observed in concurrently run sister-chromatid exchange studies. In the absence of S9 treatment, cells were exposed to benzene for 10 hours. Colcemid was added 2-3 hours prior to cell harvest. In the presence of S9, cells were treated with benzene and S9 for 2 hours. The cells were then washed, returned to complete medium and incubated for 11 hours, colcemid being added 2 hours before harvest.

Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade; >=99% pure.

(527) (528)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 1.33 mg/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: After a treatment time of 1 hour, 20.9 and 33.0% survival was seen in the cells treated without and with S9 respectively. Single-strand breaks were greatly increased after 30 min, 1 and 2 hours treatment in the absence of S9; the increase being time-dependent. No such effect was noted in the presence of S9.
The metabolic activation system was the S9 fraction from Aroclor-1254-induced rat liver. Benzene was dissolved in dimethylsulphoxide. The cells were incubated in the presence of benzene for 30 min, 1 or 2 hours. Metabolic activation was only added in the 1 hour study. Survival of the cells was also checked. The single-strand DNA breaks were detected by high-salt alkaline sucrose-gradient sedimentation. Untreated cells acted as the negative control and ethylmethanesulphonate and diethyl-nitrosamine treated cells as the positive controls.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(529)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 0.39-7.81 mg/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide before addition to the cells. Metabolic activation system was derived from the liver of Aroclor-1254 induced Sprague-Dawley rats (S9 mix). Cells were treated for 1 hour with benzene, washed and subjected to alkaline sucrose sedimentation analysis in order to assess the DNA single-strand breaks. Prior to testing benzene, its cytotoxicity was determined by examining inhibition of cell growth.
The cytotoxicity threshold, that is, the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 µg/ml when tested in the presence and absence of S9. In the mutagenicity study, benzene was tested at levels of 0.39-7.81 mg/ml. No single-strand breaks were found at levels up to 2.34 mg/ml tested with or without S9. Above this, single-strand breaks increased but this was thought to be due to the toxic effects of benzene. Other investigators have reported

similar negative results using the alkaline elution technique in Chinese hamster V79 cells in the presence and absence of metabolic activation (Swenberg J.A. et al. Biochem. biophys. Res. Commun. 72, 732-738, 1976) and rat hepatocytes in the absence of activation (Bradley M.O. Prog. Mutation Res. 5, 353-357, 1985; Sina J.F. et al. Mutation Res. 113, 357-391, 1983).

Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade; purity unspecified.

(530)

Type: Cytogenetic assay

System of testing: Syrian hamster embryo (SHE) cells

Concentration: 20-500 µg/ml

Metabolic activation: without

Result: positive

Method: other

Year: **GLP:** no data

Test substance: other TS

Remark: Lethally X-irradiated SHE cells were seeded and 24 hours later target cells were added. After 24 hours, benzene was added and the cells were incubated for 6 days. The medium was then removed, the cells rinsed and benzene added for a further 24 hours. The cells were then washed and transformation activity assessed. Benzene was dissolved in dimethylsulphoxide. Benzo(a)pyrene was used as the positive control.
Positive results were obtained at concentrations of 20 µg/ml and above. However, at concentrations higher than 300 µg/ml, the transformation frequency decreased. Similar positive results in the absence of activation have been reported in this cell line by Barrett J.C. & Lamb P.W. Prog. Mutation Res. 5, 623-628, 1985. Amacher and Zelljadt reported a low transformation frequency in SHE cells when tested in the absence of metabolic activation (Amacher D.E. & Zelljadt I. Carcinogenesis 4, 291-295, 1983) and a negative result has been reported in the SHE cell-transformation enhancement assay using SA7 viruses, again in the absence of activation (Hatch G.G. & Anderson T.M. Prog. Mutation Res. 5, 629-638, 1985).

Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade; 99% pure.

(531)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 7.81-7810 µg/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide before addition to the culture medium. Aroclor-1254-induced Sprague-Dawley rat-liver S9 was used as the metabolic activation system. A preliminary cytotoxicity test was conducted in which cells were incubated with a range of benzene concentrations with and without S9 for 24 hours. From this test, the concentrations to be used in the micronucleus assay were selected. In the micronucleus assay, cells were treated for 1 hour, washed and incubated in normal medium for 24 hours. The micronucleus frequency was then assessed. Historic dimethylsulphoxide control data were used as the background readings.
In the preliminary study, the cytotoxicity threshold, that is the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 µg/ml when in both the absence and presence of S9. In the micronucleus assay, no evidence of mutagenicity was seen with and without S9 at concentrations of 7.81 mg/ml to 7.81 µg/ml.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(530)

Type: Cytogenetic assay
System of testing: Chinese hamster V79 cells
Concentration:
Metabolic activation: no data
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Induction of micronuclei was reported in cells treated with anti-benzene-diol-epoxide, quinone, hydroquinone, catechol, phenol, 1,2,4-trihydroxybenzene, 1,2,3-trihydroxybenzene and duroquinone. The potency of this effect varied between the chemicals. No such effect was seen with syn-benzene-diol-epoxide and trans-benzene-1,2-dihydrodiol. Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compound for 24 hours. They were then harvested and scored for micronuclei. Compounds were tested up to their cytotoxic limits.

Source: BP Chemicals Ltd. London

(532)

Type: Cytogenetic assay
System of testing: human lymphocytes
Concentration: 9, 44, 88 ug/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about mitogen, harvest time, mitotic index
results: In the cytotoxicity study, a 50-80% reduction in mitosis was seen in cultures treated with 88 ug/ml when compared with the negative control. The mutagenicity test was conducted at benzene concentrations of 9, 44 and 88 ug/ml. A dose-related increase in chromosomal aberrations was noted in both the male and female donated lymphocytes treated in the absence and presence of S9. The incidence of all aberrations, excluding chromatid gaps, was statistically significantly increased over the negative controls, with all treatment regimes; this being more pronounced in the males than females (not significant in females at the low dose +/- S9 and at mid dose -S9).
A second set of investigators reported chromosomal aberrations in human lymphocytes treated in the absence of metabolic activation (Morimoto K., Jap. J. ind. Hlth 18, 23-34, 1976), however, negative results in the absence of activation have also been reported (Gerner-Smidt P. & Friedrich U., Mutation Res. 58, 313-316, 1978).
The method of Richardson C.R. & Wildgoose J. Mutation Res. 100, 287-293, 1982 was followed. Benzene was dissolved in dimethylsulphoxide before use and dimethyl-sulphoxide was used to treat the negative control cultures. Metabolic activation system was derived from the liver of Aroclor-1254-induced rats. Blood was withdrawn from a donor of each sex who had a previously established low incidence of chromosomal damage. Preliminary cytotoxicity tests were performed in the absence and presence of S9. In the chromosome aberration test, the lymphocyte cultures were treated with benzene, 44 hours after initiation for 3 hours. When activation was used, benzene and the relevant controls were incubated for 30 min with S9 before being added to the blood cultures. The cultures exposed to benzene were agitated throughout the exposure period to maintain homogenicity of the chemical through the culture. Mitomycin C was used as the positive control substance in the absence of S9 and cyclophosphamide in the presence of S9.
Lymphocytes were treated with colchicine before analysis.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(526)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 200, 600, 1800 ug/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Comparable to guideline study
results:
In cultures fixed after 3 hours, significant increases in the frequency of chromosomal aberrations were seen in the absence and presence of S9 at 1800 and 600 ug/ml respectively. No such effects were found after 12 hours and after 18 hours, significant increases were observed at 200 and 600 ug/ml in the presence of S9. A second study conducted on a Chinese hamster lung fibroblast cell line also showed benzene-induced chromosome aberrations when tested in the presence but not the absence of S9 (Ishidate M. Jr & Sofuni T., Prog. Mutation Res. 5, 427-432, 1985). The metabolic activation system (S9 mix) was prepared from the livers of phenobarbital- and beta-naphthoflavone-induced male Sprague-Dawley/CD rats. Benzene was dissolved in dimethylsulphoxide to achieve the required concentration. The negative control consisted of both untreated and solvent treated cultures. Cyclophosphamide and aflatoxin B1 were used as the positive controls. Benzene was tested at concentrations of 200, 600 and 1800 ug/ml, the top dose giving approximately 50% inhibition of the mitotic index. Cells were treated for 3 hours, the medium aspirated and cultures washed. Some cultures were fixed while others were resuspended in fresh medium and incubated for 6 or 12 hours. Colchicine was added to these cultures and 3 hours later they were fixed.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(525)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: not specified
Metabolic activation: with and without
Result: negative
Method: other
Year: 1985 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about mitotic index, single harvest time
results:
Benzene failed to induce chromosome aberrations when in either the absence or presence of S9. Similar negative

findings have also been reported in this same cell line by Natarajan A.T. et al. Prog. Mutation Res. 5, 433-437, 1985; in a Chinese hamster fibroblast cell line in the absence of a metabolic activation system by Danford N. ibid. 5, 397 411, 1985; and in Wistar rat liver cells in the absence of metabolic activation by Priston R.A.J. & Dean B.J. ibid. 5, 387-395, 1985.
The method of Galloway S.M. et al. Envir. Mutagen. 7, 1-51, 1985 was followed. Benzene was dissolved in dimethylsulphoxide to the required test concentration. The metabolic activation system (S9) was prepared from Aroclor-1254-induced male Sprague-Dawley rats. The dose levels used in the study were selected based on the toxicity of benzene observed in concurrently run sister-chromatid exchange studies. In the absence of S9 treatment, cells were exposed to benzene for 10 hours. Colcemid was added 2-3 hours prior to cell harvest. In the presence of S9, cells were treated with benzene and S9 for 2 hours. The cells were then washed, returned to complete medium and incubated for 11 hours, colcemid being added 2 hours before harvest.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; >=99% pure.

(527) (533)

Type: Cytogenetic assay
System of testing: Chinese hamster fibroblast cell line CH1-L
Concentration: 25, 62, 125, 250 ug/ml
Metabolic activation: without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: method: preliminary cytotoxicity test; triplicate test/concentration; positive and negative solvent control (DMSO); incubation with the test substance for 36 h, colcemid added last 3 h; 2 independent replicates; 300 cells analysed (pos. control 200).
results: significant hypo- and hyper-diploidy at 62 ug/ml, highly significant hyper-ploidy at the top concentration.
Reliability: 1 (valid)
Comparable to guideline study
single harvest time
Source: Deutsche Shell Chemie GmbH Eschborn

(534)

Type: Cytogenetic assay
System of testing: human lymphocytes cultures
Concentration: 4E-5 M and 3E-3 M
Metabolic activation: no data
Result: positive
Method: other: see ref.
Year: 1976 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(535)

Type: Gene mutation in Saccharomyces cerevisiae
System of testing: Saccharomyces cerevisiae strains XV185-14C and RM52
Concentration: 274.7, 549.4, 1098.8, 2197.5 µg/ml
Metabolic activation: with and without
Result: positive
Method: OECD Guide-line 480 "Genetic Toxicology: Saccharomyces cerevisiae Gene Mutation Assay"
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: In a buffer medium at pH7.0, benzene induced histidine point mutations in the presence of S9 in XV185-14C. No tryptamine point mutations in XV185-14C were recorded. With treatment in a yeast extract peptone dextrose medium at pH6.3, arginine, histidine and tryptamine point mutations were induced in XV185-14C in the absence of S9; histidine point mutations were also present with S9 treatment. No histidine point mutations were found in RM52. A second set of investigators reported point mutations in S. cerevisiae strain D7 when treated with but not without S9 (Parry J.M. & Eckardt F. Prog. Mutation Res. 5, 261 269, 1985). Negative point mutation results have also been reported in strains PV-1, PV-2, PV-3 (Inge-Vechtomov S.G. et al. ibid. 5, 243-255, 1985) and D7 (Arni P. ibid. 5, 217-224, 1985), all tested in the presence and absence of metabolic activation. The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative control. The cultures were incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(536)

Type: Gene mutation in Saccharomyces cerevisiae
System of testing: Saccharomyces cerevisiae strains XV185-14C and RM52
Concentration: 274.7, 549.4, 1098.8, 2197.5 ug/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about 2nd independent experiment, 4 instead of 5 recommended concentrations
results:
In a buffer medium at pH7.0, benzene induced histidine point mutations in the presence of S9 in XV185-14C. No tryptamine point mutations in XV185-14C were recorded. With treatment in a yeast extract peptone dextrose medium at pH6.3, arginine, histidine and tryptamine point mutations were induced in XV185-14C in the absence of S9; histidine point mutations were also present with S9 treatment. No histidine point mutations were found in RM52. A second set of investigators reported point mutations in S. cerevisiae strain D7 when treated with but not without S9 (Parry J.M. & Eckardt F. Prog. Mutation Res. 5, 261 269, 1985). Negative point mutation results have also been reported in strains PV-1, PV-2, PV-3 (Inge-Vechtomov S.G. et al. ibid. 5, 243-255, 1985) and D7 (Arni P. ibid. 5, 217-224, 1985), all tested in the presence and absence of metabolic activation. The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative solvent control. The cultures were incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(536)

Type: HGPRT assay
System of testing: Chinese hamster V79 cells
Concentration: 0.1, 0.3, 1.0, 2.0 mg/ml
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 476 "Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests"
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Preliminary cytotoxicity studies were carried out using this same cell-line, with and without 5% foetal calf serum. Benzene was added over a concentration range of 5 µg/ml to 10 mg/ml in the presence and absence of a metabolic

activation system (S9 mix). Cells were examined microscopically at intervals up to 4 hr for evidence of damage. Based on the results of this study, a dose range was chosen for use in assays for inhibition of colony-forming ability. This was conducted in the presence and absence of serum and the presence and absence of S9 mix for periods of up to 4 hr. For the mutagenicity assay, the cells were exposed to benzene for 1 hr in the absence of serum and the presence and absence of S9 mix. Cyclophosphamide and ethylmethanesulphonate were used as positive controls, in the presence and absence of S9 mix respectively. The concentrations of benzene tested included at least one dose that induced measurable cytotoxicity and one higher dose. The S9 mix used was derived from livers of male albino rats treated with phenobarbital and ß-naphthoflavone.
The maximum non-toxic benzene dose in the cytotoxicity assay was 0.31 mg/ml when tested for 4 hr in the presence of serum and the presence and absence of S9 or in the absence of serum and the presence of S9. When tested without S9, in the absence of serum, the maximum non-toxic dose was 0.63 mg/ml. In the colony forming assay, the highest non-toxic dose when tested with and without serum and with and without S9 mix was 0.003 mg/ml and the concentration which killed 50% of the cells in the presence of serum and the absence and presence of S9 mix was 1 mg/ml and >1 mg/ml in the absence of serum. In the mutagenicity assay, the mutation frequency was evaluated 5 and 8 days after exposure. No mutagenic activity was found. A similar study conducted at benzene concentrations of 0.1 to 0.5 mg/ml and in the absence of a metabolic activation system, also failed to show any mutagenic activity (Elmore E. et al. Prog. Mutation Res. 5, 597-612, 1985).

Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(537)

Type: HGPRT assay
System of testing: Chinese hamster V79 cells
Concentration: 1, 10, 50, 100, 500, 1000 µg/ml
Metabolic activation: with and without
Result: positive
Method: OECD Guide-line 476 "Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests"
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: A preliminary cytotoxicity study was carried out using this same cell line treated with 1, 10, 50, 100 and 500 µg/ml for 3 hours. The treated cells were then rinsed with Hanks' solution and incubated in normal medium for a further 7 days before the colony-forming activity was evaluated. The mutagenic activity was determined by the 'replating' method. Cells were treated for 3 hours with benzene, rinsed with Hanks' solution and then incubated in normal medium for 6 days. They were then treated with trypsin, replated in

medium containing 6-thioguanine and incubated for 14 days. The number of 6-thioguanine-resistant colonies was scored. The assay was conducted in the absence and presence of a metabolic activation system (S9 mix) which had been obtained from male Sprague-Dawley rats treated with an intraperitoneal injection of phenobarbital and 5,6-benzoflavone. Benzene was dissolved in dimethylsulphoxide before use and diluted with Hanks' solution to obtain the appropriate concentration for treatment of cells. The vehicle alone was tested as the 0 µg benzene/ml level and ethyl-methanesulphonate was the positive control chemical.
Cells treated with benzene at 100 and 500 µg/ml had slightly decreased colony-forming activities, showing slight cytotoxicity. A slight mutagenic effect was noted at 50 and 100 µg/ml, tested in the absence of S9 mix. No mutations were noted at any concentration in the presence of S9 mix.

Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(538)

Type: HGPRT assay
System of testing: Chinese hamster V79 cells
Concentration: see remarks
Metabolic activation: no data
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
no data about metabolic activation, concentration used, test substance, survival, positve control, number of tests
results: Mutations to 6-thioguanine resistance were induced by quinone and hydroquinone (potent inducer), anti-benzene-diol-epoxide, catechol, trans-benzene-1,2-dihydrodiol and 1,2,4-trihydroxybenzene. The cytotoxicity and potency of the mutagenic effect varied between the chemicals. Phenol, the dihydroxybiphenyls and duroquinone showed no mutagenic effect. Anti-benzene-diol epoxide induced a significant but weak mutation to ouabain resistance.
Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxybiphenyl, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone and anti-benzene-diol-epoxide were also included. Compounds were tested up to their cytotoxic limits. Cells were mixed with the test compound for 24 hours and mutants were determined after an expression period of 6 days.
Source: Deutsche Shell Chemie GmbH Eschborn

(532)

Type: HGPRT assay
System of testing: Chinese hamster V79 cells
Concentration: 0.1, 0.3, 1.0, 2.0 mg/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Preliminary cytotoxicity studies were carried out using this same cell-line, with and without 5% foetal calf serum. Benzene was added over a concentration range of 5 g/ml to 10 mg/ml in the presence and absence of a metabolic activation system (S9 mix). Cells were examined microscopically at intervals up to 4 hr for evidence of damage. Based on the results of this study, a dose range was chosen for use in assays for inhibition of colony-forming ability. This was conducted in the presence and absence of serum and the presence and absence of S9 mix for periods of up to 4 hr. For the mutagenicity assay, the cells were exposed to benzene for 1 hr in the absence of serum and the presence and absence of S9 mix. Cyclophosphamide and ethylmethanesulphonate were used as positive controls, in the presence and absence of S9 mix respectively. The concentrations of benzene tested included at least one dose that induced measurable cytotoxicity and one higher dose. The S9 mix used was derived from livers of male albino rats treated with phenobarbital and beta-naphthoflavone.
Reliability: 1 (valid)
Comparable to guideline study
results: The maximum non-toxic benzene dose in the cytotoxicity assay was 0.31 mg/ml when tested for 4 hr in the presence of serum and the presence and absence of S9 or in the absence of serum and the presence of S9. When tested without S9, in the absence of serum, the maximum non-toxic dose was 0.63 mg/ml. In the colony forming assay, the highest non-toxic dose when tested with and without serum and with and without S9 mix was 0.003 mg/ml and the concentration which killed 50% of the cells in the presence of serum and the absence and presence of S9 mix was 1 mg/ml and >1 mg/ml in the absence of serum. In the mutagenicity assay, the mutation frequency was evaluated 5 and 8 days after exposure. No mutagenic activity was found.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(537)

Type: HGPRT assay
System of testing: Chinese hamster V79 cells
Concentration: 1, 10, 50, 100, 500, 1000 ug/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: A preliminary cytotoxicity study was carried out using this same cell line treated with 1, 10, 50, 100 and 500 ug/ml for 3 hours. The treated cells were then rinsed with Hanks' solution and incubated in normal medium for a further 7 days before the colony-forming activity was evaluated. The mutagenic activity was determined by the 'replating' method. Cells were treated for 3 hours with benzene, rinsed with Hanks' solution and then incubated in normal medium for 6 days. They were then treated with trypsin, replated in medium containing 6-thioguanine and incubated for 14 days. The number of 6-thioguanine-resistant colonies was scored. The assay was conducted in the absence and presence of a metabolic activation system (S9 mix) which had been obtained from male Sprague-Dawley rats treated with an intraperitoneal injection of phenobarbital and 5,6-benzoflavone. Benzene was dissolved in dimethylsulphoxide before use and diluted with Hanks' solution to obtain the appropriate concentration for treatment of cells. The vehicle alone was tested as the 0 ug benzene/ml level and ethyl-methanesulphonate was the positive control chemical.
Reliability: 1 (valid)
Comparable to guideline study
results: Cells treated with benzene at 100 and 500 ug/ml had slightly decreased colony-forming activities, showing slight cytotoxicity. A slight mutagenic effect was noted at 50 and 100 ug/ml, tested in the absence of S9 mix. No mutations were noted at any concentration in the presence of S9 mix.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(538)

Type: Mammalian cell gene mutation assay
System of testing: Chinese hamster V79 cells
Concentration:
Metabolic activation: no data
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Mutations to 6-thioguanine resistance were induced by quinone (a potent inducer), anti-benzene-diol-epoxide, hydroquinone, catechol, trans-benzene-1,2-dihydrodiol and 1,2,4-trihydroxybenzene. The cytotoxicity and potency of the mutagenic effect varied between the chemicals. Phenol,

the dihydroxybiphenyls and duroquinone showed no mutagenic effect. Anti-benzene-diol epoxide induced a significant but weak mutation to ouabain resistance.
Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxybiphenyl, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone and anti-benzene-diol-epoxide were also included. Compounds were tested up to their cytotoxic limits. Cells were mixed with the test compound for 24 hours and mutants were determined after an expression period of 6 days.

Source: BP Chemicals Ltd. London

(532)

Type: Mammalian cell gene mutation assay
System of testing: human lymphoblast cell lines TK6 and AHH-1
Concentration: 0.5, 1, 1.5, 2 mg/ml
Metabolic activation: without
Result: positive
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: method: preliminary cytotoxicity determination; at least 1 independent replicate; positive and negative solvent control; TK6 cells with metabolic activation (exposure 3 h), AHH-1 cells without (exposure to benzene for 28 h); phenotypic expression period 3 d for thymidine kinase locus in TK6 and 6 d for HGPRT locus in AHH-1 cells.
results: significantly positive results at >= 1 mg/ml in AHH-1 cells.
Reliability: 1 (valid)
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn

(539)

Type: Mitotic recombination in Saccharomyces cerevisiae
System of testing: Saccharomyces cerevisiae strain D7-144
Concentration: 274.7, 549.4, 1098.8, 2197.5 µg/ml
Metabolic activation: with and without
Result: positive
Method: OECD Guide-line 481 "Genetic Toxicology: Saccharomyces cerevisiae Mitotic Recombination Assay"
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: In a buffer medium at pH7.0, no gene conversions were found. With treatment in a yeast extract peptone dextrose medium at pH6.3, gene conversion occurred in the presence and absence of S9. A second set of investigators reported mitotic gene conversions in S. cerevisiae strain D7 when treated with but not without S9 (Parry T.M. & Eckardt F. Prog. Mutation Res. 5, 261-269, 1985). Negative mitotic gene conversion results have also been reported in strains D7 (Arni P. et al. ibid.

5, 217-224, 1985), JDI (Brooks T.M. et al. ibid. 5, 225-228, 1985), PV-2 and PV-3 (Inge-Vechtomov S.G. et al. ibid. 5, 243-255, 1985), all treated with and without activation.
The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative control. The culture was incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.

Source: BP Chemicals Ltd. London
Test substance: 99% pure.

(536)

Type: Mitotic recombination in Saccharomyces cerevisiae
System of testing: Saccharomyces cerevisiae strain D7-144
Concentration: 274.7, 549.4, 1098.8, 2197.5 ug/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about 2nd independent experiment, 4 instead of 5 recommended concentrations tested
results:
In a buffer medium at pH7.0, no gene conversions were found. With treatment in a yeast extract peptone dextrose medium at pH6.3, gene conversion occurred in the presence and absence of S9. A second set of investigators reported mitotic gene conversions in S. cerevisiae strain D7 when treated with but not without S9 (Parry T.M. & Eckardt F. Prog. Mutation Res. 5, 261-269, 1985). Negative mitotic gene conversion results have also been reported in strains D7 (Arni P. et al. ibid. 5, 217-224, 1985), JDI (Brooks T.M. et al. ibid. 5, 225-228, 1985), PV-2 and PV-3 (Inge-Vechtomov S.G. et al. ibid. 5, 243-255, 1985), all treated with and without activation.
The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative solvent control. The culture was incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99% pure.

(536)

Type: Mouse lymphoma assay
System of testing: L5178Y mouse lymphoma cells
Concentration: 66, 88, 132, 176, 352, 528 µg/ml
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 476 "Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests"
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Cells treated for 2 hours in the absence and presence of S9 showed 66.6 and 1% survival respectively at a benzene concentration of 352 µg/ml. The cells were completely killed at concentrations of 704 and 528 µg/ml when treated in the absence and presence of S9 respectively. In the mutagenicity assays, benzene was tested at concentrations of 88, 176, 352 and 528 µg/ml in the absence of any activation; 88, 176 and 352 µg/ml in the presence of S9; and at 66 and 132 µg/ml in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. No evidence of mutagenicity at the thymidine kinase locus or the hypoxanthine guanine phosphoribosyltransferase locus was seen in any of the systems. Similar negative findings have been reported by Amacher D.E. & Turner G.N. Prog. Mutation Res. 5, 487-496, 1985; Myhr B et al. ibid. 5, 555-568, 1985; Oberly T.J. et al. Mutation Res. 125, 291-306, 1984. Preliminary cytotoxicity tests were conducted in the presence and absence of metabolic activation. The metabolic activation systems were liver enzymes from Aroclor-1254-induced male SPF Wistar rats (S9 mix), primary chick embryo hepatocytes, and Syrian hamster embryo cells. The results of this test were used to select the concentrations to be used in the mutagenicity assay, with the lowest concentration showing no toxicity and the highest giving a survival of about 15% immediately after treatment. In the mutagenicity test, cells were treated for 2 hours in the absence or presence of S9 and for 20 hours in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. After treatment, the cells were seeded for survival or propagated for several days in standard medium to allow for the expression of induced mutants. Cloning efficiency and selection of mutants were made and mutant frequency assessed. Benzene was dissolved or diluted to the appropriate test concentration in dimethylsulphoxide and this solvent was included in each experiment at 1% as the negative control. Ethylnitrosourea, diethylnitrosamine and benzo(a)pyrene acted as the positive controls in the experiments with no activation, activated with S9 and activated with Syrian hamster-embryo cells and primary chick-embryo hepatocytes respectively.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(540)

Type: Mouse lymphoma assay
System of testing: L5178Y mouse lymphoma cells
Concentration: 500-1200 µg/ml and 100-800 µg/ml
Metabolic activation: with and without
Result: positive
Method: OECD Guide-line 476 "Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests"
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Based on former toxicity data, concentrations of 500-1200 µg/ml and 100-800 µg/ml were selected for the nonactivated and activated tests respectively. In the absence of S9, 1000 µg/ml killed all the test culture and 49% survival was noted at 600 µg/ml. In the presence of S9, 8% survival was seen at a benzene concentration of 800 µg/ml and 45% survival occurred at 100 µg/ml. In the mutagenicity test, benzene was found not to be mutagenic in the absence of S9. In the presence of S9, cultures exposed to 100, 300, 400 and 500 µg/ml showed 2-3-fold increases in mutant frequency over the background. Survival in these cultures exceeded 10% and benzene was, therefore, judged to be mutagenic in the presence of activation. A similar result was reported by Styles J.A. et al. Prog. Mutation Res. 5, 587-596, 1985. In the presence of S9, benzene produced an unequivocal positive result at the oua locus and a weak result at the TK locus. No such effect was found in the absence of S9 but this was thought to be due to excessive toxicity of benzene. Benzene was tested dissolved in dimethylsulphoxide. The metabolic activation system (S9) was prepared from the livers of male Fischer 344 rats pretreated with Aroclor-1254. Preliminary toxicity studies previously conducted at the same laboratory were used to determine the concentrations to be used in the mutagenicity assay. The highest dose selected was that concentration causing a 50-90% inhibition of growth in the preliminary study. In the mutagenicity study, cells were treated with benzene for 4 hours, washed, resuspended in normal medium and incubated for a further 48 hours. Mutant frequency was then evaluated after a further 12-day incubation period. This procedure was repeated with the addition of S9. Dimethylsulphoxide (1%) was used as the negative solvent control and ethylmethanesulphonate and 3-methyl-cholanthrene as the positive controls in the nonactivated and activated tests respectively.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(541)

Type: Mouse lymphoma assay
System of testing: L5178Y mouse lymphoma cells
Concentration: 12.5-200 µg/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1976 **GLP:** no data
Test substance: other TS
Remark: From the preliminary study, a dose range of 12.5-200 µg/ml was determined. The mutant frequency was statistically significantly increased in the 6-thioguanine test in the absence of S9.
The method of Cole J. et al. Mutation Res. 41, 377-386, 1976 was followed. Benzene was dissolved in dimethylsulphoxide and diluted to obtain the required concentration. Cells were treated in the presence and absence of S9 mix (derived from Aroclor-1254-induced rats) for 2 hours. Survival was determined initially at the various concentrations tested without S9. In the mutagenicity assay, after treatment, the cells were cultured for 48 hours, mixed with ouabain and incubated for 2 weeks or cultured for 7 days, mixed with 6-thioguanine and incubated for 2 weeks. Benzo(a)pyrene and 4-nitroquinoline-1-oxide were the positive control chemicals for the assays conducted with and without S9 respectively. Dimethylsulphoxide was the negative solvent control.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade.

(542)

Type: Mouse lymphoma assay
System of testing: L5178Y mouse lymphoma cells
Concentration: 66, 88, 132, 176, 352, 528 ug/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Preliminary cytotoxicity tests were conducted in the presence and absence of metabolic activation. The metabolic activation systems were liver enzymes from Aroclor-1254-induced male SPF Wistar rats (S9 mix), primary chick embryo hepatocytes, and Syrian hamster embryo cells. The results of this test were used to select the concentrations to be used in the mutagenicity assay, with the lowest concentration showing no toxicity and the highest giving a survival of about 15% immediately after treatment. In the mutagenicity test, cells were treated for 2 hours in the absence or presence of S9 and for 20 hours in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. After treatment, the cells were seeded for survival or propagated for several days in standard medium to allow for the expression of induced

mutants. Cloning efficiency and selection of mutants were made and mutant frequency assessed. Benzene was dissolved or diluted to the appropriate test concentration in dimethylsulphoxide and this solvent was included in each experiment at 1% as the negative control. Ethylnitrosourea, diethylnitrosamine and benzo(a)pyrene acted as the positive controls in the experiments with no activation, activated with S9 and activated with Syrian hamster-embryo cells and primary chick-embryo hepatocytes respectively.
Reliability: 1 (valid)
Comparable to guideline study
no data about 2nd independent experiment
results: Cells treated for 2 hours in the absence and presence of S9 showed 66.6 and 1% survival respectively at a benzene concentration of 352 ug/ml. The cells were completely killed at concentrations of 704 and 528 ug/ml when treated in the absence and presence of S9respectively. In the mutagenicity assays, benzene was tested at concentrations of 88, 176, 352 and 528 ug/ml in the absence of any activation; 88, 176 and 352 ug/ml in the presence of S9 and at 66 and 132 ug/ml in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. No evidence of mutagenicity at the thymidine kinase locus or the hypoxanthine guanine phosphoribosyltransferase locus was seen in any of the systems. Similar negative findings at the thymidine kinase locus have been reported by Amacher D.E. & Turner G.N. Prog. Mutation Res. 5, 487-496, 1985; Myhr B et al. ibid. 5, 555-568, 1985; Oberly T.J. et al. Mutation Res. 125, 291-306, 1984.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Laboratory reagent grade; 99% pure.

(540)

Type: Mouse lymphoma assay

System of testing: L5178Y mouse lymphoma cells

Concentration: 500-1200 ug/ml and 100-800 ug/ml

Metabolic activation: with and without

Result: positive

Method: other

Year: 1984 **GLP:** no data

Test substance: other TS

Remark: Benzene was tested dissolved in dimethylsulphoxide. The metabolic activation system (S9) was prepared from the livers of male Fischer 344 rats pretreated with Aroclor-1254. Preliminary toxicity studies previously conducted at the same laboratory were used to determine the concentrations to be used in the mutagenicity assay. The highest dose selected was that concentration causing a 50-90% inhibition of growth in the preliminary study. In the mutagenicity study, cells were treated with benzene for 4 hours, washed, resuspended in normal medium and incubated for a further 48 hours. Mutant frequency at the thymidine kinase locus was then evaluated after a further 12-day incubation period. This procedure was repeated with the addition of S9. Dimethylsulphoxide (1%) was used as the

negative solvent control and ethylmethanesulphonate and 3-methyl-cholanthrene as the positive controls in the nonactivated and activated tests respectively.
Reliability: 1 (valid)
Comparable to guideline study
no data about 2nd independent experiment
results: Based on former toxicity data, concentrations of 500-1200 ug/ml and 100-800 ug/ml were selected for the nonactivated and activated tests respectively. In the absence of S9, 1000 ug/ml killed all the test culture and 49% survival was noted at 600 ug/ml. In the presence of S9, 8% survival was seen at a benzene concentration of 800 ug/ml and 45% survival occurred at 100 ug/ml. In the mutagenicity test, benzene was found not to be mutagenic in the absence of S9. In the presence of S9, cultures exposed to 100, 300, 400 and 500 ug/ml showed 2-3-fold increases in mutant frequency over the background. Survival in these cultures exceeded 10% and benzene was, therefore, judged to be mutagenic in the presence of activation.
A similar positive result was reported by Styles J.A. et al. Prog. Mutation Res. 5, 587-596, 1985: In the presence of S9, benzene produced an unequivocal positive result at the Na+/K+ ATPase locus (selected by resistance to ouabain) and a weak result at the thymidine kinase locus (no such effect was found in the absence of S9 but this was thought to be due to excessive toxicity of benzene).
Matthews, E.J. et al., Prog. Mutat. Res. 5, 639-650 (1985), reported also increased mutation frequency to ouabain resistance at the Na+/K+ ATPase locus.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(541)

Type: Mouse lymphoma assay
System of testing: L5178Y mouse lymphoma cells
Concentration: 12.5-200 ug/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about mean value and deviation, 2 plates per dose, no 2nd independent experiment
results: From the preliminary study, a dose range of 12.5-200 ug/ml was determined. The mutant frequency was statistically significantly increased in the 6-thioguanine test in the absence of S9. No increased mutations to ouabain resistance were detected.
The method of Cole J. et al. Mutation Res. 41, 377-386, 1976 was followed. Benzene was dissolved in dimethylsulphoxide and diluted to obtain the required concentration. Cells were treated in the presence and absence of S9 mix (derived from Aroclor-1254-induced rats)

for 2 hours. Survival was determined initially at the various concentrations tested without S9. In the mutagenicity assay, after treatment, the cells were cultured for 48 hours, mixed with ouabain and incubated for 2 weeks or cultured for 7 days, mixed with 6-thioguanine and incubated for 2 weeks. Benzo(a)pyrene and 4-nitroquinoline-1-oxide were the positive control chemicals for the assays conducted with and without S9 respectively. Dimethylsulphoxide was the negative solvent control.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade.

(542)

Type: Salmonella typhimurium reverse mutation assay
System of testing: Salmonella typhimurium strain TM667
Concentration: <=13mM (1014 mg/l)
Metabolic activation: with
Result: negative
Method: other
Year: 1978 **GLP:** no data
Test substance: other TS
Remark: The mutation assay was carried out according to Skopek T.R. et al. Proc. natn. Acad. Sci. U.S.A. 75, 410-414 & 4470-4473, 1978. Bacteria exposed for 2 hr in the presence of 10% of a postmitochondrial supernatant, prepared as a 25% liver homogenate of phenobarbital- or Aroclor-pretreated male Sprague-Dawley rats. Resistance to 8-azaguanine was used as the genetic marker.
Source: BP Chemicals Ltd. London
Test substance: Supplied by Aldrich Chemical Co., Milwaukee, Wisconsin; purity unspecified.

(543)

Type: Salmonella typhimurium reverse mutation assay
System of testing: Salmonella typhimurium strains TA97, 98, 100, 102, 104, 1535
Concentration: 3-1000ppm (converts to 0.009- 3.26 mg/l)
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was found to be mutagenic in the presence but not the absence of S9. Strain TA1535 was the most responsive. A benzene concentration as low as 0.03 mg/l caused a two-fold increase in the number of mutants above the control. In this same strain, the metabolites trans-benzene-1,2-dihydrodiol in the presence of S9 and anti-benzene-diol-epoxide and syn-benzene-diol-epoxide in the absence of S9 induced mutations. No other metabolite, including catechol to which trans-benzene-1,2-dihydrodiol is converted by cytosolic dihydrodiol dehydrogenase, gave a positive result in strain TA1535. Mutagenic responses, some of them weak, were noted in other strains treated with

1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, catechol, quinone, hydroquinone, syn-benzene-diol-epoxide and anti-benzene-diol-epoxide.
Tests were conducted on benzene and its following metabolites: benzene oxide, phenol, hydroquinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxy-biphenyl, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-tri-hydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. S. typhimurium was exposed to benzene vapour in desiccators to allow for longer exposure periods. S9 mix derived from rat and mouse liver homogenate was used as the activation system.

Source: BP Chemicals Ltd. London

(532)

Type: Sister chromatid exchange assay
System of testing: human peripheral lymphocytes
Concentration: 10, 100, 500, 600 µg/ml
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 479 "Genetic Toxicology: In vitro Sister Chromatid Exchange Assay in Mammalian Cells"
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide and added to the cultures for 48 hours. Diepoxybutane was used as the positive control and untreated cells acted as the negative control. Colcemid was added at the end of the treatment period and preparations made to assess SCE. In the studies using metabolic activation, cells were exposed to S9 derived from Aroclor-induced rat liver, at the same time as exposure to the highest benzene concentration tested in the study without activation. After 1 hour exposure, cells were washed and reincubated in complete medium for 24 hours. Colcemid was added and preparations made to assess SCE. Cyclophosphamide was used as the positive control in the presence of S9.
Benzene was tested at 10, 100, 500 and 600 µg/ml in the absence of S9 and at 600 µg/ml in its presence. No increase in SCE rate was noted in any of the benzene treated cultures. Similar negative results, in the absence of activation have been reported by Gerner-Smidt P. & Friedrich U. Mutation Res. 58, 313-316, 1978; Morimoto K. & Wolff S. Cancer Res. 40, 1189-1193, 1980.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(544)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary cells
Concentration: 16, 50, 160, 500, 1600, 5000 µg/ml
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 479 "Genetic Toxicology: In vitro Sister Chromatid Exchange Assay in Mammalian Cells"
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide. Metabolic activation (S9) was derived from the liver of Aroclor-1254-induced male Sprague-Dawley rats. Cells were treated with benzene for 2 hours, bromodeoxyuridine was added and incubation continued for 24 hours. The cells were then placed in fresh medium with colcemid and incubated for 2-3 hours. When S9 was present, cells were incubated for 2 hours as above except foetal bovine serum was omitted to prevent the binding of serum proteins to short-lived, highly reactive intermediates. After the 2 hours, cells were washed, suspended in complete medium and incubated for 26 hours, colcemid being added for the final 2-3 hours. Assessment of SCEs for both treatment regimes were made. Cyclophosphamide and mitomycin C were used as positive controls in the presence and absence of S9 respectively. Benzene was tested at levels of 16, 50, 160 and 500 µg/ml in the absence of S9 and at 16, 50, 160, 500, 1600 and 5000 µg/ml in the presence of S9. No increase in the number of SCEs were found. Subsequent analyses of the data at benzene levels of 100, 250, 500, 750 and 1000 µg/ml in the absence of S9 showed an increase in SCEs. Other investigators have reported negative SCE results in this cell line when treated in the absence and presence of a metabolic activation system (Douglas G.R. et al. Prog. Mutation Res. 5, 359-366, 1985; Lane A.M. et al. ibid. 5, 451-455, 1985; Natarajan A.T. et al. ibid. 5, 433-4370, 1985), in Chinese hamster V79 cells in the absence and presence of activation (van Went G.F. ibid. 5, 469 477, 1985) and in rat liver cells (Priston R.A.J. & Dean B.J. ibid. 5, 387-395, 1985).
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; >=99% pure.

(527) (533)

Type: Sister chromatid exchange assay
System of testing: human peripheral lymphocytes
Concentration: 16, 78, 391 mg/l
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: An increased number of SCEs were found in cultures treated in the presence of 10% S9 mix. In the absence of S9 and at S9 concentrations of 1 or 90% no increase in the frequency

of SCEs was noted. 10% S9 mix is thought to be the optimal concentration for converting benzene into the active metabolites that might be responsible for the induction of SCEs. When the cells were exposed to benzene concentrations of 2 x 10-4, 1 x 10-3 and 5 x 10-3M (approximately 16, 78 and 391 mg/l), a dose-related increase in SCEs was seen when the appropriate activation concentration was used. S9 mix at 10-30% converted benzene into active forms that were cytotoxic and delayed cell turnover times. Further examination of this suggested that the metabolites responsible for cell division delay may be different from those which induce SCE. The addition of GSH to the culture caused a dose-dependent decrease in SCEs in cells exposed to benzene and S9 mix. The addition of GSH also completely prevented the induction of SCEs by catechol and hydroquinone, two major phenolic metabolites of benzene and potent inducers of SCEs.
Blood was obtained from healthy adult men. Benzene was dissolved in serum-free culture medium. Cells were suspended in the serum-free culture medium containing benzene and the metabolic activation system (S9 mix derived from rat liver) and incubated for 2 hours. The flask was agitated to ensure even distribution of active metabolites among the cells. After incubation, the cells were washed, resuspended in the same medium and incubated further. SCEs were analysed.

Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; purity not specified.

(545)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster V79 cells
Concentration:
Metabolic activation: no data
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: A statistically significant increase in the number of SCEs was found in cultures treated with anti-benzene-diol-epoxide, syn-benzene-diol-epoxide, hydroquinone, catechol, 1,2,4- and 1,2,3-trihydroxybenzene. No such effect was seen with quinone, phenol and trans-benzene-1,2-dihydrodiol.
Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compounds. After 1 hour, bromodeoxyuridine was added and after 23 hours, colcemid was added. Cells were harvested and the number of SCEs scored.
Source: BP Chemicals Ltd. London

(532)

Type: Sister chromatid exchange assay
System of testing: human peripheral lymphocytes
Concentration: 16, 78, 391 mg/l
Metabolic activation: with and without
Result: positive
Method: other
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: An increased number of SCEs were found in cultures treated in the presence of 10% S9 mix. In the absence of S9 and at S9 concentrations of 1 or 90% no increase in the frequency of SCEs was noted. 10% S9 mix is thought to be the optimal concentration for converting benzene into the active metabolites that might be responsible for the induction of SCEs. When the cells were exposed to benzene concentrations of 2 x 10-4, 1 x 10-3 and 5 x 10-3M (approximately 16, 78 and 391 mg/l), a dose-related increase in SCEs was seen when the appropriate activation concentration was used. S9 mix at 10-30% converted benzene into active forms that were cytotoxic and delayed cell turnover times. Further examination of this suggested that the metabolites responsible for cell division delay may be different from those which induce SCE. The addition of GSH to the culture caused a dose-dependent decrease in SCEs in cells exposed to benzene and S9 mix. The addition of GSH also completely prevented the induction of SCEs by catechol and hydroquinone, two major phenolic metabolites of benzene and potent inducers of SCEs.
Blood was obtained from healthy adult men. Benzene was dissolved in serum-free culture medium. Cells were suspended in the serum-free culture medium containing benzene and the metabolic activation system (S9 mix derived from rat liver) and incubated for 2 hours. The flask was agitated to ensure even distribution of active metabolites among the cells. After incubation, the cells were washed, resuspended in the same medium and incubated further. SCEs were analysed.
Reliability: 1 (valid)
Comparable to guideline study
no positive control
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; purity not specified.

(545)

Type: Sister chromatid exchange assay
System of testing: human peripheral lymphocytes
Concentration: 10, 100, 500, 600 ug/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide and added to the cultures for 48 hours. Diepoxybutane was used as the positive control and untreated cells acted as the negative control. Colcemid was added at the end of the treatment period and preparations made to assess SCE. In the studies using metabolic activation, cells were exposed to S9 derived from Aroclor-induced rat liver, at the same time as exposure to the highest benzene concentration tested in the study without activation. After 1 hour exposure, cells were washed and reincubated in complete medium for 24 hours. Colcemid was added and preparations made to assess SCE. Cyclophosphamide was used as the positive control in the presence of S9.
Reliability: 1 (valid)
Comparable to guideline study
results:
Benzene was tested at 10, 100, 500 and 600 ug/ml in the absence of S9 and at 600 ug/ml in its presence. No increase in SCE rate was noted in any of the benzene treated cultures. Similar negative results, in the absence of activation have been reported by Gerner-Smidt P. & Friedrich U. Mutation Res. 58, 313-316, 1978; Morimoto K. & Wolff S. Cancer Res. 40, 1189-1193, 1980.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(544)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary cells
Concentration: 16, 50, 160, 500, 1600, 5000 ug/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide. Metabolic activation (S9) was derived from the liver of Aroclor-1254-induced male Sprague-Dawley rats. Cells were treated with benzene for 2 hours, bromodeoxyuridine was added and incubation continued for 24 hours. The cells were then placed in fresh medium with colcemid and incubated for 2-3 hours. When S9 was present, cells were incubated for 2 hours as above except foetal bovine serum was omitted to prevent the binding of serum proteins to short-lived, highly reactive intermediates. After the 2 hours, cells were

washed, suspended in complete medium and incubated for 26 hours, colcemid being added for the final 2-3 hours. Assessment of SCEs for both treatment regimes were made. Cyclophosphamide and mitomycin C were used as positive controls in the presence and absence of S9 respectively.
Reliability: 1 (valid)
Comparable to guideline study
results:
Benzene was tested at levels of 16, 50, 160 and 500 ug/ml in the absence of S9 and at 16, 50, 160, 500, 1600 and 5000 ug/ml in the presence of S9. No increase in the number of SCEs were found. Subsequent analyses of the data at benzene levels of 100, 250, 500, 750 and 1000 ug/ml in the absence of S9 showed an increase in SCEs (but judged by the authors as negative result).
Other investigators have reported negative SCE results in this cell line when treated in the absence and presence of a metabolic activation system (Douglas G.R. et al. Prog. Mutation Res. 5, 359-366, 1985; Lane A.M. et al. ibid. 5, 451-455, 1985; Natarajan A.T. et al. ibid. 5, 433-4370, 1985), in Chinese hamster V79 cells in the absence and presence of activation (van Went G.F. ibid. 5, 469 477, 1985) and in rat liver cells (Priston R.A.J. & Dean B.J. ibid. 5, 387-395, 1985).

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; >=99% pure.

(527) (533)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster V79 cells
Concentration:
Metabolic activation: no data
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
no data about metabolic activation, 2nd independent experiment, positive control, partly test conditions, test substance
results:
A statistically significant increase in the number of SCEs was found in cultures treated with anti-benzene-diol-epoxide, syn-benzene-diol-epoxide, hydroquinone, catechol, 1,2,4- and 1,2,3-trihydroxybenzene. No such effect was seen with quinone, phenol and trans-benzene-1,2-dihydrodiol.
Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compounds. After 1 hour, bromodeoxyuridine was added and after 23

hours, colcemid was added. Cells were harvested and the number of SCEs scored.
Source: Deutsche Shell Chemie GmbH Eschborn

(532)

Type: Sister chromatid exchange assay
System of testing: Human lmphocytes
Concentration: 1, 10, 25, 50, 100 & 250 ug/ml
Metabolic activation: no data
Result: positive
Method: other: see ref.
Year: 1973 **GLP:** no data
Test substance:
Source: REPSOL PETROLEO,S.A. MADRID

(546)

Type: Unscheduled DNA synthesis
System of testing: HeLa S3 cells
Concentration: 0.05, 0.075, 0.1, 0.15, 0.188, 0.2, 0.375, 0.75, 1.5, 3.0 µg/ml
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 482 "Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells in vitro"
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Benzene did not induce an increase in UDS in this study. The investigators thought that this absence of benzene-induced DNA-repair activity may be due to an insensitivity to the type of genotoxicity shown by benzene, an inadequate or inappropriate activation system for the production of a DNA-reactive metabolite or an inability of any reactive metabolite to live long enough to reach the DNA of the target cells.
The metabolic activation system (S9 mix) was derived from the liver of Aroclor-1254-induced male Wistar rats. Cells were incubated in growth medium for 4 days, followed by growth-limiting medium for 72 hours. Hydroxyurea was added to maximise inhibition of background DNA synthesis. The cells were incubated for 1 hour and S9 mix was added, if required. Benzene was added and the mixture incubated for 2.5 hours. At the end, the cells were washed, DNA extracted and assessed. Dimethyl-sulphoxide was used as the negative, solvent control and 2 acetaminofluorene as the positive control chemical in the presence of S9 and 4-nitroquinoline-N-oxide and N-methyl-N'-nitro-N-nitroso-guanidine in the absence of S9.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(547)

Type: Unscheduled DNA synthesis
System of testing: adult male albino rat hepatocytes
Concentration: 0.078-3900 µg/ml
Metabolic activation: without
Result: positive
Method: other
Year: 1982 **GLP:** no data
Test substance: other TS
Remark: The method of Althaus F.R. et al. Cancer Res. 42, 3010-3015, 1982 was followed. The hepatocytes were plated in normal medium which was changed after 4 hours. Benzene, dissolved in ethanol or dimethylsulphoxide, was added 18 hours before harvesting. Hydroxyurea was added 1 hour before benzene, to reduce thymidine incorporation from replicative DNA synthesis. Thymidine was added immediately after benzene. At the end of exposure, the cells were washed and assayed for UDS. Initial studies were conducted to identify the maximum effective dose.
The optimal concentration for benzene was found to be 78.1 µg/ml. At this dose, a statistically significant increase ($p<0.05$) in UDS was induced. Other investigators have, however, reported negative results in rat hepatocyte UDS assays (Probst G.S. & Hill L.E. Prog. Mutation Res. 5, 381-386, 1985; Probst G.S. et al. Envir. Mutagen. 3, 11-32, 1981; Williams G.M. et al. Prog. Mutation Res. 5, 341-345, 1985).
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(548)

Type: Unscheduled DNA synthesis
System of testing: HeLa S3 cells
Concentration: 0.05, 0.075, 0.1, 0.15, 0.188, 0.2, 0.375, 0.75, 1.5, 3.0 ug/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: 1986 **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Comparable to guideline study
results:
Benzene did not induce an increase in UDS in this study. The investigators thought that this absence of benzene-induced DNA-repair activity may be due to an insensitivity to the type of genotoxicity shown by benzene, an inadequate or inappropriate activation system for the production of a DNA-reactive metabolite or an inability of any reactive metabolite to live long enough to reach the DNA of the target cells.
The metabolic activation system (S9 mix) was derived from the liver of Aroclor-1254-induced male Wistar rats. Cells were incubated in growth medium for 4 days, followed by

growth-limiting medium for 72 hours. Hydroxyurea was added to maximise inhibition of background DNA synthesis. The cells were incubated for 1 hour and S9 mix was added, if required. Benzene was added and the mixture incubated for 2.5 hours. At the end, the cells were washed, DNA extracted and assessed. Dimethyl-sulphoxide was used as the negative, solvent control and 2 acetaminofluorene as the positive control chemical in the presence of S9 and 4-nitroquinoline-N-oxide and N-methyl-N'-nitro-N-nitroso-guanidine in the absence of S9.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(547)

Type: Unscheduled DNA synthesis
System of testing: adult male albino rat hepatocytes
Concentration: 0.078-3900 ug/ml
Metabolic activation: without
Result: positive
Method: other
Year: 1982 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, no independent 2nd test, no data about cytotoxicity
results:
The optimal concentration for benzene was found to be 78.1 ug/ml. At this dose, a statistically significant increase ($p<0.05$) in UDS was induced. Other investigators have, however, reported negative results in rat hepatocyte UDS assays (Probst G.S. & Hill L.E. Prog. Mutation Res. 5, 381-386, 1985; Probst G.S. et al. Envir. Mutagen. 3, 11-32, 1981; Williams G.M. et al. Prog. Mutation Res. 5, 341-345, 1985).
The method of Althaus F.R. et al. Cancer Res. 42, 3010-3015, 1982 was followed. The hepatocytes were plated in normal medium which was changed after 4 hours. Benzene, dissolved in ethanol or dimethylsulphoxide, was added 18 hours before harvesting. Hydroxyurea was added 1 hour before benzene, to reduce thymidine incorporation from replicative DNA synthesis. Thymidine was added immediately after benzene. At the end of exposure, the cells were washed and assayed for UDS. Initial studies were conducted to identify the maximum effective dose.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(548)

Type: Yeast Cytogenetic assay
System of testing: Saccharomyces cerevisiae strains D6 and D61-M
Concentration:
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Aneuploidy occurred in D6 cells treated, in the absence of S9, 4 hours after inoculation into high glucose medium and in D61-M cells treated, in the absence and presence of S9, immediately after inoculation into high glucose medium. No effect was seen when this inoculation was delayed 4 hours. Cells were exposed to benzene either at the time of inoculation into high glucose medium or 4 hours after inoculation. All cultures were then grown for 18 hours on a shaker. The cells were then washed and plated on selective media to detect cell viability and mitotic aneuploidy. Benzene was dissolved in dimethylsulphoxide. Cyclophosphamide and chenodeoxycholic acid were used as the positive controls. The metabolic activation system (S9) was derived from the liver or Aroclor-induced rats.
Source: BP Chemicals Ltd. London
Test substance: Lancaster synthesis, purity not specified.

(549)

Type: Yeast Cytogenetic assay
System of testing: Saccharomyces cerevisiae strains D6 and D61-M
Concentration: see remarks
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Cells were exposed to benzene either at the time of inoculation into high glucose medium or 4 hours after inoculation. All cultures were then grown for 18 hours on a shaker. The cells were then washed and plated on selective media to detect cell viability and mitotic aneuploidy. Benzene was dissolved in dimethylsulphoxide. Cyclophosphamide and chenodeoxycholic acid were used as the positive controls. The metabolic activation system (S9) was derived from the liver or Aroclor-induced rats.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no data about concentration used
results:
Aneuploidy occurred in D6 cells treated, in the absence of S9, 4 hours after inoculation into high glucose medium and in D61-M cells treated, in the absence and presence of S9, immediately after inoculation into high glucose medium. No

effect was seen when this inoculation was delayed 4 hours.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Lancaster synthesis, purity not specified.

(549)

Type: other
System of testing: Salmonella typhimurium strain TM667
Concentration: <=13mM (1014 mg/l)
Metabolic activation: with
Result: negative
Method: other
Year: 1978 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no concurrent negative control, no data about cytotoxicity
The mutation assay was carried out according to Skopek T.R. et al. Proc. natn. Acad. Sci. U.S.A. 75, 410-414 & 4470-4473, 1978. Bacteria exposed for 2 hr in the presence of 10% of a postmitochondrial supernatant, prepared as a 25% liver homogenate of phenobarbital- or Aroclor-pretreated male Sprague-Dawley rats. Exposure followed by 2 d postexposure incubation period. Resistance to 8-azaguanine was used as the genetic marker.
results: No evidence of mutagenicity was seen when benzene was tested up to a concentration of 13 mM in this strain in the presence of metabolic activation systems. Similar results were reported by Seixas, G.M. et al., Mutat. Res. 102, 201-212 (1982, Reliability: 2 (valid with restriction))
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Supplied by Aldrich Chemical Co., Milwaukee, Wisconsin; purity unspecified.

(543)

Type: other
System of testing: Salmonella typhimurium strains TA98, 100, 104, 1535
Concentration: 3-1000ppm (converts to 0.009- 3.26 mg/l)
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no positive control, TA1537 not tested; no data about cytotoxicity, test substance, standard deviation
results: Benzene was found to be mutagenic in the presence but not the absence of S9. Strain TA1535 was the most responsive. A benzene concentration as low as 0.03 mg/l

caused a two-fold increase in the number of mutants above the control. In this same strain, the metabolites trans-benzene-1,2-dihydrodiol in the presence of S9 and anti-benzene-diol-epoxide and syn-benzene-diol-epoxide in the absence of S9 induced mutations. No other metabolite, including catechol to which trans-benzene-1,2-dihydrodiol is converted by cytosolic dihydrodiol dehydrogenase, gave a positive result in strain TA1535. Mutagenic responses, some of them weak, were noted in other strains treated with 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, catechol, quinone, hydroquinone, syn-benzene-diol-epoxide and anti-benzene-diol-epoxide.
Tests were conducted on benzene and its following metabolites: benzene oxide, phenol, hydroquinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxy-biphenyl, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-tri-hydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. S. typhimurium was exposed to benzene vapour in desiccators to allow for longer exposure periods. S9 mix derived from rat and mouse liver homogenate was used as the activation system. Metabolites also tested with strains TA97 and 102.

Source: Deutsche Shell Chemie GmbH Eschborn

(532)

Type: other
System of testing: Chinese hamster ovary cells
Concentration: 1.33 mg/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Metabolic activation system was the S9 fraction from Aroclor-1254-induced rat liver. Benzene was dissolved in dimethylsulphoxide. The cells were incubated in the presence of benzene for 30 min, 1 or 2 hours. Metabolic activation was only added in the 1 hour study. Survival of the cells was also checked. The single-strand DNA breaks were detected by high-salt alkaline sucrose-gradient sedimentation. Untreated cells acted as the negative control and ethylmethanesulphonate and diethyl-nitrosamine treated cells as the positive controls.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
single concentration, no solvent control, no data about 2nd independent experiment, no negative control with S9
results:
After a treatment time of 1 hour, 20.9 and 33.0% survival was seen in the cells treated without and with S9 respectively. Single-strand breaks were greatly increased after 30 min, 1 and 2 hours treatment in the absence of S9;

the increase being time-dependent. No such effect was noted in the presence of S9.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(529)

Type: other
System of testing: Chinese hamster ovary cells
Concentration: 0.39-7.81 mg/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide before addition to the cells. Metabolic activation system was derived from the liver of Aroclor-1254 induced Sprague-Dawley rats (S9 mix). Cells were treated for 1 hour with benzene, washed and subjected to alkaline sucrose sedimentation analysis in order to assess the DNA single-strand breaks. Prior to testing benzene, its cytotoxicity was determined by examining inhibition of cell growth.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no positive control, single exposure time, no data on negative control and 2nd independent experiment
results:
The cytotoxicity threshold, that is, the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 g/ml when tested in the presence and absence of S9. In the mutagenicity study, benzene was tested at levels of 0.39-7.81 mg/ml. No single-strand breaks were found at levels up to 2.34 mg/ml tested with or without S9. Above this, single-strand breaks increased but this was thought to be due to the toxic effects of benzene. Other investigators have reported similar negative results using the alkaline elution technique in Chinese hamster V79 cells in the presence and absence of metabolic activation (Swenberg J.A. et al. Biochem. biophys. Res. Commun. 72, 732-738, 1976) and rat hepatocytes in the absence of activation (Bradley M.O. Prog. Mutation Res. 5, 353-357, 1985; Sina J.F. et al. Mutation Res. 113, 357-391, 1983).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; purity unspecified.

(530)

Type: other
System of testing: Balb/c-3T3 mouse embryo cells
Concentration: 0.5, 1.67, 2.0, 3.33, 4.0, 5.0 mg/ml
Metabolic activation: with and without
Result: positive
Method: other
Year: 1988 **GLP:** no data
Test substance: other TS
Remark: A cytotoxicity study was initially carried out to determine the benzene doses to be tested in the cell transformation assay. Doses causing 10-100% survival of the cells were used. In the nonactivation assay cells were treated with benzene for 72 hours, washed and incubated for a further 24 days. Transformed foci were scored microscopically. For metabolic activation primary rat-liver cells were prepared from Fischer-344 rats and lethally X-irradiated. These feeder cells were added to the mouse embryo cells for 3 hours before treatment with benzene for 48 hours. The cultures were then treated with 12-O-tetra-decenoylphorbol-13-acetate biweekly for 3 weeks, starting 1-2 days after benzene treatment. Dimethylnitrosamine and 3-methylcholanthrene were used as the positive controls in the tests conducted with and without the addition of the rat liver cells respectively. Untreated cells were the negative controls. The tests were conducted in closed tissue culture flasks.
Reliability: 1 (valid)
Comparable to guideline study
no data about 2nd independent experiments
results:
In the cytotoxicity test, 59% survival was seen at doses of 3.0 and 3.33 mg/ml in the absence and presence of activation respectively. All cells were killed at 6.0 mg/ml in the absence of activation and 0.3% survival was found at 5.0 mg/ml with activation. In the transformation assay, doses of 0.5, 2.0 and 4.0 mg/ml and 1.67, 3.33 and 5.0 mg/ml were tested without and with activation respectively. In the absence of activation, no transformation activity was found. In the presence of activation, statistically significant effects were noted at all dose levels, the increases in the numbers of transformed cell foci being dose-related. A second set of investigators also reported a lack of transforming activity in this cell line when benzene was tested in the absence of activation. The test was not conducted, in this case, with added activation (Rundell J.O. et al. Envir. Sci. Res. 27, 309-324, 1983).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; purity 99%.

(523)

Type: other
System of testing: C3H/10T. mouse embryonic fibroblast cells
Concentration: 50, 80, 100, 160, 200, 320 ug/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: 1988 **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved and diluted in dimethylsulphoxide before use. The metabolic activation system (S9) was obtained from the liver of Aroclor-1254-treated male rats. A dose-ranging study was conducted to determine the doses to be used in a preliminary cytotoxicity test. The latter was conducted to establish the range of concentrations to be used in the cell transformation assay. In the transformation assay, the cells were exposed to benzene for 24 hours in the presence or absence of S9 mix. One of the treated cultures/dose was used to assess cell survival in the study. In the remaining cultures, the medium was changed twice weekly until the cells reached confluence and weekly thereafter. After an 8-week incubation period, the cultures were stained and assessed for cell transformation. Dimethylsulphoxide was used as the negative control chemical and cyclophosphamide as the positive control in the presence of S9. In the absence of the activation mix, 3-methylcholanthrene acted as the positive control.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no data on 2nd independent experiment, cytotoxicity not specified, no data on statistical evaluation, no data how test results judged to be positive
results:
Based on the preliminary toxicity tests, benzene was tested at concentrations of 50, 100 and 200 ug/ml in the absence and 80, 160 and 320 ug/ml in the presence of S9. No transformed foci were identified at any benzene concentration in the presence or absence of S9. A negative or marginally significant positive result in this cell line in the absence of activation was reported by Nesnow S. et al. Prog. Mutation Res. 5, 659-664, 1985.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(524)

Type: other
System of testing: Syrian hamster embryo (SHE) cells
Concentration: 20-500 ug/ml
Metabolic activation: with
Result: positive
Method: other
Year: 1988 **GLP:** no data
Test substance: other TS
Remark: Lethally X-irradiated SHE cells were seeded and 24 hours later target cells were added. After 24 hours, benzene was added and the cells were incubated for 6 days. The medium was then removed, the cells rinsed and benzene added for a further 24 hours. The cells were then washed and transformation activity assessed. Benzene was dissolved in dimethylsulphoxide. Benzo(a)pyrene was used as the positive control.
Reliability: 1 (valid)
Comparable to guideline study
results:
Positive results were obtained at concentrations of 20 ug/ml and above. However, at concentrations higher than 300 ug/ml, the transformation frequency decreased. Similar positive results in the absence of activation have been reported in this cell line by Barrett J.C. & Lamb P.W. Prog. Mutation Res. 5, 623-628, 1985. Amacher and Zelljadt reported a low transformation frequency in SHE cells when tested in the absence of metabolic activation (Amacher D.E. & Zelljadt I. Carcinogenesis 4, 291-295, 1983) and a negative result has been reported in the SHE cell-transformation enhancement assay using SA7 viruses, again in the absence of activation (Hatch G.G. & Anderson T.M. Prog. Mutation Res. 5, 629-638, 1985).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(531)

Type: other
System of testing: Chinese hamster ovary cells
Concentration: 7.81-7810 ug/ml
Metabolic activation: with and without
Result: negative
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in dimethylsulphoxide before addition to the culture medium. Aroclor-1254-induced Sprague-Dawley rat-liver S9 was used as the metabolic activation system. A preliminary cytotoxicity test was conducted in which cells were incubated with a range of benzene concentrations with and without S9 for 24 hours. From this test, the concentrations to be used in the micronucleus assay were selected. In the micronucleus assay, cells were treated for 1 hour, washed and incubated in normal medium for 24 hours.

The micronucleus frequency was then assessed. Historic dimethylsulphoxide control data were used as the background readings.
Reliability: 3 (not valid)
Significant methodological deficiencies
no positive control, no statistical evaluation, no data about mitotic index and 2nd independent experiment, insufficient presentation of results
results:
In the preliminary study, the cytotoxicity threshold, that is the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 ug/ml when in both the absence and presence of S9. In the micronucleus assay, no evidence of mutagenicity was seen with and without S9 at concentrations of 7.81 mg/ml to 7.81 ug/ml.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(530)

Type: other
System of testing: Chinese hamster V79 cells
Concentration:
Metabolic activation: no data
Result: positive
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 3 (not valid)
Documentation insufficient for assessment
no data about metabolic activation, number of experiments/dose, survival, positive control, test substance
results:
Induction of micronuclei was reported in cells treated with anti-benzene-diol-epoxide, quinone, hydroquinone, catechol, phenol, 1,2,4-trihydroxybenzene, 1,2,3-trihydroxybenzene and duroquinone. The potency of this effect varied between the chemicals. No such effect was seen with syn-benzene-diol-epoxide and trans-benzene-1,2-dihydrodiol. Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compound for 24 hours. They were then harvested and scored for micronuclei. Compounds were tested up to their cytotoxic limits.
Source: Deutsche Shell Chemie GmbH Eschborn

(532)

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 1, 10, 100, 1000 ppm (converts to 0, 0.003, 0.033, 0.33, 3.26 mg/l)
Result:
Method: OECD Guide–line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test – Chromosomal Analysis"
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Only male animals were tested. Groups of eight rats were exposed at each benzene concentration and the control group contained 12 animals. Controls were exposed to clean dry air. The benzene exposure concentrations were measured. Animals received colchicine intra–peritoneally 2 hours before sacrifice, they were then killed, their femurs removed and the bone marrow harvested. Slides were prepared and examined for abnormalities.
Result: Analysis of the test atmospheres gave benzene concentrations of 0.0026 ± 0.0006, 0.032 ± 0.006, 0.44 ± 0.12 and 3.56 ± 0.34 mg/l. The breathing of animals at the top dose was very rapid immediately after the exposure period. This was not reported at the lower doses. There was a statistically significant increase in the percentage of cells with abnormalities, including or excluding gaps, in animals exposed to 0.33 and 3.26 mg/l. Elevated levels of abnormal cells were seen at 0.003 and 0.033 mg/l, these being dose–related but not statistically significant. Positive results in rats inhaling benzene have been reported by other investigators (Anderson D. & Richardson C.R. Mutation Res. 90, 261–272, 1981.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; purity not specified.

(550)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 10.2, 25.6, 64, 160, 400, 2500 µl/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was administered in peanut oil. Groups of ten male and ten female Swiss Lane Petter mice were exposed to each benzene dose. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24–hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were

removed and processed for metaphase analysis.
Result: The frequency of chromosome aberrations was increased in the benzene treated animals, this being significant at 56.3 mg/kg bw/day and above. The male animals were more sensitive than the females. At doses up to 56.3 mg/kg bw/day, the abnormalities consisted of chromatid gaps and single breaks. At 140 mg/kg bw/day, multiple breaks, as well as gaps and single breaks, occurred and at the higher doses, cells showing pulverisation of chromosomes were also seen. Chromosomal damage has also been reported in Swiss CD-1 mice administered benzene by gavage on two or three days by Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984 and Meyne J. & Legator M.S. Envir. Mutagen 2, 43-50, 1980.
Source: BP Chemicals Ltd. London
Test substance: "chemically pure" benzene tested.

(551)

Type: Cytogenetic assay
Species: hamster **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were removed and processed for metaphase analysis.
Result: In the males, a dose of 8.8 g/kg bw/day induced a significant increase in the frequency of chromosome aberrations, including gaps. In the low-dose group, the frequency of aberrations did not differ significantly from the controls. No significant effect was seen in the females at either dose level.
Source: BP Chemicals Ltd. London
Test substance: "Chemically pure" benzene tested.

(551)

Type: Cytogenetic assay
Species: mouse **Sex:** male
Strain: ICR
Route of admin.: gavage
Exposure period: 2 weeks
Doses: 0, 36.6, 73.2, 146.4 mg/kg bw/day
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of five mice were administered benzene in olive oil daily for 2 weeks excluding days 5 and 10. The mice were killed on day 15 and spleen lymphocytes were isolated and cultured. Slides were prepared and analysed for the presence of chromosome aberrations.
Result: Significant dose–dependent increases in both chromatid breaks and abnormal cells were found in the top two dose groups. An increased frequency of breaks and abnormal cells was also recorded at 36.6 mg/kg bw/day but this was not significantly different from the control group. The frequency of cells with multiple breaks was increased at 73.2 and 146.4 mg/kg bw/day and there was a significant increased frequency of polyploid cells at all dose levels.
Source: BP Chemicals Ltd. London

(552)

Type: Cytogenetic assay
Species: rat **Sex:** male/female
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure period: 13 weeks
Doses: 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Report of a study conducted for the American Petroleum Institute.
Result: No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or less. Slight increases in aberrations were seen at 0.98 mg/l but these were not statistically significant.
Source: BP Chemicals Ltd. London

(553) (554)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: DBA
Route of admin.: inhalation
Exposure period: 4 hours
Doses: 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six animals/sex. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrow flushed out, slides prepared and chromosomal aberration frequency assessed.
Result: Benzene alone did not increase the frequency of chromosomal aberrations. Treatment with phenobarbital before benzene exposure caused a statistically significant increase in chromatid-type aberrations. This was not seen in control mice pretreated with phenobarbital.
Source: BP Chemicals Ltd. London

(555)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 22 hours per day/7 days per week/6 weeks
Doses: 0, 40, 100, 1000 ppb (converts to 0, 0.13, 0.33, 3.26 µg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of 14 male and 14 female mice/dose were used. After benzene treatment, the animals were exposed to vinblastine sulphate for 4 hours before sacrifice. A further group of 12 mice/sex were exposed to 0, 0.33 and 3.26 µg/l and injected, 4 hours before sacrifice, with colchicine. After the mice were killed, lymphocytes were obtained from the spleen for cytogenetic analysis.
Result: No overt signs of toxicity were seen during the study. Dose-dependent increases in chromosome aberration frequencies were observed in both sexes at the top two doses, this being statistically significant. In the females, the increase after exposure to 3.26 mg/l was much moderated compared to the increase in the 0.33 mg/l dose group. On repeating the experiment and including exposure to 0.13 mg/l, statistically significant increases in the frequencies of aberrations were again seen. However, in both sexes, those in the top-dosed groups were lower than the frequencies seen in mice exposed to the lower benzene

concentrations. Other positive results in mice inhaling benzene have been reported by Au W.W. et al. Envir. Mutagen. 9(Suppl.8), 8, 1987 (Abstract 16); Tice R.R. et al. Envir. Sci. Res. 25, 257-275, 1982; Zhurkov V.S. et al. Bull. exp. Biol. Med. 96, 1741-1743, 1983.

Source: BP Chemicals Ltd. London

(556)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 13 weeks
Doses: 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l0
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Report of a study conducted for the American Petroleum Institute.
Result: No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or below. Slight increases in aberrations were seen at 0.98 mg/l but these were only statistically significant in the females.
Source: BP Chemicals Ltd. London

(553) (557)

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: i.p.
Exposure period: single dose
Doses: 878 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of five male Wistar-derived, outbred, Alderely Park rats were injected with benzene or, in the case of the control group, water. The animals were killed 24 hours later, the bone marrow samples prepared according to the method of Sugiyama T. J. natn. Cancer Inst. 47, 1267-1275, 1971 but with slight modifications and the slides examined for any chromosome abnormalities.
Result: Benzene caused a statistically significant increase in the numbers of cells with abnormalities and chromatid and chromosome gaps and breaks.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; purity not specified.

(558)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: 3 days
Doses: 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was diluted in corn oil to the required concentration and administered to five males and five females/dose. The control group received corn oil. The mice were treated 2 hours after the final treatment with colchicine and killed 2 hours later. The femurs were removed and the bone marrow prepared and assessed.
Result: An increased incidence of chromosome aberrations were seen in the benzene treated mice, the majority of the aberrations being chromatid breaks. The males were more susceptible at all doses than the females. Not all of the increased incidences were found to be statistically significant. Gad-El-Karim and co-workers also reported an increase in chromosome damage in this strain of mouse injected with 2 doses of 440 mg/kg bw (Gad-El-Karim M.M. et al. Mutation Res. 135, 225 243, 1984).
Source: BP Chemicals Ltd. London
Test substance: >99% pure.

(559)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 10.2, 25.6, 64, 160, 400, 2500 ul/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)
Result:
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Benzene was administered in peanut oil. Groups of ten male and ten female Swiss Lane Petter mice were exposed to each benzene dose. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were removed and processed for metaphase analysis.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, one sampling time
Result: The frequency of chromosome aberrations was increased in the benzene treated animals, this being significant at 56.3 mg/kg bw/day and above in males and females. The male animals were more sensitive than the females. At doses up to 56.3 mg/kg bw/day, the abnormalities consisted of

chromatid gaps and single breaks. At 140 mg/kg bw/day, multiple breaks, as well as gaps and single breaks, occurred and at the higher doses, cells showing pulverisation of chromosomes were also seen. Chromosomal damage has also been reported in Swiss CD-1 mice administered benzene by gavage on two or three days by Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984 and Meyne J. & Legator M.S. Envir. Mutagen 2, 43-50, 1980.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: "chemically pure" benzene tested.

(551)

Type: Cytogenetic assay
Species: Chinese hamster **Sex:** male/female
Strain:
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were removed and processed for metaphase analysis.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, one sampling time, 2 doses

Result: In the males, a dose of 8.8 g/kg bw/day induced a significant increase in the frequency of chromosome aberrations, including gaps. In the low-dose group, the frequency of aberrations did not differ significantly from the controls. No significant effect was seen in the females at either dose level.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: "Chemically pure" benzene tested.

(551)

Type: Cytogenetic assay
Species: mouse **Sex:** male
Strain: ICR
Route of admin.: gavage
Exposure period: 2 weeks
Doses: 0, 36.6, 73.2, 146.4 mg/kg bw/day
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of five mice were administered benzene in olive oil daily for 2 weeks excluding days 5 and 10. The mice were killed on day 15 and spleen lymphocytes were isolated and cultured. Slides were prepared and analysed for the presence of chromosome aberrations.
Reliability: 4 (validity cannot be judged)
Original reference not yet available
Result: Significant dose–dependent increases in both chromatid breaks and abnormal cells were found in the top two dose groups. An increased frequency of breaks and abnormal cells was also recorded at 36.6 mg/kg bw/day but this was not significantly different from the control group. The frequency of cells with multiple breaks was increased at 73.2 and 146.4 mg/kg bw/day and there was a significant increased frequency of polyploid cells at all dose levels.
Source: Deutsche Shell Chemie GmbH Eschborn

(552)

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 1, 10, 100, 1000 ppm (converts to 0, 0.003, 0.033, 0.33, 3.26 mg/l)
Result:
Method: other
Year: 1984 **GLP:** no data
Test substance: other TS
Remark: Only male animals were tested (justified by the authors). Groups of eight rats were exposed at each benzene concentration and the control group contained 12 animals. Controls were exposed to clean dry air. The benzene exposure concentrations were measured. Animals received colchicine intra–peritoneally 2 hours before sacrifice, they were then killed, their femurs removed and the bone marrow harvested. Slides were prepared and examined for abnormalities.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, single sampling time
Result: Analysis of the test atmospheres gave benzene concentrations of 0.0026 $ 0.0006, 0.032 $ 0.006, 0.44 $ 0.12 and 3.56 $ 0.34 mg/l. The breathing of animals at the top dose was very rapid immediately after the exposure period. This was not reported at the lower doses. There was a statistically significant increase in the percentage of cells with

abnormalities, including or excluding gaps, in animals exposed to 0.33 and 3.26 mg/l. Elevated levels of abnormal cells were seen at 0.003 and 0.033 mg/l, these being dose-related but not statistically significant. Positive results in rats inhaling benzene have been reported by other investigators (Anderson D. & Richardson C.R. Mutation Res. 90, 261-272, 1981.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: analytical reagent grade; purity not specified.

(550)

Type: Cytogenetic assay
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 13 weeks
Doses: 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Report of a study conducted for the American Petroleum Institute.
Result: No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or less. Slight increases in aberrations were seen at 0.98 mg/l but these were not statistically significant.
Source: Deutsche Shell Chemie GmbH Eschborn

(553) (554)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: inhalation
Exposure period: 4 hours
Doses: 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six animals/sex. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrow flushed out, slides prepared and chromosomal aberration frequency assessed.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, one sampling time, no data about the

test substance
Result: Benzene alone did not increase the frequency of chromosomal aberrations. Treatment with phenobarbital before benzene exposure caused a statistically significant increase in chromatid-type aberrations. This was not seen in control mice pretreated with phenobarbital.
Source: Deutsche Shell Chemie GmbH Eschborn

(555)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: inhalation
Exposure period: 22 hours per day/7 days per week/6 weeks
Doses: 0, 40, 100, 1000 ppb (converts to 0, 0.13, 0.33, 3.26 ug/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of 14 male and 14 female mice/dose were used. After benzene treatment, the animals were exposed to vinblastine sulphate for 4 hours before sacrifice. A further group of 12 mice/sex were exposed to 0, 0.33 and 3.26 ug/l and injected, 4 hours before sacrifice, with colchicine. After the mice were killed, lymphocytes were obtained from the spleen for cytogenetic analysis. Analytical concentrations: 0, 34, 88, 858 ppb (deviation < 5%). 2 independent experiments. At least 6 mice/dose/sex were analysed for chromosomal aberration.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no positive control, no data about test substance
Result: No overt signs of toxicity were seen during the study. Dose-dependent increases in chromosome aberration frequencies (chromatid breaks) were observed in both sexes at the two top doses, this being statistically significant. In the females, the increase after exposure to 3.26 mg/l was much moderated compared to the increase in the 0.33 mg/l dose group. On repeating the experiment and including exposure to 0.13 mg/l, statistically significant increases in the frequencies of aberrations were again seen. However, in both sexes, those in the top-dosed groups were lower than the frequencies seen in mice exposed to the lower benzene concentrations. Other positive results in mice inhaling benzene have been reported by Au W.W. et al. Envir. Mutagen. 9(Suppl.8), 8, 1987 (Abstract 16); Tice R.R. et al. Envir. Sci. Res. 25, 257-275, 1982; Zhurkov V.S. et al. Bull. exp. Biol. Med. 96, 1741-1743, 1983.
Source: Deutsche Shell Chemie GmbH Eschborn

(556)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 13 weeks
Doses: 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l0
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Original reference not yet available
Report of a study conducted for the American Petroleum Institute.
Result: No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or below. Slight increases in aberrations were seen at 0.98 mg/l but these were only statistically significant in the females.
Source: Deutsche Shell Chemie GmbH Eschborn
(553) (557)

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: i.p.
Exposure period: single dose
Doses: 878 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of five male Wistar-derived, outbred, Alderely Park rats were injected with benzene or, in the case of the control group, water. The animals were killed 24 hours later, the bone marrow samples prepared according to the method of Sugiyama T. J. natn. Cancer Inst. 47, 1267-1275, 1971 but with slight modifications and the slides examined for any chromosome abnormalities.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, one sampling time, one sex, one dose
Result: Benzene caused a statistically significant increase in the numbers of cells with abnormalities and chromatid and chromosome gaps and breaks.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; purity not specified.
(558)

Type: Cytogenetic assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 3 days
Doses: 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was diluted in corn oil to the required concentration and administered to five males and five females/dose. The control group received corn oil. The mice were treated 2 hours after the final treatment with colchicine and killed 2 hours later. The femurs were removed and the bone marrow prepared and assessed.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no positive control, one sampling time
Result: A significantly increased incidence of chromosome aberrations were seen in the benzene high–dosed females and in the mid– and high–dosed male mice, the majority of the aberrations being chromatid breaks. The males were more susceptible at all doses than the females.
Gad–El–Karim and co–workers also reported an increase in chromosome damage in this strain of mouse injected with 2 doses of 440 mg/kg bw (Gad–El–Karim M.M. et al. Mutation Res. 135, 225 243, 1984).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99% pure.

(559)

Type: Cytogenetic assay
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: inhalation
Exposure period: a) single exposure for 2 h , b) 6 h per day for 5 days
Doses: 0, 750 or 7500 ppm (2.4 or 24 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of at least 7 male Wistar–derived, outbred, Alderely Park rats were exposed to benzene or, in the case of the control group, to air. The animals were killed 6 (b) or 24 (a and b) hours after last exposure, the bone marrow samples prepared according to the method of Sugiyama T. J. natn. Cancer Inst. 47, 1267–1275, 1971 but with slight modifications and the slides examined for any chromosome abnormalities.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no positive control, one sex
Result: Benzene caused a statistically significant clastogenic effect in all treatment groups.
Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Laboratory reagent grade; purity not specified.

(558)

Type: Cytogenetic assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: Continuous exposure for 7 days
Doses: 9.7, 13.9, 36.8, 73.7 mg/m3
Result:
Method: other
Year: 1983 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Remark: Colchicine was administered by i.p. 2 hours prior to sacrifice. One hundred metaphases were evaluated per animal. Benzene concentration had no effect on mitotic activity in the bone marrow. However, the frequency of cells with chromosomal aberrations increased with increasingbenzene concentration. The minimal active concentration of benzene was 36.8 mg/m3. The maximum inactive concentration was 13.9 mg/m3. The principal chromosomal aberrations were single and paired fragments. Single chromatid exchanges were observed. However, there were no chromosomal exchanges. The calculated allowable concentration of benzene that would cause the spontaneous mutation rate to increase by 1% compared to controls equalled 0.06 mg/m3. Inaccordance with the formula MAD/200 proposed by Zhurkov, theallowable concentration of benzene by inhalation was 0.18 mg/m3. [MAD = the minimal allowable dose of mutagen causinga significant increase in the mutation level in the experimental group compared to control.]
Source: Deutsche Shell Chemie GmbH Eschborn

(560)

Type: Cytogenetic assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: gavage
Exposure period: 10 consecutive days
Doses: 5, 20 and 80 mg/kg in vegetable oil
Result:
Method: other
Year: 1983 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Remark: Colchicine was administered by i.p. 2 hours prior to sacrifice. One hundred metaphases were evaluated per animal. Benzene concentration had no effect on mitotic activity in bone marrow. However, the frequency of cells with chromosomal aberrations increased with an increase in benzene concentration. The minimal active dose was 20 mg/kg, and the maximal inactive dose was 5 mg/kg. The principal chromosomal aberrations were single and paired fragments. Single chromatid exchanges were observed. Therewere no chromosomal exchanges. The calculated allowable concentration of benzene that would cause the spontaneous mutation rate to increase by 1% compared to

controls equalled 0.07 mg/kg. In accordance with the formula MAD/200proposed by Zhurkov, the allowable concentration of benzene by oral route was 0.1 mg/kg. [MAD = the minimal allowable dose of mutagen causing a significant increase in the mutation level in the experimental group compared to control.]

Source: Deutsche Shell Chemie GmbH Eschborn

(560)

Type: Cytogenetic assay
Species: mouse **Sex:** no data
Strain: no data
Route of admin.: i.p.
Exposure period: no data
Doses: 0.2 to 2.0 ml/Kg/day
Result:
Method: other: see ref.
Year: 1971 **GLP:** no data
Test substance: no data
Remark: Studies of chromosomal aberrations in bone-marrow cells
Result: Most of the induced aberrations were breaks or deletions
Source: REPSOL PETROLEO,S.A. MADRID

(561)

Type: Dominant lethal assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: gavage
Exposure period: no data
Doses: up to 320 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was administered in sunflower-seed oil to SHR strain mice at four doses ranging from 0.001-0.2 LD50. No further details were available from the abstract.
Result: Benzene did not induce dominant lethal mutations. A negative result was also apparently obtained after male rats were injected intraperitoneally with 0.5 ml benzene/kg bw [approximately 440 mg/kg bw] (Lyon J.P. PhD Thesis, University of California, 1975 cited in Dean B.J. Mutation Res. 47, 75-91, 1978). There is one report of dominant lethal induction in mouse spermatogonia following intraperitoneal injection of 3000 mg/kg bw (Pavlenko G.I. et al. Toksikol. Nov. Prom. Khim. Veshchestv. 15, 30-33, 1979 cited in IPCS, 1991).
Source: BP Chemicals Ltd. London

(562) (563) (564)

Type: Dominant lethal assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: gavage
Exposure period: 5 weeks
Doses: up to 320 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was administered in sunflower-seed oil to SHR strain mice at four doses ranging from 0.001-0.2 LD50. No further details were available from the abstract.
Reliability: 4 (validity could not be judged)
Original reference in Russian
data from abstract or data from secondary literature
Result: Benzene did not induce dominant lethal mutations. A negative result was also apparently obtained after male rats were injected intraperitoneally with 0.5 ml benzene/kg bw [approximately 440 mg/kg bw] (Lyon J.P. PhD Thesis, University of California, 1975 cited in Dean B.J. Mutation Res. 47, 75-91, 1978). There is one report of dominant lethal induction in mouse spermatogonia following intraperitoneal injection of 3000 mg/kg bw (Pavlenko G.I. et al. Toksikol. Nov. Prom. Khim. Veshchestv. 15, 30-33, 1979 cited in IPCS, 1991).
Source: Deutsche Shell Chemie GmbH Eschborn
(563) (564)

Type: Dominant lethal assay
Species: mouse **Sex:** male
Strain: NMRI
Route of admin.: oral unspecified
Exposure period: einmalige Applikation
Doses: 1758; 3516 mg/kg
Result:
Method: other: nach Roehrborn und Vogel, Deutsche med. Wochenzeitschrift, 92, 2315-2321, (1967)
Year: **GLP:** no
Test substance: as prescribed by 1.1 - 1.4
Result: Die Versuchs- und Kontrollgruppen bestanden aus je 20 maennl. Tieren.
Die behandelten Tiere wurden ca. 20 Stunden nach der Applikation mit 3 unbehandelten weibl. Tieren fuer die Dauervon 7 Tagen verpaart, danach wurden den maennl. Tieren 7 malfuer die Dauer von je 7 Tagen je 3 weibl. Tiere zugesetzt.
In der niederen Dosisgruppe zeigten die Tiere voruebergehendeinen geringfuegigen Gewichtsverlust. In der hohen Dosisgruppe wurde neben dem zeitweisen Gewichtsverlust, Zittern, Unruhe und Apathie bei den behandelten Tieren festgestellt. Die Konzeptionsrate war in beiden Dosisgruppenin der ersten Paarungswoche verringert. Es wurden keine Veraenderungen bezueglich des Mutagenitaetsindex festgestellt.
Source: BASF AG Ludwigshafen
Reliability: (2) valid with restrictions

Bewertung und Dokumentation nachvollziehbar und akzeptabel
(565)

Type: Dominant lethal assay
Species: mouse **Sex:** male
Strain: NMRI
Route of admin.: oral unspecified
Exposure period: einmalige Applikation
Doses: 1758; 3516 mg/kg
Result:
Method: other: nach Standard Protocol for the Dominant Lethal Test on Male Mice, Arch. Toxicol., 39, 173-185, (1978)
Year: **GLP:** no
Test substance: as prescribed by 1.1 - 1.4
Result: Die Kontroll- und Versuchsgruppen bestanden aus je 45 maennlichen Tieren. Diese wurden ca. 20 Stunden nach der Applikation mit je einem unbehandelten Weibchen fuer 4 Tage verpaart. Danach wurden den gleichen maennlichen Tieren, in 11 weiteren aufeinanderfolgenden Versuchsserien von je 4 Tagen Dauer, jeweils ein neues weibl. Tier zugesetzt.
In der niederen Dosisgruppe starb ein Tier, in der hohen Dosisgruppe 4 Tiere waehrend der Versuchsdauer. Bei den Tieren der hohen Dosisgruppe wurde voruebergehend eine Gewichtsabnahme festgestellt. Bis auf eine erniedrigte Konzeptionsrate im ersten Paarungsintervall in der hohen Dosisgruppe wurden keine Effekte auf die Konzeptionsraten, die durchschnittliche Zahl der Implantate, den prozentualen Anteil lebender Foeten bzw. abgestorbener Implantate festgestellt. Der Mutagenitaetsindex und Dominant-Letal-Faktor waren aufgrund der Behandlung nicht veraendert.
Source: BASF AG Ludwigshafen
Reliability: (1) valid without restriction
Guideline-aehnliche Studie nach Standard-Vorschrift
(566)

Type: Drosophila SLRL test
Species: Drosophila melanogaster **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: 1 hour
Doses: 0, 27000 ppm (converts to 0, 88 mg/l)
Result:
Method: OECD Guide-line 477 "Genetic Toxicology: Sex-linked Recessive Lethal Test in Drosophila melanogaster"
Year: 1984 **GLP:** no data
Test substance: no data
Remark: Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. They were then mated daily to 5 Basc virgins for 12 days to obtain 12 successive one-day broods. The progeny were pair mated separately for each brood and these pair matings were scored for the presence or absence of lethals. All suspected lethals were confirmed by testing for one more generation. Laboratory cumulative control data were used as

the comparison.
Result: Benzene did not induce any sex-linked recessive lethal mutations.
Source: BP Chemicals Ltd. London
(567)

Type: Drosophila SLRL test
Species: Drosophila melanogaster **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: 1 hour
Doses: 0, 27000 ppm (converts to 0, 88 mg/l)
Result:
Method: other
Year: 1984 **GLP:** no data
Test substance: no data
Remark: Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. They were then mated daily to 5 Basc virgins for 12 days to obtain 12 successive one-day broods. The progeny were pair mated separately for each brood and these pair matings were scored for the presence or absence of lethals. All suspected lethals were confirmed by testing for one more generation. Laboratory cumulative control data were used as the comparison.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about test substance, one dose
Result: Benzene did not induce any sex-linked recessive lethal mutations.
Source: Deutsche Shell Chemie GmbH Eschborn
(567)

Type: Heritable translocation assay
Species: Drosophila melanogaster **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: 1 hour
Doses: 0, 27000 ppm (converts to 0, 88 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. For the detection of heritable translocations, the treated males were individually mated to ten virgin females for 2 days to obtain one brood. Six such broods were cultured and the F1 males individually back crossed to one virgin. These pair matings were scored for brown-and-scarlet or white-eye coloured males.
Result: A 1-hour exposure to 88 mg/l kills approximately 80% of the exposed flies in 2 or 3 days. Benzene did not induce translocations in the spermatozoon, spermatid or spermatocyte chromosomes when compared with the controls.

Source: BP Chemicals Ltd. London
(567)

Type: Heritable translocation assay
Species: Drosophila melanogaster **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure period: 1 hour
Doses: 0, 27000 ppm (converts to 0, 88 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. For the detection of heritable translocations, the treated males were individually mated to ten virgin females for 2 days to obtain one brood. Six such broods were cultured and the F1 males individually back crossed to one virgin. These pair matings were scored for brown-and-scarlet or white-eye coloured males.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no data about the TS
Result: A 1-hour exposure to 88 mg/l kills approximately 80% of the exposed flies in 2 or 3 days. Benzene did not induce translocations in the spermatozoon, spermatid or spermatocyte chromosomes when compared with the controls.
Source: Deutsche Shell Chemie GmbH Eschborn
(567)

Type: Mammalian germ cell cytogenetic assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: i.p.
Exposure period: 5 days
Doses: 0, 0.1, 0.25, 0.4, 0.5, 0.6, 0.8, 1.0 ml/kg bw/day (converts to 0, 88, 220, 352, 440, 527, 703, 880 mg/kg bw/day)
Result:
Method: other
Year: 1980 **GLP:** no data
Test substance: other TS
Remark: The method of Topham J.C. Mutation Res. 69, 149-155, 1980 was followed. The animals tested were the F1 generation derived from mating CBA male mice with BALB/c females. Groups of five were exposed to each dose. Five weeks after the final dose, smears of caudal sperm were examined for head abnormalities. Cyclophosphamide monohydrate was used as the positive control. Benzene was administered in corn oil and negative control animals were treated with corn oil alone.
Result: Benzene administration caused an increase in sperm-head abnormalities. Although this occurred over a limited dose range, it was reproducible in repeat experiments.
Source: BP Chemicals Ltd. London

Test substance: Laboratory reagent grade; purity not specified.
(568)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 10.2, 25.6, 64, 160, 400, 2500 µl/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten male and ten female Swiss Lane Petter mice were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The mice were given an intraperitoneal injection of colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determinations.
Result: The mean numbers of polychromatic erythrocytes with micronuclei increased with dose from 22.5 mg/kg bw/day. The number of polychromatic erythrocytes was reduced at 2198 mg/kg bw/day and, therefore, the reading of smears was unsatisfactory. Male mice were more sensitive to micronucleus induction than the females. These investigators have previously reported positive micronuclei tests in mice (Siou G. & Conan L. Cah. Notes Doc. 89, 443-444, 1977; Siou G. & Sourdeix D. ibid. 101, 531-534, 1980).
Source: BP Chemicals Ltd. London
Test substance: "Chemically pure" benzene tested.
(551)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: ICR
Route of admin.: gavage
Exposure period: 2 weeks
Doses: 0, 36.6, 73.2, 146.4 mg/kg bw/day
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten mice were administered benzene in olive oil daily for 2 weeks with no treatment on days 5 and 10. Control animals received olive oil only. Blood was withdrawn to assess micronucleus frequency on days 3, 6, 9, 12 and 15. Half the animals were maintained after treatment and sampled eight times, the last sample being obtained on day 60 after treatment.
Result: Benzene induced significant dose- and time-dependent increases in micronucleus frequency during treatment. In the mid- and high-dosed groups, increases were noted from day 9 and in the low-dosed group, the micronucleus frequency

was similar to the control levels until day 15. After day 11 post-treatment, the micronuclei levels began to decline but remained significantly higher than in the control group. In the treatment groups, erythropoiesis was dose-dependently suppressed during treatment. The levels of polychromatic erythrocytes in each dose group began to increase from day 9 and reached normal levels by day 15 of treatment.

Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; thiophene-free benzene tested.

(569)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: gavage
Exposure period: 5 days per week/6-8 weeks
Doses: 0.03, 0.06, 0.125, 0.250, 0.5 ml/kg bw/day (converts to 26, 53, 110, 220, 440 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was administered in olive oil. Peripheral blood smears from the tail vein were prepared and the incidence of micronuclei scored. Micronuclei induction was also determined in bone marrow cells after a single treatment of 220 mg/kg bw/day. Control animals were treated with olive oil alone.
Result: No evidence of toxicity was seen in animals treated for up to 6 weeks except for decreased body weight and polychromatic/normochromatic erythrocytes in the top-dosed males. A significant increase in micronuclei in normochromatic erythrocytes was seen after 21 days in the high-dosed males and females, the females being less sensitive. A dose-response relationship was seen when treatment was for longer than 21 days. A decline of micronuclei after longer periods was also observed. The investigators thought this may be due to a selection of resistant cells, a decreased ability to metabolise benzene towards genotoxic metabolites or an increased capability in detoxification of active benzene metabolites. The frequency of micronucleated polychromatic erythrocytes obtained in bone marrow cells was increased after a single benzene dose. In splenectomized male mice treated with 220 mg/kg bw/day, micronucleated circulating normochromatic erythrocytes were increased after 21 days of treatment followed by a significant decrease after 6 weeks. No spontaneous increase was noted in the control group. Other investigators have also reported positive findings in CD1 mice (Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984; Hite M. et al. ibid. 77, 149-155, 1980; Meyne J. & Legator M.S. Envir. Mutagen. 2, 43-50, 1980).
Source: BP Chemicals Ltd. London
Test substance: "Purest grade" benzene tested.

(570)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: ICR
Route of admin.: oral unspecified
Exposure period: 1 day
Doses: 0, 220, 440, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of five or more mice were treated with single doses of benzene alone or coadministered with pyridine, aniline or naphthalene to modify benzene's metabolism, all in olive oil. In one group, Ó–naphthoflavone was injected intraperitoneally 1 hour before benzene treatment. Some groups were induced with Aroclor–1254 or 3–methylcholanthrene. Animals were sacrificed 24 hours after benzene treatment and the numbers of micronuclei in the femoral bone marrow assessed. Urinary samples were collected to measure the benzene metabolites.
Result: Pyridine administered at 50 mg/kg bw and above inhibited benzene's clastogenicity when benzene was given at 880 mg/kg bw. The clastogenicity was partially blocked at this dose by 5 mg/kg bw pyridine while 0.5 mg/kg bw was ineffective. Urinary analysis showed pyridine to completely block benzene metabolism. Aniline or naphthalene coadministration enhanced the effect of benzene in the micronucleus assay. Urinary analysis in the aniline/benzene treated mice showed increased levels of hydroquinone in mice treated with 220 mg benzene/kg bw and 125 mg aniline/kg bw. As the aniline dose increased, the levels of hydroquinone and catechol decreased and the amount of phenol increased slightly. As the benzene dose increased, aniline did not affect its metabolism or clastogenicity. In the presence of naphthalene, the amounts of urinary hydroquinone and catechol were decreased. Pretreatment with Ó–naphthoflavone totally blocked both benzene's metabolism and clastogenicity when injected at high levels of 170 mg/kg bw. 3–Methylcholanthrene and phenobarbital increased the metabolism of benzene but clastogenicity was increased only by the former.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; thiophene–free benzene tested.

(571)

Type: Micronucleus assay
Species: hamster **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24–hour intervals. The hamsters were injected intraperitoneally with colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determination.
Result: No dose–related increase in the numbers of polychromatic erythrocytes with micronuclei were found. In the females given 2.2 g/kg bw/day, there was a slight increase in the mean number of micronuclei but this was not seen at the higher dose or in the male animals.
Source: BP Chemicals Ltd. London
Test substance: "Chemically pure" benzene tested.

(551)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD–1
Route of admin.: gavage
Exposure period: single dose
Doses: 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was administered in distilled water to male, female and, on day 13 of gestation, pregnant mice. At different periods after treatment, four animals were killed. Foetal liver and adult bone marrow cells were obtained and assessed for micronuclei. Further groups of animals were exposed to the benzene metabolites, catechol, p–benzoquinone, phenol, 1,2,4–benzenetriol, hydroquinone, o,o'–biphenol and p,p'–biphenol.
Result: Micronucleus induction was statistically significantly elevated in the males, females and pregnant females at each monitoring period. The males were more susceptible than the females and the virgin females more so than the pregnant females. Micronuclei were also induced in the foetal liver cells together with a high cell toxicity. All of the tested benzene metabolites induced micronuclei and, with the exception of catechol and 1,2,4–benzene–triol, produced myelotoxicity. The investigators concluded that the induction of micronuclei by benzene cannot be attributed solely to any particular benzene metabolite tested in this study. Hydroquinone and, to a lesser extent,

p-benzoquinone, catechol and o,o'-biphenol were active in inducing micronuclei and toxic effects in the foetal liver cells. p-Benzo-quinone, 1,2,4-benzenetriol and p,p'-biphenol produced toxic effects alone in the foetal cells.

Source: BP Chemicals Ltd. London

(572)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: gavage
Exposure period: single dose
Doses: 0, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone.
Result: Benzene induced micronuclei with peak activity after 24 hours. The genotoxicity then rapidly decreased but still caused significant effects after 48 hours. Bone marrow depression increased slowly, becoming evident and significant after 42 hours and reaching a maximum after 48 hours. All of the metabolites tested induced micronuclei although phenol and hydroquinone had weak effects. Bone marrow depression occurred in the phenol, p-benzoquinone and catechol treated animals. Hydroquinone produced negligible bone marrow depression until 42 hours after treatment.
Source: BP Chemicals Ltd. London

(573)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: DBA
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 10, 100, 1000 ppm (converts to 0, 0.03, 0.33, 3.26 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene concentrations were analysed hourly. Groups of five DBA/2 mice/dose were exposed. The control group consisted of ten animals exposed to air. Femoral bone marrow was prepared and stained 18 hours after exposure by a modification of the technique by Schmid W. In: Chemical Mutagens: Principles and Methods for their Detection. Vol.4. p.31-53. Edited by A. Hollaender. Plenum Press, New York, 1976. Four slides were prepared per animal.¿
Result: A statistically significant, dose-related increase in the number of bone marrow polychromatic erythrocytes containing

micronuclei was found. The top dose caused a 13.4-fold increase in the control level.
Source: BP Chemicals Ltd. London

(574)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: up to 16 weeks
Doses: 10, 25, 100, 300, 400 ppm (converts to 0.033, 0.083, 0.33, 0.98, 1.3 mg/l)
Result:
Method: other
Year: 1982 **GLP:** no data
Test substance: no data
Remark: The method of Schlegel R. & MacGregor J.T. Mutation Res. 104, 367-369, 1982 was followed. C57bl/6 mice were exposed for 9 days to 0.033, 0.083, 0.33 and 1.30 mg/l and peripheral blood smears were prepared 1 day later to analyse the frequency of micronuclei. For the 0.98 mg/l dose level, mice were exposed for 2, 4, 8 and 16 weeks and samples were taken 3 days, 2, 4, 8 and 16 weeks following cessation of exposure.
Result: The micronucleus frequency was statistically significantly increased in a dose-dependent manner in normochromatic erythrocytes of mice exposed at all the 9-day levels. Similar increases wee seen in the polychromatic erythrocytes of animals exposed to 0.083, 0.33 and 1.30 mg/l. When the micronucleus frequency was examined after different exposure and recovery times, the results suggested a lack of residual bone marrow damage and an alteration in the sensitivity of the animals to benzene with increasing duration of the exposure.
Source: BP Chemicals Ltd. London

(575)

Type: Micronucleus assay
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 0.1, 0.3, 1, 3, 10, 30 ppm (converts to 0, 0.0003, 0.001, 0.003, 0.01, 0.033, 0.098 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: The top two benzene concentrations were analysed hourly, the other concentrations being analysed two or three times per hour. The control group consisted of ten to 20 animals exposed to air. Groups of five animals/dose were exposed to benzene. Femoral bone marrow was prepared and stained 18 hours after exposure by a modification of the technique by Schmid W. In: Chemical Mutagens: Principles and Methods for their Detection. Vol.4. p.31-53. Edited by A. Hollaender. Plenum Press, New York, 1976. Four slides were prepared per

animal.
Result: Benzene induced statistically significant, dose-dependent increases in the numbers of polychromatic erythrocytes containing micronuclei at concentrations of 0.003 to 0.098 mg/l. There was no observable effect at the lower two doses.
Source: BP Chemicals Ltd. London

(574)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: 3 days
Doses: 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene diluted in corn oil. Control animals received corn oil only. Groups of five males and five females were exposed at each dose level. The animals were sacrificed 4 hours after the final dose, the femurs removed and bone marrow used for cytogenetic preparations.
Result: No increase in frequency of micronuclei was seen in the low-dosed females and no significant increase in the mid- and high-dosed females. In the male mice, a dose-related increase in the frequency of micronuclei was noted; the increases being significant at the two higher doses.
Source: BP Chemicals Ltd. London
Test substance: >99% pure.

(576)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: i.p.
Exposure period: single dose
Doses: 0, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone.
Result: A significant increase of micronuclei was seen after 18 hours. Bone marrow depression was also noted and remained constant up to 48 hours. Phenol produced immediate genotoxic effects which were no longer significant after 42 hours. Bone marrow depression also occurred. Genotoxicity which peaked at 18 hours and was not significant after 42 hours was seen with hydroquinone. Bone marrow depression

induction was rapid, peaking after 18 hours and returning to normal after 48 hours. p-Benzoquinone did not induce a significant micronucleus increase but high levels of toxicity were seen. Significant genotoxic effects were seen 24 hours after catechol treatment and bone marrow depression was evident after 18 hours.

Source: BP Chemicals Ltd. London

(573)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 10.2, 25.6, 64, 160, 400, 2500 ul/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)
Result:
Method: other
Year: 1985 **GLP:** no data
Test substance: other TS
Remark: Groups of ten male and ten female Swiss Lane Petter mice were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The mice were given an intraperitoneal injection of colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determinations.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no positive control, single sampling time
Result: The mean numbers of polychromatic erythrocytes with micronuclei increased significantly with dose from 56.3 mg/kg bw/day.
The number of polychromatic erythrocytes was reduced at 2198 mg/kg bw/day and, therefore, the reading of smears was unsatisfactory. Male mice were more sensitive to micronucleus induction than the females. These investigators have previously reported positive micronuclei tests in mice (Siou G. & Conan L. Cah. Notes Doc. 89, 443-444, 1977; Siou G. & Sourdeix D. ibid. 101, 531-534, 1980).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: "Chemically pure" benzene tested.

(551)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: gavage
Exposure period: 2 weeks
Doses: 0, 36.6, 73.2, 146.4 mg/kg bw/day
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten mice were administered benzene in olive oil daily for 2 weeks with no treatment on days 5 and 10. Control animals received olive oil only. Blood was withdrawn to assess micronucleus frequency on days 3, 6, 9, 12 and 15. Half the animals were maintained after treatment and sampled eight times, the last sample being obtained on day 60 after treatment.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions one sex, no positive control, no toxicity data
Result: Benzene induced significant dose- and time-dependent increases in micronucleus frequency during treatment. In the mid- and high-dosed groups, increases were noted from day 9 and in the low-dosed group, the micronucleus frequency was similar to the control levels until day 15. After day 11 post-treatment, the micronuclei levels began to decline but remained significantly higher than in the control group. In the treatment groups, erythropoiesis was dose-dependently suppressed during treatment. The levels of polychromatic erythrocytes in each dose group began to increase from day 9 and reached normal levels by day 15 of treatment.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; thiophene-free benzene tested.

(569)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: gavage
Exposure period: 5 days per week, 6-8 weeks
Doses: 0.03, 0.06, 0.125, 0.250, 0.5 ml/kg bw/day (converts to 26, 53, 110, 220, 440 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was administered in olive oil. Peripheral blood smears from the tail vein were prepared and the incidence of micronuclei scored. Micronuclei induction was also determined in bone marrow cells after a single treatment of 220 mg/kg bw. Control animals were treated with olive oil alone.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions one dose, no data about PCE/NCE ratio in bone marrow experiment, no positive control, 3 mice per group

Result: No evidence of toxicity was seen in animals treated for up to 6 weeks except for decreased body weight and polychromatic/normochromatic erythrocytes in the top-dosed males. A significant increase in micronuclei in normochromatic erythrocytes was seen after 21 days in the high-dosed males and females, the females being less sensitive. A dose-response relationship was seen when treatment was for longer than 21 days. A decline of micronuclei after longer periods was also observed. The investigators thought this may be due to a selection of resistant cells, a decreased ability to metabolise benzene towards genotoxic metabolites or an increased capability in detoxification of active benzene metabolites. The frequency of micronucleated polychromatic erythrocytes obtained in bone marrow cells was increased after a single benzene dose. In splenectomized male mice treated with 220 mg/kg bw/day, micronucleated circulating normochromatic erythrocytes were increased after 21 days of treatment followed by a significant decrease after 6 weeks. No spontaneous increase was noted in the control group. Other investigators have also reported positive findings in CD1 mice (Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984; Hite M. et al. ibid. 77, 149-155, 1980; Meyne J. & Legator M.S. Envir. Mutagen. 2, 43-50, 1980).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: "Purest grade" benzene tested.

(570)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: ICR
Route of admin.: other
Exposure period: once
Doses: 0, 220, 440, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of five or more mice were treated with single doses of benzene alone or coadministered with pyridine, aniline or naphthalene to modify benzene's metabolism, all in olive oil. In one group, -naphthoflavone was injected intraperitoneally 1 hour before benzene treatment. Some groups were induced with Aroclor-1254 or 3-methylcholanthrene. Animals were sacrificed 24 hours after benzene treatment and the numbers of micronuclei in the femoral bone marrow assessed. Urinary samples were collected to measure the benzene metabolites.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, one sex, one sampling time, no data about PCE/NCE ratio
Result: Benzene induced significant dose dependent increase in micronucleus frequency.
Pyridine administered at 50 mg/kg bw and above inhibited benzene's clastogenicity when benzene was given at 880 mg/kg bw. The clastogenicity was partially blocked at this dose

by 5 mg/kg bw pyridine while 0.5 mg/kg bw was ineffective. Urinary analysis showed pyridine to completely block benzene metabolism. Aniline or naphthalene coadministration enhanced the effect of benzene in the micronucleus assay. Urinary analysis in the aniline/benzene treated mice showed increased levels of hydroquinone in mice treated with 220 mg benzene/kg bw and 125 mg aniline/kg bw. As the aniline dose increased, the levels of hydroquinone and catechol decreased and the amount of phenol increased slightly. As the benzene dose increased, aniline did not affect its metabolism or clastogenicity. In the presence of naphthalene, the amounts of urinary hydroquinone and catechol were decreased. Pretreatment with –naphthoflavone totally blocked both benzene's metabolism and clastogenicity when injected at high levels of 170 mg/kg bw. 3-Methylcholanthrene and phenobarbital increased the metabolism of benzene but clastogenicity was increased only by the former.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Laboratory reagent grade; thiophene-free benzene tested.

(571)

Type: Micronucleus assay
Species: Chinese hamster **Sex:** male/female
Strain:
Route of admin.: gavage
Exposure period: 24 hours
Doses: 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The hamsters were injected intraperitoneally with colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determination.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no positive control, one sampling time, no data about PCE/NCE ratio

Result: No dose-related increase in the numbers of polychromatic erythrocytes with micronuclei were found. In the females given 2.2 g/kg bw/day, there was a slight increase in the mean number of micronuclei but this was not seen at the higher dose or in the male animals.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: "Chemically pure" benzene tested.

(551)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: gavage
Exposure period: single dose
Doses: 0, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene was administered in distilled water to male, female and, on day 13 of gestation, pregnant mice. At different periods after treatment, four animals were killed. Foetal liver and adult bone marrow cells were obtained and assessed for micronuclei. Further groups of animals were exposed to the benzene metabolites, catechol, p-benzoquinone, phenol, 1,2,4-benzenetriol, hydroquinone, o,o'-biphenol and p,p'-biphenol.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, no data about TS, one dose, 4 instead of 5 recommended mice/group
Result: Micronucleus induction was statistically significantly elevated in the males, females and pregnant females at each monitoring period. The males were more susceptible than the females and the virgin females more so than the pregnant females. Micronuclei were also induced in the foetal liver cells together with a high cell toxicity. All of the tested benzene metabolites induced micronuclei and, with the exception of catechol and 1,2,4-benzene-triol, produced myelotoxicity. The investigators concluded that the induction of micronuclei by benzene cannot be attributed solely to any particular benzene metabolite tested in this study. Hydroquinone and, to a lesser extent, p-benzoquinone, catechol and o,o'-biphenol were active in inducing micronuclei and toxic effects in the foetal liver cells. p-Benzo-quinone, 1,2,4-benzenetriol and p,p'-biphenol produced toxic effects alone in the foetal cells.
Source: Deutsche Shell Chemie GmbH Eschborn

(572)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: gavage
Exposure period: single dose
Doses: 0, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions one sex, no positive control, one dose, 4 instead of 5 recommended mice/group
Result: Benzene induced micronuclei with peak activity after 24 hours. The genotoxicity then rapidly decreased but still caused significant effects after 48 hours. Bone marrow depression increased slowly, becoming evident and significant after 42 hours and reaching a maximum after 48 hours. All of the metabolites tested induced micronuclei although phenol and hydroquinone had weak effects. Bone marrow depression occurred in the phenol, p-benzoquinone and catechol treated animals. Hydroquinone produced negligible bone marrow depression until 42 hours after treatment.
Source: Deutsche Shell Chemie GmbH Eschborn

(573)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 10, 100, 1000 ppm (converts to 0, 0.03, 0.33, 3.26 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene concentrations were analysed hourly. Groups of five DBA/2 mice/dose were exposed. The control group consisted of ten animals exposed to air. Femoral bone marrow was prepared and stained 18 hours after exposure by a modification of the technique by Schmid W. In: Chemical Mutagens: Principles and Methods for their Detection. Vol.4. p.31-53. Edited by A. Hollaender. Plenum Press, New York, 1976. Four slides were prepared per animal.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, no data about TS, one sex, one sampling time, no data about toxicity and PCE/NCE ratio
Result: A statistically significant, dose-related increase in the number of bone marrow polychromatic erythrocytes containing

micronuclei was found. The top dose caused a 13.4-fold increase in the control level.
Source: Deutsche Shell Chemie GmbH Eschborn

(574)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: up to 16 weeks
Doses: 10, 25, 100, 300, 400 ppm (converts to 0.033, 0.083, 0.33, 0.98, 1.3 mg/l)
Result:
Method: other
Year: 1982 **GLP:** no data
Test substance: no data
Remark: Reliability: 4 (validity cannot be judged)
Abstract
The method of Schlegel R. & MacGregor J.T. Mutation Res. 104, 367-369, 1982 was followed. C57bl/6 mice were exposed for 9 days to 0.033, 0.083, 0.33 and 1.30 mg/l and peripheral blood smears were prepared 1 day later to analyse the frequency of micronuclei. For the 0.98 mg/l dose level, mice were exposed for 2, 4, 8 and 16 weeks and samples were taken 3 days, 2, 4, 8 and 16 weeks following cessation of exposure.
Result: The micronucleus frequency was statistically significantly increased in a dose-dependent manner in normochromatic erythrocytes of mice exposed at all the 9-day levels. Similar increases wee seen in the polychromatic erythrocytes of animals exposed to 0.083, 0.33 and 1.30 mg/l. When the micronucleus frequency was examined after different exposure and recovery times, the results suggested a lack of residual bone marrow damage and an alteration in the sensitivity of the animals to benzene with increasing duration of the exposure.
Source: Deutsche Shell Chemie GmbH Eschborn

(577)

Type: Micronucleus assay
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 0.1, 0.3, 1, 3, 10, 30 ppm (converts to 0, 0.0003, 0.001, 0.003, 0.01, 0.033, 0.098 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, no data about TS (but concurrent analysis), one sex, one sampling time, no data about toxicity and PCE/NCE ratio
The top two benzene concentrations were analysed hourly, the other concentrations being analysed two or three times per

hour. The control group consisted of ten to 20 animals exposed to air. Groups of five animals/dose were exposed to benzene. Femoral bone marrow was prepared and stained 18 hours after exposure by a modification of the technique by Schmid W. In: Chemical Mutagens: Principles and Methods for their Detection. Vol.4. p.31-53. Edited by A. Hollaender. Plenum Press, New York, 1976. Four slides were prepared per animal.

Result: Benzene induced statistically significant, dose-dependent increases in the numbers of polychromatic erythrocytes containing micronuclei at concentrations of 0.003 to 0.098 mg/l. There was no observable effect at the lower two doses.

Source: Deutsche Shell Chemie GmbH Eschborn

(574)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: gavage
Exposure period: 3 days
Doses: 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene diluted in corn oil. Control animals received corn oil only. Groups of five males and five females were exposed at each dose level. The animals were sacrificed 4 hours after the final dose (2 h after colchicine treatment, assay combined with metaphase analysis), the femurs removed and bone marrow used for cytogenetic preparations. 5 male and 5 female mice per group.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, no toxicity data, one sampling time

Result: Significant increase in frequency of micronuclei was seen in the in the mid- and high-dosed male and female mice.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99% pure.

(576)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: CD–1
Route of admin.: i.p.
Exposure period: single dose
Doses: 0, 880 mg/kg bw
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0–48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p–benzoquinone, phenol and hydroquinone.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no positive control, one sex, 4 instead of 5 recommended animals per group
Result: A significant increase of micronuclei was seen after 18 hours. Bone marrow depression was also noted and remained constant up to 48 hours. Phenol produced immediate genotoxic effects which were no longer significant after 42 hours. Bone marrow depression also occurred. Genotoxicity which peaked at 18 hours and was not significant after 42 hours was seen with hydroquinone. Bone marrow depression induction was rapid, peaking after 18 hours and returning to normal after 48 hours. p–Benzoquinone did not induce a significant micronucleus increase but high levels of toxicity were seen. Significant genotoxic effects were seen 24 hours after catechol treatment and bone marrow depression was evident after 18 hours.
Source: Deutsche Shell Chemie GmbH Eschborn

(573)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD–1
Route of admin.: gavage
Exposure period: 5 days/week, for 42 days
Doses: 0.00, 0.03, 0.06, 0.125, 0.250, 0.500 ml/kg body weight
Result:
Method: other
Year: 1980 **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Remark: The study methodology is based on the hypothesis that, unlike man and the rat, mouse damaged erythrocytes are not eliminated by the spleen. Both normal and splenectomized mice were administered benzene in corn oil by oral gavage (3/sex/dose group). Peripheral blood was evaluated weekly for micronucleus formation. A total of 3000 normochromatic erythrocytes were evaluated per animal. A slight decrease in body weight and decreased polychromatic (PCE): NCE ratiowas evident in high dose males. A significant increase in micronuclei formation was only observed in male and female mice (with intact spleens) after Day 21 at the

highest dose groups (0.250, 0.50 ml/kg body weight). A dose-response wasevident following exposure Day 21, reaching a steady state after Day 28 in females and Day 42 in males (depending on dose level). However, females were less sensitive to micronuclei formation than were their male counterparts. Anincrease in micronuclei formation was also observed in splenectomized mice exposed to 0.25 ml/kg benzene after Day 21, followed by a significant decrease in micronuclei at Day42.

Result: Results: positive
Source: Deutsche Shell Chemie GmbH Eschborn

(570) (578)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: gavage
Exposure period: Daily for 2 consecutive days; sacrificed at 6 hours to 16 days post-dose
Doses: 0.0625, 0.125, 0.25, 0.5, 1.0 or 2.0 ml/kg/day. Positive control: 0.26 mg/ml methyl methanesulfonate (i.p.). Negative control: corn oil (p.o.).
Result:
Method: other
Year: 1980 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Remark: Mice (4-5/sex/benzene group) were sacrificed 6, 18, 24 or 48hours, or 5, 9 or 16 days post exposure. Bone marrow was evaluated for micronuclei formation. Approximately 3000 polychromatic (PCE) and normochromatic (NCE) erythrocytes were evaluated per animal. Responses were similar for both sexes. The number of micronuclei in NCE's showed no relationship to treatment. A significant increase in micronucleated PCE's was observed at 6 hours, at doses greater than or equal to 0.25 ml/kg/day; and at 18 or 24 hours in mice exposed at 0.125 mg/kg/day or higher benzene. However, in a second experiment, mice exposed to 0.0625, 0.125, or 0.25 mg/kg/day benzene failed to show increased micronuclei formation at the 24 hour sacrifice. Mice exposed at concentrations greater than or equal to 0.125 mg/kg/day and sacrificed 5 days post exposure failed to showa significant increase in micronucleated PCE's. By Days 9 or 16 of sacrifice, mice exposed at the highest concentrations (0.5, 1.0 or 2.0 mg/kg/day) exhibited micronuclei values that were similar to negative controls.
Result: Results: positive
Source: Deutsche Shell Chemie GmbH Eschborn

(579)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: NMRI
Route of admin.: inhalation
Exposure period: Continuous exposure for 10 days
Doses: 0, 1, 10, 14, 21, 50, 95 ppm
Result:
Method: other
Year: 1982 **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Remark: Continuous exposure of mice (5/group) to benzene for a period of 4 – 10 days at levels as low as 21 ppm significantly affected the number of nucleated cells/tibia, the number of colony forming granulopoietic stem cells (CFU–C)/tibia and the number micronucleated polychromatic erythrocytes (PCE's) in the bone marrow. At lower levels (10 and 1 ppm) no reproducible effects on cellularity or CFU–C/tibia could be demonstrated even after 8 weeks of exposure. However, at 14 ppm the frequency of micronucleated PCE's was slightly elevated after only 1 weekof exposure but did not continue to increase with greater exposure periods.
Result: Results: positive
Source: Deutsche Shell Chemie GmbH Eschborn

(580)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: NMRI
Route of admin.: inhalation
Exposure period: 8 hours/day, 5 days/week for 2 weeks
Doses: 0, 1.0, 10.5, 21, 50, 95, and 107 ppm
Result:
Method: other
Year: 1982 **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Remark: Intermittent exposure of mice (5 males/group) to benzene resulted in a significant decrease in the number of colony forming granulopoietic stem cells (CFU–C)/tibia and a significant increase in micronuclei frequency compared to controls at concentrations exceeding 10.5 ppm. The number of nucleated cells/tibia was significantly different from controls at concentrations exceeding 21 ppm.
Result: Results: measurable toxicity
Source: Deutsche Shell Chemie GmbH Eschborn

(580)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: unspecified
Exposure period: 0, 15, 30, 60, 90 days
Doses: 1, 10, 30, 300 pmm
Result:
Method: other: see ref.
Year: 1985 **GLP:** no data
Test substance: as prescribed by 1.1 - 1.4
Result: Exposure to 3000 ppm benzene caused a significant increase in micronucleated polychromatic and monochromatic erytrocytes in male and female mice at all simples times
Source: REPSOL PETROLEO,S.A. MADRID

(581)

Type: Sister chromatid exchange assay
Species: mouse **Sex:** male/female
Strain: DBA
Route of admin.: inhalation
Exposure period: 4 hours
Doses: 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six males and six females. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrows flushed out and SCE frequency assessed.
Result: Benzene significantly increased the level of SCEs in bone marrow cells when compared with the control group. There was no significant difference between the treated males and females. Phenobarbital pretreatment enhanced the benzene-increased SCE frequency in female but not male mice.
Source: BP Chemicals Ltd. London

(582) (583) (584) (585) (555)

Type: Sister chromatid exchange assay
Species: mouse **Sex:** male
Strain: DBA
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 10, 100, 1000 ppm (converts to 0, 0.033, 0.33, 3.26 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Each dose group consisted of five DBA/2 mice and the control group of ten mice, exposed to room air. Exposure chamber atmospheres were analysed hourly. Blood was withdrawn 18 hours after benzene exposure and lymphocytes were cultured. 5–Bromo–2′–deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 60 hours. Two or three slides were prepared per animal for SCE analysis.
Result: Benzene induced significant, dose–related increases in the SCE frequencies at all the dose levels. A significant dose–related decrease in the mitotic activity was seen but there were no significant effects on leucocyte counts.
Source: BP Chemicals Ltd. London

(574)

Type: Sister chromatid exchange assay
Species: rat **Sex:** male
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 0.1, 0.3, 1, 3, 10, 30 ppm (converts to 0, 0.0003, 0.001, 0.003, 0.01, 0.033, 0.098 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Each dose group consisted of five animals and the control groups of ten or twenty mice, exposed to room air. Exposure chamber atmospheres were analysed hourly for the top two benzene concentrations and two to three times per hour for the other doses. Blood was withdrawn 18 hours after benzene exposure and lymphocytes were cultured.
5–Bromo–2′–deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 52 hours. Two or three slides were prepared per animal for SCE analysis.
Result: Benzene induced significant dose–dependent increases in the SCE frequency at 0.01 mg/l and above. The significance of the effect seen at 0.003 mg/l was borderline and dependent on the statistical test chosen, although the SCE frequency falls in line between the SCE frequencies observed at 0.001 and 0.01 mg/l and is higher in all exposed rats when compared with the controls. Benzene concentrations of 0.0003 and 0.001 mg/l did not significantly affect the SCE frequency. Mitotic activity was significantly decreased at 0.01 and 0.098 mg/l but not at 0.033 mg/l. No significant effect was seen on leucocyte count.
Source: BP Chemicals Ltd. London

(574)

Type: Sister chromatid exchange assay
Species: mouse **Sex:** male/female
Strain: other
Route of admin.: inhalation
Exposure period: 4 hours
Doses: 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six males and six females. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrows flushed out and SCE frequency assessed.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no positive control, one sampling time, single dose (exposure period)
Result: Benzene significantly increased the level of SCEs in bone marrow cells when compared with the control group. There was no significant difference between the treated males and females. Phenobarbital pretreatment enhanced the benzene-increased SCE frequency in female but not male mice.
Source: Deutsche Shell Chemie GmbH Eschborn
(586) (585) (555)

Type: Sister chromatid exchange assay
Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 10, 100, 1000 ppm (converts to 0, 0.033, 0.33, 3.26 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Each dose group consisted of five DBA/2 mice and the control group of ten mice, exposed to room air. Exposure chamber atmospheres were analysed hourly. Blood was withdrawn 18 hours after benzene exposure and lymphocytes were cultured. 5-Bromo-2'-deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 60 hours. Two or three slides were prepared per animal for SCE analysis.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no positive control, no data about the TS (
Result: Benzene induced significant, dose-related increases in the

SCE frequencies at all the dose levels. A significant dose-related decrease in the mitotic activity was seen but there were no significant effects on leucocyte counts.

Source: Deutsche Shell Chemie GmbH Eschborn

(574)

Type: Sister chromatid exchange assay
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 6 hours
Doses: 0, 0.1, 0.3, 1, 3, 10, 30 ppm (converts to 0, 0.0003, 0.001, 0.003, 0.01, 0.033, 0.098 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Each dose group consisted of five animals and the control groups of ten or twenty mice, exposed to room air. Exposure chamber atmospheres were analysed hourly for the top two benzene concentrations and two to three times per hour for the other doses. Blood was withdrawn 18 hours after benzene exposure and lymphocytes were cultured.
5-Bromo-2'-deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 52 hours. Two or three slides were prepared per animal for SCE analysis.
Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no positive control, no data about the TS
Result: Benzene induced significant dose-dependent increases in the SCE frequency at 0.01 mg/l and above. The significance of the effect seen at 0.003 mg/l was borderline and dependent on the statistical test chosen, although the SCE frequency falls in line between the SCE frequencies observed at 0.001 and 0.01 mg/l and is higher in all exposed rats when compared with the controls. Benzene concentrations of 0.0003 and 0.001 mg/l did not significantly affect the SCE frequency. Mitotic activity was significantly decreased at 0.01 and 0.098 mg/l but not at 0.033 mg/l. No significant effect was seen on leucocyte count.
Source: Deutsche Shell Chemie GmbH Eschborn

(574)

Type: Somatic mutation assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 22 hours per day/7 days per week/6 weeks
Doses: 0, 40, 100, 1000 ppb
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of 14 mice/sex were exposed to benzene in whole-body inhalation chambers. At sacrifice, the spleen of each mouse was removed and the lymphocytes released. The numbers of hypoxanthine-guanine phosphoribosyl transferase (hprt) mutant (variant) cells were determined according to an autoradiographic hprt mutant lymphocyte assay. Control groups of mice were either exposed to purified air or housed in standard plastic cages.
Result: Benzene caused a dose-dependent increase in the frequencies of hprt variant (mutant) lymphocytes in both male and female mice at concentrations of 40 and 100 ppb. At 1000 ppb, the frequency was also increase when compared with the controls but this was less than in the 40 and 100 ppb groups. Females were more sensitive than males.
Source: BP Chemicals Ltd. London

(587)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** male/female
Strain: other
Route of admin.: oral feed
Exposure period: up to 96 hours
Doses: 0.88, 4.4, 8.8 mg/ml
Result:
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: The method of Wurgler F.E. et al. Mutation Res. 122, 321-328, 1983 was followed. Benzene was administered to mei-9 and +/mei-9 strain Drosophila as a solution in 5% Tween 80 and 5% ethanol. Larvae aged 48 or 72 hours were exposed for 2 hours to benzene concentrations of 4.4 and 0.88 mg/ml respectively. In some cases, larvae were treated during the entire larval life (96 hours) to 4.4 or 8.8 mg/ml. After exposure, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and the frequency and size of the spots were recorded. Control experiments were conducted by exposing larvae of the same age as those in the treatment groups to the 5% Tween 80 and 5% ethanol vehicle for the same length of time as the corresponding treated larvae.
Result: A benzene concentration of 8.8 mg/ml was lethal and 4.4 mg/ml toxic when given for the entire larval life. A significant increase in the frequency of small single spots and/or total single spots was seen in the acute and chronic studies in the excision-repair-defective larvae (mei-9

strain). The acute result was only marginally positive. No such effect was seen when benzene was given to excision-repair-proficient larvae (+/mei-9 strain).
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(588)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** male
Strain: other
Route of admin.: oral feed
Exposure period: 96 hours
Doses: 0, 0.5, 1, 2 % (converts to 0, 4.4, 8.8, 17.6 mg/ml)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in 40% ethanol. Male and female C(1)DX,yf/Y/sc z w+ mei-9a Drosophila were mated for 4-5 days and allowed to ovipost for 24 hours. The resultant eggs were allowed to develop for 24 hour and the newly hatched larvae exposed to benzene or the vehicle as the control. Benzene was added (1 ml) to the culture medium and the larvae were allowed to develop throughout the larval period (roughly 96 hours). Eyes of adult males were examined for colour mutations.
Result: No significant increase in mutation frequency over the control levels was seen. Benzene, however, caused a dose-dependent toxic effect on the survival of the male larvae, with 50% survival occurring at the top dose. A similar study using the genotype sc z w+ and benzene concentrations of 8.8 and 17.6 mg/ml reported similar decreases in survival and a lack of mutagenic effect (Nylander P.-O. et al. Mutation Res. 57, 163-167, 1978).
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(589)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** female
Strain: other
Route of admin.: oral feed
Exposure period: 10-11 days
Doses: 156 mg/l
Result:
Method: other
Year: 1957 **GLP:** no data
Test substance: other TS
Remark: The method of Becker H.J. Z. Indukt. Abstammungs-Vererbungsl. 88, 333-373, 1957 was followed. Benzene was prepared in a mixture of 3% ethanol and 1% Tween 60 before being added to the food. Virgin females of the genotype wcosn2; se h were crossed with w; se h males and allowed to lay eggs for a period of 4 days on the food supplemented with benzene. The control larvae were exposed to the solvent. The developing culture was incubated for 10-11 days and the hatching females were scored for genetic

changes which produced w clones or wco//w twin spots in their eyes.
Result: No increase in the number of mosaic twin spots was observed. However, the numbers of mosaic single spots and twin and single spots were significantly increased. The authors regarded these as inconclusive as more data are needed for their final classification.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(590)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** male/female
Strain: other
Route of admin.: inhalation
Exposure period: 1 hour
Doses: 0, 0.05, 0.2, 0.3 ml/1150ml (converts to 0, 38, 153, 229 mg/l)
Result:
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: The method of Wurgler F.E. et al. Mutation Res. 122, 321-328,1983 was followed. mwh+/+flr and mei+9 strain larvae were exposed at an age of 48 or 72 hours. They were placed in a 1150 ml airtight container and benzene was injected and allowed to volatilize to give the required concentration. After treatment, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and frequency and size of the spots were recorded. Control groups were exposed to air at the same age and for the same length of time as the larvae in the treatment groups.
Result: An increase in the frequency of small single spots and/or total single spots was seen in excision-repair-proficient larvae (mwh+/+flr) exposed to 229 mg/l but not 153 mg/l and in excision-repair-defective larvae (mei-9) exposed to 38 mg/l, the only concentration tested.
Source: BP Chemicals Ltd. London
Test substance: Laboratory reagent grade; 99% pure.

(588)

Type: Somatic mutation assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 22 hours per day/7 days per week/6 weeks
Doses: 0, 40, 100, 1000 ppb
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Groups of 14 mice/sex were exposed to benzene in whole-body inhalation chambers. At sacrifice, the spleen of each mouse was removed and the lymphocytes released. The numbers of hypoxanthine-guanine phosphoribosyl transferase (hprt) mutant (variant) cells were determined according to an autoradiographic hprt mutant lymphocyte assay. Control

groups of mice were either exposed to purified air or housed in standard plastic cages.
Reliability: 4 (validity could not be judged)
Original reference not yet available

Result: Benzene caused a dose-dependent increase in the frequencies of hprt variant (mutant) lymphocytes in both male and female mice at concentrations of 40 and 100 ppb. At 1000 ppb, the frequency was also increase when compared with the controls but this was less than in the 40 and 100 ppb groups. Females were more sensitive than males.

Source: Deutsche Shell Chemie GmbH Eschborn

(587)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** male/female
Strain: other
Route of admin.: oral feed
Exposure period: up to 96 hours
Doses: 0.88, 4.4, 8.8 mg/ml
Result:
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
one dose tested per experimental design
The method of Wurgler F.E. et al. Mutation Res. 122, 321-328, 1983 was followed. Benzene was administered to mei-9 and +/mei-9 strain Drosophila as a solution in 5% Tween 80 and 5% ethanol. Larvae aged 48 or 72 hours were exposed for 2 hours to benzene concentrations of 4.4 and 0.88 mg/ml respectively. In some cases, larvae were treated during the entire larval life (96 hours) to 4.4 or 8.8 mg/ml. After exposure, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and the frequency and size of the spots were recorded. Control experiments were conducted by exposing larvae of the same age as those in the treatment groups to the 5% Tween 80 and 5% ethanol vehicle for the same length of time as the corresponding treated larvae.

Result: A benzene concentration of 8.8 mg/ml was lethal and 4.4 mg/ml toxic when given for the entire larval life. A significant increase in the frequency of small single spots and/or total single spots was seen in the acute (larvae aged 72 h) and chronic studies in the excision-repair-defective larvae (mei-9 strain). The acute result was only marginally positive. No such effect was seen when benzene was given to excision-repair-proficient larvae (+/mei-9 strain).

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(588)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** male
Strain: other
Route of admin.: oral feed
Exposure period: 96 hours
Doses: 0, 0.5, 1, 2 % (converts to 0, 4.4, 8.8, 17.6 mg/ml)
Result:
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Benzene was dissolved in 40% ethanol. Male and female C(1)DX,yf/Y/sc z w+ mei-9a Drosophila were mated for 4-5 days and allowed to ovipost for 24 hours. The resultant eggs were allowed to develop for 24 hour and the newly hatched larvae exposed to benzene or the vehicle as the control. Benzene was added (1 ml) to the culture medium and the larvae were allowed to develop throughout the larval period (roughly 96 hours). Eyes of adult males were examined for colour mutations.
Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Result: No significant increase in mutation frequency over the control levels was seen. Benzene, however, caused a dose-dependent toxic effect on the survival of the male larvae, with 50% survival occurring at the top dose. A similar study using the genotype sc z w+ and benzene concentrations of 8.8 and 17.6 mg/ml reported similar decreases in survival and a lack of mutagenic effect (Nylander P.-O. et al. Mutation Res. 57, 163-167, 1978).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; 99% pure.

(589)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** female
Strain: other
Route of admin.: oral feed
Exposure period: 10-11 days
Doses: 156 mg/l
Result:
Method: other
Year: 1957 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
one dose
The method of Becker H.J. Z. Indukt. Abstammungs-Vererbungsl. 88, 333-373, 1957 was followed. Benzene was prepared in a mixture of 3% ethanol and 1% Tween 60 before being added to the food. Virgin females of the genotype wcosn2; se h were crossed with w; se h males and allowed to lay eggs for a period of 4 days on the food supplemented with benzene. The control larvae were exposed to the solvent. The developing culture was incubated for

10-11 days and the hatching females were scored for genetic changes which produced w clones or wco//w twin spots in their eyes.

Result: No increase in the number of mosaic twin spots was observed. However, the numbers of mosaic single spots and twin and single spots were significantly increased. The authors regarded these as inconclusive as more data are needed for their final classification.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Laboratory reagent grade; 99% pure.

(590)

Type: Somatic mutation assay
Species: Drosophila melanogaster **Sex:** male/female
Strain: other
Route of admin.: inhalation
Exposure period: 1 hour
Doses: 0, 0.05, 0.2, 0.3 ml/1150ml (converts to 0, 38, 153, 229 mg/l)
Result:
Method: other
Year: 1983 **GLP:** no data
Test substance: other TS
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
max. 2 doses tested per experimental design
The method of Wurgler F.E. et al. Mutation Res. 122, 321-328,1983 was followed. mwh+/+flr and mei+9 strain larvae were exposed at an age of 48 or 72 hours. They were placed in a 1150 ml airtight container and benzene was injected and allowed to volatilize to give the required concentration. After treatment, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and frequency and size of the spots were recorded. Control groups were exposed to air at the same age and for the same length of time as the larvae in the treatment groups.

Result: An increase in the frequency of small single spots and/or total single spots was seen in excision-repair-proficient larvae (mwh+/+flr) exposed to 229 mg/l but not 153 mg/l and in excision-repair-defective larvae (mei-9) exposed to 38 mg/l, the only concentration tested.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Laboratory reagent grade; 99% pure.

(588)

Type: other
Species: Drosophila melanogaster **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: 45 minutes
Doses: 0, 27000 ppm (converts to 0, 88 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: This test was conducted to examine the ability of benzene to induce crossing-over in Drosophila strain vg bw/++ males. Benzene (0.1 ml) was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. For the detection of induced crossing over, the five treated males were then individually back crossed to ten vg bw virgins for 18 days to obtain nine two-day broods. Broods were then scored for presence of crossovers. Laboratory cumulative control data were used as the comparison.
Result: Benzene induced crossing-over in spermatogonia but not in spermatozoa, spermatids or spermatocytes. The data were heavily clustered with more than half the crossovers coming from one individual treated male. The clusters were due to mitotic multiplication of crossover chromosomes in gonial cells and the investigators concluded that the observations could not be used for quantitative estimation of crossing-over induction. The crossovers were derived from four males and the difference when compared with the control group was found to be statistically significant. All four males found positive for induction of crossing-over in spermatogonia were sterile in the next brood.
Source: BP Chemicals Ltd. London

(567)

Type: other
Species: mouse **Sex:** male
Strain: other
Route of admin.: i.p.
Exposure period: once daily for 5 days
Doses: 0, 0.1, 0.25, 0.4, 0.5, 0.6, 0.8, 1.0 ml/kg bw/day (converts to 0, 88, 220, 352, 440, 527, 703, 880 mg/kg bw/day)
Result:
Method: other
Year: 1980 **GLP:** no data
Test substance: other TS
Remark: Reliability: 1 (valid)
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
The method of Topham J.C. Mutation Res. 69, 149-155, 1980 was followed. The animals tested were the F1 generation derived from mating CBA male mice with BALB/c females. Groups of five were exposed to each dose. Five weeks after the final dose, smears of caudal sperm were examined for head abnormalities. Cyclophosphamide monohydrate was used as the positive control. Benzene was administered in corn

oil and negative control animals were treated with corn oil alone.
Result: Benzene administration caused an significant increase in sperm-head abnormalities. Although this occurred over a limited dose range, it was reproducible in repeat experiments.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Laboratory reagent grade; purity not specified.

(568)

Type: other
Species: Drosophila melanogaster **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: 45 minutes
Doses: 0, 27000 ppm (converts to 0, 88 mg/l)
Result:
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 2 (valid with restriction)
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
no data about the TS
This test was conducted to examine the ability of benzene to induce crossing-over in Drosophila strain vg bw/++ males. Benzene (0.1 ml) was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. For the detection of induced crossing over, the five treated males were then individually back crossed to ten vg bw virgins for 18 days to obtain nine two-day broods. Broods were then scored for presence of crossovers. Laboratory cumulative control data were used as the comparison.
Result: Benzene induced crossing-over in spermatogonia but not in spermatozoa, spermatids or spermatocytes. The data were heavily clustered with more than half the crossovers coming from one individual treated male. The clusters were due to mitotic multiplication of crossover chromosomes in gonial cells and the investigators concluded that the observations could not be used for quantitative estimation of crossing-over induction. The crossovers were derived from four males and the difference when compared with the control group was found to be statistically significant. All four males found positive for induction of crossing-over in spermatogonia were sterile in the next brood.
Source: Deutsche Shell Chemie GmbH Eschborn

(567)

Type: other
Species: mouse **Sex:** male/female
Strain: CD–1
Route of admin.: gavage
Exposure period: 30 to 54 hours
Doses: 440 mg/kg
Result:
Method: other
Year: 1984 **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Remark: The effects of benzene on bone marrow was studied in the micronucleus assay and by metaphase analysis. Male and female CD–1 mice were treated with 2 doses of benzene (440 mg/kg) or toluene (860 or 1720 mg/kg) or both 24 hours apart, and sacrificed 30 hours (or 54 hours) after the firstdose.

Benzene treated animals were pretreated with phenobarbital, 3–methylcholanthrene, SKF–525A or Aroclor 1254 by i.p. injection. Toluene showed no clastogenic activity and reduced the clastogenic effect of benzene when the mixture was given. None of the pretreatments protected against the clastogenic effects of benzene. However, pretreatment with 3–methylcholanthrene greatly promoted the clastogenic effects of benzene on bone marrow. Females were consistently less sensitive to benzene than males. Dose–response curves in benzene–treated mice were much steeper with 3–MCA induction than without. Chromosomal damage was higher with p.o. than i.p. administration of benzene.
Source: Deutsche Shell Chemie GmbH Eschborn

(591)

Type: other
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 120 days to 2 years
Doses:
Result:
Method:
Year: **GLP:** yes
Test substance: as prescribed by 1.1 – 1.4
Remark: Retrospective analysis of peripheral blood obtained during the NTP Cancer Bioassay of Benzene demonstrates that the frequency of micronuclei in circulating normochromatic erythrocytes (NCE’s) reflects the clastogenic effect of benzene and sex sensitivity in mice. Peripheral blood was evaluated from mice subject to the following exposure schemes. In subchronic studies, benzene was administerd by gavage to mice (5/group), at doses of 0, 25, 50, 100, 200, 400 or 600 mg/kg benzene in corn oil, 5 days/week for a 120 day period. In chronic studies, mice (50/group) were exposed at 0, 25, 50 or 100 mg/kg benzene in corn oil for 24months. An additional 10 mice/group were sacrificed at 12 months. In all cases, a total of 1000 NCE’s were scored peranimal.

Study results demonstrated a statistically significant dose dependent increase in micronucleated NCE's at all sampling times. The frequency of micronucleated NCE's was higher in male than in female mice. In treated males, the frequency was highest in the 120–day samples. In females, the responses to long term exposure were to weak to permit comparison around time points. Evaluation of the control animals from 120 day study found no statistical increase in the frequency of micronucleated polychromatic erythrocytes (PCE's) versus NCE's. This finding suggests that micronucleated NCE's are not selectively removed from peripheral circulation in B6C3F1 mice.

Source: Deutsche Shell Chemie GmbH Eschborn

(592)

5.7 Carcinogenicity

Species: rat **Sex:** male/female
Strain: Sprague–Dawley
Route of admin.: gavage
Exposure period: 52 weeks
Frequency of treatment: daily on 4 or 5 days each week
Post. obs. period: untill spontaneous death or killed at 144 weeks
Doses: 50 or 250 mg/Kg
Result:
Control Group: yes, concurrent vehicle
Method: other: no data
Year: 1979 **GLP:** no data
Test substance: other TS: benzene+ olive oil
Result: Zymbal gland carcinomas, mammary gland carcinomas and leukemia
Source: REPSOL PETROLEO,S.A. MADRID
Test substance: benzene + olive oi

(593)

Species: rat **Sex:** male/female
Strain: Sprague–Dawley
Route of admin.: gavage
Exposure period: 52 weeks
Frequency of treatment: 4–5 days/week
Post. obs. period: until spontaneous death
Doses: 50, 250 mg/kg bw/day; 30 and 35 rats/sex respectively
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 30 rats/sex administered olive oil. Benzene was administered in olive oil. During the experiment, the animals were examined every 2 weeks, weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. Complete

autopsy was performed on each animal with histological examination being carried out on all the major organs.

Result: The experiment ended after 144 weeks. A dose-related decrease in survival was seen both during the exposure period and the postexposure observation period. In the females, a dose-related increase in Zymbal gland carcinomas (incidences = 0, 7.1 and 28.6% for the control, low- and high-dosed groups respectively) and mammary gland carcinomas (13.3, 14.3 and 25.0% respectively) were noted. Oral cavity tumours were also reported in the high-dosed females (0, 0 and 9.5% respectively). The high-dosed males and all dosed females had elevated haemolymphoreticular neoplasias (0, 0 and 16.7% for the males and 3.3, 7.1 and 3.4% for the females). There was one hepatoma and one subcutaneous angiosarcoma both occurring at the high-dose level. These tumours are rarely seen in this strain of animal. In general, the incidences of total malignant tumours were clearly and dose-relatedly greater in the treated when compared with the control animals (incidences = 4.5, 4.3 and 33.3% for males; 23.3, 35.7 and 72.4% for females and 15.4, 21.6 and 54.7% when the two groups were combined for control, low-dosed and high-dosed groups respectively).

Source: BP Chemicals Ltd. London

Test substance: 99.93% pure.

(594) (595)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 days/week
Post. obs. period: 1 week
Doses: 50, 100, 200 mg/kg bw/day for males; 25, 50, 100 mg/kg bw/day for females; 50 rats/sex/dose
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn oil. Blood was withdrawn from ten randomly preselected animals from each sex and dose group at 12, 15, 18 and 21 months. Blood was also taken from moribund animals before they were killed and all animals at terminal kill. Groups of ten animals/sex were administered these same benzene doses for 51 weeks; blood was withdrawn at 0, 3, 6, 9 and 12 months; the animals were killed at 12 months and necropsies were performed. All animals were observed twice daily and weighed weekly for 13 weeks and monthly thereafter. All gross lesions and tissue masses were examined histologically together with all the major organs.

Result: See section 5.4 for general systemic effects. The high-dosed male and mid- and high-dosed female groups had significantly decreased survival rates when compared to the control groups. The female control group, however,

apparently had exceptionally good survival when compared with historic control data for the same strain and sex of animal. Zymbal gland carcinomas occurred in both the male and female treated rats with a significant positive trend, the incidences in the mid- and high-dosed males and all the dosed females being significantly greater than in the vehicle control group (incidences = 6, 13, 24 and 40% in males and 0, 13, 11 and 30% in females for the control and three dose groups respectively). A significant positive trend was seen for the number of benzene-treated rats with squamous cell papillomas or carcinomas of the palate, lip and tongue when considered either separately (in the case of the males) or combined (in both the males and females) (combined incidence = 2, 18, 32 and 46% in males and 2, 10, 24 and 20% in females for the four groups respectively). The individual or combined incidences of skin squamous cell papillomas and carcinomas were increased in the male rats with a significant positive trend, the incidences in the high-dosed animals being significantly greater than in the controls (incidence = 0, 4, 2 and 10% for squamous cell papillomas and 0, 10, 6 and 16% for squamous cell carcinomas in the four groups respectively). A significant positive trend was found for endometrial stromal polyps of the uterus, the high-dose incidence being significantly greater than in the controls (incidence = 14, 14, 14 and 28% for the four groups respectively).

Source: BP Chemicals Ltd. London
Test substance: >99.7% pure.

(514)

Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: gavage
Exposure period: 104 weeks
Frequency of treatment: 4-5 days/week
Post. obs. period: until spontaneous death
Doses: 500 mg/kg bw/day; 40 rats/sex
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group of 40 rats of each sex administered olive oil. Benzene administered in olive oil. The status and behaviour of the animals were examined three times daily and they were examined clinically for gross changes every 2 weeks. The animals were weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. A full necropsy was conducted on each animal.
Result: A higher mortality rate was seen in both sexes treated with benzene and body weight, particularly in the males, was affected. When considering each sex individually and as a combined group, increased incidences were recorded for total malignant tumours (combined incidence = 50% compared with 22.5% in the control group), Zymbal gland squamous cell

carcinomas (incidence = 16.2% compared with 0%), oral cavity squamous cell carcinomas (incidence = 7.5% compared with 1.2%) and nasal cavity indifferentiated carcinomas (incidence = 3.7% compared with 0%).
Source: BP Chemicals Ltd. London
Test substance: 99.93% pure.

(596)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: 104 weeks
Frequency of treatment: 4-5 days/week
Post. obs. period: until spontaneous death
Doses: 500 mg/kg bw/day; 40 rats/sex
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 50 animals/sex administered olive oil. Benzene was administered in olive oil. During the experiment, the animals were examined every 2 weeks, weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal with histological examination being carried out on all the major organs.
Result: No details on survival were given however an interim report notes decreases in body weight and total red blood cell and total white blood cell counts after 92 weeks of the study, the latter being mainly due to lymphocytopenia. In the full term study, when the sexes were considered individually and combined, increases were seen in the incidences of Zymbal gland carcinomas (combined incidence = 43.0 compared with 1.1% in the controls), oral cavity carcinomas (incidence = 52.6 compared with 0%), nasal cavity carcinomas (incidence = 6.1 compared with 0%), forestomach dysplasias/acanthomas (incidence = 21.8 compared with 0%), liver angiosarcomas (incidence = 8.6 compared with 0%) and total malignant tumours (incidences = 174.4 compared with 26.7% in the males; 147.5 compared with 22.4% in the females and 160.8 compared with 24.5% when the two groups are combined). The males also showed increased skin carcinomas (incidence = 28.1 compared with 0%) and the females, forestomach carcinomas (incidence = 31.6 compared with 0%) and hepatocarcinomas (incidence = 7.1 compared with 0%); the latter being based on only one case. A number of other tumours were reported which are, apparently, rarely seen in historic control data for this breed and strain of rat, for example lung adenocarcinomas and soft tissue liposarcomas were seen in one and two males respectively.
Source: BP Chemicals Ltd. London
Test substance: 99.93% pure.

(597) (594)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: 52 weeks
Frequency of treatment: once daily, 4-5 days/week
Post. obs. period: until spontaneous death
Doses: 50, 250 mg/kg bw/day; 30 and 35 rats/sex respectively
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 30 rats/sex administered olive oil. Benzene was administered in olive oil. During the experiment, the animals were examined every 2 weeks, weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal with histological examination being carried out on all the major organs.
Reliability: 3 (not valid)
Significant methodological deficiencies
low number of animals, 2 doses, no statistical evaluation, limited histopathology
Result: The experiment ended after 144 weeks. A dose-related decrease in survival was seen both during the exposure period and the postexposure observation period. In the females, a dose-related increase in Zymbal gland carcinomas (incidences = 0, 7.1 and 28.6% for the control, low- and high-dosed groups respectively) and mammary gland carcinomas (13.3, 14.3 and 25.0% respectively) were noted. Oral cavity tumours were also reported in the high-dosed females (0, 0 and 9.5% respectively). The high-dosed males and all dosed females had elevated haemolymphoreticular neoplasias (0, 0 and 16.7% for the males and 3.3, 7.1 and 3.4% for the females). There was one hepatoma and one subcutaneous angiosarcoma both occurring at the high-dose level. These tumours are rarely seen in this strain of animal. In general, the incidences of total malignant tumours were clearly (males and females) and dose-relatedly (only females) greater in the treated when compared with the control animals (incidences = 4.5, 4.3 and 33.3% for males; 23.3, 35.7 and 72.4% for females and 15.4, 21.6 and 54.7% when the two groups were combined for control, low-dosed and high-dosed groups respectively).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.93% pure.

(594) (595)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 days/week
Post. obs. period: 1 week
Doses: 50, 100, 200 mg/kg bw/day for males; 25, 50, 100 mg/kg bw/day for females; 50 rats/sex/dose
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn oil. Blood was withdrawn from ten randomly preselected animals from each sex and dose group at 12, 15, 18 and 21 months. Blood was also taken from moribund animals before they were killed and all animals at terminal kill. Groups of ten animals/sex were administered these same benzene doses for 51 weeks; blood was withdrawn at 0, 3, 6, 9 and 12 months; the animals were killed at 12 months and necropsies were performed. All animals were observed twice daily and weighed weekly for 13 weeks and monthly thereafter. All gross lesions and tissue masses were examined histologically together with all the major organs.
Reliability: 1 (valid)
Comparable to guideline study
Result: See section 5.4 for general systemic effects. The high–dosed male and mid– and high–dosed female groups had significantly decreased survival rates when compared to the control groups. The female control group, however, apparently had exceptionally good survival when compared with historic control data for the same strain and sex of animal. Zymbal gland carcinomas occurred in both the male and female treated rats with a significant positive trend, the incidences in the mid– and high–dosed males and all the dosed females being significantly greater than in the vehicle control group (incidences = 6, 13, 24 and 40% in males and 0, 13, 11 and 30% in females for the control and three dose groups respectively). A significant positive trend was seen for the number of benzene–treated rats with squamous cell papillomas or carcinomas of the palate, lip and tongue when considered either separately (in the case of the males) or combined (in both the males and females) (combined incidence = 2, 18, 32 and 46% in males and 2, 10, 24 and 20% in females for the four groups respectively). The individual or combined incidences of skin squamous cell papillomas and carcinomas were increased in the male rats with a significant positive trend, the incidences in the high–dosed animals being significantly greater than in the controls (incidence = 0, 4, 2 and 10% for squamous cell papillomas and 0, 10, 6 and 16% for squamous cell carcinomas in the four groups respectively). A significant positive trend was found for endometrial stromal polyps of the uterus, the high–dose incidence being significantly greater

than in the controls (incidence = 14, 14, 14 and 28% for the four groups respectively).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99.7% pure.

(514)

Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: gavage
Exposure period: 104 weeks
Frequency of treatment: once daily, 4-5 days/week
Post. obs. period: until spontaneous death
Doses: 500 mg/kg bw/day; 40 rats/sex
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group of 40 rats of each sex administered olive oil. Benzene administered in olive oil. The status and behaviour of the animals were examined three times daily and they were examined clinically for gross changes every 2 weeks. The animals were weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. A full necropsy was conducted on each animal.
Reliability: 3 (not valid)
Significant methodological deficiencies
one dose tested, no data about number of animals for final determination of specific tumor incidences, no statistical evaluation, low number of animals at start of experiments
Result: A higher mortality rate was seen in both sexes treated with benzene and body weight, particularly in the males, was affected (no further data). When considering each sex individually and as a combined group, increased incidences were recorded for total malignant tumours (combined incidence = 50% compared with 22.5% in the control group), Zymbal gland squamous cell carcinomas (incidence = 16.2% compared with 0%), oral cavity squamous cell carcinomas (incidence = 7.5% compared with 1.2%) and nasal cavity indifferentiated carcinomas (incidence = 3.7% compared with 0%).
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.93% pure.

(596)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: 104 weeks
Frequency of treatment: once daily, 4-5 days/week
Post. obs. period: until spontaneous death
Doses: 500 mg/kg bw/day; 40 rats/sex
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 50 animals/sex administered olive oil. Benzene was administered in olive oil. During the experiment, the animals were examined every 2 weeks, weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal with histological examination being carried out on all the major organs.
Reliability: 3 (not valid)
Significant methodological deficiencies
low number of animals in treatment group, one dose, no statistical evaluation, limited histopathology

Result: An interim report notes decreases in body weight and total red blood cell and total white blood cell counts after 92 weeks of the study, the latter being mainly due to lymphocytopenia. Survival was decreased in females (60% versus 66% in controls) but elevated in males (72.5 versus 58%) after 92 weeks. In the full term study, when the sexes were considered individually and combined, increases were seen in the incidences of Zymbal gland carcinomas (combined incidence = 43.0 compared with 1.1% in the controls), oral cavity carcinomas (incidence = 52.6 compared with 0%), nasal cavity carcinomas (incidence = 6.1 compared with 0%), forestomach dysplasias/acanthomas (incidence = 21.8 compared with 0%), liver angiosarcomas (incidence = 8.6 compared with 0%) and total malignant tumours (incidences = 174.4 compared with 26.7% in the males; 147.5 compared with 22.4% in the females and 160.8 compared with 24.5% when the two groups are combined). The males also showed increased skin carcinomas (incidence = 28.1 compared with 0%) and the females, forestomach carcinomas (incidence = 31.6 compared with 0%) and hepatocarcinomas (incidence = 7.1 compared with 0%); the latter being based on only one case. A number of other tumours were reported which are, apparently, rarely seen in historic control data for this breed and strain of rat, for example lung adenocarcinomas and soft tissue liposarcomas were seen in one and two males respectively.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.93% pure.

(597) (594)

Species: mouse **Sex:** male/female
Strain: Swiss
Route of admin.: gavage
Exposure period: 78 weeks
Frequency of treatment: 4–5 days/week
Post. obs. period: until spontaneous death
Doses: 500 mg/kg bw/day; 40 mice/sex
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group of 40 mice of each sex administered olive oil. Benzene administered in olive oil. The status and behaviour of the animals were examined three times daily and they were examined clinically for gross changes every 2 weeks. The animals were weighed every 2 weeks during the exposure period and every 8 weeks during the post exposure observation period. A full necropsy was conducted on each animal.
Result: No effects were seen on the survival of the benzene–treated animals but body weight, particularly in the males, was affected. When considering each sex individually and as a combined group, increased incidences were recorded for total benign and malignant tumours combined (combined incidence = 70% compared with 38.7% in the control group), total malignant tumours (incidence = 52.5% compared with 25%), lung adenomas (incidence = 22.5% compared with 7.5%), lung atypic adenomas (incidence = 15.0% compared with 1.2%), Zymbal gland dysplasias (incidence = 8.7% compared with 0%) and Zymbal gland carcinomas (incidence = 6.2% compared with 0%). Mammary carcinomas were increased in the females (incidence = 47.5% compared with 5%) and lung adenomatous hyperplasia and adenocarcinomas were increased in the males (incidences = 2.5% for each compared with 0% for each in the control group).
Source: BP Chemicals Ltd. London
Test substance: 99.93% pure.

(596)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 days/week
Post. obs. period: 1 week
Doses: 25, 50, 100 mg/kg bw/day; 50 mice/sex/dose
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn oil. Blood was withdrawn from ten randomly preselected animals from each sex and dose group at 12, 15, 18 and 21 months. Blood was also taken from moribund animals before they were killed and all animals at terminal kill. Groups of ten animals/sex were administered these same benzene doses for 51 weeks; blood was withdrawn at 0, 3, 6, 9 and 12 months; the animals were killed at 12 months and necropsies were performed. All animals were observed twice daily and weighed weekly for 13 weeks and monthly thereafter. All gross lesions and tissue masses were examined histologically together with all the major organs.
Result: See section 5.4 for general systemic effects. The survival rates were significantly decreased in the high-dosed male and female animals. Zymbal gland squamous cell carcinomas occurred in both sexes of mice with positive trends, the incidences in the mid- and high-dosed males and high-dosed females being significantly greater than in the controls (incidence = 0, 3, 10 and 54% in males and 0, 0, 3 and 10% in females for the four groups respectively). All carcinomas and in particular squamous cell carcinomas were seen with increased incidence in the preputial gland of male mice, the incidences of each in the mid- and high-dosed groups being significantly greater than in the controls (incidences = 0, 7, 3 and 8% for all carcinomas and 0, 11, 62 and 78% for squamous cell carcinomas in the four groups respectively). In the females, granulosa cell tumours and benign mixed tumours occurred with significant positive trends in the ovaries, those in the high-dosed and mid- and high-dosed animals respectively being significantly increased (incidences = 2, 2, 12 and 14% for granulosa cell tumours and 0, 2, 24 and 14% for benign mixed tumours for the four groups respectively). Carcinomas and carcinosarcomas of the mammary gland were seen in female mice, those in the mid- and high-dosed and high-dosed animals respectively being significant (incidences = 0, 4, 10 and 16% for carcinomas and 0, 0, 2 and 8% for carcinosarcomas). Harderian gland adenomas and carcinomas occurred in the males and females respectively, the incidences in all the treated males (0, 17, 27 and 22%) were significantly and in the high-dosed females (0, 0, 0, 8%) marginally greater than the controls. Increased dose-related incidences of alveolar/bronchiolar adenomas and

carcinomas were seen in both sexes when the tumour types were considered either separately or combined (combined incidences = 20, 33, 38 and 43% for males and 8, 12, 20 and 27% for females). The incidences of total malignant lymphomas were significantly increased in all the treated males and females (8, 21, 20 and 31% and 31, 56, 52 and 45% respectively). A significant positive trend in squamous cell papillomas of the forestomach occurred in both sexes of treated mice. The incidences, however, were not statistically significant, indicating a weak carcinogenic activity at this site. In the females, the incidences of hepatocellular adenomas in the low-dosed group (2, 18, 10 and 8%) and, when combined with hepatocellular carcinomas, in the low- and mid-dosed groups (8, 27, 26 and 14%) were significantly elevated over the controls. Pheochromocytomas of the adrenal gland were seen in significant excess in the mid-dosed males (incidence = 2, 2, 14 and 2%).

Source: BP Chemicals Ltd. London
Test substance: >99.7% pure.

(598)

Species: mouse **Sex:** male/female
Strain: Swiss
Route of admin.: gavage
Exposure period: 78 weeks
Frequency of treatment: once daily, 4-5 days/week
Post. obs. period: until spontaneous death
Doses: 500 mg/kg bw/day; 40 mice/sex
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group of 40 mice of each sex administered olive oil. Benzene administered in olive oil. The status and behaviour of the animals were examined three times daily and they were examined clinically for gross changes every 2 weeks. The animals were weighed every 2 weeks during the exposure period and every 8 weeks during the post exposure observation period. A full necropsy was conducted on each animal.
Reliability: 3 (not valid)
Significant methodological deficiencies
one dose tested, no data about number of animals for final determination of specific tumor incidences, no statistical evaluation, low number of animals at start of experiments
Result: No effects were seen on the survival of the benzene-treated animals but body weight, particularly in the males, was affected (no further data). When considering each sex individually and as a combined group, increased incidences were recorded for total benign and malignant tumours combined (combined incidence = 70% compared with 38.7% in the control group), total malignant tumours (incidence = 52.5% compared with 25%), lung adenomas (incidence = 22.5% compared with 7.5%), lung atypic adenomas (incidence = 15.0%

compared with 1.2%), Zymbal gland dysplasias (incidence = 8.7% compared with 0%) and Zymbal gland carcinomas (incidence = 6.2% compared with 0%). Mammary carcinomas were increased in the females (incidence = 47.5% compared with 5%) and lung adenomatous hyperplasia and adenocarcinomas were increased in the males (incidences = 2.5% for each compared with 0% for each in the control group).

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.93% pure.

(596)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 days/week
Post. obs. period: 1 week
Doses: 25, 50, 100 mg/kg bw/day; 50 mice/sex/dose
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** yes
Test substance: other TS
Remark: Benzene administered in corn oil. Blood was withdrawn from ten randomly preselected animals from each sex and dose group at 12, 15, 18 and 21 months. Blood was also taken from moribund animals before they were killed and all animals at terminal kill. Groups of ten animals/sex were administered these same benzene doses for 51 weeks; blood was withdrawn at 0, 3, 6, 9 and 12 months; the animals were killed at 12 months and necropsies were performed. All animals were observed twice daily and weighed weekly for 13 weeks and monthly thereafter. All gross lesions and tissue masses were examined histologically together with all the major organs.
Reliability: 1 (valid)
Comparable to guideline study
Result: See section 5.4 for general systemic effects. The survival rates were significantly decreased in the high-dosed male and female animals. Zymbal gland squamous cell carcinomas occurred in both sexes of mice with positive trends, the incidences in the mid- and high-dosed males and high-dosed females being significantly greater than in the controls (incidence = 0, 3, 10 and 54% in males and 0, 0, 3 and 10% in females for the four groups respectively). All carcinomas and in particular squamous cell carcinomas were seen with increased incidence in the preputial gland of male mice, the incidences of each in the mid- and high-dosed groups being significantly greater than in the controls (incidences = 0, 7, 3 and 8% for all carcinomas and 0, 11, 62 and 78% for squamous cell carcinomas in the four groups respectively). In the females, granulosa cell tumours and benign mixed tumours occurred with significant positive trends in the ovaries, those in the high-dosed and mid- and

high-dosed animals respectively being significantly increased (incidences = 2, 2, 12 and 14% for granulosa cell tumours and 0, 2, 24 and 14% for benign mixed tumours for the four groups respectively). Carcinomas and carcinosarcomas of the mammary gland were seen in female mice, those in the mid- and high-dosed and high-dosed animals respectively being significant (incidences = 0, 4, 10 and 16% for carcinomas and 0, 0, 2 and 8% for carcinosarcomas). Harderian gland adenomas and carcinomas occurred in the males and females respectively, the incidences in all the treated males (0, 17, 27 and 22%) were significantly and in the high-dosed females (0, 0, 0, 8%) marginally greater than the controls. Increased dose-related incidences of alveolar/bronchiolar adenomas and carcinomas were seen in both sexes when the tumour types were considered either separately or combined (combined incidences = 20, 33, 38 and 43% for males and 8, 12, 20 and 27% for females). The incidences of total malignant lymphomas were significantly increased in all the treated males and females (8, 21, 20 and 31% and 31, 56, 52 and 45% respectively). A significant positive trend in squamous cell papillomas of the forestomach occurred in both sexes of treated mice. The incidences, however, were not statistically significant, indicating a weak carcinogenic activity at this site. In the females, the incidences of hepatocellular adenomas in the low-dosed group (2, 18, 10 and 8%) and, when combined with hepatocellular carcinomas, in the low- and mid-dosed groups (8, 27, 26 and 14%) were significantly elevated over the controls. Pheochromocytomas of the adrenal gland were seen in significant excess in the mid-dosed males (incidence = 2, 2, 14 and 2%).

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: >99.7% pure.

(598)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 15 weeks
Frequency of treatment: 4 hours per day/5 days per week/7 weeks and then 7 hours per day/5 days per week/8 weeks
Post. obs. period: until spontaneous death
Doses: 200 ppm (converts to 0.65 mg/l); 70 males and 59 females
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 158 male and 149 female rats exposed in utero (as 12-day-old embryos) and postnatally to air for a total of 104 weeks. Twelve-day-old embryos were exposed in utero and postnatally to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 8 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during

the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The rats may also have had some oral exposure through their mothers' milk at weaning.

Result: No effects were seen on survival rate or body weight. When the sexes were considered both individually and combined, increases were seen in the incidences of Zymbal gland carcinomas (combined incidence = 3.9 compared with 0.7% in the control group), oral cavity carcinomas (incidence = 19 compared with 0%), nasal cavity carcinomas (incidence = 15 compared with 2%), hepatomas (incidence= 5.7 compared with 0.3%) and total malignant tumours (incidence = 31.4, 51.7 and 40.6% in the treated males, females and when combined respectively compared with 18.5, 17.6 and 18.0 in the control groups respectively). In the females, alone, increases were seen in malignant mammary tumours (incidence = 13.8 compared with 5.4%) and haemolymphoreticular neoplasias other than thymomas (incidence = 6.9 compared with 0.7%).

Source: BP Chemicals Ltd. London

Test substance: 99.93% pure.

(595) (599) (600)

Species: rat **Sex:** female

Strain: Sprague-Dawley

Route of admin.: inhalation

Exposure period: 104 weeks

Frequency of treatment: 4 hours per day/5 days per week/7 weeks and then 7 hours per day/ 5 days per week/97 weeks

Post. obs. period: until spontaneous death

Doses: 200, 300 ppm (converts to 0.65, 0.98 mg/l); 54 rats

Result:

Control Group: yes, concurrent no treatment

Method: other

Year: **GLP:** no data

Test substance: other TS

Remark: Control group consisted of 60 female rats exposed from day 12 of pregnancy to air for a total of 104 weeks. The benzene-treated group was exposed, from day 12 of pregnancy, to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 12 weeks and finally to 0.98 mg/l, 7 hours/day, 5 days/week for 85 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The offspring from these dams were used in the 12 day-old embryo in utero exposure studies reported in the same references.

Result: The survival rate was slightly decreased after 118 weeks but not 104 weeks. No effect on body weight was noted. An increase was seen in Zymbal gland carcinomas (incidence = 5.7 compared with 1.7% in the control group), malignant mammary tumours (incidence = 11.1 compared with 3.3%) and total malignant tumours (incidence = 29.6 compared with 16.7%). Hepatomas and oral and nasal cavity carcinomas were

slightly increased, being seen in one, two and one animals respectively and in none of the controls (incidence = 2.1, 40.0 and 16.7% respectively).
Source: BP Chemicals Ltd. London
Test substance: 99.93% pure.

(595) (599) (600)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 104 weeks
Frequency of treatment: 4 hours per day/5 days per week/7 weeks and then 7 hours per day/ 5 days per week/97 weeks
Post. obs. period: until spontaneous death
Doses: 200, 300 ppm (converts to 0.65, 0.98 mg/l); 75 male and 65 female rats
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 158 male and 149 female rats exposed in utero (as 12-day-old embryos) and postnatally to air for a total of 104 weeks. Twelve-day-old embryos were exposed in utero and postnatally to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 12 weeks followed finally by exposure to 0.98 mg/l, 7 hours/day, 5 days/week for 85 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The rats may also have had some oral exposure through their mothers' milk at weaning.
Result: Decreased survival rate and mean body weights were noted in both the treated males and females after 104 weeks. An interim report notes a decrease in the numbers of white blood cells, due mainly to lymphocytopenia, after 91 weeks of the study. When the males and females were considered separately and combined, increased incidences were seen in Zymbal gland carcinomas (combined incidence = 10 compared with 0.7% in the control), oral cavity carcinomas (incidence = 33.3 compared with 0%), nasal cavity carcinomas (incidence = 7.5 compared with 0%), hepatomas (incidence = 6.8 compared with 0.3%) and total malignant tumours (incidence = 56.4 compared with 18.0%). Leukaemias were increased in the males (incidence = 2.7 compared with 0.6%) and malignant mammary tumours (incidence = 13.8 compared with 5.4%) and forestomach carcinomas (incidence = 60 compared with 0%) increased in the females.
Source: BP Chemicals Ltd. London
Test substance: 99.93% pure.

(595) (599) (600)

Species: rat **Sex:** male
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure period: life–time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 100, 300 ppm (converts to 0.33, 0.98 mg/l); 40 and 45 rats respectively
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 40 and 27 rats for the two exposure groups respectively, exposed to air. Semi–monthly haematologic studies were conducted on ten test and ten control animals.
Result: One animal exposed to 0.33 mg/l developed chronic myelogenous leukaemia. Although not statistically significant when compared with the control group, where no such leukaemias were seen, the fact that myelogenous leukaemia has not been reported in historic control animals of this same strain is suggestive of a causative role for benzene.
Source: BP Chemicals Ltd. London

(601)

Species: rat **Sex:** male/female
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure period: 15 weeks
Frequency of treatment: 4 hours per day/5 days per week/7 weeks and then 7 hours per day/5 days per week/8 weeks
Post. obs. period: until spontaneous death
Doses: 200 ppm (converts to 0.65 mg/l); 70 males and 59 females
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 158 male and 149 female rats exposed in utero (as 12–day–old embryos) and postnatally to air for a total of 104 weeks. Twelve–day–old embryos were exposed in utero and postnatally to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 8 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The rats may also have had some oral exposure through their mothers' milk at weaning.
Benzene concentration analysed by GC.
Reliability: 3 (not valid)

Significant methodological deficiencies
no data about analytical concentration, one concentration tested, controls not sham-exposed for the same period, low number of animals (minimal 21/sex in treatment or 11/sex in control group) for final determination of specific tumor incidence, no statistical evaluation, limited histopathology

Result: No effects were seen on survival rate or body weight. When the sexes were considered both individually and combined, increases were seen in the incidences of Zymbal gland carcinomas (combined incidence = 3.9 compared with 0.7% in the control group), oral cavity carcinomas (incidence = 19 compared with 0%), nasal cavity carcinomas (incidence = 15 compared with 2%), hepatomas (incidence= 5.7 compared with 0.3%) and total malignant tumours (incidence = 31.4, 51.7 and 40.6% in the treated males, females and when combined respectively compared with 18.5, 17.6 and 18.0 in the control groups respectively). In the females, alone, increases were seen in malignant mammary tumours (incidence = 13.8 compared with 5.4%) and haemolymphoreticular neoplasias other than thymomas (incidence = 6.9 compared with 0.7%).

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.93% pure.

(595) (599) (600)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 104 weeks
Frequency of treatment: see remarks
Post. obs. period: until spontaneous death
Doses: 200, 300 ppm (converts to 0.65, 0.98 mg/l); 54 rats
Result:
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 60 female rats exposed from day 12 of pregnancy to air for a total of 104 weeks. The benzene-treated group was exposed, from day 12 of pregnancy, to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 12 weeks and finally to 0.98 mg/l, 7 hours/day, 5 days/week for 85 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The offspring from these dams were used in the 12 day-old embryo in utero exposure studies reported in the same references. Benzene concentration measured by GC.
Reliability: 3 (not valid)
Significant methodological deficiencies
no data about analytical concentration, one treatment group, one sex, no statistical evaluation, limited histopathology

Result: The survival rate was slightly decreased after 118 weeks but

not 104 weeks. No effect on body weight was noted. An increase was seen in Zymbal gland carcinomas (incidence = 5.7 compared with 1.7% in the control group), malignant mammary tumours (incidence = 11.1 compared with 3.3%) and total malignant tumours (incidence = 29.6 compared with 16.7%). Hepatomas and oral and nasal cavity carcinomas were slightly increased, being seen in one, two and one animals respectively and in none of the controls.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.93% pure.

(595) (599) (600)

Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 104 weeks
Frequency of treatment: see remarks
Post. obs. period: until spontaneous death
Doses: 200, 300 ppm (converts to 0.65, 0.98 mg/l); 75 male and 65 female rats
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 158 male and 149 female rats exposed in utero (as 12-day-old embryos) and postnatally to air for a total of 104 weeks. Twelve-day-old embryos were exposed in utero and postnatally to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 12 weeks followed finally by exposure to 0.98 mg/l, 7 hours/day, 5 days/week for 85 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The rats may also have had some oral exposure through their mothers' milk at weaning. Benzene concentration measured by GC.
Reliability: 3 (not valid)
Significant methodological deficiencies
one treatment group, no data about analytical concentration, low number of animals (minimal 5/sex in treatment group) for final determination of specific tumor incidence, no statistical evaluation, limitedhistopathology
Result: Decreased survival rate and mean body weights were noted in both the treated males and females after 104 weeks. An interim report notes a decrease in the numbers of white blood cells, due mainly to lymphocytopenia, after 91 weeks of the study. When the males and females were considered separately and combined, increased incidences were seen in Zymbal gland carcinomas (combined incidence = 10 compared with 0.7% in the control), oral cavity carcinomas (incidence = 33.3 compared with 0%), nasal cavity carcinomas (incidence = 7.5 compared with 0%), hepatomas (incidence = 6.8 compared with 0.3%) and total malignant tumours (incidence = 56.4

compared with 18.0%). Leukaemias were increased in the males (incidence = 2.7 compared with 0.6%) and malignant mammary tumours (incidence = 13.8 compared with 5.4%) and forestomach carcinomas (incidence = 60 compared with 0%) increased in the females.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.93% pure.

(595) (599) (600)

Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 100, 300 ppm (converts to 0.33, 0.98 mg/l); 40 and 45 rats respectively
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 40 and 27 rats for the two exposure groups respectively, exposed to air. Semi-monthly haematologic studies were conducted on ten test and ten control animals.
Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, no data about measured benzene concentration and test substance, insufficient documentation of methods, low number of animals, results limited to leukaemia
Result: One animal exposed to 0.33 mg/l developed chronic myelogenous leukaemia. Although not statistically significant when compared with the control group, where no such leukaemias were seen, the fact that myelogenous leukaemia has not been reported in historic control animals of this same strain is suggestive of a causative role for benzene.
Source: Deutsche Shell Chemie GmbH Eschborn

(601)

Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: inhalation
Exposure period: 10 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 1200 ppm (converts to 3.92 mg/l); 80 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 80 males exposed to filtered, conditioned air in chambers, 6 hours/day, 5 days/week for 10 weeks and observed until spontaneous death. Benzene concentrations in the exposure chambers were monitored at ½-hour intervals during the daily exposures. Blood was withdrawn from ten exposed and ten control animals every second week during the exposure period and monthly thereafter. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically.
Result: The mortality rate was increased by benzene exposure and intense lymphocytopenia and mild anaemia were noted during the exposure period. The blood counts returned to normal when exposure ceased. When the tumour types were grouped together, statistically significant increases were seen in the incidences of total, malignant and benign tumour-bearing animals, lung adenomas and Zymbal gland carcinomas when compared with the untreated controls (numbers = 45, 24, 35, 33 and 4 of the 71 treated mice compared with 36, 22, 21, 17 and 0 in the control group of 71 mice, for each tumour class respectively). No increase in the numbers of leukaemias or lymphomas were reported. The incidences of tumours in the individual tissue types were not reported.
Source: BP Chemicals Ltd. London
Test substance: Chromatoquality grade benzene was tested.

(602)

Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 10 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 1200 ppm (converts to 3.92 mg/l); 80 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: C57Bl/6J strain mice were used. Control group consisted of 80 males exposed to filtered, conditioned air in chambers, 6 hours/day, 5 days/week for 10 weeks and observed until spontaneous death. Benzene concentrations in the exposure chambers were monitored at ½-hour intervals during the daily exposures. Blood was withdrawn from ten exposed and ten control animals every second week during the exposure period and monthly thereafter. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically.
Result: The mortality rate was increased by benzene exposure and intense lymphocytopenia and mild anaemia were noted during the exposure period. The blood counts returned to normal when exposure ceased. When the tumour types were grouped together, an increase in the numbers of Zymbal gland carcinomas were found (4 of the 68 treated mice compared with 0 in the control group of 67 mice). No increases in total, malignant or benign tumour-bearing animals, lung adenomas and leukaemias or lymphomas were noted. The incidences of tumours in the individual tissue types were not recorded.
Source: BP Chemicals Ltd. London
Test substance: Chromatoquality grade benzene was tested.

(602)

Species: mouse **Sex:** female
Strain: C57BL
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 300 ppm (converts to 0.98 mg/l); 118 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: C57Bl/6 strain mice were used. Control group consisted of 116 females exposed to air. On days 3, 28, 56, 112, 168 and 179 after the last exposure, five mice (three on day 179) from each group were killed for haematopoietic stem cell assays. Necropsy was performed on all animals and gross and microscopic appearance of the thymus, lungs, spleen, liver, kidneys and bone marrow evaluated.
Result: An increase in mortality was seen 48 weeks after the end of the exposure period in the benzene-treated animals. Of the ten animals which died, six were found to have thymic lymphoma and two, unspecified lymphomas. Of the remaining two deaths, one mouse was killed in a moribund condition and found to be leukaemia free while the other was undiagnosed due to autolysis and partial cannibalism. This increase in leukaemia was statistically significantly greater than the incidence seen in the control group (one control mouse was killed moribund and had no histologic evidence of lymphoma-leukaemia) or in a group of 354 recent historical controls of this same sex and strain from the same laboratory.
Source: BP Chemicals Ltd. London
Test substance: Reagent grade, thiophene-free benzene was tested.

(603)

Species: mouse **Sex:** female
Strain: C57BL
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: 16 weeks
Doses: 300 ppm (converts to 0.98 mg/l); 89 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: C57Bl/6 strain mice were used. Control group consisted of 88 females exposed to conditioned air. At various times during and after the exposure period, five to ten mice were removed from both the treated and control groups and the blood, bone marrow and spleens removed and examined.

Result: Early mortality and an increase in mortality rate were noted in the benzene-treated group. All leukaemias and, in particular, thymic and nonthymic lymphocytic neoplasms, benign and malignant Zymbal gland tumours and ovarian tumours were increased in the treated animals when compared with the control animals (incidence = 22.5, 11.2, 6.7, 18.0, 9.0 and 4.5% in the treated and 9.1, 1.1, 2.3, 1.1, 0 and 2.3% in the controls for the tumour types respectively).
Source: BP Chemicals Ltd. London

(604)

Species: mouse **Sex:** male
Strain: CBA
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: 18 months
Doses: 300 ppm (converts to 0.98 mg/l); 125 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: CBA/Ca strain mice were used. Control group consisted of 125 animals exposed to conditioned, filtered air. Benzene concentrations in the exposure chambers were monitored twice per hour during the exposures. The experiment was terminated when the survival of the benzene-exposed group reached 20% (22 months from the start of exposure). Blood and bone marrow smears were examined from the 24 benzene-exposed mice alive at the terminal kill and 24 randomly selected controls. The major organs were examined histologically.
Result: A statistically significant increase in the mortality rate was seen in the benzene-treated group. Benzene-induced malignant lymphoma with metastasis was a significant cause of early mortality with 11 deaths due to lymphoma occurring in the first 9 months postexposure. For the total exposure period, the incidence of malignant lymphoma was significantly greater in the treated (14 cases) than the control (2 cases) group. Increased incidences of preputial gland squamous cell carcinomas (60% in treated, 0% in control group), lung adenomas (36 and 14% respectively), Zymbal gland carcinomas (11 and 1% respectively), forestomach squamous cell carcinomas (7 and 0% respectively) and granulocytic hyperplasias of the bone marrow (36 and 8% respectively) and the spleen (6 and 0% respectively) were found. Of the 24 treated and control mice alive at the terminal kill, no cytologic lesions were found in the bone marrow smears. Minimal to marked granulocytic hyperplasia was present in the bone marrow of 14 of the exposed animals and minimal granulocytic hyperplasia in two of the controls.¿
Source: BP Chemicals Ltd. London
Test substance: 100% pure.

(605)

Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 100 ppm (converts to 0.33 mg/l); 50 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: AKR/J strain mice were used. Control group consisted of 50 males exposed to filtered, conditioned air under the same exposure regime as above. Benzene concentrations in the exposure chambers were determined every ½-hour during the daily exposures. Animals were observed daily for evidence of morbidity and weighed weekly for the first 4 weeks and biweekly thereafter. Blood was withdrawn from ten test and ten control mice every other week throughout the study. The lung, liver, spleen, kidney, bone marrow and any abnormally appearing organs were examined histologically. Exposure ended after 505 days when the last test animal died.
Result: No statistically significant difference in median survival time or on the rate of weight gain was noted. Haematological studies were suspended after week 37 as too few animals remained due to advancing mortality. Statistically significant lymphocytopenia was observed throughout the study, from week 1. Red blood cell levels were consistently depressed and there was some tendency toward neutrophilia throughout the study. Little or no morphological differences were seen in the peripheral blood. Malignant lymphoma occurred in 29 of the 49 treated and 24 of the 50 control animals. The predominant type in both groups was disseminated, lymphocytic lymphoma with thymic involvement. Of the 29 treated mice with lymphoma, 15 had lymphoma cell infiltrates in the bone marrow, 7 had bone marrow hypoplasia and 7 had essentially normocellular bone marrows. The corresponding figures in the 24 control animals were 5, 1 and 18 respectively. In the treated group as a whole, ten of the animals (20%) had bone marrow hypoplasia in comparison with one of the control mice, a statistically significant increase.
Source: BP Chemicals Ltd. London

(606)

Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week/1 week in every 3
Post. obs. period: 2 weeks observation after each week of exposure
Doses: 300 ppm (converts to 0.98 mg/l); 60 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 60 males exposed to filtered conditioned air in chambers, using the same intermittent exposure regime. Benzene concentrations in the exposure chambers were monitored at ½-hour intervals during the daily exposures. Blood samples were taken from ten exposed and ten control animals at the end of each week of exposure. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically. Total number of days exposed was 129.
Result: The mortality rate was increased by benzene exposure and lymphocytopenia and anaemia were seen throughout the study. When the tumour types were grouped together, all the incidences were increased; that is, the numbers of total, malignant and benign tumour-bearing animals, lung adenomas, leukaemias or lymphomas and Zymbal gland carcinomas (25, 12, 15, 14, 7 and 2 of the 54 treated mice and 4, 1, 3, 3, 1 and 0 of the 46 control animals for each tumour class respectively). The incidences of tumours in the individual tissue types were not recorded.
Source: BP Chemicals Ltd. London
Test substance: Chromatoquality grade benzene was tested.

(607)

Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week/1 week in every 3
Post. obs. period: 2 weeks observation after every week of exposure
Doses: 300 ppm (converts to 0.98 mg/l); 60 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: C57Bl/6J strain mice were used. Control group consisted of 60 males exposed to filtered, conditioned air in chambers, using the same intermittent exposure regime. Benzene concentrations in the exposure chambers were monitored at

½-hour intervals during the daily exposures. Blood samples were taken from ten exposed and ten control animals at the end of each week of exposure. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically. Total number of days exposed was 181.

Result: The mortality rate was increased by benzene exposure and lymphocytopenia and anaemia were seen throughout the study. When the tumour types were grouped together, statistically significant increases were seen in the numbers of total and malignant tumour-bearing animals, leukaemias or lymphomas and Zymbal gland carcinomas (25, 24, 3 and 19 of the 54 treated mice compared with 8, 2, 1 and 0 of the 46 control animals, for each tumour class respectively). No increase in the numbers of benign tumour-bearing animals or lung adenomas were recorded. The incidences of tumours in the individual tissue types were not recorded.

Source: BP Chemicals Ltd. London

Test substance: Chromatoquality grade benzene was tested.

(607)

Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 300 ppm (converts to 0.98 mg/l); 40 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 40 mice exposed to air. Semi-monthly haematologic studies were conducted on ten test and ten control animals.

Result: Three mice showed myeloproliferative disorders, including one case of acute myelogenous leukaemia, one case of chronic myelogenous leukaemia and a third in which granulocytic hyperplasia was present. No such effects were seen in the control group. Although the increased incidences are not statistically significant when compared with the control group, the fact that myelogenous leukaemia has not been reported in historic control data on this strain of mouse is suggestive of a causative role for benzene.

Source: BP Chemicals Ltd. London

(601)

Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 300 ppm (converts to 0.98 mg/l); 40 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: C57Bl/6J strain mice were used. Control group consisted of 40 males exposed to filtered, conditioned air under the same exposure regime as above. Benzene concentrations in the exposure chambers were determined every ½-hour during the daily exposures. Animals were observed daily for evidence of morbidity and weighed weekly for the first 4 weeks and biweekly thereafter. Blood was withdrawn from ten test and ten control mice every other week throughout the study. The lung, liver, spleen, kidney, bone marrow and any abnormally appearing organs were examined histologically. Exposure ended after 488 days when the last test animal died.
Result: Median survival time and body weight gain were decreased in treated mice. Haematological studies were suspended after 61 weeks as too few animals were alive in the test group. Statistically significant increases in lymphocytopenia and anaemia were seen throughout the study and neutrophilia was evident after 17 weeks. Changes were observed in the peripheral blood cell morphology of the treated animals including anisocytosis (variation in red blood cell size), poikilocytosis (variation in red blood cell shape), giant platelets and, concurrent with neutrophilia, hyperlobulated, mature neutrophils and neutrophilic left shift. On histopathologic examination, the incidences of haematopoietic neoplasms and bone marrow and splenic hyperplasias (without evidence of haematopoietic neoplasm) were statistically significantly increased when compared with the control group (8/40, 13/32 and 10/32 in the treated and 2/40, 0/38 and 2/38 in the control group for the three tumours respectively). Six of the eight test animals showing haematopoietic neoplasms had lymphocytic lymphoma with thymic involvement, a significant increase when compared with the two control animals showing lymphocytic lymphoma without thymic involvement.
Source: BP Chemicals Ltd. London

(606)

Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: inhalation
Exposure period: 10 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 1200 ppm (converts to 3.92 mg/l); 80 mice; mean analytical daily concentration 1195 +/- 41 ppm
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 80 males exposed to filtered, conditioned air in chambers, 6 hours/day, 5 days/week for 10 weeks and observed until spontaneous death. Benzene concentrations in the exposure chambers were monitored at half-hour intervals during the daily exposures. Blood was withdrawn from ten exposed and ten control animals every second week during the exposure period and monthly thereafter. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically.
Reliability: 3 (not valid)
Significant methodological deficiencies
one concentration tested, one sex, limited histopathology
Result: The mortality rate was increased by benzene exposure and intense lymphocytopenia and mild anaemia were noted during the exposure period. The blood counts returned to normal when exposure ceased. When the tumour types were grouped together, statistically significant increases were seen in the incidences of total, malignant and benign tumour-bearing animals, lung adenomas and Zymbal gland carcinomas when compared with the untreated controls (numbers = 45, 24, 35, 33 and 4 of the 71 treated mice compared with 36, 22, 21, 17 and 0 in the control group of 71 mice, for each tumour class respectively). No increase in the numbers of leukaemias or lymphomas were reported. The incidences of tumours in the individual tissue types were not reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Chromatoquality grade benzene was tested.

(602)

Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 10 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 1200 ppm (converts to 3.92 mg/l); 80 mice; mean analytical daily concentration 1195 +/– 41 ppm
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: C57Bl/6J strain mice were used. Control group consisted of 80 males exposed to filtered, conditioned air in chambers, 6 hours/day, 5 days/week for 10 weeks and observed until spontaneous death. Benzene concentrations in the exposure chambers were monitored at half–hour intervals during the daily
exposures. Blood was withdrawn from ten exposed and ten control animals every second week during the exposure period and monthly thereafter. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically.
Reliability: 3 (not valid)
Significant methodological deficiencies
one concentration tested, one sex, limited histopathology
Result: The mortality rate was increased by benzene exposure and intense lymphocytopenia and mild anaemia were noted during the exposure period. The blood counts returned to normal when exposure ceased. When the tumour types were grouped together, an increase in the numbers of Zymbal gland carcinomas were found (4 of the 68 treated mice compared with 0 in the control group of 67 mice) but this was not statistically significant. No increases in total, malignant or benign tumour–bearing animals, lung adenomas and leukaemias or lymphomas were noted. The incidences of tumours in the individual tissue types were not recorded.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Chromatoquality grade benzene was tested.

(602)

Species: mouse **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 300 ppm (converts to 0.98 mg/l); 118 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: C57Bl/6 strain mice were used. Control group consisted of 116 females exposed to air. On days 3, 28, 56, 112, 168 and 179 after the last exposure, five mice (three on day 179) from each group were killed for haematopoietic stem cell assays. Necropsy was performed on all animals and gross and microscopic appearance of the thymus, lungs, spleen, liver, kidneys and bone marrow evaluated. Chamber concentration measured by GC.
Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, one dose, limited histopathology, no data about clinical parameters
Result: An increase in mortality was seen 48 weeks after the end of the exposure period in the benzene-treated animals. Of the ten animals which died, six were found to have thymic lymphoma and two, unspecified lymphomas. Of the remaining two deaths, one mouse was killed in a moribund condition and found to be leukaemia free while the other was undiagnosed due to autolysis and partial cannibalism. This increase in leukaemia was statistically significantly greater than the incidence seen in the control group (one control mouse was killed moribund and had no histologic evidence of lymphoma-leukaemia) or in a group of 354 recent historical controls of this same sex and strain from the same laboratory.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Reagent grade, thiophene-free benzene was tested.

(603)

Species: mouse **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: until spontaneous death
Doses: 300 ppm (converts to 0.98 mg/l); 89 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 88 females exposed to conditioned air.
Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, one dose, no statistical evaluation, methods insufficiently reported, no data about chamber concentration analysis and test substance
Result: Early mortality and an increase in mortality rate were noted in the benzene-treated group. All leukaemias and, in particular, thymic and nonthymic lymphocytic neoplasms, benign and malignant Zymbal gland tumours and ovarian tumours were increased in the treated animals when compared with the control animals (incidence = 22.5, 11.2, 6.7, 18.0, 9.0 and 4.5% in the treated and 9.1, 1.1, 2.3, 1.1, 0 and 2.3% in the controls for the tumour types respectively).
Source: Deutsche Shell Chemie GmbH Eschborn

(604)

Species: mouse **Sex:** male
Strain: CBA
Route of admin.: inhalation
Exposure period: 16 weeks
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: 18 months
Doses: 300 ppm (converts to 0.98 mg/l); 125 mice; mean analytical chamber concentration 300+/-3 ppm
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: CBA/Ca strain mice were used. Control group consisted of 125 animals exposed to conditioned, filtered air. Benzene concentrations in the exposure chambers were monitored twice per hour during the exposures. The experiment was terminated when the survival of the benzene-exposed group reached 20% (22 months from the start of exposure). Blood and bone marrow smears were examined from the 24 benzene-exposed mice alive at the terminal kill and 24 randomly selected controls. The major organs were examined histologically.

Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, one concentration tested, limited histopathology

Result: A statistically significant increase in the mortality rate was seen in the benzene-treated group. Benzene-induced malignant lymphoma with metastasis was a significant cause of early mortality with 11 deaths due to lymphoma occurring in the first 9 months postexposure. For the total exposure period, the incidence of malignant lymphoma was significantly greater in the treated (14 cases) than the control (2 cases) group. Significantly increased incidences of preputial gland squamous cell carcinomas (60% in treated, 0% in control group), lung adenomas (36 and 14% respectively) and not significantly increased Zymbal gland carcinomas (11 and 1% respectively), forestomach squamous cell carcinomas (7 and 0% respectively) and granulocytic hyperplasias of the bone marrow (36 and 8% respectively) and the spleen (6 and 0% respectively) were found. Of the 24 treated and control mice alive at the terminal kill, no cytologic lesions were found in the bone marrow smears. Minimal to marked granulocytic hyperplasia was present in the bone marrow of 14 of the exposed animals and minimal granulocytic hyperplasia in two of the controls.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 100% pure (analysed)

(605)

Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 100 ppm (converts to 0.33 mg/l); 50 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data

Remark: Control group consisted of 50 males exposed to filtered, conditioned air under the same exposure regime as above. Benzene concentrations in the exposure chambers were determined every 0.5 h during the daily exposures. Animals were observed daily for evidence of morbidity and weighed weekly for the first 4 weeks and biweekly thereafter. Blood was withdrawn from ten test and ten control mice every other week throughout the study. The lung, liver, spleen, kidney, bone marrow and any abnormally appearing organs were examined histologically. Exposure ended after 505 days when the last test animal in the treatment group died.
Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, one concentration tested, no data about measured benzene concentration and test substance, limited histology, partly insufficient documentation of methods, no statistical

evaluation of tumor incidences

Result: No statistically significant difference in median survival time or on the rate of weight gain was noted. Haematological studies were suspended after week 37 as too few animals remained due to advancing mortality. Statistically significant lymphocytopenia was observed throughout the study, from week 1. Red blood cell levels were consistently depressed and there was some tendency toward neutrophilia throughout the study. Little or no morphological differences were seen in the peripheral blood. Malignant lymphoma occurred in 29 of the 49 treated and 24 of the 50 control animals. The predominant type in both groups was disseminated, lymphocytic lymphoma with thymic involvement. Of the 29 treated mice with lymphoma, 15 had lymphoma cell infiltrates in the bone marrow, 7 had bone marrow hypoplasia and 7 had essentially normocellular bone marrows. The corresponding figures in the 24 control animals were 5, 1 and 18 respectively. In the treated group as a whole, ten of the animals (20%) had bone marrow hypoplasia in comparison with one of the control mice, a statistically significant increase.

Source: Deutsche Shell Chemie GmbH Eschborn

(606)

Species: mouse **Sex:** male
Strain: CD–1
Route of admin.: inhalation
Exposure period: life–time
Frequency of treatment: 6 hours per day/5 days per week/1 week interrupted by 2 weeks of non–exposure
Post. obs. period: discontinuous exposure until death of last mice
Doses: 300 ppm (converts to 0.98 mg/l); 60 mice; mean analytical benzene concentration 298+/–12.4 ppm
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 60 males exposed to filtered conditioned air in chambers, using the same intermittent exposure regime. Benzene concentrations in the exposure chambers were monitored at half–hour intervals during the daily exposures. Blood samples were taken from ten exposed and ten control animals at the end of each week of exposure. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically. Total number of days exposed was 129.
Reliability: 3 (not valid)
Significant methodological deficiencies
one concentration tested, one sex, limited histopathology

Result: The mortality rate was increased by benzene exposure and lymphocytopenia and anaemia were seen throughout the study. When the tumour types were grouped together, all the

incidences were increased; that is, the numbers of total, malignant and benign tumour-bearing animals, lung adenomas, leukaemias or lymphomas (not significant) and Zymbal gland (not significant) carcinomas (25, 12, 15, 14, 7 and 2 of the 54 treated mice and 4, 1, 3, 3, 1 and 0 of the 46 control animals for each tumour class respectively). The incidences of tumours in the individual tissue types were not recorded.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Chromatoquality grade benzene was tested.

(607)

Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week/1 week interrupted by 2 weeks of non-exposure
Post. obs. period: discontinuous exposure until death of last mice
Doses: 300 ppm (converts to 0.98 mg/l); 60 mice; mean analytical benzene concentration 300+/-9.5 ppm
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: C57Bl/6J strain mice were used. Control group consisted of 60 males exposed to filtered, conditioned air in chambers, using the same intermittent exposure regime. Benzene concentrations in the exposure chambers were monitored at 0.5 h intervals during the daily exposures. Blood samples were taken from ten exposed and ten control animals at the end of each week of exposure. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically. Total number of days exposed was 181.
Reliability: 3 (not valid)
Significant methodological deficiencies
one concentration tested, one sex, limited histopathology
Result: The mortality rate was increased by benzene exposure and lymphocytopenia and anaemia were seen throughout the study. When the tumour types were grouped together, statistically significant increases were seen in the numbers of total and malignant tumour-bearing animals, and Zymbal gland carcinomas (25, 24, and 19 of the 54 treated mice compared with 8, 2, and 0 of the 46 control animals, for each tumour class respectively). No increase in the numbers of benign tumour-bearing animals or lung adenomas were recorded. The incidences of tumours in the individual tissue types were not recorded.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: Chromatoquality grade benzene was tested.

(607)

Species: mouse **Sex:** male
Strain: CD-1
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 300 ppm (converts to 0.98 mg/l); 40 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 40 mice exposed to air. Semi-monthly haematologic studies were conducted on ten test and ten control animals.
Reliability: 3 (not valid)
Significant methodological deficiencies
one sex, no data about measured benzene concentration and test substance, insufficient documentation of methods, low number of animals, one concentration tested, results limited to leukaemia
Result: Three mice showed myeloproliferative disorders, including one case of acute myelogenous leukaemia, one case of chronic myelogenous leukaemia and a third in which granulocytic hyperplasia was present. No such effects were seen in the control group. Although the increased incidences are not statistically significant when compared with the control group, the fact that myelogenous leukaemia has not been reported in historic control data on this strain of mouse is suggestive of a causative role for benzene.
Source: Deutsche Shell Chemie GmbH Eschborn

(601)

Species: mouse **Sex:** male
Strain: other
Route of admin.: inhalation
Exposure period: life-time
Frequency of treatment: 6 hours per day/5 days per week
Post. obs. period: none
Doses: 300 ppm (converts to 0.98 mg/l); 40 mice
Result:
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: C57Bl/6J strain mice were used. Control group consisted of 40 males exposed to filtered, conditioned air under the same exposure regime as above. Benzene concentrations in the exposure chambers were determined every 0.5 h during the daily exposures. Animals were observed daily for evidence of morbidity and weighed weekly for the first 4 weeks and biweekly thereafter. Blood was withdrawn from ten test and ten control mice every other week throughout the study. The

lung, liver, spleen, kidney, bone marrow and any abnormally appearing organs were examined histologically. Exposure ended after 488 days when the last test animal died.
Reliability: 3 (not valid)
Significant methodological deficiencies
low number of animals at start of experiment, one sex, one concentration tested, no data about measured benzene concentration and test substance, limited histology, partly insufficient documentation of methods

Result: Median survival time and body weight gain were decreased in treated mice. Haematological studies were suspended after 61 weeks as too few animals were alive in the test group. Statistically significant increases in lymphocytopenia and anaemia were seen throughout the study and neutrophilia was evident after 17 weeks. Changes were observed in the peripheral blood cell morphology of the treated animals including anisocytosis (variation in red blood cell size), poikilocytosis (variation in red blood cell shape), giant platelets and, concurrent with neutrophilia, hyperlobulated, mature neutrophils and neutrophilic left shift. On histopathologic examination, the incidences of haematopoietic neoplasms and bone marrow and splenic hyperplasias (without evidence of haematopoietic neoplasm) were statistically significantly increased when compared with the control group (8/40, 13/32 and 10/32 in the treated and 2/40, 0/38 and 2/38 in the control group for the three tumours respectively). Six of the eight test animals showing haematopoietic neoplasms had lymphocytic lymphoma with thymic involvement, a significant increase when compared with the two control animals showing lymphocytic lymphoma without thymic involvement.

Source: Deutsche Shell Chemie GmbH Eschborn

(606)

Species: mouse **Sex:** female
Strain: no data
Route of admin.: oral unspecified
Exposure period: 2 years
Frequency of treatment: no data
Post. obs. period: no data
Doses: TDLo: 18250 mg/Kg
Result:
Control Group: no data specified
Method: other: no data
Year: 1986 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(608)

5.8 Toxicity to Reproduction

Type: Fertility
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure Period: 10 weeks
Frequency of treatment: 6hr/day, 5 days/week
Duration of test: 20 weeks
Doses: 30,300ppm
Control Group: yes, concurrent no treatment
Method: other: no data
Year: 1980 **GLP:** no data
Test substance: no data
Result: Testicular lessions.
Source: REPSOL PETROLEO,S.A. MADRID

(609)

Type: Fertility
Species: rat **Sex:** female
Strain: no data
Route of admin.: inhalation
Exposure Period: 15 D pre/1-22 preg
Frequency of treatment: no data
Duration of test: no data
Doses: 670mg/m3/24h
Control Group: no data specified
Method: other: no data specified
Year: 1968 **GLP:** no data
Test substance: no data
Result: Effects on female fertility index
Source: REPSOL PETROLEO,S.A. MADRID

(610)

Type: Fertility
Species: rat **Sex:** female
Strain: no data
Route of admin.: inhalation
Exposure Period: 31-36 days
Frequency of treatment: daily/24 hours per day
Premating Exposure Period female: 10-15 days
Duration of test: 4.5-5 weeks
Doses: 0.3-20 ppm, 210 ppm (converts to 0.001-0.063 mg/l, 0.67 mg/l respectively); group size unspecified
Control Group: no data specified
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Females were exposed continuously for 10-15 days before mating with untreated males and for 3 weeks after mating.
Result: There was an absence of litters in the females but it is not known if this was due to failure to mate, infertility or early implantation losses of the fertilized ova.

Source: BP Chemicals Ltd. London
(611) (612) (582)

Type: Fertility
Species: rat **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure Period: gestation days 6–13
Frequency of treatment: daily/24 hours per day
Duration of test: gestation day 21
Doses: 0.15, 0.45, 1.5, 3.0 mg/l; groups of 20, 20, 22 and 22 rats respectively
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: CFY strain rats were used. Control group consisted of 48 females exposed to clean air. Body weights were recorded weekly. On day 21, dams were sacrificed, caesarean sections performed and number and position of live, dead and resorbed foetuses noted. Foetuses and placentae were weighed and macroscopically examined. Half the foetuses were dissected under the microscope while the other half were investigated for skeletal abnormalities. In each group, the organs of five foetuses and five dams were studied microscopically.
Result: No maternal deaths were seen at 0.15 mg/l, however 1–3 animals/ group died in the other treatment groups after completion of the exposures. Dissection revealed fresh haemorrhages in the lungs and kidneys and multiple incipient necroses in the liver, indicating ischaemia. Maternal weight gain and placental weight were statistically significantly decreased in all the treatment groups and liver/body weight ratio significantly increased at 0.45 mg/l and above. Increases were seen in the numbers of non–pregnant females at 1.5 and 3.0 mg/l and in the numbers of dams with total resorptions at 0.45 mg/l and above. Postimplantation foetal loss was statistically significantly increased at 0.45 mg/l and above as a result of both early and late foetal mortality increases. See section 5.9 for developmental effects.
Source: BP Chemicals Ltd. London
Test substance: p.a. grade benzene was tested.
(613)

Type: Fertility
Species: rat **Sex:** female
Strain: no data
Route of admin.: inhalation
Exposure Period: 31-36 days
Frequency of treatment: daily/24 hours per day
Premating Exposure Period female: 10-15 days
Duration of test: 4.5-5 weeks
Doses: 0.001, 0.0056, 0.0204, 0.0566, 0.0633, 0.67 mg/l; group size unspecified
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Females were exposed continuously for 10-15 days before mating with untreated males and for 3 weeks after mating. Reliability: 4 (validity cannot be judged) data from secondary literature and partly from English abstract of the original article in Russian
Result: There was an absence of litters in the females but it is not known if this was due to failure to mate, infertility or early implantation losses of the fertilized ova.
Source: Deutsche Shell Chemie GmbH Eschborn

(611) (612) (582)

Type: Fertility
Species: rabbit **Sex:** female
Strain: New Zealand white
Route of admin.: inhalation
Exposure Period: gestation days 6-19
Frequency of treatment: daily/24 hours per day
Duration of test: gestation day 29
Doses: 0.5, 1.0 mg/l; 11 and 15 rabbits respectively
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 60 dams exposed to air. Dams were killed on day 29 and foetuses examined as described by Ungvary G. Munkavédelem, Munka-és Uzemegészségügy 29, 207-216, 1983.J. Hyg. Epidem. Microbiol. Immun. 24, 363-371, 1980.
Result: The top dose caused statistically significant decreases in maternal weight gain and mean foetal weight and a statistically significant increase in the number of dead or resorbed foetuses, mainly arising from abortion. At 0.5 mg/l, a slight non-significant decrease in maternal weight gain and increase in the number of dead or resorbed foetuses was reported.
Source: BP Chemicals Ltd. London

(614)

Type: other
Species: rat **Sex:** male/female
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure Period: 13 weeks
Frequency of treatment: 6 hours per day/5 days per week
Duration of test: 13 weeks
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose
Control Group: yes, concurrent no treatment
NOAEL Parental: = 300 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 50 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 10 rats/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high–dosed groups sectioned and examined microscopically.
Result: No exposure related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. No effects were recorded on the testes or ovary weights and no histological changes were seen on microscopic examination of the tissues from these organs.
Source: BP Chemicals Ltd. London
Test substance: >99.9% pure.

(615)

Type: other
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: inhalation
Exposure Period: up to 212 days
Frequency of treatment: 7 hours per day/5 days per week
Duration of test: up to 212 days
Doses: 0.28, 7, 14, 21, 30 mg/l; 10–25 rats/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.
Result: See section 5.4 for general systemic effects. A moderate increase in testicular weight was seen in animals exposed for 93 days to 21 mg/l. No other effects on the testes were

reported.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Type: other
Species: rat **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure Period: up to 212 days
Frequency of treatment: 7 hours per day/5 days per week
Duration of test: up to 212 days
Doses: 0.28, 7, 14, 21, 30 mg/l; 10-25 rats/dose; low dose with both sexes
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.
Reliability: 3 (not valid)
Unsuitable test system
no reproduction toxicity study, only additional comment
Result: See section 5.4 for general systemic effects. A moderate increase in testicular weight was seen in animals exposed for 93 days to 21 mg/l. No other effects on the testes were reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.98% pure.

(491)

Type: other
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure Period: 13 weeks
Frequency of treatment: 6 hours per day/5 days per week
Duration of test: 13 weeks
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose
Control Group: yes, concurrent vehicle
NOAEL Parental: = 300 ppm
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 50 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 10 rats/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund

condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups sectioned and examined microscopically.
Reliability: 3 (not valid)
Unsuitable test system
no reproduction toxicity study, only additional comment

Result: No exposure related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. No effects were recorded on the testes or ovary weights and no histological changes were seen on microscopic examination of the tissues from these organs.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: >99.9% pure.

(615)

Type: other
Species: mouse **Sex:** female
Strain: CD-1
Route of admin.: gavage
Exposure Period: gestation days 12-15
Frequency of treatment: daily
Duration of test: no data
Doses: 1.0 ml/kg bw (converts to 880 mg/kg bw); group size unspecified
Control Group: no data specified
NOAEL Parental: > 880 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene administered in cotton seed oil.
Result: A significant increase in maternal lethality and embryonic resorption was seen. Foetal weight was significantly reduced.
Source: BP Chemicals Ltd. London

(616)

Type: other
Species: mouse **Sex:** female
Strain: CD-1
Route of admin.: gavage
Exposure Period: gestation days 6-15
Frequency of treatment: daily
Duration of test: no data
Doses: 0.3, 0.5, 1.0 ml/kg bw/day (converts to 264, 440, 880 mg/kg bw/day); group sizes unspecified
Control Group: no data specified
NOAEL Parental: = 264 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene administered in cotton seed oil.
Result: At the mid- and high-dose levels, a significant increase in maternal lethality and in embryonic resorption was seen.

Foetal weight was significantly reduced at all three doses.
Source: BP Chemicals Ltd. London

(616)

Type: other
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure Period: 13 weeks
Frequency of treatment: 6 hours per day/5 days/per week
Duration of test: 13 weeks
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 150 mice/sex/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: Control group consisted of 150 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 20 mice/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups in the interim kills and from all treated groups at the final sacrifice sectioned and examined microscopically.
Result: No exposure-related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. A statistically significant, exposure-related decrease in the mean testis weight was seen at the top-dose on days 28, 56 and 91, corresponding to a decreased mean testis/body weight ratio on days 56 and 91. No effect on the ovary weight was noted. The top-dosed animals, dosed for the full 13 week period, were found to have moderate-moderately severe bilateral testicular atrophy/degeneration (7 mice), a moderate-moderately severe decrease in spermatozoa in the epididymal ducts (6 mice), a minimal-moderate increase in abnormal sperm forms (9 mice) and bilateral ovarian cysts (4 mice). Similar lesions, reported to be of doubtful biological significance, were seen in both sexes at lower doses [no details available]. Some animals tended to have decreased lymphoid infiltrates in many tissues including the testes.
Source: BP Chemicals Ltd. London
Test substance: >99.9% pure.

(615)

Type: other
Species: mouse **Sex:** female
Strain: CD-1
Route of admin.: gavage
Exposure Period: 269 days
Frequency of treatment: 7 hours per day/5 days per week
Duration of test: up to 269 days
Doses: 0.28 mg/l; 5-10 guinea pigs
Control Group: no data specified
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.
Reliability: 3 (not valid)
Unsuitable test system
no reproduction toxicity study, only additional comment
Result: See section 5.4 for general systemic effects. A slight increase in testicular weight was noted. No other testicular effects were reported.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 99.98% pure.

(491)

Type: other
Species: mouse **Sex:** female
Strain: CD-1
Route of admin.: gavage
Exposure Period: 243 days
Frequency of treatment: 7 hours per day/5 days per week
Duration of test: 243 days
Doses: 0.26 mg/l; 1 or 2 rabbits
Control Group: no data specified
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.
Reliability: 3 (not valid)
Unsuitable test system
no reproduction toxicity study, only additional comment
Result: See section 5.4 for general systemic effects. Slight histopathological testicular changes (degeneration of the germinal epithelium) were noted. It is difficult to draw any conclusions from this study due to low numbers of animals tested and the single dose level used.
Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: 99.98% pure.

(491)

Type: other
Species: rabbit **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure Period: 243 days
Frequency of treatment: 7 hours per day/5 days per week
Duration of test: 243 days
Doses: 0.26 mg/l; 1 or 2 rabbits
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.
Result: See section 5.4 for general systemic effects. Slight histopathological testicular changes (degeneration of the germinal epithelium) were noted. It is difficult to draw any conclusions from this study due to low numbers of animals tested and the single dose level used.
Source: BP Chemicals Ltd. London
Test substance: 99.98% pure.

(491)

Type: other
Species: rabbit **Sex:** female
Strain: New Zealand white
Route of admin.: inhalation
Exposure Period: 13 weeks
Frequency of treatment: 6 hours per day/5 days/per week
Duration of test: 13 weeks
Doses: 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 100 mice/sex/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 100 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 20 mice/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups in the interim kills and from all treated groups at the final sacrifice sectioned and examined microscopically.
Reliability: 3 (not valid)

Unsuitable test system
no reproduction toxicity study, only additional comment

Result: No exposure-related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. A statistically significant, exposure-related decrease in the mean testis weight was seen at the top-dose on days 28, 56 and 91, corresponding to a decreased mean testis/body weight ratio on days 56 and 91. No effect on the ovary weight was noted. The top-dosed animals, dosed for the full 13 week period, were found to have moderate-moderately severe bilateral testicular atrophy/degeneration (7 mice), a moderate-moderately severe decrease in spermatozoa in the epididymal ducts (6 mice), a minimal-moderate increase in abnormal sperm forms (9 mice) and bilateral ovarian cysts (4 mice). Similar lesions, reported to be of doubtful biological significance, were seen in both sexes at lower doses [no details available]. Some animals tended to have decreased lymphoid infiltrates in many tissues including the testes.

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: >99.9% pure.

(615)

Type: other
Species: guinea pig **Sex:** male
Strain: no data
Route of admin.: inhalation
Exposure Period: 269 days
Frequency of treatment: 7 hours per day/5 days per week
Duration of test: 269 days
Doses: 0.28 mg/l; 5-10 guinea pigs
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

Result: See section 5.4 for general systemic effects. A slight increase in testicular weight was noted. No other testicular effects were reported.

Source: BP Chemicals Ltd. London

Test substance: 99.98% pure.

(491)

5.9 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure period: Days 6–15 gestation
Frequency of treatment: 6h/day
Duration of test: no data
Doses: 300,2200
Control Group: yes
Method: other: no data
Year: 1978 **GLP:** no data
Test substance: no data
Source: REPSOL PETROLEO,S.A. MADRID

(617)

Species: rat **Sex:** female
Strain: Sprague–Dawley
Route of admin.: inhalation
Exposure period: Days 6–15 gestation
Frequency of treatment: 6h/day
Duration of test: 20 days gestation
Doses: 0,1,10,40 and 100ppm
Control Group: yes, concurrent no treatment
Method: other: no data
Year: 1986 **GLP:** no data
Test substance: as prescribed by 1.1 – 1.4
Source: REPSOL PETROLEO,S.A. MADRID

(618)

Species: rat **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure period: gestation days 6–13
Frequency of treatment: daily/24 hours per day
Duration of test: gestation day 21
Doses: 0.15, 0.45, 1.5, 3.0 mg/l/day; groups of 20, 20, 22, 22 rats respectively
Control Group: yes, concurrent no treatment
NOAEL Teratogen.: = 3 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: CFY strain rats were used. Control group consisted of 48 females exposed to clean air. Body weights were recorded weekly. On day 21, dams were sacrificed, caesarean sections performed and number and position of live, dead and resorbed foetuses noted. Foetuses and placentae were weighed and macroscopically examined. Half the foetuses were dissected under the microscope while the other half were investigated for skeletal abnormalities. In each group, the organs of five foetuses and five dams were studied microscopically.
Result: See section 5.8 for reproductive effects. Mean foetal weight was decreased in all the exposed groups, this being

statistically significant, and half to two-thirds of the foetuses showed retarded development at 0.45 mg/l and above, as seen by weights of less than 3.3 g. Skeletal retardation (missing or poorly ossified sternebrae, bipartite vertebral centres and shortened 13th rib) were slightly increased at 0.15 mg/l, the incidence being significantly higher in the other treatment groups. There was an increasing non-significant trend in the incidence of skeletal anomalies (irregular, fused sternebrae and supernumerary ribs) on exposure to 1.5 and 3.0 mg/l. External malformations were seen in three of the 653 foetuses (one case of missing tail at 0.45 mg/l and 2 cases of syndactylia at 1.5 mg/l) and none of the controls. No increase over the control group was seen in internal malformations and three foetuses had skeletal malformations (one case of missing sacral vertebrae at 0.45 mg/l; one case of fused metacarpal and carpal bones at 1.5 mg/l and one case of missing orbita). The incidence of these malformations was not considered to have been increased and the investigators concluded that benzene was not teratogenic.

Source: BP Chemicals Ltd. London

Test substance: Supplied by Reanal, Budapest; p.a. grade.

(619)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: gestation day 20
Doses: 1, 10, 40, 100 ppm/day (converts to 0.003, 0.033, 0.12, 0.33 mg/l/day); 40 rats/dose
Control Group: yes, concurrent no treatment
NOAEL Maternalt.: = 100 ppm
NOAEL Teratogen.: = 100 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Two control groups consisting of 40 females were exposed to filtered room air. Animals were examined prior to, during and after daily exposure and weighed at intervals. On day 20 dams were sacrificed and necropsied. Foetuses were removed by caesarian section and weighed and measured. Numbers of corpora lutea/ovary, implantation sites, resorptions and live and dead foetuses were recorded for each dam. Foetuses were examined for gross external variations and either sectioned to examine for visceral abnormalities or examined for skeletal abnormalities. Benzene vapour concentrations were determined daily and the mean ranges by infrared and gas chromatographic analyses found to be 1.02, 8.36-11.02, 37.36-41.21 and 96.59-104.15 ppm (0.003, 0.028-0.036, 0.12-0.14 and 0.32-0.34 mg/l) for the four groups respectively.
Result: No deaths and no treatment-related clinical signs or gross pathology were noted in the dams and the pregnancy rate was within the expected range. Average maternal body weight

gain was very slightly lower than the controls in the top-dose but this was not statistically significant. The average number of implantation sites, number of resorptions, resorption incidence and number of live foetuses were similar in all groups. A small, statistically significant decrease in the average male and female foetal body weight and non-significant, slight decrease in mean foetal body length was recorded at 0.33 mg/l. A slight, non-significant increase in dilation of the renal pelvis and ureters was seen in the lowest- and top-dose groups and a small, non-significant decrease in the average number of sternebrae and caudal vertebrae occurred at 0.33 mg/l. No benzene-induced skeletal variants or soft tissue anomalies were reported. The investigators concluded that benzene was slightly foetotoxic as shown by reduced foetal weights and lengths and a statistically non-significant delay in ossification of extremities and sternebrae at 0.33 mg/l.

Source: BP Chemicals Ltd. London

(620)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/7 hours per day
Duration of test: gestation day 20
Doses: 10, 50, 500 ppm (converts to 0.033, 0.16, 1.63 mg/l); 18, 20, 19 rats respectively
Control Group: yes, concurrent no treatment
NOAEL Teratogen.: = 50 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 17 females exposed to filtered room air. On day 20 females were sacrificed, caesarian sections were performed and numbers of ovarian corpora lutea, resorption sites and live and dead foetuses examined. Each foetus was examined externally and weighed. Whole-body transverse sections were made and examined microscopically for visceral changes. The skeleton was examined microscopically for anomalies and ossification. Blood samples were obtained on days 5 and 20 and erythrocyte and leukocyte counts determined. Benzene vapour concentrations were determined daily and the means found to be 9.75, 52.9 and 513.9 ppm (0.032, 0.17 and 1.70 mg/l) for the three groups respectively.
Result: No deaths, observable illness or signs of compound-induced toxicity were noted in the dams and the pregnancy rate was within the expected range. Maternal body weight was decreased in the mid- and high-dosed dams and transient changes in mean body weight gain were noted at various stages throughout the study at all doses. No treatment-related effects were seen in any of the haematology parameters, at necropsy or on the number of implantation sites, resorbed and dead foetuses, incidence of live foetuses or sex distribution of the foetuses.

Statistically significant decreases in the mean crown-rump distance of the high-dosed foetuses and mean body weights of the mid- and high-dosed live foetuses were noted when compared with the controls. Skeletal variants (excencephaly, angulated ribs and ossification of the forefeet out of sequence in one, one and two rats respectively) were seen at 1.63 mg/l and evidence of lagging ossification was recorded in the rib cage and extremities of the mid-dosed group and, together with the skull, vertebral column and pelvic girdle, the high-dosed group. A significantly fewer number of caudals compared to the controls were seen at 1.63 mg/l and a dose-related decrease in the mean number of metatarsals and phalanges occurred. Visceral examination revealed slight dilation of the ventricles in the brain in five mid- and four high-dosed animals. The investigators concluded that benzene was foetotoxic to rats at concentrations of 0.16 and 1.63 mg/l and it manifests a teratogenic potential at 1.63 mg/l.

Source: BP Chemicals Ltd. London

(621)

Species: rat **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure period: gestation days 6-13
Frequency of treatment: daily/24 hours per day
Duration of test: gestation day 21
Doses: 0.15, 0.45, 1.5, 3.0 mg/l/day; groups of 20, 20, 22, 22 rats respectively
Control Group: yes, concurrent no treatment
NOAEL Teratogen.: = 3 mg/l
Method: other
Year: **GLP:** no data
Test substance: other TS
Remark: CFY strain rats were used. Control group consisted of 48 females exposed to clean air. Body weights were recorded weekly. On day 21, dams were sacrificed, caesarean sections performed and number and position of live, dead and resorbed foetuses noted. Foetuses and placentae were weighed and macroscopically examined. Half the foetuses were dissected under the microscope while the other half were investigated for skeletal abnormalities. In each group, the organs of five foetuses and five dams were studied microscopically. Benzene concentration analysed by GC.
Reliability: 1 (valid)
Comparable to guideline study
exposure period gestation day 6-13 instead the recommended day 6-15, result of benzene concentration analysis not reported
Result: Mean foetal weight was decreased in all the exposed groups, this being statistically significant, and half to two-thirds of the foetuses showed retarded development at 0.45 mg/l and above, as seen by weights of less than 3.3 g. Skeletal retardation (missing or poorly ossified sternebrae, bipartite vertebral centres and shortened 13th rib) were slightly increased at 0.15 mg/l, the incidence being

significantly higher in the other treatment groups. There was an increasing non-significant trend in the incidence of skeletal anomalies (irregular, fused sternebrae and supernumerary ribs) on exposure to 1.5 and 3.0 mg/l. External malformations were seen in three of the 653 foetuses (one case of missing tail at 0.45 mg/l and 2 cases of syndactylia at 1.5 mg/l) and none of the controls. No increase over the control group was seen in internal malformations and three foetuses had skeletal malformations (one case of missing sacral vertebrae at 0.45 mg/l; one case of fused metacarpal and carpal bones at 1.5 mg/l and one case of missing orbita). The incidence of these malformations was not considered to have been increased and the investigators concluded that benzene was not teratogenic.
No maternal deaths were seen at 0.15 mg/l, however 1-3 animals/ group died in the other treatment groups after completion of the exposures. Dissection revealed fresh haemorrhages in the lungs and kidneys and multiple incipient necroses in the liver, indicating ischaemia. Maternal weight gain and placental weight were statistically significantly decreased in all the treatment groups and liver/body weight ratio significantly increased at 0.45 mg/l and above. Increases were seen in the numbers of non-pregnant females at 1.5 and 3.0 mg/l and in the numbers of dams with total resorptions at 0.45 mg/l and above. Postimplantation foetal loss was statistically significantly increased at 0.45 mg/l and above as a result of both early and late foetal mortality increases.

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: Supplied by Reanal, Budapest; p.a. grade.

(619)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: gestation day 20
Doses: 1, 10, 40, 100 ppm/day (converts to 0.003, 0.033, 0.12, 0.33 mg/l/day); 40 rats/dose
Control Group: yes, concurrent vehicle
NOAEL Maternalt.: = 100 ppm
NOAEL Teratogen.: = 100 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Reliability: 1 (valid)
Comparable to guideline study
no data about test substance but concurrent analysis
Two control groups consisting of 40 dams were exposed to filtered room air. Animals were examined prior to, during and after daily exposure and weighed at intervals. On day 20 dams were sacrificed and necropsied. Foetuses were removed by caesarian section and weighed and measured. Numbers of corpora lutea/ovary, implantation sites,

resorptions and live and dead foetuses were recorded for each dam. Foetuses were examined for gross external variations and either sectioned to examine for visceral abnormalities or examined for skeletal abnormalities. Benzene vapour concentrations were determined daily and the mean ranges by infrared and gas chromatographic analyses found to be 1.02, 8.36–11.02, 37.36–41.21 and 96.59–104.15 ppm (0.003, 0.028–0.036, 0.12–0.14 and 0.32–0.34 mg/l) for the four groups respectively.

Result: Benzene chamber concentration variability ranged from 3–13%. No deaths and no treatment-related clinical signs or gross pathology were noted in the dams and the pregnancy rate was within the expected range. Average maternal body weight gain was very slightly lower than the controls in the top-dose but this was not statistically significant. The average number of implantation sites, number of resorptions, resorption incidence and number of live foetuses were similar in all groups. A small, statistically significant decrease in the average male and female foetal body weight and non-significant, slight decrease in mean foetal body length was recorded at 0.33 mg/l. A slight, non-significant increase in dilation of the renal pelvis and ureters was seen in the lowest- and top-dose groups and a small, non-significant decrease in the average number of sternebrae and caudal vertebrae occurred at 0.33 mg/l. No benzene-induced skeletal variants or soft tissue anomalies were reported. The investigators concluded that benzene was slightly foetotoxic as shown by reduced foetal weights and lengths and a statistically non-significant delay in ossification of extremities and sternebrae at 0.33 mg/l.

Source: Deutsche Shell Chemie GmbH Eschborn

(620)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: gestation days 6–15
Frequency of treatment: daily/7 hours per day
Duration of test: gestation day 20
Doses: 10, 50, 500 ppm (converts to 0.033, 0.16, 1.63 mg/l); 18, 20, 19 rats respectively
Control Group: yes, concurrent vehicle
NOAEL Teratogen.: = 50 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 17 females exposed to filtered room air. On day 20 females were sacrificed, caesarian sections were performed and numbers of ovarian corpora lutea, resorption sites and live and dead foetuses examined. Each foetus was examined externally and weighed. Whole-body transverse sections were made and examined microscopically for visceral changes (1/3 of fetuses/group). The skeleton of the remaining fetuses were examined microscopically for anomalies and ossification. Maternal blood samples were obtained on days 5 and 20 and erythrocyte and leukocyte

counts determined. Benzene vapourconcentrations were determined daily and the means found to be 9.75, 52.9 and 513.9 ppm (0.032, 0.17 and 1.70 mg/l) for the three groups respectively (variation in concentration max. 10%).
Reliability: 1 (valid)
Comparable to guideline study
no data about test substance but concurrent GC analysis

Result: No deaths, observable illness or signs of compound-induced toxicity were noted in the dams and the pregnancy rate was within the expected range. Maternal body weight was significantly decreased in the mid- and high-dosed dams, significantly increased in the low-dose group and transient changes in mean body weight gain were noted at various stages throughout the study at all doses. No treatment-related effects were seen in any of the haematology parameters, at necropsy or on the number of implantation sites, resorbed and dead foetuses, incidence of live foetuses or sex distribution of the foetuses. Statistically significant decreases in the mean crown-rump distance of the high-dosed foetuses and mean body weights of the mid- and high-dosed live foetuses were noted when compared with the controls. Skeletal variants (exencephaly, angulated ribs and ossification of the forefeet out of sequence in one, one and two rats respectively) were seen at 1.63 mg/l and evidence of lagging ossification was recorded in the rib cage and extremities of the mid-dosed group and, together with the skull, vertebral column and pelvic girdle, the high-dosed group. A significantly fewer number of caudals compared to the controls were seen at 1.63 mg/l and a dose-related decrease in the mean number of metatarsals and phalanges occurred. Visceral examination revealed slight dilation of the ventricles in the brain in five mid- and four high-dosed animals. The investigators concluded that benzene was foetotoxic to rats at concentrations of 0.16 and 1.63 mg/l and it manifests a teratogenic potential at 1.63 mg/l.

Source: Deutsche Shell Chemie GmbH Eschborn

(621)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6–15
Frequency of treatment: daily/6 hours per day
Duration of test: gestation day 16
Doses: 5, 10, 20 ppm/day (converts to 0.017, 0.033, 0.066 mg/l/day); 5–10 mice/dose
Control Group: yes, concurrent no treatment
NOAEL Teratogen.: = 20 ppm
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed pregnant mice were sacrificed, their uteri removed and the number of live, dead and resorbed foetuses recorded. Two male and two female foetuses/litter were weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells.
Result: Litter size, foetal weights and the numbers of dead, resorbed and malformed foetuses were within the control limits. In the foetuses, changes in the numbers of both erythroid precursor cells (i.e. colony forming unit–erythroid; CFU–E; and burst forming unit–erythroid; BFU–E) were observed, the more mature, CFU–E, cells being affected to a greater extent by all exposure concentrations. The number of foetal CFU–E cells were increased at 0.017 and 0.033 mg/l and decreased at 0.066 mg/l.
Source: BP Chemicals Ltd. London

(622)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6–15
Frequency of treatment: daily/6 hours per day
Duration of test: 2–day–old neonates
Doses: 5, 10, 20 ppm/day (converts to 0.017, 0.033, 0.066 mg/l/day); 5–10 mice/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. Two male and two female neonates/litter were selected at 2 days of age, weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells.
Result: No difference in CFU–E numbers were noted between the low–dose offspring and the controls. Offspring exposed, in utero, to 0.033 mg/l had either increased or decreased CFU–E numbers and those in male offspring exposed in utero to

0.066 mg/l were increased. The numbers of granulocytic/macrophage colony forming cells were increased in the male and female offspring from the top-dosed group and decreased in the male offspring in the mid-dosed group.

Source: BP Chemicals Ltd. London

(622)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: 6-week-old offspring
Doses: 5, 10, 20 ppm/day (converts to 0.017, 0.033, 0.066 mg/l/day); 5-10 mice/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 6 weeks of age, one male and one female offspring were selected from each litter, sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells.
Result: CFU-E numbers were equivalent to the control values except in the males exposed to 0.033 mg/l in utero which displayed significant variations from the controls.
Source: BP Chemicals Ltd. London

(622)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: 12-week-old offspring
Doses: 10 ppm/day (converts to 0.033 mg/l/day); 5-10 mice/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 10 weeks of age, the offspring [numbers unspecified] were re-exposed for 2 weeks to 0.033 mg/l. After the 2 weeks, the animals were sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells. Control offspring exposed in utero to air were similarly exposed at 10 weeks of age to 0.033 mg/l benzene for 2 weeks.
Result: The in utero benzene-exposed offspring showed a marked reduction in the numbers of bone marrow CFU-E on re-exposure. No such effect was seen in the in utero air-exposed animals. Both groups of offspring had decreased

splenic granulocyte/macrophage colony forming unit-culture numbers on re-exposure but this was more severe in the in utero benzene-exposed offspring.

Source: BP Chemicals Ltd. London

(622)

Species: mouse **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure period: gestation days 5-14
Frequency of treatment: daily/three 4-hour exposures per day
Duration of test: gestation day 17
Doses: 0.5, 1.0 mg/l; 15 mice/dose
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: CFLP (LATI-Gödöllö, Hungary) strain mice were used. Control group consisted of 115 females exposed to air. Dams were killed on day 17 and foetuses examined as described by Hudak A. & Ungvary G. Toxicology 11, 55-63, 1978.
Result: No effects were seen on the numbers of live or dead or resorbed foetuses or on the incidence of minor anomalies or total malformations. The incidences of weight and skeletal retarded foetuses were increased, this being statistically significant.
Source: BP Chemicals Ltd. London

(623)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: gestation day 16
Doses: 5, 10, 20 ppm (converts to 0.017, 0.033, 0.066 mg/l); 5-10 mice/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed pregnant mice were sacrificed, their uteri removed and the number of live, dead and resorbed foetuses recorded. Two male and two female foetuses/litter were weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells.
Reliability: 3 (not valid)
Significant methodological deficiencies predominantly effects on hematopoietic system studied (low number of animals, duration of experiment, only gross external malformations studied in 4 fetuses)
Result: Litter size, foetal weights and the numbers of dead, resorbed and malformed foetuses were within the control

limits. In the foetuses, changes in the numbers of both erythroid precursor cells (i.e. colony forming unit–erythroid, CFU–E; and burst forming unit–erythroid, BFU–E) were observed, the more mature, CFU–E, cells being affected to a greater extent by all exposure concentrations. The number of foetal CFU–E cells were significantly increased at 0.017 and 0.033 mg/l and significantly decreased at 0.066 mg/l.

Source: Deutsche Shell Chemie GmbH Eschborn

(622)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6–15
Frequency of treatment: daily/6 hours per day
Duration of test: 2–day–old neonates
Doses: 5, 10, 20 ppm (converts to 0.017, 0.033, 0.066 mg/l); 5–10 mice/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. Two male and two female neonates/litter were selected at 2 days of age, weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells. Benzene chamber concentration measured by infrared analyser.
Reliability: 3 (not valid)
Significant methodological deficiencies predominantly effects on hematopoietic system studied (low number of animals, duration of experiment, only gross external malformations studied in 4 mice)
Result: No difference in CFU–E numbers were noted between the low–dose offspring and the controls. Offspring exposed, in utero, to 0.033 mg/l had either increased or decreased CFU–E numbers and those in male offspring exposed in utero to 0.066 mg/l were increased. The numbers of granulocytic/macrophage colony forming cells were increased in the male and female offspring from the top–dosed group and decreased in the male offspring in the mid–dosed group.
Source: Deutsche Shell Chemie GmbH Eschborn

(622)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: 6-week-old offspring
Doses: 5, 10, 20 ppm (converts to 0.017, 0.033, 0.066 mg/l); 5-10 mice/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 6 weeks of age, one male and one female offspring were selected from each litter, sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells.
Reliability: 3 (not valid)
Significant methodological deficiencies
only effects on hematopoietic system studied
Result: CFU-E numbers were equivalent to the control values except in the males exposed to 0.033 mg/l in utero which displayed significant variations from the controls.
Source: Deutsche Shell Chemie GmbH Eschborn

(622)

Species: mouse **Sex:** female
Strain: Swiss Webster
Route of admin.: inhalation
Exposure period: gestation days 6-15
Frequency of treatment: daily/6 hours per day
Duration of test: 12-week-old offspring
Doses: 10 ppm (converts to 0.033 mg/l); 5-10 mice/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 10 weeks of age, the offspring [numbers unspecified] were re-exposed for 2 weeks to 0.033 mg/l. After the 2 weeks, the animals were sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells. Control offspring exposed in utero to air were similarly exposed at 10 weeks of age to 0.033 mg/l benzene for 2 weeks.
Reliability: 3 (not valid)
Significant methodological deficiencies
only effects on hematopoietic system studied
Result: The in utero benzene-exposed offspring showed a marked reduction in the numbers of bone marrow CFU-E on re-exposure. No such effect was seen in the in utero air-exposed animals. Both groups of offspring had decreased splenic granulocyte/macrophage colony forming unit-culture

numbers on re-exposure but this was more severe in the in utero benzene-exposed offspring.

Source: Deutsche Shell Chemie GmbH Eschborn

(622)

Species: mouse **Sex:** female
Strain: other
Route of admin.: inhalation
Exposure period: gestation days 5-14
Frequency of treatment: daily/three 4-hour exposures per day
Duration of test: gestation day 17
Doses: 0.5, 1.0 mg/l; 15 mice/dose
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: CFLP (LATI-Goedoelloe, Hungary) strain mice were used. Control group consisted of 115 females exposed to air. Dams were killed on day 17 and foetuses examined as described by Hudak A. & Ungvary G. Toxicology 11, 55-63, 1978.
Reliability: 3 (not valid)
Significant methodological deficiencies
no data about test substance, partly insufficient documentation of methods, 2 concentrations tested, low number of pregnant mice in the treatment group, short post treatment period
Result: No effects were seen on the numbers of live or dead or resorbed foetuses or on the incidence of minor anomalies or total malformations. The incidences of weight and skeletal retarded foetuses were increased, this being statistically significant.
Source: Deutsche Shell Chemie GmbH Eschborn

(623)

Species: rabbit **Sex:** female
Strain: New Zealand white
Route of admin.: inhalation
Exposure period: gestation days 6-19
Frequency of treatment: daily/24 hours per day
Duration of test: gestation day 29
Doses: 0.5, 1.0 mg/l; 11 and 15 rabbits respectively
Control Group: yes, concurrent no treatment
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 60 dams exposed to air. Dams were killed on day 29 and foetuses examined as described by Ungvary G. Munkavédelem, Munka-és Uzemegészségügy 29, 207-216, 1983.
Result: The top dose caused statistically significant decreases in maternal weight gain and mean foetal weight and statistically significant increases in the number of dead or resorbed foetuses, mainly arising from abortion, and the percentage of minor anomalies. At 0.5 mg/l, slight non-significant decreases in maternal weight gain and

increases in the numbers of dead or resorbed foetuses and minor anomalies was reported. The investigators did not consider benzene to be teratogenic.

Source: BP Chemicals Ltd. London

(623)

Species: rabbit **Sex:** female
Strain: New Zealand white
Route of admin.: inhalation
Exposure period: gestation days 6–19
Frequency of treatment: daily/24 hours per day
Duration of test: gestation day 29
Doses: 0.5, 1.0 mg/l; 11 and 15 rabbits respectively
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Control group consisted of 60 dams exposed to air. Dams were killed on day 29 and foetuses examined as described by Ungvary G. Munkavedelem 29, 207–216, 1983.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions no data about test substance, partly insufficient documentation of methods, 2 concentrations tested
Result: The top dose caused statistically significant decreases in maternal weight gain and mean foetal weight and statistically significant increases in the number of dead or resorbed foetuses, mainly arising from abortion, and the percentage of minor anomalies. At 0.5 mg/l, slight non–significant decreases in maternal weight gain and increases in the numbers of dead or resorbed foetuses and minor anomalies was reported. The investigators did not consider benzene to be teratogenic.
Source: Deutsche Shell Chemie GmbH Eschborn

(623)

Species: mouse **Sex:** female
Strain: CD–1
Route of admin.: gavage
Exposure period: gestation days 12–15
Frequency of treatment: daily
Duration of test: no data
Doses: 1.0 ml/kg bw/day (converts to 880 mg/kg bw/day); group size unspecified
Control Group: no data specified
NOAEL Maternalt.: = 880 mg/kg bw
NOAEL Teratogen.: = 880 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene administered in cotton seed oil.
Result: No statistically significant benzene–related change was seen in the incidence of malformations.
Source: BP Chemicals Ltd. London

(624)

Species: mouse **Sex:** female
Strain: CD–1
Route of admin.: gavage
Exposure period: gestation days 6–15
Frequency of treatment: daily
Duration of test: no data
Doses: 0.3, 0.5, 1.0 ml/kg bw/day (converts to 264, 440, 880 mg/kg bw/day); group sizes unspecified
Control Group: yes, concurrent vehicle
NOAEL Maternalt.: = 880 mg/kg bw
NOAEL Teratogen.: = 880 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene administered in cotton seed oil.
Result: No statistically significant benzene–related change was seen in the incidence of malformations.
Source: BP Chemicals Ltd. London

(624)

Species: mouse **Sex:** female
Strain: NMRI
Route of admin.: gavage
Exposure period: 6.- 15. Tag der Traechtigkeit
Frequency of treatment: einmal taeglich
Duration of test: bis zum 18. Tag der Traechtigkeit
Doses: 563; 1688 mg/kg
Control Group: yes, concurrent vehicle
NOAEL Teratogen.: 563 mg/kg bw
Method: other: nach den Richtlinien der FDA (1966)
Year: **GLP:** no
Test substance: as prescribed by 1.1 - 1.4
Result: Die Versuchs- und Kontrollgruppen bestanden aus je 19 bis 23 Muttertieren.
Bereits in der niederen Dosisgruppe wurde maternale Toxizitaet in Form von verringerter Gewichtszunahme und, ab dem 11. Versuchstag, Unruhe, Zittern oder Apathie festgestellt. In der hohen Dosisgruppe traten diese Symptomebereits ab dem 8. Versuchstag auf. Ein Tier hatte einen Abort von 8 Feten.
In der niederen Dosisgruppe wurden weder embryo- noch fetotoxischen Wirkungen festgestellt, auch traten keine vermehrten Missbildungen auf.
Die hohe Dosis wirkte embryotoxisch, 23 % der Keime waren abgestorben (Kontrollgruppen 14 %). Das Gewicht der Feten war verringert. Die Feten wiesen vermehrt (bei 12/175 Feten) Missbildungen auf.(In einem Wurf wurde 6 mal verkuerzter Schwanz, sowie Spina bifida bei 3 Feten festgestellt, 1 Knickschwanz in einem anderen Wurf.)
Source: BASF AG Ludwigshafen
Reliability: (3) invalid
entspricht nicht den heutigen Kriterien; Dosierungen hoeher als der Limit-Test; Maeuse weisen bei hoher Maternaltoxizitaet vermehrt Missbildungen auf

(494)

Species: mouse **Sex:** female
Strain: CD–1
Route of admin.: gavage
Exposure period: gestation days 12–15
Frequency of treatment: daily
Duration of test: no data
Doses: 1.0 ml/kg bw/day (converts to 880 mg/kg bw/day); group size unspecified
Control Group: no data specified
NOAEL Teratogen.: = 880 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene administered in cotton seed oil.
Reliability: 4 (validity cannot by judged)
Abstract
Result: A significant increase in maternal lethality and embryonic resorption was seen. Foetal weight was significantly reduced.
No statistically significant benzene–related change was reported in the incidence of malformations.
Source: Deutsche Shell Chemie GmbH Eschborn
(624)

Species: mouse **Sex:** female
Strain: CD–1
Route of admin.: gavage
Exposure period: gestation days 6–15
Frequency of treatment: daily
Duration of test: no data
Doses: 0.3, 0.5, 1.0 ml/kg bw/day (converts to 264, 440, 880 mg/kg bw/day); group sizes unspecified
Control Group: yes, concurrent vehicle
NOAEL Teratogen.: = 880 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: no data
Remark: Benzene administered in cotton seed oil.
Reliability: 4 (validity cannot by judged)
Abstract
Result: At the mid– and high–dose levels, a significant increase in maternal lethality and in embryonic resorption was seen.
Foetal weight was significantly reduced at all three doses.
No statistically significant benzene–related change was seen in the incidence of malformations.
Source: Deutsche Shell Chemie GmbH Eschborn
(624)

5.10 Other Relevant Information

Type: adsorption
Remark: method: percutaneous absorption of 14C-labelled benzene in male hairless mice was measured, following 4 h administration of a) undiluted benzene or b) 0.5% benzene in rubber solvent using special skin-depot attachement.
results: a) 0.89%, b) 0.88% absorbed. a) 33% of absorbed dose excreted (urine, faeces), 40% expired, 23% in carcass and 5% at application site; similar reults in b). Estimation by the authors: dermal absorption could contribute 20-40% of the total benzene dose of workers in rubber industry (1 ppm benzene in air).
Reliability: 1
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn

(625)

Type: Behaviour
Remark: method: groups of 8 male Sprague-Dawley rats received 3 i.p. injections of 550 mg/kg/d benzene in corn oil or the vehicle on day 9, 11, 13 postpartum.
results: no effects in body weight gain, food and water consumption; significantly impaired learning ability when tested on problems of the Hebb-Williams closed-field maze-learning task on day >= 54.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: purity >99%

(626)

Type: Biochemical or cellular interactions
Remark: Inhaled 3H- or C14-labelled benzene had been found to bind to adult male SIV50 rat liver DNA to the extent of 2.40 or 2.38 umoles benzene per mole DNA phosphate.
Seven deoxyguanosine adducts and one deoxyadenine adduct have been detected in rabbit bone marrow mitoblast DNA (incubation 1st. with 3H-labelled dGTP or dATP, than with C14-benzene).
Source: Deutsche Shell Chemie GmbH Eschborn

(627) (628)

Type: Biochemical or cellular interactions
Remark: The hemoglobin adduct, S-phenylcysteine, was detected in the blood of mice and rats exposed to benzene either by inhalation or by gavage.
Both covalently bound and soluble metabolites accumulated in bone marrow, liver, and kidney over a 24-h-period after a single s.c. administration of 3H-labelled benzene (440 or 880 mg/kg) to male mice; the highest level of covalent binding were seen in kidney and liver after 3 d of dosing at 880 mg/kg, 2 doses per day.
Source: Deutsche Shell Chemie GmbH Eschborn

(629) (630)

Type: Distribution
Remark: Rats were exposed to 500ppm benzene for 30 min to 8h. Benzene concentrations reached steady state within 4h in blood, 6h in fat and 2h in bonr narrow. Lesser concn. were detected in the kidney, lung, liver, brain and spleen.
Source: REPSOL PETROLEO,S.A. MADRID

(631)

Type: Distribution
Remark: method: 4 pregnant C57BL mice per group were exposed to 2000 ppm (6.4 mg/l) benzene for 10 min.; mice were sacrified 0, 0.5, 1, 4, and 24 h after inhalation for whole-body autoradiography and liquid scintillation methods.
results: immediately after inhalation high concentration in lipid-rich tissue (brain, fat) and well perfused organs (lung, liver , kidney) but rapidly eliminated resulting in low concentrations at 1 h in all maternal tissues, except fat; volatile radioactivity was observed in placenta and fetuses at 0, 0.5, and 1 h after inhalation; fetal levels much lower than in maternal tissue.
Reliability: 1
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn

(632)

Type: Immunotoxicity
Remark: methods: effects of benzene on murine resistance to an infectious agent (bacteria), Listeria monocytogenes, studied by exposure of 5-7 male C57Bl/6J mice/group to 0, 10, 31, 100, or 298 ppm (analytical concentration) for 6 h/d for a) 5 d prior to infection or b) 5 d prior to infection plus 7 d during infection.
results: significantly increased number of bacteria on day 4 of infection at 300 ppm in a) and in b) at all doses except the lowest; B and T lymphocytes significantly reduced at >= 31 ppm in a) and b);
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: no data

(633)

Type: Immunotoxicity
Remark: method: 5 male CD-1 mice/group were exposed to 0, 31, 166 or 790 mg/l in the drinking water for 28 d (see also chapter 5.4); mice sensitized by i.p. injection with sheep red blood cell suspension on day 24 were sacrified on day 28 and spleen lymphocytes cultered for further examinations.
results: see also chapter 5.4; lymphocyte proliferation to both B and T cell mitogens was enhanced at the low dose and depressed in the mid- and high-dosed group; similar biphasic effects with cell-mediated immunity to allogeneic cells and with cytotoxic T lymphocyte activity to YAC-1 tumor cells; antibody production as assessed by enumeration of the sheep cell-specific plaque forming cells indicated a significant suppression at 166 and 790 ppm.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: purity 99.9%

(518)

Type: Immunotoxicity
Remark: methods: at least 5 male C57Bl mice per group were exposed to 0, 10, 31, 100 or 300 ppm 6 h/d for 6 d.
results: even exposure to 10 ppm resulted in significant depression in femoral lipopolysaccharide-induced B-colony-forming ability, while total number of B-lymphocytes were not significantly depressed; splenic phytohemagglutinin- induced blastogenesis was significantly depressed at 31 ppm without a depression in numbers of T-lymphocytes.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: no data

(634)

Type: Immunotoxicity
Remark: method: groups of 10 male C57Bl/6J mice were exposed to 300 ppm for 6, 30, 115 exposures (6 h/day, 5 d/week).
results: number of B-lymphocytes in bone marrow and spleen and number of T-lymphocytes in thymus and spleen significantly reduced after all 3 periods; progressive depression of mitogen-induced proliferation of bone marrow and splenic B-lymphocytes, also splenic T-cell mitogen-induced proliferation depressed but not progressively; marked increase in thymic and bone marrow cellularity.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: no data

(635)

Type: Immunotoxicity
Remark: methods: 15 female Swiss mice per group were exposed to 50, 200, or 400 ppm (6 h/d, 5 d per week) for 5, 12, or 22 exposures; after these exposure periods mice were immunized with 2 different tetanus toxoids.
results: no effects at 50 ppm; inhibited primary antibody formation after 10 or 20 exposures to 200 ppm; 400 ppm increasingly suppressed primary antitoxin response to both antigens;
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: no data

(636)

Type: Metabolism
Remark: Metabolic products in rat are phenol, hydroquinone, cathecol, hydroxyhydroquinone and phenylmercapturic acid.
Source: REPSOL PETROLEO,S.A. MADRID

(637)

Type: Metabolism
Remark: Benzene is well absorbed in humans and experimental animals after oral and inhalation exposures, but in humans dermal absorption is poor. Approximately 50% absorption occurs in humans during continuous exposures to 163-326 mg/m3 for several hours. After a 4-hr exposure to 170-202 mg/m3, retention in the human body was approximateky 30%, with 16% of the retained dose having been excreted as unchanged benzene in expired air. Women may retain a greater

percentage of inhaled benzene than men. Benzene tends to accumulate in tissues containing high amounts of lipids, and it crosses the placenta.

Benzene metabolism occurs mainly in the liver, is mediated primarily through the cytochrome P-450 IIE1 enzyme system and involves the formation of a series of unstable reactive metabolites. In rodents, the formation of two putative toxic metabolites, benzoquinone and muconaldehyde, appears to be saturable. This may have important implications for dose-response relationships, because a higher proportion of the benzene will be converted to toxic metabolites at low doses than at high doses. The metabolic products are excreted primarily in the urine. Appreciable levels of the known metabolites phenol, catechol and hydroquinone are found in bone marrow. Phenol is the predominant urinary metabolite in humans and is mainly found as an ethereal sulphate conjugate until the levels approach 480 mg/l, at which time glucuronides are detected. Recent studies suggest that benzene toxicity is the result of the interactive effects of several benzene metabolites formed in both the liver and the bone marrow.

Inhaled benzene had been found to bind to rat liver DNA to the extent of 2.38 µmoles/mole DNA phosphate. Seven deoxyguanosine adducts and one deoxyadenine adduct have been detected in rabbit bone marrow mitochondrial DNA.

Source: BP Chemicals Ltd. London

(638)

Type: Metabolism

Remark: Reliability: 4 (validity cannot be judged)
data from secondary literature, but validated (WHO, 1993) by an expert group (WHO task group)
The major metabolism of benzene in vivo takes place in liver and, to lesser extent, in bone marrow; first step is oxidative, yielding hydroxylated compounds (phenol, catechol, hydroquinone, 1,2,4-trihydroxy-benzene) which are excreted in the urine as ethereal sulfates and glucuronides; Conjugation with glutathione and urinary excretion of the mercapturic acid is an additional pathway that detoxifies benzene oxide (initial intermediate formed during metabolism to phenol); urinary metabolite muconic acid is the only known ring-opened metabolite, a detoxification product of trans,trans-muconaldehyde; further metabolism of the metabolite phenol lead to formation of hydroquinone, catechol and 1,2,4-trihydroxy benzene; further metabolism of catechol leads to o-benzoquinone and hydroquinone leads to p-benzoquinone.
In summary, benzene metabolism occurs mainly in the liver, is mediated primarily through the cytochrome P-450 IIE1 enzyme system and involves the formation of a series of unstable reactive metabolites. In rodents, the formation of two putative toxic metabolites, benzoquinone and muconaldehyde, appears to be saturable. This may have important implications for dose-response relationships, because a higher proportion of the benzene will be converted

to toxic metabolites at low doses than at high doses. The metabolic products are excreted primarily in the urine. Appreciable levels of the known metabolites phenol, catechol and hydroquinone are found in bone marrow. Phenol is the predominant urinary metabolite in humans and is mainly found as an ethereal sulphate conjugate until the levels approach 480 mg/l, at which time glucuronides are detected. Recent studies suggest that benzene toxicity is the result of the interactive effects of several benzene metabolites formed in both the liver and the bone marrow.

Source: Deutsche Shell Chemie GmbH Eschborn

(639) (640) (638)

Type: Metabolism

Remark: method: a) male F344/N rats or male B6C3F1 mice inhaled 50 ppm 3H-labelled benzene for 6 h and 0.5, 2, 4, 6, 7.5, 9, 11, 14 h after start of exposure 4 animals of each species were sacrified and metabolites determined; b) both species received i.p. injections of 0.5, 5, 15, 50, 150 mg/kg benzene or inhaled 11, 130, 930 ppm for 6 h and metabolites were determined during a 48-h-period after dosing.
results: a) in rats the metabolites (measured as areas under the curve in liver, lung and blood over the 14 h period) were mainly phenyl sulfate, muconic acid, phenyl mercapturic acid and in small quantities phenol, catechol, hydrochinon; in mice the main metabolites were muconic acid, phenyl sulfate, phenyl mercapturic acid, hydroquinone glucuronide, phenyl glucuronide, hydroquinone and small amounts catechol and phenol; mice converted more inhaled benzene to putative toxic metabolites, benzoquinone and muconaldehyde, than rats did. b) the effect of increasing doses was to increase the proportion of the total metabolites that were products of detoxification pathways relative to the products of pathways leading to putative toxic metabolites in both species. This indicates high-affinity, low-capacity pathways leading to putative toxic metabolites.
Reliability: 1 (valid)
Comparable to guideline study

Source: Deutsche Shell Chemie GmbH Eschborn

(641) (642) (643) (644)

Type: Metabolism

Remark: Monoclonal antibody-directed characterization of benzene oxidative metabolism in rat liver microsomes showed that benzene is a substrate for the cytochrome P450IIE1 and at higher benzene concentrations for P450IIB.
Reliability: 1
Comparable to guideline study

Source: Deutsche Shell Chemie GmbH Eschborn

(645)

Type: Metabolism
Remark: Bone marrow, bone marrow-derived macrophages, and stromal fibroblasts were isolated from male B6C3F1 mice in order to more completely characterize the enzymology contributing to the metabolic activation and deactivation in bone marrow-derived macrophages and fibroblastoid stromal cells. The investigators examined the levels of gluthathione (GSH) and the activities of phase II metabolic pathways that are critical in the deactivation of benzene-derived phenolic metabolites and quinones formed by further oxidation of these phenolics. Specifically, the activities of UDP-glucuronosyltransferase (UDP-GT); (EC 2.4.1.17), PAPS-dependent arylsulfotransferase (EC.2.8.2.1), and gluthathione-S-transferase (EC 2.5.1.18) were examined in unpurified white bone marrow, bone marrow-derived macrophages, and stromal fibroblasts. Fibroblastoid stromalcells had elevated glutathione-S-transferase and DT-diaphorase activity relative to macrophages, whereas macrophages demonstrated increased UDP-GT and peroxidase activity relative to stromal fibroblasts. UDP-GT and glutathione-S-transferase activities in macrophages and fibroblasts, respectively, were significantly greater than those in unpurified white marrow. Because UDP-GT activity is high in macrophages, these data suggest DT-diaphorase levels would be rate limiting in the detoxification of benzene-derived quinones in bone marrow macrophages. The peroxidase responsible for bioactivation of benzene-derived phenolic metabolites in bone marrow macrophages is unknown but has been suggested to be prostaglandin H synthase (PGS).Hydrogen peroxide, but not arachidonic acid, supported metabolism of hydroquinone to reactive species in bone marrow-derived macrophage lysates. These data do not support a major role for PGS in peroxidase-mediated bioactivation of hydroquinone in bone marrow-derived macrophages. Peroxidase-mediated interactions between phenolic metabolites of benzene occurred in bone marrow-derived macrophages. Bioactivation of hydroquinone to species that would bind to acid-insoluble cellular macromolecules was increased by phenol and was markedly stimulated by catechol. Bioactivation of catechol was also stimulated by phenol, but was inhibited by hydroquinone. These data define the enzymology and the cell-specific metabolism of benzene metabolites in bone marrow stroma and demonstrate that interactions between phenolic metabolites may contribute to the toxicity of benzene in this critical bone marrow compartment.
Source: Deutsche Shell Chemie GmbH Eschborn

(646)

Type: Metabolism
Remark: This study investigates the prostaglandin H synthase (PHS) catalyzed oxidation of the benzene metabolite hydroquinone and examines the sulfhydryl-binding and DNA-damaging effectsof the reactive metabolite generated during this reaction. The study also compares the activation of hydroquinone by PHS to those of other purified peroxidase systems. Hydroquinone was oxidized by PHS to

1,4-benzoquinone, which was measured by HPLC with reductive electrochemistry. Hydroquinone metabolism in the presence of cysteine generated a thiol adduct, which was identified as the monosubstituted cysteine conjugate of hydroquinone by HPLC with oxidative electrochemical and radiochemical detection. The PHS-catalyzed activation of hydroquinone to 1,4-benzoquinone or its thiol conjugate required the presence of either arachidonic acid or hydrogen peroxide. The oxidative metabolism of hydroquinone also resulted in the formation of a reactive product(s) that irreversibly bound the DNA. This binding was time dependent and did not occur in the presence of heat-inactivated PHS. Metabolite(s) generated during hydroquinone oxidation also induced single-strand breaks in Bluescript plasmid DNA. ThePHS/arachidonic acid catalyzed metabolism of hydroquinone to1,4-benzoquinone and to product(s) that bound to sulfhydrylsand DNA and caused strand breaks in DNA was prevented by indomethacin, an inhibitor of PHS cyclooxygenase. Because prostaglandin synthesis is elevated in bone marrow followingbenzene exposure and inhibitors of PHS cyclooxygenase prevent benzene-induced myelotoxicity, the activation of hydroquinone by PHS represents a possible mechanism for benzene's effects.

Source: Deutsche Shell Chemie GmbH Eschborn

(647)

Type: Metabolism

Remark: In this study, benzene metabolism was investigated using twopurified rat hepatic mixed function oxidase (MFO) systems containing either cytochrome P450 2B1 or cytochrome P450 2E1. Studies performed over a wide substrate concentration range indicate that cytochrome P450 2B1 represents a relatively low-affinity form of cytochrome P450 with respectto benzene metabolism while cytochrome P450 2E1 is substantially more efficient at low benzene concentrations. Cytochrome b5 stimulated benzene metabolism by both cytochromes P450 2B1 and P450 2E1. With cytochrome P450 2E1the stimulation of benzene metabolism by cytochrome b5 was very pronounced at low concentrations of benzene and was most effective with respect to formation of hydroquinone. The metabolites observed in these studies were phenol and hydroquinone. Cytochrome P450 2E1 metabolized phenol with an affinity and capcity comparable to those of benzene. Hydroquinone was the major product formed at all substrate concentrations, while some catechol was formed at higher concentrations of phenol. Phenol metabolism was also stimulated by cytochrome b5. The metabolism of benzene by cytochrome P450 2E1 in the presence of the major microsomal epoxide hydrolase, mEHb yielded phenol, hydroquinone, and benzene dihydrodiol. The addition of glutathione transferases plus glutathione (GSH) did not yield GSH conjugates during benzene metabolism. However, metabolism of phenol by cytochrome P450 2E1 in the presence of glutathione yielded a nonenzymatically formed glutathione conjugate derived from hydroquinone or from an oxidative product of hydroquinone. Studies of the metabolic pathway of benzene and its control are important as they may

demonstrate that the development of benzene toxicity can be altered by changing the rate of production of mixtures of toxic metabolites.

Source: Deutsche Shell Chemie GmbH Eschborn

(648)

Type: Metabolism

Remark: Benzene metabolism is a requirement for toxicity and the phenolic metabolites of benzene have been repeatedly implicated in hematotoxicity. Peroxidase–mediated metabolism of benzene–derived phenolic compounds generates reactive and cytotoxic quinones which have been proposed to be responible for benzene–induced toxicity. Human as well as animal (i.e. rat and mouse) bone marrow contains high peroxidase activity and can readily bioactivate phenolic metabolites of benzene in a peroxidase–mediated process. Thus, bone marrow cells containing significant peroxidase activity can be considered as potential targets of benzene toxicity. In this study, the authors examined the peroxidase activity of murine hematopoietic progenitor cells (HPC) by two different approaches. Conventional biochemical techniques were used to quantitatively determineperoxidase activity in enriched progenitor cell populations, purified as lineage–negative cells using a cocktail of antibodies to remove cells with lineage–specificmarkers. Peroxidase activity in enriched progenitor cells and in whole bone marrow was also measured directly using flow cytometry. In addition, the peroxidase activity of HPCdefined as CD34–enriched populations was also measured. Thedata demonstrate that both murine and human HPC contain peroxidase activity and thus may be potential targets of xenobiotics that are bioactivated by peroxidases.

Source: Deutsche Shell Chemie GmbH Eschborn

(649)

Type: Neurotoxicity

Remark: methods: narcotic properties of benzene studied on 10 rabbits exposed to 3.5–4.5% benzene vapor in air.
results:

symptoms	average time of occurrence in minutes
light anesthesia, relaxed	3.7
excitation (running movement, tremor, chewing)	5.0
pupillary reflex lost	6.5
loss of blink reflex to tactual stimulus	11.4
pupillary contraction	12.0
involuntary blinking	15.6
death	36.2

Source: Deutsche Shell Chemie GmbH Eschborn

Test substance: no data

(650)

Type: Neurotoxicity
Remark: method: 5 male CD-1 mice per group were fed drinking water containing 0, 31, 166, or 790 mg/l benzene (analytical) for 28 d; at termination monoamine neurotransmitter concentrations in different brain regions were determined.
results: no significant effects on body weight gain, water and food consumption; benzene doses correspond to 0, 8, 40, 180 mg/kg/d, respectively; dose dependent significant increase in norepinephrine (hypothalamus, medulla oblongata, cerebellum), dopamine (corpus striatum, hypothalamus) and serotonin (hypothalamus, midbrain, corpus striatum, cerebral cortex, medulla oblongata) levels; also increased levels of catecholamine metabolites indicating induction of synthesis and catabolism of monoamine transmitters by benzene ingestion.
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: purity 99.9%

(651)

Type: Toxicokinetics
Remark: In summary, benzene is well absorbed in humans and experimental animals after oral and inhalation exposures, but in humans dermal absorption is poor. Approximately 50% absorption occurs in humans during continuous exposures to 163-326 mg/m3 for several hours. After a 4-hr exposure to 170-202 mg/m3, retention in the human body was approximately 30%, with 16% of the retained dose having been excreted as unchanged benzene in expired air. Women may retain a greater percentage of inhaled benzene than men. Benzene tends to accumulate in tissues containing high amounts of lipids, and it crosses the placenta. Benzene is eliminated by exhalation of unmetabolized benzene from the lungs and by metabolism in the liver (lesser extend by bone marrow) and following excretion via urine.
Reliability: 4 (validity cannot be judged)
data from secondary literature, but validated (WHO, 1993) by an expert group (WHO task group)
Source: Deutsche Shell Chemie GmbH Eschborn

(639) (638)

Type: Toxicokinetics
Remark: method: 6 male and 6 female human volunteers inhaled 52-62 ppm benzene for 4 h; benzene concentration was measured in 1-h-intervals by GC and spectrophotometry.
results: retention decreased with duration of exposure and reached a constant level (30%, estimated) after 2 h (no differences between men and women); absorption was greatest during 1st 5 minutes of exposure; after 4 h 46.9% of the dose was taken up by the subjects, 16.8% was excreted as unchanged benzene in the expired air; metabolites of benzene were excreted in the urine mainly in form of sulfate and glucuronide conjugates of phenol.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about test substance
Source: Deutsche Shell Chemie GmbH Eschborn

(652) (653)

Type: Toxicokinetics
Remark: method: rabbits received 340, 400 or 500 mg/kg 14C–radiolabelled benzene by oral gavage and excretion in the urine and expired air was determined for up to 7 d after dosing.
results: 90% of total radioactivity of applied dose was absorbed and eliminated in air and urine; oral absorption occurs readily and rapidly; 46.5% of the label was recovered as unchanged benzene in expired air (70 h, 400 mg/kg); 34.8% of the applied dose (48 h, 340 mg/kg) were excreted via urine, mainly in form of conjugated phenol (23.5%); other metabolites excreted were 4.8% hydroquinone, 2.2% catechol, 0.3% 1,2,4–trihydroxybenzene, 1.3% muconic acid and 0.5% L–phenylmercapturic acid; 5 or 10% (500 or 340 mg/kg, respectively) of the labelled benzene remained in the tissues or were excreted in the faeces (0.88% or 0.3%, respectively) 48 h after dosing.
Reliability: 2 (valid with restriction)
Comparable to guideline study with acceptable restrictions
no data about number of animals
Source: Deutsche Shell Chemie GmbH Eschborn

(654)

Type: Toxicokinetics
Remark: method: male F344/N rats or male B6C3F1 mice (n=3–4/dose) were a) gavaged with 0.5, 5, 15, 50, 150 or 300 mg/kg benzene (high dose only rats) and excretion determined next 48 h or b) were exposed to 33, 75, 340, 680, 2260 mg/m3 (rats) or 29, 75, 350, 2570 mg/m3 (mice) for 6 h and excretion measured for up to 56 h after termination of exposure.
results: a) absorption > 97% in mice and rats for doses 0.5–150 mg/kg; at < 15 mg/kg > 90% excreted in urine, at > 15 mg/kg increasing percentage exhaled unchanged (saturation of metabolic pathways); at > 50 mg/kg total metabolites not linearly related to administered dose; metabolites per unit body weight equal in mice and rats at doses up to 50 mg/kg, total metabolites not increased at higher dose in mice.
b) absorption 33% and 15% in rats (low dose and high dose, respectively) and 50% and 10% in mice; total metabolite formation exponentially related to benzene concentration with one–half max. amount of metabolic formation occurring at 220 mg/m3 for mice and 650 mg/m3 for rats; mainly due to higher amount inhaled the total metabolites were higher in mice than in rats at any concentration.
Reliability: 1
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 14C–labelled benzene, purity > 99%

(643)

Type: Toxicokinetics
Remark: method: 5 male Sprague-Dawley rats received dermal applications of 14C-labelled benzene under a glass cap (evaporation prevented) for 72 h; benzene blood levels measured at 2, 4, 8, 12, 24, 30, 48, and 72 h; excretion via urine, faeces and expired air determined.
results: max. plasma concentration at 8-24 h (ca. 2000 DPM/ml); absorption half-live 3.1 h and elimination half-live 23 h in rat plasma; excretion of radioactivity 86% in the urine, 0.2% in the faeces and 13% via expired air at 0-48 h; in 0-12 h urine 37.7% of total radioactivity excreted as phenol, 13.1% as catechol, 19.0% as hydroquinone and 5.4% as 1,2,4-trihydroxybenzene; after 48 h highest radioactivity was found in the kidney (0.026%), followed by 0.013% in the liver and 0.011% in the treated skin.
Reliability: 1
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn

(655)

Type: Toxicokinetics
Remark: method: 3 female Sprague-Dawley rats per group received a single application of 0.15, 1.5, 15, 150, or 500 mg/kg 14C-benzene by gavage and were sacrified 1, 3, 6, 9, 12, and 24 h after administration for radioactivity analysis and determination of metabolites.
results: highest levels of radioactivity in all tissues at 1 h (peak probably earlier); at the 2 lowest doses highest levels in liver and kidney (brain and fat not determined), intermediate level in blood and low levels in zymbal gland, nasal and oral cavity, and mammary gland; at 15 mg/kg disproportionate increases in mammary gland and bone marrow; biphasic elimination of the radioactivity, half-live in the rapid phase (determined at 0.15 mg/kg) between 2.2 h (blood) and 4.2 h (liver) and in the slow phase between 11 h (bone marrow) and 29 h (blood); slow phase half-live reduced with higher doses (analysed in blood and zymbal gland); no accumulation of radioactivity in zymbal gland with increasing doses; metabolites determined 1 h after dosing at 15 mg/kg (relative percentage): main metabolites in blood 83% phenyl sulfate and 64% hydroquinone, in liver 89% hydroquinone and 26% phenylsulfate, in kidney 65% hydroquinone, 26% phenol, 23% phenyl sulfate and 15% muconic acid.
Reliability: 1
Comparable to guideline study
Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: 14C-labelled benzene, purity >99%

(656)

Type: Toxicokinetics
Remark: method: 3 male F344 rats per group were exposed to a) 484 ppm (analytical concentration) for 0.5, 1, 1.5, 2, 4, 6, 8 h and sacrificed for steady state determination or they were b) exposed to 490 ppm (analytical conc.) for 6 h and sacrified after 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9 h post exposure period for elimination study.

results: benzene concentration reached steady state within 4 h in blood, 6 h in fat, and < 2 h in bone marrow; highest steady state concentration in fat (164 ug/g) followed by bone marrow (37 ug/g) and kidney (25 ug/g); similar elimination half-lifes in all tissues (0.4-0.8 h) exept fat (1.6 h); benzene eliminated in the expired air in a biphasic manner, half-lifes 0.7 h and 13.1 h; phenol disappeared rapidly from blood and bone marrow, whereas catechol and hydroquinone remained fairly constant over the time period studied.
Reliability: 1
Comparable to guideline study

Source: Deutsche Shell Chemie GmbH Eschborn
Test substance: analytical grade

(657)

Type: other
Remark: This paper examined the effect of benzene and its metabolites, (phenol, hydroquinone and catechol) alone and in combination, on bone marrow function in female mice using59Fe uptake as a measure of erythropoiesis. Benzene treatment at doses of 150 and 300 mg/kg resulted in dosage dependent reductions in radioiron uptake to 63% and 24%, respectively. Hydroquinone was marginally effective, but the inhibtion occurred only at the highest dose tested (100 mg/kg). The combination of phenol and hydroquinone was moreeffective in reducing erythrocyte production than either chemical given alone. Catechol given alone was not inhibitory but when phenol was added to catechol, erythropoiesis was suppressed, as observed for the phenol and hydroquinone combination. These data suggest that benzene toxicity may be the result of cooperative inhibitoryeffects produced by its metabolites.
Source: Deutsche Shell Chemie GmbH Eschborn

(658)

Type: other
Remark: The present study evaluated whether other benzene metabolites including muconaldehyde (MUC), hydroquinone (HQ)and p-benzoquinone (BQ) could effect hematopoiesis either singly or in combination. Using radio-iron uptake into erythrocytes as a measure of hematopoiesis, it was demonstrated that BQ and MUC are potent inhibitors of bone marrow function in female mice. These two benzene metabolites reduced iron uptake at dosages of < 5-6 mg kg-1.The combination of MUC and HQ (100 mg kg-1) was additive, reducing iron incorporation to an extent that was the sum ofthe effect of each chemical given alone. The combined effect of MUC and BQ was significantly less than additive, demonstrating antagonism in the response. Multiple regression was used to study the contributions of the components of binary mixtures of the benzene metabolites. The study results support the hypothesis that the toxic effects of benzene are produced by several metabolites acting interactively.
Source: Deutsche Shell Chemie GmbH Eschborn

(659)

Type: other
Remark: This study analyzed the effects of benzene on the morphologyand functional activity of bone marrow phagocytes. Male Balb/c mice were treated with benzene (660 mg/kg) once per day for 3 days. Bone marrow cells were then isolated and fractionated by density gradient centrifugation. Using flowcytometry/cell sorting, three distinct populations of bone marrow cells that differed with respect to size and density could be separated. Monoclonal antibody binding and cell sorting revealed a large, dense population that consisted predominatly of granulocytes, a smaller, less dense population of lymphocytes, and a population of intermediate size and density consisting of mononuclear phagocytes and precursor cells. Differential staining of sorted mononuclear phagocytes revealed that benzene treatment of mice caused a marked increase in the number of mature, morphologically activated macrophages in the bone marrow. Benzene treatment of mice also resulted in enhanced chemotaxis and production of hydrogen peroxide by bone marrow granulocytes and mononuclear phagocytes. In contrast, treatment of mice with the combination of hydroquinone and phenol (50 mg/kg each, 1x/day, 3 days), two metabolites of benzene, resulted in a significant depression of granulocyte chemotaxis and had no effect on hydrogen peroxide production by bone marrow phagocytes compared to cells from control animals. Taken together these results demonstrate that benzene causes increased differentiation and/or activation of phagocytes in the bone marrow.
Source: Deutsche Shell Chemie GmbH Eschborn

(660)

Type: other
Remark: This study evaluated the effects of benzene (BZ) and hydroquinone (HQ), a BZ metabolite, on the human HL-60 promyelocytic leukemic cell line. Because the HL-60 cell isbipotential and can be induced to differentiate to monocytesor granulocytes, it has been used in many studies as a surrogate for the granulocyte/macrophage committed cell, GM-CFU. Treatment of HL-60 cells with BZ specifically induced differentiation along the granulocytic lineage as measured by morphology, induction of superoxide production and chloroacetate esterase activity and the appearance of the L12-2 surface antigen. Differentiation was induced via the activation of protein kinase C and phosphorylation of several proteins known to be involved in HL-60 cell differentiation. Subsequent to kinase C activation, arachidonic acid was released and the 5-lipoxygenase pathwaywas activated for the production of leukotriene D4 (LTD4) required for granulocytic differentiation. BZ induction of granulopoiesis was prevented by preincubation of HL-60 cellswith inhibitors of protein kinase C. Treatment of HL-60 cells with tetraphorbol myristate acetate (TPA), 1 alpha, 25-dihydroxyvitamin D3 (1,25-(OH2)D3) or interleukin-1 (IL-1) induced HL-60 cells to differentiate to monocytes/macrophages. Hydroquinone prevented induction to monocytes/macrophages induced by TPA or 1 alpha, 25-dihydroxyvitamin D3, but not by IL-1 and significantly,

had no effect on the induction of granulocytic differentiation by BZ or any other inducer. Taken together,these results indicate that BZ induces differentiation of HL-60 cells specifically to granulocytes by the activation of the lipoxygenase pathway and the production of LTD4. HQ prevents differentiation to monocytes/macrophages. Consequently, in the bone marrow, BZ could increase the number of granulocytic precursors and even granulocytes while at the same time HQ prevents the development of monocytes and macrophages. Although the findings have been obtained with a single cell line, they may provide some insight into the processes that occur during the differentiation of the GM-CFU, the normal bipotential myeloid committed progenitor.

Source: Deutsche Shell Chemie GmbH Eschborn

(661)

Type: other

Remark: The stromal macrophage, a target of benzene (BZ) toxicity, is involved in hematopoietic regulation through synthesis ofseveral cytokines, including interleukin-1 (IL-1), which synergizes with IL-3 to promote development of the pluripotent stem cell to myeloid and lymphoid stem cells. Monocyte dysfunction and decreased IL-1 production have beenshown to be involved in aplastic anemia in humans. This study demonstrated that hydroquinone (HQ), a toxic bone marrow (BM) metabolite of BZ produced a time-and concentration-dependent inhibition of the processing of the 34-Kd pre-interleukin-1 alpha (IL-1 alpha) to the 17-Kd mature cytokine in murine P388D1 macrophages and BM stromal macrophages, as measured by Western immunoblots of cell lysate proteins using a polyclonal rabbit anti-murine IL-1 alpha antibody. HQ over a 10-fold concentration range had no effect on the lipopolysaccharide (LPS)-induced productionof pre-IL-1 alpha precursor or on cell viability or DNA and protein synthesis. Stromal macrophages obtained from the femoral BM of C57B1/6 mice exposed to BZ (600 or 800 mg/kg) body weight for 2 days were incapable of processing the 34-KD pre-IL-1 alpha to the mature 17-Kd cytokine when stimulated in culture with LPS. Stromal macrophages from mice coadministered BZ and indomethacin, a prostaglandin H synthase (PHS) inhibitor that has been shown to prevent BZ-induced myelotoxic and genotoxic effects in mice when coadministered with benzene were able to convert the pre-IL-1 alpha to mature cytokine. Adminstration of recombinant murine IL-1 alpha to mice before a dose of BZ that causes severe depression of BM cellularity completely prevents BM depression, most probably by bypassing the inability of the stromal macrophage in BZ-treated animals toprocess pre-IL-1 alpha to the mature cytokine.

Source: Deutsche Shell Chemie GmbH Eschborn

(662)

Type: other
Remark: Leukemogenesis is recognized to be a multifactorial process involving more than a single direct cytotoxic effect on a cycling progenitor cell. In this paper, the authors hypothesize that a distinguishing characteristic of agents with leukemogenic potential is their ability to produce intrinsic alterations in the regulation of stem cell differentiation in addition to targeting dividing cells. Benzene metabolism is a requirement for toxicity, and the phenolic metabolites of benzene have been repeatedly implicated in hematotoxicity. In this paper the authors studied the effects of in vitro pretreatment with benzene metabolites on colony-forming response of murine bone marrowcells stimulated with recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF). Pretreatment with hydroquinone (HQ) at concentrations ranging from picomolar to micromolar for 30 minutes resulted in a 1.5 to 4.6-fold enhancement in colonies formed in response to rGM-CSF that was due to an increase in granulocyte/macrophage colonies. Optimal enhancement was obtained with 1 micromolar HQ and was largely independent of the concentration of rGM-CSF. Pretreatment with other authentic benzene metabolites, phenol and catechol, and the putative metabolite trans,trans-muconaldehyde did not enhance growth factor response. Coadministration of phenol and HQ did not enhancethe maximal rGM-CSF response obtained with HQ alone but shifted the optimal concentration to 100 picomolar. The mechanisms underlying the HQ-induced enhanced response to GM-SCF need to be further evaluated.
Source: Deutsche Shell Chemie GmbH Eschborn

(663)

Type: other
Remark: The importance of cell proliferation in chemical carcinogenesis has been appreciated for over a decade. In tissues such as bone marrow with normally high rates of celldivision, proliferation is tightly coordinated with cell differentiation. It has been established that survival, proliferation and differentiation of early hematopoietic progenitor cells (HPC) depends on the growth factors, interleukin 3 (IL-3) and/or granulocyte-macrophage colony stimulating factor (GM-CSF) and their synergism with other cytokines. In this study using both in vivo and in vitro methods, the authors demonstrated increased recruitment of cells responsive to rGM-CSF following exposure to a variety of agents with documented leukemogenic potential. These findings support the possibility that transient alterations in HPC differentiation may be an important factor in the early stages of development of leukemia secondary to chemical or drug exposure.
Source: Deutsche Shell Chemie GmbH Eschborn

(664)

Type: other: absortion
Remark: Benzene is readily absorbed via lung and about 40-50% is retained. It is taken up preferentially by fatty and nervous tissues and about 30-50% is excreted unchanged via lung; a 3-phase excretion pattern is seen at approx. 0.7-1.7h, 3-4h and 20-30h.
Source: REPSOL PETROLEO,S.A. MADRID

(665)

5.11 Experience with Human Exposure

Remark: Epidemiological studies have been conducted on workers in the tire industry (Mc. Mitchael et Al. 1975) and in the shoe factories (Aksoy et Al., 1974) where benzene is used extensively. Among workers who died from exposure to benzene , death was caused by either leukaemia or aplastic anemia, in aprox. equal prportions.
Source: REPSOL PETROLEO,S.A. MADRID

(666)

Remark: A hematological investigation was carried out on 147 workers (employed for +1year) exposed to high benzene levels (320-470ppm). Abnormalities were noted in at least one parameter in 73%, the most common one being thrombocytopenia, wich occurred in 62% followed by anemia 35% and leucopenia 32%.
Source: REPSOL PETROLEO,S.A. MADRID

(667)

Remark: ACUTE TOXICITY
The acute toxicity to humans when benzene is inhaled has been described as being as follows:

20,000 ppm 5-10 minutes exposure can give fatalities
7,5000 ppm 30 minutes exposure may be fatal
1500 ppm 60 minutes exposure causes serious symptoms
500 ppm 60 minutes exposure leads to symptoms of illness
50-150 ppm 5 hours exposure causes headache, lassitude and weakness
25 ppm 8 hours exposure causes no clinical effect.

Signs of toxicity following exposures are CNS depression, cardiac arrhythmia and respiratory failure resulting in death. At lower levels of exposure CNS effects are rapidly reversible.

The acute lethal dose of benzene to humans when ingested has been estimated to be about 10 mls (8.8g). The signs and symptoms of toxicity are typically those of narcotic solvent type materials leading to profound CNS depression, collapse and death (WHO, 1993).
Source: BP Chemicals Ltd. London

(638)

Remark: BONE MARROW DEPRESSION
Bone marrow depression results in a spectrum of haematological changes depending upon the degree of exposure and to individual susceptibility. Thrombocytopaenia, pancytopaenia, and aplastic anaemia may result.

Aksoy (1972) reported on 32 individuals who had been exposed to 150-650 ppm benzene for periods of 4 months-15 years and who had pancytopaenia with hypoplastic, hyperplastic or normoplastic marrow. Eight of the 32 were thrombocytopaenic with associated haemorrhage and infection. Severe or fatal aplastic anaemia seems to be associated with high exposure (>200 ppm) (Aksoy, 1972; Vigliani & Forni, 1976).

Much of the literature on haematological effects of benzene relates to exposures that took place in the 1930s and 1940s and consequently had poor characterization of benzene exposure (WHO, 1993). The haematotoxic effects of benzene appear to occur with exposures to >30 ppm (ECETOC, 1984). More recent studies show no (or marginal effects) after 8 hour TWA exposures of 2-30 ppm, <0.1-25 ppm, <1.0-10 ppm and 0.01-1.4 ppm (Townsend et al. 1978; Tsai et al. 1983; Yardley-Jones et al. 1988; Collins et al. 1991).

As benzene is toxic to bone marrow it would not be unexpected that it could have an effect on immune function. Studies of immune function in workers have however usually been compromised by confounding exposures (WHO, 1993).

Source: BP Chemicals Ltd. London
(668) (669) (670) (671) (672) (673) (638) (674)

Remark: GENOTOXICITY IN HUMANS
The finding of both structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells of those occupationally exposed to benzene has led to general acceptance that benzene can be a human clastogen (WHO, 1993). It is however acknowledged that methodological difficulties (e.g. adequate sample size and control groups, confounding by other factors especially age and smoking) make this a difficult area to study (Ashby & Richardson, 1985).

Chromosome aberrations have been reported in individuals who have previously suffered benzene induced haemopathy resulting from high benzene exposures (125-532 ppm for 1-22 years; Forni et al. 1971). Some studies have indicated genotoxic effects in individuals exposed to lower concentrations of benzene. Picciano (1979) has reported increases in chromosomal aberrations in workers exposed to concentrations <10 ppm and Sarto et al. (1984) reported an increased frequency of chromosome aberrations but not of sister chromatid exchanges (SCEs) in benzene production workers exposed to 0.2-12.4 ppm. Yardley-Jones et al. (1990) have reported a slight increase in lymphocyte chromosomal aberrations in oil refinery workers (48 workers and 29 controls) largely exposed to <1 ppm 8-hour TWA benzene. There was however no increase in SCEs

(Yardley-Jones et al. 1988).

In contrast, other studies of workers exposed to benzene have shown no excess of chromosome aberrations (Watanabe et al. 1980; De Jong et al. 1988) or SCEs (Seiji et al. 1990; Watanabe et al. 1980). The finding of an excess of chromosomal aberrations in milk road tanker drivers (not specifically exposed to benzene) compared to petrol tanker crews (exposed to 6.5 ppm 8-hour TWA benzene) illustrates the difficulties of execution and interpretation of human cytogenic studies in the occupational toxicology field (Berlin, 1985).

Source: BP Chemicals Ltd. London
(675) (676) (677) (678) (679) (680) (681) (638) (674) (682)

Remark: CARCINOGENIC EFFECTS

Introduction

Populations exposed occupationally to benzene have been widely studied. Both case studies and epidemiological studies have been reported leading to the conclusion that benzene is a human leukaemogen (IARC, 1982; IARC, 1987; WHO, 1993). On the basis of such evidence benzene has been classified as a category 1 carcinogen within the EC and as a group 1 carcinogen ("The agent is carcinogenic to humans") by IARC.

The evidence is clear that at high concentrations (>100 ppm) exposure to benzene for a number of years can lead to the development of acute non-lymphocytic leukaemia (ANLL), predominantly the acute myeloid form.

The evidence that benzene might induce other haematopoietic and lymphatic malignancies is however very much weaker. Lymphopoietic malignancies have also been associated with benzene exposure but often under circumstances in which confounding factors have been involved or in which the evidence has been very much weaker than for ANLL. Associations have been reported between benzene exposure and both Hodgkins and non-Hodgkins lymphomas (Young, 1989; Vianna & Polan, 1979). However, a lack of an association between lymphoma and benzene work has also been shown (Smith & Lickiss, 1980). The cohort studies examining benzene exposure have not identified notably increased incidences of lymphoma.

Cases of benzene exposed workers developing multiple myeloma (Goldstein, 1990; Aksoy, 1980) have been reported but the reported excesses of multiple myeloma reported in the cohort studies of Rinsky (Rinsky et al. 1987) have not been confirmed in other cohort studies.

Investigation of workers heavily exposed to benzene when it was used as a solvent in the manufacture of shoes and other leather goods gave evidence that bone marrow supression due to benzene was sometimes followed by the development of

leukaemia (Aksoy et al. 1972; Vigliani & Forni 1976; Aksoy 1978). Although acute non–lymphocytic leukaemia (ANLL) has been the predominant form reported there have been reports of acute lymphocytic leukaemia and chronic leukaemias (Aksoy, 1978, Aksoy et al. 1976).

Much of the basic evidence for the link between high benzene exposures and ANLL came from the large worker population in the Turkish leather working industry. Of an estimated 28,500 workers reported in the shoe, slipper and handbag industry in Istanbul, in which benzene was used as a solvent, 26 were admitted to hospital between 1967 and 1973 with acute leukaemia or preleukaemia. The working conditions in the industry were poor with small, unhygienic and badly ventilated workshops and the concentrations of benzene were reported to reach a maximum of 210–650 ppm when benzene containing glues were being used. Acute myeloblastic leukaemia was seen in 14 of these 26 patients who had been exposed for 1–15 years (mean of 9.7 years). The annual incidence of acute or preleukaemia was at least 13/100,000 which was statistically significantly greater (P>0.02) than the overall incidence of leukaemia in the general population (6/100,000) (Aksoy et al. 1974).

A number of cohort studies have been carried out on worker populations that have been exposed to benzene. These have however generally had limitations, many of which have been beyond the control of the investigators. Such limitations have been imposed for example by the difficulties in estimating historic exposures.

The principal studies are outlined in the following entries.

Source: BP Chemicals Ltd. London
(683) (684) (685) (686) (687) (688) (689) (690) (638) (691)

Remark: DOW CHEMICAL WORKERS

The mortality rates for 594 Michigan workers exposed to various compounds, including benzene, on or after January 1, 1940, were followed to the end of 1973 and compared with those for the US white male population adjusted for age, cause and calendar–year. The estimated benzene exposure concentrations ranged from 0.3–35.5 ppm.

No statistically significant increases were recorded for total death rate or any specific causes of death (Ott et al. 1978). This study was updated to the end of 1982. The original cohort of 594 workers exposed for at least 1 month between 1940 and 1970 was extended to include an additional 362 workers exposed up to 1978. The duration of exposure ranged from <1 to >= 15 years with a mean of 7 years. Those lost to follow–up (15) were withdrawn on the date they were last known to be alive. Data were analysed both with and without data from workers with possible confounding exposures to arsenic, asbestos and high levels of vinyl chloride. Excluding those with these possible confounding exposures, death from all causes was not increased

(O/E=200/242.3; SMR=83; 95% CI=72-95) but a significant excess of skin cancer death was recorded (O/E=4/0.9; SMR=441; 95% CI=121-1138). The excess of skin cancer however was suggested not to be related to benzene exposure on account of the cases all being in the lowest exposure category, with no common characteristic suggesting a link to exposure and no parallel findings in other cohort studies. No significant results were found for other specific causes of death.

Leukaemia was increased (O/E=3/1.9; SMR=162; 95% CI=33-461) but this was not statistically significant (Bond et al. 1986).

Source: BP Chemicals Ltd. London

(692) (693)

Remark: CMA CHEMICAL WORKERS

Wong (1987a,b) has reviewed the mortality experience of 3536 workers from seven plants in the USA, who were continuously exposed to benzene for at least six months between 1946 and 1976. Estimates of benzene exposure data were based, where possible, upon industrial hygiene data and workers were assigned to three exposure classes based on cumulative estimated 8-hour TWA values (<15 ppm years, 15-59.9 ppm years and >=60 ppm years).

Mortality for lymphatic and haematopoietic cancer combined showed five deaths in each exposure category and increasing SMRs with increasing estimates of cumulative dose (35, 91, 147 and 175 respectively for the control low, medium and high exposure groups; p=0.02).

Only six leukaemia deaths were observed and none of these was from acute myeloid leukaemia. The leukaemia SMRs were 0, 97, 78 and 275 for the control low, medium and high exposure groups respectively (p=0.02). The exposure response relationship, between cumulative benzene exposure and non-Hodgkin's lymphoma was of marginal statistical significance.

Source: BP Chemicals Ltd. London

(694) (695)

Remark: OTHER CHEMICAL WORKERS

In a retrospective cohort study of 259 men employed in a chemical plant for at least a year between 1947 and 1960, four deaths from lymphatic and haematopoietic cancer were observed. When analysed as combined lymphatic/haematopoietic cancer, this was statistically significant (SMR=377; 95% CI=109-1024). Three of these were due to leukaemia (of which two were ANLL and one chronic lymphatic leukaemia) and one was due to multiple myeloma. SMRs were not given for leukaemia and multiple myeloma. No cases of aplastic anaemia were found and benzene exposures were not quantified. They were however likely to be high in some workers (Decoufle et al. 1983).

Source: BP Chemicals Ltd. London

(696)

Remark: PLIOFILM RUBBER WORKERS

The vital status of a cohort of 1165 white males occupationally exposed for at least 1 day between January 1, 1940 and December 31, 1965 in the production of a natural rubber cast film at two localities in Ohio, was followed to December 31, 1981. The expected numbers of cause-specific deaths within 5-year age and 5-year calendar periods were based on death rates for the US white male population specific for the same 5-year age and calendar periods. At follow-up, 16 members of the cohort (1.4%) were unaccounted for but were assumed to be alive on December 31, 1981.

No statistically significant increases in total mortality (O/E=330/331.6; SMR=99.5) or deaths from malignant neoplasms (O/E=69/66.8; SMR=103) were found. Deaths from all lymphatic and haemopoietic neoplasms were significantly in excess (O/E=15/6.6; SMR=227; 95% CI=127-376); this being due mainly to excess numbers of leukaemia deaths (O/E=9/2.7; SMR=337; 95% CI=154-641) and multiple myeloma (O/E=4/1; SMR=409; 95% CI=110-1047). The leukaemia deaths were of heterogenous histological types (1 chronic myelogenous, 2 unspecified myelogenous and 6 acute myelogenous [ANLL]).

Industrial hygiene records gave background information on the atmospheric concentrations of benzene after 1946 and, for the most part, employees' 8-hr time-weighted average exposures appeared to be within the limits of the standard in effect at any given time (10-100 ppm). Occasional increases above these levels were reported, however, workers were required to wear respirators when the recommended limit was exceeded. The efficiency of these respirators was not reported.

The cumulative exposure for each cohort member was estimated from historical air-sampling data or from interpolation on the basis of existing data. The cohort was then divided according to levels of cumulative exposure; a level of 400 ppm-years equating to a mean annual exposure of 10 ppm over a 40-year working life. The SMRs for leukaemia deaths (SMRs=109, 322, 1186 and 6637) were found to markedly and progressively increase with a corresponding increase in cumulative exposure (1 ppm-day -39.99 ppm-years; 40-199.99 ppm-years; 200-399.99 ppm-years and >400 ppm-years respectively). These estimated cumulative exposure sub-groups contained 2, 2, 2 and 3 leukaemia deaths respectively. When halving or doubling these exposure ranges, the resultant SMRs continued to show a strongly positive trend of increasing risk with increasing exposure. The time to death ranged from <5 to >30 years but no apparent pattern was evident.

A case-control analysis, conducted by comparing each cohort leukaemia death with ten controls matched for year of birth

and first year of employment, indicated that the mean cumulative exposure was higher for the cases than the controls (254 and 50 ppm–years respectively), the average duration of exposure was longer (8.7 and 2.6 years respectively) and there was a difference in the rates of exposure (approximate averages of 24 and 16.5 ppm benzene/day respectively). It was suggested that the controls were selected from cohort members still alive at the time of death of the corresponding case.

The authors noted that, although hydrochloric acid, soda ash, natural rubber and small amounts of plasticizers were used, benzene was the only chemical in the plants that could reasonably be associated with haematologic toxicity (Rinsky et al. 1987). This study is an update of previous work (Infante et al. 1977; Rinsky et al. 1981).

Partly on the basis that it is a study in which benzene exposures appear not to have been confounded by other significant chemical exposures, the Rinsky et al. 1987 study has been widely cited. It has also been the basis of a number of quantitative risk assessment extrapolations which have attempted to project the leukaemia risk of benzene. However, this study has a number of limitations, largely concerning the estimation of worker exposure to benzene. No industrial hygiene data were available for the period 1940–1946 during which 6/9 leukaemia case workers were employed. It has been suggested that the Rinsky et al. 1987 estimates of exposure prior to 1946 were severe underestimates because of the following factors (Paustenbach et al. 1992; Crump & Allen, 1984; Cody et al. 1993; CEFIC 1988):–

An extensive exhaust ventilation system was installed at one plant in 1946. Exposures would have been expected to have been higher without such a system than were subsequently shown by industrial hygiene monitoring and used as a basis for determining worker exposures.

Although mortality from aplastic anaemia was not reported in the study, blood dyscrasias including aplastic anaemia were not uncommon amongst the work force at the Pliofilm plants indicating that some high exposures had taken place.

Review of worker haematological data and comparison with individual "pre–employment" data suggest that workers in the 1940s experienced clinically detectable bone marrow depression during their initial period of work in benzene areas of the plant. This information supports the view that benzene exposures were higher during this period than those estimated by Rinsky et al. 1987.

Account had not been taken of the possible effect of short term peak exposures to benzene.

No account was taken of dermal exposure to benzene.

Tyre building work was carried out at one Pliofilm site with the possibility that benzene exposures had been higher there.

War production work during the period 1940-1945 is likely to have been associated with higher exposures and longer working hours.

It has also been suggested that the case control study may be compromised by poor matching of the exposure history of the control group compared to the cases (cases mean period exposed 8.9 years, controls mean period exposed 2.6 years) (CEFIC, 1988).

Source: BP Chemicals Ltd. London
(697) (698) (699) (700) (701) (702) (703) (704)

Remark: CHINESE INDUSTRIAL WORKERS

A retrospective study of 28,460 workers selected from 233 painting, shoe-making, rubber synthesis, and adhesives and organic synthesis factories in 12 cities in China who had worked for at least 6 months between January 1, 1972 and December 31, 1981, was conducted. The control cohort of 28,257 individuals who had worked in the same cities for at least 6 months, was chosen from 83 machine production, textile and cloth factories in which there was no known exposure to benzene or other occupational carcinogens. Thirty cases of leukaemia (of whom 25 had died) were identified in the benzene cohort and four (all of whom had died) in the control group, giving mortality rates from leukaemia of 14/100,000 person-years and 2.01/100,000 person-years respectively. The numbers of leukaemia deaths were significantly in excess for both male and female workers [SMR=501 (95% CI=292-802) and 830 (95% CI=358-1635) respectively]. The mean age of the leukaemia patients was lower in the exposed group and the average latency period was 11.4 years (ranging form 0.8-49.5 years). The benzene concentrations, as determined in a grab sampling programme, ranged from 7-1100 mg/m3 (~3-340 ppm) but were mainly in the range of 50-500 mg/m3 (~16-160 ppm). Chronic benzene poisoning and aplastic anaemia were identified in 196 exposed workers, seven of whom died from leukaemia. The leukaemia mortality rate among these poisoning cases was 700.7/100,000 person-years; 49 times greater than that in the benzene workers (Yin et al. 1987). It has been commented that little detail has been given of the methods used to take account of the possible confounding effects of smoking (WHO, 1993).

Source: BP Chemicals Ltd. London
(638) (705)

Remark: PETROLEUM INDUSTRY WORKERS

A number of cohort studies have investigated oil refining and petroleum distribution workers but few have focussed specifically on benzene exposure.

A large cohort study of British refinery workers (34,781 workers employed for at least a year between 1950–1975) showed no excess mortality from leukaemia (Rushton & Alderson, 1981a). No benzene exposure measurements were made but when leukaemia cases and controls were qualitatively assigned to low, medium or high benzene exposure groups on the basis of job category in a nested case–control study, a significant association (p=0.05) was reported between leukaemia and combined high or medium exposure compared to the low exposure group (RR=3.0, 95% CI=1.2–7.2) (Rushton & Alderson, 1981b).

The 39 year follow up of this study has shown that in 34,569 refinery workers there was still no leukaemia excess (68 observed compared to 70 expected). In the follow up of the 23,306 distribution workers there were 61 leukaemias observed compared to 57 expected (SMR=108, 95% CI=83–140) (Rushton, 1993).

The effects of occupational exposure to lower concentrations of benzene has been the subject of much study. The availability of data from further large cohorts occupationally exposed to benzene is relevant to defining the dose effect relationship at well characterized low exposures. Such data is likely to be forthcoming from the UK oil distribution cohort. This is currently the subject of a nested cohort study, due to report early in 1995.

Tsai et al. (1983) reported on 454 US refinery workers employed from 1952 until 1978 and found no deaths from leukaemia (0.4 expected). Personal samples indicated however that during the period 1973–1982 only 16% of 1394 samples indicated exposure to air concentrations (TWA) of >1 ppm.

Another US refinery study was apparently set up because of a suspected excess of leukaemia in the work force (McCraw et al. 1985). Fourteen leukaemia deaths were observed (6.6 expected) of which 8 were acute myeloid leukaemia (2.0 expected). A later nested case–control study found no association with potential exposure to benzene (Austin et al. 1986).

Source: BP Chemicals Ltd. London

(706) (707) (708) (709) (710) (672)

Remark: COKE OVEN WORKERS

A number of cohort studies have looked at mortality in coke oven workers. No excesses of leukaemias and lymphomas combined or of leukaemias respectively were found in North American or Dutch coke oven workers (Redmond et al. 1972; Swaen et al. 1991). Exposures to benzene were not reported in these studies.

Preliminary results from a total 6520 British male coke plant workers have been reported (Hurley et al. 1991). Personal air sampling from two cohorts of benzole workers

(84 and 307 workers respectively) showed benzene concentrations of <0.19–15 ppm. Contemporary mean 8 hour time weighted averages for similar workers in the UK during the 1980s were 1.3 ppm. No increased risk of mortality from leukaemia has been observed in either cohort (SMR=98, 95% CI=2–429, 1 death and SMR=76, 95% CI=1–192, 1 death). For two cohorts of coke oven workers (total 2349 workers) leukaemia mortality had SMRs of 34, 95% CI=O–186, 1 death and 35 95% CI=1–192, 1 death respectively.

Source: BP Chemicals Ltd. London

(711) (712) (713)

Remark: Chromosomenuntersuchungen bei 10 gegenueber Benzol exponierten Beschaeftigten in der Benzol–Fabrikation zeigten 1–3 % Chromosomenaberationen. Die Phenolausscheidung im Urin lag bei 31–65 mg/l.

Source: BASF AG Ludwigshafen

(714)

Remark: Von 1994–09/96 wurde ein Fall von Haut– und Augenreizung nach lokaler akzidentieller Einwirkung von Benzol beobachtet, der zur weiteren Behandlung in die Klinik eingewiesen wurde.

Source: BASF AG Ludwigshafen

(715)

Remark: A cluster of ten cases of Hodgkin‘s disease was identified within the active workforce of a large chemical manufacturing firm over a 23–year period. In the total workforce of 62000 person–years, an expected number of 2.01 cases was calculated, giving a standardized incidence ratio of 497 (95 % CI 238–915). A nested case–control study was undertaken with 200 controls. For 11 seleceted chemicals (incl. benzene) exposure odds ratios were calculated. No substance emerged as a likely candidate for explaining the observed Hodgkin‘s disease cluster.

Source: BASF AG Ludwigshafen

(716)

Remark: ACUTE TOXICITY
The acute toxicity to humans when benzene is inhaled has been described as being as follows (Gerarde, 1960):

20,000 ppm 5–10 minutes exposure can give fatalities
7,5000 ppm 30 minutes exposure may be fatal
1500 ppm 60 minutes exposure causes serious symptoms
500 ppm 60 minutes exposure leads to symptoms of illness
50–150 ppm 5 hours exposure causes headache, lassitude and weakness
25 ppm 8 hours exposure causes no clinical effect.

Signs of toxicity following exposures are CNS depression, cardiac arrhythmia and respiratory failure resulting in death. At lower levels of exposure CNS effects are rapidly reversible.

The acute lethal dose of benzene to humans when ingested has

been estimated to be about 10 ml (8.8 g). The signs and symptoms of toxicity are typically those of narcotic solvent type materials leading to profound CNS depression, collapse and death (WHO, 1993).

Avis & Hutton (1993) reported of 3 fatalities of acute benzene poisoning. Victims were exposed to benzene fumes for a few minutes after opening a valve on a chemical cargo ship. Findings at autopsy: 2nd degree chemical burns to face, trunk and limbs (20-70% burn), hemorrhagic airless lungs with alveolar hemorrhage and pulmonary edema, brains with vascular congestion, benzene concentration in blood 30-120 mg/l and in brain 58-63 mg/l.

Source: Deutsche Shell Chemie GmbH Eschborn

(717) (718) (638)

Remark: BONE MARROW DEPRESSION
Bone marrow depression results in a spectrum of haematological changes depending upon the degree of exposure and to individual susceptibility. Thrombocytopaenia, pancytopaenia, and aplastic anaemia may result.

Aksoy (1972) reported on 32 individuals who had been exposed to 150-650 ppm benzene for periods of 4 months-15 years and who had pancytopaenia with hypoplastic, hyperplastic or normoplastic marrow. Eight of the 32 were thrombocytopaenic with associated haemorrhage and infection. Severe or fatal aplastic anaemia seems to be associated with high exposure (>200 ppm) (Aksoy, 1972; Vigliani & Forni, 1976).

Much of the literature on haematological effects of benzene relates to exposures that took place in the 1930s and 1940s and consequently had poor characterization of benzene exposure (WHO, 1993). The haematotoxic effects of benzene appear to occur with exposures to >30 ppm (ECETOC, 1984). More recent studies show no (or marginal effects) after 8 hour TWA exposures of 2-30 ppm, <0.1-25 ppm, <1.0-10 ppm and 0.01-1.4 ppm (Townsend et al. 1978; Tsai et al. 1983; Yardley-Jones et al. 1988; Collins et al. 1991).

As benzene is toxic to bone marrow it would not be unexpected that it could have an effect on immune function. Studies of immune function in workers have however usually been compromised by confounding exposures (WHO, 1993).

Source: Deutsche Shell Chemie GmbH Eschborn

(668) (669) (670) (671) (672) (673) (638) (674)

Remark: GENOTOXICITY IN HUMANS
The finding of both structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells of those occupationally exposed to benzene has led to general acceptance that benzene can be a human clastogen (WHO, 1993). It is however acknowledged that methodological difficulties in elder (1965-1984) cytogenetic studies (e.g. adequate sample size and control groups, confounding by other factors especially age and smoking) make this a difficult area to study (Ashby & Richardson, 1985).

Chromosome aberrations have been reported in individuals who have previously suffered benzene induced haemopathy resulting from high benzene exposures (125-532 ppm for 1-22 years; Forni et al. 1971). Some studies have indicated genotoxic effects in individuals exposed to lower concentrations of benzene. Picciano (1979) has reported significant increases in chromosomal aberrations in 52 workers exposed to concentrations < 10 ppm (control n=44) and Sarto et al. (1984) reported an increased frequency of chromosome aberrations but not of sister chromatid exchanges (SCEs) in benzene production workers exposed to 0.2-12.4 ppm. Yardley-Jones et al. (1990) have reported a slight increase (significant using Fisher's exact test) in lymphocyte chromosomal aberrations in oil refinery workers (48 workers and 29 controls) largely exposed to <10 ppm 8-hour TWA benzene. There was however no increase in SCEs (Yardley-Jones et al. 1988).

The distribution of the breakpoints in the karyotypes of 56 workers occupationally exposed to benzene (<10 ppm, 6 h/d for 10-20 years) was investigated in cultured lymphocytes. A significant increase (control n=20) in chromosome aberrations was observed. The breakpoints in the karyotypes accumulated mainly on chromosome 2, 4, and 7 (Sasiadek, 1992).

In contrast, other studies of workers exposed to benzene have shown no excess of chromosome aberrations (Watanabe et al. 1980; De Jong et al. 1988) or SCEs (Seiji et al. 1990; Watanabe et al. 1980). The finding of an excess of chromosomal aberrations in milk road tanker drivers (not specifically exposed to benzene) compared to petrol tanker crews (exposed to 6.5 ppm 8-hour TWA benzene) illustrates the difficulties of execution and interpretation of human cytogenic studies in the occupational toxicology field (Berlin, M., Am. J. Ind. Med. 7, 365-373 (1985)).

Source: Deutsche Shell Chemie GmbH Eschborn
(675) (676) (677) (678) (679) (719) (680) (681) (674) (682)

Remark: CARCINOGENIC EFFECTS

Introduction

Populations exposed occupationally to benzene have been widely studied. Both case studies and epidemiological studies have been reported leading to the conclusion that benzene is a human leukaemogen (IARC, 1982; IARC, 1987; WHO, 1993). On the basis of such evidence benzene has been classified as a category 1 carcinogen within the EC and as a group 1 carcinogen ("The agent is carcinogenic to humans") by IARC.

The evidence is clear that at high concentrations (>100 ppm) exposure to benzene for a number of years can lead to the development of acute non-lymphocytic leukaemia (ANLL), predominantly the acute myeloid form.

The evidence that benzene might induce other haematopoietic and lymphatic malignancies is however very much weaker. Lymphopoietic malignancies have also been associated with benzene exposure but often under circumstances in which confounding factors have been involved or in which the evidence has been very much weaker than for ANLL. Associations have been reported between benzene exposure and both Hodgkins and non–Hodgkins lymphomas (Young, 1989; Vianna & Polan, 1979). However, a lack of an association between lymphoma and benzene work has also been shown (Smith & Lickiss, 1980). The cohort studies examining benzene exposure have not identified notably increased incidences of lymphoma.

Cases of benzene exposed workers developing multiple myeloma (Goldstein, 1990; Aksoy, 1980) have been reported but the reported excesses of multiple myeloma reported in the cohort studies of Rinsky (Rinsky et al. 1987) have not been confirmed in other cohort studies.

Investigation of workers heavily exposed (150–650 ppm, 4 months–15 years) to benzene when it was used as a solvent in the manufacture of shoes and other leather goods gave evidence that bone marrow supression due to benzene was sometimes followed by the development of leukaemia (Aksoy et al. 1972; Vigliani & Forni 1976; Aksoy 1978). Although acute non–lymphocytic leukaemia (ANLL) has been the predominant form reported there have been reports of acute lymphocytic leukaemia and chronic myeloid leukaemias (Aksoy, 1978, Aksoy et al. 1976).

Much of the basic evidence for the link between high benzene exposures and ANLL came from the large worker population in the Turkish leather working industry. Of an estimated 28,500 workers reported in the shoe, slipper and handbag industry in Istanbul, in which benzene was used as a solvent, 26 were admitted to hospital between 1967 and 1973 with acute leukaemia or preleukaemia. The working conditions in the industry were poor with small, unhygienic and badly ventilated workshops and the concentrations of benzene were reported to reach a maximum of 210–650 ppm when benzene containing glues were being used. Acute myeloblastic leukaemia was seen in 14 of these 26 patients who had been exposed for 1–15 years (mean of 9.7 years). The annual incidence of acute or preleukaemia was at least 13/100,000 which was statistically significantly greater (P>0.02) than the overall incidence of leukaemia in the general population (6/100,000) (Aksoy et al. 1974).

A number of cohort studies have been carried out on worker populations that have been exposed to benzene. These have however generally had limitations, many of which have been beyond the control of the investigators. Such limitations have been imposed for example by the difficulties in estimating historic exposures.

The principal studies are outlined in the following entries.

Source: Deutsche Shell Chemie GmbH Eschborn
(683) (684) (685) (686) (687) (688) (689) (690) (638) (691)

Remark: DOW CHEMICAL WORKERS

The mortality rates for 594 Michigan workers exposed to various compounds, including benzene, on or after January 1, 1940, were followed to the end of 1973 and compared with those for the US white male population adjusted for age, cause and calendar-year. The estimated TWA benzene exposure concentrations ranged from 0.3-35.5 ppm.

No statistically significant increases were recorded for total death rate or any specific causes of death (Ott et al. 1978). This study was updated to the end of 1982. The original cohort of 594 workers exposed for at least 1 month between 1940 and 1970 was extended to include an additional 362 workers exposed up to 1978. The duration of exposure ranged from <1 to >= 15 years with a mean of 7 years. Those lost to follow-up (15) were withdrawn on the date they were last known to be alive. Data were analysed both with and without data from workers with possible confounding exposures to arsenic, asbestos and high levels of vinyl chloride. Excluding those with these possible confounding exposures, death from all causes was not increased (O/E=200/242.3; SMR=83; 95% CI=72-95) but a significant excess of skin cancer death was recorded (O/E=4/0.9; SMR=441; 95% CI=121-1138). The excess of skin cancer however was suggested not to be related to benzene exposure on account of the cases all being in the lowest exposure category, with no common characteristic suggesting a link to exposure and no parallel findings in other cohort studies. No significant results were found for other specific causes of death.
In the cohort less exclusions (exposure to arsenic, asbestos, or high levels of vinyl chloride) leukaemia was increased (O/E=3/1.9; SMR=162; 95% CI=33-461) but this was not statistically significant (Bond et al. 1986).

Source: Deutsche Shell Chemie GmbH Eschborn
(692) (693)

Remark: CMA CHEMICAL WORKERS

Wong (1987a,b) has reviewed the mortality experience of 3536 workers from seven plants in the USA, who were continuously exposed to benzene for at least six months between 1946 and 1976. Estimates of benzene exposure data were based, where possible, upon industrial hygiene data and workers were assigned to three exposure classes based on cumulative estimated 8-hour TWA values (<15 ppm years, 15-59.9 ppm years and >=60 ppm years).

Mortality for lymphatic and haematopoietic cancer combined showed five deaths in each exposure category and increasing SMRs with increasing estimates of cumulative dose (35, 91, 147 and 175 respectively for the control low, medium and high exposure groups; p=0.02).

Only six leukaemia deaths were observed and none of these was from acute myeloid leukaemia. The leukaemia SMRs were 0, 97, 78 and 275 for the control low, medium and high exposure groups respectively (p=0.02). The exposure response relationship, between cumulative benzene exposure and non-Hodgkin's lymphoma was of marginal statistical significance.

Source: Deutsche Shell Chemie GmbH Eschborn

(694) (695)

Remark: OTHER CHEMICAL WORKERS

In a retrospective cohort study of 259 men employed in a chemical plant for at least a year between 1947 and 1960, four deaths from lymphatic and haematopoietic cancer were observed. When analysed as combined lymphatic/haematopoietic cancer, this was statistically significant (SMR=377; 95% CI=109-1024). Three of these were due to leukaemia (of which two were ANLL and one chronic lymphatic leukaemia) and one was due to multiple myeloma. SMRs were not given for leukaemia and multiple myeloma. No cases of aplastic anaemia were found and benzene exposures were not quantified. They were however likely to be high in some workers (Decoufle et al. 1983).

Source: Deutsche Shell Chemie GmbH Eschborn

(696)

Remark: Partly on the basis that it is a study in which benzene exposures appear not to have been confounded by other significant chemical exposures, the Rinsky et al. 1987 study has been widely cited. It has also been the basis of a number of quantitative risk assessment extrapolations which have attempted to project the leukaemia risk of benzene. However, this study has a number of limitations, largely concerning the estimation of worker exposure to benzene. No industrial hygiene data were available for the period 1940-1946 during which 6/9 leukaemia case workers were employed. It has been suggested that the Rinsky et al. 1987 estimates of exposure prior to 1946 were severe underestimates because of the following factors (Paustenbach et al. 1992; Crump & Allen, 1984; Cody et al. 1993; CEFIC 1988):

An extensive exhaust ventilation system was installed at one plant in 1946. Exposures would have been expected to have been higher without such a system than were subsequently shown by industrial hygiene monitoring and used as a basis for determining worker exposures.

Although mortality from aplastic anaemia was not reported in the study, blood dyscrasias including aplastic anaemia were not uncommon amongst the work force at the Pliofilm plants indicating that some high exposures had taken place.

Review of worker haematological data and comparison with individual "pre-employment" data suggest that workers in the 1940s experienced clinically detectable bone marrow

depression during their initial period of work in benzene areas of the plant. This information supports the view that benzene exposures were higher during this period than those estimated by Rinsky et al. 1987.

Account had not been taken of the possible effect of short term peak exposures to benzene.

No account was taken of dermal exposure to benzene.

Tyre building work was carried out at one Pliofilm site with the possibility that benzene exposures had been higher there.

War production work during the period 1940–1945 is likely to have been associated with higher exposures and longer working hours.

It has also been suggested that the case control study may be compromised by poor matching of the exposure history of the control group compared to the cases (cases mean period exposed 8.9 years, controls mean period exposed 2.6 years) (CEFIC, 1988).
PLIOFILM RUBBER WORKERS

The vital status of a cohort of 1165 white males occupationally exposed for at least 1 day between January 1, 1940 and December 31, 1965 in the production of a natural rubber cast film at two localities in Ohio, was followed to December 31, 1981. The expected numbers of cause–specific deaths within 5–year age and 5–year calendar periods were based on death rates for the US white male population specific for the same 5–year age and calendar periods. At follow–up, 16 members of the cohort (1.4%) were unaccounted for but were assumed to be alive on December 31, 1981.

No statistically significant increases in total mortality (O/E=330/331.6; SMR=99.5) or deaths from malignant neoplasms (O/E=69/66.8; SMR=103) were found. Deaths from all lymphatic and haemopoietic neoplasms were significantly in excess (O/E=15/6.6; SMR=227; 95% CI=127–376); this being due mainly to excess numbers of leukaemia deaths (O/E=9/2.7; SMR=337; 95% CI=154–641) and multiple myeloma (O/E=4/1; SMR=409; 95% CI=110–1047). The leukaemia deaths were of heterogenous histological types (1 chronic myelogenous, 2 unspecified myelogenous and 6 acute myelogenous [ANLL]).

Industrial hygiene records gave background information on the atmospheric concentrations of benzene after 1946 and, for the most part, employees' 8–hr time–weighted average exposures appeared to be within the limits of the standard in effect at any given time (10–100 ppm). Occasional increases above these levels were reported, however, workers were required to wear respirators when the recommended limit was exceeded. The efficiency of these respirators was not reported.

The cumulative exposure for each cohort member was estimated from historical air-sampling data or from interpolation on the basis of existing data. The cohort was then divided according to levels of cumulative exposure; a level of 400 ppm-years equating to a mean annual exposure of 10 ppm over a 40-year working life. The SMRs for leukaemia deaths (SMRs=109, 322, 1186 and 6637) were found to markedly and progressively increase with a corresponding increase in cumulative exposure (1 ppm-day -39.99 ppm-years; 40-199.99 ppm-years; 200-399.99 ppm-years and >400 ppm-years respectively). These estimated cumulative exposure sub-groups contained 2, 2, 2 and 3 leukaemia deaths respectively. When halving or doubling these exposure ranges, the resultant SMRs continued to show a strongly positive trend of increasing risk with increasing exposure. The time to death ranged from <5 to >30 years but no apparent pattern was evident.

A case-control analysis, conducted by comparing each cohort leukaemia death with ten controls matched for year of birth and first year of employment, indicated that the mean cumulative exposure was higher for the cases than the controls (254 and 50 ppm-years respectively), the average duration of exposure was longer (8.7 and 2.6 years respectively) and there was a difference in the rates of exposure (approximate averages of 24 and 16.5 ppm benzene/day respectively). It was suggested that the controls were selected from cohort members still alive at the time of death of the corresponding case.

The authors noted that, although hydrochloric acid, soda ash, natural rubber and small amounts of plasticizers were used, benzene was the only chemical in the plants that could reasonably be associated with haematologic toxicity (Rinsky et al. 1987). This study is an update of previous work (Infante et al. 1977; Rinsky et al. 1981).

Source: Deutsche Shell Chemie GmbH Eschborn
(697) (698) (699) (700) (701) (702) (703) (704)

Remark: CHINESE INDUSTRIAL WORKERS

A retrospective study of 28,460 workers selected from 233 painting, shoe-making, rubber synthesis, and adhesives and organic synthesis factories in 12 cities in China who had worked for at least 6 months between January 1, 1972 and December 31, 1981, was conducted. The control cohort of 28,257 individuals who had worked in the same cities for at least 6 months, was chosen from 83 machine production, textile and cloth factories in which there was no known exposure to benzene or other occupational carcinogens. Thirty cases of leukaemia (of whom 25 had died) were identified in the benzene cohort and four (all of whom had died) in the control group, giving mortality rates from leukaemia of 14/100,000 person-years and 2.01/100,000 person-years respectively. The numbers of leukaemia deaths were significantly in excess for both male and female workers [SMR=501 (95% CI=292-802) and 830 (95% CI=358-1635)

respectively]. The mean age of the leukaemia patients was lower in the exposed group and the average latency period was 11.4 years (ranging form 0.8-49.5 years). The benzene concentrations, as determined in a grab sampling programme, ranged from 7-1100 mg/m3 (3-340 ppm) but were mainly in the range of 50-500 mg/m3 (16-160 ppm). Chronic benzene poisoning and aplastic anaemia were identified in 196 exposed workers, seven of whom died from leukaemia. The leukaemia mortality rate among these poisoning cases was 700.7/100,000 person-years; 49 times greater than that in the benzene workers (Yin et al. 1987). It has been commented that little detail has been given of the methods used to take account of the possible confounding effects of smoking (WHO, 1993).

Source: Deutsche Shell Chemie GmbH Eschborn

(638) (705)

Remark: PETROLEUM INDUSTRY WORKERS

A number of cohort studies have investigated oil refining and petroleum distribution workers but few have focussed specifically on benzene exposure.

A large cohort study of British refinery workers (34,781 workers employed for at least a year between 1950-1975) showed no excess mortality from leukaemia (Rushton & Alderson, 1981a). No benzene exposure measurements were made but when leukaemia cases and controls were qualitatively assigned to low, medium or high benzene exposure groups on the basis of job category in a nested case-control study, a significant association (p=0.05) was reported between leukaemia and combined high or medium exposure compared to the low exposure group (RR=3.0, 95% CI=1.2-7.2) (Rushton & Alderson, 1981b).

The 39 year follow up of this study has shown that in 34,569 refinery workers there was still no leukaemia excess (68 observed compared to 70 expected). In the follow up of the 23,306 distribution workers there were 61 leukaemias observed compared to 57 expected (SMR=108, 95% CI=83-140) (Rushton, 1993).

The effects of occupational exposure to lower concentrations of benzene has been the subject of much study. The availability of data from further large cohorts occupationally exposed to benzene is relevant to defining the dose effect relationship at well characterized low exposures. Such data is likely to be forthcoming from the UK oil distribution cohort. This is currently the subject of a nested cohort study, due to report early in 1995.

Tsai et al. (1983) reported on 454 US refinery workers employed from 1952 until 1978 and found no deaths from leukaemia (0.4 expected). Personal samples indicated however that during the period 1973-1982 only 16% of 1394 samples indicated exposure to air concentrations (TWA) of >1 ppm.

Another US refinery study (retrospective follow-up study, 3976 white men) was apparently set up because of a suspected excess of leukaemia in the work force (McCraw et al. 1985). Fourteen leukaemia deaths were observed (6.6 expected) of which 8 were acute myeloid leukaemia (2.0 expected, significant increase).

14 cases of leukemia (8 acute myelogenous leukemia) occurred among the work force of an oil refinery (8 cases expected); in a case-control study (50 matched controls) no particular job or department in this refinery was identified as the likely source of the excess leukemia deaths (Austin et al. 1986).

Source: Deutsche Shell Chemie GmbH Eschborn
(706) (707) (708) (709) (710) (672)

Remark: COKE OVEN WORKERS

A number of cohort studies have looked at mortality in coke oven workers. No excesses of leukaemias and lymphomas combined or of leukaemias respectively were found in North American or Dutch coke oven workers (Redmond et al. 1972; Swaen et al. 1991). Exposures to benzene were not reported in these studies.

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Source: Deutsche Shell Chemie GmbH Eschborn
(711) (712) (713)

Remark: UPDATE OF THE PLIOFILM COHORT STUDY
method: study based on mortality data of a cohort of 1717 white male workers employed in pliofilm operations for at least 1 d and included 577 workers in jobs not considered by the studies of Rinsky et al. 1981 and 1987; mortality experience of the remaining 1140 workers was followed through 31 Dec. 1987
results: significant excess of acute myelocytic or acute monocytic leukemia with strong dose-response trend; quadratic models predicted an additional lifetime risk of a benzene-related death from 45 years of exposure to 1 ppm of between 0.020-0.036 per thousand, linear models predicted risks of between 1.6 and 3.1 per thousand.

Source: Deutsche Shell Chemie GmbH Eschborn
(720)

Remark: Odor threshold = 1.5 ppm
Source: Deutsche Shell Chemie GmbH Eschborn
(721)

Remark: Hematological abnormalities in 217 workers exposed to benzene were compared to abnormalities in 100 hospital patients and personnel, and medical students of similar age. Workplace benzene concentrations ranged from 30-210 ppm, and length of exposure from 3 months to 17 years. Hematological abnormalities attributable to benzene (e.g., leucopenia, thrombocytopenia, pancytopenia, lymphocytopenia,giant platelets, eosinophilia, basophilia) were detected in 24% of the workers. No apparent association with duration of exposure was detected. About 1/3 of the workers were anemic, but when 1/3 of these anemic workers were treated with iron, the anemia disappeared so the anemia was not attributed to benzene alone. The authors conclude that the harmful effects of benzene are primarily on the leukocytes, with inconsistent findings of eosinophilia and basophilia, and thrombocytopenia and pancytopenia as secondary effects.
Source: Deutsche Shell Chemie GmbH Eschborn
(722)

Remark: The authors studied 32 pancytopenic patients (n=30 males, n=2 females, age 15-57) who had been exposed to benzene concentrations ranging from 150 to 650 ppm for 4 months to 15 years. Analyses of a number of hematologic and bone marrow parameters were performed. Categorization of patients based on their bone marrow findings showed large variations in both the clinical and hematologic findings, ranging from asymptomatic to very severe. The authors conclude that a common basis in all cases is that benzene isthe causative agent.
Source: Deutsche Shell Chemie GmbH Eschborn
(723)

Remark: This is a review of hematotoxic and carcinogenic properties of benzene, supplemented with a summary of case or series findings from the author's patients. The suggested effects of benzene exposure and topics discussed include: aplastic anemia or pancytopenia; benzene - hepatitis - aplastic anemia syndrome; leukemia; leukemia in pancytopenia patientswith chronic benzene toxicity; genetic relationships and individual susceptibility in leukemia; other cancers - malignant lymphomas, multiple myeloma, lung cancer. The conclusions of the author are suggestive rather than definitive.
Source: Deutsche Shell Chemie GmbH Eschborn
(724)

Source: Deutsche Shell Chemie GmbH Eschborn
(725)

Remark: The authors describe clinical findings of ten Dow Chemical Company employees exposed for several years to over 25 ppm, 8-hour TWA benzene. The initial high exposure of these workers had started more than 16 years previous to this report. Some alterations in hemoglobin and mean corpuscularvolume were observed, but no other significant blood or bonemarrow abnormalities. Since the time of high exposures in 1963, the clinical findings have generally tended to return to normal values. All ten employees were included in the mortality and morbidity studies conducted at Dow and published as separate reports.
Source: Deutsche Shell Chemie GmbH Eschborn

(726)

Remark: This is a cross-sectional study of 332 workers in three fast-press rotogravure printing plants in NYC in 1938. Benzene concentrations ranged from 11 to 1060 ppm in the three plants. There were 48 area samples analyzed and were probably 8-hour TWAs, although methods are not specified in this report. There was also dermal exposure as the workers used benzene to wash the ink off their hands and arms. Physical examination and complete blood studies were completed on 102 benzene workers and partial studies on the remainder; there were 81 nonbenzene exposed workers available for some comparisons. There were 130/332 cases with variable degrees of benzene poisoning. The findings from the blood studies are described.
Source: Deutsche Shell Chemie GmbH Eschborn

(727)

Remark: This is an analysis of white and red cell counts as well as hemoglobin of 459 benzene-exposed workers between 1940 and 1975. These workers are a subset of about 7200 rubber workers from the Pliofilm cohort at St. Mary's in Akron, Ohio. Positive correlations between fluctuations in blood count and benzene exposure were reported between 1940-1948 (mean estimated exposure = 75 ppm) but not from 1948-1975 (estimated exposure = 15-20 ppm). The authors suggest examination of the blood is unlikely to detect abnormalitiesat lower benzene exposures.
Source: Deutsche Shell Chemie GmbH Eschborn

(728)

Remark: This report is a literature review of benzene. Topics discussed include animal models for benzene-induced leukemia, mechanism of benzene-induced toxicity and the roleof benzene metabolites, studies in mutagenicity and teratogenicity, toxicity and carcinogenicity in humans, population exposures to benzene (especially exposure throughwater), and risk assessment. The review is particularly useful for historical purposes as the older literature is included. There are 13 pages of references, two of which goback to 1880.
Source: Deutsche Shell Chemie GmbH Eschborn

(729)

Remark: This short report summarizes the results from a cohort of shoe workers in Florence, Italy exposed to benzene between 1953-1964. Follow-up was through 1984. SMRs for males were19.4 (66 observed) for aplastic anemia and 4.8 (6 observed) for leukemia. There was no apparent increase in mortality with increasing latency or duration of employment for eithercause of death. Peak workroom exposure was in 1960 and averaged about 60 ppm, ~40 ppm from about 1953-1960, and dropping to zero between 1960 and 1964.
Source: Deutsche Shell Chemie GmbH Eschborn

(730)

Remark: Ten workers were exposed to a benzene spill that resulted insubstantial excretion of phenol. Three months later, genotoxic bnormalities in peripheral lymphocytes of benzene exposed workers and 11 controls were compared. Lymphocytes were examined for chromosomal damage (e.g., polyploidy, gaps, chromatid breaks, acentric fragments) and the frequency of sister chromatid exchange. Controls had slightly more chromosomal damage, but all values were in thenormal range. Benzene-exposed workers had slightly more SCEwith a slight exposure-response trend based on maximum phenol excretion shortly after the spill. The authors concluded there was no evidence of any lasting chromosomal damage resulting from the acute benzene.
Source: Deutsche Shell Chemie GmbH Eschborn

(731)

Remark: Peripheral blood lymphocytes of 34 workers exposed to benzene and toluene at a rotogravure plant and 34 controls matched by sex and age were analyzed from chromosomal aberrations. All chromosomal analyses were carried out blindly by the authors, and persons with viral diseases and recent vaccinations were excluded. Benzene concentrations ranged from 131 ppm to 532 ppm. Among 10 workers exposed tobenzene before 1952 and then to toluene, the proportion of unstable and stable chromosomal aberrations were statistically significantly higher compared with workers exposed only to toluene. The frequencies of chromosomal aberrations were not statistically significantly different among workers exposed only to toluene and controls.
Source: Deutsche Shell Chemie GmbH Eschborn

(732)

Remark: Chromosomal aberrations in cultured lymphocytes were studiedin six groups of 65 workers exposed to benzene in motor fuels and a younger group of 15 patient controls. A moderate but statistically significant increase in frequencyof aberrations was found in road tank drivers and workers atindustrial gasworks, but not in ship tanker crews and employees at petrol filling stations. The range of TWA concentrations of benzene in each group were as follows: 0.02-0.31 ppm for petrol station employees; 0.01-4.13 ppm for road tank drivers delivering petrol; 0.33-23 ppm for crew members loading ship tankers (but frequency of exposurewas less than road tanker drivers); 5-10 ppm for gasworks workers, but there were only occasional short-term

exposures. No estimates were available for road tanker drivers delivering milk or for controls. The authors consider it unlikely that benzene is the main reason for aberrations in the road tanker drivers since there was no difference between those delivering petrol and those delivering milk.

Source: Deutsche Shell Chemie GmbH Eschborn

(733)

Remark: The white blood cell count and frequency and size of lymphocyte micronuclei of 15 gasoline pump mechanics were compared to 15 construction worker controls. The male subjects in the two groups were similar in age and smoking. Pump mechanics were exposed to 1 mg/m3 benzene (8-hour TWA, 10-minute peaks as high as 20 mg/m3). The leukocyte count was higher in the exposed group. Beta-cell micronuclei werelarger and more frequent compared to controls, but there wasno apparent occupational effect on T- cell lymphocyte micronuclei. Most human lymphocytic tumors originate from Beta-lymphocytes and appear more sensitive to mutagens than T-cells.

Source: Deutsche Shell Chemie GmbH Eschborn

(734)

Remark: Lymphocytes from peripheral blood of 52 benzene exposed workers and 44 controls were scored for cytogenetic changes.Exposed and controls were matched on sex and time period of analysis, but were not matched on age or smoking. There were no apparent differences in the percentage of chromatid breaks and total abnormal cells between the two groups. However, the benzene exposed group had twice the percentage of chromosome breaks, three times the percentage of marker chromosomes, and 10 times the percentage of both breaks and marker chromosomes. Data collected between 1945 and 1978 suggest 8-TWA concentrations were about 2-3 ppm benzene (25 ppm for 15 minutes and 50 ppm for 2-3 minutes samples duringspecific operations, and possibly > 100 ppm.). Ethylbenzeneconcentrations were about 2-10 ppm TWA, 25 ppm ceiling, and > 100 ppm excursions.

Source: Deutsche Shell Chemie GmbH Eschborn

(735)

Remark: Chromosomes from peripheral blood leukocytes of three groupsof factory workers exposed to benzene were examined. Results were compared with on-site as well as population controls without benzene exposure. The exposed workers in Group 1 had a higher proportion of stable chromosomal abnormalities than controls, but there were no apparent differences in Groups 2 and 3. Population controls had fewer stable chromosomal abnormalities than both exposed andcontrols in Group 2. There was no apparent differences in unstable chromosomal abnormalities between exposed and controls in any of the groups. Rings and dicentrics were lower in the popluation controls than in workers, were highest in Group 2 exposed and controls, and higher in Group1 exposed than controls. Actual exposures to benzene were not known, but were probably less in Group 3. The

reasons for the observed differences could be due to environmental factors, but differences in age, small numbers of counted cells, and perhaps inadequate comparison groups might explain some of these results.

Source: Deutsche Shell Chemie GmbH Eschborn

(736)

Remark: Results of cytogenetic analyses of peripheral blood lymphocytes from 58 benzene exposed shoe workers and 20 population controls without exposure to mutagenic or carcinogenic agents were compared. The exposed group had a higher percentage of smokers (68% vs. 35%) and alcohol drinkers (39% vs. 5%). The exposed group had higher frequencies of aberrations (breaks, gaps, rearrangements). Working period, smoking, and alcohol intake were thought to be unrelated to chromosomal changes in these groups (except for alcohol intake which was associated with increased frequency of breaks). No benzene exposure measurements weretaken, but in Turkey the MAC is 20 ppm, and Aksoy indicated that maximum benzene exposure for Turkish shoemakers was between 210-610 ppm.

Source: Deutsche Shell Chemie GmbH Eschborn

(737)

Remark: The authors calculated the incidence of leukemia among 28,500 shoeworkers chronically exposed to benzene in Istanbul. The maximum benzene concentrations to which theseworkers were exposed was 210-650 ppm. During the time period from 1967 to September 1973, 26 cases of acute leukemia or preleukemia were admitted to several clinics. Among these patients the duration of benzene exposure rangedfrom 1-15 years. The incidence of leukemia was calculated to be 13 per 100,000 workers, which is statistically significantly greater compared with the general population. The authors conclude that these data provide evidence for a leukemogenic effect of benzene in man.

Source: Deutsche Shell Chemie GmbH Eschborn

(738)

Remark: Two cases of acute leukemia (lymphoblastic and myeloblastic)are presented. The commonality of these cases is that the two are related (one is the paternal uncle of the other) andboth had chronic exposure to benzene from working as shoemakers. The thesis of this report is that genetic predisposition may be significant in susceptibility to benzene poisoning, and that some genetic factors were triggered by the 4-6 years of benzene exposure experienced at work by these patients.

Source: Deutsche Shell Chemie GmbH Eschborn

(739)

Remark: This is a review of benzene as a carcinogen and covers some of the same issues covered in Aksoy (1989). Topics include:incidence of leukemia among Turkish shoemakers; types of leukemia (distribution of types of leukemia -- AML most common; leukemia preceeded by pancytopenia; duration of exposure -- no clear relationship between duration of benzene exposure and type of leukemia; genetics and individual susceptibility; safe workplace exposure limits for benzene -- ~1 ppm); other malignancies due to benzene --malignant lymphoma, multiple myeloma, lung cancer.
Source: Deutsche Shell Chemie GmbH Eschborn

(740)

Remark: This is a review of hematotoxic and carcinogenic properties of benzene, supplemented with a summary of case or series findings from the author's patients. The suggested effects of benzene exposure and topics discussed include: aplastic anemia or pancytopenia; benzene – hepatitis – aplastic anemia syndrome; leukemia; leukemia in pancytopenia patientswith chronic benzene toxicity; genetic relationships and individual susceptibility in leukemia; other cancers -- malignant lymphomas, multiple myeloma, lung cancer. The conclusions of the author are suggestive rather than definitive.
Source: Deutsche Shell Chemie GmbH Eschborn

(741)

Remark: This is a nested case-control study of lymphocytic leukemia.The 15 cases and 30 matched controls were identified from a cohort of rubber workers. Potential for exposure to benzenewas based on occupational titles and types of solvents used from records of product specifications and standard operating procedures. Cases were 4.5 times more likely thancontrols to have had direct exposure to benzene and other solvents. Cases were 6.7 times more likely than controls tohave been exposed to coal-based solvents, and 1.5 times morelikely than controls to have been exposed to petroleum-basedsolvents. Coal-based xylene appeared more potent than coal-based benzene with odds ratios of 5.5 and 3.0, respectively.
Source: Deutsche Shell Chemie GmbH Eschborn

(742)

Remark: Mortality among more than 15,000 male employees of the Australian petroleum industry was examined from 1981 to 1989. All employees had at least 5 years of employment in the industry. Standardized mortality ratio (SMR) analysis indicated total cancer mortality was slightly lower comparedwith the national population. However, mortality due to lymphohematopoietic cancers, primarily leukemia (SMR=1.6; 95% confidence interval [CI] 0.6-3.4), was elevated, but this finding was not statistically significant. Mortality due to cancers of the pleura was also elevated (SMR=3.9; 95%CI=0.8-11.0), although two of the three cases were likely exposed to asbestos during employment in other industries.
Source: Deutsche Shell Chemie GmbH Eschborn

(743)

Remark: This study examined mortality among the Pliofilm cohort (n=1212 white male "wetside" workers) using an additional 6 years of follow-up (extended through December 31, 1987). Three different sets of job-, plant-, and year-specific exposure estimates were used in the analysis. The updated findings showed no additional cases of multiple myeloma; this does not support earlier Pliofilm reports of elevated mortality due to this disease. Extension of mortality follow-up did not substantially alter earlier estimates of leukemia risk associated with benzene exposure, and the choice of exposure estimated did not alter the conclusion. The results did, however, confirm the link between high dosebenzene exposures and leukemia.
Source: Deutsche Shell Chemie GmbH Eschborn

(744)

Remark: Using updated mortality information for the Pliofilm cohort (n=1868 Pliofilm workers from all departments), the authors performed proportional hazards modelling to estimate the dose-response for leukemia and occupational benzene exposure. Based on exposure estimates of Crump and Allen orof Paustenbach et al., 0.3-0.5 additional leukemia deaths per thousand workers with 45 ppm-years of cumulative benzeneexposure are predicted. These estimates are thought to be improved over earlier estimates by Rinsky et al., because proportional hazards modelling uses more of the available exposure data compared with the logistic modelling methods used by Rinsky et al.
Source: Deutsche Shell Chemie GmbH Eschborn

(745)

Remark: This is a case-control study nested within a cohort of men employed at 8 oil refineries in the U.K. Thirty-six leukemia cases were matched with two sets of 108 controls. The matching criteria included refinery and year of birth inone set, and in the second set of controls, length of service was an added criterion. Exposure to benzene was based on job history and qualitative ranking of low, medium,and high. The risk for men with medium/high benzene exposure was about twice that of the risk of men with low exposure. The authors conclude that if there is an increased risk of leukemia due to benzene exposure in oil refineries, only a very small proportion of workers could beaffected.
Source: Deutsche Shell Chemie GmbH Eschborn

(746)

Remark: This nested case-control study examined lymphohematopoietic cancer (LHC) mortatlity risk and longer term, lower benzene exposures among male marketing/distribution, marine and pipeline workers. Among the 31 cases studied, 16 had leukemia, 7 multiple myeloma and 8 non-Hodgkin's lymphoma. For each case, four male controls were selected from recordsfrom the same cohort, frequency matched to cases by decade of birth. Industrial hygienists estimated workplace

exposures for benzene and total hydrocarbons for every job/location/era combination. For leukemia, family history of cancer and cigarette smoking were the two strongest risk factors, with cumulative benzene exposure showing no additional effect when considered in the same models. No exposure-repsonse relationships were seen when benzene was categorized by several exposure metrics. The numbers of multiple myeloma and non-Hodgins lymphoma cases were too small to reliably charaterize risk with logistic modelling.

Source: Deutsche Shell Chemie GmbH Eschborn

(747)

Remark: This is a review of benzene leukemias and a compilation of cases of leukemia and anemia associated with benzene exposure. The number of cases is from the published literature, unpublished reports from Northern Italy, and theauthors experience in Milan. A synopsis of five new cases of benzene seen at the Institute of Occupational Health of Milan from 1964-1974 is provided.

Source: Deutsche Shell Chemie GmbH Eschborn

(748)

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(31) TRGS 900- Grenzwerte (Stand Januar 1994) BArbBl. Nr.2/1993 S. 57, Nr.9/1993 S.76, Nr. 11/1993 S.46, Nr. 1/1994 S.64

(32) Deutsche Forschungsgemeinschaft: MAK- und BAT-Werte-Liste 1993. VCH Verlagsgesellschaft mbH, Weinheim (1993)

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Note:
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7.1 Risk Assessment

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NICNAS

National Industrial Chemicals Notification and Assessment Scheme

Benzene

Priority Existing Chemical Assessment Report No. 21

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Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with Environment Australia (EA) and the Therapeutic Goods Administration (TGA), which carry out the environmental and public health assessments, respectively.

NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia. Chemicals selected for assessment are referred to as Priority Existing Chemicals.

This Priority Existing Chemical report has been prepared by the Director (Chemicals Notification and Assessment) in accordance with the Act. Under the Act manufacturers and importers of Priority Existing Chemicals are required to apply for assessment. Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made. Where variations are requested the Director's decision concerning each request is made available to each respondent and to other interested parties (for a further period of 28 days). Notices in relation to public comment and decisions made appear in the *Commonwealth Chemical Gazette.*

In accordance with the Act, publication of this report revokes the declaration of this chemical as a Priority Existing Chemical, therefore manufacturers and importers wishing to introduce this chemical in the future need not apply for assessment. However, manufacturers and importers need to be aware of their duty to provide any new information to NICNAS, as required under section 64 of the Act.

Copies of this and other Priority Existing Chemical reports are available from NICNAS either by using the prescribed application form at the back of this report, the website www.nicnas.gov.au or ordering directly from the following address:

GPO Box 58
Sydney
NSW 2001
AUSTRALIA

Overview

Benzene (CAS No. 71-43-2) was declared a Priority Existing Chemical on 7 April 1998 in response to occupational and public health concerns.

Benzene occurs naturally in fossil fuels and is produced incidentally in the course of natural processes and human activities that involve the combustion of organic matter such as wood, coal and petroleum products. The main industrial use of benzene is as a starting material for the synthesis of other chemicals. Most benzene feedstock is imported, but some is manufactured at an Australian steelworks as a by-product of coal coking. Large quantities of benzene are produced during the refining of petroleum and retained as a component of petrol. Petrol vehicle emissions are the predominant source of benzene in the environment.

Benzene is volatile and water-soluble and is considered biodegradable. Its major release is to the atmosphere, where it will break down in a matter of weeks. Direct release to the aquatic compartment is expected to be minor and significant removal will occur from volatilisation. Benzene release to soil is likely to be marginal. Concentrations in aquatic systems are expected to be far lower than of concern and a low aquatic risk is predicted. Due to the low expected exposure, a low environmental risk to terrestrial organisms is predicted. The short atmospheric lifetime of benzene indicates concentrations will not occur at levels harmful to the atmosphere. While widespread transport within the troposphere is possible, the chemical is not expected to reach the stratosphere and therefore would not have an influence on global warming or ozone depletion.

In animals and humans, benzene is absorbed by all routes of exposure, although dermal absorption is limited by its rapid evaporation from the skin. It is metabolised in the liver and several other organs, including the bone marrow. The parent molecule is eliminated with exhaled air. The metabolites are excreted in the urine.

In animals, benzene is not highly acutely toxic. Chronic exposure can result in central nervous system depression, immunosuppression, bone marrow depression, degenerative lesions of the gonads, foetal growth retardation, damage to genetic material and solid tumours in several organs.

In humans, acute exposure to high concentrations of benzene vapours can result in irritation of the skin, eyes and respiratory system and in central nervous system depression. Chronic exposure can result in bone marrow depression and leukaemia, particularly acute myeloid leukaemia, and possibly an increased risk of non-Hodgkin's lymphoma and multiple myeloma. Structural and numerical chromosome aberrations have been detected in peripheral blood cells of workers exposed to high levels of benzene. For bone marrow depression, the lowest observed adverse effect level in humans is 7.6 parts per million (ppm), based on minimal blood count changes in otherwise healthy workers. No threshold has been established for the genotoxic and carcinogenic effects of benzene. Epidemiological evidence indicates that the risk of leukaemia increases with exposure and is significantly elevated at cumulative exposures above 50 ppm-years, corresponding to an 8-hour time-weighted average exposure above 1.25 ppm over a working life of 40 years.

Chronic benzene toxicity has been attributed to the formation of reactive metabolites that appear to exert their toxic effect in combination, with no one metabolite accounting for all of the observed effects.

Benzene is currently listed in the NOHSC *List of Designated Hazardous Substances* with the following classification: 'Flammable', 'Carcinogen, Category 1' and 'Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed'. Category 1 carcinogens are those substances known to be carcinogenic to humans. Based on the assessment of health effects, this report has concluded that benzene also meets the NOHSC *Approved Criteria for Classifying Hazardous Substances* for classification as a skin, eye and respiratory system irritant and as a mutagenic substance in Category 3.

The public is exposed to benzene through the inhalation of indoor, in-vehicle and outdoor air contaminated with the chemical through releases that predominantly derive from vehicle exhaust, petrol evaporation and tobacco smoke. The 24-hour average lifetime exposure in the Australian urban population is estimated at 5.2 parts per billion (ppb). It is one-fifth higher in passive smokers exposed to tobacco smoke at home, at work and in their cars (6.1 ppb) and almost three times as high (15.2 ppb) in the average smoker.

Benzene-induced bone marrow depression is not expected to present a significant public health risk. Based upon low-dose extrapolation of relevant quantitative risk estimates and the above-mentioned exposure estimate, the excess lifetime risk of benzene-induced leukaemia in the Australian urban population is estimated to be in the order of one case per 10,000 with increased risk in sensitive subpopulations or at higher exposure levels. However, the estimated excess risk is based on substantial uncertainties in the exposure assessment which should be validated through collection of monitoring data.

As benzene is an established human carcinogen for which no safe level of exposure has been established, it is recommended that any increase in public exposure be avoided and that measures be taken to reduce exposure where this is practicable. The establishment of a national ambient air benzene level would facilitate these objectives.

Occupational exposure to benzene is predominantly by the inhalation route. It occurs primarily in the petroleum, steel, chemical and associated industries and in laboratories using the chemical for research or analysis. Occupational exposure to benzene can also result from the contamination of workplace environments with petrol vapours, engine exhaust or tobacco smoke, for example, in vehicle mechanics, professional drivers and hospitality workers. It is estimated that current long-term occupational exposures to benzene are less than or equal to 0.7 ppm in the steel and associated industries and during maintenance of phenol plants; less than 0.1 ppm in the upstream petroleum industry (oil and gas production); less than 0.5 ppm in the chemical industry and in laboratory workers; less than 0.2 ppm in vehicle mechanics; less than 0.7 ppm in the downstream petroleum industry (refining, distribution and marketing of petroleum products); and less than 0.05 ppm in people who work in roadside or in-vehicle environments contaminated with vehicle exhaust or in indoor environments contaminated with tobacco smoke.

The occupational risk characterisation found no cause for concern about acute health effects or bone marrow depression, given the control measures which are already in place in Australian workplaces. However, there is cause for concern about the risk for leukaemia in all workers with repeated occupational exposure to benzene. There is no known threshold for the carcinogenic effects of benzene, but because the risk for leukaemia increases with exposure, it can be reduced by controlling exposure to the highest practicable standard.

With regard to occupational health and safety, it is recommended that the national exposure standard for benzene be revised. It is recommended that an eight-hour time-weighted average of 0.5 ppm be adopted. It is further recommended that the current hazard classification be amended to include classification as 'Irritating to eyes, respiratory system and skin' (risk phrase R36/37/38) and as a mutagenic substance in Category 3 (risk phrase

R40: ‘Possible risks of irreversible effects, Mutagen Category 3’). Occupational exposures to benzene should be minimised by improving workplace control measures and by using the best available technology.

This report has identified the need to reduce public exposure to air benzene levels as much as practicable. Public health recommendations include measures to reduce indoor benzene levels, such as proper sealing of attached garages and minimising environmental tobacco smoke. In order to better characterise the risk to the public from benzene exposure, personal and ambient air monitoring is recommended and a national ambient air standard should be set.

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Abbreviations and Acronyms

ACGIH	American Conference of Governmental Industrial Hygienists
ADG	Australian Dangerous Goods
AIP	Australian Institute of Petroleum
ALC	absolute lymphocyte count
ALL	acute lymphatic leukaemia
AML	acute myeloid leukaemia
ANLL	acute non-lymphocytic leukaemia
atm	atmosphere
Avgas	light aircraft gasoline
BCF	bioconcentration factor
BP	benzene poisoning
BTX	benzene/toluene/xylenes
BW	body weight
C	centigrade
CAS	Chemical Abstracts Service
CFU-E	colony forming units of erythrocyte progenitor cells
CFU-M	colony forming units of granulocyte/macrophage progenitor cells
CI	confidence interval
CLL	chronic lymphatic leukaemia
cm	centimetre
cm^2	square centimetre
cm^3	cubic centimetre
CMA	Chemical Manufacturers Association
CML	chronic myeloid leukaemia
CNS	central nervous system
CSF	colony stimulating factor
CYP	cytochrome-P450
DNA	deoxyribonucleic acid
EA	Environment Australia
EC_{50}	median effective concentration
EINECS	European Inventory of Existing Chemical Substances
EPA	Environment Protection Authority
ETS	environmental tobacco smoke
g	gram
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GD	gestation day
GLP	Good Laboratory Practices
GM-CSF	granulocyte/macrophage colony stimulating factor
GSH	glutathione
GST	glutathione-S-transferase
h	hour
ha	hectare
Hb	haemoglobin
Hct	haematocrit

IARC	International Agency for Research on Cancer
IL-1	interleukin-1
IPCS	International Programme on Chemical Safety
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union for Pure and Applied Chemistry
K	Kelvin
kg	kilogram
K_m	Michaelis-Menten constant
km	kilometre
km^2	square kilometre
K_{oc}	sorption coefficient
kPa	kilopascal
kt	kilotonne
L	litre
LC	lymphocyte
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest observed adverse effect level
LP	leaded petrol
LPG	liquid pressurised gas
m^3	cubic meter
MCV	mean corpuscular volume
MDS	myelodysplastic syndrome
mg	milligram
mL	millilitre
ML	megalitre
mM	millimolar
MM	multiple myeloma
MN	micronucleus
mol	mole
MSDS	material safety data sheet
NADPH	nicotinamide adenine dinucleotide phosphate
NEPC	National Environment Protection Council
NEPM	National Environment Protection Measures
ng	nanogram
NHIS	National Health Interview Survey
NHL	non-Hodgkin's lymphoma
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute of Occupational Safety and Health
nm	nanometre
nmol	nanomole
nM	nanomolar
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOHSC	National Occupational Health and Safety Commission
NPI	National Pollutant Inventory
NQO1	NAD(P)H:quinone oxidoreductase
NSAID	non-steroidal anti-inflammatory drug
OECD	Organisation for Economic Co-Operation and Development
OR	odds ratio

OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PGE_2	prostaglandin E_2
PKC	protein kinase C
Plt	blood platelet
PNEC	predicted no-effect concentration
$P_{o/w}$	octanol/water partition coefficient
POP	persistent organic pollutant
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
PULP	premium unleaded petrol
RBC	red blood cell (erythrocyte)
RNA	ribonucleic acid
RR	relative risk
s	second
SAb	spontaneous abortion
s-AML	secondary acute myeloid leukaemia
SC	subcutaneous
SCE	sister chromatid exchange
SGA	small-for-gestational age
SIR	standardised incidence rate
SMA	State marketing area
SMR	standardised mortality rate
STP	sewage treatment plant
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
t	tonne
TGD	Technical Guidance Document
TWA	time-weighted average
TWA_8	8-h time-weighted average
ULP	unleaded petrol
UN	United Nations
USEPA	United States Environmental Protection Agency
$UTL_{95\%,95\%}$	upper tolerance limit of a distribution's 95th percentile
v/v	volume/volume
VOC	volatile organic chemical
w/w	weight/weight
WBC	white blood cell (leukocyte)
WHO	World Health Organization
y	year
µg	microgram
µL	microlitre
µM	micromolar
µmol	micromole
8-OHdG	8-hydroxydeoxyguanosine

1. Introduction

1.1 Declaration

The chemical benzene (CAS No. 71-43-2) was declared a Priority Existing Chemical for full assessment under the *Industrial Chemicals (Notification and Assessment) Act 1989* on 7 April 1998. It was nominated by the public because of concerns about its human health effects and the adequacy of the current Australian occupational exposure standard.

1.2 Objectives

The objectives of this assessment were to:

- characterise the properties of benzene;
- determine the uses of benzene in Australia;
- determine the extent of occupational, public and environmental exposure to benzene;
- characterise the intrinsic capacity of benzene to cause adverse effects on persons or the environment;
- characterise the risks to humans and the environment resulting from exposure to benzene; and
- determine the extent to which any risk is capable of being reduced.

1.3 Sources of information

Consistent with the objectives, this report presents a summary and critical evaluation of relevant information relating to the potential health and environmental hazards from exposure to benzene. Relevant scientific data were submitted by the applicants listed in Section 3, obtained from published papers identified in a comprehensive literature search of several online databases up to December 2000, or retrieved from other sources such as the reports and resource documents prepared for the health surveillance program of the Australian Institute of Petroleum (AIP) and the Illawarra leukaemia cluster investigation. Due to the availability of several peer-reviewed overseas assessment reports, not all primary sources of data were evaluated. However, relevant studies published since the cited reviews were assessed on an individual basis.

The characterisation of health and environmental risks in Australia was based upon information on use patterns, product specifications, occupational exposure and emissions to the environment made available by applicants and relevant State authorities. Information to assist in the assessment was also obtained through site visits and telephone interviews. The site visits included two petroleum refineries, two petrol terminals, a steelworks, a coal tar distillery, a bulk liquid storage facility and three chemical plants.

1.4 Peer review

During all stages of preparation, the report has been subject to internal peer review by NICNAS, Environment Australia (EA) and the Therapeutic Goods Administration (TGA). Selected parts of the report were also peer reviewed by Professor Tom Beer, CSIRO Atmospheric Research (Sections 8 and 15); Dr Stephen Corbett, New South Wales Department of Health (Section 11); Dr Andrea Hinwood, Department of Environmental Protection, Western Australia (Sections 9, 11 and 13); and Professor Martyn T. Smith, University of California (Sections 9 and 12).

2. Background

2.1 Introduction

Benzene is a naturally occurring, hazardous, volatile organic compound which is ubiquitous in the environment. It is formed from biomass under the impact of heat, pressure and geological time. As such, it is present in fossil fuels which may release it to air when unearthed and, in particular, when heated to combustion. Benzene is also a product of natural processes and human activities that involve the instantaneous thermal degradation of organic matter. These sources of entry include bush fires, crop residue and forest management burning, petroleum refining, petrol combustion, wood and charcoal fires, fumes from heated cooking oils, tobacco smoke, incense, and waste incineration. In addition, benzene enters the environment in emissions and waste streams from industrial processes and waste disposal facilities.

2.2 International perspective

Benzene was first isolated in 1825 and gradually became widely used as a solvent and starting material for the synthesis of a number of organic chemicals (Folkins, 1984). Benzene also became recognised as a valuable constituent of petrol because of its antiknock properties and ability to increase the octane rating of automotive fuels.

Until World War II, benzene was isolated from light oil, which is a by-product of the carbonisation of coal to produce gas for heating or coke for the blast furnaces of the steel industry. Beginning in the 1930s, new catalytic and thermal processes for the production of aromatic hydrocarbons from crude oil were discovered and commercialised in the petroleum industry. With the advent of natural gas in the 1960s, worldwide coal gas production started to diminish. Simultaneously, the introduction of modern steel processing methods decreased coke production and made it attractive to burn the light oil as fuel rather than segregate it into benzene and other products. In consequence, the petroleum industry is now the predominant source of benzene.

In recent years, the use of benzene-containing solvents has been practically eliminated because of the toxicity of the chemical. Current worldwide consumption of benzene is 30-35 million metric tonnes (t) per annum, primarily as chemical feedstock in the production of large-scale intermediates such as ethyl benzene, cumene and cyclohexane (Chemistry & Industry News, 1996). This figure does not include benzene produced by the petroleum industry and retained as a petrol component.

Commercial low-grade qualities are sometimes referred to as benzol. Benzene is not to be confused with benzine, which is a mixture of several low-boiling hydrocarbons obtained in the distillation of petroleum.

2.3 Australian perspective

Developments in Australia have followed the general pattern outlined above, albeit with a delay of 1-2 decades. The recovery of benzene from coal gas is now limited

to the steelworks at Port Kembla in New South Wales and Whyalla in South Australia.

There are eight petroleum refineries in Australia: two in Brisbane and Sydney and one in Adelaide, Geelong, Melbourne and Perth. Since the 1970s, close to 100% of local demand for petrol has been met from crude which is low in aromatic fractions (Tresider, 1998). As such, all Australian petroleum refineries have processes in place to increase the content of aromatic hydrocarbons including benzene in their petrol blendstock. Petroleum-derived benzene feedstock for the chemical industry is not produced in Australia.

As of 1986, new petrol-driven cars had to be fitted with catalytic converters and use unleaded fuel. An Australian Standard for petrol for motor vehicles was established in 1990 and limited the benzene content to a maximum of 5% v/v (Standards Australia, 1990). In 1998, the average benzene content in Australian petrol was 2.9, 2.6 and 3.3% v/v in leaded petrol (LP), unleaded petrol (ULP) and premium unleaded petrol (PULP) respectively (AIP, 1998b). The *Fuel Quality Standards Act 2000* enables the Commonwealth to make mandatory national quality standards for fuel supplied in Australia. Among others, these will include a maximum content of benzene in petrol of 1% v/v from January 1 2006. Meanwhile, Western Australia and Queensland have introduced regulations limiting the benzene content in petrol to 1% and 3.5% respectively (EA, 2000b).

In 1980, AIP contracted The University of Melbourne to set up an epidemiological health surveillance program called Health Watch. The program covers about 95% of the industry's 18,000 employees in refineries, natural gas plants, distribution terminals and production sites. It consists of a prospective cohort study of all-cause mortality and cancer incidence, in addition to a case-control study of lympho-haematopoietic cancers and benzene exposure established in 1988 (Glass et al., 1998, 2000; Health Watch, 1998).

Air pollution became a major concern in the 1990s and prompted environment and health authorities from the Commonwealth, States and Territories to initiate several research projects into ambient air quality. Early results of this research resulted in the inclusion of benzene in the National Pollutant Inventory (NPI), which was established by the National Environment Protection Council (NEPC) in 1998. The NPI currently comprises 36 chemicals of health and environmental concern which must be reported to EA if the quantity used or handled per site exceeds a threshold limit, which for benzene is 10 t per year (EA, 1999b). More recently, the Australian and New Zealand Environment and Conservation Council contracted the Victorian Environment Protection Authority (EPA) to assess the available air level data and derive a risk-based rank order of hazardous air pollutants according to their priorities for further research (EPA Victoria, 1999). Based on a scoring system as well as on professional judgement, benzene came first among 15 chemicals recommended for general urban air monitoring. Benzene is also the subject of a publication in the series of National Environmental Health Forum Monographs, which are intended to provide plain language information about important, topical environmental health matters (Wadge & Salisbury, 1997). Current EA initiatives such as the Fuel Quality Review and Living Cities – Air Toxics Program both address a number of environmental aspects relating to benzene (EA, 2000a, 2000b).

Public concern about exposure to benzene reached a peak in 1996, when a cluster of leukaemia cases was identified in people living in the suburbs adjacent to the coke ovens and coal gas by-product plant at the Port Kembla steelworks. A committee reporting to the New South Wales Department of Health was set up to

investigate the matter. It concluded that based on the available data, it was not possible to ascribe the cluster to a particular exposure (including benzene). The investigation produced several useful publications relating to benzene and the risk of leukaemia (ILISC, 1997; Westley-Wise et al, 1999).

2.4 Assessments by other national or international bodies

Although there have been restrictions on the manufacture, handling, storage and use of benzene in Australia since 1978, this report represents the first comprehensive risk assessment by a national agency.

Benzene has been assessed by several overseas or international bodies involved in the review or evaluation of data pertaining to the health and/or environmental hazards posed by chemicals. Of these, the most noteworthy are:

- The Advisory Committee to the German Chemical Society on Existing Chemicals of Environmental Relevance (GDCh, 1988);
- The Agency for Toxic Substances and Disease Registry under the US Department of Health and Human Services (ATSDR, 1997);
- The Commission of the European Communities (EC, 1989, 2000);
- Environment and Health Canada (Government of Canada, 1993);
- The International Agency for Research on Cancer (IARC, 1982a, 1987);
- The International Programme on Chemical Safety (IPCS, 1993);
- The UK Department of the Environment (DoE, 1994);
- The US Environmental Protection Agency (USEPA, 1985, 1998a, 1998c); and
- The OECD SIDS International Assessment Report (draft) (OECD, 2000).

3. Applicants

Following the declaration of benzene as a Priority Existing Chemical, 21 companies or organisations applied for assessment of the chemical. The applicants supplied information on the properties, import and manufacturing quantities and uses of benzene and, in some cases, on occupational exposures and releases to the environment. In accordance with the *Industrial Chemicals (Notification and Assessment) Act 1989*, NICNAS provided the applicants with a draft copy of the report for comments during the corrections/variation phase of the assessment. The applicants were as follows:

Alltech Associates (Australia) Pty Ltd
PO Box 6005
Baulkham Hills NSW 2153

Australian Institute of Petroleum
GPO Box 279
Canberra ACT 2601

Australian Council of Trade Unions
393 Swanston Street
Melbourne VIC 3000

Australian Manufactures Workers Union
3/440 Elizabeth Street
Melbourne VIC 3000

Bio-Scientific Pty Ltd
PO Box 78
Gymea NSW 2227

BP Australia Holding Limited
360 Elizabeth St
Melbourne VIC 3000

Caltex Petroleum Australia Pty Ltd
19-29 Martin Pl
Sydney NSW 2000

Crown Scientific Pty Ltd
Private Mail Bag 4
Moorebank NSW 2170

Huntsman Chemical Company Australia Pty Ltd
PO Box 62
West Footscray VIC 3012

ICN Biomedicals Australasia
PO Box 187
Seven Hills NSW 2147

Whyalla Steelworks (OneSteel Manufacturing)
PO Box 21
Whyalla SA 5600

Koppers Coal Tar Products Pty Ltd
PO Box 23
Mayfield NSW 2304

Merck Pty Ltd
207 Colchester Rd
Kilsyth VIC 3137

Mobil Oil Australia Pty Ltd
417 St Kilda Rd
Melbourne VIC 3004

BHP Steel – Flat Products
PO Box 1854
Wollongong NSW 2505

Qenos Pty Ltd
Private Bag 3
Altona VIC 3018

Selby-Biolab
Private Bag 24
Mulgrave North VIC 3170

Sigma-Aldrich Pty Ltd
PO Box 970
Castle Hill NSW 2154

Terminals Pty Ltd
PO Box 268
Footscray VIC 3011

3M Australia Pty Ltd
PO Box 144
St Marys NSW 2760

Trafigura Fuels Australia Pty Ltd
Unit 2, 47 Epping Rd
North Ryde NSW 2113

4. Chemical Identity and Composition

4.1 Chemical name (IUPAC)

Benzene

4.2 Registry numbers

Benzene is listed on the Australian Inventory of Chemical Substances (AICS) as *benzene.*

CAS number	71-43-2
EINECS number	200-753-7
UN number	1144

4.3 Other names

Annulene

Benzol(e)

Bicarburet of hydrogen

Carbon oil

Coal naphtha

Cyclohexatriene

Mineral naphtha

Motor benzol

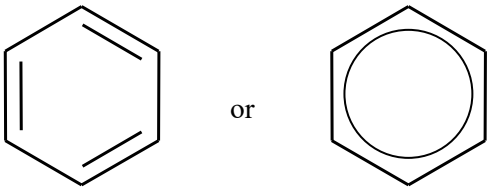
Phenyl hydride

Pyrobenzol(e)

4.4 Molecular formula

C_6H_6

4.5 Structural formula



or

4.6 Molecular weight

78.11

4.7 Composition of commercial grade product

Several different grades of benzene are commercially available. The principal impurities are toluene, xylenes and other hydrocarbons with boiling points similar to that of benzene. The higher the grade, the lower the content of thiophene (thiofuran) and other sulfur compounds, which foul many catalysts used in reactions of benzene (Fruscella, 1992). The specifications for two typical import grades and the benzene/toluene/xylenes (BTX) mixture produced at the Port Kembla steelworks are shown in Table 4.1.

Table 4.1: Raw material specifications for some commercially available benzene grades

Test	Pure benzene	Crude benzene	BTX
Benzene (% v/v)	>99	≥95	≥80
C_9 & higher (% v/v)	-	<1.5	<1.6
Carbon disulfide (ppm)	-	<50	<4000
H_2S & SO_2	None	-	-
Non-aromatic C_5-C_6 (% v/v)	<0.15	<0.7	<1.5
Styrene (% v/v)	-	-	<1.8
Thiophene (ppm)	<1	<6000	<6000
Toluene (% v/v)	-	-	<12.5
Total sulfur (ppm)	-	-	<6000
Xylenes & styrene (% v/v)	-	-	<3.8

5. Physical and Chemical Properties

5.1 Physical state

Benzene is a volatile, colourless and flammable liquid with a characteristic, sweet aromatic odour (Budavari, 1996). The odour threshold ranges from 0.8-160 ppm (AIHA, 1989); 50% of the population can identify the odour at 2 ppm and 100% at 5ppm (Verscheuren, 1996). The physical properties of benzene are summarised in Table 5.1.

Conversion factors (at 25°C):

1 mg/m^3 = 0.31 ppm *and* 1 ppm = 3.2 mg/m^3 (Cavender, 1994).

5.2 Physical properties

Table 5.1: Physical properties

Property	Value	Reference
Melting point	5.53ºC	Folkins (1984)
Boiling point	80.1ºC	Folkins (1984)
Density • at 15ºC • at 20ºC • at 25ºC	 0.885 kg/L 0.879 kg/L 0.874 kg/L	 Fruscella (1992)
Vapour density	2.8 (relative to air = 1)	Cavender (1994)
Vapour pressure • at 0ºC • at 20ºC • at 25ºC • at 40ºC • at 50ºC	 3.47 kPa 9.97 kPa 12.6 kPa 24.2 kPa 35.8 kPa	 Folkins (1984), Fruscella (1992)
Water solubility (at 25ºC)	1.80 g/L	IPCS (1993)
Henry's Law constant (at 20ºC)	0.56 kPa.m^3/mol	Mackay & Leinonen (1975)
Partition coefficient (log $P_{o/w}$)	1.56-2.15	IPCS (1993)
Sorption coefficient (log K_{oc})	1.8-1.9	IPCS (1993)
Flash point (closed cup)	– 11ºC	Fruscella (1992)
Autoignition temperature	560ºC	Fruscella (1992)
Explosive limits • lower • upper	 1.4% v/v (45 g/m^3) 7.9% v/v (250 g/m^3)	 Cavender (1994)

5.3 Chemical properties

The six carbon atoms of benzene form a regular hexagon and all 12 atoms lie in a single plane, with all bond angles being exactly 120° (Fruscella, 1992). The molecule is traditionally depicted as having alternating single and double bonds (see structure (1) in Section 4). However, as the six carbon-carbon bonds are

physically and chemically identical and intermediate in length between single and double bonds (as indicated by structure (2) in Section 4), benzene does not react as a typical unsaturated compound.

Benzene has great thermal stability and elevated temperatures are required for its decomposition. It undergoes substitution and addition reactions and ring cleavage. For industrial applications, the most important reactions are alkylation with ethylene or propylene to produce ethyl benzene or cumene, hydrogenation to cyclohexane, nitration and sulfonation to form nitrobenzene and benzenesulfonic acid, and halogenations. Benzene cannot be hydrolysed.

Benzene is miscible with numerous other organic solvents including alcohol, acetone, diethyl ether, ethyl acetate, chloroform, carbon disulfide, glacial acetic acid and oils (Budavari, 1996). Its solubility in water ranges from 1.13% v/v at 25°C to 5.07% v/v at 107°C (Folkins, 1984). Benzene forms binary and tertiary azeotropes with water and a large number of organic substances (for examples, see Folkins (1984)).

Benzene is highly flammable and potentially explosive. Combustion products include carbon dioxide, water vapour and carbon monoxide. With a deficiency of air or oxygen, partial decomposition and soot deposition occur (Folkins, 1984). Vapours burn with a sooty flame.

6. Methods of Detection and Analysis

6.1 Characterisation

Benzene can be characterised by infrared, ultraviolet and mass spectrometry and nuclear magnetic resonance techniques (Fruscella, 1992).

6.2 Detection and analysis

A time-honoured spot test for benzene in the workplace or surroundings involves the treatment of a sample with nitric acid followed by ether extraction and dissolution in a mixture of alcohol and methyl ethyl ketone. Benzene is converted to *m*-dinitrobenzene which imparts a persistent red colour to the solution (Dolin, 1943, cited in Fruscella, 1992).

Standard analytical methods for benzene in air, water, soil, foods, smoke, biological samples, petroleum products etc. rely on gas chromatography (GC) with flame or photo ionisation detection, or on gas chromatography-mass spectrometry (GC-MS) (Fruscella, 1992; IPCS, 1993). Benzene in water, soil and food is usually measured by a purge and trap method by bubbling an inert gas through the sample and collecting the chemical on an absorbent. Benzene is then desorbed and determined. The best available GC methods are able to detect benzene at 0.1 ppb in air or 1 ng/kg in liquid or solid media, although 3 ppb in air and 1μg/L in water are the limits of detection in routine analysis (IPCS, 1993; NHMRC, 1996). The GC-MS method is not quite as sensitive, but more reliable in the case of samples with multiple components with retention times similar to that of benzene (IPCS, 1993).

6.3 Atmospheric monitoring methods

In the environment

The methods commonly used for measuring the concentration of benzene in ambient air fall into the following two categories (EPA Victoria, 1999):

(1) discrete air sampling with subsequent laboratory analysis; or

(2) continuous or semi-continuous in-field analysis.

Among the former, the most widely used method involves the collection of air into a stainless steel canister over a predetermined period of time such as 24 h, followed by analysis of a concentrate of the air sample by GC or GC-MS. This method is described in more detail by DEP Western Australia (2000).

A commonly used continuous method for in-field analysis utilises an optical remote sensing system to determine the concentration of the chemical by means of the differential absorption of transmitted light by gaseous compounds along the light path. The system consists of a light transmitter and sensor placed at a given distance apart at the monitoring site.

Alternatively, the concentration in air can be analysed by semi-continuous gas chromatography. Samples of air are collected directly onto solid absorbents,

desorbed thermally onto the GC column and analysed while the next sample is collected.

The analytical limit of detection of the above methods typically ranges from 0.003-0.1 ppb. All of the methods allow for the simultaneous determination of several other gaseous air pollutants in the same sample. Discrete sampling methods determine average pollutant levels over the sample collection time. Continuous or semi-continuous methods enable more detailed information about concentration variations to be obtained.

In the workplace

This section summarises the methods commonly used for the measurement of benzene in the workplace. Other past and present techniques are described in a recent review by Verma & des Tombe (1999a, 1999b).

For personal monitoring during full shifts or tasks, workers are equipped with a charcoal tube or badge placed in the breathing zone. For area monitoring, the tube or badge is placed at a fixed location in the workplace environment. Tubes are connected with a portable metering pump, whereas badges sample the air by diffusion. At the end of the sampling period, the tube or badge is sealed and transferred to a laboratory, where the chemical is liberated from the absorbent by elution or thermal desorption and quantified by GC (NIOSH, 1994). The result is expressed as a time-weighted average (TWA) concentration in ppm or mg/m^3 over the duration of the sampling period. The analytical detection limit depends on the airflow across the absorbent and the duration of the sampling period. The detection limit using charcoal tube sampling and analysis according to the NIOSH method is 0.02 ppm.L, or 0.004 ppm for a sample collected over 60 min at a pump speed of 80 mL/min (IPCS, 1993). The agreement between the tube and badge methods is not perfect, but the differences are generally of little importance (Hotz et al., 1997; Purdham et al., 1994).

'Grab sampling' or instantaneous measurement of the concentration of airborne benzene is conducted with colorimetric detector tubes. These are glass tubes sealed at both ends with a graduated concentration scale etched into the outer surface. The tubes contain a carrier material covered with chemical reagents that react with benzene to produce a colour change whose end-point is read against the scale. Prior to use, the seals are broken, the tube is connected to a hand pump and the pump is operated to draw a defined amount of air through the tube. A colorimetric detector has become available which can measure benzene at 0.2 ppm in the presence of other hydrocarbons, with a measuring time of 8 minutes. Non-selective photoionisation detectors can be used for instantaneous measurement of benzene concentrations with a detection limit of approximately 0.1 ppm but, because the detector also responds to volatile organic chemicals, they are limited to situations where the vapour is known to be pure benzene. Recently, a benzene-selective photoionisation detector has become available which is claimed to be able to measure benzene at concentrations of 0.1 ppm – 200 ppm in the presence of other hydrocarbons within approximately 1 minute. The detector uses a single use pre-treat tube to filter out interfering hydrocarbons except for $\leq C_3$ alkanes and must be calibrated against 5 ppm benzene prior to use.

A less widely used method is continuous area monitoring, which is performed by pumping air collected at one or more fixed locations through an auto analyser equipped with an ultraviolet spectrometer. This method delivers readings for TWA

as well as peak concentrations and has a limit of detection of approximately 0.2 ppm (IPCS, 1993).

6.4 Biological monitoring methods

Biological monitoring for benzene exposure involves the measurement of unmetabolised benzene in blood, urine or breath samples, of benzene metabolites in the urine, or of protein adducts with the benzene oxide metabolite.

The concentration of benzene in venous blood and urine can be determined by GC, with a detection limit of around 0.5 μg/L (IPCS, 1993). For the determination of benzene in breath air, an end-exhaled sample is collected and analysed by GC-MS, with a detection limit of 3-6 ppb (Money & Gray, 1989). However, these methods are only suitable for research purposes, as great care must be exercised to avoid contamination of the samples with ambient benzene.

Various metabolites are excreted in the urine, including phenol, hydroquinone and catechol conjugates, S-phenylmercapturic acid and *trans,trans*-muconic acid (see Section 9.3), although none of them is formed exclusively from benzene. These metabolites can be quantified by GC, GC-MS or high-performance liquid chromatography as described by Ducos et al. (1990), Hotz et al. (1997), Lee et al. (1993), Popp et al. (1994) and others. Whereas the urinary concentration of phenol has been widely used as an index of benzene exposure, the background levels make it unreliable at exposures <5-10 ppm. The concentration of S-phenylmercapturic acid or muconic acid relative to creatinine in an end-of-shift urine sample has been shown to be a fairly good indicator of exposure in the 0.25-1 ppm range, even in smokers (Ghittori et al, 1995; Hotz et al, 1997; Ong et al, 1996).

The metabolite benzene oxide binds to nucleophilic sites and forms phenyl cysteine residues with proteins such as haemoglobin and albumin (see Section 9.3.1). The concentration of such adducts in blood correlates with benzene exposure. However, high background levels severely limit the practical use of the S-phenyl cysteine adduct as a biological marker for benzene uptake (Yeowell-O'Connell et al, 1998).

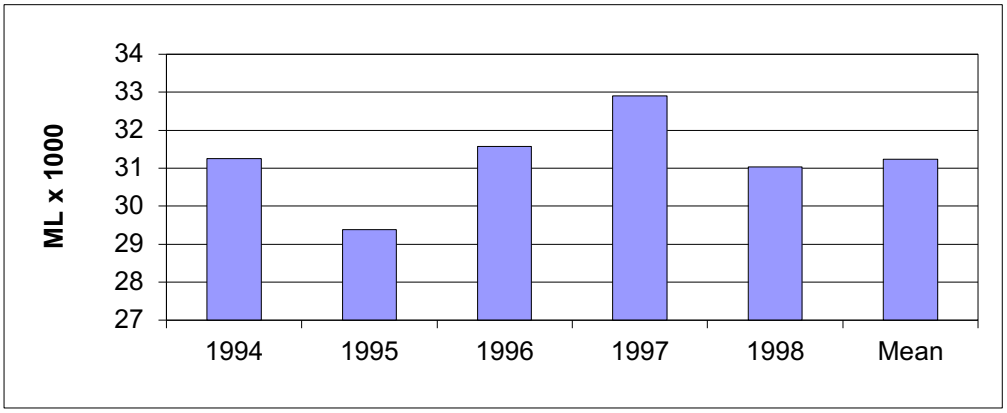
7. Manufacture, Importation and Use

7.1 Manufacture and importation

Benzene is introduced into Australia through extraction, importation and manufacture.

The total Australian production of crude in 1994-98 is shown in Figure 7.1. Crude includes unrefined oil as well as condensate, which is a liquid mixture of hydrocarbons recovered from gas wells. The mean annual and 1998 volumes are both approximately 31,000 ML. Australian crude is reported to have a low benzene content, estimated at about 0.1% v/v (Glass et al, 1998). As such, the annual extraction of benzene from Australian oil and gas fields can be estimated at approximately 31 ML. As the density of benzene is around 0.88 kg/L, this corresponds to a quantity of 27 kilotonnes (kt) pure benzene.

Figure 7.1: Australian production of crude oil and condensate 1994-98 (AIP, 1999b)



The throughput of crude at Australian refineries in 1998 was 44,678 ML, of which 62% was of non-Australian origin (AIP, 1999a). Most of the imports come from oil fields in the Pacific basin and contain approximately the same concentration of benzene as Australian crude, that is, about 0.1% v/v (Glass et al, 1998). This corresponds to a total input of 45 ML or 39 kt pure benzene.

From these figures, it can be concluded that Australia is a net importer of benzene in crude to the tune of approximately 12 kt per annum.

Table 7.1 shows the throughput of benzene-containing gasoline products, that is, leaded petrol (LP), unleaded petrol (ULP), premium unleaded petrol (PULP) and light aircraft gasoline (Avgas), at Australian refineries in 1998. The table also gives the average content of benzene and the corresponding quantities of pure benzene. Data for Avgas are estimates, but have little impact on the total. Other petroleum products such as liquefied petroleum gas, kerosene, civil aviation jet fuel, diesel oil, fuel and heating oils and lubricants contain no or practically no (<0.02% v/v) benzene (AIP, 1999a; IARC, 1989; Potter & Simmons, 1998).

The volume of pure benzene in petrol produced at Australian refineries in 1998 was 484 ML, corresponding to 426 kt pure benzene. As the throughput crude contained

approximately 39 kt pure benzene, it can be concluded that the production of benzene in the Australian petroleum industry amounted to 387 kt in 1998.

Table 7.1: Throughput of LP, ULP, PULP and Avgas at Australian refineries in 1998 (AIP, 1998b, 1999b)

	LP	ULP	PULP	Avgas	Total
Petrol (ML)	4965	12,218	640	100	17,923
Benzene (% v/v)	2.9	2.6	3.3	1.0	-
Pure benzene (ML)	144	318	21	1	484

Petrol is also imported in finished form. From January 1994 through August 1999, petrol imports averaged 680 ML per annum (DISR, 1999). There is no information on the benzene content of imported petrol, but it is unlikely to differ much from that of Australian ULP, which averages 2.6% v/v. As such, annual imports of benzene as an ingredient in petrol purchased overseas is estimated at 18 ML corresponding to approximately 15 kt pure benzene.

The Port Kembla steelworks produces 20-22 ML per annum of a commercial low-grade benzene product called BTX. As the specifications stipulate a benzene content of ≥80% v/v (Table 4.1), this corresponds to approximately 14.0-15.5 kt pure benzene per annum. The Whyalla steelworks no longer produce BTX, however, 0.12kt of benzene per annum, from the naphthalene still, is reinjected into the fuel gas stream.

Benzene is also produced as a by-product stream component at two olefins (pyrolysis) plants belonging to Qenos Pty Ltd. The total quantity amounts to approximately 15 kt per annum, all of which is exported for use or further processing overseas.

The only importer of benzene feedstock is Huntsman Chemical Company, whose annual imports are stable at about 50 kt pure benzene and 30 kt crude benzene (≥95% v/v), that is, approximately 80 kt pure benzene per annum.

Minor quantities not exceeding 1 t in the aggregate are imported for laboratory and other small-scale uses, as described below.

7.2 Manufacturing processes and end use

7.2.1 Petroleum industry

Table 7.2 provides an overview of the location and ownership of the currently operating oil refineries in Australia, as well as a summary of the processes employed to produce benzene, their capacity, and the benzene content of locally produced petrol. The latter is taken from a 1994 survey, which reported the concentration in % w/w (Tresider, 1998). As such, it is not directly comparable to the data provided in Table 7.1[1]. The process technologies and end use of the benzene produced are described below.

[1] The factor needed to convert % w/w to % v/v varies with the density of the petrol. Based on the average density of Australian petrol in 1999, multiplication of the concentration in % w/w with 0.84 will give an approximate concentration in % v/v (Exxonmobile personal communication, 2001).

Table 7.2: Benzene processes at Australian oil refineries (AIP, 1997; Mobil, 2000; Tresider, 1998)

State	Location	Owner	Benzene technology	Capacity (kt/y)*	Benzene in LP/ULP/PULP (% w/w)
NSW	Clyde	Shell	Reforming Cracking	890 1558	2.7/ 2.4/3.6
	Kurnell	Caltex	Reforming Cracking	1344 2024	2.3/2.3/4.7
QLD	Bulwer Island	BP	Reforming Cracking	588 912	2.5/3.7/5.0
	Lytton	Caltex	Reforming Cracking	1157 1469	2.4/2.5/4.4
SA	Port Stanvac	Mobil	Reforming	1157	2.3/2.0/2.5
VIC	Altona	Mobil	Reforming Cracking	1380 1246	4.8/4.5/5.5
	Geelong	Shell	Reforming Cracking	1380 1780	3.6/3.6/5.4
WA	Kwinana	BP	Reforming Cracking	979 1456	1.9/2.0/2.2

* The capacity is calculated on the basis of 350 stream days per year and refers to the quantity of raw material processed, not benzene produced.

Based on more recent information (AIP average data for 1999), ULP contains approximately 3.01% (w/w) benzene, PULP contains 4.02% (w/w) and LP contains 3.46 (w/w) (Exxonmobile Personal Communication, 2001).

Petroleum refining

Petroleum refining involves a series of continuous, enclosed processes designed to convert crude oil and condensate into end products such as liquefied petroleum gas, Avgas, petrol, jet fuel, diesel, heating oil, lubricants and bitumen. The main processes designed to augment the content of aromatics such as benzene in petrol are shown in the flowchart in Figure 7.2 and summarised below.

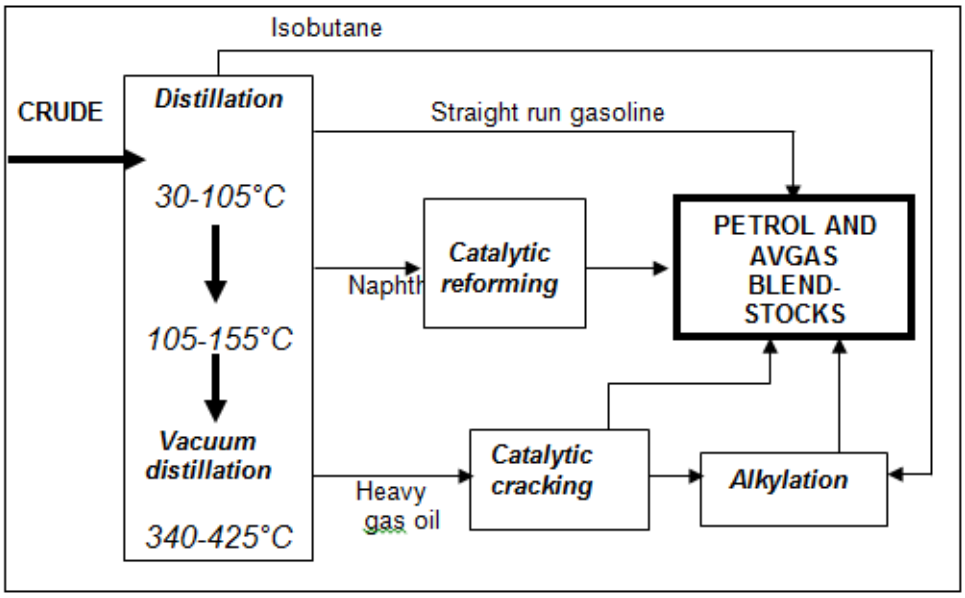


Figure 7.2: Benzene production in petroleum refineries

At all refineries, crude oil is first separated into a number of fractions by atmospheric and vacuum distillation. Petrol is a blend of butane, refined naphthas, isomerate, reformate, cracked gasoline and alkylate. Avgas is primarily made from alkylate although reformate can also be used.

The straight run gasoline fraction contains a 5- to 10-fold concentrate of all the benzene that was present in the crude, corresponding to a benzene concentration of 0.5-1% v/v.

The naphtha fraction, which contains many cyclic, saturated hydrocarbons, undergoes catalytic reforming in a process using heat, pressure and a platinum catalyst to convert a portion of the feedstock to aromatic compounds. The resulting reformate typically contains 4-8% v/v of benzene (Audrey, 1994).

At the Mobil Altona refinery, part of the heavy gas oil fraction is piped to a nearby petrochemical plant for steam cracking, as described below. This process gives rise to a by-product known as steam cracked naphtha or pyrolysis gasoline, which contains 6-8% benzene. This by-product stream is piped back to the oil refinery where it is stored in floating-roof tanks and eventually exported to overseas customers by shipping tanker.

The heavy gas oil fraction, which contains large, high boiling hydrocarbon molecules, is cracked to a mixture of lower molecular weight compounds by means of heat, pressure and a silica/aluminium oxide or zeolite catalyst. The benzene content of cracked gasoline rarely exceeds 1-2% v/v, but varies depending on the composition of the feedstock, the nature of the catalyst and the temperature and pressure conditions. As shown in Figure 7.2, some of the output from the cracking process is reacted with isobutane to form larger branched-chain molecules (isoparaffins) that increase the octane rating of the final petrol blend. The alkylation process does not augment benzene content.

Eventually, the various petrol feedstock qualities are blended to produce end products with the desired specifications. These vary according to the likely ambient temperatures in the area and season in question and generally require higher concentrations of aromatic components such as benzene in colder climates.

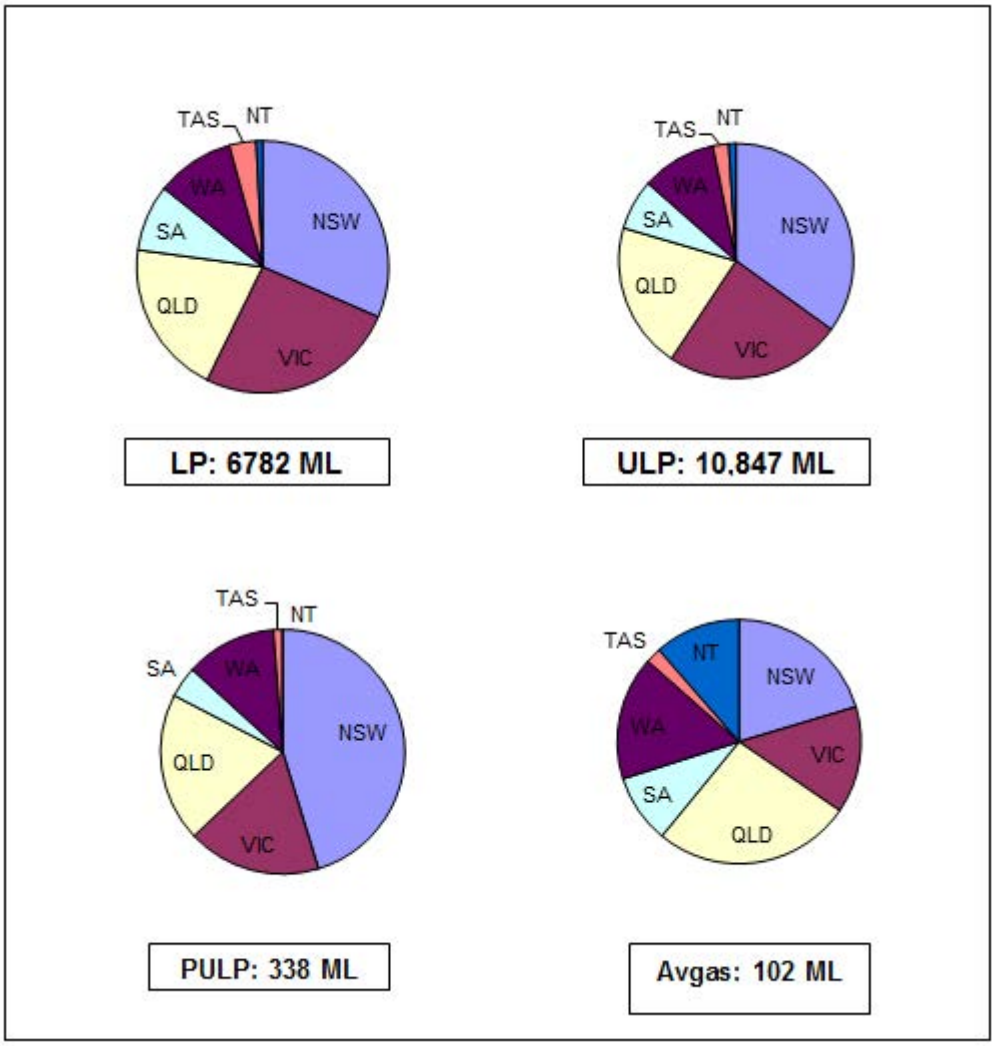
Feedstock and end product are stored in tanks equipped with floating roofs or connected to vapour recovery systems. The end products are distributed to larger terminals by pipeline, in coastal tankers or bottom-loaded rail tankers and/or to local depots and service stations in road tankers, the majority of which are bottom loaded. In rural areas not all road tankers are bottom loaded. Terminals in Sydney, Melbourne and Perth have vapour recovery systems to minimise vapour emissions during truck filling operations. There were 8233 petrol retail outlets in Australia at the end of 1998 (AIP, 2000).

End use

In Australia, all benzene produced by petroleum refiners is retained as one of several aromatic components in automotive petrol and Avgas; most of this benzene is burnt during normal engine operation. Figure 7.3 shows the total demand for benzene-containing petroleum products in 1996, and its breakdown by State marketing area[2].

[2] The State marketing area (SMA) of Queensland includes the Murwillumbah district of NSW, which is supplied from the refineries in Brisbane. The SMA of South Australia includes the Broken Hill-

Figure 7.3: Demand for petrol in 1996 (AIP, 1997)



As expected, the demand for petrol is highest in the most populous States of New South Wales and Victoria. Queensland, Western Australia and the Northern Territory account for more than one-half of the total demand for Avgas.

Since catalytic converters became mandatory on new cars in 1986, there has been a steady increase in the demand for ULP and a corresponding decline in the demand for LP. However, although LP contains more benzene than ULP, the corresponding fall in benzene consumption has been counterbalanced by an overall growth in petrol demand. PULP, which was first produced in significant quantities in 1989 and has the highest concentration of benzene (Table 7.1), accounted for only 2% of total petrol sales in 1996, almost half of which was generated in New South Wales. However, PULP demand is expected to grow as LP is phased out nationally by 2002 and pre-1986 cars will need to run on either PULP or lead replacement petrol, that is, PULP pre-blended with an anti-valve seat recession additive.

Wilcannia district of NSW and the Murrayville district of Victoria, which are supplied from the refinery at Port Stanvac. The SMA of Victoria includes the Riverina district of New South Wales, which is supplied from the refineries at Altona and Geelong. The SMA of New South Wales includes the Australian Commonwealth Territory.

The likely impact of the predicted changes in demand on the use of benzene in petrol can be estimated on the basis of AIP's petrol sales forecasts for the decade 1998-2007 (AIP, 1998a). If current benzene concentrations are assumed to remain unchanged throughout the period, total benzene use in petrol is estimated to increase from 434 kt/y in 1998 to 461 kt/y in 2007. However, if a nationwide standard is introduced limiting the maximum content in petrol to 1%, total benzene use in petrol is estimated to fall to 176 kt/y in 2007.

Independent petrol retailers

The main imports of petrol are marketed by independent chains or supermarkets such as Trafigura (formerly Burmah) Fuels, Liberty and Woolworths, who had 564 service stations between them at the end of 1998 (AIP, 2000). Their imports pass through the terminals of Vopak (formerly Van Ommeren) at Port Botany in Sydney and Hastings Point near Melbourne, Gull at Kwinana in Western Australia, and Fletcher Challenge in Brisbane. These terminals have a petrol storage capacity of 95, 70, 53 and 13 ML respectively (DISR, 1999; Vopak, 2000).

7.2.2 Steel and associated industries

BTX

In the steel industry, BTX is a by-product from volatile fractions produced in the coking ovens. It contains ≥80% v/v benzene (see Table 4.1) and is recovered in an enclosed process which yields from 3-5 kg pure benzene per t coke produced.

The coking ovens are arranged in batteries, each of which may contain in the region of 60 units. The ovens are sequentially charged by means of mechanical hopper systems through special lidded holes that are closed and sealed to keep out air during the coking cycle. This cycle takes place at 900-1100°C for 12-24 h. On completion of the cycle, the hot coke is removed mechanically through doors on the sides of the oven and sprayed with flushing liquor (a dilute solution of ammonia in water) to quench combustion upon exposure to air.

The coke oven gas contains hydrogen, methane, carbon monoxide and light oil, which is a mixture of various aliphatic and aromatic hydrocarbons, including benzene. The overhead gas is collected into a pipe that runs along the length of each battery and propelled to the by-product plant. At emission, it has a temperature of approximately 600°C which is brought down to 80°C by spraying with flushing liquor. It is further cooled to 38°C, passed through an electrostatic precipitator and an acid scrubber where tar and ammonia are removed and cooled to a final temperature of 20°C. The gas then passes to a system of light oil scrubbers, where most of the C_5 and higher hydrocarbons are recovered by counter-current absorption using a high-boiling (300-400°C) petroleum fraction. BTX is recovered from the absorbent oil by steam stripping and separated from the water by distillation. At Port Kembla, the distillation process is continuous and BTX is collected and piped to a storage tank. At the smaller Whyalla steelworks, BTX is separated by batch distillation and returned directly to the gas system. At both sites, the refined coke oven gas is stored in a gasholder and used for heating.

Process waste water, which is mainly from the flushing liquor circuit and contains a number of contaminants, including benzene, is passed through a water/oil separator and discharged to a biological treatment plant. All storage tanks are eventually vented to the atmosphere, but may be connected to a vent header with a sealpot

arrangement to prevent emission unless there is a build-up of pressure. Excess gas is flared off.

All BTX produced at Port Kembla is transported by road to Huntsman Chemical Company in Melbourne for use as chemical feedstock.

Coal tar

Tar condensed from the coke oven gas and flushing liquor circuit is collected in a system of decanting tanks and pumped to a wet tar storage tank. This tank decants excess liquor back to the liquor circuit and transfers the tar sediment to a dry tar tank farm. The residue from the BTX distillation, which is known as naphthalene oil, is also pumped to the dry tar tank farm. The dry tar, which contains ≤0.16% residual benzene, is shipped to Koppers Coal and Tar Products at Mayfield, Newcastle, New South Wales, in splash top loaded rail or road tankers, or by sea tanker.

The Newcastle plant receives in the order of 125 kt crude tar per annum containing about 145 t residual benzene. The plant comprises two interconnected, fully enclosed systems which separate the tar in a series of continuous distillation processes. The distillation products include solvent naphtha (4% benzene), distilled tar (0.5% benzene), creosote oil (0.2% benzene), naphthalene (no measurable benzene) and coal tar pitch (no measurable benzene). Most of the solvent naphtha representing about 80% of the benzene received is burnt as fuel. The remainder is blended into creosote oil which is used in solvent-based industrial timber preservatives or as feedstock in the manufacture of carbon black. Distilled tar is used in the coatings industry. Naphthalene is exported and coal tar pitch onsold to the aluminium industry for the manufacture of carbon electrodes.

Process waste water from the Koppers coal tar plant is passed through a water/oil separator and discharged to a biological treatment plant. All storage tanks are connected to fume scrubbing systems. Off-gases from the stills are burnt as fuel.

7.2.3 Chemical industry

Qenos

Ethane and naphtha (gas oil) cracking

The olefins plant at the Qenos site at Altona in Melbourne produces 10-12 kt benzene per year as a by-product of the steam cracking of ethane and naphtha (gas oil) to ethylene, propylene and butadiene, which are then converted into plastics and rubbers. The Qenos (formerly Orica) olefins plant at Botany in Sydney produces 2-3 kt benzene per year as a by-product of the steam cracking of ethane to ethylene.

Steam cracking is a continuous, fully enclosed process which produces a variety of products by free radical reactions. Steam and hydrocarbon feedstock are mixed and subjected to a brief surge of extreme heat (750-900°C). The effluent is rapidly cooled, compressed, purified in a caustic washer, dried, chilled and fractionated in a train of distillation columns.

At the Altona plant, the by-product streams from the ethane and naphtha steam cracking processes are combined and purified by distillation to produce a pyrolysis

gasoline containing 6-8% benzene, which is piped to the Mobil Altona refinery and eventually exported for use overseas (Section 7.2.1).

At the Botany plant, the heavier molecules produced in the cracking process are collected in a feed tank and further processed in a fully enclosed system which is on stream for approximately 60 days per annum. In the process, the by-product stream is hydrogenated and then distilled to remove light ends, which are returned to the ethane cracking system. The end product is a pyrolysis gasoline containing approximately 55% aromatics including 35-36% benzene. This is stored on site in a floating-roof tank. At intervals of 5-6 months, it is piped to the bulk liquid terminal at Port Botany and shipped overseas for further processing.

Butadiene rubber manufacture

Qenos' Altona facility also uses about 40 t benzene per year as a solvent component in the manufacture of butadiene rubber. The benzene is purchased from Huntsman (see below) and supplied by dedicated road tanker. It is stored in a nitrogen-blanketed tank and pumped to the butadiene rubber plant via sealed pipes.

In the plant, butadiene is polymerised in solution in a fully enclosed batch process. The solvent contains cyclohexane and benzene in a ratio of about 2:1 and is not consumed in the reaction. The polymerisation process is strongly exothermic and the reactor temperature is kept at approximately 20°C by ammonia cooling. The reaction is stopped with an antioxidant. The solvent is removed from the rubber-solvent solution by steam stripping and is then condensed, purified and recycled to the beginning of the process for feedstock blending. Waste water is steam stripped to remove dissolved benzene prior to discharge to sewer. Off-gases containing benzene are sent to a thermal oxidiser for destruction.

Huntsman Chemical Company

The Huntsman (formerly Chemplex) plant at West Footscray in Melbourne converts about 80 kt benzene per annum to ethyl benzene and 10-15 kt to cumene (isopropyl benzene). Ethyl benzene is further processed to styrene, which is used in the production of polystyrene polymers and unsaturated polyester and vinyl ester resin solutions. Cumene is oxidised to acetone and phenol. The phenol is used on site in the production of phenol-formaldehyde resins. The acetone is onsold in bulk to other manufacturers.

Huntsman purchases about 15% of their requirements for benzene in the form of BTX produced at the Port Kembla steelworks. The remainder is imported from Indonesia, Japan, Korea and Singapore in chemical tankers. The bulk chemical is unloaded at Terminals Pty Ltd on Coode Island on Melbourne's waterfront where it is kept in storage before being transported to Huntsman by dedicated road tanker. A small part is trucked to Qenos' Altona facility for use as a solvent component in the manufacture of butadiene rubber.

Styrene manufacture

The styrene plant was commissioned in 1977 and operates a series of four continuous, fully enclosed processes, namely, ethylene, Litol, alkylation and dehydrogenation. The principal feedstocks are ethane, pure benzene and BTX.

In the Litol plant, BTX is vaporised with hot hydrogen and passed through fixed bed catalytic reactors to hydro-dealkylate toluene and xylenes to benzene and destroy heterocyclic compounds. Pure benzene is recovered by fractional

distillation. By-product hydrocarbon gases, excess hydrogen and heavy distillation residues are used as fuel. A waste stream rich in hydrogen sulfide is incinerated.

The alkylation plant makes ethyl benzene from benzene and ethylene produced on site by the cracking of ethane. In the first of two reactors, the alkylation is carried out in the presence of a homogenous acidic catalyst prepared separately from aluminium chloride. In the second reactor, recycled polyethyl benzene is trans-alkylated to ethyl benzene. The remaining undesired components from the dilute ethylene benzene stream are recovered, neutralised and used as fuel. Catalyst is removed from the alkylated liquor in a 3-stage wash system. The aqueous wash liquors containing aluminium and sodium chlorides and some hydrocarbon contaminants are treated aerobically at the site effluent treatment plant before discharge to sewer. The alkylation liquor is then refined in a 3-column distillation train. Pure ethyl benzene is recovered for subsequent use in the dehydrogenation plant. Excess benzene and the polyethyl benzene are recovered and recycled to the reactors. The heavy distillation residue is utilised elsewhere in the complex to lower the viscosity of other residue streams and ultimately utilised as fuel.

In the dehydrogenation plant, ethyl benzene is dehydrogenated to styrene at high temperature and low pressure in the presence of steam. The dehydrogenated mixture is condensed and cooled, the water is separated out, and the stream is refined in a 3-column distillation train. Hydrogen and other gases produced in the reactor are used as fuel. By-product benzene and toluene are recovered and sent to the Litol plant for conversion to pure benzene. Unreacted ethyl benzene is recovered and returned to the dehydrogenation reactor. Pure styrene is distilled and dosed with a polymerisation inhibitor prior to storage.

Tanks containing benzene are vented to a carbon bed vapour emission control system that recovers about 60 t of benzene per annum and returns it to the styrene plant.

Phenol manufacture

The phenol plant was commissioned in 1968. It produces phenol and acetone in a continuous, fully enclosed process. Cumene is formed by the reaction of pure benzene and propylene in a fixed-bed reactor using a phosphoric acid catalyst on a solid support. The pure benzene feedstock is either imported or produced in the styrene plant and piped to the phenol plant. A 3-column refining section recovers gaseous components from the cumene stream. Unreacted benzene is recycled into the process, and cumene is sent on to the oxidation reactor. Heavy distillation residue is utilised as fuel or as an aromatic feedstock in the Litol plant. The purified cumene stream is partially oxidised with air to cumene hydroperoxide, which is cleaved by acid to a mixture of crude phenol and acetone. The mixture is split into phenol and acetone, which are purified by distillation in a 7-column refining train.

Heavy distillation residue is subjected to a 2-column system, where some additional phenol is recovered via pyrolysis and distillation for recycling to the refining train. Residue from this system is utilised as fuel. Spent air from the oxidation reactor is chilled to remove most of the organic substances and then passed through activated carbon beds before release to the atmosphere. A combined aqueous waste stream is treated in the site effluent treatment plant prior to discharge to sewer.

7.2.4 Laboratory uses

Seven of the applicants listed in Section 3 identified themselves as occasional importers of reagent grade benzene. Between them, they imported approximately 500 kg benzene in 1999, which was onsold to a total of 55 end users. Of these, 27 were commercial enterprises such as contract and company in-house analytical laboratories. Twenty-one belonged to the science or medical faculties of 15 different universities. Seven were State or Commonwealth laboratories. The quantities purchased by individual laboratories in 1999 ranged from 0.1-150 L (0.88-130 kg), with a mean of 10 L (8.8 kg) and a median of 2.5 L (2.2 kg).

Benzene is also present in some ready-made liquid or gaseous standards for the calibration of gas chromatographs and other analytical instruments. The quantity of benzene consumed through the use of such standards is estimated at less than 1 kg per annum.

7.2.5 Coincidental production

Benzene is formed coincidentally during the burning of aromatic and non-aromatic organic compounds contained in biomass such as crops, wood and humus, in fossil fuels such as black and brown coal, and in petroleum products including diesel and jet engine fuel which have a negligible benzene content prior to combustion. These processes are important sources of entry into the environment and will be considered in detail in subsequent sections.

7.3 Summary

Table 7.3 summarises the industrial mass balance of benzene in Australia and the available information on its major manufacturers, importers and users, and most significant end uses. These figures are approximate and give a general indication of industrial use of benzene in Australia. Benzene produced coincidentally in the course of human activities or natural processes and products containing benzene as an impurity are not accounted for.

Table 7.3: Benzene mass balance and major manufacturers, importers and end users in Australia in 1998-99

	Kilotonnes/year (rounded)						
Industry or company	Extrac-tion	Manu-facture	Import	Total	Export	Con-sumption	End use
Petroleum	30	385	25	440	-	440	Petrol
Huntsman	-	-	80	80	-	95	Feedstock
Steel	15	-	-	15	-	0.2	Fuel
Qenos	-	15	-	15	15	0.040	Solvent
Others	-	-	0.0005	0.0005	-	0.0005	Reagent
TOTAL	45	400	105	550	15	535	-

In 1998-99, total benzene consumption in Australia was in the order of 535 kt per year. Of this quantity, 105 kt were imported, 45 kt were extracted from crude oil and coal gas, and the remainder produced at eight oil refineries. Petrol accounted for approximately 82%, chemical synthesis for 18% and all other uses combined for less than 1% of total consumption.

8. Environmental Release, Fate and Effects

As no environmental fate and toxicity studies were submitted for assessment, this section is based on international, peer-reviewed reports such as GDCh (1988), Government of Canada (1993) and IPCS (1993), the International Uniform Chemical Information Database (IUCLID) and the USEPA's ECOTOX database (USEPA, 2000). Data within these three reports are largely the same and also appear in the databases.

IUCLID contains non-confidential data supplied by industry to the European Commission. They have not undergone peer review and are therefore only reported where they are not described elsewhere but nonetheless give guidance to the fate and effects of benzene in the environment. Results in the ECOTOX database have been published and are generally considered reliable.

8.1 Environmental release

Benzene is ubiquitous in the environment, with numerous sources of entry including bush fires, crop residue and forest management burning, petrol combustion, wood fires, tobacco smoking and emissions and waste streams from various industries. Due to the nature of benzene being produced incidentally during natural processes and human activities, it is not possible to obtain accurate figures in estimating national releases. However, several point source releases provided in NPI reports for the first reporting year of 1998/99 are described in Section 15, which also gives an estimation of diffuse releases in a model urban environment.

Overall, release of benzene will primarily be to the atmosphere through emissions in exhaust during petrol combustion in motor vehicles, followed by releases to air from point sources in the petroleum, steel, aluminium, chemical and other industries. By contrast, releases to water and soil are expected to be relatively minor, as borne out in NPI reports where the highest annual release from a single point source to water and soil was 1100 kg and 45 kg respectively, compared with 130,000 kg to air from an oil and gas extraction plant.

8.2 Environmental fate

The Trent University (1999) Level 1 Fugacity Based Environmental Equilibrium Model indicates that in the order of 99% of benzene will partition to air, with 0.88% and 0.05% partitioning to water and soil respectively. Negligible amounts are expected to partition to sediments, suspended sediments, biota and aerosols.

8.2.1 Atmospheric fate

The water solubility of benzene suggests that one removal mechanism from the atmosphere is through returning to the terrestrial and aquatic compartments in rainwater. However, the Henry's Law constant and volatility of benzene indicate that the chemical would rapidly volatilise back into the atmosphere where it would be available for abiotic breakdown.

Direct photolysis

IPCS (1993) reports that direct photolysis of benzene in the troposphere is unlikely since the UV-visible spectrum of benzene shows no appreciable absorbance at wavelengths >260 nm. According to GDCh (1988), direct photolysis is of minor importance for the same reason.

IUCLID provides test results for a smog chamber experiment in which light with a wavelength >290 nm corresponding to tropospheric sunlight was used with benzene at a concentration of 100 ppm (0.32 mg/L). Although the validity of this test cannot be judged due to insufficient documentation, the outcome showed no evidence of benzene degradation. After the addition of chemicals producing active species, benzene half-lives were between 4-5 h. Using light with a higher intensity (wavelengths >230 nm), a half-life of 6.5 h was detected. These findings indicate that direct photolysis is minimal at environmentally significant wavelengths, stated by Howard et al. (1991) to be >290 nm, and thus confirm that this process will not be a major removal process for benzene in the troposphere.

Indirect photolysis

IUCLID provides details for several studies on indirect photolysis. The results of those where a half-life was determined are presented in Table 8.1. In all tests, hydroxyl radicals were used as the reactant, with air as the medium. Not all studies had temperature reported. However, where available, it was 25ºC. While the validity of these studies is uncertain, it is well accepted that indirect photolysis through reaction with hydroxyl radicals is the major degradation pathway for benzene in air (GDCh, 1988; Government of Canada, 1993; IPCS, 1993).

Table 8.1: Half-life of benzene in the atmosphere where degraded by hydroxyl radicals

Light source	Hydroxyl concentration (radicals/cm^3)	Rate constant (cm^3/(molecule.s)	Half-life (days)
Sun light	5×10^5	1.2×10^{-12}	13.4
Other*	5×10^5	1.2×10^{-12}	13.4
Sun light	7.5×10^6	1.3×10^{-12}	19
Sun light	1.1×10^6	1.3×10^{-12}	5.6
Sun light	1.2×10^6	$1.2\text{-}1.6 \times 10^{-12}$	5.3

* Hydroxyl radicals produced by flash photolysis and using a resonance fluorescence method.

The Dutch Environment Ministry calculated a half-life of benzene in the atmosphere of 5.3 days assuming an average hydroxyl radical concentration of 1.25×10^6 molecules/cm^3 over the Netherlands with a rate constant of 1.3×10^{-12} cm^3/(molecule.s). This is reported in both IPCS (1993) and GDCh (1988), although neither report describes the basis for the assumed hydroxyl radical concentration. The global 24-h average hydroxyl radical concentration has been reported to be around 5×10^5 molecules/cm^3 (Calamari, 1993; GDCh, 1988). Additionally, using the OECD Environment Monograph No. 61 (OECD, 1993), a rate constant for benzene can be calculated at 2×10^{-12} cm^3/(molecule.s) (contrary to the range of $0.8\text{-}1.4 \times 10^{-12}$ cm^3/(molecule.s) quoted in GDCh (1988)). Applying the global average hydroxyl radical concentration and rate constant from the OECD monograph and following the methodology in this monograph, gives an estimated half-life of 8 days. This is more in agreement with the Canadian authorities where a half-life attributable to reactions with hydroxyl radicals was calculated to be 9 days

under typical urban atmospheric conditions, although the hydroxyl radical concentration and rate constant were not reported (Government of Canada, 1993).

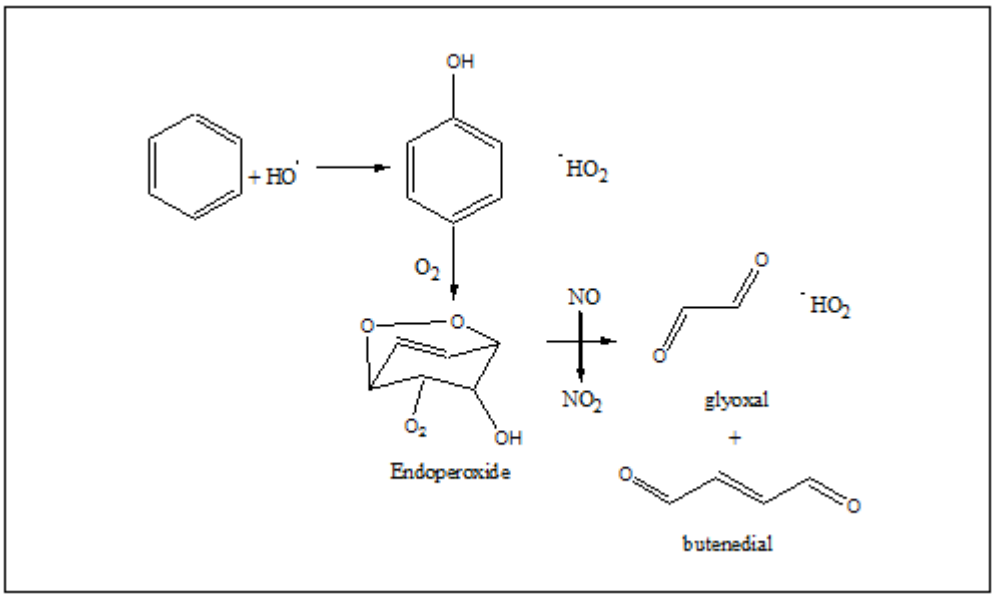
The global concentration used above applies to the average for the whole troposphere. In the lower troposphere where benzene and hydroxyl radicals occur at higher concentrations, the benzene half-life would be expected to be lower, and is reported as 3-10 days (GDCh, 1988). Additionally, in districts with high traffic density, where there is a higher concentration of hydroxyl radicals because of higher concentrations of precursors, a lower atmospheric half-life can be expected, and again 3-10 days is reported (GDCh, 1988).

For the purposes of this assessment, an atmospheric half-life of 8 days will be used based on the globally accepted tropospheric average for the concentration of hydroxyl radicals and the methodology and rate constant prescribed in the OECD monograph.

These results given above are all within the range predicted in Howard et al. (1991) where the photooxidation half-life in air has been calculated to fall between 50.1 h (2.09 days) and 501 h (20.9 days).

The proposed degradation pathway through reaction with hydroxyl radicals is shown in Figure 8.1 (Verscheuren, 1996).

Figure 8.1: Proposed degradation pathway of benzene in the atmosphere



In the IUCLID database one study is described where ozone was used as a reactant. In this test, air was the medium and the light source was chemiluminescence. A sensitiser concentration of 3 x 10^{12} molecules/cm^3 and a rate constant of 1 x 10^{-22} cm^3/(molecule.s) were used. In an urban atmosphere, the half-life for the reaction of benzene with ozone was calculated to be 105 years. In a rural atmosphere, the half-life would be 327 years, using an atmospheric concentration for ozone of 9.6 x 10^{11} molecules/cm^3. Therefore, photolysis through reaction with ozone is not expected to be a major removal process for benzene in the atmosphere.

One test is described where atomic oxygen was used as the reactant at a concentration of 7.2 x 10^4 molecules/cm^3 with a rate constant of 2.8 x 10^{-14} cm^3/(molecule.s). The half-life for this reaction between benzene and atomic oxygen was calculated to be 10.9 years, indicating that this reaction will not be a major removal process of benzene from the atmosphere.

One test describes the photodegradation using sulphur dioxide as the reactant. The test was performed in air with sunlight as the light source (light spectrum >290 nm). Benzene was present at a concentration of 100 ppm (0.32 mg/L). No further information is available on the method, so the validity of this test is unknown. However, sulphur dioxide was present at a concentration of 10-110 ppm (0.026-0.288 mg/L). Photodegradation was observed. IUCLID states that approximately 2 days was required for 50% degradation to CO_2, although the half-life for photodegradation of benzene is stated as 6 h.

8.2.2 Aquatic fate

Photolysis in water

Direct photolysis of benzene in aqueous solution was investigated with half-lives observed varying from 9-673 days. However, the authors concluded that the test method was not suitable for poorly soluble, volatile substances (GDCh, 1988).

Two direct photolysis studies are reported in IUCLID where benzene was tested adsorbed on silica gel. In both tests the concentration of benzene was 0.32 mg/L and the light source was not stated. Few details are reported and the validity of the tests is unknown. However, one was irradiated at >230 nm (not environmentally significant), and resulted in a half-life of 6.5 h. The other was irradiated at tropospheric wavelengths (>290 nm) and showed that 5% had photomineralised to CO_2 after 17 h.

Hydrolysis

IUCLID provides two reports for abiotic degradation of benzene, both concluding that hydrolysis is not expected to be a significant process for removing benzene. Few details are available for these tests and validity cannot be assumed. However, degradation by this route is not expected, as benzene has no hydrolysable groups.

Volatilisation

Volatility from water to air is summarised in several reports in IUCLID.

Based on a reported Henry's Law constant of 0.0053 atm.m^3/mol and a model river 1 m deep flowing at 1 m/s with a wind velocity of 3 m/s, the half-life of benzene was 2.7 h at 20°C.

The half-life for the evaporation of benzene from seawater was investigated in a mesocosm containing planktonic and microbial communities. Half lives for summer, spring and winter were reported as 3.1, 23 and 13 days respectively.

The half-life for evaporation of benzene from a 1 m thick still water column was 4.8 and 5 h at 25 and 10°C respectively by thermodynamic calculations. The residence half-time for well-mixed water was 37 min. This half-life of 4.8 h is also included in the IPCS (1993) and Government of Canada (1993) reports.

An experiment in a wind-wave tank 6 m long, 0.61 m deep and 0.6 m wide with wind velocities of around 6-13 m/s at a temperature of 20.7°C is described. The testing period was >50 h so that an approximate 10-fold change of solute concentration (which was measured by gas chromatography) would occur. The mass transfer coefficients of benzene at the water-air interface were 11.4-34 cm/h dependent on wind velocity. The volatilisation is of first order kinetics. For a wind speed of 7.09 m/s, a half-life of 5.2 h can be calculated.

While the reliability of these results is unclear, they support that rapid volatilisation from water will occur.

8.2.3 Terrestrial fate

Adsorption

Documentation on the adsorption of benzene to soil is limited as the exposure of the terrestrial compartment is likely to be low.

The Government of Canada (1993) report cites K_{oc} values for benzene ranging from 12-213, indicating the chemical to be moderately to highly mobile in soil.

IUCLID reports a calculated soil absorption coefficient of 71, using equations developed by Kenaga & Goring and published by the American Society for Testing and Materials. While this reference has not been obtained, an experimental soil absorption coefficient value of 83 is reported in IUCLID as well as in IPCS (1993).

IPCS (1993) cites a rounded log K_{oc} range of 1.8-1.9 (K_{oc} = 60-83), indicating fair mobility in soil, and states that benzene is not expected to adsorb to bottom sediments based on its K_{oc}, solubility and volatility.

K_{oc} values provided in IUCLID indicate that benzene may exhibit high mobility in soils and may migrate to groundwater. Several tests are reported and are generally described as valid, or valid with restrictions. They can be used to provide a guide as to the adsorptive behaviour of benzene.

One report gave the results of adsorption tests using radioactive labelled test substance on aquifer material. This test is described as valid with IUCLID noting that the test procedure was in accordance with generally accepted scientific standards and described in sufficient detail. The results provided log K_{oc} values between 2.09 and 3.01 (K_{oc} 123-1023). Experiments were carried out in capped glass centrifuge tubes on two American groundwater aquifer materials with the following characteristics:

Material:	Sand (%):	Silt (%):	Clay (%):	Organic matter (%):	pH:
A	90	8.0	2.0	4.4	3.8
B	70.4	24.0	5.6	2.2	5.5

Both these materials were acidic, with material A being quite acidic. It cannot be concluded from the IUCLID summary whether K_{oc} was a function of pH, although this is not expected to be the case since benzene is a neutral molecule. These results suggest that benzene is moderately mobile.

A water-soil adsorption coefficient of 18.2 provided in IUCLID was measured in soil-solution mixtures which were equilibrated for 24 h at 20°C in capped centrifuge tubes. Losses by volatilisation were avoided by sampling through the septum of the caps. The substance amounts were corrected by the airspace of the tubes under consideration of air volume and Henry's Law constant. Soil characteristics were reported as 9% sand; 68% silt; 21% clay and 1.9% organic matter. pH was not stated.

While IUCLID also provides some calculated results, these are not reported here as measured values are considered more reliable and the terrestrial compartment is not expected to be a significant sink for benzene.

Volatilisation

The primary mechanisms responsible for loss of benzene from soil are volatilisation to the atmosphere and runoff to surface water. Benzene released below the soil surface may leach to groundwater (Government of Canada, 1993).

The volatility of benzene from soil to air is summarised in two reports in IUCLID. In one report, the half-lives of volatilisation, without water evaporation, of benzene uniformly distributed at a rate of 1 kg/ha to 1 and 10 cm in soil with an organic carbon content of 1.25% were 7.2 and 38.4 days respectively. The second report is from a model developed to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank. It estimated that some 67% of the benzene would volatilise from the soil within 17 months, with 29% leaching to groundwater and the remainder associating with the soil.

8.2.4 Biodegradation

IPCS (1993) provides the following insight into the biodegradation of benzene:

- In surface and ground water benzene is biodegradable by microorganisms under both aerobic and anaerobic conditions with the mechanism of biodegradation seeming to involve the formation of catechol via *cis*-1,2-dihydroxy-1,2-dihydrobenzene followed by ring cleavage.
- One study on the aerobic biodegradation of benzene in groundwater utilised a mixed bacterial culture from groundwater and soil bacteria capable of using gasoline as a sole carbon source. Under closed agitated conditions without added nutrients the half-life appeared to be <48 h with benzene levels falling from 480 to 218 μg/L in this time. When ammonium nitrate was added, the reaction was much faster, with benzene levels decreasing to 35 μg/L in 20 h.
- The biodegradation of benzene in ground and river waters appears to follow first-order rate kinetics with reported half-lives of 28 and 16 days respectively.

IUCLID provides results from several biodegradation studies which generally agree that a significant degree of biodegradation occurs under aerobic conditions. Some tests classify the substance as readily biodegradable. However, many of the tests are not ready biodegradation tests and the results do not indicate degradation that would be fast enough for benzene to be classed as readily biodegradable. While validity cannot be assumed, they may be used to provide guidance as to the biodegradability of benzene. As such, for the purposes of this assessment, the chemical will be considered at least inherently biodegradable.

The majority of tests summarised in IUCLID for anaerobic degradation indicate degradation is very slow to non-existent. This is supported in the GDCh (1988) report where it is stated that degradation of benzene has not yet been detected in anaerobic conditions. However, IPCS (1993) describes a report where samples of landfill leachate incubated under methogenic conditions in an anaerobic glove box showed a 72% reduction in benzene concentrations after 40 weeks, although no significant benzene biodegradation occurred during the first 20 weeks of incubation. In another study using anaerobic digesting sludge under methano-trophic conditions, benzene was undegraded after 11 weeks. It is also reported that no toxic effects of benzene on the anaerobic digestion of sewage sludges were observed until levels of 50-200 mg/L had been reached.

8.2.5 Bioaccumulation

Benzene is not expected to bioconcentrate to any significant degree in aquatic or terrestrial organisms given the reported values for log $P_{o/w}$ of 1.56-2.15 (GDCh, 1988; ICPS, 1993). IPCS (1993) also reports a bioconcentration factor (BCF) for freshwater algae of 30, for water fleas of 153-225 depending on the concentration of benzene in their food, and for goldfish of 4.3.

GDCh (1988) reports measured BCF values in *Clupea harengus* (herring) of 2-6 in most organs, and 31 in the gall bladder. One study outlined in this document claims no significant biological accumulation in algae or fish. For fish, the BCF was in the range of 1-10 after 3 days.

These conclusions are largely supported by data available from USEPA (2000) and IUCLID. Results are available for several species of fish including *Anguilla japonica* (Japanese eel), *Leuciscus idus melanotus* (golden orfe), *Morone saxatilis* (striped bass), *Salmo gairdneri* (rainbow trout) and *Engraulis mordax* (Northern anchovy). BCF values were all under 100, with the exception of the Northern anchovy. This species provided BCF values of 113-505, with an outlying result of 8450. There is not enough detail to determine whether these factors are for specific organs or the whole organism, which would impact on the analysis.

Nonetheless, based on the scale provided in Mensink et al. (1995), benzene can be classed as slightly to moderately concentrating in fish. No data are available on depuration rates.

Benzene appears to be more concentrating in invertebrates with results generally indicative of a moderately concentrating chemical. Several species of invertebrates have test results reported by USEPA (2000). An 8-day static and 9-day flow through test on *Brachionus plicatilis* (rotifer) showed BCF values of 100-1000 under static conditions and 10,000 under flow through conditions. The maximum concentration tested was 900 µg/L, well within the limit of solubility. Three results are available for *Daphnia pulex* with BCF values ranging from 153-225.

Several results in IUCLID were also reported by USEPA and have not been duplicated here. IUCLID provides information on depuration from *Daphnia pulex*. Daphnids were exposed to water dosed with 10 µg/L benzene, water containing algae preloaded by incubation with 50 µg/L benzene, or both dosed water and preloaded algae. The reported BCF values were 225 for exposure to just dosed water, 203 for feeding on preloaded algae and 153 after incubation in dosed water with preloaded algae. Where exposure was through dosed water only, clearance was 88% after 72 h. Where daphnids were exposed to dosed water and preloaded algae, 83% clearance was reported when moved to fresh water with unloaded algae, although the time involved in this depuration was not stated.

Limited data are available for algae, but suggest bioaccumulation will be slight, with the ECOTOX database (USEPA, 2000) reporting BCF values of 30 for the green algae *Chlorella fusca* and *Chlorella fusca vacuolata*.

8.3 Effects on organisms in the environment

Of the three assessments listed at the beginning of this section, only GDCh (1988) has any detailed discussion of the effects of benzene on organisms in the environment. As this publication is relatively old, the USEPA's ECOTOX database

was interrogated for more recent results (USEPA, 2000). In addition, IUCLID was consulted where limited data were reported in the other two sources, but as this is largely unvalidated, is used for guidance only. Not all available information is reported here due to its volume. However, the range for each trophic level will be given with an indication of where the majority of results fall.

8.3.1 Aquatic organisms

Fish

The majority of reported results come from tests performed under static conditions. GDCh (1988) provides 96-h results for 7 freshwater species including *Leuciscus idus melanotus* (golden orfe), *Lepomis macrochirus* (sun perch), *Pimephales promelas* (fathead minnow), *Lebistes reticulatus* (guppy), *Carassius auratus* (goldfish), *Gambusia affinis* (mosquito fish) and *Ictalurus punctatus* (channel catfish), with LC_{50} values ranging from 15-430 mg/L. Most of these fall in the 10-100 mg/L range, indicating slight toxicity to fish. This is largely supported by more recent data from USEPA (2000) where a 48-h LC_{50} to *Mugil curema* (white mullet) of 22 mg/L, 96-h LC_{50} to fathead minnow of 12.6-24.6 mg/L and 96-h LC_{50} to *Poecilia reticulata* (guppy) of 28.6 are reported. These results are all indicative of slight toxicity.

Flow through tests provide more sensitive results. All flow through results are for rainbow trout. Two tests reported in GDCh (1988) give 96-h LC_{50} values of 5.3 and 9.2 mg/L, while one more recent result gives a 96-h LC_{50} of 5.9 mg/L (USEPA, 2000). These results are indicative of moderate toxicity.

GDCh (1988) reports a 96-h LC_{50} of 5.8 mg/L in the saltwater species *Morone saxatilis* (striped bass), which is indicative of moderate toxicity. The test conditions are not known.

As such, benzene can be considered moderately to slightly toxic to fish under acute exposure.

Chronic and sub-chronic data for fish appear limited with only one study reported in GDCh (1988). Following 14 days exposure of *Lebistes reticulatus* (guppy) under static conditions, a LC_{50} of 63 mg/L was determined.

The Government of Canada (1993) report highlights an investigation into the chronic toxicity of benzene to the early life stages of rainbow trout, leopard frog and the Northeastern salamander. Eggs of each species were exposed continuously to benzene from within 30 minutes of fertilisation (embryos) through to 4 days post-hatch (larvae). This resulted in continuous exposures of 27 days for rainbow trout, 9 days for leopard frog and 9.5 days for Northeastern salamander. The corresponding LC_{50} values were 8.3, 3.7 and 5.2 mg/L respectively.

IUCLID provides results of tests in *Pimephales promelas* (fathead minnow), *Morone saxatilis* (striped bass) and *Clupea harengus* (pacific herring) for 7-day, 28-day and 17-day exposure periods respectively. The striped bass was exposed under flow through conditions. A no observed effect concentration (NOEC) of 10.2, 3.1 and 0.49-0.88 mg/L was reported for fathead minnow, striped bass and pacific herring respectively, although those for pacific herring were the highest concentrations tested on these fish so no real conclusions can be drawn from the results.

Overall, these results indicate that benzene is of very low toxicity to fish from chronic exposure.

GDCh (1988) lists some toxic effects of benzene on developmental stages and behaviour of fish. Pacific herring demonstrated a decrease of survival time of eggs after 48-h exposure of sexually mature females at 0.7 mg/L. However, this study was conducted in a polluted region so other chemicals may have been responsible. Other tests on pacific herring showed unspecified developmental abnormalities at 31-40 mg/L. Also, 24-h exposure of embryos to sublethal concentrations (up to 1.85 mg/L) under static conditions showed an effect on metabolism. Significantly less growth of the embryos, altered oxygen consumption and greater food intake in larvae were reported.

Sublethal effects were reported in the coho salmon at 1.8 mg/L and an increase in the respiratory rate of chinook salmon was found at 4.4 mg/L. This effect was also observed at the same concentration in striped bass.

Invertebrates

Invertebrates appear to be the largest group tested. GDCh (1988) provides results for four freshwater species, of which *Daphnia magna, Daphnia pulex* and *Daphnia cucullata* all had 48-h EC_{50} values >100 mg/L. One freshwater invertebrate, *Aedes aegypti* (mosquito larva) had a 24-h LC_{50} of 59 mg/L. Of the saltwater species reported in GDCh (1988), benzene could be considered moderately toxic to four species: *Artemia salina* (salt water shrimp), *Crango franciscorum* (bay shrimp), *Nitroca spinepes* and *Palaemonetes pugio* (grass shrimp), with 24- to 96-h LC_{50} values ranging from 20-82 mg/L. Two salt water species, *Cancer magister* (Dungeness crab) and *Crassostrea gigas* (oyster), had 96-h LC_{50} values >100 mg/L.

More recently published data from the ECOTOX database (USEPA, 2000) largely confirm the moderate to slight toxicity of benzene to aquatic invertebrates outlined above. Moderate toxicity is reported for *Ceriodaphnia dubia* (water flea; 24-h EC_{50} = 18.4 mg/L), *Gammarus fossarum* (scud; 96-h LC_{50} = 53 mg/L) and *Corixa punctata* (water boatman; 48-h LC_{50} = 48 mg/L), with LC_{50} values >100 mg/L reported for a further three species: *Daphnia magna*, *Lymnaea stagnalis* (great pond snail) and *Viviparus bengalensis* (snail).

However, one crab species (*Scylla serrata*) was relatively sensitive to benzene, with three 96-h LC_{50} results (mortality as the end point) of 3.7, 6.1 and 7.7 mg/L. While the majority of results indicate benzene is only moderately to slightly toxic to aquatic invertebrates, this species shows benzene may be considered toxic to some aquatic invertebrates.

GDCh (1988) only describes one study where chronic effects were investigated in *Daphnia magna*. In a lifetime and partial lifetime test, no toxic effect of benzene was found at a concentration of 98 mg/L.

Only one chronic test is available in IUCLID where sufficient detail is presented. This test on a crab species (*Cancer magister*) indicates slight toxicity to aquatic invertebrates. Larval stages of the crab were continuously exposed after hatching in a flowing water laboratory culture system at benzene levels of 0.17-0.18, 1.1-1.2 and 6.5-7.0 mg/L. Benzene had little effect on the duration of the larval stages and no effect on the size of surviving larvae. At the lowest concentration, there was no effect on survival. At the other two concentrations, benzene led to an accelerated mortality rate compared to untreated controls. After 10 days of exposure at the

highest concentration, most larvae died. At the middle concentration, most larvae died before day 20 of exposure. Therefore, the 20-day NOEC was 0.17 mg/L.

Algae

Data covered in GDCh (1988) suggest benzene is only slightly to very slightly toxic to algae. A 24-h EC_{50} on the green algae *(Chlorella vulgaris*) based on cell division was >>100 mg/L.

Three 72-h EC_{50} values are reported for sea algae with cell division as the end point. The green algae (*Dunaliella tertiolecta*), siliceous algae (*Skeletonema costratum*) and yellow-green algae *(Cricopshaera carterae*) all had EC_{50} results >100 mg/L. In a 3-day test in the dinoflagellata *Amphidinium carterae,* the EC_{50} with cell division as the end point was reported to be 50 mg/L, indicating slight toxicity (GDCh, 1988).

More recent data from the ECOTOX database (USEPA, 2000) appear more indicative of slight toxicity than the earlier studies reported in GDCh (1988). One 72-h EC_{50} in *Selanastrum capricornutum* of 29 mg/L and a 24-h EC_{50} for the diatom *Thalassiosira pseudonana* of 40 mg/L are reported.

In summary, benzene can be classed as slightly to very slightly toxic to algae under acute exposure.

In 8-day tests, >1400 mg/L benzene had no detectable effect on biomass in the freshwater species *Scenedesmus quadricauda* and the blue alga *Microcystis aeruginosa* (GDCh, 1988). With growth as the end point, the more sensitive species *Selenastrum capricornutum* provided an 8-day EC_{50} of 41 mg/L, although a 14-day EC_{50} of 292 mg/L is also reported for this species (USEPA, 2000).

Predicted No-Effect Concentration (PNEC) in the aquatic environment

As there are results available for both acute and chronic exposure in three trophic levels, the lowest NOEC for chronic exposure, in this case to the aquatic invertebrate crab species *Cancer magister*, will be used with an assessment factor of 10. While this result (NOEC = 0.17 mg/L) is based on unvalidated results from IUCLID, it is considered that there are sufficient data available from published and peer-reviewed sources for this test to be accepted for use in a worst-case PNEC and that there is sufficient detail in the IUCLID report to support the results.

Therefore, the PNEC for the aquatic environment is 0.17/10 = 0.017 mg/L, or 17 µg/L.

8.3.2 Terrestrial organisms

A study in *Eisenia fetida* (earthworm) is reported in the ECOTOX database (USEPA, 2000), in which an LC_{50} of 98 µg/cm^2 was determined in adult worms weighing 300-500 mg placed for 48 h on filter paper impregnated with a solution of benzene in water, acetone and trichloromethane.

According to GDCh (1988), use of benzene as a solvent for plant protection agents in bioassay tests showed that it is slightly toxic to various insect species. The LD_{50} for the house fly (*Musca domestica*) was 0.8 mg per animal. Exposure to benzene in the vapour phase exhibited toxic action in the grain weevil (*Calandra granaria*), although the concentration is not reported. Benzene acted as a repellent to the adults of certain species of flies (*Diptera*).

In plants, air concentrations >50 mg/m^3 (>15.5 ppm) have a lethal effect. However, all plant species investigated recovered from sublethal effects. In water, higher concentrations of 0.9-1.3 g/L have a growth-inhibiting effect (GDCh, 1988).

It is difficult to translate the earthworm measurement to an application rate likely to lead to adverse impacts in soil and a PNEC cannot be determined from these data.

8.4 Summary

Benzene is expected to partition predominantly to the atmosphere, with the primary route of degradation coming from indirect photolysis through reaction with hydroxyl radicals. Direct photolysis or reactions with oxygen or ozone are not expected to be major removal processes from the atmosphere. Based on the accepted global concentration of hydroxyl radicals, the degradation half-life of benzene from the atmosphere is calculated at 8 days.

Benzene is largely abiotically stable in water, with the major removal process expected to be volatilisation. The high water solubility and relatively low log $P_{o/w}$ indicate that benzene will not adsorb significantly to organic matter and sediments.

When released to the terrestrial compartment, benzene may be relatively mobile and may leach to groundwater if released underground, for example, from leaking storage tanks. The chemical is unlikely to adsorb readily to soils and may readily volatilise from soil surfaces.

Benzene may be considered biodegradable under aerobic conditions, although under anaerobic conditions, biodegradation may be expected to be very slow. Based on the chemical's low log Pow and experimental results, bioaccumulation is not expected to any significant degree, and at worst, benzene can be described as moderately concentrating.

Aquatic organisms exhibit only a low level of sensitivity to benzene, with the chemical being slightly toxic to fish following acute exposure under static conditions and moderately toxic under flow through conditions. Chronic exposure of fish to benzene gave results indicative of slight toxicity. Invertebrates appear to be the largest group of aquatic organisms tested. For the majority of species tested, benzene was only slightly to very slightly toxic. However, one crab species was relatively sensitive, with results in the range of a moderately toxic chemical. Chronic results show benzene to be slightly toxic to aquatic invertebrates. Benzene may also be classified as slightly to very slightly toxic to algae. The PNEC for the aquatic environment is 17 μg/L.

Limited data on the toxicity of benzene to terrestrial organisms show the chemical to be slightly toxic to various insect species and the earthworm. In plants, high concentrations in air have a lethal effect, although all plants investigated recovered from sublethal effects.

9. Kinetics and Metabolism

The toxicokinetics and metabolism of benzene have been extensively investigated in several animal species and, to a lesser extent, in humans. Studies relevant to the toxicokinetics of benzene have been reviewed and summarised in this section. The metabolites and their modes of action are further discussed in Section 12. A number of reviews of benzene toxicokinetics and metabolism are available, including IPCS (1993) and ATSDR (1997).

9.1 Absorption

9.1.1 Animal studies

Inhalation

Schrenk et al. (1941) found the absorption of benzene vapour by dogs after inhalation exposure to be rapid. Inhalation of the vapour (800 ppm) over 4-7 h resulted in the concentration of benzene in arterial blood approaching equilibrium conditions by 30 minutes. Although considerable inter-animal variation was noted, a linear relationship was demonstrated between the concentration of benzene in air over the range from 200-1300 ppm and the equilibrium concentration in blood. In another study, the absorbed dose after inhalation (nose-only) of [^{14}C]-benzene (approximately 10-1000 ppm) for 6 h by rats (F344) and mice (B6C3F$_1$) was found to be non-linear. The percentage of benzene absorbed decreased from 33% to 15% in rats and from 50% to 10% in mice as the exposure concentration increased from 10 to 1000 ppm. Due to apparent physiological differences in respiration between the two species, mice inhaled approximately twice the amount of benzene compared to rats (Sabourin et al, 1987). Similarly, Eutermoser et al. (1986) found that the absorption rate of benzene vapour (300 ppm) by male rats (Sprague-Dawley) decreased with increasing duration of exposure. When determined after 1, 3 and 6 h of continuous exposure and compared to baseline values, benzene absorption decreased to 33, 22 and 9% respectively. Male mice (Swiss) when exposed to benzene (310 ppm) for 1, 3 and 6 h of continuous exposure and compared to baseline values gave values of 65, 76 and 81% respectively. Thus after the first hour, the rate of benzene uptake by rats decreased significantly compared to mice.

Dermal

Dermal absorption of liquid benzene was investigated by Maibach & Anjo (1981) using intact and abraded skin of rhesus monkeys. Under conditions where evaporative losses were allowed, the application of a single dose of benzene to intact skin resulted in absorption of 0.17% of the dose while the application of multiple doses (11 exposures with an interval of 15 min) resulted in 0.85% of the dose being absorbed. In contrast, abraded skin resulted in 0.91% of the applied dose being absorbed. Similar results were obtained by Franz (1984) who found that after a single dermal application of benzene, 0.14% and 0.09% was absorbed by rhesus monkeys and miniature pigs respectively. It was concluded from the in vitro studies that the major factor influencing percutaneous absorption of benzene was its contact time with the skin. Susten et al. (1985) reported similar findings with

hairless mice (HRS/J) using a skin chamber where less than 1% of the applied dose was absorbed.

Adsorption of benzene onto soil matrices (sandy or clay soil) was found to modify the dermal absorption of [^{14}C]-benzene when applied topically to male rats (Sprague-Dawley) over a 72-h period using a glass skin chamber. While the peak plasma level of radioactivity after exposure to benzene adsorbed onto sandy soil was comparable to that obtained for pure benzene, a statistically significant lower plasma level was obtained for benzene adsorbed onto clay, however, neither soil type altered the time to reach peak plasma levels which was 12 h (Skowronski et al, 1988).

Dermal absorption of benzene vapour has also been addressed. Dermal absorption (whole-body) of benzene vapour over 2, 4 or 6 h was investigated by the use of male nude mice attached to respirators. The dermal absorption rates for exposures of 200, 1000 and 3000 ppm were 4.11, 24.2 and 75.5 nmol/cm^2/h (0.3, 1.9 and 5.9 μg/cm^2/h) respectively, demonstrating a linear relationship between the two parameters. Dermal absorption was also found to be linear with respect to exposure time. The dermal absorption coefficient for the mouse was determined to be 0.619 cm/h (Tsuruta, 1989). McDougal et al. (1990) exposed male rats (F344; whole-body) to benzene vapour (40,000 ppm) for periods up to 4 h. The rats were shaved of all fur prior to exposure and provided with latex face masks attached to a fresh air supply during exposure. Benzene blood levels at 0.5 h were 8 μg/mL and rose to 11 μg/mL at 4 h indicating that benzene is rapidly absorbed by the dermal route. The dermal absorption rate was determined to be 0.0191 mg/cm^2/h and the dermal absorption coefficient 0.152 cm/h.

Oral

Following the administration by gavage of [^{14}C]-benzene (340-500 mg/kg) to rabbits, approximately 80% of the ingested radiolabel was recovered in exhaled breath and urine indicating substantial gastrointestinal absorption at these dose levels (Parke & Wiliams, 1953). Similar results were obtained by the administration of lower doses of [^{14}C]-benzene (0.5-150 mg/kg) to rats (F344 and Sprague-Dawley) and mice (B6C3F$_1$) where it was determined that >97% of the dose was absorbed (Sabourin et al, 1987).

9.1.2 Human studies

Inhalation

In a study of 23 subjects exposed to benzene vapour (47-110 ppm) over 2-3 h, maximal absorption (70-80% of dose) occurred within 5 min of initial exposure. Subsequent absorption declined rapidly and reached a plateau at 15 min. Absorption remained constant for the remainder of the exposure duration at approximately 50% (range: 20-60%) of the exposure dose (Srbova, et al, 1950). Comparable results have been obtained in a number of other studies. Nomiyama & Nomiyama (1974a) exposed 6 volunteers (3 males and 3 females) to benzene vapour (52 to 62 ppm) for 4 h and showed that after an initially high absorption rate (50-60%), the rate decreased to reach a plateau of approximately 30-40% after 3 h of exposure. The mean retention of benzene, after allowances for respiratory excretion, was determined to be 30.2% for a 3-h exposure. Similarly, Snyder et al. (1981) demonstrated that during continuous exposure to benzene vapour approximately 50% of the dose is absorbed by the lungs. Pekari et al. (1992)

exposed 3 non-smoking volunteers to benzene vapour (1.6-9.4 ppm) for 4 h. The absorbed dose was estimated to be 52% and 48% for the low and high dose exposure respectively based on the average difference in concentration between the inhaled and exhaled air.

Further evidence for the absorption of benzene by inhalation is provided by studies of cigarette smokers. Analysis of cigarette smoke has shown the presence of substantial amounts of benzene, with the yield within the range of 0.4-104 μg/cigarette (see Section 16.1). Analysis of breath samples from 198 smokers and 322 non-smokers showed significantly higher ($p < 0.001$) benzene concentrations in the breath of smokers (16 $\mu g/m^3$) compared to non-smokers (2.5 $\mu g/m^3$). Benzene breath levels were significantly correlated ($p < 0.01$) with the number of cigarettes smoked per day (Wallace & Pelizzari, 1986; Wallace et al, 1987). Pekari et al. (1992) found 6 non-smokers to have venous blood benzene levels of <1-2 nM compared to 3 smokers (1 pack/day) with 4-13 nM in the morning and 5-8 nM in the afternoon. Cessation of smoking for a period (duration not stated) resulted in a reduction of blood benzene levels to <2 nM. In a similar study, it was found that the mean venous blood benzene levels of 14 smokers were significantly higher (7.0 nM; range 3.7-12.1 nM) compared to 13 non-smokers (2.8 nM; range 1.4-5.8 nM); however, the number of cigarettes consumed were not stated (Hajimiragha et al, 1989). With the exception of cigarette smoking, there were no other known activities undertaken by the subjects that may have resulted in benzene exposure prior to or during either study.

Dermal

A number of studies indicate that benzene is absorbed via the dermal route in humans. A study of 2 men exposed to benzene (approximately 0.06 g/cm^2 applied to the forearm, 35-43 cm^2, under occluded conditions for 1.25-2 h) determined the dermal absorption rate to be 0.4 $mg/cm^2/h$ based on urinary excretion of phenol (Hanke et al, 1961). Approximately 0.05% of the applied dose of [^{14}C]-benzene (0.0026 mg/cm^2) was absorbed when applied to the forearm skin of 4 volunteers. Absorption was determined by urinary excretion of radiolabel which indicated that absorption was rapid. Evaporative losses during the absorption period were not accounted for (Franz, 1984).

The absorption of benzene due to dermal exposure to petrol has been studied in car mechanics having direct skin contact with petrol for 30-150 min during work on car fuel systems, with the concentration of benzene in the breathing zone ranging from 0.2 ppm (detection limit) to 3.7 ppm averaged over the duration of the task. Blood benzene levels determined 2-9 h after exposure ranged from 3-16 nM. Based on expected benzene blood levels derived from the airborne concentrations, it was estimated that dermal absorption accounted for up to 80% of the total absorbed dose of benzene. The mechanics did not wear protective gloves (Laitinen et al, 1994). However, the estimation assumed that the mechanics were exposed to non-detectable benzene air concentrations during the remainder of the working day and would therefore have overestimated the extent of skin absorption if this were not the case.

In an in vitro study, benzene (pure benzene, air saturated with benzene vapour or a saturated aqueous solution of benzene) was shown to diffuse across hydrated stratum corneum prepared from human skin. Absorption, initially preceded by a lag phase (range: <1-1.5 h), was linear over the duration of the experiment (4 h). The rates of benzene absorption due to pure benzene and air saturated with benzene

vapour were 2.1 and 1.0 μL/cm^2/h (1.8 and 0.88 mg/cm^2/h) respectively. It was further demonstrated that the barrier characteristics of human skin alter in response to the presence of other solvents (Blank & McAuliffe, 1985). Lodén (1986) determined the amount of benzene absorbed by excised human skin to be 0.17 mg/cm^2 after 0.5 h and 0.93 mg/cm^2 at steady state (13.5 h). The total absorption of benzene over 13.5 h in skin and receptor fluid was 1.92 mg/cm^2 and the resorption rate (that is, the amount of substance migrating to the receptor fluid below the skin) was determined to be 99 μg/cm^2/h.

Oral

No studies were identified addressing the absorption of benzene by the oral route in humans. Cases of accidental or intentional ingestion indicate that benzene is readily absorbed by the gastrointestinal tract, with a dose of 125 mg/kg proving fatal (see Section 11.1).

9.2 Distribution

9.2.1 Animal studies

Inhalation

Schrenk et al. (1941) observed that in dogs continuously exposed to the vapour, benzene preferentially partitions to the organs and tissues with a higher fat content, although considerable inter-animal variation was noted. The establishment of an equilibrium between most tissues (except fat) and blood levels appeared to be rapid (≤15.5 h). When exposed to various concentrations of benzene vapour (850-1320 ppm) for periods ranging from 0.65-5 days, benzene levels were highest in bone marrow (57.6-64.1 mg/100 g tissue) followed by peritoneal fat (40.3-61.4 mg/100 g tissue) and subcutaneous fat (39.9-48.6 mg/100 g tissue). All other tissues or organs had substantially (generally 20-fold) lower levels of benzene. A comparable distribution pattern was observed when dogs were exposed to 800 ppm benzene vapour for 8 h/day for 38-272 days. Rickert et al. (1979) studied the distribution and residence times of benzene and three major metabolites, phenol, hydroquinone and catechol, in male rats (F344) exposed to benzene vapour (500 ppm) for up to 8 h. The steady-state benzene concentrations at 6 h were determined for the following tissues: fat (164.4 μg/g), bone marrow (37.0 μg/g), kidney (25.3 μg/g), lung (15.1 μg/g), liver (9.9 μg/g), brain (6.5 μg/g) and spleen (4.9 μg/g), while blood contained 11.5 μg/mL. The half-times for tissues to reach steady-state levels for benzene were essentially the same for all tissues (0.9-2.0 h) as were the elimination times (0.4-0.8 h), with the exception of fat which was 1.6 h. The concentrations of phenol in blood and bone marrow were maximal within 2 h after cessation of exposure and declined rapidly thereafter. Hydroquinone and catechol concentrations were sustained for 9 h after exposure with higher concentrations found in bone marrow.

Ghantous & Danielsson (1986) demonstrated the transplacental distribution of benzene and its metabolites in mice following inhalation exposure to [^{14}C]-benzene. Benzene was detected in the placenta and the foetus immediately following and for up to 1 h after exposure, as were benzene metabolites. The metabolites did not reach the same tissue concentrations as in maternal tissues and no metabolites were retained in the placenta or the foetus.

Dermal

Susten et al. (1985) examined the distribution of radiolabel after dermal application of undiluted [^{14}C]-benzene and a 0.5% (v/v) solution in rubber solvent using a skin chamber attached to male hairless mice (HRS/J) for 4 h. Approximately 5% and 8% of the benzene in the pure sample and rubber solvent respectively remained associated with the site of application while approximately 23% and 22% respectively was associated with the carcass. Skowronski et al. (1988) examined the tissue distribution of radiolabel in male rats (Sprague-Dawley) 48 h following the topical application of benzene (300 μL) using a glass skin chamber. The highest levels of radiolabel (expressed as % of initial dose per g of tissue) were found in the kidneys (0.026%), liver (0.013%) and treated skin (that is, below the site of application; 0.011%). Untreated skin gave a value of 0.002%. Subcutaneous fat from below the area of application gave 0.008% while subcutaneous fat from a different site gave 0.005% as did bone marrow. All other tissues and organs examined (including the brain) accounted for less than 0.04% of the initial dose.

Oral

Analysis of rabbit tissues and organs (1 animal) 2 days after dosing by gavage with [^{14}C]-benzene (500 mg/kg) showed the highest level of radioactivity to occur in muscle (1.6%), fat (1.5%), liver (0.07%), stomach (0.05%), testes (0.02%) and kidneys (0.015%). No radioactivity was detected in the brain, spinal cord or blood (Parke & Wiliams, 1953). Low et al. (1989) found that the distribution of radiolabel in female rats (Sprague-Dawley) varied with the dose of [^{14}C]-benzene administered. At 1 h after a single dose of 0.15 or 1.5 mg/kg by gavage, radiolabel was highest in the liver and kidneys (0.198-2.043 μg/g tissue), intermediate in blood (0.086-0.769 μg/mL), and lowest in the Zymbal gland, nasal cavity tissue, oral cavity tissue, mammary gland and bone marrow (0.034-0.547 μg/g tissue). In contrast, at 15 mg/kg, the amount of radiolabel found in the mammary gland and bone marrow had substantially increased in comparison to other tissues. At the highest dose, bone marrow and adipose tissue had the highest concentrations of benzene.

9.2.2 Human studies

Studies of the distribution of benzene in humans are generally limited to a number of fatal cases of accidental or deliberate benzene exposure. Autopsy data from such cases indicate that benzene preferentially partitions into lipid-rich tissues.

Analysis of tissue and fluid samples from a youth who died after sniffing benzene (reagent grade) showed the following order for tissue benzene content: brain, 39 mg/kg; abdominal fat, 22.3 mg/kg; blood, 0.02 mg/mL; kidneys, 19 mg/kg; liver, 16 mg/kg; bile, 0.011 mg/mL; stomach, 10 mg/kg and urine, 0.0006 mg/mL (Winek & Collom, 1971). Similar findings were demonstrated at autopsy of 3 cases of acute industrial benzene poisoning indicating that benzene preferentially distributes to lipid-rich tissues such as body fat (range: 68->120 mg/kg) and brain tissue (range: 58-63 mg/kg) with lesser amounts in blood (range: 30-129 mg/mL), liver (range: 15-38 mg/kg), lungs (positive findings) and bile (range: trace to 45 mg/mL) (Avis & Hutton, 1993). In a similar industrial accident involving acute fatal benzene poisoning analysis of tissue and fluid samples revealed the following benzene concentrations: blood, 0.0317 mg/mL; brain, 178.7 mg/kg; lungs, 22.2 mg/kg; heart, 182.6 mg/kg; liver, 378.6 mg/kg; kidneys, 75.2 mg/kg and urine, 0.0023 mg/ml (Barbera et al, 1998). In the above case reports the individuals are

believed to have inhaled benzene vapour for some time before death occurred. In one case in which an individual died suddenly during an industrial accident involving benzene, precluding extensive inhalation of the vapour, autopsy findings revealed the following benzene concentrations: blood, 0.0038 mg/mL; brain, 13.8 mg/kg; liver, 2.6 mg/kg (Tauber, 1970).

Limited data indicate that developing foetuses and infants may be exposed to benzene as a result of maternal exposure. Benzene can cross the placenta and concentrations in umbilical cord blood have been shown to be equal to or greater than in maternal blood (Dowty et al, 1976). Due to the richly perfused nature of breast tissue and the high fat content of human milk (approximately 4%), benzene is expected to partition from blood into human milk from which it can transfer to nursing infants (Fisher et al, 1997). Qualitative analysis of 12 human milk samples revealed the presence of benzene in 8 of them (Pellizzari et al, 1982).

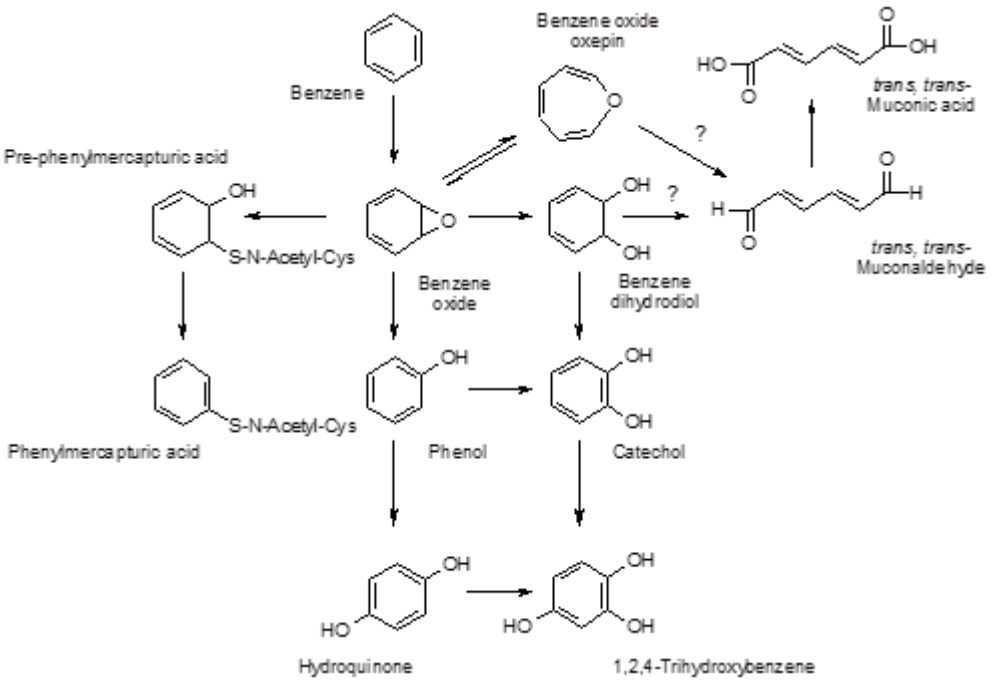
9.3 Metabolism

The metabolism of benzene has been extensively investigated in several species of animals including humans. Benzene toxicity has been attributed to the formation of reactive metabolites that appear to exert their toxic effect in combination, with no one metabolite accounting for all of the observed effects. The metabolism of benzene has been reviewed by Ross (1996) and Snyder & Hedli (1996).

9.3.1 General metabolic pathways

Urinalysis of several species exposed to benzene has demonstrated qualitative similarities in the spectrum of metabolites produced, indicating that the metabolism of benzene follows similar pathways between species. Urinary benzene metabolites identified from rabbits, rats, mice, monkeys and humans include conjugates of phenol, hydroquinone, catechol and 1,2,4-trihydroxybenzene while phenylmercapturic acid and *trans*,*trans*-muconic acid have also been identified. The conjugates are principally glucuronides and sulfates (Parke & Williams, 1953; Rothman et al, 1998; Sabourin et al, 1988, 1992). Analysis of rat and human blood samples has further revealed the presence of benzene oxide and its S-phenylcysteine adducts following benzene exposure (Bechtold et al, 1992a, 1992b; Lovern et al, 1997). The general metabolic pathways for benzene metabolism are provided in Figure 9.1. The initial step in the formation of toxic metabolites is the conversion of benzene to the benzene oxide/oxepin which can be further metabolised to phenolic compounds or cleaved to give *trans*,*trans*-muconaldehyde. Detoxification pathways primarily involve conversion of benzene oxide to pre-phenylmercapturic acid and phenylmercapturic acid while the phenolic compounds form glucuronide and sulfate conjugates.

Figure 9.1. The metabolism of benzene, with question marks indicating suspected pathways for which definitive evidence is lacking (after Sabourin et al. (1988) and Schlosser et al. (1993))



The primary site for benzene metabolism is the liver. It has been observed that animals that have undergone partial hepatectomy metabolise less benzene and exhibit reduced benzene-mediated toxicity compared to animals with intact livers (Sammet et al, 1979). The initial biotransformation of benzene involves oxidation by the action of a cytochrome-P450 (CYP) to produce the benzene oxide/oxepin intermediate (Jerina et al, 1968). Studies of liver microsomal preparations from rats and rabbits, using inhibitor and immunochemical techniques, have identified the cytochrome as CYP2E1 (Johansson & Ingelman-Sundberg, 1988; Koop et al, 1989; Nakajima et al, 1989). Similar studies with human liver microsomal preparations have shown CYP2E1 to be the major cytochrome involved in the metabolism of benzene by humans (Guengerich et al, 1991). Valentine et al. (1996) confirmed the role of CYP2E1 in the in vivo metabolism of benzene using transgenic CYP2E1 knockout mice (*cyp2e1*$^{-/-}$). Analysis of urine samples after exposure to [^{14}C]-benzene by nose-only inhalation showed reduced levels of urinary metabolites compared to wild-type mice (*cyp2e1*$^{+/+}$). The study further demonstrated that, while oxidative metabolism of benzene occurs primarily through CYP2E1, other cytochromes are involved.

Studies of rat liver microsomes have shown there to be high affinity (K_m = 20 μM) and low affinity (K_m = 0.3 mM) binding sites for benzene (Johansson & Ingelman-Sundberg, 1988). Nakajima et al. (1989), using monoclonal antibodies, identified two distinct rat enzymes, a high affinity and a low affinity binding type involved in benzene oxidation. Subsequent studies of rat microsomal P450 isozymes, CYP2E1, CYP2C11/6, CYP1A1/2 and CYP2B1/2, by Nakajima et al. (1992) using monoclonal antibodies showed that all four isozymes are involved in the initial oxidation of benzene. However, while CYP2E1 has been characterised as a high affinity enzyme with respect to benzene metabolism, CYP2B1/2 exhibits low affinity but high capacity (Gut et al, 1996; Nakajima et al, 1989) and CYP2C11/6

and CYP1A1/2 exhibit low affinity and low efficiency towards benzene (Nakajima et al., 1992).

Several studies have demonstrated the inducible nature of CYP2E1 and subsequent enhancement of benzene metabolism by phenobarbital, acetone or ethanol treatment of rats (Johansson & Ingelman-Sundberg, 1988; Koop et al., 1989; Nakajima et al., 1989). In addition, it has been demonstrated that benzene is able to stimulate its own metabolism by inducing CYP2E1 activity (Arinç et al., 1991; Gut et al., 1993). However, it has also been demonstrated in mice that repeated oral exposure to benzene can diminish CYP2E1 activity (Daiker et al., 1996). One postulated mechanism for reduced cytochrome activity, demonstrated in vitro, requires inactivation of the cytochrome by quinones formed by oxidation of hydroquinone, catechol and 1,2,4-trihydroxybenzene (Soucek et al., 1994).

The initial oxidation product of benzene, benzene oxide, has been estimated to have a half-life, in vitro, of approximately 8 min in blood (Lindstrom et al., 1997). Thus the oxide has sufficient stability to allow it to participate in a variety of reactions. Minor reactions of benzene oxide include alkylation with DNA and RNA (Mueller et al, 1987) and proteins (Bechtold et al, 1992a, 1992b) while epoxide hydrolase converts it to benzene dihydrodiol (1,2-dihydroxycyclohexadiene).

The presence of benzene oxide in blood has been detected by the presence of S-phenylcysteine adducts of haemoglobin and albumin (Bechtold et al, 1992a; 1992b). Haemoglobin adducts were detected in the blood of rats (F344) and mice ($B6C3F_1$) after inhalation exposure (600 ppm, 6 h/day, 5 days/week for 2 weeks) or gavage (rats only; 0, 100, 1000 or 10,000 μmol/kg). Albumin adducts were also detected in the plasma of rats exposed to benzene vapour (Bechtold & Henderson, 1993). While the haemoglobin adduct has been found to be relatively stable in the rat (F344) with decay rates consistent with the life-span of erythrocytes (approximately 60 days), albumin adducts were found to have a half-life of 0.4 days compared to unmodified albumin (half-life approximately 3 days) (Troester et al, 2000). Lindstrom et al. (1998) estimated the half-life of benzene oxide in blood, under *in vitro* conditions, to be approximately 6.6 min in mice ($B6C3F_1$), 7.9 min in rats (F344) and 7.2 min in humans. When benzene oxide (0-184 μM) was incubated with mouse, rat or human blood for 3 h it was observed that haemoglobin adduct formation was proportional to the oxide concentration. The order of reactivity for the oxide with haemoglobin was rat >> mouse > human. Negligible haemoglobin adduct formation was observed with human blood. All three species formed albumin adducts with the order being rat ≈ human > mouse.

9.3.2 Formation of phenolic metabolites

Studies with microsomal preparations, which preclude conjugation (detoxification) pathways, indicate that the major pathway for the further metabolism of benzene oxide involves the spontaneous rearrangement to phenol (Jerina & Daly, 1974; Jerina et al, 1968). It has been demonstrated, using liver microsomes and reconstituted enzyme systems, that phenol can also arise by the direct oxidation of benzene by hydroxyl radicals derived from the reduction of molecular oxygen by cytochrome P450 activity (Johannson & Ingelman-Sundberg, 1983). However, Gorsky & Coon (1985) observed that when benzene is present at concentrations approaching the K_m of CYP2E1 for benzene, hydroxyl radicals do not contribute significantly to benzene oxidation. The further oxidation of phenol by cytochrome P450 results in hydroquinone (Koop et al, 1989; Valentine et al, 1996) and catechol while 1,2,4-trihydroxybenzene arises from the P450-mediated oxidation of either

hydroquinone or catechol (Schlosser et al., 1993). In addition, catechol can be produced from benzene dihydrodiol by the action of dihydrodiol dehydrogenase (Bolcsak & Nerland, 1983).

The hydroquinone species derived from benzene, that is, hydroquinone, catechol and 1,2,4-trihydroxybenzene, readily undergo autoxidation to their respective semiquinone and quinone forms and the presence of peroxidases facilitate the oxidation process (Schlosser et al., 1989; Smith et al., 1989). Quinones are chemically reactive and capable of forming adducts with macromolecules. Further discussion of the secondary metabolism of benzene-derived phenol and hydroquinone species, along with their biological effects, is presented in Section 12.

The detoxification of the phenolic benzene metabolites occurs primarily by conjugation to glutathione (GSH), glucuronide or sulfate (Parke & Williams, 1953). Conjugation of benzene oxide with GSH by glutathione-S-transferase (GST) results in the formation of pre-phenylmercapturic acid and, by dehydrogenation, phenylmercapturic acid, both of which have been identified as urinary metabolites. The major metabolites, phenol, hydroquinone, catechol and 1,2,4-trihydroxybenzene, all form glucuronide and sulfate conjugates (Parke & Williams, 1953; Sabourin et al., 1988).

9.3.3 Formation of *trans,trans*-muconaldehyde

Cleavage of the oxidised aromatic ring results in the formation of *trans,trans*-muconaldehyde which is subsequently converted to *trans*,*trans*-muconic acid prior to excretion. A number of in vivo studies of several animal species, including humans, have shown *trans*,*trans*-muconic acid to be an end-stage product of benzene metabolism (Parke & Williams, 1953; Rothman et al., 1998; Sabourin et al., 1988). While the opening of the benzene ring and subsequent formation of muconic acid have been shown to occur in isolated perfused rat livers, as has the conversion of *trans*,*trans*-muconaldehyde to *trans*,*trans*-muconic acid (Grotz et al., 1994), the precise mechanism of ring opening remains elusive. It has been proposed that benzene oxide, while in the oxepin state, undergoes secondary oxidation by cytochrome P450 to produce *trans*,*trans*-muconaldehyde (Davies & Whitham, 1977) and small quantities of *trans*,*trans*-muconaldehyde have been found to be produced by mouse liver microsomal preparations on incubation with benzene (Latriano et al, 1986). However, hydroxyl radicals have also been implicated in the formation of *trans*,*trans*-muconaldehyde. Incubation of benzene with Fenton's reagent, which produces reactive oxygen species, results in the formation of *cis,trans*-muconaldehyde which, through a series of rearrangements, yields the *trans*,*trans*-isomer (Zhang et al., 1995). An alternative proposed mechanism for aldehyde formation requires cleavage of benzene dihydrodiol (Latriano et al., 1986), however, the aldehyde was not produced when benzene dihydrodiol was incubated with Fenton's reagent (Zhang et al., 1995). The subsequent conversion of *trans*,*trans*-muconaldehyde to *trans*,*trans*-muconic acid involves several steps requiring the action of an aldehyde dehydrogenase (Kirley et al, 1989; Zhang et al, 1993). Conjugation of *trans*,*trans*-muconaldehyde with GSH via hepatic GST has been demonstrated as a detoxification pathway for this metabolite (Goon et al., 1993a, 1993b).

9.4 Elimination and excretion

9.4.1 Animal data

Inhalation

Benzene was detected in the urine of dogs exposed to benzene vapour (850-1320 ppm) at levels ranging from 29.3-48.3 mg/100 g (Schrenk et al, 1941). Sabourin et al. (1989) identified the urinary metabolites of benzene metabolism in rats and mice following inhalation exposure to benzene vapour (nose-only) for 6 h. The results are presented in Table 9.1.

Table 9.1: Major urinary metabolites after inhalation of benzene, expressed as a percentage of total urinary metabolites from 24-h samples (adapted from Sabourin et al. (1989))

Species	Dose (ppm)	Phenol conjugates	Hydroquinone conjugates	Catechol conjugates	Pre-phenyl-mercapturic acid	Muconic acid
Rat (F344)	5	57	5.7	ND*	9.5	19
	600	74	2.0	ND	17	4.0
Mouse ($B6C3F_1$)	5	37	33	ND	6.0	23
	600	67	11	ND	15	5.0

* ND = not detected.

Dermal

Franz (1984) observed that peak excretion of radioactivity in the urine of rhesus monkeys after the application of 0.5 mL of [^{14}C]-benzene occurred during the first 2 h and decreased rapidly thereafter but remained detectable for up to 30 h. Skowronski et al. (1988) found that after the topical application of 300 µL of [^{14}C]-benzene to male rats (Sprague-Dawley) by means of a glass skin chamber, the major excretory route for radiolabel was in the urine, with substantially lesser amounts in faeces and expired air. Excretion in the urine was greatest during the 12- to 24-h interval after application, accounting for 58.8% of the initial dose with 68.4% recovered in 24 h and 86.2% after 48 h. In contrast, 0.2% of the initial dose was recovered over 48 h in the faeces. Expired air accounted for 12.0% in the first 24 h with the following 24 h accounting for only a further 0.8%.

Oral

Analysis of urinary benzene metabolites from several species following administration of [^{3}H]-benzene by gavage has shown similar profiles of metabolites. As indicated in Table 9.2, the principal urinary metabolites for the species shown are conjugates (glucuronides and sulfates) of phenol and, to a lesser extent, hydroquinone. The mouse is quantitatively different from the other species with a substantially higher production of hydroquinone conjugates and *trans,trans*-muconic acid. In addition to species differences, the table shows the effect of changes in dose levels on urinary metabolites for rats and mice. Increasing the dose of benzene leads to an increase in the excretion of phenol conjugates and a decrease in hydroquinone conjugates by the mouse but no substantial change in the rat. In contrast, *trans,trans*-muconic acid excretion is diminished in both the rat and the mouse at higher doses.

Table 9.2: Major urinary metabolites after oral administration of benzene, expressed as a percentage of total urinary metabolites from 24-h samples (adapted from Parke & Williams (1953), and Sabourin et al. (1989, 1992))

Species	Dose (mg/kg)	Phenol conjugates	Hydroquinone conjugates	Catechol conjugates	Pre-phenyl-mercapturic acid	Muconic acid
Rat (F344)	1	70	4.0	ND*	11	13
	10	71	2.8	ND	15	10
	200	75	2.0	ND	18	5.0
Mouse ($B6C3F_1$)	1	30	47	ND	2.7	20
	10	38	39	1.0	4.5	16
	200	63	16	ND	11	9.0
Rabbit	340	24	4.8	2.2	No data	1.3

* ND = not detected.

Within 2 days of administering [^{14}C]-benzene (0.34-0.5 g/kg) to rabbits by gavage, approximately 45% of the dose was detected in expired air (43% as unchanged benzene and 1.5% as carbon dioxide) and approximately 35% appeared in the urine. Urinary radiolabel was predominantly in the form of conjugated phenols, with phenol comprising approximately 23% of the administered dose and with hydroquinone, catechol and 1,2,4-benzenetriol making up 4.8%, 2.2% and 0.3% respectively (as conjugates). Approximately 1.3% of the dose was recovered as *trans,trans*-muconic acid and a further 0.5% as phenylmercapturic acid. No diphenyl or its derivatives were detected in the urine. The residual radioactivity (5% to 10%) was associated with the tissues and faeces (Parke & Williams, 1953).

The elimination of benzene by the metabolic route appears to be saturable. Oral doses of [^{14}C]-benzene ≤15 mg/kg resulted in the excretion in the urine over 48 h of >89% of the administered radioactivity by rats (F344 or Sprague-Dawley). Doses ≥50 mg/kg bw resulted in a dose-dependent reduction in urinary excretion and a corresponding dose-dependent increase in exhaled ^{14}C, predominantly as the parent molecule. At all doses, residual ^{14}C in the carcass amounted to less than 8%. Excretion in the faeces did not exceed 11% of the administered dose up to the maximum dose of 300 mg/kg. Mice (B6C3F1) demonstrated similar elimination characteristics to rats (Sabourin et al, 1987).

Other routes

Analysis of urine from male cynomolgus monkeys administered [^{14}C]-benzene (5, 50 and 500 mg/kg) by intraperitoneal injection revealed that urinary excretion of radiolabel diminished with increasing dose. At 5 mg/kg an average of 56% of the administered dose was recovered in the urine compared to 13% at 500 mg/kg over a 95-h period. In contrast, recovery of radiolabel from the urine of chimpanzees administered a dose of benzene (1 mg/kg) by intravenous injection was complete after 24 h with >90% of the radiolabel collected within the first 8 h. As shown in Table 9.3, phenyl sulfate was found to be the major metabolite (45-74% of total urinary metabolites) for all doses. Lesser amounts of hydroquinone glucuronide, muconic acid, phenyl glucuronide, hydroquinone sulfate and catechol sulfate were also present. No unconjugated metabolites were detected. The amount of excreted hydroquinone sulfate and muconic acid decreased and phenyl glucuronide and catechol glucuronide increased as the benzene dose increased. A similar urinary profile of metabolites was obtained with female chimpanzees administered an intravenous dose of [^{14}C]-benzene (1 mg/kg), although the formation of catechol conjugates was not detected (Sabourin et al, 1992).

Table 9.3: Major urinary metabolites after intraperitoneal administration of benzene, expressed as a percentage of total urinary metabolites from 24-h samples (adapted from Sabourin et al. (1992))

Species	Dose (mg/kg)	Phenol conjugates	Hydroquinone conjugates	Catechol conjugates	Pre-phenyl-mercapturic acid	Muconic acid
Cynomolgus monkey	5	61	27	8.0	No data	4.4
	50	73	15	6.0	No data	3.1
	500	78	8.9	9.9	No data	1.3
Chimpanzee	1	75	8.0	ND*	0.5	5.5

* ND = not detected.

9.4.2 Human data

Inhalation

The elimination of benzene across the lungs of 10 subjects was studied. Subjects inhaled benzene (47-84 ppm) for 2-3 h after which breath samples were taken over a further 5-7 h. The results showed 16.4-41.6% of the absorbed benzene to be exhaled with the greatest rate occurring during the first hour. Excretion in the urine accounted for a maximum of 0.2% of the absorbed dose (Srbova et al, 1950). It appears that urinary benzene metabolites were not measured by the protocol employed. Comparable results were produced after a 4-h exposure to benzene vapour (52-62 ppm) where 6 volunteers (3 males and 3 females) were shown to exhale 16.3% (men) and 17.2% (females) of the inhaled benzene (Nomiyama & Nomiyama, 1974a). The ratio of respiratory elimination of non-metabolised benzene to retained benzene was determined to be 114.8% for males (considered by the authors to be unreliable) and 39.8% for females (Nomiyama & Nomiyama, 1974b). Using 4 volunteers (male non-smokers) exposed to benzene vapour (mean daily exposure 26.2-42.2 ppm) for 5 consecutive daily 6-h periods, Berlin et al. (1980) showed the clearance of benzene across the lungs to be biphasic with a half-time of 2.6 h for the rapid phase and 24 h for the slow phase. At higher benzene concentrations (99 ppm for 1 h), Sherwood (1988) identified one individual with an initial rapid phase and 2-3 slower phases while urinary excretion displayed a biphasic pattern.

Ghittori et al. (1993) found a linear correlation between benzene in the breathing zone and unmetabolised benzene in the urine of workers. Subsequently, Ghittori et al. (1995) identified a linear relationship between benzene in the breathing zone of workers and urinary levels of *trans*,*trans*-muconic acid and phenylmercapturic acid.

Dermal and oral

Peak excretion of radioactivity in the urine of 4 human volunteers after the application of 0.4 ml of [^{14}C]-benzene to the ventral forearm occurred rapidly within the first 2 h and decreased rapidly thereafter but remained detectable for up to 30 h (Franz, 1984).

No studies were identified that address the elimination of benzene or its metabolites from humans after exposure by the oral route.

9.5 Comparative kinetics and metabolism

As discussed above, CYP2E1 is a high affinity, low capacity enzyme. Consequently, the pathway for the hepatic metabolism of benzene becomes saturated at relatively low doses. Henderson et al. (1989) found a single oral dose of benzene of 50 mg/kg or greater, when administered to rats or mice, resulted in saturation of the metabolic pathway with consequent loss of unmetabolised benzene by exhalation. Rats and mice administered an oral dose of 150 mg/kg exhaled approximately 50% and 85% respectively as unmetabolised benzene. This loss of benzene by exhalation becomes a limiting factor in the maximal tissue concentrations of metabolites that can be achieved following oral dosing. However, higher tissue concentrations of benzene metabolites can be achieved by the inhalation route. It has been observed that mice accumulate substantially more benzene metabolites than rats during inhalation exposure. This appears to be due to physiologic and metabolic differences between the two species. Mice have a higher respiratory minute volume per kg body weight compared to rats allowing for the blood benzene level to achieve equilibrium faster than in the rat (Sabourin et al, 1989, 1990). Mice also have a higher metabolic rate, based on increased oxygen consumption (approximately 1.8 times greater than the rat), resulting in faster removal of benzene from the circulation. This allows for higher levels of metabolites to accumulate within body tissues compared to the rat. Doses of benzene that lead to metabolic saturation also produce changes in the metabolic profile of benzene metabolites (Sabourin et al, 1989; Daiker et al, 1996).

9.5.1 Oral studies

The content of water-soluble benzene metabolites in bone marrow has been examined following oral administration of benzene to male rats and mice. Table 9.4 shows that phenol conjugates accounted for the major proportion of metabolites in the rat and remained relatively constant over the dose range as did the mercapturic acid derivatives. The *trans*,*trans*-muconic acid content diminished with increasing doses of benzene and hydroquinone conjugates remained at relatively low levels. In contrast, the mouse produced comparable bone marrow levels of phenol and hydroquinone conjugates at the lowest dose with the amount of hydroquinone conjugates decreasing as the benzene dose increased. Both phenyl mercapturic acid and *trans*,*trans*-muconic acid increased with the dose of benzene (Sabourin et al, 1989).

Table 9.4: Major bone marrow metabolites after oral administration of benzene, expressed as a percentage of total water-soluble metabolites from pooled samples (adapted from Sabourin et al. (1989))

Species	Dose (mg/kg)	Phenol conjugates	Hydroquinone conjugates	Pre-phenyl-mercapturic acid	Phenyl-mercapturic acid	Muconic acid
Rat (F344)	1	75	ND*	15	ND	10
	10	74	3.0	15	4.0	4.0
	200	84	2.3	13	ND	0.2
Mouse ($B6C3F_1$)	1	50	50	ND	ND	ND
	10	52	35	ND	13	ND
	200	56	9.0	ND	27	8.0

* ND = not detected.

9.5.2 Inhalation studies

Animal data

The profile of metabolites produced by male rats (F344) and mice ($B6C3F_1$) exposed (nose-only) to benzene vapour for 6 h at 5 ppm or 600 ppm is presented in Table 9.1. Phenol conjugates account for the major proportion of metabolites produced at either concentration by both species. At 5 ppm, hydroquinone conjugates and *trans,trans*-muconic acid are present in higher amounts than at 600 ppm while pre-phenylmercapturic acid increases with the administered dose (Sabourin et al, 1989).

Tissue and blood levels of non-conjugated benzene metabolites were determined in male rats (F344) and mice ($B6C3F_1$) after inhalation of benzene vapour (50 ppm) for 6 h. While phenol, hydroquinone and catechol could not be detected in the liver, lung or blood of the rat, detectable levels of phenol and hydroquinone were found in the mouse with catechol detected only in the liver. The data for male rats and mice are presented in Table 9.5.

Table 9.5: Major non-conjugated benzene metabolites (nmol/g tissue) in rat and mouse tissues (adapted from Sabourin et al. (1988))

	F344 rats			$B6C3F_1$ mice		
Metabolite	**Liver**	**Lung**	**Blood**	**Liver**	**Lung**	**Blood**
Phenol	ND*	ND	ND	0.3	0.6	1.3
Hydroquinone	ND	ND	ND	2.1	1.2	4.3
Catechol	ND	ND	ND	0.3	ND	ND

* ND = not detected.

In contrast, conjugated derivatives of phenol, hydroquinone and catechol were detected in substantially greater amounts in the tissues and blood of both species (Table 9.6).

Table 9.6: Major conjugated benzene metabolites (nmol/g tissue) in rat and mouse tissues (adapted from Sabourin et al. (1988))

	F344 rats			$B6C3F_1$ mice		
Metabolite	**Liver**	**Lung**	**Blood**	**Liver**	**Lung**	**Blood**
Phenylglucoronide	ND*	ND	ND	6.3	1.2	ND
Cathecholglucoronide	1.0	ND	ND	0.8	0.5	ND
Hydroquinoneglucoronide	0.4	ND	ND	26	15	12
Phenylsulfate	1.5	15	20	28	36	36
Hydroquinone monosulfate	ND	ND	ND	2.8	0.6	ND
Pre-phenylmercapturic acid	6.9	0.9	ND	44	2.1	2.3
Phenylmercapturic acid	ND	ND	ND	ND	ND	ND
Muconic acid	8.4	1.9	0.7	228	18	1.0

* ND = not detected.

As shown in the table, the level of *trans,trans*-muconic acid in the mouse liver was very much greater than observed in lung tissue or blood for either species (Sabourin et al, 1988). Henderson et al. (1989) observed that the mouse metabolised more of an inhaled dose of benzene than the rat under comparable conditions and that a greater proportion was converted to the putative toxic metabolites. It was found that

detoxification (conjugation) pathways were low-affinity, high-capacity whereas toxic metabolite formation appeared to be high-affinity, low capacity.

A short-term benzene inhalation study (exposure for 6 h/day for 6 days) with male Swiss mice showed that at 199 ppm or less, the major metabolite in blood was phenylsulfate while above 199 ppm a dose-dependent increase in phenyl-glucuronide occurred. At all benzene concentrations, the blood phenol level increased in a dose-dependent manner (Wells & Nerland, 1991).

Human data

Studies of humans are generally limited to analysis of urine or blood samples of workers occupationally exposed to benzene.

Bechtold and Henderson (1993) conducted analyses on the urine and blood of non-smoking female workers exposed to benzene vapour. Five women exposed to approximately 4.4 ppm for 8 h showed the presence of elevated urinary levels of *trans,trans*-muconic acid (6.2 μg/mg creatinine) compared to 8 females with no known exposure (0.27 μg/mg creatinine). Blood samples from 10 women exposed to benzene vapour (0-23.1 ppm) showed a linear relationship between benzene exposure levels and albumin-S-phenylcysteine adducts, however, no haemoglobin-S-phenylcysteine adducts were detected.

9.5.3 Dermal studies

Comparative studies of the metabolism of benzene after dermal absorption were not identified.

9.5.4 In vitro studies

The metabolism of low levels of benzene by microsomes prepared from 10 human liver samples was investigated. When [^{14}C]-benzene (3.4 μM) was incubated with microsomal preparations, the major metabolites were phenol and hydroquinone accounting for up to 48% of the recovered radiolabel while minor metabolites were catechol and 1,2,4-trihydroxybenzene which accounted for <2% and 0.2% respectively. A further metabolite, tentatively identified as 2,2'-biphenol, accounted for approximately 4% of radiolabel. The CYP2E1 activities of the individual liver samples were found to vary 13-fold as determined by a standard hydroxylation assay with activities ranging between 0.253 to 3.266 nmol/mg/min. When benzene was used as the substrate, a 6-fold difference between liver samples was noted that ranged from 10% to 59%. Comparison of individual liver samples over a 16-min incubation period showed that phenol was the major metabolite formed with the exception of two samples where hydroquinone predominated. These latter two samples had higher CYP2E1 activities and the sample with the highest activity produced equal quantities of phenol and hydroquinone. The rate of benzene metabolism by each of the 10 liver samples correlated with their CYP2E1 activity (Seaton et al, 1994). In a subsequent report by Seaton et al. (1995) addressing the *in vitro* sulfonation of phenol and glucuronidation of hydroquinone by human liver cytosolic and microsomal preparations from 10 donors, there was a 3-fold difference in the rates of conjugation for each reaction.

9.6 Summary

Benzene is readily absorbed by the inhalation, oral and dermal routes in all animal species tested. In humans, the absorption of benzene by the inhalation route is maximal within minutes of exposure and subsequently declines to a constant level. Dermal absorption is generally low compared to inhalation due to volatilisation, with less than 1% of an applied dose being absorbed unless skin exposure is prolonged. The variation in benzene absorption between individuals following inhalation is high. Partitioning of benzene is expected to occur into lipid-rich tissues due to the lipophilic nature of benzene. Several studies have confirmed that benzene accumulates in the adipose tissue, bone marrow and brain of animals and humans.

The metabolism of benzene is qualitatively similar between various animal species, including humans, and proceeds predominantly by hepatic CYP2E1-mediated oxidation of the aromatic ring to yield benzene oxide/oxepin. Subsequent pathways for metabolism of the oxide/oxepin include spontaneous rearrangement to phenol or ring cleavage to give *trans*,*trans*-muconaldehyde. Phenol can be further oxidized to polyphenols (hydroquinone, catechol and 1,2,4-trihydroxybenzene). Detoxification pathways involve conjugation of benzene oxide or *trans*,*trans*-muconaldehyde with GSH while the phenolic metabolites are conjugated to either glucuronate or sulfate. The metabolism of benzene is rapid with water-soluble metabolites appearing in the urine within 2 h of exposure. The major urinary metabolites from several species are conjugates of phenol followed by lesser and variable amounts of hydroquinone conjugates and of pre-phenylmercapturic acid and *trans*,*trans*-muconic acid. Conjugates of catechol have been detected in small amounts in the urine of mice, rabbits and primates.

Due to the limited capacity of hepatic CYP2E1 to metabolise benzene, a substantial proportion of absorbed benzene is eliminated unchanged in exhaled air, with the remainder being eliminated via the urine, principally as metabolites. Urinary excretion appears to be biphasic with a fast phase followed by a prolonged phase, suggesting the slow removal of benzene from adipose tissue. Due to the readily saturable nature of benzene metabolism, exposure at higher doses results in greater elimination of unmetabolised benzene via exhalation.

While comparative studies of urinary benzene metabolites have shown common pathways for benzene metabolism to exist between various species, physiological as well as metabolic differences contribute to some of the observed differences. The easily saturated nature of benzene metabolic pathways and greater respiratory minute volume of the mouse allow the mouse to expire more of an oral dose of benzene compared to the rat. Similarly, respiratory differences and the greater metabolic rate of the mouse allow tissue levels of benzene metabolites to reach higher levels compared to the rat.

10. Effects on Laboratory Mammals and Other Test Systems

The aim of this section is to describe the toxic effects and corresponding effect levels of benzene in animals. In the case of end points studied extensively in humans (mainly haematological and genetic toxicity), the assessment is based on recent reviews by ATSDR (1997) and USEPA (USEPA 1998a, 1998c). For other adverse effects, individual animal studies have been reviewed for this assessment. These include all carcinogenicity studies as well as investigations of the toxic effects of benzene on the central nervous system (CNS), immune function, reproductive organs and foetus.

Most of the available studies do not comply with Good Laboratory Practices (GLP) or international standards such as the OECD Test Guidelines. In consequence, all available publications with a relevant end point have been included in the review irrespective of their compliance with formal quality criteria. However, studies providing insufficient scientific detail to permit a critical appraisal of their findings are clearly identified as such. Unless otherwise indicated, only effects that were statistically different ($p < 0.05$) from controls have been considered.

10.1 Acute toxicity

The acute toxicity of benzene in experimental animals is summarised in Table 10.1, which includes the highest and the lowest values reported in the published literature. Mortality is due to cardio-respiratory arrest from severe CNS depression and/or cardiac arrhythmia (Nahum & Hoff, 1934).

Table 10.1: Acute toxicity of benzene

Route	Species	Measure*	Results	Sex	Reference
Inhalation	Mouse	$LC_{50(7\ h)}$	9980 ppm	Not specified	Svirbely et al. (1943)
	Rat	$LC_{50(4\ h)}$	13,700 ppm	Females	Drew & Fouts (1974)
		$ALD_{(4\ h)}$	16,000 ppm	Males	Smyth et al. (1962)
	Rabbit	$LC_{100(30\ min)}$	45,000 ppm	Both sexes	Carpenter et al. (1944)
Oral	Mouse	LD_{50}	4700 mg/kg	Not specified	RTECS (2000)
			6500 mg/kg	Males	Spanò et al. (1989)
	Rat	LD_{50}	810 mg/kg	Males	Cornish & Ryan (1965)
			5600 mg/kg	Males	Wolf et al. (1956)
			9900 mg/kg	Males	Smyth et al. (1962)
Dermal	Guinea pig, rabbit	LD_{50}	>8200 mg/kg	Male guinea pigs Male and female rabbits	Roudabush et al. (1965)
SC	Mouse	ALD	3500 mg/kg	Males	Watanabe & Yoshida (1970)

* ALD = approximate lethal dose; LC_{50} = median lethal concentration; LC_{100} = concentration leading to 100% mortality; LD_{50} = median lethal dose; SC = subcutaneous.

10.2 Irritation and corrosivity

Several rabbit tests for skin and eye irritation have been reported. From 10-20 daily applications of undiluted benzene to the skin caused redness, oedema, skin peeling and blistering (Wolf et al, 1956). The chemical was also reported to cause skin irritation in a test according to OECD Test Guideline No. 404, however, further details were not provided (Jacobs, 1992, as cited in OECD, 2000). One or two drops of undiluted benzene applied to the eye produced moderate irritation of the conjunctiva and very slight, transient corneal injury (Wolf et al, 1956). Smyth et al. (1962) reported similar skin and eye lesions rated as grade 3 on a 10-point scale.

Rats exposed to benzene vapours for 6 h/day, 5 days/week for 10 weeks exhibited lacrimation during the first 3 weeks of exposure to levels ≥10 ppm (Shell, 1980, as cited in ATSDR, 1997).

10.3 Sensitisation

There are no studies of skin or respiratory sensitisation to benzene in animals.

10.4 Repeated dose toxicity (other than carcinogenicity)

10.4.1 Short-term exposure

The toxic effects of benzene have been investigated in numerous short-term studies in mice and rats. In these studies, benzene was administered orally in vegetable oil or drinking water for 2 days to 24 weeks, or by whole-body exposure to vapours, usually for 6 h/day, 5 days/week. The dose levels tested ranged from 1-600 mg/kg/day by mouth and from 0.44-6600 ppm by inhalation. Repeated dose dermal studies could not be identified.

Neurotoxicity

Evans et al. (1981) exposed male CD-1 and C57BL mice to inhalation of 0, 300 or 900 ppm benzene for 6 h/day. After the 5th exposure, the mice were observed and scored for 7 behavioural categories at 30 and 75 min post-exposure. In both strains, there were increases in the frequency of eating and grooming, and a decrease in sleeping and resting. These stimulatory effects were more pronounced at 75 than at 30 min post-exposure and in the 300 than in the 900 ppm exposure groups, indicating an association with brain concentrations below a certain level.

Immediately following single and repeated 6-h exposures of male mice to 100, 300, 1000 or 3000 ppm benzene, there were increased milk licking at 100 ppm and a reduction in hind limb grip strength at ≥1000 ppm, but no effects on locomotor activity (Dempster et al, 1984). In the absence of motor disturbances, hind limb grip strength is a test for unconditional reflexes. Milk licking, however, may be influenced by hunger and mucosal membrane irritation.

In groups of 10 adult male mice exposed to 0, 0.78, 3.13 or 12.52 ppm benzene for 2 h/day, 6 days/week for 30 days, tests for behaviour (time taken to run to a safety area in a Y-maze following an electric shock) and forelimb grip strength showed stimulation at 0.78 ppm and depression at 12.52 ppm, but no effect on locomotor activity (Li et al, 1992). Compared to unexposed controls, the average change in the frequency of rapid shock responders was +30% at 0.78 ppm and –24% at 12.5 ppm. For grip strength, it was +77% and –11% respectively. As the concentration

of benzene in the air was not checked after the first three days of the experiment and there were extraordinary changes in bone marrow morphology on day 30, actual benzene exposure may have been higher than reported (USEPA, 1998c). On the other hand, the changes in behaviour were observed already on the first 1-2 days of exposure when air level monitoring did take place.

Tegeris & Balster (1994) evaluated the acute behavioural effects in mice of a 20-min inhalation exposure to 2000, 4000 and 8000 ppm benzene and five derivatives (toluene, ethyl benzene, propyl benzene, *m*-xylene and cumene). All six chemicals produced a nearly identical profile of CNS depressant effects that paralleled those of the anaesthetic drug pentobarbital, except that they were short-lived, with recovery beginning within minutes of cessation of exposure.

In mice, administration for 4 weeks of 8-180 mg/kg/day benzene in drinking water had no behavioural effects, but induced a dose-related increase in the level of noradrenaline, dopamine, serotonin and their metabolites in a number of brain regions (Hsieh et al, 1988b). There was also a dose-dependent stimulation of hypothalamic-pituitary-adrenocortical activity (Hsieh et al, 1991). Changes in brain noradrenaline, dopamine, serotonin and/or their metabolites were also found in rats 2 h after a single oral dose of 950 mg/kg benzene or following inhalation of 1500 ppm benzene, 6 h per day for 3 days (Andersson et al, 1983; Kanada et al, 1994). The most consistent finding in these studies was an increase in noradrenaline and dopamine levels in the hypothalamus and other subcortical brain regions. In a rat inhalation study, benzene also induced noradrenaline release from post-ganglionic sympathetic nerves in the ovaries and uterus (Ungváry & Donáth, 1984).

A 30-day drinking water study found a reduction in brain weight in mice at 350 but not at 195 mg/kg/day (Shell 1992, as cited in ATSDR, 1997). No studies were identified that specifically looked for benzene-induced morphological changes in nervous organs or tissues.

Immunotoxicity

When administered to mice by inhalation or in drinking water, benzene suppressed a number of lymphocyte (LC) functions. These included T- and B-LC response to mitogens; interleukin-2 production in T-helper LC; the activity of cytotoxic, alloreactive and suppressive T-LC; B-LC antibody production; and T-LC and macrophage resistance to intracellular infection with *Listeria monocytogenes* (Fan, 1992, as cited in USEPA, 1998c; Hsieh et al, 1988a; Irons et al, 1983; Rosenthal & Snyder, 1985, 1987; Rozen et al, 1984; Rozen & Snyder, 1985; Stoner et al, 1981, as cited in IPCS, 1993; White et al, 1984, as cited in USEPA, 1998c). Based on the above effects, the lowest observed adverse effect level (LOAEL) was 10 ppm by inhalation (Rozen et al, 1984) and 12 mg/kg/day by the oral route (White et al, 1984, as cited in USEPA, 1998c). A no observed adverse effect level (NOAEL) was not achieved, although Hsieh et al. (1988a) found a bimodal response with a reduction in LC proliferation at 40 mg/kg/day and an increase at 8 mg/kg/day, the lowest dose tested. However, Daiker et al. (2000) recently found no changes in spleen LC cellularity, subtype profile or function in mice exposed to inhalation of 0.44 ppm benzene for 7 h/day, 5 days/week for 6 weeks.

In these studies in mice, immunosuppression generally occurred at exposure levels which were also associated with reduced absolute lymphocyte counts (ALC). However, in one 30-day study, mitogen-stimulated LC proliferation was decreased at 12 mg/kg/day (the lowest dose tested) in the absence of any other blood or bone marrow toxicity (White et al, 1982, as cited in USEPA, 1998c). There is no

evidence of specificity with respect to antigen or immune response type. Overall, these findings indicate that benzene-induced immunosuppression is the outcome of a general impairment of the ability of LC to respond to antigenic stimuli by rapid clonal expansion, with little, if any, interference with antigen recognition.

In wild cotton rats given three consecutive intraperitoneal injections of benzene at a dosage of 0, 100, 300, 600 or 1000 mg/kg/day and a battery of tests for cellular and humoral immune functions on days 1-9 after the last treatment, there was no evidence of immunosuppression in any of the treatment groups (McMurry et al, 1991).

In a subacute inhalation study in male Sprague-Dawley rats exposed to 0, 30, 200 or 400 ppm benzene for 6 h/day, 5 days/week for 2-4 weeks, Robinson et al. (1997) determined a NOAEL/LOAEL of 200/400 ppm based on spleen weight, cellularity, total T-LC, T-helper LC, antigen-stimulated and unstimulated B-LC content, and thymus weight. As such, rats appear to be less sensitive than mice to the immunotoxic effects of benzene.

Effects on blood and blood forming organs

ATSDR (1997) and USEPA (1998c) have reviewed a large number of published and unpublished study reports that address the effects of short-term exposure to benzene on the blood and blood forming organs of mice and rats. Based on these reviews, the overall findings can be summarised as follows:

- In peripheral blood, there was a decrease in the quantity of some or all of the formed elements, including red blood cells (RBC), white blood cells (WBC), LC and blood platelets (Plt). In some studies, there was also a reduction in haemoglobin (Hb) levels and the average RBC size (mean corpuscular volume (MCV)) was either increased or decreased.
- There was bone marrow hypoplasia, with decreased numbers of multipotential stem cells and cells that differentiate into RBC, WBC and macrophages, but an increase in immature RBC such a micronucleated polychromatic and normochromatic RBC.
- In the spleen, there was a decrease in the number of all LC types, but an increase in haematopoiesis in general[3].
- There was a decrease in the weight of the thymus, which is the main site of T-LC proliferation.

The order of susceptibility to these effects was male mice > female mice > rats. In terms of target organ, it was spleen ≥ peripheral blood ≥ bone marrow > thymus. In mice, the most sensitive indicators of benzene toxicity were spleen weight, bone marrow cellularity, and WBC and RBC counts, one or more of which were affected at concentrations around 10 ppm and above for inhalation and at 8 mg/kg/day and above for oral exposure. In rats, the most sensitive end points were ALC and WBC counts, which were affected at 100 ppm by inhalation and 25 mg/kg/day by oral administration.

[3] Haematopoiesis is the sum of processes involved in the production and development of the blood cells. The haematopoietic processes are usually confined to the bone marrow, but may also take place in the spleen and liver, for example, in foetuses and newborn animals, or when there is a substantial increase in the demand for blood cells.

Two 2-16 week studies in the mouse showed that all haematological abnormalities returned to normal or near-normal within 4-25 weeks post-exposure (Cronkite et al, 1985; Snyder et al, 1988).

Other effects

There are no consistent reports of respiratory, cardiovascular, gastro-intestinal, hepatic or renal effects of short-term exposure to benzene by any route (ATSDR, 1997). Effects on reproductive organs are reviewed in Section 10.5. Mortality was generally low and only a few studies reported decreases in body weight (BW) gain.

Other experimental animals

There is limited evidence that airborne exposure to benzene for 3-4 weeks induces leukopoenia in guinea pigs and lymphocytopoenia, leukopoenia and impaired cellular immunity in pigs at levels ≥88-100 ppm (Dow, 1982, as cited in ATSDR, 1997; Wolf et al, 1956).

10.4.2 Long-term exposure

Blood and blood forming organs

ATSDR (1997) and USEPA (1998c) have reviewed more than a dozen published reports which describe the results of nine separate studies of the long-term effects of benzene exposure on blood and blood forming organs. In these studies, mice or rats were exposed to benzene for ≥26 weeks by oral administration in vegetable oil or by whole-body inhalation for 5-6 h/day, 4-5 days/week, at dose levels ranging from 1-500 mg/kg/day by mouth and from 88-300 ppm by inhalation.

The most consistent long-term effect on the blood was a reduction in RBC, WBC and LC counts. In some studies, the number of neutrophilic granulocytes and reticulocytes (young RBC) was increased. The bone marrow and the spleen showed hypoplasia in some studies and an increase in haematopoietic tissue in others.

Haematological effects were recorded at the lowest dose level examined in all long-term tests, except for one poorly reported rat study in which the NOAEL was 1 mg/kg/day by mouth (Wolf et al, 1956). This study also recorded a lower LOAEL than any of the other available studies, namely 88 ppm by inhalation based on WBC count and spleen weight and 10 mg/kg/day by oral administration based on WBC count. In more adequately reported studies, the LOAEL was 100 ppm by inhalation and 25 mg/kg/day by mouth in both mice and rats (ATSDR, 1997; USEPA, 1998c).

Other effects

There are no consistent reports of non-neoplastic cardiovascular, liver or kidney abnormalities from long-term exposure to benzene by any route (ATSDR, 1997). There was chronic irritation of the forestomach epithelium in male rats and mice and hyperplasia of the Harderian gland[4] and pulmonary alveolar epithelium in male and female mice in 2-year, but not in 17-week oral gavage studies (NTP, 1986[5]). Lesions occurred at 200 mg/kg/day in rats and at dose levels ≥25 mg/kg/day in

[4] A tear gland in the median angle of the eye which is rudimentary in humans.
[5] The major findings in the studies conducted by the National Toxicology Program (NTP) have been published by Huff et al. (1989).

mice. Reproductive effects are described below. The median survival time and BW gain were generally reduced in a dose-dependent manner.

Other experimental animals

There is limited evidence that airborne exposure to benzene for 35-38 weeks induces leukopoenia and increased spleen weight in guinea pigs and leukopoenia in rabbits at dose levels ≥80-88 ppm (Wolf et al, 1956).

10.5 Reproductive toxicity

10.5.1 Effects on fertility and lactation

Data on fertility and lactation are available from three repeated-dose toxicity tests, three one-generation fertility studies and a limited number of other studies.

Repeated-dose toxicity and one-generation fertility studies

In a 13-week inhalation study, Ward et al. (1985) exposed groups of 150 mice and 50 rats per sex to inhalation of 0, 1, 10, 30 or 300 ppm benzene for 6 h/day, 5 days/week. There was clear evidence of haematological toxicity at the highest dose level in both species and sexes, but no consistent exposure-related trends in mortality, clinical observations or mean BW data. The testes and ovaries from 20 mice/sex/group and from 10 rats/sex exposed to either 0 or 300 ppm benzene were examined microscopically. In mice from the highest exposure group (300 ppm), there were 4 animals with cystic ovaries, 7 with bilateral testicular atrophy or degeneration, 6 with decreases in the number of spermatozoa in the epididymal ducts and 9 with an increase in abnormal sperm forms. Similar lesions of doubtful biological significance were seen in both sexes at lower dose levels. No abnormalities were found in the gonads of rats exposed to 300 ppm benzene.

A study in mice administered benzene at 25, 50 or 100 mg/kg/day by gavage for 2 years found the following number of animals with epithelial hyperplasia or follicular atrophy of the ovaries (NTP, 1986):

Ovarian lesions:	0 mg/kg/day	25 mg/kg/day	50 mg/kg/day	100 mg/kg/day
No. of mice examined	47	44	49	48
Epithelial hyperplasia	12	39	31	29
Senile atrophy	15	35	32	22

The statistical significance of these findings is not reported. However, when analysed for this assessment, the increase in the incidence of epithelial hyperplasia was significant at all dose levels, whereas the incidence of senile atrophy was significantly elevated at 25 and 50, but not at 100 mg/kg/day ($p < 0.05$; test for exact confidence limits). Testes were not examined microscopically, as they had no grossly visible lesions. A parallel study in rats found no macroscopic abnormalities in the gonads, even at the highest dose level tested (100 mg/kg/day in females, 200 mg/kg/day in males) (NTP, 1986).

In an early inhalation study, Wolf et al. (1956) observed sedation, growth depression, mortality and a moderate increase in testis weight in male rats exposed to 6600 ppm benzene for 13 weeks. The testes were normal in rats exposed to 88 or 2200 ppm for 30 weeks. At these dose levels there were mild lesions of the blood and lymphatic system, but no mortality. In guinea pigs and rabbits exposed to 80-88 ppm benzene for 35-38 weeks, there was no mortality, mild haematological

changes and a slight increase in testis weight and in mild degenerative lesions of the seminiferous tubules. Further details were not reported and it is unclear whether these findings were statistically significant.

In female mice, a single intraperitoneal injection of benzene at the maximum tolerated dose of 1250 mg/kg did not reduce the number of offspring and litters produced in a reproductive capacity test involving the observation of 35 treated and untreated breeding pairs for 347 days (Bishop et al, 1997).

In a one-generation fertility study, groups of 26 female rats were exposed to 0, 1, 10, 30 or 300 ppm for 6 h/day, 5 days/week for 10 weeks prior to mating and then daily on days 0-20 of gestation and days 5-20 of lactation (Kuna et al, 1992). In the dams, there was no exposure-related effect on BW gain, clinical observations or necropsy findings and no fertility-related effects. When the neonates were examined at weaning on day 21 postpartum, there was a dose-related, 6-9% increase in relative kidney weight in female offspring of dams exposed at 10 ppm and above. Female offspring of dams exposed to 300 ppm benzene also had a 10% BW reduction and a 14% reduction in absolute liver weight.

In another fertility study, female rats were kept in inhalation chambers where they were exposed for 24 h/day to 0, 0.3, 1.6, 6.3, 15, 18, 20 or 200 ppm benzene (Gofmekler, 1968). Exposure began 10-15 days prior to mating, when males were introduced into the chambers for 6-10 days, and continued throughout the entire pregnancy period until spontaneous delivery. There were no pregnancies at the highest dose level. In dams exposed to 0.3-20 ppm benzene, the average litter size was 7.5 compared to 8.4 in the controls, but there was no exposure-related effect on the birth weight of the pups.

Other studies

In early experimental studies, benzene caused degenerative changes in the testes and severely hypoplastic ovaries, degenerated ovarian follicles and chromosomal damage and mitotic interruption in the ova when administered by subcutaneous injection or inhalation to male and female mice and female rabbits (Hett & Mark, 1938; Vara & Kinnunen, 1946). The dose levels used in mice were not given, but were high enough to induce marked leukopoenia. Rabbits were administered 1000 mg/kg/day for 10 days.

In a study in adult mice, testicular germ cell suspensions were examined for DNA content by flow cytometry at 1, 2, 3, 4 and 10 weeks after a single sublethal dose of 1-7 mL (880-6160 mg) benzene/kg administered by oral gavage (Spanò et al, 1989). These doses had no effect on body or testis weight, but resulted in a dose-dependent reduction in the relative cell count in the primary spermatocyte and spermatid fractions. The primary spermatocyte fraction was most affected at 2 weeks, the round spermatid fraction at 3 weeks and the elongated spermatid fraction at 4 weeks post-treatment, as one would expect from a cytotoxic insult resulting in a transient reduction in the number of differentiating spermatogonia.

Conclusions

Overall, the above studies indicate that benzene exposure may cause degenerative changes in the gonads of mice, whereas there is insufficient evidence of similar effects in other species. There was also epithelial hyperplasia in the ovaries of mice in the NTP (1986) 2-year oral bioassay. However, this is likely to represent a preneoplastic lesion as ovarian tumours occurred with a significant positive trend in

this study and epithelial hyperplasia was found in other organs with neoplastic lesions, namely the Harderian gland and the lungs.

Compound-related testicular atrophy or degeneration was observed in male mice exposed to 300 ppm benzene by inhalation. Ovarian atrophy was observed in mice at 25 mg/kg/day by mouth and cystic ovaries at 300 ppm by inhalation. In both sexes, these lesions occurred at dose levels that were associated with haematological effects, but not with mortality or other signs of generalised toxicity.

The available data on reproductive capacity are inconclusive.

The changes in body, liver and relative kidney weights observed by Kuna et al. (1992) in 21-day old female neonates of rats exposed to inhalation of benzene during pregnancy and lactation are modest, but nonetheless indicative of developmental toxicity. Because of the study design it cannot be determined whether these effects were lactational or the result of exposure *in utero.*

10.5.2 Developmental toxicity

Standard tests

Developmental toxicity tests have been conducted in mice, rats and rabbits exposed to benzene by inhalation, mouth or subcutaneous injection during the gestation period (Table 10.2). All foetuses were examined for external defects and in all but two studies (Exxon Chemical Company, 1986, as cited in USEPA, 1998c; Watanabe & Yoshida, 1970) for visceral and skeletal abnormalities as well.

Overall, there were no major structural abnormalities in the foetuses, except in one study in the mouse in which a single SC injection of a maternally toxic dose of 2600 mg/kg benzene on GD 13 was associated with cleft palate and jaw malformations (Watanabe & Yoshida, 1970). However, several inhalation and oral studies conducted in mice or rats found evidence of other foetal effects at dose levels where no toxic effects were recorded in the dams. These include a small (4-6%), but statistically significant reduction in foetal BW (Coate et al, 1984; Murray et al, 1979; Seidenberg et al, 1986) and a significant increase in the frequency of minor skeletal abnormalities (Green et al, 1978; Murray et al, 1979). Moreover, in two studies on which little experimental detail is available, there was an increase in resorptions in rats (Litton Bionetics, 1977, as cited in USEPA, 1998c) and a decrease in foetal BW in mice (Nawrot & Staples, 1979), in both cases in the absence of any signs of maternal toxicity.

In rabbits, continuous inhalation of 310 ppm benzene was associated with abortions, an increase in resorptions or foetal deaths, a decrease in foetal BW and an increased incidence of minor abnormalities in the presence of maternal toxicity (reduced BW gain), whereas 155 ppm had neither foetal nor maternal effects (Ungváry & Tátrai, 1985).

Table 10.2: Summary of developmental toxicity tests*

Species	Study design	Daily dose	Foetal effects†	Maternal effects†	Reference
Mouse	SC injection on GD 8-9 or GD 12-13	1760 mg/kg	None	Decreased Hgb	Matsumoto et al. (1975)
	8-11 pregnancies per group	3520 mg/kg	Decreased BW (3%) Decreased placenta weight (5%) in GD 12-13 group Delayed ossification in GD 12-13 group	Decreased Hgb Decreased WBC count	
	Inhalation, 7 h/day, GD 6-15 26-30 pregnancies/group	500 ppm	Decreased BW (6%) Unspecified increase in ' minor skeletal variants'	None	Murray et al. (1979)
	Oral gavage 3 times daily, GD 6-15 No. of pregnancies not given	800 mg/kg	Decreased BW	None	Nawrot & Staples (1979)
		1300 mg/kg	Increase in resorptions Decreased BW	Increase in mortality	
		2600 mg/kg	Increase in resorptions Decreased BW	Increase in mortality	
	Oral gavage 3 times daily,GD 12-15 No. of pregnancies not given	2600 mg/kg	Increase in late resorptions Decreased BW	Increase in mortality	Nawrot & Staples (1979)
	Inhalation, 24 h/day, GD 6-15 15 exposed pregnancies 115 control pregnancies	155 ppm	' Weight retardation' (25 vs. 7% of foetuses) Delayed ossification (10 vs. 5% of foetuses)	No information	Ungváry & Tátrai (1985)
		310 ppm	' Weight retardation' (27 vs. 7% of foetuses) Delayed ossification (11 vs. 5% of foetuses)	No information	
	Oral gavage, GD 8-12 28 pregnancies/group	1300 mg/kg	Decreased BW (4%)	None	Seidenberg et al. (1986)
	Single SC injection on GD 11, 12, 13, 14 or 15 15 pregnancies per group No controls External foetal examination only	2600 mg/kg	Increased incidence of cleft palate and jaw malformations in offspring of dams injected on GD 13 compared to foetuses of dams injected on GD 11-12 or 14-15	Decrease in WBC count in all dams No difference in fall in WBC count or in BW gain between dams with or without malformed foetuses	Watanabe & Yoshida (1970)

Table 10.2: Continued

Species	Study design	Daily dose	Foetal effects†	Maternal effects†	Reference
Rat	Inhalation, 6 h/day, GD 6-15 26-31 pregnancies/group	10 ppm	Increase in resorptions	None	Litton Bionetics (1977), as cited in EPA (1998c)
		40 ppm	Increase in resorptions	None	
	Inhalation, 7 h/day, GD 6-15 14-18 pregnancies/group	100 ppm	Missing sternebrae (9/18 vs. 1/16 litters)	None	Green et al. (1978)
		300 ppm	Delayed ossification of sternebrae (10 vs. 2% of female foetuses)	None	
		2200 ppm	Decreased BW (10%) Decreased crown-rump length (5%) Delayed ossification of sternebrae (11 vs. 1% of female foetuses) Missing sternebrae (11/15 vs. 2/14 litters)	Decreased BW gain Lethargy	
	Inhalation, 24 h/day, GD 9-14 19 exposed pregnancies 28 controls	313 ppm	Decreased BW (12%) Delayed ossification (11 vs. 0% of foetuses) Fused sternebrae and extra ribs (9 vs. 1% of foetuses)	Decreased BW gain (57%)	Hudák & Ungváry (1978)
	Inhalation, 24 h/day, GD 7-14 17-20 pregnancies/ exposure group 46 control pregnancies	50 ppm	Decreased BW (5%)	Decreased BW gain (27%) Decreased placenta weight (9%)	Tátrai et al. (1980)
		150 ppm	Resorbed or dead foetuses (42 vs. 6%) Decreased BW (28%) Skeletal abnormalities (57 vs. 5% of foetuses)	Mortality (3/20 vs. 0/48) Decreased BW gain (45%) Increased relative liver weight (9%) Decreased placenta weight (7%)	
		500 ppm	Resorbed or dead foetuses (32 vs. 6%) Decreased BW (20%) Skeletal abnormalities (66 vs. 5% of foetuses)	Mortality (1/22 vs. 0/48) Decreased BW gain (55%) Increased relative liver weight (14%) Decreased placenta weight (16%)	
		1000 ppm	Resorbed or dead foetuses (29 vs. 6%) Decreased BW (22%) Skeletal abnormalities (55 vs. 5% of foetuses)	Mortality (3/22 vs. 0/48) Decreased BW gain (41%) Increased relative liver weight (10%) Decreased placenta weight (20%)	

Table 10.2: Continued

Species	Study design	Daily dose	Foetal effects†	Maternal effects†	Reference
Rat	Inhalation, 7 h/day, GD 6-15 14-15 pregnancies/ exposure group 11 control pregnancies	10 ppm	None	Increased BW gain (33%) on GD 15-20	Kuna & Kapp (1981)
		50 ppm	Decreased BW (14%) Increase in foetuses with skeletal and/or visceral variations (18 vs. 3%)	Decreased BW gain (34%) on GD 5-15	
		500 ppm	Decreased BW (18%) Decreased crown-rump length (7%) Increase in foetuses with skeletal and/or visceral variations (21 vs. 3%)	Decreased BW gain (37%) on GD 5-15 Increased BW gain (40%) on GD 15-20	
	Inhalation, 7 h/day, GD 6-15 32-38 pregnancies/group 2 control groups	1 ppm	None	None	Coate et al. (1984)
		10 ppm	None	None	
		40 ppm	None	None	
		100 ppm	Decreased BW (6%)	None	
	Oral gavage, GD 6-15 20-22 pregnancies/group External foetal examination only	50 mg/kg	None	None	Exxon Chemical Company (1986), as cited in EPA (1998c)
		250 mg/kg	None	Decreased feed consumption	
		500 mg/kg	Decreased BW	Decreased BW gain Decreased feed consumption	
		1000 mg/kg	Decreased BW	Decreased BW gain Decreased feed consumption Alopecia	
Rabbit	Inhalation, 7 h/day, GD 6-18 18-19 pregnancies/group	500 ppm	None	Increased feed and water consumption	Murray et al. (1979)
	Inhalation, 24 h/day, GD 7-20 11-15 exposed pregnancies/ group 60 control pregnancies	155 ppm	None	None	Ungváry & Tátrai (1985)
		310 ppm	Abortions (6/15 vs. 0/60 dams) Resorbed or dead foetuses (16 vs. 5%) Decreased BW (17%) ‘ Minor anomalies’ (86 vs. 34% of foetuses)	Decreased BW gain (62%; not corrected for the effect of abortions) Increased relative liver weight (17%)	

* BW = body weight; GD = gestation day; Hgb = haemoglobin; SC = subcutaneous; WBC = white blood cell.
† Where available, information on the incidence or magnitude of effects compared to non-exposed controls is shown in brackets.

Other studies

According to Ungváry (1985), continuous inhalation of benzene (125 ppm), benzene plus toluene, or benzene plus xylenes had foetotoxic effects in rats, but only in the presence of maternal toxicity. Exposure to 830 ppm benzene over 48 h on GD 10-13 increased the severity of maternal toxicity and incidence of malformations induced by a single oral dose of 250-500 mg/kg acetyl salicylic acid administered at the end of the exposure period.

Keller & Snyder (1986, 1988) investigated the effects of low level maternal exposure to benzene on the blood and blood forming organs of mouse foetuses, neonates and young adults. Groups of 5-10 dams were exposed to inhalation of 0, 5, 10, or 20 ppm benzene for 6 h/day on GD 6-15. Tests on the progeny comprised RBC, WBC, differential blood cell count, and blood cell morphology; Hb and haematocrit (Hct); quantification of colony forming units of erythrocyte (CFU-E) and granulocyte/macrophage (CFU-GM) progenitor cells; and microscopic examination of blood forming tissue in the liver, bone marrow and spleen. The number of progeny examined included 2/sex/litter/dose on GD 16, 2/sex/litter/dose on day 2 after birth, and 1/sex/ litter/dose at 6 weeks after birth.

In 16-day old foetuses exposed to 20 ppm benzene *in utero*, liver CFU-E was depressed in both sexes. In peripheral blood from the 2-day old neonates, there was a dose-related, marked decrease in the number of nucleated RBC. At 20 ppm, there was also an increase in CFU-E (males only), CFU-GM, non-dividing and dividing granulocytes in hematopoietic liver tissue, and in non-dividing granulocytes in peripheral blood. In 6-week old young adult progeny, bone marrow CFU-E was depressed and spleen CFU-E increased in males exposed to 10 but not to 20 ppm *in utero*. In the 20 ppm group, there was a decrease in early nucleated RBC in the bone marrow and an increase in blast cells in the spleen. There were no effects on BW or BW gain, feed consumption and clinical signs in the dams, or on BW and structural abnormalities in the foetuses at any dose level.

In a subsequent study on the interaction between alcohol and inhaled benzene in mice, CFU-E was significantly depressed in the liver of 16-day old male (but not female) foetuses exposed *in utero* to 10 ppm benzene for 6 h/day on GD 6-15 (Corti & Snyder, 1996). This study comprised a total of 9 exposed and 12 control litters.

The embryotoxicity of benzene and its major metabolites has also been investigated *in vitro* in GD 10-12 rat conceptuses. There were no toxic effects at concentrations of 0.4-0.8 mM (32-64 mg/L) benzene (Brown-Woodman et al, 1994; Chapman et al, 1994). Phenol was not toxic at 1.6 mM, but caused 100% lethality at 0.2 mM in the presence of several CP450-dependent bioactivating systems. In the absence of metabolic activation, catechol, hydroquinone, and quionone each produced 100% lethality at 0.1 mM and the combination of phenol and hydroquinone showed a greater than additive effect (Chapman et al, 1994).

Conclusions

In several studies in pregnant animals exposed to benzene by inhalation or ingestion, there was a small, but statistically significant decrease in foetal BW and an increase in the incidence of minor skeletal abnormalities at dose levels at which there was no evidence of maternal toxicity. Major structural abnormalities and abortions only occurred at dose levels that also caused marked toxicity in the dams. As such, benzene can be characterised as foetotoxic, but not teratogenic. Based on

adequately reported rat studies that found foetal effects in the absence of any signs of maternal toxicity, the inhalation NOAEL for foetal growth disturbances is 40 ppm (Coate et al, 1984), with a LOAEL of 100 ppm (Coate et al, 1984; Green et al, 1978). Reliable oral effect levels cannot be determined from the available data.

In a small number of pregnant mice, inhalation of 10-20 ppm benzene resulted in specific adverse effects on multipotential haematopoietic stem cells (colony forming units) and other blood cells in the liver, bone marrow or spleen of the offspring (Corti & Snyder, 1996; Keller & Snyder, 1986, 1988). These effects occurred in the absence of any other signs of developmental toxicity and at levels similar to those that are known to be toxic to the blood and blood forming organs of adult mice (Section 10.4).

In vitro studies indicate that some benzene metabolites, including catechol, hydroquinone and quinone (but not phenol) are substantially more toxic to rat embryos than benzene itself.

10.6 Genotoxicity

The toxic effects of benzene on human genetic material have been investigated in numerous in vivo studies which are addressed in Section 11.6 below. As such, the assessment of studies conducted in animals and various in vitro systems is limited to an overview of the most significant findings. Unless otherwise indicated, the information presented is summarised from ATSDR (1997), IARC (1987), IPCS (1993) and USEPA (1998a).

In vitro tests

Tests with benzene itself have predominantly produced negative results in conventional in vitro gene mutation assays in bacteria and mammalian cell systems, with and without metabolic activation. In vitro assays for chromosome aberrations have also generally been negative, unless special precautions were taken to prevent the evaporation of benzene from the test system (Randall et al, 1993). Likewise, conventional in vitro tests for DNA breaks, unscheduled DNA synthesis and DNA synthesis inhibition have produced inconsistent results. However, sister chromatid exchanges (SCE), micronuclei (MN) and unscheduled DNA synthesis have been induced in vitro by metabolites such as catechol, hydroquinone and/or quinone, and DNA adducts with phenol, hydroquinone and quinone have been detected in a number of in vitro systems. Benzene itself has recently been shown to induce morphological transformation, gene mutations through base substitutions, and aneuploidy in Syrian hamster embryo cells, but is less potent than its metabolites (Tsutsui et al, 1997). In the alkaline single cell gel electrophoresis (Comet) assay, pronounced, contact time-dependent DNA damage has been detected in non-cycling (G_0) human LC after treatment not only with catechol, hydroquinone, quinone, 1,2,4-trihydroxybenzene or muconic acid, but also with benzene itself (Anderson et al, 1995).

Tests in *Drosophila*

Benzene was consistently negative in the sex-linked recessive lethal test in *Drosophila melanogaster*, which is a specific, but insensitive test for the potential of chemicals to cause heritable gene mutations and chromosome aberrations. However, benzene has been shown to induce a statistically significant number of

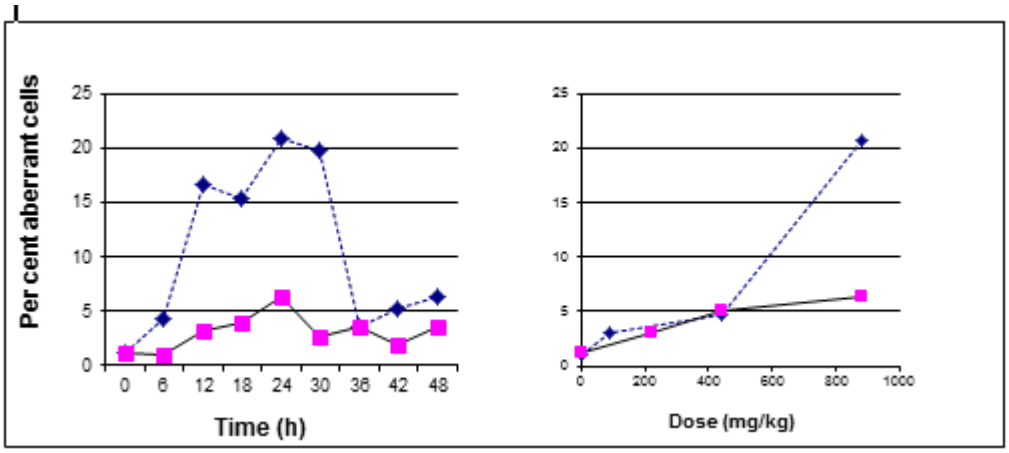
so-called delayed lethal mutations, which may be the result of heritable mutations in one rather than in both DNA strands of the X chromosome (Kale & Kale, 1995).

In vivo tests in rodents

There is ample evidence that benzene is genotoxic in a broad spectrum of *in vivo* tests in rodents, in which the chemical was administered by inhalation, oral gavage or parenteral injection. These include tests for SCE and MN induction in peripheral blood cells, bone marrow cells, foetal liver cells, lung fibroblasts (Ranaldi et al, 1998), and Zymbal gland cells (Angelsanto et al, 1996); gene mutations in LC, lung and spleen cells; chromosome aberrations in LC, bone marrow cells, spleen cells, and spermatogonia; and DNA adducts in nucleated blood and bone marrow cells. Furthermore, many of these effects have been shown to be mitigated by inhibitors of benzene metabolism and reproduced by benzene metabolites such as hydroquinone and 1,2,4-trihydroxybenzene.

In an in vivo chromosome aberration study in male mice, the sensitivity and dose response to a single oral dose of benzene was found to differ markedly between bone marrow cells and differentiating spermatogonia, as illustrated in Figure 10.1 (Ciranni et al, 1991).

Figure 10.1: Chromatid aberrations excluding gaps in mouse bone marrow cells (broken lines) and spermatogonia (solid lines) at 6-48 h after oral treatment with 880 mg/kg benzene (left) and at 24 h after oral treatment with 88, 220, 440 or 880 mg/kg benzene (right) (Ciranni et al, 1991)



10.7 Carcinogenicity

Table 10.3 highlights the principal findings in the 23 carcinogenicity tests that have been reported in the open literature. They include oral gavage studies in B6C3F1, RF/J and Swiss mice and F344, Sprague-Dawley and Wistar rats and inhalation studies in AKR, C57BL, CBA, CD-1 and HRS mice and Sprague-Dawley rats.

Table 10.3: Principal findings in inhalation (I) and oral gavage (O) carcinogenicity studies in mice and rats

Strain	I/O	Protocol	Principal findings*	Reference
Mice				
AKR†	I	0, 100 ppm for 6 h/day, 5	No increase in tumour incidence	Snyder et al.

		days/week for life (72 weeks)		(1980)
	I	0, 300 ppm for 6 h/day, 5 days/week for life (28 weeks)	No increase in tumour incidence	Snyder et al. (1978)
$B6C3F_1$	O	0, 25, 50, 100 mg/kg/day for 5 days/week, 103 weeks	Harderian gland tumours, lung tumours, lymphoma, mammary gland carcinoma, ovarian granulosa cell and mixed benign tumours, preputial gland carcinoma, Zymbal gland carcinoma	NTP (1986)
C57BL‡	I	0, 300 ppm 6 h/day, 5 days/week for 16 weeks, with life-long observation (about 110 weeks)	Lymphoma, ovarian tumours, Zymbal gland carcinoma (no statistical analysis)	Cronkite et al. (1985)
	I	0, 300 ppm for 6 h/day, 5 days/week for life (70 weeks)	Lymphoma	Snyder et al. (1980)
	I	0, 300 ppm 6 h/day, 5 days/week, 1 out of every 3 weeks for life (118 weeks)	Zymbal gland carcinoma	Snyder et al. (1988)
	I	0, 1200 ppm 6 h/day, 5 days/week for 10 weeks, with life-long observation (about 146 weeks)	No increase in tumour incidence	Snyder et al. (1988)
CBA§	I	0, 100 ppm for 6 h/day, 5 days/week for 16 weeks, with life-long observation (about 135 weeks)	Unspecified, non-hepatic, non-haematopoietic tumours	Cronkite et al. (1989)
	I	0, 300 ppm for 6 h/day, 5 days/week for 16 weeks, with life-long observation (about 115 weeks)	Non-hepatic, non- haematopoietic tumours (including Harderian gland carcinoma, lung adenocarcinoma, mammary gland carcinoma, neoplasms resembling acute myeloblastic and chronic granulocytic leukaemia, Zymbal gland carcinoma)	Cronkite et al. (1989)
	I	0, 300 ppm for 6 h/day, 5 days/week for 16 weeks, with life-long observation (78 weeks)	Lung adenoma, lymphoma, preputial gland carcinoma	Farris et al. (1993)
CD-1	I	0, 300 ppm for 6 h/day, 5 days/week for life (not further specified)	Sporadic cases of suspected myeloid leukaemia ($p = 0.147$)	Goldstein et al. (1982)
	I	0, 300 ppm for 6 h/day, 5 days/week 1 out of every 3 weeks for life (about 60 weeks)	Lung adenoma	Snyder et al. (1988)
	I	0, 1200 ppm for 6 h/day, 5 days/week for 10 weeks, with life-long observation (about 130 weeks)	Lung adenoma, Zymbal gland carcinoma	Snyder et al. (1988)
HRS	I	0, 400 ppm for 6 h/day, 5 days/week for 26 weeks	No leukaemia or lymphoma in either hr/hr (leukaemia-prone) or hr/– (leukaemia-resistant) strains	Stoner et al. (1980), as cited in Cronkite et al. (1985)
RF/J	O	0, 500 mg/kg/day for 5 days/week, 52 weeks	Lymphatic neoplasms, lung tumours, mammary gland carcinoma (no statistical analysis)	Maltoni et al. (1989)
Swiss	O	0, 500 mg/kg/day for 5 days/week, 78 weeks	Lung adenomas, mammary gland carcinoma, Zymbal gland carcinoma (no statistical analysis)	Maltoni et al. (1989)

Table 10.3: Continued

Strain	I/O	Protocol	Principal findings	Reference
Rat				

F344	O	0, 50, 100, 200 mg/kg/day in males and 0, 25, 50, 100 mg/kg/day in females for 5 days/week, 103 weeks	Oral cavity tumours, skin tumours, Zymbal gland carcinoma	NTP (1986)
Sprague-Dawley	I	0, 100 ppm for 6 h/day, 5 days/week for life (123 weeks)	No increase in tumour incidence	Snyder et al. (1984)
	I	0, 300 ppm for 6 h/day, 5 days/week for life (99 weeks)	No increase in tumour incidence	Snyder et al. (1978)
	I	0, 200-300 ppm for 4-7 h/day, 5 days/week, 104 week	Oral cavity carcinoma, Zymbal gland carcinoma (no statistical analysis)	Maltoni et al. (1989)
	O	0, 50, 250 mg/kg/day, 5 days/week, 52 weeks	Mammary gland tumours (lowest dose level only), Zymbal gland carcinoma in females (no statistical analysis)	Maltoni et al. (1989)
	O	0, 500 mg/kg/day, 5 days/ week, 104 weeks	Forestomach carcinomas, liver angiosarcomas, nasal and oral cavity carcinomas, skin carcinomas, Zymbal gland carcinoma (no statistical analysis)	Maltoni et al. (1989)
Wistar	O	0, 500 mg/kg/day, 5 days/week, 104 weeks	Nasal and oral cavity carcinoma, Zymbal gland carcinoma (no statistical analysis)	Maltoni et al. (1989)

* Positive findings were statistically significant ($p < 0.05$) unless otherwise indicated.
† Carries a virus causing spontaneous lymphoma in 90% of animals by 52 weeks of age.
‡ Carries a virus yielding a high incidence of lymphoma from exposure to radiation, immunosuppression and certain carcinogens.
§ Highly susceptible to radiation-induced thymic lymphoma.

There was a frequent association between benzene exposure and the occurrence of solid tumours in epithelia of the mouth, nasal cavities, lung alveoli, Harderian, Zymbal, preputial and mammary glands, and the ovary.

With regard to the blood and lymphatic system, the incidence of lymphoma was elevated in several studies conducted in B6C3F1, C57BL, CBA and RF/J mice. There was also a statistically significant increase in the incidence of lesions resembling acute myeloblastic and chronic granulocytic leukaemia in a study in CBA mice exposed to 300 ppm benzene for 16 weeks (Cronkite et al, 1989). In addition, 3/40 CD-1 mice exposed to 300 ppm and 1/40 Sprague-Dawley rats exposed to 100 ppm benzene developed suspected myeloid leukaemia after 27-38 weeks of exposure (Goldstein et al, 1982; Snyder et al, 1984). However, the increase in lymphoma incidence was limited to strains where this is a common spontaneous tumour type and the lesions resembling leukaemia may not have been malignant but rather an intense proliferation of myeloid cells caused by infections or necrotic processes in benzene-induced tumours in other organs (Farris et al, 1993). Furthermore, early findings of lymphoma or leukaemia-like lesions in a given strain have not been consistently reproduced in later studies (Farris et al, 1993; Snyder et al, 1988).

Some of the target organs in rodents such as the forestomach, Harderian, Zymbal and preputial glands have no anatomical equivalent in humans. Moreover, human exposure to benzene is not associated with tumours of the mouth, nasal cavities or lung alveoli (see Section 11). However, as there is limited evidence of an elevated risk of malignant melanoma and breast cancer in humans exposed to benzene-containing products, skin and mammary tumours are further analysed below.

Skin tumours

The incidence of skin tumours was increased in F344 and Sprague-Dawley rats in 2-year oral gavage studies (NTP, 1986; Maltoni et al, 1989). By contrast, no skin tumours developed in groups of 10 mice after oral, subcutaneous or topical application of 800 mg/kg benzene followed 4 weeks later by topical application of the tumour promoter 12-*o*-tetradecanoylphorbol-13-acetate 3 times a week for 20 weeks (Bull et al, 1986). Furthermore, although benzene was once widely used as a solvent in tests for skin cancer induction in mice resulting in large numbers of controls being topically exposed to benzene alone, there has been no indication that it induced skin tumours in these models (IARC, 1982a).

In F344 rats, skin tumours were found on the face, back, flank, and other locations. Microscopically, they represented a spectrum from pure squamous cell papillomas or carcinomas to mixed tumours containing basal cell, sebaceous gland or hair follicle elements. By incidental tumour tests, the incidence was elevated in male rats at 200 mg/kg/day (12/50 vs. 1/50 in controls; $p < 0.01$), but not at 50 mg/kg/day (7/50) or 100 mg/kg/day (5/50), or in female rats treated with 25, 50 or 100 mg/kg/day. Based on mortality and BW data, high dose male rats were probably exposed to benzene levels that exceeded the maximal tolerated dose (NTP, 1986).

In Sprague-Dawley rats, skin carcinomas (not further specified) occurred in 9/40 male animals administered 500 mg/kg/day by oral gavage for 2 years. The incidence was zero in male controls and treated females (Maltoni et al, 1989). The authors did not comment on the statistical significance of these results, however, when analysed for this assessment, the difference in incidence between exposed and control males was statistically significant ($p < 0.05$; test for exact confidence limits). Compared to their controls, male rats had an increased survival rate but a reduction in BW that ranged from 6-18% during the course of the study (Maltoni et al, 1983).

Mammary gland tumours

Mammary tumours have been found in $B6C3F_1$, CBA, RFJ and Swiss mice and Sprague-Dawley rats (Cronkite, 1986; NTP, 1986; Maltoni et al, 1989).

In a 2-year oral bioassay in $B6C3F_1$ mice, benzene induced a significantly elevated incidence of carcinomas and carcinosarcomas in mid- and high-dose females, with a trend for dose-dependence (Table 10.4). The carcinomas often showed extensive squamous cell metaplasia, whereas the carcinosarcomas contained a prominent spindle-cell component resembling malignant fibroblasts. The historical incidence of mammary gland carcinoma in this strain is approximately 1% (NTP, 1986).

Table 10.4: Mammary gland lesions in female $B6C3F_1$ mice in a 2-year oral carcinogenicity study (NTP, 1986)

Lesions	Controls	25 mg/kg/day	50 mg/kg/day	100 mg/kg/day
Hyperplasia	2/49 (4%)	4/45 (9%)	2/50 (4%)	1/49 (2%)
Carcinoma	0/49 (0%)	2/45 (4%)	5/50 (10%)*	10/49 (20%)†
Carcinosarcoma	0/49 (0%)	0/45 (0%)	1/50 (2%)	4/49 (8%)*

* $p < 0.05$ (incidental tumour tests).
† $p < 0.01$ (incidental tumour tests).

Among male and female CBA mice exposed to 100 ppm benzene for 6 h/day, 5 days/week for 16 weeks, 20% had developed mammary gland tumours at follow-up

102 weeks after the last exposure (Cronkite, 1986). Details on tumour incidence in concurrent or historical controls were not given and the histopathology of the tumours was not described, although a later publication refers to them as adenocarcinomas (Cronkite et al, 1989).

In female RF/J mice administered 500 mg/kg/day by oral gavage for 52 weeks, the incidence of mammary carcinomas was 22.5%, compared to 2.5% in controls. In female Swiss mice receiving the same treatment for 78 weeks, the incidence was 47.5% compared to 5.0% in controls (Maltoni et al, 1989). The statistical significance of these findings is not discussed in the paper. When analysed for this assessment, the incidence in exposed females was significantly different from controls ($p < 0.05$; test for exact confidence limits) in Swiss but not in RF/J mice.

In female Sprague-Dawley rats given benzene by oral gavage for 1 year, the incidence of total/malignant mammary tumours was 53.3/13.3% in controls, 73.3/13.3% in animals treated with 50 mg/kg/day, and 45.7/20.0% in animals treated with 250 mg/kg/day. In female rats given 500 mg/kg/day for 2 years, the incidence was 32.5/17.5% compared to 42.0/14.0% in controls (Maltoni et al, 1989). The tumours comprised fibroadenomas, adenocarcinomas and carcino-sarcomas similar to the spontaneous mammary gland tumours commonly found in ageing female Sprague-Dawley rats (Maltoni et al, 1983). The investigators did not report on the statistical significance of their findings. When analysed for this assessment, there was no difference between any of the groups in the incidence of either total or malignant mammary tumours ($p > 0.05$; test for exact confidence limits).

Conclusions

The available carcinogenicity studies provide clear evidence of a causal relationship between benzene exposure and malignant neoplasms in mice and rats. The tissues most commonly involved are various glandular or non-glandular epithelia of the oral cavity, nasal cavity, lungs and skin (Table 10.3). The incidence of lymphoma was increased in several studies, but only in mice where this is a common spontaneously occurring tumour type. In one study in mice, there was a significant increase in bone marrow lesions described as resembling myeloblastic or granulocytic leukaemia (Cronkite et al, 1989), but this may have been the result of an intense inflammatory response (Farris et al, 1993). As such, a proven reproducible animal model for benzene-induced leukaemia is not available.

The lowest exposure levels associated with an increase in tumour incidence in rodents was 100 ppm by inhalation for 16 weeks in CBA mice and 25 mg/kg/day in a 2-year oral gavage test in $B6C3F_1$ mice and F344 rats (Cronkite, 1989; NTP, 1986). However, there was no increase in tumour incidence in two out of three inhalation tests in rats exposed to 100-300 ppm benzene for 99-123 weeks (Maltoni et al, 1989; Snyder et al, 1978, 1984).

There was an increased incidence of epithelial skin tumours in male rats in two 2-year oral bioassays. In both studies, however, the increase only occurred at the highest dose level tested (200 and 500 mg/kg/day respectively), which may have exceeded the maximum tolerated dose. Mammary gland carcinomas were increased in female mice at 50 and 100 mg/kg/day in a 2-year and at 500 mg/kg/day in a 78-week test.

10.8 Summary and conclusions

Taken together, the tests summarised above clearly demonstrate that benzene is not highly acutely toxic to experimental animals, whereas it is a potent, multi-organ toxicant by repeated administration. The target organs include the CNS, skin, eyes, immune system, blood and blood forming organs, gonads and developing foetus. Benzene is also toxic to genetic material and induces a variety of solid tumours, including mammary cancer in female mice.

The only consistently reported acute systemic effects are CNS depression and cardio-respiratory arrest. In rats, the median lethal dose is 810-9900 mg/kg by mouth and 13,700 ppm by 4-h inhalation.

Topically, benzene appears to be irritating to the skin and eyes.

Of the available repeated dose oral studies, only the US National Toxicology Program's 2-year bioassays in mice and rats have been conducted and reported in full compliance with GLP and other internationally recognised quality standards (NTP, 1986). In these studies, benzene administered by oral gavage induced leukopoenia and lymphocytopoenia and an increase in the incidence of malignant tumours at the lowest dose level tested, namely 25 mg/kg/day in male and female mice and female rats and 50 mg/kg/day in male rats. In other oral studies of a lesser quality, benzene produced leukopoenia in mice and rats and signs of immunosuppression in mice at dose levels from 8-12 mg/kg/day.

With regard to repeated exposure by inhalation, which is the predominant route in humans, the studies available for assessment were either poorly reported or inadequate for the determination of dose-response relationships for other reasons, such as an insufficient number of animals or range of exposure levels. Nonetheless, the weight of evidence indicates that the following approximate effect levels are likely to apply:

- In mice but not in rats, subtle signs of neurobehavioural stimulation may be detectable at vapour concentrations around 1 ppm, whereas gross CNS impairment only occurs at and above 1000 ppm. ;
- There are functional disturbances of the immune system at and above 10 ppm in mice, but no such effects in rats below 400 ppm. The NOAELs were determined to be 0.44 and 200 ppm for mice and rats respectively;
- Abnormal blood counts and morphological abnormalities in blood forming organs are found at and above 10 ppm in mice (including mouse foetuses exposed *in utero*) and at and above 100 ppm in rats. As effects were observed at all concentrations tested, a NOAEL could not be determined;
- There are degenerative changes in the gonads at 300 ppm in mice, but not in rats. The NOAEL was determined to be 30 ppm for mice ;
- Benzene is foetotoxic, but not teratogenic in rats and mice exposed during pregnancy at levels in the 100-500 ppm range, with an inhalation NOAEL for foetotoxicity of 40 ppm in rats; and
- The incidence of solid tumours is increased in mice exposed to 100-300 ppm benzene for 16 weeks, but not consistently in mice exposed to 1200 ppm for 10 weeks or in rats exposed to 100-300 ppm for 99-123 weeks.

The relevance of these findings for human risk characterisation will be examined in Section 13, in the context of the interspecies variations in benzene metabolism

addressed in Section 9, the human health effects reviewed in Section 11, and the molecular mechanisms of action discussed in Section 12.

11. Human Health Effects

The literature on human health effects of benzene is extensive and contains data on hundreds of thousands of people. This section summarises and reviews studies that are relevant to the characterisation of the toxic effects of benzene and the corresponding effect levels. Because of the nature of the available studies, the review is predominantly based on findings in people who were exposed to benzene at work or held jobs with the potential for exposure to the chemical.

The findings reported below must be interpreted with caution, as they rely on inherently uncertain information about the exposure of individuals or populations to benzene, which was either inferred or, at best, estimated from limited monitoring data. Furthermore, in the vast majority of cases there was co-exposure to other chemicals. These may be hazardous in their own right or inhibit the metabolism of benzene to toxic metabolites, thus resulting in either an over- or underestimation of the toxic potential of benzene. For example, the aromatic organic solvent toluene may interfere with the metabolism of benzene as well as cause brain atrophy and developmental toxicity (IPCS, 1985; Wilkins-Haug, 1997); some of the polycyclic aromatic hydrocarbons (PAHs) that occur in petroleum, coal gas, coal tar and vehicle exhaust are genotoxic and cause anaemia, immunosuppression and non-melanoma skin cancer (IPCS, 1998); and 1,3-butadiene found in vehicle exhaust is genotoxic and may increase the risk of blood and lymphatic cancers (IARC, 1999). Moreover, many studies are not controlled for confounding by smoking, although tobacco smoke contains benzene (see Section 16.1) and several studies have found an association between active smoking and leukaemia and reproductive effects such as semen quality and pregnancy outcome (Brownson et al, 1993; Vine, 1996; Werler, 1997).

Unless otherwise mentioned, all results were statistically significant in comparison with unexposed controls ($p < 0.05$). In occupational studies, chronic inhalation exposures refer to 8-h TWA (TWA_8) concentrations. Technical terms used to describe epidemiological study designs and statistics have the meaning given in Last (1995).

11.1 Acute toxicity

Cases of acute intoxication have occurred because of workplace accidents and in persons sniffing benzene-containing products for recreational purposes (Avis & Huton, 1993; Barbera et al, 1998; Tauber, 1970; Winek & Collum, 1971). The approximate lethal dose is 20,000 ppm by inhalation for 5-10 min, or 125 mg/kg by ingestion, whereas exposure to 25 ppm for 8 h is reported to be without clinical effects (Gerarde, 1960; Thienes & Haley, 1972, as cited in IPCS, 1993). No adverse effects were reported in three kinetic studies in healthy volunteers exposed to benzene levels of 26-42 ppm for 6 h, 52-62 ppm for 4 h or 47-110 ppm for 2-3 h (Berlin et al, 1980; Nomiyama & Nomiyama, 1974a; Srbova et al, 1950). Clinical signs at higher exposure levels include generalised symptoms such as dizziness, headache and vertigo at levels of 250-3000 ppm, leading to drowsiness, tremor, delirium and loss of consciousness at 700-3000 ppm (ATSDR, 1997; USEPA, 1998c). Unless fatal, the CNS symptoms are reversible following cessation of exposure. Autopsy findings are typical of cardio-respiratory arrest.

11.2 Irritation

Aspiration of liquid benzene has been observed to cause immediate pulmonary oedema and bleeding at the site of contact (Gerarde, 1960). Benzene vapours have been reported to cause eye and mucous membrane irritation in workers exposed at 33-59 ppm and irritation of the skin, nose, mouth and throat at levels ≥60 ppm (Midzenski et al, 1992; Yin et al, 1987a). Acute tracheitis, laryngitis, bronchitis and massive haemorrhage of the lungs were observed in a youth who died from an overdose of intentionally inhaled benzene (Winek & Collum, 1971). Second degree burns to the face, trunk and limbs were reported in chemical cargo ship crew accidentally exposed to fumes at a concentration resulting in death within minutes (Avis & Hutton, 1993).

11.3 Sensitisation

There are no reports of skin or respiratory sensitisation to benzene in humans.

11.4 Repeated dose toxicity (other than carcinogenicity)

11.4.1 Neurological effects

Yin et al. (1987a) found a dose-dependent increase in the prevalence of dizziness and headache in a survey of female Chinese workers in the footwear and printing industries. This study included 87 unexposed controls and two groups exposed to benzene at levels ranging from 1-40 ppm (40 cases) or 41-210 ppm (47 cases). In the two groups combined, benzene levels averaged 59 ppm. Both groups were co-exposed to low levels of toluene (≤16 ppm).

Peripheral neuropathy was reported in a small number of Turkish workers with benzene-induced aplastic anaemia or preleukaemia (Baslo & Aksoy, 1982). At a benzene-manufacturing petrochemical plant in Estonia, frequent headaches at the end of the shift, tiredness, sleep disturbances and memory loss occurred in 61% of workers exposed to levels in the 2-16 ppm range for several years (Kahn & Muzyka, 1973). In a survey of deck crew on nine Norwegian petroleum product tankers, headache, dizziness or nausea were reported by 5/11 workers exposed to >0.3 ppm benzene whereas there were no CNS complaints in 10 workers exposed to ≤0.3 ppm (Moen et al, 1995). Psychological examinations in 28 men exposed to a mixture of benzene (0.56-1.8 ppm), toluene (2.1-9.8 ppm) and xylenes (0.43-12 ppm) indicated diminished function of some cortical centres and impaired motor reaction time (Sikora & Langauer-Lewowicka, 1998). Varelas et al. (1999) used computed tomography imaging to visualise abnormal calcifications and cortical atrophy in the brains of 122 petrol station workers, taxi and bus drivers in central Athens. The subjects had been in their present employment for a minimum of 3 and an average of 16-17 years. Whereas blood lead levels were unremarkable in all three groups, there was mild to moderate cortical atrophy in 19/37 petrol station workers, 14/44 taxi drivers and 14/41 bus drivers. The prevalence in petrol station workers was higher than in taxi and bus drivers and unrelated to smoking or alcohol habits. None of these studies included an unexposed control group.

11.4.2 Effects on the immune system

There was a decrease in circulating IgA and IgG immunoglobulins, accompanied by an increase in IgM and an elevated occurrence of leukocyte auto-antibodies, in

painters co-exposed to benzene, toluene and xylenes at air levels ranging from 3-57, 21-71 and 27-680 ppm respectively (Lange et al, 1973a, 1973b). In workers co-exposed to benzene, toluene and xylenes at air levels that averaged from 1-35, 2-32 and 4-28 ppm respectively over an 11-year period, total LC and T-LC counts were slightly lower in workers exposed for 55-122 months than in an unexposed control group, whereas there were no differences in LC function as determined by LC transformation and tuberculin tests (Moszczynsky & Lisiewicz, 1984).

11.4.3 Cardiovascular effects

Kotseva & Popov (1998) conducted a routine cardiological examination of a sample of male and female petrochemical workers aged 20-60 years. It included 118 workers concomitantly exposed to ≤20 ppm benzene and low levels of toluene and petrol as well as 154 workers concomitantly exposed to <3 ppm benzene, ≤32 ppm xylenes and low levels of toluene and petrol. Compared to unexposed controls matched for age, sex, salt intake, smoking and body mass index, the prevalence of arterial hypertension and minor electrocardiographic abnormalities was approximately twice as high in the exposed groups.

11.4.4 Haematological effects

Benzene has been known to be toxic to the blood for more than a hundred years and in the past was sometimes given orally to leukaemia patients to reduce WBC count (ATSDR, 1997; Landrigan, 1996).

Occupational exposure

Table 11.1 summarises a number of surveys of non-cancerous blood disorders in workers exposed to airborne benzene. Overall, these studies point to a strong association between recent or current exposure to airborne benzene and the occurrence of decreased ALC, WBC, RBC and Plt counts, Hb and haematocrit (Hct), and an increase in MCV. Such cases are sometimes described as ‘benzene poisoning’ (BP). Depending on the pattern and magnitude of these changes and the histological findings in a bone marrow biopsy, they may be clinically diagnosed as lymphocytopoenia, leukopoenia, anaemia, thrombocytopoenia, pancytopoenia, agranulocytosis, myelofibrosis, or aplastic anaemia. They may be accompanied by clinical signs such as paleness, increased susceptibility to infections, and a tendency to bruising and bleeding. BP is generally reversible upon cessation of exposure, except aplastic anaemia which may be fatal or progress to acute myeloid leukaemia (AML) (Aksoy, 1989).

Among the studies summarised in Table 11.1, three surveys comprising a total of 795 workers found no adverse haematological effects from long-term benzene exposure at levels averaging 0.55, 0.81 and 0.53 ppm respectively (Collins et al, 1997; Khuder et al, 1999; Tsai et al, 1983). These studies have limitations with respect to blood analysis methodology, exposure assessment and/or control for confounders such as smoking and co-exposure to other chemicals. Nevertheless, taken together they indicate that the NOAEL for bone marrow toxicity is likely to be >0.5 ppm.

Table 11.1: Summary of haematological effects in workers exposed to airborne benzene

Industry	Country	Benzene exposure (TWA_8)*	Condition(s) observed†	Comments	Reference
Chemical	USA	Range = 0.01-1.40 ppm for an average of 7.3 years	In 200 exposed compared to 268 non-exposed workers there was no consistent benzene-related effect on haematology surveillance indicators		Collins et al. (1991)
	USA	Mean (range) = 0.55 (0.01-88) ppm, with <5% of workers exposed to levels >2 ppm	In 387 exposed compared to 553 non-exposed workers there was no difference in the prevalence of decreased ALC, RBC, WBC or Plt counts, decreased Hb levels, or increased MCV values		Collins et al. (1997)
	USA	Mean >24 ppm for an average of 9.6 years	10/10 workers had increased MCV values and 9/10 also had decreased Hb levels		Fishbeck et al. (1978)
	USA	From <2 to about 30 ppm for 1-20 years	Marginally lower RBC count and total bilirubin in 282 exposed workers compared to an equal number of matched controls	2/282 exposed workers died from leukaemia during the study period (1967-74)	Townsend et al. (1978)
Coke oven by-products	USA	0.1-31.4 ppm	No differences in WBC, RBC or Hb values between groups of 17-37 workers with no, low (<2 ppm-years), intermediate (2-20 ppm-years) or high (>20 ppm-years) exposure		Hancock et al. (1984)
Footwear manufacturing	Turkey	15-210 ppm for 3 months to 17 years	Increased incidence of reduced WBC and/or Plt or CBC counts in 217 workers compared to 100 controls matched for sex, age and general living conditions	Exposed to adhesives. No correlation with duration of exposure	Aksoy et al. (1971)
	Croatia	Median (range) = 5.9 (1.9-14.8) ppm (area monitoring)	Decreased mean Hb concentration and percentage of B-LC and increased MCV and band neutrophils in 49 exposed females compared to 27 unexposed controls	Benzene contaminated glues, cleaners and paints; co-exposed to 11-50 ppm toluene	Bogadi-Šare et al. (1997, 2000)
Miscellaneous uses of benzene-based solvents	Italy	>20 ppm	Of 301 workers referred to an occupational health clinic with suspected benzene intoxication, 153 had transient and 39 progressive bone marrow abnormalities; 11 died from aplastic anaemia and 21 developed cancers of the blood and lymphatic system	There was a highly significant correlation between severity of bone marrow disease and current or recent exposure	Vai et al. (1989)
	China	Median (range) current personal exposure = 31 (1.6-328.5) ppm, with an average duration of exposure of 6.3 years	In 44 exposed workers compared to an equal number of controls matched for sex, age, cigarette and alcohol consumption, WBC, ALC, Plt, RBC and Htc levels were reduced and MCV values increased. In 11 workers exposed to a median (range) level of 7.6 (1-20) ppm, only ALC was decreased	No correlation between any haematological parameter and cumulative exposure	Rothman et al. (1996a, 1996b)
	China	Mean (range) = 5.8 (0.7-139) ppm (assessment method not specified)	26% of 326 exposed workers had leukopoenia (WBC count <4.5 x 10^9/L) compared to 8.9% of 236 non-exposed workers		Xia et al. (1995)
	China	Mean (maximum) = 59.2 (210) in women and 47.9 (210) ppm in men, for an average of 5 years	Decrease in ALC in 83 exposed women compared to 85 unexposed controls, but no differences between 61 exposed men and 44 unexposed controls	Co-exposed to 6-7 ppm toluene	Yin et al. (1987a)

Table 11.1: Continued

Industry	Country	Benzene exposure (TWA_8)	Condition(s) observed	Comments	Reference
Petroleum refining	Canada	Mean (range) = 0.81 (0.14-2.08) ppm for an average of 10 years	In 105 exposed workers, levels of WBC, RBC, Hb, MCV and Plt were generally in the low normal range. MCV and Plt values were negatively correlated with duration of employment, but not with individual benzene exposure		Khuder et al. (1999)
	USA	Median = 0.53 ppm	All haematological parameters generally within normal limits in 303 workers followed from 1959-1980		Tsai et al. (1983)
	UK	≤ 10 ppm	In 66 exposed compared to 33 non-exposed workers, there was no difference in various unspecified haematology and serum biochemistry values, except for a small increase in MCV values in exposed workers	All absolute MCV values were within the normal clinical range	Yardley-Jones et al. (1988)
Printing	USA	11-1060 ppm for 3-5 years	130/332 exposed workers showed signs of intoxication, including anaemia, increased MCV, reduced Plt counts and/or reduced WBC counts	No cases of relapse after benzene use was discontinued	Greenburg et al. (1939)
Rubber manufacturing	USA	Median exposure estimated at 30-54 ppm	In 161 workers hired between 1946-49, there was a 10% decline in WBC counts over the first 4 months of employment, but no consistent changes in RBC levels	Pliofilm cohort (Section 11.6.1)	Cody et al. (1993)
	USA	Mean estimated at 75 ppm during 1940-48 and at 15-20 ppm during 1949-78	In a longitudinal study of 459 workers, WBC, RBC and Hb levels decreased with total exposure between 1940-48, but showed no persistent trends over the ensuing 25 years	Pliofilm cohort (Section 11.6.1)	Kipen et al. (1988, 1989)
	USA	Range estimated at <5-34 ppm	Haematological screening data for 657 workers exposed between 1939 to 1975 showed a relationship between benzene exposure and the risk of a low WBC or RBC count which was stronger for WBC, with no evidence for a threshold exposure level	Pliofilm cohort (Section 11.6.1)	Ward et al. (1996)
	USA	Mean and range estimated at 100 and 50-500 ppm respectively	Following complaints of malaise, nausea, vomiting, and bleeding, blood counts were done on 1104 workers. ALC was abnormally low in 83 of them and 25 had severely reduced WBC, RBC and Plt counts. Of these, 9 were hospitalised, where aplastic anaemia was diagnosed by bone marrow biopsy; 3 died	Outbreak coincided with large war orders for synthetic rubber	Wilson (1942)
Ship repair	USA	60-600 ppm	9/15 workers exposed over several days from the degassing of shipboard tanks developed abnormal WBC, ALC, Hb, Plt and/or MCV values within 4 months. At 12 months, 7/15 still had one or more abnormal values	No relationship between blood changes and duration of exposure	Midzenski et al. (1992)
Tyre cord manufacturing	Turkey	0-110 ppm (area monitoring)	Decreased WBC count in 9, decreased Plt count in 4 and decreased WBC, RBC and Plt count in 1 of 231 exposed workers	Used thinners and solvents containing up to 6-8% benzene	Aksoy et al. (1987)

* TWA_8 = 8-h time-weighted average.
† CBC = complete blood cell count; for other abbreviations, see text.

In 44 Chinese workers exposed to a median benzene concentration of 31 ppm, with a range from 1.6-328.5 ppm, Rothman et al. (1996a, 1996b) found a decrease in WBC, RBC and Htc, an increase in MCV and an inverse correlation between ALC and benzene exposure. In a subgroup of these workers with a median exposure level of 7.6 ppm (range: 1-20 ppm), the lowest exposure group examined, the only haematological finding was a 16% decrease in ALC (1.6 x 10^9/L compared to 1.9 x 10^9/L in controls; p = 0.03). This study is small, but had a well-matched control group, minimal exposure to other chemicals (toluene and xylenes) and a dose-response relationship was established between ALC and benzene exposure as measured by repeated personal monitoring as well as with benzene metabolites in the urine.

Three other studies reported haematological effects in workers whose exposure was stated to range from 1.9-14.8 (median: 5.9), <5-34 and 0.7-139 (mean: 5.8) ppm respectively (Bogardi-Ŝare et al, 1997; Ward et al, 1996; Xia et al, 1995). However, these studies assessed exposure by means of area monitoring, a job-exposure matrix or unspecified methods and are therefore less suitable for dose-response characterisation.

Repeated exposure at higher levels was usually associated with clear signs of BP in some workers, with little or no relationship with cumulative exposure (Aksoy et al, 1971; Midzenski et al, 1992; Rothman et al, 1996a, 1996b; Vai et al, 1989).

Dosemeci et al. (1997) evaluated the statistical relationship between a clinical diagnosis of BP and benzene exposure in a subgroup of 412 cases drawn from a large Chinese cohort study (Hayes et al, 1997), which is described in detail in Section 11.6.1. The cumulative incidence of BP (defined as (1) a WBC count <4 x 10^9/L or a WBC count <4.5 x 10^9/L and a Plt count <80 x 10^9/L over several months, (2) occupational benzene exposure for ≥6 months and (3) exclusion of other causes of abnormal blood counts) rose sharply with increasing estimated intensity of benzene exposure over a period of 18 months prior to diagnosis, as shown by the following relative risks (RRs):

Exposure	<5 ppm	5-19 ppm	20-39 ppm	≥40 ppm
RR (95% CI)[6]	1.0 (reference level)	2.2 (1.7-2.9)	4.7 (3.4-6.5)	7.2 (5.3-9.8)

The clear trend with the level of exposure is noteworthy, even if the absolute exposure levels may have been underestimated, as discussed in Section 11.6.1 below.

The risk of BP developing into cancer was assessed in a subgroup of 11,177 benzene-exposed workers from the same Chinese cohort, 103 of whom had BP as defined above (Rothman et al, 1997). At follow-up 4-14 years after diagnosis, three of the cases had developed acute non-lymphocytic leukaemia (ANLL), non-Hodgkin's lymphoma (NHL) and myelodysplastic syndrome (MDS) respectively, compared to 6 cases of cancer of the blood and lymphatic system (including 2 with ANLL) and 1 case of MDS among the 11,074 workers without a diagnosis of BP (Table 11.2)[7]. The corresponding RRs indicate that a diagnosis of BP is associated with a 42-fold increase in the risk of pre-cancer or cancer of the blood and lymphatic system and with a 71-fold increase in the risk for ANLL/MDS. The RRs

[6] Throughout this section, ranges in brackets immediately following a relative risk (RR), odds ratio (OR), standardised mortality rate (SMR) or standardised incidence rate (SIR) represent the 95% confidence interval (CI) of the statistic.

[7] ANLL comprises all acute leukaemias other than acute lymphocytic leukaemia and can usually be equated to AML. MDS is a term that encompasses a variety of preleukaemic disorders.

changed little upon adjustment for cumulative exposure, indicating that the elevated cancer risk was not due to a higher cumulative exposure in the 103 BP cases.

Table 11.2: Benzene poisoning and subsequent risk of blood and lymphatic system cancer and related disorders (Rothman et al, 1997)

Parameter*	Without benzene poisoning	With benzene poisoning
Person-years of follow-up	122,620	848
RR (95% CI) of all pre-cancer or cancer of the blood and lymphatic system	1.0	42.3 (10.7-167.0)
RR (95% CI) of ANLL/MDS	1.0	70.6 (11.4-439.3)
RR (95% CI) of all pre-cancer or cancer of the blood and lymphatic system, adjusted for cumulative benzene exposure	1.0	47.4 (11.7-191.9)
RR (95% CI) of ANLL/MDS, adjusted for cumulative benzene exposure	1.0	61.3 (9.8-384.3)

* ANLL = acute non-lymphocytic leukaemia; CI = confidence interval; MDS = myelodysplastic syndrome; RR = relative risk.

Similarly, Vai et al. (1989) reported 28 cases of fatal blood cancer among 304 workers in Northern Italy who were hospitalised 15-35 years earlier with suspected BP, representing a 13.3-fold increase over the incidence in the general population in the region.

Public exposure

In the 1980s, the US Federal Department of Health and Human Services created a National Exposure Registry to assess the health consequences to the general population from long-term, low-level exposure to specific substances in the environment. A Benzene Subregistry was established in 1991 based on a population health survey in a community in Texas, USA, where tap water from the public water system was known to have contained ≤66 μg/L benzene since 1 January 1979 (Burg & Gist, 1998). The survey included 1,143 persons who had used contaminated water as the sole source of drinking, bathing and cooking for at least 30 consecutive days. These persons were administered a questionnaire and follow-up telephone interviews were conducted one and two years later. The questions asked were similar to those used in the National Health Interview Survey (NHIS) conducted every year in USA, except that the benzene questionnaire contained a qualifier relating to professional rather than self diagnosis of ailments, so as to minimise reporting bias. Findings were compared with concurrent NHIS data subsets matched for demographic variables and current and ever smoking rates. The initial response rate was 97%. There was a loss of 9% for each follow-up from the previous data collection.

The outcome of anaemia and related blood disorders within the last 12 months was reported in excess at all three data collections, with 40 observed vs. 14.1 expected cases at baseline, 32 observed vs. 11.6 expected cases at one year ($p < 0.01$), and 28 observed vs. 11.9 expected cases at two years. There was no difference in the reporting of cancer.

Conclusions

Several occupational surveys show that chronic exposure to benzene may lead to bone marrow depression, with manifestations that range from small reductions in blood count parameters to aplastic anaemia. The available data indicate that both the incidence and severity of this effect is dose-related. In a small, but reliable study, the only haematological effect in workers with a median (range) exposure of 7.6 (1-20) ppm (TWA_8) was a modest reduction in ALC. As this was the lowest exposure group examined, 7.6 ppm (TWA_8) is currently considered the best estimate for a LOAEL which may be close to the point of departure for the onset of haematological effects (USEPA, 1998c). An appropriate NOAEL has not been determined, but studies with various limitations indicate that it is likely to be >0.5 ppm (TWA_8).

The only available epidemiological study in the general population found an excess occurrence of anaemia and related disorders in a community whose tap water contained ≤66 μg/L benzene.

There is some evidence that bone marrow depression is associated with a substantially increased risk for ANLL/MDS.

11.4.5 Reproductive effects

Effects on fertility

Vara & Kinnunen (1946) reported a variety of gynaecological disorders in 12 female rubber workers who were exposed to unspecified levels of benzene on a daily basis. All 12 women had menstruation disorders, with sparse bleeding being the most common complaint. Although most of the women practised regular unprotected intercourse, only two of them had conceived since they started working and both pregnancies ended in spontaneous abortions (SAb) by the first trimester. Five had ovarian hypoplasia. Other common findings included excessive bruising, tiredness, dizziness, headaches and abnormal haematological findings, particularly low WBC and Plt counts.

Menstruation abnormalities have also been reported in surveys of female workers exposed to mixed aromatic hydrocarbons including benzene or to benzene, petroleum and chlorinated hydrocarbons in Poland and Russia in the 1960s (Michon, 1965; Mukhametova & Vozovaya, 1972; both as cited in ATSDR, 1997) and in female workers at a large petrochemical company in China (Thurston et al, 2000). Huang (1991) reported menstruation disorders in 49% of 223 Chinese leather footwear workers co-exposed to an average of 29 (range: 1-132) ppm benzene and 19 (range: 1-136) ppm toluene compared to a prevalence of 16% in unexposed controls ($p < 0.01$). There is no information on the smoking habits of the study population.

Benzene exposure as a risk factor for fecundability (time to pregnancy) was assessed in a Norwegian case-control study in 558 female dental surgeons and 450 high school teachers with at least one child (Dahl et al, 1999). Forty percent of the dentists reported daily exposure to a now discontinued disinfectant containing 0.25% v/v benzene. There was no difference in fecundability between dental

surgeons exposed to benzene and the controls. Potential confounders were considered, but the level of benzene exposure resulting from the disinfectant was not assessed.

In males, De Celis et al. (2000) studied the sexual functioning and semen profile of 48 Mexican rubbers workers exposed to a mixture of benzene (10-15 ppm), ethyl benzene (~50 ppm), toluene (~50 ppm) and xylenes (~12 ppm) for ≥2 years. Mean sperm count and the mean percentage of motile and normal sperm forms were reduced by 78, 62 and 24% respectively, compared to a group of 42 age-matched controls. There was no correlation between smoking or alcohol intake and alterations in the semen profile. Longer abstinence intervals may have contributed to the reduced sperm concentration and motility in exposed workers as they also had an increased prevalence of reduced libido.

Effects on pregnancy outcome

Huang (1991) investigated pregnancy outcome in 106 Chinese leather footwear workers co-exposed to an average of 29 ppm (range: 1-132) ppm benzene and 19 (range: 1-136) ppm toluene and 209 unexposed controls. Exposure to benzene and toluene was associated with an elevated incidence of SAb (5.8 vs. 2.4%; RR = 2.4; $p < 0.01$), whereas there was no difference in the incidence of preterm delivery or stillbirth. There is no information on the smoking habits of the study population.

Lindbohm et al. (1991) used Finnish census data, hospital records and industry-wide air monitoring results collected in 1975-82 to study the outcome of 11,570 pregnancies with potential paternal exposure to hazardous chemicals compared with a control group of 87,616 unexposed pregnancies. The RR for SAb was elevated for paternal exposure to solvents used in petroleum refineries, but did not differ significantly from unity when analysed separately for exposure to benzene. Although not quantified, benzene exposure levels were estimated to be low.

In a case-control study of female workers in the Finnish pharmaceutical industry which included 44 cases of SAb and 130 matched controls, Taskinen et al. (1986) found a non-significant association between abortion risk and benzene exposure (OR = 2.4 (0.5-12.0)).

The effects of parental occupational exposures on foetal development were investigated in an exploratory case-control study based on probability samples of live births and foetal deaths obtained by the US National Natality and Fetal Mortality survey conducted in 1980 among married women (Savitz et al, 1989). The samples included case groups of stillbirths (2096 mothers, 3170 fathers), preterm deliveries at <37 weeks of pregnancy (363 mothers, 552 fathers) and small-for-gestational age (SGA) infants (218 mothers, 371 fathers). Control pregnancies were drawn from the same survey. Occupational exposures within the last 12 months were defined by industry of employment and relative levels of exposure to individual agents estimated on the basis of a job-exposure linkage system. In computing the OR, adjustments were made for known confounding factors for each pregnancy outcome, such as child's race, receipt of prenatal care, mother's age, number of previous miscarriages, previous induced abortions and maternal smoking and alcohol consumption.

Overall, this study found a significantly elevated SGA risk in the offspring of fathers exposed to benzene at work (OR = 1.5 (1.1-2.3)), with a strong dose-response gradient. Benzene-exposed fathers of SGA infants included a large percentage of engine mechanics and repairers, welders and flame cutters. Maternal

benzene exposure showed a marginally significant association with stillbirths (OR = 1.3 (1.0-1.8)), which was supported by the demonstration of a dose-response gradient. In these mothers, benzene exposure was attributed mainly to work in the textile industry, barbering and cosmetology, with smaller contributions from the chemical, pharmaceutical and paint industries.

Stücker et al. (1994) evaluated the risk of SAb before 28 weeks among the spouses of 1077 male workers at two organic chemical factories in France, on the basis of exposures estimated by plant occupational physicians and questionnaires administered to the men and their wives. Medical records of the women were not examined. There was a total of 1739 pregnancies, of which 171 (9.8%) ended in SAb. The abortion rate was 8.8% in the wives of unexposed workers. Workers were divided into low (<5 ppm) and high (≥5 ppm) exposure categories depending on their estimated past exposure to benzene. After adjustment for maternal tobacco consumption, age and pregnancy order, the risk of SAb did not differ from unity in either of the two exposure groups. Similar results were obtained in analyses of first pregnancies only, and when pregnancy outcome was examined against a more detailed exposure graduation.

A Finnish case-control study of 206 cases of SAb in laboratory workers and 329 individually matched controls identified 11 cases of benzene exposure in the SAb group compared to 25 among the controls and concluded that benzene exposure was not a significant risk factor (Taskinen et al, 1994).

In a Chinese study, the overall risk of SAb in 3070 non-smoking, primiparous women employed at a large petrochemical complex and married to male workers at the same facility was 8.8% in chemical compared to 2.2% in non-chemical workers (Xu et al, 1998a). Benzene, toluene, xylenes and styrene exposure levels in 38 breathing zone samples collected throughout the complex averaged 0.86, 0.40, 0.50 and 0.03 ppm respectively. In analyses for exposure to specific chemicals during the first trimester of pregnancy, the estimated ORs of SAb were significantly elevated for benzene (2.5 (1.7-3.7)) and petrol (1.8 (1.1-2.9)).

Chen et al. (2000) conducted a prospective study of pregnant workers at a Chinese petrochemical plant producing benzene, toluene, xylenes, styrene and phenol. Compared with 459 mothers not exposed to organic solvents, there was a small reduction in birth weight (–58 g; 95% CI = –115 to –2 g) among 366 mothers exposed to 0.02-0.2 ppm benzene with or without other exposures.

Conclusions

There are several reports of menstruation disturbances in female workers and one of reduced semen quality in male workers exposed to benzene.

The available studies of pregnancy outcome have produced mixed results with regard to the risk for SAb. One study found an elevated SGA risk for fathers with occupational exposure to benzene. In another study, there was a marginally significant reduction in birth weight in infants whose mothers had been exposed to low levels of benzene at work.

However, all of the available studies have one or more limitations, such as multiple exposures, inadequate adjustment for other confounders and/or inadequately quantified exposure to benzene as well as other chemicals. Therefore, there is at present no convincing evidence from human studies that benzene may have adverse effects on reproduction.

11.4.6 Other health effects

Chronic tiredness and headache and large, spreading bruises on the arms and legs have been described in a number of workers exposed to benzene air levels in the order of 100-200 ppm (Helmer, 1944).

Yin et al. (1987a) conducted a survey of the prevalence of symptoms of intoxication in Chinese factory workers exposed to high levels of benzene or benzene and toluene for up to 40 years. There was a slight decrease in ALC in both groups. Sore throat and episodes of nose bleeding were common in all exposed workers and their frequency was related to benzene exposure levels.

In an uncontrolled case report, Davidoff et al. (1998) described a group of workers who began complaining about petrol odour and symptoms of nausea, headache, throat and eye irritation, and cough while tunnelling underneath a former service station site. An air sample from the tunnel contained 60 ppm benzene. Eight out of 30 randomly selected workers subsequently investigated in detail reported the post-incident onset of chemical hypersensitivities and other characteristics which, according to the authors, fitted conservative criteria for the diagnosis of multiple chemical sensitivities syndrome.

11.5 Genotoxic effects

Several occupational studies conducted over the past 30 years point to a link between a number of unstable or stable, numerical or structural chromosome aberrations and benzene exposure (ATSDR, 1997; IPCS, 1993). In most cases, these studies were conducted in workers exposed to benzene levels >10 ppm. However, Tompa et al. (1994) analysed whole blood metaphase spreads from workers employed in an environment where improved working conditions over a 3-year period reduced average peak exposures from 21 ppm in 1990 to 8.4 ppm in 1991 and 5.7 ppm in 1992. As shown in Figure 11.1, the reduction in benzene levels was paralleled by a decrease in the frequency of chromosome aberrations, but not in SCEs. These findings provide evidence of a direct relationship between benzene exposure and the extent of chromosome damage, but do not establish a threshold level for the effect.

Figure 11.1: Changes in the frequency of SCEs and chromosome aberrations (excluding gaps) in workers exposed to progressively reduced benzene levels (Tompa et al, 1994)

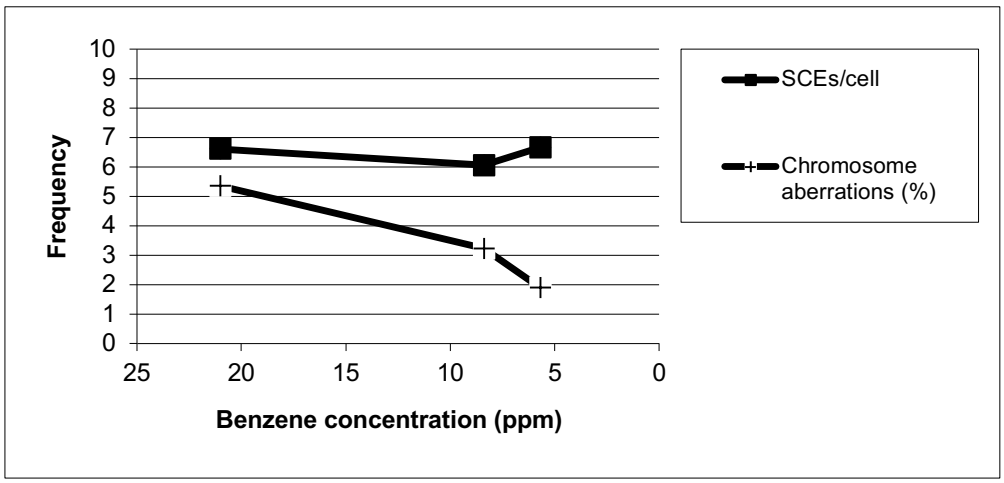


Table 11.3 summarises a number of recent occupational studies which used personal air monitoring to measure benzene exposure and modern cytogenetic techniques such as polymerase chain reaction methods, ^{32}P-postlabelling, fluorescence *in situ* hybridization and alkaline single cell gel electrophoresis to determine the genotoxic effects in various cell samples.

Table 11.3: Genotoxic effects in workers exposed to airborne benzene

Study population (number)			
Exposed	**Controls**	**Effects and effect levels (TWA_8)***	**Reference**
Petrol station attendants (12)	Matched for sex, age and smoking habits (12)	Excess of overall DNA damage and highly damaged cells in freshly isolated non-cycling peripheral blood LC in subjects exposed to a mean air level of 0.11 ppm (range: 0.03-3.0 ppm) (Lagorio et al, 1997)	Andreoli et al. (1997)
Petrol station attendants (12)	Matched for sex, age and smoking habits (12)	No evidence of numerical aberrations involving chromosomes 7, 11, 18 or X in peripheral blood LC of subjects exposed to an average air level of 0.1 ppm	Carere et al. (1998)
Styrene plant workers (25)	Matched for sex and age (25)	Increase in kinetochore-positive MN in T-LC, but no changes in DNA adducts in MC or in DNA single strand breaks, SCE or total MN in LC at average exposure levels corresponding to 0.24 ppm benzene and 0.31 ppm styrene (as well as toluene, xylenes and ethylbenzene)	Holz et al. (1995)
Coke gas plant workers (56)	Matched for age (28)	No increases in the frequency of MN, MN harbouring whole chromosomes or acentric chromosomal fragments or chromosome 9 numerical abnormalities in LC and buccal cells at exposure levels from 0.5-1.2 ppm	Surrallés et al. (1997)
Coke gas plant workers (12) and oven operators (5)	Unmatched inhabitants of neighbouring rural village (8)	Small but statistically significant increase in centromeric breakage of chromosomes 1 and 9 in interphase LC in benzene workers exposed to a geometric average of 1.3 ppm benzene, but not in coking oven workers exposed to a geometric average of 1.0 ppm	Marcon et al. (1999)
Workers using benzene-based solvents (24)	Matched for sex, age, smoking, drinking and obesity (23)	In heterozygous individuals, the frequency of NN but not Nø GPA mutants was doubled in peripheral RBC and strongly correlated with lifetime cumulative benzene exposure, at a mean exposure level of 72 ppm (range: 2-301 ppm)	Rothman et al. (1995, 1996b)
Workers using benzene-based solvents (43)	Matched for sex and age (44)	There was a dose-related increase in hyperdiploidy at chromosomes 8 and 21 and in hypodiploidy at chromosome 8 in LC of workers with a median exposure level of 31 ppm (range not specified). There was also a 15-fold increase in t(8;21) (27 versus 2% LC) and a doubling of t(8;?) and t(21;?) in LC at exposures >31 ppm. All increases were related to current but not to cumulative exposure	Smith et al. (1998)
		Increased frequency of hyperdiploidy at chromosome 9, mainly trisomy, in LC at exposure levels >31 ppm, which correlated with ALC decreases	Zhang et al. (1996)
		Increased frequency of monosomy at chromosomes 5 and 7, in trisomy and tetrasomy at chromosomes 1, 5 and 7, and a dose-dependent, up to 3.5-fold increase in long arm deletions of chromosomes 5 and 7 in whole blood metaphase spreads, at a median exposure level of 31 ppm (range: 2-329 ppm)	Zhang et al. (1998)

* ALC = absolute lymphocyte count
GPA = glycophorin A
LC = lymphocytes
MC = monocytes
MN = micronuclei
RBC = red blood cells
SCE = sister chromatid exchange
t(a;b) = translocations between chromosomes a and b
TWA_8 = 8-h time-weighted average
? = unidentified chromosome.

The studies of Andreoli et al. (1997) and Carere et al. (1998) of petrol station attendants exposed to benzene concentrations that averaged around 0.1 ppm are

difficult to interpret as there is no information on the nature and extent of co-exposure to other chemicals in petrol or vehicle exhaust fumes, which would include 1,3-butadiene and a number of genotoxic PAHs (IARC, 1999; IPCS, 1998). Furthermore, Carere et al. (1998) investigated only one of the six chromosomes in which aberrations have been found at high levels of benzene exposure.

Holz et al. (1995) reported kinetochore-positive (that is, whole chromosome) MN in workers with an average exposure of 0.24 ppm benzene and 0.31 styrene. However, styrene alone is known to cause chromosome damage in human lymphocytes at low concentrations (IARC, 1994).

In coal gas by-product workers, Surrallés et al. (1997) found no chromosome aberrations at benzene levels ≤1.2 ppm, whereas Marcon et al. (1999) found a small increase in centromeric breakages in chromosomes 1 and 9 at 1.3 ppm, but not in coke oven workers exposed to a slighter lower level averaging 1.0 ppm. However, coke oven and coal gas by-product workers are co-exposed to numerous PAHs, many of which have a variety of genotoxic effects at low concentrations (IPCS, 1998).

The main findings at exposure levels ≥31 ppm benzene were aneuploidy, long-arm deletions and translocations involving chromosomes 1, 5, 7, 8, 9 and 21 and gene duplication in nucleated RBC stem cells at the glycophorin A locus on chromosome 4 (Rothman et al, 1995, 1996b; Smith et al, 1998; Zhang et al, 1996, 1998). The subjects of these studies were co-exposed to toluene and xylenes, which may inhibit the metabolism of benzene, but have not been shown to cause chromosome lesions that resemble the above (IPCS, 1997; McGregor, 1994).

As such, whereas studies using modern cytogenetic techniques have shown a clear association between extensive chromosome damage and exposure to high benzene levels, they have not contributed to the definition of a threshold level for genotoxic effects in humans.

11.6 Carcinogenicity

11.6.1 Cohort studies

Cohort studies with poorly characterised benzene exposure levels

Table 11.4 summarises a number of occupational cohort studies with a combined study population approaching 450,000 workers holding jobs with the potential for exposure to benzene, mainly in the petroleum industry. They include the ongoing, prospective Health Watch (1998) cohort study, which covers about 95% of the Australian petroleum industry's 18,000 employees in refineries, natural gas plants, distribution terminals and production sites. They also include two meta-analyses based on a large number of petroleum industry cohorts (Raabe & Wong, 1995; Wong & Raabe, 1996, 2000). The most important limitation of these studies and meta-analyses is their lack of adequate data on benzene exposure levels.

Table 11.4: Summary of cohort studies in workers exposed to poorly characterised benzene levels

Exposed population	Controls	Health outcome*	Ratio (95% CI) †	Comments	Reference
Chemical industry					
259 workers ever employed at a US benzene alkylation plant from 1947-60 and followed up for 17-30 years	National population	Any death rate	No difference	In 194 workers employed for ≥ 12 months, SMR for CBLS = 3.77 (1.09-10.24)	Decouflé et al. (1983)
822 chemists graduated from university in Stockholm, Sweden, between 1930-50 and followed up till the end of 1974	Architects from same school	All-cause mortality All cancer mortality All CBLS	RR = 1.14 (0.91-1.37) RR = 2.54 (p <0.05) RR = ∞ (p = 0.02)	There were 10 cases of CBLS among the chemists compared to 0 among the architects; nine were organic chemists	Olin & Ahlbom (1980)
Coke oven and coal gas by-product workers					
2708 men employed by British Steel Corporation at 14 coke works in the UK in 1967 and followed up for 20 years	Regional population	*Leukaemia:* All workers By-product workers Coke oven workers	 SMR = 0.41 (0.05-1.47) SMR = 0.98 (0.02-5.57) SMR = 0.35 (0.01-1.92)	Benzene breathing zone levels were reported to average 1.3 ppm in by-product and 0.3 ppm in coke oven workers in the 1980s	Hurley et al. (1991)
3812 men employed by National Smokeless Fuels Ltd at 13 coke works in the UK in 1967 and followed up for 20 years	Regional population	*Leukaemia:* All workers By-product workers Coke oven workers Maintenance workers	 SMR = 0.42 (0.09-1.23) SMR = 0.76 (0.02-4.29) SMR = 0.34 (0.00-1.86) SMR = 0.58 (0.01-3.28)		
5659 coke oven workers employed for ≥ 6 months from 1945-69 at a Dutch coke plant and followed up for 15-40 years	National population	All-cause mortality, all cancer, liver cancer, and respiratory disease	SMR >1.00 (p< 0.05)	Among 222 benzene plant workers, death rates were similar to the expected figures	Swaen et al. (1991)
Footwear manufacturing					
2013 men and women ever employed at an Italian shoe manufacturing plant from 1939-64 and followed up for 20-45 years	National population	*Male workers (n = 1008):* GI disease and accidents Blood disease All cancer Stomach cancer Leukaemia *Female workers (n = 1005):* Any cause of death	 SMR <1.00 (p <0.05) SMR = 15.66 (5.47-32.64) SMR = 1.40 (1.09-1.81) SMR = 2.40 (1.37-3.78) SMR = 4.00 (1.46-8.70) No difference	Workers in some departments were exposed to glues containing >70% benzene All non-cancer blood disease cases were aplastic anaemia No relationship between leukaemia risk and duration of exposure No information on job categories, which likely may have explained negative findings in female workers	Paci et al. (1989)

Table 11.4: Continued

Exposed population	Controls	Health outcome*	Ratio (95% CI) †	Comments	Reference
Highway maintenance workers					
4849 men employed for at least 1 year between 1945-84 as highway maintenance workers by the Department of Transportation in Minnesota, USA	Regional white population	All-cause mortality and all cancer	SMR <1.00 (p <0.05)	In workers with 30-39 years of employment, the SMR for leukaemia was 4.25 (1.71-8.76)	Bender et al. (1989)
		All CBLS	SMR = 0.95 (0.66-1.33)		
		Leukaemia	SMR = 1.07 (0.62-1.71)	No observed deaths from melanoma compared to 2.9 expected	
Petrol and petroleum distribution					
18,969 men and women employed as petrol station attendants on the day of the 1970 censuses in Denmark, Norway, Sweden and Finland and followed up for deaths and incident cancer cases for 15-20 years	National population of gainfully employed	*Male workers (n = 16,524):*		No information on employment status before or after the census date and hence no adjustment for person-years at risk	Lynge et al. (1997)
		All malignant neoplasms	SIR = 1.1 (1.0-1.1)		
		Cancer of the nose	SIR = 3.1 (1.5-5.7)		
		Lung cancer	SIR = 1.3 (1.1-1.4)		
		Non-Hodgkin's lymphoma	SIR = 1.1 (0.8-1.5)		
		Hodgkin's disease	SIR = 1.0 (0.5-1.8)		
		Multiple myeloma	SIR = 0.6 (0.3-1.1)		
		Leukaemia	SIR = 0.9 (0.6-1.3)		
		Female workers (n = 2445):			
		All malignant neoplasms	SIR = 1.0 (0.8-1.1)		
		Cancer of the nose	SIR = 8.0 (1.0-28.9)		
		Non-Hodgkin's lymphoma	SIR = 0.6 (0.0-2.0)		
		Hodgkin's disease	SIR = IC		
		Multiple myeloma	SIR = IC		
		Leukaemia	SIR = 0.7 (0.1-2.4)		
2665 petrol station workers in the Latium (greater Rome) region in Italy in 1980 and followed up for 10 years	Regional population	All-cause mortality, all cancer, and CV disease	SMR <1.00 (p <0.05)	Benzene exposure levels were reported to be in the order of 0.2 ppm	Lagorio et al. (1994a)
		All CBLS	SMR = 0.40 (0.07-1.26)		
23,306 workers employed for ≥ 1 continuous year between 1950-75 at UK oil distribution centres and followed up for 15-40 years	Regional populations	All-cause mortality, respiratory, liver and kidney disease, all cancer and cancer of the oesophagus, lung and pleura	SMR <1.00 (p <0.05)		Rushton (1993b)
		Leukaemia	SMR = 1.08 (0.83-1.40)		

Table 11.4: Continued

Exposed population	Controls	Health outcome*	Ratio (95% CI) †	Comments	Reference
Petrol and petroleum distribution: Continued					
18,135 US workers employed for ≥ 1 year at land-based petrol terminals or on marine petrol tankers from 1946-1985 and followed up for 5-55 years	National population	All-cause mortality, all cancer and circulatory, respiratory and liver disease	SMR <1.00 (p <0.01)		Wong et al. (1993)
		Leukaemia	SMR = 0.89 (0.59-1.29) SMR = 0.70 (0.42-1.09)	Land-based workers Marine workers	
		Lymphoma	SMR = 0.75 (0.58-0.97) SMR = 0.61 (0.43-0.83)	Land-based workers Marine workers	
Petroleum production, refining and distribution					
1583 workers ever employed at an Italian refinery from 1949-82 and followed up for 10-42 years	National population	All-cause mortality, all CV disease, stroke, respiratory disease, liver, and GI disease	SMR <1.00 (p <0.05)	Limited monitoring data indicate that a substantial fraction of the workforce had been exposed to benzene levels >1 ppm	Consonni et al. (1999)
		CBLS (all workers)	SMR = 1.79 (1.00-2.95)		
		CBLS (employment >15 years) Leukaemia (employment >15 years)	SMR = 2.71 (1.09-5.59) SMR = 3.77 (1.01-9.65)		
		CBLS (employment pre-1961)	SMR = 2.82 (1.13-5.81)		
		Lymphoma (employment pre-1961)	SMR = 4.02 (1.08-10.28)		
15,732 male workers employed for ≥ 5 years in the Australian petroleum industry from 1981-96 and followed up for 5-15 years	National population	All-cause mortality, ischaemic heart disease, stroke, and respiratory, liver and GI disease	SMR <1.0 (p <0.05)	Elevated incidence of leukaemia mainly accounted for by refinery and terminal workers; no definite relationship with length of employment	Health Watch (1998)
		Bladder cancer Multiple myeloma Leukaemia Lymphocytic leukaemia Myeloid leukaemia	SIR = 1.4 (1.0-1.9) SIR = 1.9 (1.0-3.3) SIR = 2.0 (1.3-2.9) SIR = 2.0 (1.0-3.5) SIR = 2.2 (1.2-3.6)	Estimated long-term benzene exposure levels were ≤ 5 ppm in all cases; estimated cumulative exposures ranged from 0.005-50.9 ppm-years (Glass et al, 1998)	
		Melanoma	SIR = 1.6 (1.3-1.9)	No excess mortality from melanoma (SMR = 0.7 (0.4-1.4)	

Table 11.4: Continued

Exposed population	Controls	Health outcome*	Ratio (95% CI) †	Comments	Reference
Petroleum production, refining and distribution: Continued					
4319 Swedish refinery operators and distribution workers employed for ≥ 1 year between 1958-91 and followed up for 5-35 years	National population	*All male workers (n = 4128):* All-cause mortality, CV disease, and lung cancer	SMR <1.00 (p <0.05)		Järvholm et al. (1997)
		Refinery operators (n = 1339): Leukaemia	SIR = 3.6 (1.5-7.0)		
		Distribution workers (n = 1391): All cancer and lung cancer Leukaemia	SIR <1.00 (p <0.05) SIR = IC (0-2.0)		
34,560 workers employed ≥ 1 year in refinery, petrochemical, distribution, marketing, production, drilling and pipeline locations throughout Canada from 1964-83 and followed up for 11-31 years	National population	*Male workers (n = 26,322):* All-cause mortality, endocrine, circulatory, respiratory and GI disease, all cancer	SMR <1.00 (p <0.05)	There was an increase in multiple myeloma (SMR = 1.94 (1.11-3.15)) in marketing and distribution workers	Lewis et al. (2000)
		Leukaemia	SMR = 0.89 (0.67-1.16)		
		Aortic aneurysm	SMR = 1.27 (1.04-1.53)		
		Female workers (n = 8238): All-cause mortality, endocrine, circulatory, respiratory and GI disease	SMR <1.00 (p <0.05)		
		Leukaemia	SMR = 0.86 (0.32-1.88)		
9187 male workers employed for ≥ 6 months at 10 US refineries from 1970-80 and followed up for 2-12 years	National population	All-cause mortality, all cancer and CV, respiratory and GI disease	SMR <1.00 (p <0.05)		Nelson et al. (1987)
		All CBLS	SMR = 0.60 (0.34-0.97)		
		Skin cancer	SMR = 2.01(1.00-3.60)	10/11 deaths from skin cancer were due to melanoma	
9454 workers employed for ≥ 3 months in 3 refineries, 1 petrochemical plant and the head office of an oil company in Finland from 1967-82 and followed up for 13-28 years	National population	*All workers:* Kidney cancer	SIR = 1.97 (1.29-2.88)	The breast cancer cases were concentrated among clerical workers (SIR = 1.70 (1.08-2.56)), particularly in the head office (SIR = 2.29 (1.25-3.84)), and the SIRs did not differ from those found in other studies of Finnish women in office jobs	Pukkala (1998)
		Male workers (n = 7512): Non-Hodgkin's lymphoma	SIR = 2.01 (1.00-3.59)		
		Female workers (n = 1942): Breast cancer	SIR = 1.50 (1.05-2.08)		

Table 11.4: Continued

Exposed population	Controls	Health outcome*	Ratio (95% CI) †	Comments	Reference
Petroleum production, refining and distribution: Continued					
34,569 men employed at 8 UK refineries for ≥ 1 continuous year between 1950-75 and followed up for 15-40 years	Regional populations	All-cause mortality, stroke all heart, respiratory and liver disease, all cancer and cancer of the mouth, pharynx, lung and pleura	SMR <1.00 (p <0.05)	The SMR for melanoma was not elevated in workers first employed before 1955, but reached 2.30 (1.05-4.37) and 4.67 (2.02-9.20) in workers first employed between 1955-64 and after 1965 respectively. It varied markedly between refinery locations and was higher among office staff than in workers employed outdoors.	Rushton (1993a)
		Diseases of the arteries	SMR = 1.20 (1.07-1.35)		
		Leukaemia	SMR = 0.97 (0.76-1.24)		
		Melanoma	SMR = 1.78 (1.20-2.54)		
Workers representing 383,276 man-years of employment in 1962-71 at 8 European affiliates of a US oil company	UK national population	All leukaemias	SMR = 0.77 (0.41-1.13)	In a subgroup of workers exposed for ≥ 5 years to streams containing ≥ 1% benzene, the SMR for leukaemia was 1.21 (0.37-	Thorpe (1974)
Meta-analysis of 19 cohorts comprising 208,741 refinery, production, pipeline and distribution workers ever employed in USA and UK from 1937-1989 and followed up for 13-50 years	Various national and regional populations	All leukaemias	MSMR = 1.02 (0.93-1.11)	Meta-analysis of leukaemia mortality by cell type; no data on other death rates	Wong & Raabe (1995); Raabe & Wong (1996)
		Acute lymphocytic leukaemia	MSMR = 1.16 (0.81-1.61)		
		Acute myeloid leukaemia	MSMR = 0.96 (0.81-1.13)		
		Chronic lymphocytic leukaemia	MSMR = 0.84 (0.67-1.04)		
		Chronic myeloid leukaemia	MSMR = 0.89 (0.68-1.15)		
Meta-analysis of 26 cohorts comprising more than 308,000 refinery, production and distribution workers ever employed in Australia, Canada, Finland, Italy, USA and UK from 1937-1996	Various national and regional populations	Non-Hodgkin' s lymphoma	MSMR = 0.90 (0.82-0.98)		Wong & Raabe (2000)
Printing					
1361 men ever employed as rotary press workers in Los Angeles from 1949-65 and followed up till 1980	National population	Kidney cancer	SMR = 3.03	Exposed to printing inks and solvents containing benzene No analysis for statistical significance	Paganini-Hill et al. (1980)
		Liver cirrhosis	SMR = 2.05		
		Leukaemia	SMR = 2.47		
Tyre manufacturing					
18,903 male workers employed for ≥ 10 years at 4 tyre manufacturing plants in Ohio and Wisconsin, USA, from 1945-1964 and followed up for 10 years	National population	All-cause mortality	SMR <1.00	No exposure assessment, but ' benzene was once the most widely used organic solvent in the industry' No analysis for statistical significance	McMichael et al. (1976)
		Stomach/colon/prostate cancer	SMR = 1.48/1.16/1.19		
		CBLS	SMR = 1.31		
		Leukaemia	SMR = 1.30		
		Lymphocytic leukaemia	SMR = 1.58		
		Lymphoma	SMR = 1.29		

Table 11.4: Continued

Exposed population	Controls	Health outcome*	Ratio (95% CI) †	Comments	Reference
Vehicle mechanics					
335 predominantly black men employed for ≥ 1 year as vehicle maintenance workers in Washington, DC, from 1977-89 and followed up for 3-15 years	Regional population	All-cause mortality and liver and GI disease CBLS (all workers) CBLS (regular contact workers)	SMR <1.00 ($p < 0.05$) SMR = 3.63 (0.75-10.63) SMR = 9.26 (1.12-33.43)	Regular contact workers used petrol to clean engine parts and wash hands or siphoned petrol by mouth	Hunting et al. (1995)
Miscellaneous industries					
74,828 male and female workers employed from 1972-87 in the painting, printing, footwear and chemical industries in China and followed up for 1-15 years	35,805 unexposed workers	All-cause mortality and all cancer deaths Fatal CBLS Fatal leukaemia Fatal lymphoma	No difference RR = ∞ (2.5-∞) RR = 2.3 (1.1-5.0) RR = 4.5 (1.3-28.4)		Yin et al. (1996)

* CBLS = cancer of the blood and lymphatic system; CV = cardiovascular; GI = gastrointestinal; GU = genitourinary.
† IC = incalculable (no observed cases); MSRM = meta-SMR; SIR = standardised incidence ratio; SMR = standardised mortality ratio; RR = relative risk; ∞ = indefinite (no cases in the unexposed group).

Cohort studies with detailed benzene exposure assessments

There are four occupational cohort studies in which the exposure to benzene has been assessed in detail.

The Chinese cohort

The US National Cancer Institute and the Chinese Academy of Preventive Medicine have collaborated to follow up on a large cohort study commenced in 1982 to assess the risks of specific bone marrow disorders in relationship to occupational benzene exposure (Hayes et al, 1997). The final cohort comprises 74,828 male and female benzene-exposed workers employed from 1972 to 1987 in 672 factories in 12 cities in China and 35,805 unexposed workers. The subjects were followed until the end of 1987, for an average of approximately 11 years. RRs were determined for incident cancer of the blood and lymphatic system, NHL, leukaemia, ANLL, a diagnosis of either ANLL or MDS, and leukaemia other than ANLL, with stratification by age and sex. Smoking or other potential confounders were not considered. The exposed workers held permanent jobs in the painting, printing, footwear, rubber and chemical industries. Exposure levels were estimated from available area monitoring data, detailed production and process information, and employee records.

There were 58 specified cancers of the blood and lymphatic system and 18 other bone marrow disorders (2 cases of agranulocytosis, 9 of aplastic anaemia and 7 of MDS) in the cohort, compared to 13 and 0 respectively in the control group.

When the cohort was divided into three categories according to the estimated average benzene exposure level, the RRs for all cancer of the blood and lymphatic system and ANLL/MDS were elevated in all categories, with a positive trend for increasing average exposure, as shown below:

	Estimated average exposure:			
Cancer type/R:	<10 ppm	10-24 ppm	≥25 ppm	Trend
Blood and lymphatic system	2.2 (1.1-4.2)	3.1 (1.5-6.5)	2.8 (1.4-5.7)	p = 0.003
ANLL/MDS	3.2 (1.0-10.1)	5.8 (1.8-18.8)	4.1 (1.2-13.2)	p = 0.01

The RR for NHL was 4.7 (1.2-18.1) in workers exposed to ≥25 ppm, but was not elevated in the lower average exposure categories.

When the cohort was divided into three categories according to the estimated cumulative benzene exposure level, the RR for all cancer of the blood and lymphatic system was elevated in all categories, whereas the RRs for leukaemia and ANLL/MDS were elevated at cumulative exposures ≥40 ppm-years:

	Estimated cumulative exposure			
Cancer type/RR	<40 ppm-years	40-99 ppm-years	≥100 ppm-years	Trend
Blood and lymphatic system	2.2 (1.1-4.5)	2.9 (1.3-6.5)	2.7 (1.4-5.2)	p = 0.004
Leukaemia	1.9 (0.8-4.7)	3.1 (1.2-8.0)	2.7 (1.2-6.0)	p = 0.04
ANLL/MDS	2.7 (0.8-9.5)	6.0 (1.8-20.6)	4.4 (1.4-13.5)	p = 0.01

The RR for NHL was not elevated in any of the three cumulative exposure categories.

Only NHL was linked to duration of exposure. None of the RRs were related to the year of initial employment in the study factories. ANLL/MDS was linked to recent exposure (<10 years prior to diagnosis), whereas NHL was linked to distant exposure (≥10 years prior to diagnosis).

The authors concluded that the results suggest an association between benzene exposure and a spectrum of blood cancers and related disorders, with an increase in cancer risk at cumulative exposures <40 ppm-years and a tendency, although not strong, for the risk to rise with increasing levels of exposure.

It should be noted that personal monitoring in a subset of the Chinese cohort measured current exposure levels which were reported to be 'much higher than expected' compared to the estimates that were made in the course of the main study (Rothman et al, 1995, 1996b). As such, the historical exposure levels used to determine the dose-response relationship may have been grossly underestimated (Budinsky et al, 1999; EPA, 1998a; Wong, 1999).

Overall mortality rates in the Chinese cohort have been reported by Yin et al. (1996) and are summarised in Table 11.4. The average latency period of fatal leukaemia in benzene-exposed workers was estimated at 11-12 years, with a range from 10 months to 50 years (Yin et al, 1987b).

The CMA cohort

The Chemical Manufacturers Association (CMA) sponsored a study of 4602 male chemical workers who were employed for ≥6 months from 1946-75 at 7 US plants (Wong, 1987a, 1987b). Two comparison groups were used: the general US population and 3074 unexposed male workers employed at the same plants at the same time as the cohort. Smoking or other potential confounders were not considered. The vital status of all subjects was followed up until the end of 1987 and the findings compared to average and peak exposures as determined from available air monitoring data and employment records obtained from the participating companies.

There were 19 deaths from cancer of the blood and lymphatic system in the exposed workers compared to 3 in the unexposed group. In the exposed group, 7 of the observed cases were diagnosed with leukaemia and the remaining 12 with lymphoma. The subjects with leukaemia comprised 1 case with acute lymphatic leukaemia (ALL), 2 with chronic lymphatic leukaemia (CLL), 1 with unspecified lymphatic leukaemia, 2 with chronic myeloid leukaemia (CML) and 1 with unspecified acute leukaemia. In the unexposed workers, all 3 cases were diagnosed with lymphoma. The SMRs for all cancers of the blood and lymphatic system/leukaemia reached 0.91/0.97, 1.47/0.78 and 1.75/2.76 for cumulative exposures of <180, 180-719 or ≥720 ppm-months respectively, but none of the ratios was significantly different from unity. The RRs for all cancers of the blood and lymphatic system were 2.10, 2.95 and 3.93 respectively for the same cumulative exposure groups, with $p = 0.02$ for trend. The RRs for leukaemia were indefinite as there were no cases in the unexposed workers, with $p = 0.01$ for trend with cumulative exposure. There was no correlation with peak levels or duration of exposure.

Based on the RRs and their trend with cumulative exposure, the author concluded that workers exposed to benzene exhibited a significant excess of deaths from leukaemia as well as from the broader category of all cancers of the blood and

lymphatic system when compared with workers who were not exposed to the chemical.

Ireland et al. (1997) conducted an extended mortality study in production personnel from one of the plants included in the CMA-sponsored study. The workers were stratified into three categories based on cumulative exposure: <12 ppm-months (n = 666), 12-72 ppm-months (n = 378) and ≥72 ppm-months (n = 164). Compared to the regional population, the SMR for leukaemia was 2.5 (0.3-8.9) in the lowest, incalculable (0.0-5.9) in the middle, and 4.6 (0.9-13.4) in the highest exposure category, with no clear dose-response relationship.

The Dow Chemical cohort

This cohort comprised 956 male chemical workers employed at a single site in Michigan, USA, between 1940 and 1982. The workers were exposed to benzene in chlorobenzene or alkylation plants which used benzene as a raw material, or in an ethyl cellulose plant where benzene was used as a solvent (Bond et al, 1986; Ott et al, 1978). They were followed up until the end of 1982. The average exposure duration and length of follow-up were 7 and 26 years respectively. Each job entry was assigned an exposure intensity level on the basis of job classification and representative personal air monitoring data.

The analysis accounted for co-exposure to arsenic, asbestos or high levels of vinyl chloride. Smoking or other potential confounders were not considered. There were 6 deaths from cancer of the blood and lymphatic system against 4.8 expected, including 4 cases of myelogenous leukaemia against 0.9 expected, and 4 from skin cancer (3 melanomas and 1 squamous cell carcinoma) against 0.9 expected, using concurrent US white male mortality rates as reference values. The excess of myelogenous leukaemia was statistically significant (p = 0.011; SMR and 95% CI not stated) and the risk for skin cancer was significantly elevated (SMR = 4.41 (1.21-11.38)). There were no significant trends with either work area, cumulative exposure or duration of exposure. Of the 6 cases of blood and lymphatic system cancer, 4 had been exposed to <500 ppm-months and 2 to ≥1000 ppm-months. In the case of myelogenous leukaemia, cumulative exposures varied from 18-4211 ppm-months. The 4 cases of skin cancer all occurred in workers with exposures <500 ppm-months, but otherwise had no unusual or common characteristics.

The authors concluded that their study provided support for an association between exposure to benzene and myelogenous leukaemia.

The Pliofilm cohort

An excess incidence of leukaemia in rubber workers at two Goodyear facilities in Ohio, USA was reported in a preliminary paper by Infante et al. (1977) and in more detail by Rinsky et al. (1981). Depending on its definition, this cohort comprises 1165-1212 male workers employed from 1936-75 in the manufacture of Pliofilm, which is a material made from rubber hydrochloride (Paxton et al, 1994a; Rinsky et al, 1987). The manufacturing process used large volumes of benzene as a solvent and there was no exposure to other known carcinogenic substances. The last worker joined the cohort in 1965 and the most recent follow-up was in 1987.

Excluding deaths before 1950, Rinsky et al. (1987) identified 15 deaths from lymphatic and haematopoietic cancers versus 6.6 expected (SMR = 2.27 (1.27-3.76)) and 9 deaths from leukaemia versus 2.7 expected (SMR = 3.37 (1.54-6.41)). In a later analysis that included deaths between 1940-50, Paxton et al. (1994a)

identified 21 deaths from lymphatic and haematopoietic cancers versus 9.51 expected (SMR = 2.21 (1.37-3.38)) and 14 deaths from leukaemia versus 3.89 expected (SMR = 3.60 (1.97-6.04)). Neither of these analyses considered smoking or other potential confounders.

The individual exposure histories of the cohort members were reconstructed after the plants closed in 1975, from fairly detailed monitoring and health surveillance data and other information on record.

Based on unpublished exposure estimates, Rinsky et al. (1987) found SMRs for leukaemia of 1.09 (0.12-3.94) at a cumulative exposure <40 ppm-years, 3.22 (0.36-11.65) at 40-200 ppm-years, 11.86 (1.33-42.85) at 200-400 ppm-years and 66.37 (13.34-193.93) at >400 ppm-years.

Paxton et al. (1994a) recalculated the SMRs for a different set of cumulative exposure categories and compared them with similar statistics derived from independent, more detailed exposure estimates produced by Crump & Allen (unpublished report prepared for the Occupational Safety and Health Administration in 1984) and Paustenbach et al. (1992), as shown in Table 11.5[8]. The results reproduced in the table suggest a strong dose-response relationship of risk increasing with cumulative exposure, no matter which estimate is used, and indicate that there is a significantly elevated risk for leukaemia (according to 2 of the 3 available exposure estimates) at a cumulative dose >50 ppm-years, corresponding to a long-term average exposure of 1.25 ppm over 40 years.

Table 11.5: SMRs (95% CI) for leukaemia in the Pliofilm cohort, analysed by cumulative exposure as estimated by Crump & Allen (1984, unpublished), Paustenbach et al. (1992) and Rinsky et al. (1987)(from Paxton et al, 1994a)

Cumulative exposure (ppm-years)	Exposure estimate		
	Crump & Allen	Paustenbach et al.	Rinsky et al.
0-5	0.88 (0.02-4.89)	1.33 (0.03-7.43)	1.97 (0.41-5.76)
>5-50	3.25 (0.88-8.33)	1.79 (0.22-6.45)	2.29 (0.47-6.69)
>50-500	4.87 (1.79-10.63)*	2.80 (0.76-7.16)	6.93 (2.78-14.28)†
>500	10.34 (2.13-30.21)†	11.86 (4.76-24.44)†	20.00 (0.51-111.4)

* p <0.05
† p <0.01

As the SMR was not significantly different from unity at cumulative exposures ≤50 ppm-years for any of the three exposure estimates, the authors concluded that the results of the analysis were consistent with a threshold model for benzene-induced leukaemia. However, the power of the analysis was insufficient to support this conclusion. The upper 95% confidence limits given in Table 11.5 range from 6.45-8.33 in the >5-50 ppm-year exposure category and from 4.89-7.43 the 0-5 ppm-year category. In either case, the upper limits are well above unity irrespective of the exposure estimate used. Therefore, it cannot be excluded that a cumulative exposure level ≤50 ppm-years is also associated with an excess mortality from leukaemia.

[8] The major distinction between the three exposure estimates is the disregard by Rinsky et al. (1987) for the likely increase in exposure levels during and in the aftermath of World War II because of wartime conditions and longer working hours. In addition, only Paustenbach et al. (1992) have given consideration to the potential for dermal exposure.

Wong (1995) reanalysed the findings of Paxton et al. (1994a) by cell type (AML and multiple myeloma (MM)), using the Rinsky et al. (1987) exposure estimate which in general is the lowest of the three. He found no relationship between cumulative exposure and the risk of MM, whereas the SMR for AML showed a clear dose response, as follows:

Cumulative exposure	SMR (95% CI)	Statistical significance
<200 ppm-years	0.91 (0.02-5.11)	Not significant
200-400 ppm-years	27.21 (3.29-98.24)	p <0.01
>400 ppm-years	98.37 (20.28-287.65)	p <0.01
Total cohort	5.03 (1.84-10.97)	p <0.01

The author concluded that there was no significant increase in the risk of AML for cumulative exposure to benzene <200 ppm-years, above which the risk rose sharply to a very substantial SMR of 98.37 for >400 ppm-years. However, as the 95% upper confidence limit in the lowest exposure group was 5.11, an increase in mortality from AML at a cumulative exposure <200 ppm-years cannot be ruled out.

In another re-analysis based on the three sets of exposure estimates referred to above, Schnatter et al. (1996b) used the work history of each Pliofilm worker to define each worker's maximally exposed job/department combination over time and the long-term average benzene exposure level associated with the maximally exposed job. They then determined the number of observed and expected cases of leukaemia (all cell types) in subcategories of workers and person-years who were always exposed at levels that did not exceed specific concentrations of benzene. As shown in Table 11.6, this analysis showed that there were fewer observed than expected deaths in all subcategories that were always exposed to benzene concentrations ≤15 ppm, irrespective of the exposure estimate used. However, because of the low number of expected cases, this finding could also be due to chance.

Table 11.6: Observed and expected cases of leukaemia (all cell types) for selected cut-off points for the average long-term exposure level in the maximally exposed job (Schnatter et al, 1996b)

Long-term benzene exposure (ppm)	Exposure estimate					
	Crump & Allen		Paustenbach et al.		Rinsky et al.	
	Observed	Expected	Observed	Expected	Observed	Expected
≤1	0	0.53	0	0.07	1	1.53
≤5	0	1.01	0	0.10	1	1.72
≤10	0	1.04	0	0.11	1	2.00
≤15	0	1.28	0	0.15	1	2.00
≤20	2	1.92	0	0.21	3	2.30
≤25	2	2.13	1	0.80	7	2.92
≤30	3	2.35	1	0.90	7	3.24
≤40	5	2.73	1	1.33	10	4.04
≤50	5	2.98	3	1.96	14	4.79
≤100	8	3.98	5	3.55	14	4.87
≤200	9	4.20	14	4.70	14	4.87
≤260	14	4.87	14	4.87	14	4.87

Conclusions

Cohort studies with poorly characterised benzene exposure levels

Several of the studies summarised in Table 11.4 have associated cancer of the blood and lymphatic system (including but not limited to leukaemia) with the obsolete practice of using benzene-containing adhesives, cleaners and solvents. Some studies indicate a positive association with long-term employment at petroleum refineries, in the chemical industry or in highway maintenance. There was no excess mortality from leukaemia in three cohorts of coke plant workers.

The risk for malignant melanoma or skin cancer (mainly melanoma) was elevated in three petroleum industry cohorts (Health Watch, 1998; Nelson et al, 1987; Rushton, 1993a). The SIR for breast cancer was elevated in female workers in a Finnish oil company cohort (Pukkala, 1998). However, the elevation was mainly due to cases among clerical workers and similar in magnitude to that found in other studies of Finnish women in office jobs.

Cohort studies with detailed benzene exposure assessments

There was an excess mortality from cancer of the blood and lymphatic system in all four cohorts for which detailed benzene exposure assessments are available and a significant trend with cumulative exposure in all but the smallest cohort (the Dow Chemical cohort). As such, it is widely accepted that these studies provide sufficient evidence of a clear dose-response relationship between benzene exposure and the broad category of all cancers of the blood and lymphatic system (ATSDR, 1997; OECD, 2000; IARC, 1982a; IPCS, 1993; USEPA, 1998a). In terms of specific cancer categories, the relationship is primarily due to the risk for AML (ANLL).

In the CMA cohort, the SMR for leukaemia was elevated (2.6) in workers with a cumulative exposure of ≥720 ppm-months (that is, ≥60 ppm-years), but it was not significantly different from unity and therefore could have been due to chance. In a subset of the CMA cohort, the SMR for leukaemia was 4.6 in workers with a cumulative exposure of ≥72 ppm-months (6 ppm-years), but again did not differ significantly from unity. There was no clear dose-response relationship in the Dow Chemical cohort and there is doubt about the true exposures in the Chinese cohort. As such, the Pliofilm cohort is the most suitable for the determination of the carcinogenic potency of benzene. In addition, the Pliofilm cohort has the advantage of limited if any co-exposure to other potentially carcinogenic compounds and a very long follow-up period. However, it suffers from uncertainty about actual exposure levels, particularly prior to 1950, which is important as there are no cases of leukaemia in workers first employed after that year (USEPA, 1998a).

Based on an unpublished assessment of individual exposures in the Pliofilm cohort, Rinsky et al. (1987) determined SMRs for leukaemia that increased exponentially with cumulative exposure, starting from near unity at a cumulative exposure <40 ppm-years. More recent dose-response analyses that include other, more comprehensive exposure assessments indicate that the risk for leukaemia is significantly elevated at a cumulative exposure level above, but not below 50 ppm-years, corresponding to an average exposure level of 1.25 ppm over 40 years (Paxton, 1994b). Moreover, whatever exposure estimate was used, the number of observed cases of leukaemia was consistently below the expected number in all workers whose long-term exposure never exceeded 15 ppm (Schnatter et al, 1996b). However, because of the limited statistical power resulting from the size of

the Pliofilm cohort, these results do not rule out the possibility of an increased risk of leukaemia at exposure levels lower than those cited above.

In the Dow Chemical cohort, there was an association between benzene exposure and skin cancer. However, all cases occurred in the lowest cumulative dose group (<500 ppm-months) and there was no trend with either level or duration of exposure.

11.6.2 Case-control studies

The case-control studies reviewed below have been divided by organ system. They comprise studies based in a specific industry, such as petroleum refining, and studies conducted in a community population. Limitations in statistical power and study quality, particularly in relation to exposure assessment and/or control for potential confounders, pervade all of the studies reviewed.

Cancer of the blood and lymphatic system

Industry-based studies

A study nested within a cohort of male workers at a large tyre manufacturing plant in Ohio, USA included 11 cases of lymphocytic leukaemia and 1350 controls (Checkoway et al, 1984). The OR for direct exposure through routine use or handling of benzene or benzene-containing solutions was 4.50 (95% CI not stated), but did not reach statistical significance (p = 0.22). The ORs for exposure to acetone, carbon disulfide, carbon tetrachloride, ethyl acetate, hexane or methanol ranged from 4.3-18 (95% CIs not stated) and were all statistically significant.

Austin et al. (1986) compared 14 cases of leukaemia in white male workers at a US refinery, including 8 cases of AML, with 50 controls. Neither job category, department nor length of employment was a significant risk factor.

In an exploratory study of cancer mortality at a transformer assembly facility in Massachusetts, USA, where benzene was used for general cleaning purposes until 1950, benzene exposure was not a significant risk factor for leukaemia (OR = 1.4 (0.64-3.2)) (Greenland et al, 1994).

Sathiakumar et al. (1995) studied 69 workers with leukaemia, predominantly AML or CLL (numbers not specified), and 284 controls who had worked for the same US-based petroleum company for ≥1 year from 1976-90. Forty-four risk factors tested for included site of work, involvement in production, job category, duration and year of employment. The only risk factors identified were for AML and included length of employment, with an OR = 8.7 (2.0-37.2) in workers employed for >30 years (trend: p = 0.01), and upstream employment in crude oil production or maintenance (OR = 3.2 (1.1-9.2)).

Schnatter et al. (1996a) compared 7 cases of NHL, 7 cases of MM and 55 controls drawn from the cohort of Canadian petroleum distribution workers described by Lewis et al. (2000). Tests included several measures for benzene exposure. The only risk factors identified were a family history of cancer and cigarette smoking, with cumulative benzene exposure showing no additional risk.

A study nested in the cohort of British petroleum distribution and marketing workers described by Rushton (1993b) compared 91 cases of leukaemia, predominantly ANLL (31) and CLL (31), with 364 controls (Rushton & Romaniuk, 1997). Risk factors tested for included cumulative, mean and maximum airborne

and potential skin exposure to benzene, duration of employment, date of hire, employment as driver, socio-economic status, and age at and years from start of work. For ANLL, none of the ORs differed from unity. For CLL, the risk factors identified included duration of employment, white-collar status and years of work, but not exposure to benzene.

The case-control study nested within the cohort of Australian petroleum industry workers currently comprises 63 cases with lympho-haematopoietic cancers, mainly NHL, MM, AML and CLL, and 315 controls (Health Watch, 1998). In the analysis, the OR was used to compare groups with different levels of exposure to various potential causative agents, relative to the least exposed or baseline group. Compared to the baseline of the rate in refineries, the OR was marginally elevated for work in terminals (1.8 (1.0-3.5)). Length of employment and period of first employment were not significant risk factors. Past exposure to benzene was ranked on a scale from 1-5, depending on AIP jobcode, the company site where the job was carried out and length of service in any job. When cases and controls were compared to the highest benzene rank of any job ever held (ranks 4-5), the OR was 7.9 (1.6-39) times higher than for rank 1 (the baseline). When compared to the benzene rank of the job held longest, the OR was 3.2 (1.1-9.4) times higher than baseline for rank 3 and 6.6 (1.4-30) times higher for rank 4 (the highest rank in this test). The authors concluded that a relatively higher exposure to benzene might be the significant factor leading to an increased risk of leukaemia and MM in the cohort study.

Nilsson et al. (1998) conducted a nested case-control study of Swedish seamen with two study bases. These comprised a total of 92 men who were registered as seamen at the national censuses in Sweden in 1960 and 1970 respectively and recorded in the Swedish National Cancer Register with cancer of the blood and lymphatic system from 1971-88. The controls were 291 age-matched men registered as seamen at the same censuses. NHL (37) and leukaemia (30) accounted for most of the cases. There were no increased risks for the 1960 cohort, in which few cases were exposed to benzene or petrol. In the 1970 cohort, the OR was increased for cancer of the blood and lymphatic system (OR = 2.6 (1.1-5.9)) and for NHL (OR = 3.3 (1.1-10.6)) in seamen who had worked on deck on chemical or petroleum product tankers, but not on crude oil tankers.

Wong et al. (1999) studied 59 cases of leukaemia, including unspecified leukaemia (35), AML (13) and MM (11), and 220 controls drawn from a US-based cohort study of 18,135 petrol distribution workers (Wong et al, 1993). Test variables included duration of employment, duration of exposure, job category, cumulative exposure to hydrocarbons, cumulative frequency of peak exposure to hydrocarbons, and year of first exposure. None of these was identified as a risk factor for any of the study diagnoses.

In a study nested within the Pliofilm cohort described above, Finkelstein (2000) examined the temporal variation of leukaemia risk following exposure to benzene. Each leukaemia case was matched with 6-333 control subjects and the exposure of cases and controls were then assessed according to Rinsky et al. (1987) and compared at various times before the death of the case subject. As expected, leukaemia risk was significantly associated with cumulative exposure ($p = 0.024$). However, exposures incurred in the previous 10 years were found to account for most of the risk and there was no significant difference in the benzene exposure of cases and controls 15 or more years prior to the death of the case subject.

Community-based studies

Exposure to benzene and/or toluene was investigated in 401 cases of various serious blood disorders and 124 controls sampled from the same general hospital in Lyon, France (Girard & Revol, 1970). The prevalence of exposure was significantly higher among patients with acute leukaemia, CLL and aplastic anaemia than in the comparison group. The majority of the exposed patients had worked in small workshops where the main sources of exposure were reported to be cleaning fluids and paint and glue thinners.

Ishimaru et al. (1971) interviewed 303 matched pairs of controls and cases of leukaemia (not further specified) with onset from 1945-67 in Hiroshima or Nagasaki in Japan. The OR was 2.5 ($p < 0.01$) among those with a history of any of 11 occupations with the potential for frequent exposure to benzene or x-rays and showed a positive trend with the length of time in those occupations.

Eighteen (36%) out of 50 working men with ANLL seen at a hospital in Lund, Sweden, were occupationally exposed to petroleum products or vehicle exhaust fumes through occupation as petrol stations attendants, drivers or operators of excavators or power saws (Brandt et al, 1978). By comparison, similar exposure patterns occurred in only 10% of three outpatient control groups ($p = 0.0002$), including a group of male patients with CML or CLL ($p = 0.006$), or 10-11% of the general male population in the region.

Linos et al. (1980) compared 138 cases of acute or chronic leukaemia (not further specified) that occurred in residents in a county in Minnesota, USA between 1955-74 with 276 controls, with regard to past occupational and chemical exposure. The OR for exposure to benzene was not significantly elevated (3.34 (0.60-27.60)).

In a study of 131 cases of MM, 111 cases of CLL and 431 controls resident in a rural woodland district in central Sweden, Flodin et al. (1987, 1988) observed an association with occupational exposure to exhaust fumes from diesel and petrol engines, including tractors and chainsaws. The OR was 2.1 (1.2-3.9) for MM and 2.2 (1.2-4.2) for CLL.

Another Swedish study of 125 cases of acute leukaemia (including 97 AML and 24 ALL cases) and an equal number of controls found a large excess risk for professional painters exposed to solvents that would have contained benzene as an impurity (OR = 13 (2-554) (Lindquist et al, 1987). There was also an excess risk among professional drivers, with an OR = 3.0 (1.1-9.2). The OR reached 5.0 (95% CI not stated; $p < 0.05$) for those who had been drivers for >5 years in their lifetime or >1 year during the 5-20-year period prior to diagnosis and remained after adjustments for exposure to organic solvents, smoking and therapeutic x-ray treatment (Lindquist et al, 1991).

A study of 475 cases of lymphoma, leukaemia and MM in white male residents in Missouri, USA, and 1425 controls found an elevated risk of leukaemia in mechanics (OR = 4.79 (1.42-16.18)) (Brownson & Reif, 1988).

Richardson et al. (1992) conducted an interview study of occupational risk factors of acute leukaemia in French adults, based on 31 cases of ALL, 154 cases of AML and 513 controls. A significant relationship was observed between AML and high or medium exposure to benzene (OR = 3.6 (1.7-7.7)). For ALL and AML combined, the OR for any exposure to benzene was 1.3 (0.8-2.3), whereas it was 2.8 (1.3-5.9) for high or medium exposure.

In an interview study of 622 white males with NHL and 1245 controls drawn from the general population in Iowa and Minnesota, USA during 1980-83, there were no indications that industrial exposures were a major determinant for NHL (Blair et al, 1993). The OR for benzene exposure was close to unity, but did increase slightly with intensity of exposure (lower intensity: OR = 1.1 (0.8-1.4); higher intensity: OR = 1.5 (0.7-3.1)).

In a study of 86 cases of AML, CML or MDS in residents in Turin, Italy, there was a marginally elevated risk of leukaemia/MDS in vehicle mechanics (OR = 2.7 (0.97-7.6)) and truck and other drivers (OR = 2.7 (0.8-9.6)), but no association with exposure to benzene (Ciccone et al, 1993).

A French, hospital-based case-control study of 226 male cases of hairy cell leukaemia and 425 matched controls found no association between occupational exposure to benzene and this rare B-lymphoid chronic leukaemia (Clavel et al, 1996).

A recent review by Savitz & Andrews (1997) identified 12 additional community-based case-control studies of benzene and cancer of the blood and lymphatic system, none of which reported any association between the two.

Childhood leukaemia

Leukaemia is the most common cancer in children under the age of 15 (Shu, 1997). A number of case-control studies have explored the potential relationship between childhood leukaemia and parental exposure to agents that might be toxic to the unborn or breast-fed baby and/or the germ cells of the parents.

Some of these studies have suggested a link between childhood leukaemia and pre-conceptional occupational exposure of the father to solvents, petroleum products, motor vehicle exhaust fumes, benzene, or plastic monomers or polymers (Buckley et al, 1989; Fabia & Thuy, 1974; McKinney et al, 1991; Shu et al, 1999; Vianna et al, 1984). Others have found a weak association with maternal employment in jobs with the potential for exposure to various chemicals including benzene, petrol, solvents and thinners, paints and/or plastic monomers or polymers (Shu et al, 1988, 1999; van Steensel-Moll et al, 1985).

A study of 123 cases of childhood leukaemia and an equal number of matched controls found a significant, dose-related elevation in the risk of leukaemia for children whose parents burned incense in the house during pregnancy or lactation (Lowengart et al, 1987). Incense stick has been reported to emit the same quantity of benzene in smoke as tobacco and herbal cigarettes (Löfroth et al, 1991).

In a study of 97 cases of childhood leukaemia (78 of whom had ALL) and 259 matched controls from Denver, USA, Pearson et al. (2000) found an association between childhood leukaemia and proximal high traffic streets with traffic counts ≥20,000 vehicles per day (OR = 8.28 (2.09-32.80)).

Skin cancer

In a study of 307 cases of non-melanoma skin cancer (basal and/or squamous cell carcinoma) and 229 controls resident in Texas, USA, the most important risk factors were red hair, fair skin, outdoor sun exposure, and a family history of skin cancer (Gamble et al, 1996). Employment at any time in the petroleum industry was associated with a slightly elevated risk of developing concurrent basal and squamous cell carcinomas (OR = 2.10 (1.08-4.09)).

In a Dutch study of 140 cases with non-metastasised melanoma and 181 controls with other types of malignancy, increased risks were found for subjects ever employed in the electronics, metal and transport and communication industries (Nelemans et al, 1993). However, they were not statistically significant and there were no trends for duration of employment or latency. Also, there was no increase in risk for workers in the chemical industry.

The American Cancer Society enrolled 1.2 million randomly selected people in a study of life style and environmental factors in relation to cancer mortality, 2780 of whom had a history of or developed melanoma during the 6-year study follow-up period (Pion et al, 1995). These cases were compared with controls selected from the remaining people enrolled on a 1:3 basis and matched for age, sex, race, and geographic location. In men, the risk of melanoma was elevated in high-paying versus low-paying jobs (OR = 1.58; $p < 0.001$) and in white-collar versus blue-collar jobs (OR = 1.33; $p < 0.001$), but unrelated to outdoor versus indoor occupations. In women, the findings were inconclusive. The only specific work-related risk factor was exposure to x-rays. Other large community-based studies in Australia, Britain and Sweden came to similar results (Burnley, 1997; Vågerö et al, 1990).

Other cancers

Gérin et al. (1998) conducted a community-based case-control study of 19 specific cancers excluding leukaemia in 3730 men and 533 controls aged 35-70 years and resident in Montreal, Canada. Their exposure to various workplace chemicals including benzene, toluene, xylenes and styrene was estimated through interviews and from workplace records. There were 737 subjects, mainly mechanics, service station attendants and shoe workers, who had been exposed to benzene, usually with concomitant exposure to toluene and xylenes. However, there was no evidence that the risks of common cancers such as those of the gastrointestinal tract, lungs, prostate, bladder or kidney were related to exposure to any of the chemicals under investigation. For NHL (215 cases) and melanoma (103 cases), the ORs for benzene exposure were <1.00.

The industry-based study by Wong et al. (1999) mentioned above under cancer of the blood and lymphatic system also included 12 cases of kidney cancer. There was no difference between cases and controls with regard to duration of exposure or to cumulative or peak exposures to hydrocarbons.

Petralia et al. (1999) studied the relationship between the risk of pre-menopausal breast cancer and exposure to benzene or polycyclic aromatic hydrocarbons in 301 cases and 316 controls sampled from two counties in New York State between 1986-91. There were 55 breast cancer cases and 35 controls who had been exposed to benzene, mainly through employment as laboratory technicians, painters, sculptors, craft-artists, or assemblers in the motor vehicle industry. Following adjustment for age, years of education, age at first birth, age at menarche, history of benign breast disease, history of breast cancer in a first-degree relative, body mass index, and months of lactation, four variables relating to benzene had ORs that reached or approached statistical significance. These were duration of exposure ≥4 years (2.57 (1.23-4.73)); medium-to-high probability of exposure (1.95 (1.14-3.33)); low average exposure intensity (2.36 (1.30-4.30)); and medium-to-high cumulative exposure (1.93 (1.00-3.72)).

In Denmark, Hansen (2000) conducted a nationwide register-based case-control study on primary breast cancer in men, which included 230 cases and 12,880

controls. Allowing for a lag time ≥10 years and after adjustment for socio-economic status, the OR was 2.5 (1.3-4.5) in all men with >3 months of employment as car mechanic or petrol station worker and 5.4 (2.4-11.9) in men who were <40 years old when first employed in those occupations. Exposure to benzene was not assessed.

Conclusions

While the case-control studies reviewed above have limitations in statistical power and study quality, there are several which indicate that occupation and/or benzene exposure is associated with an increased risk of cancer of the blood and lymphatic system, including, but not limited to AML and other leukaemias. Positively identified risk factors include employment in upstream petroleum production, at petroleum terminals, on deck on chemical or petroleum product tankers, and as a mechanic, machinist, chemical worker, chemist, painter, driver or logger. The studies by Health Watch (1998) and Richardson (1992) found that the risk was significantly elevated at relatively high, but not at lower levels of exposure to benzene.

There are some indications that parental exposure to benzene and in particular maternal exposure during pregnancy may be linked to childhood leukaemia, but the overall evidence for this association is limited at present. Other tentative findings suggest a relationship between the risk of breast cancer and exposure of female workers to benzene on the one hand and between male breast cancer and exposure to petrol and vehicle exhaust on the other.

11.6.3 Ecological studies

Leukaemia and car traffic variables

Robinson (1982, 1991) found a strong relationship between leukaemia mortality and vehicle usage (as monitored by the annual rate of vehicle fatalities) in Australia, France, Germany, Italy, Japan, The Netherlands, UK and USA. In a study of all incident childhood cancer in Denver, Colerado, USA, between 1976-83, Savitz & Feingold (1989) found a statistically significant association between traffic density at the place of residence at the time of diagnosis and the risk for all leukaemia combined. In a sample of 22 British counties, Wolff (1992) found a significant correlation coefficient between the incidence of AML, lymphoma, ALL, CLL and low-grade NHL for the years 1984-88 and the number of cars per household reported in the 1981 Great Britain Population Census.

Swaen & Slangen (1995) found a non-significant, inverse relationship between leukaemia mortality and petrol consumption in 19 European countries, but a weak positive association between the incidence of myeloid leukaemia and the consumption of petrol per km^2. However, both findings could be due to unrelated factors such as changes in prognosis or country differences in leukaemia case ascertainment. As such, the authors concluded that their study did not support an association between petrol consumption and leukaemia incidence or mortality.

Nordlinder & Järvholm (1997) compared the 1975 car density in Swedish local government areas with the 1975-1985 cumulative incidence of ALL, AML, CML and NHL in persons under 25. None of these showed a significant correlation with car density, although the combined group of areas with >5 cars/km^2 had a higher rate of AML than those with <5 cars/km^2 (95% CI for the difference: 0.1-4.0 cases/10^6 person-years).

Leukaemia and industry emission variables

There was an excess mortality rate between 1950-69 from childhood leukaemia and young adult Hodgkin's disease and lymphoma among residents in the heavily industrialised New Jersey-New York-Philadelphia Metropolitan Region compared to USA as a whole (Greenberg et al, 1980). However, other early studies of populations residing in the vicinity of petroleum refineries and chemical plants have not suggested links with cancers of the blood and lymphatic system (Blot et al, 1977; Hearey et al, 1980; Hoover et al, 1975; Kaldor et al, 1984).

A more recent study of an area within a radius of 3.0 km of a large petrochemical plant in South Wales, UK, compared the 1974-91 incidence of leukaemia and lymphoma with onset before age 25 among study area residents with those in the regional population (Lyons et al, 1995). There were no statistically significant differences, although the number of observed cases was higher than expected for all disease types except myeloid leukaemia. Data from ambient monitoring for benzene around the site showed monthly peak values varying from 4-16 ppb.

11.7 The Illawarra leukaemia cluster

The Illawarra leukaemia cluster refers to a group of 12 cases of leukaemia that occurred in 1989-96 in three contiguous suburbs bordering the Port Kembla steelworks near Wollongong, New South Wales (Westley-Wise et al, 1999). There were 3 cases of AML, 3 of CML and 6 of ALL. All cases were under 44 years of age at the time of diagnosis and nine were ≤20 years of age. Four attended the same local high school in the late 1980s, three of them in the same school year. Using the rest of the region as reference population, only 3.49 cases were expected, corresponding to a SIR of 3.44 (1.42-6.92).

The regional health authority launched an investigation which examined a wide range of possible explanations for the cluster, including benzene emissions from the coking ovens and coal gas by-product plant at the steelworks. It was estimated that ambient air levels of benzene had averaged up to 3 ppb since 1970, although the mean levels measured in 1996 did not exceed 1 ppb within 1.6 km from the plant. As this is less than one-thousandth of the level at which leukaemia risk has been identified in occupational epidemiological studies, the authors concluded that the cause of the cluster was uncertain, although an association with chemical exposures could not be totally excluded. The odds that it was due to chance were calculated at 1 in 4-8000 (Westley-Wise & Hogan, 1997).

11.8 Summary and conclusions

There is anecdotal evidence that acute exposure to benzene vapours causes dizziness and other CNS effects at concentrations above 25 ppm and eye, mucous membrane and skin irritation at levels above 30-60 ppm. Furthermore, aspiration of liquid benzene has been observed to cause lung oedema and bleeding. Deaths from cardio-respiratory arrest have occurred following short-term inhalation of 20,000 ppm benzene, or from ingestion of a single dose of 125 mg benzene per kg BW.

Several studies demonstrate that repeated exposure to benzene may induce bone marrow depression, cause damage to genetic material and induce leukaemia, specifically AML. Some studies also point to an association between benzene exposure and the risk for lymphoma, specifically NHL and MM. For bone marrow depression, the best estimate for a LOAEL is 7.6 ppm (TWA_8), based on current

data. An appropriate NOAEL has not been determined, although studies with various limitations indicate that it is likely to be >0.5 ppm (TWA_8). Dose-related structural or numerical chromosome aberrations have been detected in peripheral LC of workers exposed to benzene levels above 10 ppm (TWA_8), but a threshold level has not been identified. The risk of developing leukaemia increases with exposure and has been shown to be significantly elevated above, but not below, a cumulative exposure of 50 ppm-years, corresponding to an average occupational exposure of 1.25 ppm (TWA_8) over 40 years. However, this finding derives from a single cohort study with insufficient statistical power to rule out the possibility of some increase in leukaemia risk at lower exposures.

In addition, some studies suggest an association between repeated exposure to benzene or benzene-containing products and several other adverse health effects, including menstruation disorders, spontaneous abortions, melanoma and breast cancer in adults and reduced birth weight and leukaemia in the children of exposed parents. However, considering the multiple exposure circumstances in most studies and the limited consistency of the findings reviewed above, the human database does not in itself suffice to establish a causal relationship between these effects and benzene exposure.

12. Modes of Action

While a general overview of benzene metabolism has been presented in Section 9, this section presents a review of the evidence for the molecular basis of the action of benzene metabolites. Several reviews of benzene metabolism and the proposed mechanisms of toxicity have been published (Ross, 1996; Snyder, 2000; Snyder et al, 1993; Snyder & Hedli, 1996; Yardley-Jones et al, 1991).

Exposure to benzene can result in haematotoxicity, immunotoxicity and carcinogenicity in humans and animals. Haematotoxicity resulting from chronic benzene exposure can present as anaemia, aplastic anaemia, leukopenia, lymphocytopenia, thrombocytopenia, or pancytopenia (Aksoy, 1989). The principal carcinogenic response in humans to chronic benzene exposure is leukaemia while other animals tend to produce solid tumours in specific organs. While the liver is the initial site for the biotransformation of benzene, hepatotoxicity is not a consequence of benzene exposure. However, a number of studies have shown that for benzene to produce haematotoxicity in animals it must first be metabolised by the liver (Andrews et al, 1977; Sammett et al, 1979). Subsequent accumulation of the major hepatic metabolites, phenol, hydroquinone and catechol, occurs in the bone marrow where they are known to persist for varying durations after exposure to benzene ceases (Rickert et al, 1979). Longacre et al. (1981) observed that strains of mice that exhibit greater sensitivity towards benzene accumulate more benzene metabolites (water-soluble and covalently bound) in bone marrow compared to less sensitive strains. However, administration of specific benzene metabolites to test animals has failed to reproduce the characteristic toxicity of benzene although co-administration of phenol and hydroquinone has been shown to mimic its haematotoxic effects (Eastmond et al, 1987). These data suggest that phenolic metabolites of benzene in combination and not the parent molecule are responsible for the haematotoxicity of benzene. The data further suggest that subsequent biotransformation of the hepatic metabolites to reactive intermediates is required and that this occurs within the bone marrow and those animal organs exhibiting solid tumours.

12.1 Activation of benzene metabolites

In order for the phenolic metabolites of benzene to exert their toxic effect on bone marrow, they must undergo activation to their oxidised forms. Once activated, they can participate in covalent binding reactions with macromolecules. Several studies have identified the presence of peroxidase enzymes in bone marrow and other tissues as the primary mechanism by which activation of benzene phenolic metabolites is achieved (Eastmond et al, 1986; Lévay et al, 1993). The peroxidases are a diverse class of enzymes that catalyse the general reaction:

$$\text{Donor} + H_2O_2 \rightarrow \text{oxidised donor} + 2H_2O.$$

While peroxidases generally act to detoxify peroxides, including hydrogen peroxide, that form within cells as a result of several metabolic reactions, a number of specialised peroxidases with other functions have evolved. In particular, the leukocytes of several species, including humans, have been shown to possess large amounts of a specific form, myeloperoxidase (Bainton et al, 1971; Himmelhoch et al, 1969). In concert with the leukocyte nicotinamide adenine dinucleotide

phosphate (NADPH) oxidase system that causes hydrogen peroxide to be produced (Patriarca et al, 1971), myeloperoxidase plays a crucial role in the host defence system by producing a potent microbicidal oxidant that protects the host from microorganisms. Immature leukocytes in the bone marrow generally have higher levels of myeloperoxidase than circulating mature cells (Bainton et al, 1971). Consequently, bone marrow has considerable capacity to metabolise suitable electron donors including the benzene metabolites, phenol, hydroquinone, catechol and 1,2,4-trihydroxybenzene, to reactive species.

12.1.1 Activation of phenol

Incubation of phenol with human leukocyte lysates, which contain myeloperoxidase, have demonstrated the formation of reactive intermediates that covalently bind to macromolecules in the presence of hydrogen peroxide as a co-oxidant (Eastmond et al, 1986; Smith et al, 1989). Eastmond et al. (1986) concluded that although 4,4'-biphenol and diphenoquinone were identifiable reaction products derived from the oxidation of phenol, only 6% of the covalent binding could be attributed to diphenoquinone with most of the covalent binding observed due to other reactive species, possibly the phenoxy radical or oxidation products of 2,2'-biphenol or 4,4'-biphenol. However, in the presence of hydroquinone, phenol appears to undergo a recycling process such that the initial phenoxy radical is reduced to phenol by transferring an electron to hydroquinone (Smith et al, 1989), thus limiting the formation of biphenol derivatives.

12.1.2 Activation of hydroquinone and catechol

Under physiological conditions, hydroquinone, catechol and 1,2,4-trihydroxybenzene can undergo autoxidation to their respective semiquinone and quinone forms (Brunmark & Cadenas, 1989) or their oxidation can be facilitated by the presence of a peroxidase and hydrogen peroxide (Sadler et al, 1988; Schlosser et al, 1989; Smith et al, 1989). Quinones are chemically reactive species capable of depleting intracellular glutathione, promoting lipid peroxidation and forming covalent adducts with macromolecules (Bolton et al, 2000; Irons, 1985; Monks et al, 1992). Several studies have shown that hydroquinone and catechol are readily oxidised by human myeloperoxidase (Eastmond et al, 1986; Sadler, et al, 1988) and it has been observed that the oxidation of hydroquinone to benzoquinone by peroxidase enzymes is enhanced by the presence of excess phenol which acts as a co-oxidant obviating the need for hydrogen peroxide to drive the reaction (Smith et al, 1989; Subrahmanyam, et al, 1990). Hydroquinone was found to be metabolised by activated human neutrophils to covalent-binding species and the amount of binding could be increased by approximately 70% by the addition of phenol (Eastmond et al, 1987). Subrahmanyam et al. (1990) reported that the presence of phenol enhanced the covalent binding of [^{3}H]-hydroquinone metabolites to macromolecules of mouse blood and bone marrow, but not to the kidneys or liver. It was further noted that hydroquinone enhanced binding of [^{3}H]-phenol metabolites in blood, bone marrow and the kidneys but inhibited binding in the liver. In contrast, catechol did not enhance [^{3}H]-hydroquinone metabolite binding.

Sadler et al. (1988) observed that the oxidation of catechol by human neutrophil peroxidases (myeloperoxidase) resulted in the formation of 1,2-benzosemiquinone and 1,2-benzoquinone. Bhat et al. (1988) found that the addition of [^{14}C]-catechol to rat or human bone marrow cells resulted in the formation of a glutathione-conjugate and covalent binding of radiolabel to protein. Both conjugate formation and the binding of radiolabel were substantially increased by the presence of

hydrogen peroxide or phenol, however, protein binding could be markedly decreased by the presence of exogenous glutathione (GSH) or hydroquinone.

12.1.3 Role of cyclooxygenase

In addition to activation by peroxidases, phenol and hydroquinones can be activated by prostaglandin H synthase (cyclooxygenase), an enzyme with oxygenase and endoperoxidase activity (Markey et al, 1987; Schlosser et al, 1989). Acting as an endoperoxidase, the enzyme requires an oxidant as a co-substrate which phenol or hydroquinones can replace (Markey et al, 1987). Prostaglandin H synthase is present in a number of bone marrow-derived cells including monocyte/macrophage populations and platelets and converts arachidonic acid to several prostaglandins including prostaglandin E_2 (PGE_2). PGE_2 plays a major role in the inhibition of progenitor cell proliferation and differentiation (Gentile and Pelus, 1987). In vitro, both phenol and hydroquinone are activated by cell lysates containing prostaglandin H synthase or by the purified enzyme and in the presence of arachidonic acid or hydrogen peroxide (Schlosser et al, 1989).

The role of prostaglandin H synthase activity has been demonstrated in a number of mouse strains ($B6C3F_1$, DBA/2 and C57BL/6) where it was shown that bone marrow toxicity could be reduced by prior treatment of the animals with non-steroidal anti-inflammatory drugs (aspirin, indomethacin or meclofenamate) that inhibit prostaglandin H synthase activity (Gaido and Wierda, 1987; Kalf et al, 1989). Pirozzi et al. (1989) further demonstrated that benzene-induced bone marrow depression and micronucleus formation in erythrocytes of C57B1/6 mice could be prevented by the co-administration of indomethacin and that protection was achieved at doses that did not inhibit cytochrome P450 or myeloperoxidase activity.

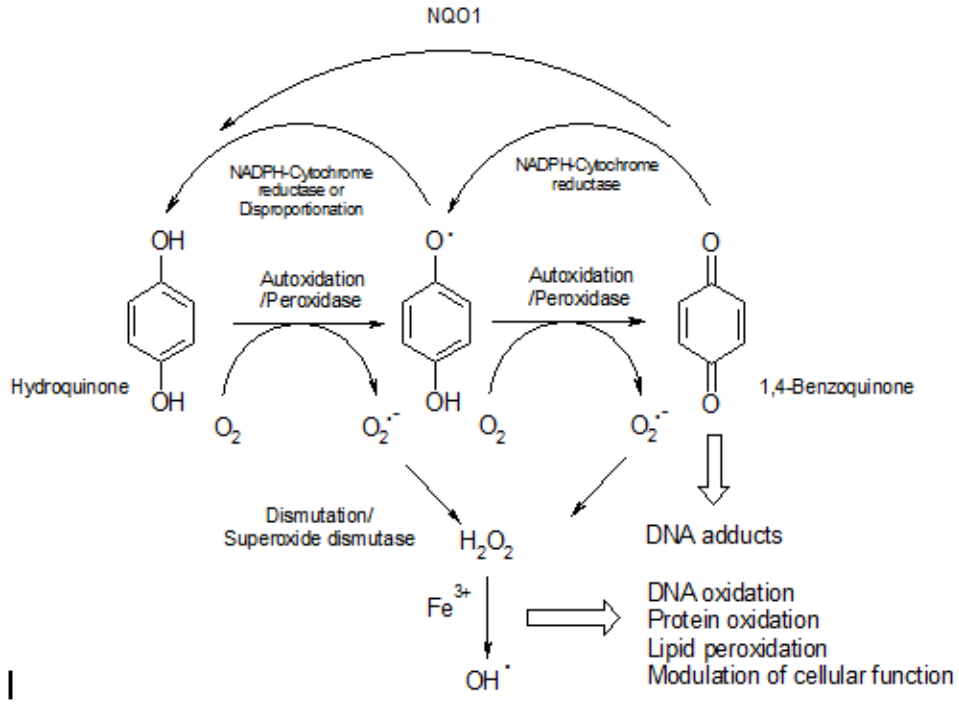
The bio-activation of catechol by prostaglandin H synthase activity in rat bone marrow appears to be limited as the addition of arachidonic acid provided only a small but significant ($p<0.05$) increase in covalent binding which was of limited duration (Bhat et al, 1988).

12.1.4 Formation of reactive oxygen species

In addition to the formation of semiquinone and quinone species, the oxidation of hydroquinones results in the formation of reactive oxygen species. Initially, molecular oxygen is reduced to superoxide anion which, by dismutation, is converted to hydrogen peroxide (Figure 12.1). In the presence of transition metal ions (for example, iron) the very reactive hydroxyl radical can form. These reactive species can promote the oxidation of protein and DNA bases, induction of chromosomal aberrations, lipid peroxidation and the modulation of cellular functions.

While hydroquinone and its semiquinone radical can both reduce molecular oxygen to superoxide, Sadler et al. (1988) found superoxide production by catechol to be limited to the first one electron reduction step to 1,2-benzosemiquinone with molecular oxygen being unable to effect the subsequent oxidation of the semiquinone to the quinone form. In contrast, 1,2,4-benzenetriol undergoes rapid autoxidation to yield hydrogen peroxide (Brunmark & Cadenas, 1988).

Figure 12.1. Redox cycle of hydroquinone with formation of reactive oxygen species and biological effects



The detoxification of quinones can be achieved by a two-electron reduction to their fully reduced forms. Two enzymes involved in the reduction of quinones are NADPH-cytochrome reductase and NAD(P)H:quinone oxidoreductase (NQO1; DT-diaphorase; Lind et al, 1982). In the case of NADPH-cytochrome reductase, reduction of the quinone to a semiquinone is achieved by a one-electron transfer to give a semiquinone and a second electron is transferred to molecular oxygen to yield the superoxide anion. The resulting semiquinone is then free to autoxidise to the quinone producing more superoxide. However, it has been proposed that redox cycling of the semiquinone could not be maintained by NADPH-cytochrome reductase at physiological pH due to protonation of the semiquinone thus minimising superoxide production (Boersma et al, 1994). In the case of NQO1, reduction to the hydroquinone is achieved by a simultaneous two-electron transfer to the quinone with no reduction of molecular oxygen. The redox cycle for hydroquinone, the role of NADPH-cytochrome reductase and NQO1 and the biological effects of these processes are illustrated in Figure 12.1.

In cells that possess both peroxidase and NQO1 activities, the ratio of the two enzymes may determine the extent to which reactive metabolites form. Thus a high intracellular myeloperoxidase/NQO1 ratio, such as occurs in human stroma and $CD34^+$ bone marrow progenitor cells, may result in a greater risk of benzene-induced cellular toxicity (Ross et al, 1996b). Although characterisation of NQO1 activity in primary cultures of mouse bone marrow stromal cells was found to be low, the enzyme was shown to be inducible and induction of the enzyme conferred protection against hydroquinone-induced toxicity (Twerdok et al, 1992).

Conjugation reactions, for example with GSH, can enhance the ability of hydroquinones to autoxidise. Glutathionyl hydroquinone, identified as a urinary benzene metabolite (Nerland & Pierce, 1990), was found by Brunmark & Cadenas (1988) to autoxidise at a rate 8-fold faster than hydroquinone. Rao (1996)

concluded that, in vitro, glutathionyl hydroquinone acted as a potent pro-oxidant based on its ability to degrade DNA.

Reactive oxygen species may also form due to the reduction of molecular oxygen by the action of cytochromes P450. Johannson & Ingelman-Sundberg (1983) observed that benzene could be directly oxidized to phenol by hydroxyl radicals derived from the reduction of molecular oxygen by microsomal cytochrome P450 activity or reconstituted enzyme systems. Similarly, Kahn et al. (1990) detected the presence of hydroxyl radicals during the NADPH-dependent metabolism of benzene by rat bone marrow microsomal preparations.

12.2 Reactivity of benzene metabolites

Results derived from in vivo and in vitro studies indicate that a number of mechanisms contribute to the cytotoxicity, genotoxicity and carcinogenicity of benzene metabolites. Cytotoxicity can arise due to depletion of intracellular GSH and changes in intracellular redox status (Ludewig et al, 1989; Rao & Snyder, 1995; Witz, 1985) and covalent binding of benzene metabolites to macromolecules (Latriano et al, 1989; Lutz and Schlatter, 1977; Mazzullo et al, 1989; Snyder et al, 1987). Metabolites suspected of contributing to the genotoxicity and carcinogenicity of benzene include benzene oxide, hydroquinone, catechol, 1,2,4-trihydroxybenzene and *trans*,*trans*-muconaldehyde. The effects induced by these metabolites comprise DNA base alterations, chromosome structural aberrations and aneuploidy. However, the metabolite concentrations at which many of these effects have been shown to occur in vitro are higher than those expected to occur in vivo.

12.2.1 Genotoxicity

Several studies have demonstrated the formation of DNA adducts after incubation of benzene metabolites with purified DNA. Hydroquinone when incubated with calf thymus DNA resulted in the formation of deoxycytidine (Pongracz et al, 1990), deoxyadenosine (Pongracz & Bodell, 1991) and deoxyguanosine adducts (Jowa et al, 1990). Gut et al. (1996) were able to demonstrate the formation of the N-7 guanine adduct on exposure of calf thymus DNA to benzene oxide under in vitro conditions while Latriano et al. (1989) found *trans,trans*-muconaldehyde to form adducts with deoxyguanosine. In addition to nuclear DNA, in vitro studies have shown mitochondrial DNA, derived from bone marrow mitoplasts, to undergo alkylation by benzene metabolites (Kalf et al, 1985; Snyder et al, 1987).

Under cellular conditions, a comparison of the ability of benzene metabolites to induce DNA adduct formation in HL-60 cells, a promyelocytic leukemia cell line, found hydroquinone to be 7-9 times more effective at inducing such adducts than catechol or 1,2,4-trihydroxybenzene and that a correlation existed between adduct formation and cytotoxicity. Co-incubation of hydroquinone with either catechol or 1,2,4-trihydroxybenzene produced a synergistic effect that was 3-6 times greater than the added effects of each metabolite. It was further observed that DNA adducts form in the presence of benzene metabolite mixtures which are not observed when cells are incubated with the individual metabolites, leading the authors to suggest that other processes leading to adduct formation may be involved (Lévay & Bodell, 1992; Lévay et al, 1991). Chenna et al. (1995) subsequently identified an enzyme with glycosylase activity that excises deoxycytidine and deoxyadenosine adducts of benzoquinone from DNA.

It is noteworthy that transfected HL-60 cells expressing a high level of NQO1 activity exhibited lower levels of DNA adduct formation when exposed to hydroquinone compared to non-transfected HL60 cells which are deficient in NQO1 activity. Similarly, C15 cells, a myeloperoxidase-deficient HL-60 subline, produced lower levels of DNA adducts with hydroquinone compared to HL-60 cells which normally express high levels of myeloperoxidase (Wiemels et al, 1999).

The ability of hydroquinone species to induce mutations has been demonstrated by Joseph et al. (1998). In a series of in vitro experiments, it was demonstrated that sequence-specific frame shift mutations could be caused by hydroquinone, but not semiquinone, benzoquinone or reactive oxygen species, in the supF tRNA gene. It was further demonstrated that BALBc/3T3 cells undergo transformation by hydroquinone (15 μM) and that the frequency of transformation could be increased by a tumour promoter. Such initiated cells produced tumours with 100% frequency when injected into severe combined immunodeficient (SCID) mice. Sakai et al. (1995) previously had shown that benzoquinone caused initiation in a two-stage model of carcinogenesis using BALB/3T3 cells.

Mueller et al. (1987) detected the alkylation product of benzene oxide, N-7-phenylguanine, in the urine of rats exposed to benzene (500 ppm) for 8 h while Norpoth et al. (1996) detected several benzene-derived urinary guanine adducts following the administration of benzene to rats. However, it should be noted that the presence of N-7-phenylguanine in the urine does not provide sufficient evidence that the adduct is derived from DNA excision-repair activities. The presence of benzene-induced DNA adducts has been detected in the tissues of rats dosed with [^{14}C]-benzene (Lutz and Schlatter, 1977; Mazzullo et al, 1989) although the nature of the adducts was not investigated. However, Reddy et al. (1989b) found only equivocal evidence for the in vivo formation of aromatic DNA adducts in the bone marrow, liver, kidney and mammary gland of benzene-treated female Sprague-Dawley rats. DNA isolated from Zymbal glands was found to contain 4 adducts per 10^9 DNA nucleotides, although the adducts did not correspond to major adducts described in in vitro studies. Thus it was concluded that DNA-quinone adduct formation in the rat is not extensive, possibly due to the efficient elimination of quinones by other mechanisms. In addition to forming DNA adducts in the bone marrow of experimental animals, benzene exposure produced DNA adducts in the livers of male mice (Lutz and Schlatter, 1977); however, liver tumours were not observed in 2-year carcinogenicity studies of these animals (NTP, 1986).

The mutation frequency of V75 Chinese hamster cells increased in a dose-dependent manner after treatment for 1 h with benzoquinone, an effect that was found to be independent of intracellular GSH status and observed at low (< 10 μM) concentrations. The frequency of micronucleated cells was also increased by benzoquinone but only at concentrations greater than 20 μM. In contrast, benzoquinone did not induce sister chromatid exchanges at any concentration up to 100 μM (Ludewig et al, 1989).

As described in Sections 10.6 and 11.5, several studies have demonstrated chromosomal aberrations in experimental animals and humans following exposure to benzene. While the administration of benzene to CD-1 mice resulted in micronuclei formation, treatment with either phenol, hydroquinone or catechol failed to induce micronuclei (Gad-el-Karim et al, 1985). Barale et al. (1990) demonstrated, in vivo, a synergistic effect on micronuclei formation in CD-1 mice bone marrow cells by the concurrent administration of hydroquinone and phenol.

Lewis et al. (1988) reported that hydroquinone, under in vitro conditions, caused DNA to form single- and double-strand breaks by a mechanism that was independent of reactive oxygen species. In contrast, catechol did not induce DNA damage. These investigators further observed that DNA could be degraded by 1,2,4-trihydroxybenzene, an effect inhibited by scavengers of reactive oxygen species. When tested together in vitro, hydroquinone and catechol produced a synergistic effect on micronuclei formation in human lymphocytes, possibly by interfering with mitotic spindle function and disturbing chromosome segregation (Robertson et al, 1991). Benzoquinone has also been reported to interfere with microtubule assembly by blocking a thiol-sensitive binding site (Irons et al, 1981).

12.2.2 Oxidative stress

The formation of reactive oxygen species is a normal part of cellular biochemistry and is considered to be an important component of intracellular signalling processes, including mediating signal transduction within haematopoietic cells initiated by growth factor signals (Sattler et al, 1999). However, exposure of biological systems to excessive levels of reactive oxygen species results in the induction of oxidative stress. Oxidative stress can induce oxidative modification of DNA bases and chromosomal abnormalities, depletion of intracellular GSH, changes in intracellular redox status, peroxidation of lipids, oxidation of proteins and modulation of cellular functions. The role of oxidative stress in benzene-mediated toxicity has been extensively reviewed by Subrahmanyam et al. (1991).

Rao & Snyder (1995) examined the effects of hydroquinone, benzoquinone and 1,2,4-trihydroxybenzene (50 μM) on several parameters of antioxidant defence function of HL-60 cells. The three metabolites did not induce the cells to generate superoxide anion or nitric oxide but did produce detectable levels of hydrogen peroxide. Intracellular GSH levels were depleted by hydroquinone and 1,2,4-trihydroxybenzene but not benzoquinone.

The presence of lipid peroxidation products was found to increase in rat tissues following the administration of benzene (Khan et al, 1984) while urinary levels of malondialdehyde, a biomarker of lipid peroxidation, were elevated in rats receiving hydroquinone (Ekström et al, 1988). The presence of intracellular peroxidation products has been detected in HL60 cells following treatment with either 1 μM benzoquinone or 10 μM hydroquinone (Hiraku & Kawanishi, 1996). Several studies have identified the presence of 8-hydroxydeoxyguanosine (8-OHdG) as a sensitive biomarker of DNA damage due to oxidative stress (Kasai & Nishimura, 1984, 1986; Shigenaga et al, 1989). In two occupational studies in workers known to have benzene exposure, a dose-response relationship was demonstrated between the exposure level and urinary 8-OHdG levels (Lagorio et al, 1994b, Nilsson et al, 1996). However, the presence of 8-OHdG in the urine is not conclusive evidence of DNA excision-repair activities in response to oxidative modification of DNA.

Benzoquinone species and *trans*,*trans*-muconaldehyde readily react with GSH (Brunmark & Cadenas, 1988; Rao et al, 1982) which can lead to depletion of intracellular GSH levels and changes in intracellular redox status. Treatment of V79 Chinese hamster cells with benzoquinone for 1 h resulted in decreased GSH, NADPH and nicotinamide adenine dinucleotide levels but only at cytotoxic concentrations at or above 100 μM (Ludewig et al, 1989). Ekström et al. (1988) found rat hepatic GSH levels to be depleted after administration of hydroquinone by gavage while the administration of *trans*,*trans*-muconaldehyde to mice for 10 or 16 days resulted in decreased hepatic sulfhydryl levels (Witz et al, 1985).

The growth of HL-60 cells was found to be stimulated by the presence of hydroquinone within the range of 10-40 μM. Similarly, the incorporation of [^{3}H]-thymidine was enhanced by hydroquinone, benzoquinone or 1,2,4-benzenetriol. However, the effects produced by the three metabolites could be eliminated if the cells were pre-incubated with catalase, an antioxidant specific for hydrogen peroxide. The effects of hydroquinone and benzoquinone could be mimicked by reactive oxygen species produced by a xanthine/xanthine oxidase system. It was further observed that while hydroquinone or benzoquinone did not reduce the cell cycle time they did increase the number of cells entering into S-phase from G_0/G_1 phase (Wiemels & Smith, 1999). The role of reactive oxygen species, and in particular hydrogen peroxide, has been further explored by Sattler et al. (1999). These investigators found haemopoietic growth factors to induce increased levels of reactive oxygen species in MO7e cells, a growth factor-dependent human megakaryocytic cell line. Treatment of these cells with either growth factors or hydrogen peroxide resulted in increased tyrosine phosphorylation of cellular proteins, a key step in intracellular signalling processes.

12.2.3 Modulation of cellular function

The mature macrophage produces interleukin-1 (IL-1), a cytokine essential for stem cell maturation. However, it has been observed that macrophages treated with hydroquinone (10 μM) produce less IL-1 than control cells (Thomas et al, 1989). This is due to the inhibition of calpain, a protease required for the conversion of pre-IL-1 to its active form, by hydroquinone (Renz & Kalf, 1991; Kalf et al, 1996). Treatment of isolated mouse bone marrow-derived macrophages with non-cytotoxic doses of hydroquinone (10 μM) resulted in a 10 to 30% reduction in total calpain activity (Miller et al, 1994). In contrast, the production of PGE_2 by bone marrow cells, in vivo, was enhanced by benzene exposure (Gaido & Wierda, 1987; Kalf et al, 1989) although it is uncertain how the release of arachidonic acid, the precursor of prostaglandins, from phospholipid stores is initiated under these conditions. However, hydroquinone and catechol have been shown to regulate protein kinase C (PKC) activity by producing a short term cytosol-to-membrane translocation of PKC (Gopalakrishna et al, 1994), a key step in the mobilisation of arachidonic acid. Da Silva et al. (1989) have further shown that benzene can directly activate PKC. PGE_2 has been identified as an inhibitor of granulocyte/macrophage progenitor cell proliferation (Gentile & Pelus, 1987).

The growth of granulocyte/macrophage colonies was stimulated in a synergistic manner by co-treatment of mouse bone marrow cells with low concentrations of hydroquinone (10^{-8} to 10^{-5} M) and recombinant granulocyte/macrophage colony stimulating factor (GM-CSF) compared to cells treated with GM-CSF alone. The maximal response was achieved with 1 μM hydroquinone but no effect was observed when phenol, catechol or *trans*,*trans*-muconaldehyde were substituted for hydroquinone (Irons et al, 1992). While treatment of HL-60 cells with hydroquinone at concentrations between 10-40 μM resulted in an increase in cell proliferation, at a concentration greater than 50 μM hydroquinone caused a decrease in cell viability (Wiemels & Smith, 1999). In a further study, Wiemels et al. (1999) demonstrated that 12 h after treatment with hydroquinone (50 μM) approximately 40% of HL-60 cells were apoptotic as determined by the terminal deoxynucleotidyl-transferase (TdT) assay. Apoptosis is a form of physiological cell death characterised by altered cell morphology including condensation of the cytoplasmic and nuclear compartments and internucleosomal DNA fragmentation. Apoptosis has been observed to occur in a dose-dependent manner when HL-60

cells are treated with hydroquinone and catechol (25 to 100 μM) but not by phenol. Similarly, hydroquinone or catechol induces $CD34^+$ human bone marrow progenitor cells to undergo apoptosis (Moran et al, 1996). These reports support an earlier study in which weak internucleosomal cleavage was observed in HL-60 cells following incubation for 4 h with 20 μM hydroquinone and 5 μM benzoquinone and pronounced cleavage observed at 50 μM hydroquinone and 10 μM benzoquinone using pulse-field gel electrophoresis (Hiraku and Kawanishi, 1996).

12.3 Critical biological effects

12.3.1 Bone marrow toxicity

The critical biological effect of benzene in all experimental species is bone marrow toxicity characterised by a reduction in bone marrow cellularity. Bone marrow consists of stromal cells (composed of macrophage and fibroblastoid cell populations) along with stem and progenitor cell populations that form a complex matrix within which are produced a number of essential regulatory growth factors. Stromal cells regulate stem and progenitor cell proliferation, differentiation and maturation by producing both inducers (colony stimulating factors (CSFs) and interleukins, particularly IL-1) and inhibitors (PGE_2) of cell growth. PGE_2 inhibits cell growth by suppressing the production of CSFs and IL-1. Benzene metabolites appear to disrupt the balance of these regulatory factors by inhibiting production of CSFs and IL-1 and increasing PGE_2 production, although low levels of hydroquinone can replace or augment, in vitro, the effects of growth factors (Wiemels & Smith, 1999). Evidence to support this hypothesis has been provided by experiments in which the co-administration of IL-1 abrogates the effects of benzene treatment. Similarly, if non-steroidal anti-inflammatory agents, which inhibit PGE_2 production and the cyclooxygenase-dependent oxidation of phenol and hydroquinone, are co-administered with benzene, haematotoxicity is not observed. Although increased apoptosis has been observed by exposing bone marrow cells to various benzene metabolites, these effects appear to occur only at high metabolite concentrations.

12.3.2 Leukaemia

Leukaemia is the progressive proliferation of abnormal and usually monoclonal leukocytes in hemopoietic tissues. Benzene-induced leukaemias are typically myelogenous in nature rather than lymphocytic. Currently, the mechanism(s) by which benzene induces leukaemia in susceptible individuals remains obscure. Clinical studies of therapy-related myelodysplastic syndromes and acute myeloid leukaemia have shown an increase in chromosomal aberrations particularly aneuploidy, long-arm deletions and translocations involving chromosomes 5, 7 and 8 (Pedersen-Bjergaard et al, 1995). Individuals with chronic exposure to benzene tend to exhibit similar changes in chromosomes 5 and 7 of peripheral blood lymphocytes (Zhang et al, 1998).

Chromosomal aberrations involving chromosomes 5, 7 and 8 of various cell lines, including blood $CD34^+$ progenitor cells, have been reported to occur, in vitro, in response to low-dose hydroquinone exposure (Smith et al, 2000; Stillman et al, 1997). In particular, $CD34^+$ bone marrow cells were observed to lose chromosome 7 accompanied by selective deletion of the long-arm of chromosome 5 (5q31) but no changes in chromosome 8 (Stillman et al, 2000). Stillman et al. (1999) have also

reported that while catechol does not alter cellular cytogenetics, a dose-dependent synergistic effect is observed between hydroquinone and catechol. The combination of metabolites induces changes in chromosome 5 not seen with hydroquinone alone, a result analogous to the synergistic effect on micronuclei formation in human lymphocytes described by Robertson et al. (1991).

A study of patients with therapy-related AML identified a close correlation between the use of drugs with DNA-topoisomerase inhibitor activity and aberrations in chromosomes 5, 7 and 8 (Super et al, 1993). Topoisomerases are a class of nuclear proteins (endonucleases) that convert one topological version of DNA into another by catalyzing the breakage and reformation of DNA phosphodiester linkages. They are involved in DNA replication and transcription, DNA repair, chromosome segregation and maintain genomic stability. Due to the sulfhydryl-dependent nature of topoisomerases and the ability of several benzene metabolites to modify sulfhydryl groups, inhibition of topoisomerase activity by benzene metabolites has been proposed as a mechanism for leukaemia formation. Chen & Eastmond (1995) found no evidence for topoisomerase I inhibition by phenol, catechol, hydroquinone, benzoquinone or 1,2,4-benzenetriol at concentrations up to 1000 μM. Similarly, topoisomerase II activity was not inhibited by benzene metabolites at concentrations less than 500 μM with the exception of 1,2,4-benzenetriol which was inhibitory at 250 μM. The activation of phenol by a peroxidase and hydrogen peroxide system resulted in inhibition at 50 μM and the products of this reaction, 2,2'-biphenol and 4,4'-biphenol were found to be inhibitory at 500 μM, whereas the peroxidase activation products of these compounds were inhibitory at 100 and 10 μM respectively. However, Parke and Williams (1953) failed to find any evidence for the in vivo formation of biphenol products after benzene exposure and there is evidence that these metabolites do not readily form in the presence of hydroquinone (Smith et al, 1989). In contrast, Hutt and Kalf (1996) found topoisomerase II activity to be inhibited by hydroquinone or benzoquinone at 6 and 3 μM respectively.

In addition to modulation of topoisomerase activity, benzene metabolites can modify other nuclear proteins including tubulin (Pfeiffer & Metzler, 1996) and produce DNA-protein cross-links (Schoenfeld & Witz, 1999) which may contribute to chromosomal aberrations and the development of leukaemia. It has been postulated that chromosomal aberrations could result in inactivation of tumour suppressor genes, such as p53, activation of proto-oncogenes and altered expression of growth-factor and growth-factor receptor genes on the aberrant chromosomes (Irons and Stillman, 1996; Smith, 1996). Similarly, the formation of apurinic sites due to depurination by N-7 guanine adducts of benzene oxide (Gut et al, 1996) could result in misreplication of DNA and contribute to the development of leukaemia, as could oxidative DNA base lesions due to benzene-induced oxidative stress.

12.3.3 Tumours in Zymbal, Harderian, lacrimal and mammary glands

In addition to haematopoietic abnormalities, rodents exposed to benzene develop solid tumours in the Zymbal, Harderian, lacrimal and mammary glands, although other organs and tissues may also be involved (Huff et al, 1989). The mechanisms by which benzene induces tumours in these glands have not been extensively investigated. Biochemical characterisation has revealed the presence of high levels of peroxidase enzymes which can activate phenolic metabolites of benzene to reactive species capable of modifying DNA and altering cellular functions as described above. Humans lack an anatomical equivalent of the Zymbal gland and

the human Harderian gland is only of rudimentary development and has not been characterised with respect to peroxidase activity.

Studies by Low et al. (1989) indicate that neither benzene nor its metabolites accumulate in the rat Zymbal gland, a sebaceous gland of the external ear duct of rodents. However, examination of Zymbal gland tissue after oral administration of benzene revealed phenol and hydroquinone to constitute 3% and 30% respectively of unconjugated metabolites. Phenyl glucuronide accounted for 35% of conjugated metabolites but phenylsulfate could not be detected. The absence of phenylsulfate was attributed to a lack of sulfotransferase activity in this tissue. In contrast, Osborne et al. (1980) found the Zymbal gland to exhibit a high level of peroxidase activity indicating that activation of phenolic benzene metabolites could occur in this organ. Reddy et al. (1989a) subsequently identified DNA adducts in excised Zymbal glands after incubation with benzene or its metabolites. The combination of low sulfotransferase and high peroxidase activity would appear to be conducive to the formation of reactive metabolites in the Zymbal gland, thus facilitating tumour formation.

Both lacrimal glands and the accessory lacrimal glands, the Harderian glands, develop tumours in response to benzene exposure. Biochemical characterisation of these glands has demonstrated the presence of high constitutive levels of lactoperoxidase (Morrison & Allen, 1966), which can activate phenolic metabolites of benzene to reactive species in the same manner as myeloperoxidase.

Mammary gland tumours have been observed in rodents in response to benzene (Huff et al, 1989) and limited epidemiological evidence suggests an association between exposure to benzene or benzene-containing products and mammary tumours in humans (Hansen, 2000; Petralia et al, 1999; see Section 11.6.2). The mechanism for the formation of these mammary tumours is uncertain. Reddy et al. (1989b) failed to detect DNA adducts associated with the mammary gland of female rats after 10 weeks of benzene exposure, suggesting an epigenetic mechanism may be involved. However, as stated in Section 9, Low et al. (1989) found the distribution of radiolabel in female rats (Sprague-Dawley) to vary depending on the dose of [^{14}C]-benzene administered. When comparing doses of benzene at 0.15, 1.5 and 15 mg/kg bw, the highest dose resulted in a substantial increase in the amount of radiolabel associated with the mammary gland and bone marrow compared to other tissues at the lesser doses. Mammary tissue is richly perfused with blood and has a high fat content which allows for the accumulation of benzene metabolites. It also contains lactoperoxidase which has been shown to metabolise phenolic compounds to reactive species (Monzani et al, 1997). Consequently, benzene metabolites may become activated within mammary tissue resulting in altered cellular function and carcinogenesis.

12.4 Interindividual variations in susceptibility

12.4.1 Gender effects

While several studies have reported gender-dependent differences in the metabolism and/or toxicity of benzene in mice, there are no reliable data to indicate that there are gender differences in humans with respect to either the metabolism of benzene or susceptibility to benzene toxicity.

Male Swiss (CD-1) mice exposed to benzene exhibited more severe benzene-related toxicity, including genotoxic effects, than females (Meyne and Legator,

1980; Ward et al, 1985). Similarly, suppression of bone marrow cellularity in male DBA/2 mice was greater than females after exposure to benzene (Luke et al, 1988a). Corti and Snyder (1996) found, using Swiss Webster mice exposed to benzene (10 ppm) for 6 h over 10 days, that the number of erythoid colony forming units (CFU-E) had decreased in the bone marrow of adult-exposed males, *in utero*-exposed males and foetal male livers compared to female adults and foetuses. It was further shown by Corti and Snyder (1998), in vitro, that isolated CFU-E derived from male mice were more susceptible to individual benzene metabolites then female isolates.

A marked gender-related difference was observed in the hepatic glutathione-S-transferase (GST) activity of CD-1 mice with the π isoform exhibiting approximately 25% greater activity towards *trans*,*trans*-muconaldehyde in the females compared to males (Goon et al, 1993). Investigations of gender-related differences in benzene metabolism by mouse bone marrow have not, as yet, been undertaken. Hu et al. (1993) observed that microsomes prepared from the kidneys (but not livers) of male mice (C3H/HeJ) possessed CYP2E1 activity up to 50-fold higher for acetaminophen metabolism compared to female mice. It was further observed that the administration of testosterone to female mice increased the CYP2E1 activity in the kidneys of females. Supporting evidence for the role of metabolic gender-differences was provided by Kenyon et al. (1995) who found that male mice ($B6C3F_1$) excreted more hydroquinone glucuronide when dosed with phenol compared to female mice. In a subsequent study, Kenyon et al. (1998) found bone marrow levels of phenol and hydroquinone to be higher in male mice ($B6C3F_1$) compared to female mice after exposure to benzene.

Attempts to demonstrate gender differences in benzene metabolism in humans by comparing urinary metabolites (*trans*,*trans*-muconic acid and phenylmercapturic acid) to benzene exposure levels have produced negative results (Inoue et al, 1989; Inoue, 2000).

12.4.2 Genetic polymorphisms

It has been observed that different strains of male mice (DBA/2, C57B1/B6 and $B6C3F_1$) exhibit differing sensitivities to benzene when exposed under identical conditions (Luke et al, 1988b; Pirozzi et al, 1989) and that the metabolic profile of urinary benzene metabolites is strain-dependent (Longacre et al, 1981). These data suggest that individual responses to benzene may be genetically determined. Johnson & Lucier (1992) concluded from an analysis of *trans,trans*-muconic acid biomarker assays in humans that genetic variability may account, in part, for the variance between benzene exposure and urinary *trans,trans*-muconic acid concentrations. Subsequently, it has been postulated that the presence of genetically determined differences in enzyme expression or activity, genetic polymorphisms, may partially account for the toxicity associated with benzene exposure (Aksoy, 1985; Moran et al, 1999; Rothman et al, 1997). Studies of cases involving familial susceptibility to benzene (Aksoy, 1985) tend to support this view. Genetic differences involved in the metabolism of benzene can modify its rate of metabolism, the profile of metabolites produced and metabolite activation/detoxification pathways. Such changes have been quantified by analysis of the urinary metabolites of benzene, phenylmercapturic acid and *trans*,*trans*-muconic acid (Rossi et al, 1999).

CYP2E1

The metabolism of benzene by hepatic CYP2E1 is the critical first step in the development of benzene toxicity as the enzyme is responsible for the formation of phenol and its secondary metabolism to hydroquinone (Guengerich et al, 1991). Genetic polymorphisms associated with CYP2E1 from different racial groups have been identified, with frequencies ranging from 2-27% (Kato et al, 1992) and changes in transcriptional activity of the enzyme in response to mutations have been described (Hayashi et al, 1991). Seaton et al. (1994) observed that the CYP2E1 activity of microsomes prepared from the livers of trauma victims varied 13-fold with respect to benzene metabolism. However, as DNA analysis was not undertaken, it is not known if the differences were genetically determined. Rothman et al. (1997) showed in a study of 50 workers exposed to benzene that CYP2E1 genetic polymorphisms were not associated with benzene toxicity. In another study of 59 workers exposed to benzene, although considerable variation in urinary metabolite markers was observed, the subjects did not exhibit polymorphisms associated with CYP2E1 (Rossi et al, 1999).

A cross-species analysis of the expression of CYP2E1 in the bone marrow of mice, rats and rabbits and human $CD34^+$ stem cells found the enzyme to be present in all species tested. While the intra- and interspecies variability between mice and rats was small with relatively low enzyme activities, rabbits exhibited enzyme activities an order of magnitude greater (Bernauer et al, 2000).

Glutathione-S-transferase

The enzymatic conjugation of GSH to a number of benzene metabolites, particularly benzene oxide, *trans*,*trans*-muconaldehyde and quinones, occurs via the action of glutathione-S-transferase (GST) (Goon et al, 1993b; Jerina et al, 1968). It has been postulated that GST genetic polymorphisms are positively correlated with increased risk of oxidative stress (Hayes & Strange, 1995) and cancer (Strange et al, 1998). Xu et al. (1998b) found a significant association ($p < 0.05$) between benzene exposure (0.71 ppm TWA), sister chromatid exchanges and the GSTT1 genotype in a study of 23 workers. Hsieh et al. (1999) examined the role of GST polymorphism in workers exposed to benzene and found that those with the GSTT1 and GSTM1 variants of the enzyme, which exhibit reduced enzymatic activity, were more likely ($p = 0.046$) to have reduced white blood cell counts on exposure to high levels of benzene.

Epoxide hydrolase

Epoxide hydrolase, which converts benzene oxide to benzene dihydrodiol, has the potential to regulate the formation of *trans*,*trans*-muconaldehyde. Analysis of 40 transplant-quality human liver samples for interindividual variation in epoxide hydrolase activity revealed an approximately 8-fold difference in enzymatic activity and microsomal epoxide hydrolase protein levels were highly correlated with that activity. In contrast, neither enzymatic activity nor microsomal epoxide hydrolase protein levels correlated with microsomal epoxide hydrolase RNA levels which varied by 49-fold. Polymorphisms in amino acid loci of epoxide hydrolase accounted, in part, for the differences in enzyme activity (Hassett et al, 1997).

NAD(P)H:quinone oxidoreductase (NQO1)

NQO1 catalyzes the two-electron reduction of quinones to their corresponding hydroquinone form (Lind et al, 1982). Twerdok et al. (1992) reported considerable

strain differences in the basal and inducible levels of NQO1 between C57B1/6 and DBA/2-derived mouse bone marrow stromal cells. The basal and maximal inducible activity of NQO1 in C57B1/6-derived stromal cells was approximately 3- and 5-fold greater respectively than that of DBA/2-derived cells.

Traver et al. (1992) identified a point mutation in the human NQO1 gene (^{609}C→T) that results in loss of enzymatic activity in the protein. Thus individuals homozygous for the mutation possess no NQO1 activity, while heterozygous individuals exhibit reduced enzymatic activity. It has been estimated, using a reference population, that the frequency of the mutation is 13% (Rosvold et al, 1995). Analysis of several ethnic groups has shown that homozygous individuals range between 5-22% and heterozygous individuals from 34-52% of the population (Kelsey et al, 1997). The study of Rothman et al. (1997) demonstrated a correlation between NQO1 genetic polymorphism and benzene toxicity among 50 workers exposed to benzene. Rossi et al. (1999) identified a high frequency of NQO1 genetic polymorphism (42.7% reduced activity and 8.3% no activity) amongst 59 workers exposed to benzene and urinary excretion of S-phenylmercapturic acid was significantly lower in individuals lacking NQO1 activity. An increased prevalence of the ^{609}C→T mutation has been found in a study of 104 patients diagnosed with myeloid leukemias (Larson et al, 1999). However, a study of a group of six related individuals predisposed to cancer showed that the NQO1 ^{609}C→T transversion did not correlate with NQO1 activity in heterozygous individuals, suggesting that either the ^{609}C→T transversion has no effect on NQO1 activity or that post-transcriptional regulation alters the activity of the modified enzyme (Kuehl et al, 1995).

Further investigations, in vitro, have found NQO1 to be inducible in wild-type (C/C) human bone marrow cells on exposure to the benzene metabolites hydroquinone and catechol. In contrast, cells homozygous for the ^{609}C→T mutation (T/T) did not express NQO1 in response to hydroquinone treatment whereas heterozygous cells (C/T) exhibited intermediate induction (Moran et al, 1999).

12.4.3 Environmental influences

In addition to genetic influences, the susceptibility of an individual to benzene toxicity may also be influenced by environmental or lifestyle factors. Generally, the role of environmental factors in modifying benzene toxicity have not been adequately studied. Reviews of environmental influences on solvent toxicity, including benzene, have been published (Medinsky et al, 1994; Sato, 1991).

Alcohol

As discussed in Section 9, CYP2E1 is the initial enzyme responsible for the metabolism of benzene to phenolic metabolites. Studies have shown a number of substances including alcohol (ethanol) to induce hepatic CYP2E1 activity (Johansson & Ingelman-Sundberg, 1988; Koop et al, 1989). Alcohol consumption by rats and rabbits resulted in increased microsomal metabolism of benzene (Johansson & Ingelman-Sundberg, 1988; Nakajima et al, 1985) and increased benzene-mediated myelotoxicity in rats (Nakajima et al, 1985). Consequently, alcohol consumption by individuals exposed to benzene may result in enhanced metabolite formation and increased risk of myelotoxicity.

Toluene

Toluene acts as a substrate for CYP2E1 (Nakajima et al, 1992) and thus, in the presence of benzene, can act as an inhibitor of benzene metabolism. Andrews et al.

(1977) demonstrated the inhibition of benzene metabolism by the co-administration of toluene to male Swiss mice. Urinary benzene metabolites were significantly decreased ($p < 0.01$) and the amount of exhaled benzene increased in toluene- and benzene-treated animals compared to control benzene-treated animals. While the tissue concentration of benzene did not alter with toluene treatment, the concentration of total benzene metabolites was significantly reduced ($p < 0.05$) in various tissues including blood and bone marrow. Inoue et al. (1989) showed that workers exposed concurrently to benzene and toluene produced significantly less ($p < 0.01$) urinary *trans*,*trans*-muconic acid compared to workers exposed only to benzene. However, in a study in which urinary phenylmercapturic acid was used as a biomarker for workers exposed to benzene, no correlation with toluene exposure was found (Inoue et al, 2000).

Non-steroidal anti-inflammatory drugs

Cyclooxygenase activity within bone marrow contributes to benzene-mediated bone marrow toxicity by participating in the oxidation of phenolic metabolites to reactive species and by the conversion of macrophage-derived arachidonic acid to PGE_2, an inhibitor of stem cell proliferation. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin are potent inhibitors of cyclooxygenase activity (Randall et al, 1980; Roth et al, 1975). The administration of NSAIDs, prior to benzene exposure, has been shown to diminish the bone marrow toxicity associated with benzene in mice (Kalf et al, 1989; Pirozzi et al, 1989). The routine use of NSAIDs may confer some protection from the effects of benzene exposure.

12.5 Summary

Exposure to benzene can result in bone marrow toxicity in several species in addition to leukaemia in humans and solid tumours in other animal species. In order for bone marrow toxicity to occur, benzene must first be metabolised by the liver to intermediate metabolites. These metabolites become localised within the bone marrow where they undergo activation by peroxidase enzymes, particularly myeloperoxidase which is found in large amounts in bone marrow, and, to a lesser extent, by cyclooxygenase. While individual benzene metabolites appear not to induce bone marrow toxicity, the combination of phenol and hydroquinone have been shown to induce the same effects on bone marrow as benzene. This effect appears to be due to the ability of phenol to act as a co-oxidant in the activation of hydroquinone to the semiquinone and benzoquinone by myeloperoxidase. Subsequent changes in cellular function result in altered growth factor production with inhibition of bone marrow stem cell proliferation, differentiation and maturation. The oxidation of hydroquinone also results in the formation of reactive oxygen species. Damage to cells by these species can result from DNA adduct formation, DNA base modification, chromosomal aberrations and changes to intracellular redox status, particularly depletion of glutathione and oxidation of protein sulfhydryl groups. Damaged cells not deleted by apoptosis and which possess activated oncogenes or damaged tumour suppressor genes may begin to proliferate as clonal lines, which may result in leukaemia in humans or solid tumours in animals.

While a number of gender-related differences have been described in the response of rodents to benzene exposure, there is no evidence for such differences in the response of humans. However, humans do exhibit differences in the expression and

activity of several enzymes involved in the metabolism of benzene, the most notable of which occurs with NQO1, an enzyme which is responsible for converting quinones to their corresponding hydroquinones and affords protection against quinone-adduct and reactive oxygen species formation within cells. Thus the expression of genetic polymorphisms may modulate the sensitivity of an individual or ethnic group to the effects of benzene exposure.

13. Health Hazard Assessment

This section compares and integrates key data on animal toxicity and human effects in order to characterise the potential adverse human health effects of benzene and their dose-response relationships. The critical studies have been described in Sections 10-11. In integrating their findings, consideration has been given to quality of data, strength of evidence and consistency of outcomes.

13.1 Acute effects

13.1.1 CNS effects

In animals and humans, acute exposure to benzene has dose-dependent CNS depressant or anaesthetic effects which have a rapid onset of action and are reversible upon cessation of exposure (unless fatal), with limited evidence of neurobehavioural stimulation at sub-anaesthetic doses.

In mice, there are clinical signs of CNS depression at exposure levels ≥1000 ppm benzene.

In humans, clinical signs include generalised symptoms such as dizziness, headache and vertigo at levels of 250-3000 ppm, leading to drowsiness, tremor, delirium and loss of consciousness at 700-3000 ppm, whereas exposure to 25 ppm benzene for 8 h is not associated with any adverse signs or symptoms.

13.1.2 Skin, eye and respiratory tract irritation

Limited, but consistent animal data show that undiluted benzene is irritating to the skin and eye. Benzene vapours have also been reported to cause lacrimation in rats. In humans, aspiration of liquid benzene has been observed to cause pulmonary oedema and bleeding. Furthermore, human case reports show that benzene vapours may induce skin, eye and/or respiratory tract lesions that vary from slight to severe irritation, depending on the vapour concentration. As such, benzene can be characterised as irritating to the skin, eyes and respiratory tract. However, there are no reports of skin, eye or respiratory tract irritation in humans exposed to benzene vapour levels <33 ppm (TWA_8).

13.2 Repeated dose effects (other than carcinogenicity)

13.2.1 CNS effects

Repeated exposure to benzene has been shown to affect the dopaminergic system in both mice and rats and one study found a reduction in brain weight in mice exposed to high doses (350 mg/kg/day) for 30 days. Although a variety of neurological effects have been attributed to repeated occupational exposure to benzene, these were predominantly recorded in studies that did not include an unexposed control group. In the only available human study with an unexposed control group, there was a dose-dependent increase in the prevalence of dizziness and headache within a dose range that averaged 59 ppm benzene. As such, it would appear that any CNS effects in humans from long-term exposure to benzene are likely to be similar to those resulting from acute exposures and occur at exposure levels that are higher

than those associated with other human health effects (such as bone marrow depression).

13.2.2 Immunosuppression

Effects suggestive of an impairment of the ability of LC to respond to antigenic and mitogenic stimuli with rapid proliferation have been consistently observed in subacute oral and inhalation studies in mice and have also been found in a subacute inhalation study in rats. The corresponding NOAEL/LOAEL values are 0.44/10 ppm in mice and 200/400 ppm in rats. These were established in studies in which blood cells from animals exposed in vivo were tested in various in vitro systems. There are no reports of similar findings in humans, although a reduction in the number of LC in peripheral blood is common in benzene-induced bone marrow depression. Changes in the blood level of some immunoglobulin classes have been reported in workers exposed to 3-57 ppm benzene, but this finding has not been confirmed in other studies.

In mice and rats, the LOAEL for immunosuppresion is similar to that for bone marrow depression. Furthermore, whereas there is no evidence that benzene compromises LC function in humans, benzene is an established cause of reduced LC counts. Therefore, it is appropriate to characterise immunosuppression as a sign of LC toxicity that is an aspect of, and included in, the wider effect of bone marrow depression discussed below.

13.2.3 Bone marrow depression

Bone marrow depression (haematotoxicity, 'benzene poisoning') from exposure to benzene has been reported in all species examined, including mice, rats, guinea pigs, rabbits, pigs and humans. Its main manifestation is a reduction in the number of one or more of the formed elements of the blood (LC, Plt, RBC, WBC) and/or in Hb, and/or an increase or decrease in RBC size (MCV). The mechanistic studies discussed in Section 12 indicate that the toxicity of benzene to haematopoietic cells in the bone marrow is due to several of its metabolites that are formed *in situ* in relatively high concentrations and act in an additive or synergistic manner to disrupt a range of mechanisms that regulate blood cell formation. Further support for this notion is provided by the order of susceptibility of mice and rats to benzene haematotoxicity (male mice > female mice > rats), which parallels the rate of formation and tissue concentrations of reactive benzene metabolites.

In animals exposed to inhalation of benzene, abnormal blood counts and morphological abnormalities in blood forming organs have been found at exposure levels ≥10 ppm in mice (including mouse foetuses exposed *in utero*) and ≥100 ppm in rats. These effects were observed even at the lowest dose tested in all long-term studies. Therefore, a NOAEL cannot be established.

In humans, several occupational studies indicate that the incidence and severity of bone marrow depression is related to recent or current exposure to benzene. It is not possible to estimate the average latency period from the available human data; however, animal studies indicate that abnormal blood counts may develop in less than a month. As described in Section 11.4.4, a LOAEL for haematological effects in humans has been determined in a study in 44 Chinese workers with long-term exposure to benzene, in which the only haematological abnormality in the lowest exposure group (n = 11; median exposure (TWA_8) = 7.6 ppm; range 1-20 ppm) was a modest decrease (16%) in ALC (Rothman et al, 1996a, 1996b). The limitations of

this study include a relatively wide exposure range and a small number of subjects of an ethnic group that may express genetic polymorphisms modulating sensitivity to the effects of benzene exposure. On the other hand, the study had a well-matched control group, minimal exposure to other chemicals (toluene and xylenes) and a dose-response relationship was established between ALC and benzene exposure as measured by repeated personal monitoring as well as with benzene metabolites in the urine. Furthermore, the prevalence of polymorphisms known to increase the susceptibility to benzene-induced bone marrow suppression (the GSTT1 and NQO1 (^{609}C→T) genotypes; see Section 12.4.2) is 3-5 times higher among Chinese than among Caucasians (Kelsey et al, 1997; Xu et al, 1998). Therefore, a LOAEL determined in Chinese subjects should also be valid for Caucasians. For these reasons, based on current human data, 7.6 ppm (TWA_8) is considered the best estimate for a LOAEL which may be close to the point of departure for the onset of haematological effects. The available human data are insufficient to establish an appropriate NOAEL, but studies with various limitations indicate that it is likely to be >0.5 ppm (TWA_8).

The only epidemiological study in the general population found an excess occurrence of anaemia and related disorders in a community whose tap water contained ≤66 μg/L benzene. However, as the equivalent dose in a 60-kg person with a daily water consumption of 2 L is <2.2 μg/kg/day or 450 times lower than the only oral NOAEL for haematotoxicity recorded in animal studies (1 mg/kg/day; Wolf et al, 1956), this finding may well have been heavily influenced by bias arising from public awareness of the pollution of the water with benzene and the health effects of the chemical.

In conclusion, there is a substantial body of animal, human and mechanistic data which supports the association between benzene exposure and bone marrow depression and indicates that this is a result of cytotoxicity and, therefore, a threshold effect. The available studies are insufficient to draw firm conclusions about the inhalation NOAEL, although human observations suggest that it is >0.5 ppm. The lowest inhalation LOAEL observed in animals (mice) is 10 ppm, which is comparable to the best estimate for a human LOAEL (7.6 ppm).

13.2.4 Fertility effects

A single oral dose of benzene has been shown to induce chromosome aberrations and have cytotoxic effects in mouse spermatogonia at high doses (≥220 and ≥880 mg/kg respectively). There is also evidence of degenerative changes in the testes (atrophy) and ovaries (atrophy or cystic lesions) of mice exposed to repeated inhalation or oral administration of high doses of benzene (300 ppm for 6 h/day and 25-50 mg/kg/day respectively). At these dose levels, there was evidence of concomitant haematotoxicity, but no mortality or other findings that would suffice to characterise the effects on the gonads as secondary to generalised toxicity.

Consistent with metabolic differences, benzene did not induce testicular or ovarian toxicity at similar exposure levels in rats. There were no fertility-related effects in female rats exposed to ≤300 ppm benzene for 6 h/day for 10 weeks prior to mating and on GD 0-20. Another study found that female rats exposed to 200 ppm benzene for 24 h/day produced no litters, but did not determine the cause of this.

In humans, there are several reports of menstruation disturbances in female and one of reduced semen quality in male workers exposed to benzene. However, the studies have several limitations and do not provide convincing evidence that benzene may have adverse effects on human fertility.

Based on a 13-week study in mice, the inhalation NOAEL for degenerative lesions in the testis and ovaries is 30 ppm, with a LOAEL of 300 ppm (Ward et al, 1985). Based on ovarian atrophy in a 2-year oral study in mice, the LOAEL is 25 mg/kg/day; a NOAEL was not determined (NTP, 1986). These effect levels are either higher than or similar to those at which there is also clear evidence of bone marrow depression.

13.2.5 Developmental effects

Based on the weight of evidence from a number of developmental toxicity studies in mice, rats and rabbits, benzene can be characterised as foetotoxic, but not teratogenic (see Section 10.5.2). The foetal effects observed in the absence of signs of maternal toxicity were a small reduction in foetal BW and an increase in the incidence of delayed ossification and other minor skeletal abnormalities. There is also limited evidence of a reduction in the number of stem cells in the blood forming tissues of mouse foetuses exposed *in utero* to benzene levels similar to those known to cause bone marrow depression in adult animals (10-20 ppm).

Studies of pregnancy outcome in humans have produced mixed results with regard to the risk for SAb. One study found an elevated SGA risk for fathers with occupational exposure to benzene. In another study, there was a marginally significant reduction in birth weight in infants whose mothers had been exposed to low levels of benzene at work. However, the studies have several limitations and do not provide convincing evidence that benzene may have foetotoxic effects in humans. There are no studies of the effect of maternal benzene exposure on the blood forming tissues of human foetuses.

In conclusion, animal and in vitro data indicate that benzene and/or its metabolites may inhibit normal foetal growth, skeletal development and possibly blood cell production. The available epidemiological data are insufficient to draw conclusions about the likelihood that benzene may have similar effects in humans.

Based on adequately reported studies in the rat, the inhalation NOAEL for foetal effects in the absence of maternal toxicity is 40 ppm, with a LOAEL of 100 ppm (Coate et al, 1984; Green et al, 1978). In other studies in the rat, 100 ppm is also the LOAEL for bone marrow depression.

13.2.6 Other non-neoplastic effects

Other non-neoplastic effects are inadequately documented to determine their significance for health hazard assessment.

13.3 Genotoxicity

The genotoxicity of benzene has been investigated extensively in a broad spectrum of in vitro and in vivo systems. Overall, the results are consistent with the conclusion that several benzene metabolites (particularly cathechol, hydroquinone and quinone) can induce a variety of lesions in genetic material, which range from DNA base alterations and single strand breaks to structural and numerical chromosome aberrations. Chromosome aberrations have also been identified in

peripheral blood cells of workers exposed to benzene, generally at exposure levels >10 ppm. Specifically, recent studies found an increase in the frequency of aneuploidy, long-arm deletions and translocations involving chromosomes 1, 5, 7, 8, 9 and 21 (see Table 11.3). As discussed below, these findings suggest that benzene exposure can lead to chromosome lesions which are similar to the specific abnormalities that are the hallmark of human leukaemia. However, there is no indication that an increase in the occurrence of such lesions in non-neoplastic peripheral blood cells is associated with any adverse health effects *per se.* Therefore, it is appropriate to characterise genotoxicity as a mode of action that is an aspect of, and included in, the wider effect of carcinogenicity addressed below.

13.4 Carcinogenicity

13.4.1 Leukaemia

In several of the occupational cohort studies reviewed in Section 11.6.1, past employment in an industry or job category with the potential for exposure to benzene was associated with a significant increase in the risk for cancer of the blood and lymphatic system and/or leukaemia. In addition, there was a significant trend with cumulative exposure, that is, a clear dose-time-response relationship in three out of four studies in which detailed exposure assessments were made. The strength of the association ranged from weak in the majority of cohorts to strong (SMR = 10-20) in Pliofilm workers with a cumulative exposure in excess of 500 ppm-years (Table 11.4; Table 11.5). With regard to leukaemia type, AML (ANLL) was found to account for most of the risk elevation and there was no evidence of a statistically significant dose-time-response relationship for CML, ALL or CLL in any of the cohorts. Several case-control studies also identified prior exposure to benzene or benzene-containing substances as a significant risk factor for a variety of lympho-haematopoietic cancers, including but not limited to acute leukaemia (see Section 11.6.2). As such, it is universally agreed that occupational exposure to benzene may induce leukaemia, specifically AML (ANLL). In the largest cohort studied to date, the latency period from first exposure to clinical diagnosis was estimated at 11-12 years, with a range from 10 months to 50 years (Yin et al, 1987b).

Human leukaemias are clonal diseases generally characterised by a large variety of acquired chromosome aberrations (including translocations, insertions, deletions and inversions) in individual bone marrow cells in the haematopoietic stem and progenitor cell compartment (Gilliland, 1998; Irons & Stillman, 1996).

As described in Section 12, although the mechanism of carcinogenesis of benzene is unknown, several benzene metabolites have been shown to cause DNA damage, including base alterations, chromosome structural abnormalities and aneuploidy.

In patients with AML secondary to treatment with alkylating agents or radiation (s-AML), there is a recurrent pattern of cytogenetic abnormalities involving loss of all or part of chromosomes 5 and 7. Furthermore, s-AML is generally preceded by a period of preleukaemia (also known as MDS) characterised by a persistent bone marrow dysplasia, which is believed to provide a microenvironment that increases the susceptibility of stem and progenitor cells to chromosome damage and/or enhances the survival and proliferation of cells with genetic abnormalities. The pattern of cytogenetic abnormalities involving chromosomes 5 and 7 is also observed in studies of leukaemia patients occupationally exposed to benzene or benzene-containing substances. It has therefore been speculated that benzene-

induced leukaemia is the result of a series of both epigenetic events that affect the microenvironment and genetic events that lead to the activation of oncogenes and/or the loss of tumour suppressor genes (Irons & Stillman, 1996) and, therefore, is a threshold rather than a non-threshold effect (CONCAWE, 1996). There is little documentation on the association between non-neoplastic bone marrow lesions such as MDS and leukaemia in benzene-exposed subjects. Of 18 incident cases of bone marrow disorders in the large cohort of benzene-exposed Chinese workers described in Section 11.6.1, 11 had bone marrow depression whereas 7 had MDS. Furthermore, in the same cohort, a diagnosis of bone marrow depression ('benzene poisoning') was associated with a 71-fold increase in the risk for ANLL/MDS which could not be attributed to cumulative benzene exposure. However, although these findings are consistent with the hypothesis that non-neoplastic bone marrow lesions precede frank leukaemia in some benzene-exposed subjects, they do not suffice to prove that the pathogenesis of benzene-induced leukaemia involves an epigenetic step.

As discussed in Section 11.6.1, only the Pliofilm cohort is suitable for the determination of the carcinogenic potency of benzene. In this cohort, the SMR for leukaemia was significantly elevated at cumulative exposures >50 ppm-years for two of the three available exposure estimates (Table 11.5), equivalent to a long-term exposure level of >1.25 ppm benzene over a working life of 40 years. However, these findings derive from a single study with insufficient statistical power to rule out the possibility of some increase in the SMR at lower exposures.

No observational studies were found which have examined the relationship between leukaemia incidence or mortality and the exposure of individual adults or children to non-occupational benzene levels.

13.4.2 Solid tumours

Lymphoma

Lymphomas are solid malignant tumours of lymphocytic origin. They include NHL and MM which are monoclonal and Hodgkin's disease, which may be either monoclonal or of mixed cellularity (Freedman & Nadler, 1998; Longo, 1998).

In mice, the incidence of lymphoma was elevated in several studies conducted in strains where this is a common spontaneous tumour.

In humans, some of the occupational cohort studies summarised in Table 11.4 link past employment in job categories with the potential for benzene exposure with a significant or marginally significant increase in the risk for all lymphoma (in Italian refinery workers employed prior to 1961 and Chinese workers in the painting, printing, footwear and chemical industries), NHL (in Finnish petroleum industry workers and Chinese workers in the painting, printing, footwear and chemical industries) or MM (in Australian petroleum industry workers). The strength of the association was weak in Australian and Finnish petroleum industry workers, intermediate in Italian refinery workers employed prior to 1961 and highest (RR = 4.7 for NHL) in Chinese workers with assessed (and probably underestimated) exposure levels ≥25 ppm benzene. However, a dose-time-response relationship has not been demonstrated. Some of the case-control studies reviewed in Section 11.6.2 also suggest an association between prior exposure to benzene or benzene-containing substances and the risk of NHL or MM.

Although it cannot be excluded that there is an association between benzene exposure and the risk for lymphoma, specifically NHL and MM, the evidence is not as conclusive as it is for leukaemia.

Mammary cancer

In female mice, there was a statistically significant, dose-related increase in the incidence of mammary gland carcinomas at dose levels ≥50 mg/kg/day in two oral carcinogenicity studies. The incidence was not increased in female rats dosed orally with up to 500 mg/kg/day for 2 years, or in a number of inhalation studies in mice and rats exposed to up to 300 ppm benzene for 2 years or longer.

In humans, the SIR for breast cancer was elevated in a cohort study including 1942 female workers in a Finnish petroleum company; however, the elevation was mainly due to cases among clerical workers and similar in magnitude to that found in other studies of Finnish women in office jobs. In addition, there is a single, but well controlled case-control study showing that the risk for breast cancer in women is weakly associated with duration of benzene exposure, probability of exposure and a crude estimate of cumulative exposure to the chemical. There was also a marginally elevated risk associated with employment as a car mechanic or petrol station worker in a case-control study of primary breast cancer in men. These findings are insufficient to determine the likelihood of a causal relationship between benzene exposure and breast cancer in humans, although it cannot be excluded.

Skin cancer

There was an increased incidence of epithelial (non-melanoma) skin tumours in male rats in two 2-year oral bioassays, but only at very high dose levels (200-500 mg/kg/day) that may have exceeded the maximum tolerated dose. Similar tumours were not seen in female rats, or in any of several carcinogenicity studies in the mouse.

In humans, the risk for melanoma or skin cancer (mainly melanoma) was elevated in three petroleum industry cohorts (Table 11.4). In addition, ever employment in the petroleum industry was a marginally significant risk factor for concurrent basal and squamous cell carcinoma in one of the case-control studies reviewed in Section 11.6.2. The risk for skin cancer (mainly melanoma) was also elevated in the Dow Chemical cohort, but there was no trend with either level or duration of exposure to benzene. As such, although these studies suggest that there may be an elevated risk for skin cancer among workers in the petroleum and chemical industries, there is no indication that this can be attributed to exposure to benzene.

13.5 Summary and conclusions

Human case reports indicate that acute exposure to benzene vapours can cause CNS depression and skin, eye and respiratory tract irritation. With regard to chronic exposure, it is well documented through numerous epidemiological studies that the principal human health hazards are bone marrow depression and leukaemia, particularly AML (ANLL). There is also some evidence of an association between chronic benzene exposure and the risk for lymphoma, specifically NHL and MM. At present, leukaemia and lymphoma must be considered non-threshold effects caused by exposure to a genotoxic carcinogen. With regard to bone marrow depression, it is reasonable to assume that this is a

threshold effect. Based on current human data, 7.6 ppm (TWA_8) is the best estimate of a LOAEL which may be close to the point of departure for the onset of haematological effects. An appropriate NOAEL has not been established; however, occupational studies with various limitations indicate that it is likely to be >0.5 ppm. Finally, it cannot be excluded that repeated exposure to benzene may be weakly associated with reproductive effects and mammary cancer in humans.

No data from human studies were found to indicate that children are more susceptible to benzene toxicity than adults or that benzene affects human males and females differently. As discussed in Section 12.4.3, experimental data indicate that alcohol consumption may increase the risk of adverse health effects from chronic exposure to benzene.

14. Classification for Occupational Health and Safety

This section discusses the classification of the health effects of benzene according to the NOHSC Approved Criteria for Classifying Hazardous Substances (the Approved Criteria) (NOHSC, 1999a) or, in the case of physicochemical hazards, the Australian Dangerous Goods (ADG) Code (FORS, 1998). The Approved Criteria are cited in the NOHSC National Model Regulations for the Control of Workplace Hazardous Substances (NOHSC, 1994c) and provide the mandatory criteria for determining whether a workplace chemical is hazardous.

Where adequate human data were unavailable, the classification for health hazards has been based on experimental studies (animal and in vitro tests). In extrapolating results from experimental studies to humans, consideration was given to relevant issues such as quality of data, weight of evidence, metabolic and mechanistic profiles, inter- and intra-species variability and relevance of exposure levels.

Benzene is currently listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999b) with the following classification: 'Flammable'; 'Carcinogen, Category 1' and 'Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed'.

14.1 Physicochemical hazards

Benzene meets the criteria of the ADG Code for classification as a flammable liquid, as it gives off flammable vapours at − 11°C and an atmosphere containing 1.4-7.9% v/v benzene is explosive (Table 5.1). Benzene is not chemically reactive under normal ambient conditions.

Classification. Benzene is already classified as 'Flammable'.

14.2 Health hazards

14.2.1 Acute toxicity

The available human data on the acute toxicity of benzene are anecdotal in nature.

As shown in Table 10.1, the 4-h inhalation LC_{50} in the rat is 13,700 ppm (43.8 mg/L). The oral LD_{50} values determined in the rat are inconsistent (810, 5600 and 9900 mg/kg). However, as the oral LD_{50} in the mouse is 4700-6500 mg/kg, the weight of evidence suggests that benzene has a low acute oral toxicity. The dermal LD_{50} in the rabbit is >8200 mg/kg.

Single exposure to benzene has been associated with CNS stimulation or depression in both animals and humans and with male germ cell toxicity in mice. However, observations indicate that these effects are reversible after a recovery period ranging from a few hours for CNS effects to several weeks for germ cell damage.

Classification. Benzene does not meet the Approved Criteria for classification for acute lethal effects or non-lethal irreversible effects after a single exposure.

14.2.2 Irritant and corrosive effects

Liquid benzene has been shown to cause skin and eye irritation in rabbits. Benzene vapours have been reported to cause eye irritation in humans and rats at concentrations ≥33 and ≥10 ppm respectively. In humans, vapour levels >60 ppm have been associated with irritation of the skin, including second degree burns, and of the mucous membranes of the nose, mouth, throat and lower airways, including signs of serious respiratory system effects such as dyspnoea, laryngitis, tracheitis, bronchitis and pulmonary haemorrhage.

Classification. Based on animal tests and practical observation in humans, benzene meets the Approved Criteria for classification as 'Irritating to skin' (risk phrase R38), 'Irritating to eyes' (R36) and 'Irritating to the respiratory system' (R37).

14.2.3 Sensitising effects

There are no animal studies or human case reports of skin or respiratory sensitisation to benzene.

14.2.4 Effects from repeated or prolonged exposure

According to the Approved Criteria, a substance is classified as hazardous when serious damage (clear functional disturbances or morphological changes which have toxicological significance) is likely to be caused by repeated or prolonged exposure by an appropriate route. In this context, haematological disturbances are considered to be particularly important if the evidence suggests that they are due to decreased bone marrow production of blood cells.

There is a substantial body of human evidence that repeated occupational exposure to benzene vapours at ≥ 7.6 ppm (0.024 mg/L) can cause bone marrow depression. This is usually a reversible condition, but may progress to aplastic anaemia or cancer (AML). Toxic effects on the blood and blood forming organs have also been found consistently in rats exposed to inhalation of benzene at concentrations ≥100 ppm (0.32 mg/L) for 6 h/day for 5 days/week, in mice exposed to inhalation of benzene at concentrations ≥10 ppm (0.032 mg/L) for 6 h/day for 5 days/week, and in rats and mice exposed to orally administered benzene at dose levels ≥25 mg/kg/day, in studies of a duration of 90 days (13 weeks) or longer.

There are no human or animal studies of the haematological effects of dermal exposure to benzene. However, as benzene is absorbed through the skin in humans, it is likely that bone marrow toxicity may also be caused by repeated or prolonged exposure by the dermal route.

Classification. Benzene meets the Approved Criteria for causing serious damage to health by repeated or prolonged exposure and is already classified as 'Toxic: Danger of serious damage to health by prolonged exposure by inhalation, in contact with skin and if swallowed' (R48/23/24/25).

14.2.5 Reproductive effects

Effects on fertility

The available human case reports and studies have several limitations and do not prove a causal relationship between benzene exposure and fertility effects in humans.

In a study of male mice, a single oral dose of 880-6160 mg/kg benzene had no effect on body or testis weight, but was toxic to differentiating spermatogonia. There is also evidence of degenerative changes in the testes (atrophy) and ovaries (atrophy or cystic lesions) of mice exposed to repeated inhalation or oral administration of high doses of benzene (300 ppm for 6 h/day and 25-50 mg/kg/day respectively). At these dose levels, there was concomitant haematotoxicity, but no mortality or other findings that would suffice to characterise the effects on the gonads as secondary to generalised toxicity.

Benzene did not induce testicular or ovarian toxicity at similar exposure levels in rats. There were no fertility-related effects in female rats exposed to ≤300 ppm benzene for 6 h/day for 10 weeks prior to mating and on GD 0-20. Another study found that female rats exposed to 200 ppm benzene for 24 h/day produced no litters, but did not determine the cause of this.

As such, benzene may have a toxic effect on the testes and ovaries in mice, but these effects were demonstrated only at high doses of doubtful relevance for humans. The available data on reproductive capacity in rats are limited and inconclusive.

Classification. The available evidence is insufficient to classify benzene as toxic to fertility under the Approved Criteria.

Developmental effects

The available human studies have several limitations and do not prove a causal relationship between benzene exposure and developmental toxicity in humans.

The available developmental toxicity studies in mice, rats and rabbits are summarised in Table 10.2 in Section 10.5.2. Based on the weight of evidence from these studies, benzene is foetotoxic, but not teratogenic. Foetotoxic effects were mainly found at dose levels associated with maternal toxicity. However, some studies in mice and rats found a small reduction in foetal BW or an increase in the incidence of delayed ossification and other minor skeletal abnormalities at dose levels at which no maternal effects were reported. These minor growth disturbances were found in the foetuses of dams exposed to ingestion or inhalation of benzene at high doses of doubtful relevance for humans (800-1300 mg/kg/day by mouth or 100-500 ppm by inhalation).

In two small studies, inhalation exposure of pregnant mice to 10-20 ppm benzene on GD 6-15 resulted in toxic effects on blood cells in haematopoietic tissue in the liver, bone marrow, or spleen of the offspring exposed *in utero*. These effects occurred in the absence of other signs of developmental toxicity and were consistent with lesions induced by benzene in the same tissue type in adult mice at similar levels of exposure. However, the studies comprised an insufficient number of animals to be entirely convincing.

Classification. The available evidence is insufficient to classify benzene as a developmental toxicant under the Approved Criteria.

Effects on lactation

Benzene has been found in human breast milk. In the rat, one study found small changes in body and organ weights in the offspring of rats exposed to inhalation of benzene during pregnancy and lactation, but did not establish whether these effects were lactational or the result of exposure *in utero*.

Classification. The available evidence is insufficient to classify benzene for effects on lactation.

14.2.6 Mutagenic effects

The Approved Criteria defines a mutation as a permanent change in the amount or structure of the genetic material in an organism, resulting in a change of the phenotypic characteristics of the organism.

To be classified in Category 1 (Substances known to be mutagenic to humans) or Category 2 (Substances which should be regarded as if they are mutagenic to humans), a substance must be known or strongly presumed to cause heritable mutations in humans, that is, changes to the genetic material that occur in germ cells and can be transmitted to the offspring. If a substance has only been shown to induce mutations in somatic cells, it is classified in Category 3.

Benzene and/or its metabolites, particularly catechol, hydroquinone and quinone, have been shown to cause DNA damage in mammalian in vitro systems as determined by sensitive test methods. Furthermore, benzene has been shown to be genotoxic to somatic cells in a broad spectrum of in vivo models in which the chemical was administered to rodents by inhalation, oral gavage or parenteral injection. These include tests for SCE and MN induction in peripheral blood cells, bone marrow cells, foetal liver cells, lung fibroblasts and Zymbal gland cells; gene mutations in LC, lung and spleen cells; chromosome aberrations in LC, bone marrow cells and spleen cells; and DNA adducts in nucleated blood and bone marrow cells. Chromosome aberrations have also been identified in peripheral blood cells of workers exposed to benzene, generally at exposure levels >10 ppm.

Published documentation on mammalian germ cells appears to be limited to two studies in the male mouse. Spanò et al. (1989) found that a single oral dose of ≥880 mg/kg benzene caused a dose-dependent reduction in the relative cell count in the primary spermatocyte and spermatid fractions, indicating that benzene and/or its metabolites have the potential to reach the germ cells (see Section 10.5.1). Ciranni et al. (1991) subsequently showed that a single oral dose of ≥220 mg/kg benzene induced chromatid aberrations in differentiating spermatogonia in a dose-dependent manner (see Section 10.6 and Figure 10.1). However, no evidence could be found in the open literature that these results have been replicated by other laboratories or are supported by findings in other appropriate tests. As such, the experimental data available at present do not satisfactorily demonstrate heritable genetic damage.

Classification. Based on the above, benzene meets the Approved Criteria for classification as a mutagenic substance in Category 3 (R40: 'Possible risk of irreversible effects').

14.2.7 Carcinogenicity

The Approved Criteria provides for the classification of carcinogenic substances into three categories. Category 1 includes substances known to be carcinogenic to humans, whereas Categories 2 and 3 in general are used where there is more or less convincing evidence from appropriate long-term animal experiments indicating that human exposure to the chemical may result in the development of cancer.

A substance is included in Category 1 if there is sufficient evidence from epidemiological studies to establish a causal association between human exposure and the development of cancer. The existence of a causal relationship would be any of the following:

- an increased incidence of one or more cancer types in an exposed population compared with a non-exposed population;
- evidence of dose-time-response relationships, that is, an increased cancer incidence associated with higher exposure levels or with increasing exposure duration;
- an association between exposure and increased risk observed in more than one study;
- demonstration of a decline in risk after reduction of exposure; and
- specificity of any association, defined as an increased occurrence at one target organ or of one morphological type.

For benzene, the strongest evidence is provided by the finding of an excess mortality from cancer of the blood and lymphatic system and a significant trend with cumulative benzene exposure in the Chinese, CMA and Pliofilm cohorts described in Section 11.6.1 (Hayes et al, 1997; Paxton et al, 1994a; Wong et al, 1987b). Further evidence is provided by two case-control studies which found a significantly elevated risk for blood and lymphatic system cancer at relatively high, but not at lower levels of exposure to benzene (Health Watch, 1998; Richardson et al, 1992). Finally, the increase in AML risk with cumulative exposure in the Pliofilm cohort is evidence of cell type specificity of the association between benzene exposure and human cancer (Wong, 1995).

Classification. Benzene meets the Approved Criteria for classification as a carcinogenic substance in Category 1 and is already classified as such and assigned risk phrase R45: ‘May cause cancer’.

14.3 Summary

Benzene is currently listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999b) with the following classification: ‘Flammable’; ‘Carcinogen, Category 1’ and ‘Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed’. Based on the information available at this time, benzene also meets the Approved Criteria for classification as ‘Irritating to eyes, respiratory system and skin’ (R36/37/38) and as a mutagenic substance in Category 3 (R40: ‘Possible risks of irreversible effects’).

15. Environmental Exposure

In this section, environmental benzene levels and major sources of entry will be discussed separately for the outdoor and indoor environment. Given the brief residence time of benzene in surface water and soil, the main emphasis is on benzene in the atmosphere.

Benzene will be released through many different activities in both a point source and diffuse manner. As such, outdoor air levels have been considered under several different scenarios for point source releases, whereas diffuse releases are addressed in the context of estimating a predicted environmental concentration (PEC) in air for urban environments.

Air monitoring data exist for several areas within Australia, both for urban and point source releases. While monitored point source releases are discussed in their relevant sections, they cannot be compared directly to predicted levels, as these are determined for points at 100 m from the point source, while the monitoring data are from a range of distances.

With regard to the indoor environment, there is documentation from studies conducted in Australia and New Zealand of the concentration of benzene in motor vehicles. However, there are few Australian measurements of benzene levels in indoor air in homes and non-residential buildings. In consequence, these have been assessed on the basis of both Australian and overseas studies and the estimated concentration of benzene in air in a model Australian urban environment.

The environmental concentration estimates developed in this section must be interpreted cautiously as they have been derived by the application of numerous assumptions and approximations to inherently uncertain databases such as the NPI.

15.1 Point source releases to air

This section will consider several different industries. The primary end use of benzene is as a component of petrol (Section 7) and as such the petroleum industry is of major importance when assessing this chemical. Additionally, other specific industries have been identified as releasing significant quantities of benzene, including but not limited to the steel, aluminium and chemical industries.

15.1.1 Petroleum industry

Section 7.2.1 provides a brief overview of the refining process. More detailed information is available from the AIP website (AIP, 2000).

Petroleum refineries

Benzene emissions from petroleum refineries can be grouped into five main categories, namely process vents, storage tanks, equipment leaks, transfer operations, and wastewater collection and treatment. USEPA (1998b) provides techniques for estimating emissions of benzene through leak emissions from refineries, which essentially consist of multiplying equipment counts, equipment leak factors and the benzene concentration in each process. While data exist on the concentration of benzene in various refinery streams, no information is available on

equipment counts and leak factors. Furthermore, leak factors are estimations in themselves so the final result would need to be treated with caution. As such, the USEPA method has been disregarded for the purposes of this assessment.

The Technical Guidance Document (TGD) (EC, 1996) may be used to estimate releases. However, production of benzene *per se* is not the objective of the refinery. It is to produce marketable petrochemical products, of which benzene is a component in various grades. As such, it is difficult to apply the correct scenario from TGD. Nevertheless, if a refinery is considered an operation that produces substances (other than intermediates) in dedicated equipment (category 1b), TGD indicates that 0.01% of available benzene will be emitted during the refining processes (based on the vapour pressure of benzene at 25°C).

AIP (1997) provides data on refinery capacity for the eight Australian refineries. For the purposes of this assessment, data from these refineries have been averaged to create a 'model refinery' with a refining capacity of 3871 kt crude oil per annum.

Benzene is expected to be available for emission during distillation activities, catalytic reforming and catalytic cracking. In order to determine the quantity of benzene available for release, the annual quantity of material passed through each of the processing stages has been averaged and multiplied by the maximum expected percentage of benzene at each stage (0.1% for crude oil undergoing distillation, 2% for material undergoing catalytic cracking and 8% for material undergoing catalytic reforming, as discussed in Section 7.2.1). It was assumed that refining operations are conducted on 300 days per annum. The release estimate of 0.01% was applied to the annual amount of benzene available for emission and converted to an expected daily release.

The following results were obtained for the 'model' refinery:

Total benzene in crude oil refined (kt/y)	Benzene in catalytic cracking (kt/y)	Benzene in catalytic re-forming (kt/y)	Total benzene production (kt/y)	Benzene release per year (kg)	Benzene release per day (kg)
3.9	22.4	72.7	99	9900	33

The Australian refineries have provided estimates of their benzene emissions from 1 July 1999 to 30 June 2000 to NPI and these appear similar to values reported for the previous year.

Table 15.1 shows that reported releases (average of 43 kg per day) are comparable with those estimated above (33 kg per day). When benzene production is calculated based on a benzene concentration of 0.1%, 2% and 8% in crude distillation, catalytic cracking and catalytic reforming processes respectively, the release percentages of benzene from Australian refineries range from 0.006 to 0.039%, with an average release of 0.016%. This compares with 0.01% used for the model refinery. These data suggest that the model underestimates releases. Therefore, when determining predicted concentrations in air, reported release figures will be used.

The PEC in air will be determined following the procedure in TGD (EC, 1996). Estimates are at 100 m from the point source and are derived as follows: PEC_{air} (mg/m^3) = emission x $Cstd_{air}$, where emission = emission rate to air (kg/d) and

$Cstd_{air}$ = standard concentration in air at source strength of 1 kg/d = 2.78 x 10^{-4} mg/m^3.

Following this calculation, the atmospheric PECs for all eight Australian refineries range from 1.6-5.5 ppb at 100 m from the point source (Table 15.1).

Table 15.1: Release of benzene from Australian petroleum refineries and predicted environmental concentrations in air at 100 m from the source

State	Refinery	Benzene release (kg/y)*	Benzene release (kg/day)	Benzene production (kt/y)	Release (%)	PEC_{air} (mg/m^3)	PEC_{air} (ppb)
NSW	Shell	11000	30	91	0.01	0.008	2.6
	Caltex	18000	49	131	0.01	0.014	4.2
QLD	BP	18000	49	59	0.03	0.014	4.2
	Caltex	5600	15	108	0.01	0.004	1.3
SA	Mobil	10000	27	82	0.01	0.008	2.4
VIC	Mobil	7000	19	120	0.01	0.005	1.7
	Shell	15000	41	129	0.01	0.011	3.5
WA	BP	19000	49	71	0.03	0.014	4.2

* As reported to NPI. N.B. BP from Western Australia is for the 1998-1999 reporting period as later results are not yet available.

In their review of studies of hazardous air pollutants performed in Australia and New Zealand, the Victorian EPA summarised measurements near two oil refineries at Lytton, Brisbane in 1992 (EPA Victoria, 1999). The mean of half-hour average measurements is stated as 0.0168 mg/m^3, or 5.2 ppb, which is within the range of the above estimated atmospheric concentrations. The highest 20 half-hour averages ranged from 0.108-1.365 mg/m^3 (or 33.5-423 ppb).

Petrol terminals

Once refined, petroleum product is transferred to the Terminals in the capital cities via pipelines, while it is shipped to Coastal Bulk Plants. The product is transferred by road to small inland bulk plants, and this is from the main terminals. Emissions reported to NPI during 1999-2000 have been recorded as fugitive emissions and tend to have been estimated rather than measured. However, the emission estimation techniques used have been stated as acceptable in the NPI database and these figures will be used to predict concentrations in air at points 100 m from the terminal sites.

When interrogating the NPI database, emissions were only considered for those sites readily identified as terminals, of which there were 28 (WA data not provided). Emissions per annum ranged from 18,000 kg from the largest terminal to 250 kg from the smallest. The average emissions from the terminals were 2706 kg per annum (with only eight of the terminals actually reporting emissions higher than this). To ensure conservatism in the assessment, two scenarios will be considered, one using the average of the full sample size (including the highest emitting terminal), and one for the highest emitting terminal. The same calculation is used as described above and it is assumed emissions occur over 365 days per annum. The resulting PECs in the atmosphere are shown in Table 15.2.

Table 15.2: Predicted environmental concentrations in air at 100 m from Australian terminals

Terminal	Release (kg/y)	Release (kg/day)	PEC_{air} (mg/m^3)	PEC_{air} (ppb)
Largest terminal	18,000	49.3	0.014	4.2
Average terminal	2,706	7.4	0.002	0.6

15.1.2 Steel and associated industries

Coke making operations

Coal is converted to coke in coking ovens. Benzene is contained in coke oven gas and, as such, may be emitted during charging of coke in ovens. Coke ovens are arranged in batteries, with operating processes as described in Section 7.2.2.

Of the two steelworks in Australia, the Port Kembla coke ovens are significantly larger. Data provided by the applicants show that in the order of 2700 kt/y of coke were produced at Port Kembla in 1994-1996, whereas at the Whyalla plant in the order of 891 kt (dry weight) coal was processed during 1999.

NPI data for 1999-2000 are available for both steel works. Port Kembla reported release of benzene to the atmosphere of 99,000 kg. This was a combination of stack emissions (24,000 kg determined through direct measurement) and fugitive emissions (75,000 kg determined through engineering calculations). Whyalla reported release of benzene to the atmosphere of 24,000 kg, a combination of stack emissions (1,100 kg determined through direct measurement) and fugitive emissions (23,000 kg determined through engineering calculations).

The PEC_{air} is calculated following the methodology described above. It was determined based on atmospheric release as reported by the Port Kembla steelworks for the 1999-2000 NPI reporting period. Based on this, 99,000 kg benzene will be expected to be released in a year. For 365 days of operation per annum, this equates to a daily release of 271 kg. Applying the formula described above, the PEC_{air} at 100 m from the coke ovens is 0.076 mg/m^3, or 23.3 ppb.

Coal gas by-product plants

A second benzene production site at an integrated steelworks is the by-product plant where BTX and coal tar are recovered from coke oven gasses as described in Section 7.2.2.

The USEPA (1998b) summary of benzene emission factors for furnace and foundry coke by-product recovery plants, covers all aspects including naphthalene separation; tar interception, dewatering, decantation and storage; light oil storage; flushing liquor circulation tank; and wash oil decanter and circulation tank. While it is expected foundry coke is used in the coking operations at Port Kembla and Whyalla, as a worst case it will be assumed that furnace coke is used. Emission factors are provided for both controlled and uncontrolled circumstances. As the level of control is uncertain, both scenarios are considered. Table 15.3 provides details (with all emission factors combined to provide a total emission factor). As described above, the Port Kembla steelworks produces in the order of 2700 kt of coke per annum. This is assumed to be the input for the determination of benzene release from the by-product plant.

Table 15.3: Estimated benzene emissions from a coal gas by-product plant

	Controlled	Uncontrolled
Emission factor	14.7 g/t	343.1 g/t
Benzene emission per annum	40 t	927 t
Benzene emission per day	133 kg	3090 kg
PEC_{air}*	11 ppb	270 ppb

* Follows methodology described above and is for points 100 m from the point source.

BHP has provided emission estimates and monitoring data from their gas processing plant at Port Kembla. Annual benzene emissions were estimated at 443 t in 1995, but are predicted to fall to 70 t once an ongoing project to control tank vent releases to air has been completed. The monitoring data show an average concentration of 174 ppb next to the plant, decreasing to 58 ppb at 200 m and suggest a concentration at 100 m from the plant to be in the order of 120 ppb. These estimates and monitoring data are within the range expressed above (Table 15.3).

In addition, ambient air benzene monitoring results are available from measurements taken from September 1996-January 1997 at several points around the by-product plant at the Port Kembla steelworks (Westley-Wise et al, 1999). Highest average concentrations of 1.08 ppb were found closest to the plant (26 samples at 1.1 km). At 1.2 km, averages were significantly less at 0.42 ppb, but higher again at 1.4 and 1.6 km (0.66 and 0.67 respectively). Control sites situated at 3.8, 5.5 and 12 km from the steelworks showed mean benzene levels of 0.68, 0.73 and 0.28 ppb respectively. These readings suggest that at distances of >1 km from the point source, levels may be expected to be close to background readings.

Coal tar distillation

Tar produced at the coal gas by-product plants at Port Kembla and Whyalla is shipped to Koppers in Newcastle for processing, as described in Section 7.2.2. Koppers has provided information showing that they receive tar in the order of 96,000 t/y from Port Kembla and 27,000 t/y from Whyalla, containing 0.107% and 0.157% benzene respectively. This equates to 145 t/y of benzene.

Koppers states that the only source of benzene emission to air is from the fume scrubbing systems' stacks. Koppers operates under a NSW EPA licence requiring the concentrations of emissions from all fume systems to be analysed each year. Annual releases of benzene from these stacks were provided for the last five years. As only concentrations were reported, stack velocities of 1 m/s and operating temperatures of 30^0C were assumed to calculate mass emissions of benzene per annum.

15.1.3 Aluminium industry

Aluminium is produced from bauxite, from which alumina (aluminium oxide) is extracted by a refining process, followed by the electrolytic reduction of the oxide to the metal in aluminium smelters.

Alumina refining

Alumina refining involves four basic stages: (1) digestion of bauxite in hot caustic soda; (2) removal of residues from the liquor stream; (3) precipitation of alumina hydrate from the clarified liquor; and (4) calcination of the precipitate to produce anhydrous aluminium oxide.

Benzene emissions from the alumina refinery at Kwinana reported to NPI are the result of 'liquor burning' processes which treat the liquor residues referred to above (Alcoa, 2000). This is presumably due to thermal degradation of organic impurities in the bauxite. Liquor treatment processes differ from plant to plant mainly due to bauxite quality differences. There are six alumina refineries in Australia, two of which have liquor burning facilities. Kwinana has the oldest liquor burning plant and does not have volatile organic chemicals (VOC) reduction equipment fitted, hence the reported benzene emissions. The other refinery with liquor burning facilities at Wagerup, Western Australia has a catalytic thermal oxidiser which removes most VOCs from the off-gas. As a result, the levels of benzene emission do not trigger NPI reporting.

Currently, the largest refinery in Australia, QAL in Gladstone, does not have liquor burning facilities. It is reasonable to assume that new technology when put in place will have VOC reduction equipment. So in determining a PEC in air, the Kwinana plant will be used as the model.

Benzene emissions reported for Kwinana were 21,000 kg during the first year of reporting to the NPI. The site produced 1900 kt of alumina indicating benzene emissions are around 0.001% of alumina production. Emissions were determined through a mixture of direct measurement (at the air stack) and engineering controls to estimate fugitive emissions and are considered reliable.

Applying the above calculation to determine a PEC_{air} at points 100 m from the point source and assuming operation on 300 days per annum, the concentration is estimated to be 0.019 mg/m^3, or 6.1 ppb.

Aluminium smelting

Metallic aluminium is produced from alumina by electrolysis. During the process, oxygen is deposited on and consumes the cell's carbon anodes, which are made from coal tar pitch and petroleum coke.

Benzene is not a component of coal tar pitch or petroleum coke, but could be released as part of the VOCs produced during smelting operations. However, it currently appears that no estimation techniques are available to predict emissions of VOCs. None of the six aluminium smelters provided releases of benzene (or VOCs) in their NPI reports. Because data were provided on other releases, it is likely that benzene was produced in quantities <10 t/y, which does not trigger reporting. Due to a lack of information, no meaningful estimations can be made with regard to benzene emissions from aluminium smelting plants.

15.1.4 Chemical industry

Bulk benzene storage

Locally produced BTX and imported benzene are stored in bulk at the Terminals Pty Ltd bulk storage facility, Coode Island - Melbourne, then distributed to the major end user of benzene feedstock, Huntsman Chemical Company in Melbourne. Emission levels sourced from the NPI database for the reporting period July 1999 to June 2000 indicated that 8.2 t of benzene were released by this facility.

Butadiene rubber manufacture

Qenos' Altona facility uses about 40 t of benzene per annum as a solvent component in the manufacture of butadiene rubber. The butadiene is polymerised in an enclosed system, as briefly described in Section 7.2.3. The benzene is a minor component in the cyclohexane based solvent and is not consumed during the reaction. Instead, the solvent is removed from the rubber-solvent solution by steam stripping, condensed, purified and recycled. No data are available to confidently predict emissions of benzene during rubber manufacture.

USEPA (1998b) does not provide any in-depth discussion on the emissions of benzene when used as a solvent as this use is expected to be eliminated in the next few years, although process vents, dryer vents and building ventilation systems are identified as emission points. However, TGD (EC, 1996) may be used to estimate emissions. Based on its vapour pressure, TGD predicts that 0.01% of benzene will be emitted to the atmosphere when used for chemical synthesis in a continuous process. With 40 t per annum, this corresponds to an annual release of 4 kg, with a daily release over 300 days of 0.013 kg, resulting in a predicted concentration in air at points 100 m from the point source of 0.004 $\mu g/m^3$, or 0.001 ppb.

Releases to water can also be predicted. Based on the solubility of benzene, TGD estimates that 0.05% will be released with wastewater. This equates to 20 kg per annum, or 0.07 kg per day.

Styrene manufacture

The Huntsman plant in Melbourne converts in the order of 80 kt benzene per annum to ethyl benzene for processing into styrene (see Section 7.2.3).

USEPA (1998b) has provided emission factors based on a controlled 300 kt/y capacity integrated ethyl benzene/styrene plant (Table 15.4). Major process emission sources are the alkylation reactor area vents, atmospheric and pressure column vents, vacuum column vents and the hydrogen separation vent. These emission factors will be used for this assessment, although actual emission factors may vary with throughput and control measures.

Table 15.4: Benzene emission factors during manufacture of styrene and phenol in a controlled 300 kt/y capacity model plant (USEPA, 1998b)

Plant	Product	Source	Emission factor (kg/t)*
Styrene plant	Ethyl benzene	Alkylation reactor vent	0.0003
		Benzene drying column	0.012-0.48
		Polyethylbenzene recovery column	0.002-0.005
		Benzene-toluene vacuum vent	0.03-1.2
	Styrene	Hydrogen separation vent	0.00003-0.0012
	Total		0.04433-1.6865
Phenol plant	Cumene	Benzene drying column	0.001
		Catalyst mix tank scrubber vent	0.00795
		Wash decant system vent	0.000392
		Benzene recovery column	8.5×10^{-4}
	Phenol	Cumene oxidation	5.82×10^{-5}
	Total		0.01025

* These emissions do not consider equipment leaks and fugitive emissions through storage and handling.

As mentioned above, in the order of 80 kt per annum benzene is used in the manufacture of styrene. By weight, benzene accounts for around 74% of styrene, that is, around 28 kt per annum ethylene is required for styrene manufacture. This indicates an annual capacity through the plant of 108 kt of materials. Applying the above emission factors to an environment where control technology is in place, between 4.8 and 182 tonnes per annum benzene would be emitted.

Annual benzene emissions from the Huntsman plant are reported to have fallen from 70 t in 1996 to 7 t in 1998 following the installation of a vapour recovery system to control emissions from tanks in the styrene plant (Huntsman, 1999). These emissions are within the range estimated above. Therefore, as estimated emissions from the phenol plant are much smaller (see below), the PEC_{air} will be calculated from the most recent Huntsman emission data, that is, 7 t/y. Assuming 365 days per annum of manufacture, this equates to 19.2 kg per day, corresponding to a PEC_{air} at 100 m from the styrene plant of 0.0054 mg/m^3, or 1.6 ppb.

Phenol manufacture

Huntsman uses up to 15 kt benzene per annum in the production of cumene (isopropyl benzene), which is then split to phenol and acetone (Section 7.2.3).

USEPA (1998b) provides emission factors for cumene and phenol production. Major process emission sources are process vents, equipment leaks, storage vessels, wastewater collection and treatment systems and product loading and transport operations. These emission factors are presented in Table 15.4.

As mentioned above, up to 15 kt per annum benzene is used in the phenol manufacture. By weight, benzene accounts for around 65% of cumene, meaning around 8 kt per annum propylene is required for the cumene manufacture. This indicates an annual capacity through the plant of 23 kt of materials. Applying the above emission factors, in an environment where control technology is in place, 235 kg per annum of benzene can be expected to be emitted. Assuming manufacture on 365 days per annum, this equates to 0.64 kg per day, corresponding to an estimated air concentration at 100 m from the plant of 0.16 μg/m^3, or 0.06 ppb.

Australian monitoring data

In their review of studies of hazardous air pollutants performed in Australia and New Zealand, the Victorian EPA summarises measurements taken from the Altona chemical complex in 1995 (EPA Victoria, 1999). Benzene-emitting industries in the area include a petroleum refinery, a petroleum terminal and the Qenos and Huntsman chemical plants. The summarised results for 3-min average measurements show an average of 2.7 ppb with a maximum 3-min average of 39 ppb. The maximum hourly average is given as 30 ppb. Measurements from four other locations show averages of 0.7 (0.2-2.5); 0.7 (0.2-1.2); 0.9 (0.3-1.6); and 0.7 (0.3-1.6) ppb (timing not specified).

Unpublished data from the Victorian EPA show benzene concentrations from 0.3-8.1 ppb in 58 air samples collected within 400-2200 m from the Huntsman facility in April-June 1997. In samples collected with a wind switch directional sampling system on four separate days at a point 600 m from the plant, benzene levels ranged from 1.5-6.4 ppb during downwind periods, with upwind (background) concentrations ranging from 0.5-4.2 ppb.

These monitored results are higher than those estimated above for butadiene rubber and phenol manufacture, but are within the range estimated for other benzene-emitting industries in the area.

15.1.5 Fossil fuel burning for power generation

The greatest sources of fossil fuels for electricity generation are black coal, brown coal and natural gas (NPI, 1999d). Electricity generation in Australia for 1996/97 saw 26% come from burning of brown coal and 58.5% from black coal (ESAA, 1999).

The combustion processes in fossil fuel power generation lead to the coincidental production of a number of NPI category 1 substances, including benzene. Generally, this coincidental production will be below NPI threshold levels. However, in the NPI emission estimation technique manual for fossil fuel electricity power generation, some emission data are available (NPI, 1999d). This manual provides an emission factor for benzene of 6.5×10^{-4} kg/t coal burnt for electricity generation.

On this basis, the NPI has estimated emissions for the largest Australian power station sites utilising black and brown coals. The largest Australian power stations utilising black coal are Eraring and Bayswater in New South Wales. These power stations use 5-6 million t of coal per annum. In estimating the emissions, a conservative assumption of 10 million t per year black coal was adopted, providing a maximum site emission estimation of 6.5 t benzene (NPI, 1999d). For brown coal, the largest single facility is Loy Yang Power, located in Victoria. This site uses an average of around 20 million t of brown coal per annum. The total emissions estimated by NPI assumed consumption of 25 million t brown coal per annum, resulting in an estimated emission of 16.3 t benzene (NPI, 1999d).

Table 15.5 shows the estimated concentrations assuming emissions occur on 365 days of the year and applying the usual formula to determine a local PEC_{air} at points 100 m from the above model power plants.

Table 15.5: Predicted environmental concentrations of benzene in air at 100 m from fossil fuel power generation plants

Fuel type	Coal consumption (kt/y)	Benzene emitted (kg/y)	Daily release (kg)	PEC (mg/m^3)	PEC (ppb)
Brown coal	25,000	16,300	45	0.013	3.9
Black coal	10,000	6500	18	0.005	1.6

15.1.6 Other point sources

Landfills

USEPA (1998b) provides an emission estimation technique for determining benzene emissions from municipal solid waste landfills. The rate of benzene emissions from a landfill is governed by gas production and transport mechanisms. Production mechanisms for benzene will include biological decomposition or chemical reaction. Transport mechanisms include transportation of benzene in its vapour phase to the surface of the landfill, through the air boundary layer above the landfill and into the atmosphere.

Uncontrolled benzene emissions from a landfill may be estimated by determining the uncontrolled methane generation rate, using this to determine the uncontrolled benzene emission rate and finally, using this to calculate the uncontrolled benzene mass emission rate.

The uncontrolled methane volumetric generation rate may be estimated for individual landfills by using a theoretical first-order kinetic model of methane production (developed by USEPA), from the equation $Q_{CH4} = L_0R(e^{-kc}-e^{-kt})$, where Q_{CH4} = methane volumetric generation rate at time T, m^3/y; L_0 = methane generation potential, m^3 methane per t refuse; R = average annual acceptance rate of degradable refuse during active life (t/y); k = methane generation rate constant, yr^{-1}; c = time since landfill closure (0 for active landfill); and t = time since initial refuse placement (years). Default values for L_0 and k are provided by USEPA. An L_0 value of 125 m^3/t refuse is stated as appropriate for most landfills. Values for k depend on moisture levels, with higher values (a suggested value is given of 0.05/y) appropriate for areas with normal or above normal precipitation. For landfills with drier waste, a k value of 0.02/y is more appropriate. For the US, an average k value is 0.04/yr. This will be used as a default value.

Information from the Australian Waste Data Base (EA, 1999a) provides average waste generation per person for selected states. The highest waste producing state was Western Australia with an average of 1.61 kg/person/day municipal waste generated over the 1995-1997 period. This compared to 1.12 kg/person/day over a six-year period (1990/91-1995/96) in New South Wales, and 1.07 kg/person/day over a 4-year period (1992/93-1995/96) in Victoria. Only municipal wastes have been considered as they are expected to be highest in degradable material.

For a worst case 'model' landfill, it will be assumed the landfill services a population of 150,000 and disposal is at a rate of 1.6 kg per person per day, 365 days of the year, giving an annual waste input into the landfill of around 88,000 t municipal waste. The landfill will be assumed to have commenced operation 10 years ago and still be in operation.

Applying these inputs to the above formula, the methane volumetric generation rate can be estimated to be 3,630,000 m^3 per annum.

Based on the methane volumetric generation rate, a volumetric emission rate for benzene can now be estimated using the equation $Q_{BZ} = 2Q_{CH4} \times C_{BZ}/(1 \times 10^6)$, where Q_{BZ} = benzene volumetric emission rate (m^3/y); Q_{CH4} = methane volumetric emission rate (m^3/y); and C_{BZ} = benzene concentration in landfill gas in ppm (USEPA, 1998b).

This model assumes that approximately 50% of landfill gas is methane. USEPA provides emission concentrations of benzene based on a landfill site's history of co-disposal with hazardous wastes. Their analysis indicates that benzene emissions vary with the amount of hazardous waste co-disposed and provides the following emission concentrations:

Type of waste disposed	Emission concentration (ppm)
Municipal waste co-disposed with hazardous waste	24.99
Municipal waste, unknown history of co-disposal with hazardous waste	2.25
Municipal waste only	0.37

With the exception of incinerators for hospital waste, there are no incinerator facilities in Australia for disposing of hazardous or municipal waste, so the main route for disposal of hazardous waste is to landfill. For this assessment, as a worst case, it is assumed that municipal waste is co-disposed with hazardous waste. Thus, a benzene emission concentration of 24.99 ppm will be used.

Applying this to the benzene volumetric emission formula above, the 'model' landfill is estimated to produce 181 m^3 benzene per annum. Based on this, the uncontrolled emission rate of benzene in kg/y can be estimated by the equation $I_{BZ} = Q_{BZ} \times 78.11/(8.205 \times 10^{-5}\ m^3.atm/(mol.°K))(1000\ g)(273 + T)$, where I_{BZ} = uncontrolled benzene mass emission rate, kg/y; Q_{BZ} = benzene volumetric emission rate, m^3/y; T = temperature of landfill gas (°C) with a default value of 25°C; and 78.11 = molecular weight of benzene (USEPA, 1998b). This gives an estimated annual emission of benzene from the 'model' landfill of 580 kg per annum.

Assuming emission over 365 days per annum, and applying the formula to determine a PEC_{air}, then at points 100 m from the landfill, the concentration of benzene in air can be expected to be 4.4×10^{-4} mg/m^3 or 0.14 ppb. As very little hazardous waste is likely to be co-disposed in Australia, this is very much a worst-case estimate.

Monitoring undertaken at the Castlereagh Waste Management Centre revealed mean benzene concentrations of 0.4-1.5 ppb at 7 sampling areas. The minimum reading was 0.3 ppb with a maximum level of 2.3 ppb detected (Dean et al, 1996). These readings are higher than the PEC determined above. However, these measurements fall within the expected urban background concentration levels for benzene (Section 15.2.2).

Waste incinerators

In the absence of data on benzene emissions from waste incinerators such as those used for hospitals and of relevant emission estimation techniques, meaningful calculations cannot be performed.

15.1.7 Summary

Table 15.6 summarises the predicted environmental concentrations of benzene in air at points 100 m from the point sources discussed above.

Benzene release from point sources is highest for coal gas by-product and coke oven plants. Coal tar distilleries and petroleum and alumina refineries may also be expected to show significant levels of benzene in surrounding air.

Table 15.6: Summary of predicted environmental concentrations of benzene in air at 100m from various point sources

Point source	PEC (mg/m^3)	PEC (ppb)
Petroleum industry		
• refineries	0.004-0.014	1.3-4.2
• terminals	0.002 (average) 0.014 (maximum)	0.6 (average) 4.2 (maximum)
Steel and associated industries		
• Coal coking	0.076	23.3
• Coke gas processing	0.04-0.86	11-270
• Coal tar distillation	0.025	7.7
Aluminium industry	0.019	6.1
Chemical industry		
• Butadiene rubber manufacture	4×10^{-6}	0.001
• Styrene manufacture	0.0054	1.6
• Phenol manufacture	1.6×10^{-4}	0.06
Fossil fuel burning for power generation	0.005-0.013	1.6-3.9
Landfills	4.4×10^{-4}	0.14

15.2 Diffuse releases to urban air

15.2.1 Emissions estimation

Within an urban environment, there will be many sources contributing to benzene levels in the atmosphere. These include point source releases as well as diffuse emissions from cars, service stations, solid fuel burning and lawn mowing.

Monitoring data for various Australian cities is considered further below. To represent other urban areas of Australia, concentrations will be modelled based upon releases to the atmospheric air column above an Australian model city located inland, with a population of 300,000, at a population density corresponding to Australia's largest city, Sydney.

To determine the land area the urban centre will cover, population density is required. The New South Wales EPA provides a 1996 population for Sydney of around 3,820,000 people. The Sydney area covers 1548 km^2 (EPA New South Wales, 1999), giving a population density approaching 2500 people/km^2. The method for determining the volume of atmospheric air columns is described in Connell & Hawker (1986). With a population of 300,000 , the urban centre used for this calculation would cover a land area of 120 km^2, with an atmospheric air column volume of 7.38×10^{11} m^3.

Service stations

According to AIP (2000), there are 8233 service stations in Australia. The model population of the model urban environment is around 2% of the Australian total. Assuming a *pro rata* allocation for service stations, this equates to around 165 service stations in the urban centre.

The NPI draft emission estimation technique manual has been used to estimate benzene emissions from service stations in the urban area (NPI, 1999c). This document provides emission rates for various stages including underground tank filling, tank breathing and emptying, vehicle refuelling and spillage. Different emission rates are suggested for tank filling and vehicle refuelling depending on methods and technology. Based on the NPI manual, this assessment will use the emission rate for submerged filling of 880 mg/L throughput and assume that displacement losses during refuelling are uncontrolled giving an emission rate of 1320 mg/L. These emission factors are for total emissions of VOCs and result in the estimation of aggregated emissions from service stations in the model urban centre shown in Table 15.7.

Table 15.7: Estimated emission of volatile organic chemicals (VOC) from service stations

Emission source	Emission factor (mg/L)	Throughput (ML/y)	VOC emissions (kg/y)
Underground tank filling	880		
Tank breathing/emptying	120		
Vehicle refuelling	1320		
Spillage	80		
Total	2400	360	864,000

NPI speciation data for VOCs emitted at service stations show benzene to be 0.9% by weight of petrol vapour. Therefore, of these emissions, 7776 kg per annum will be emitted as benzene.

Petrol engine exhaust

Benzene in petrol engine exhaust emissions is a combination of unburned benzene originally present in fuel and benzene produced as a result of incomplete combustion of other petrol components. Non-benzene aromatics in fuels can cause around 70-80% of the exhaust benzene formed and some also forms from engine combustion of non-aromatic fuel hydrocarbons (USEPA, 1993). As such, vehicles using benzene-free fuel such as LPG and diesel may still release benzene in exhaust emissions.

The quantity of benzene in exhaust varies depending on control technology and fuel composition. A catalytic converter is a device that uses a catalyst such as platinum and palladium to more fully complete the burning or oxidation of the fuel as it leaves the engine. These unburned exhaust emissions comprise hydrocarbons including benzene (in the form of unburned petrol), carbon monoxide (formed by the combustion of fuel) and nitrogen oxides (created when the heat in the engine forces nitrogen in the air to combine with oxygen). The catalyst helps to convert carbon monoxide into carbon dioxide. It also converts the hydrocarbons into carbon dioxide and water and the nitrogen oxides back into nitrogen and oxygen.

Based on the 1996 census, Canberra, which has a population similar to the model urban centre, has 118,700 households and 188,900 registered motor vehicles (predominantly passenger vehicles, but includes light commercial vehicles). These figures will be assumed for the model urban environment. Data collected by the Australian Bureau of Statistics in 1995 indicate an average distance travelled of 15,200 km per annum (including vehicles which did not travel any distance). Duffy et al. (1999) provide measurements of benzene emissions from Australian cars and state that pre-1986 cars have an average emission of 132 mg/km, while post-1985 cars average 41 mg/km benzene emission.

The Motor Vehicle Census for Australia (ABS, 2000a) was used to determine the number of vehicles expected to have control technology (catalytic converters) to those that do not. This census gives data up to the end of October 1999, and lists vehicles by fuel type, vehicle type and age. The data were divided into various age classes with any vehicle in or before the 1983-1986 age group being assumed to not have control technology.

Considering figures for petrol driven passenger motor vehicles and light commercial vehicles (these two vehicle types accounted for around 92% of the vehicle fleet), it was determined that in the order of 35.3% of the vehicles in the model urban centre would not have control technology (that is, we assume they will produce emissions in line with pre-1986 cars given above). The following emissions were calculated:

Catalytic converters	No. of vehicles	Total km/y	Emissions (kg/y)
Yes	122,218	1.86×10^9	76,166
No	66,682	1.01×10^9	133,790
Total			209,956

Under the *Fuel Quality Standards Act 2000*, the Commonwealth Government is establishing national standards prescribing a range of characteristics for petrol and diesel. Federal Cabinet has agreed that there will be a maximum benzene concentration in petrol of 1% v/v from January 1 2006. Modelling, based on an earlier proposal to reduce benzene content to 1% by 2005, predicted this would lower total benzene emissions (evaporative + exhaust) by 29% in the year 2010 (Environment Australia, 2000). Reducing the benzene content of petrol by two-thirds does not equate to a similar reduction in total benzene emissions because benzene exhaust emissions are to a large extent independent of benzene levels in petrol.

Lawn mowing

It is assumed for worst case purposes that all dwellings have lawns. NPI has an emission estimation technique manual for aggregated emissions from domestic lawn mowing (NPI, 1999a). This document provides averages for percentages of 2-stroke and 4-stroke machines and mowing times per annum per household depending on fuel type. Estimated emissions of benzene are provided for each fuel type. Based on 118,700 households, the following emissions of benzene from domestic lawn mowing can be estimated following the NPI guidelines. As for petrol engine exhaust above, the anticipated lowering of benzene in fuel to 1% will lead to expected reductions in benzene emissions.

Engine type	Petrol type	Per cent of mowers	No. of mowers	Mowing time (h/y)	Emission factor (g/h)	Emission (kg/y)
2-stroke	Leaded	22	26,114	443,938	17	5330
	Unleaded	27	32,049	512,784	17	8720
4-stroke	Leaded	18	21,366	384,588	2.3	885
	Unleaded	26	30,862	462,930	2.3	1065
Total						16,000

Industry benzene emissions

The model urban centre will be assumed to have benzene emitting industries such as a power generator and a petrol terminal. According to the estimated releases shown in Tables 15.2 and 15.6, these sources would provide an average yearly emission of around 30 t, based on the highest values. While this obviously depends on industry type and control factors, it will be used for this assessment as a worst case assumption.

Domestic solid fuel burning

While estimation techniques have been developed (NPI, 1999b), as yet no survey data appear available to determine quantities of wood burnt in different heater types or consumption of timber for heating purposes. UK data indicate that the combustion of fuels contributes around 2% of total benzene emissions (Wadge & Salisbury, 1997). This will be assumed to hold for the current calculation. Total annual emissions from other uses estimated above amount to around 273 tonnes, indicating a further 5580 kg are released through domestic solid fuel burning.

15.2.2 Predicted environmental concentration in urban air

The calculated daily releases within the model urban centre are summarised in Table 15.8.

Table 15.8: Predicted daily releases of benzene within a model urban centre

Emission source	Approximate annual emission (kg)	Average daily release (kg)	Average daily release (%)
Service stations	7800	21	2.9
Vehicle exhaust	209,956	575	78
Lawn mowers	16,000	44	6
Industry	30,000	82	11.1
Domestic solid fuel burning	5600	15	2
Total	269,356	737	100

The atmospheric component for the model urban centre is defined as 7.38×10^{11} m^3. With 737 kg entering the atmosphere daily, this equates to a concentration of 1 $\mu g/m^3$, or 0.31 ppb. This applies to a ground area of 120 km^2, with a population density of 2500 people/km^2.

This calculation only provides a daily contribution to the atmosphere and does not account for breakdown and dispersion of benzene or accumulation in the atmosphere through release occurring every day. Assuming an atmospheric half-life of 8 days (Section 8.2.1) and no dispersion from the atmospheric column above the urban centre to the surrounding atmosphere, the concentration of benzene in the atmosphere is calculated to stabilise at around 3.4 ppb. This may be considered an

overestimation, as dispersion from the atmospheric column above the urban centre will occur. However, for the purposes of this assessment it will be used as a worst case average urban atmospheric PEC.

As explained above, under the *Fuel Quality Standards Act 2000*, the maximum benzene concentration in petrol will be lowered to 1% from January 1 2006. This will lead to a significant reduction in benzene emissions, due to lower emissions from vehicles and lawn mowers and reduced releases from petroleum refineries and terminals, which will affect the PEC calculation for benzene in urban air.

Australian monitoring data

Table 15.9: Reported concentrations of benzene in urban air in Australia (from EPA Victoria (1999) and DEP Western Australia (2000))

	Concentration (ppb)	
Location and period*	**Average**	**Maximum**
Adelaide, Edwardstown (industrial) 1994	19	77
Adelaide, North Terrace (CBD) 1994	8	26
Brisbane, 3 residential areas	0.62	1.67
	0.502	1.41
	0.328	0.78
Goat Island, NSW, downwind of refinery 1979	2.6	No data
Latrobe Valley, VIC 1987/88 (summer)	~1	No data
Melbourne, Altona 1991	7.9	20
Melbourne, CBD 1983/84[1]	22.3	37.8
Melbourne, CBD 1990	15.7	19.8
Melbourne, CBD 1991 (?)	~16	No data
Perth and Kwinana, urban background 1993/94	1.63	No data
Perth, Darling Scarp 1993/94	0.45	No data
Perth, Gooseberry Hill 1997/98	0.15	0.21
Perth, Kwinana, plume 1993/94	4.7	No data
Perth, metropolitan area 1997/98	1.44	17.6
Perth, North Fremantle 1997/98	0.37	0.54
Perth, smog 1993/94	1.9	No data
Sydney, Cahill Tunnel (peak hour) 1991 (?)	7-38	38
Sydney, George Street, summer 1994	4.1	5.2
Sydney, George Street, winter 1994	7.6	9.5
Sydney, near point sources 1992 (summer)	2.5	6.8
Sydney, suburban 1995:		
• Castlereagh Llandilo Road	0.4	No data
• Castlereagh Northern Road	0.5	No data
• Earlwood	4.72	No data
• Lidcombe	1.63	No data
• North Ryde	0.90	No data
• Randwick	2.40	No data
• Rozelle	1.29	No data
• Westmead	4.90	No data

*Results may not be comparable because of differences in sampling and analytical methodology

[1] CSIRO, 1995

The Victorian EPA has undertaken a review of monitoring studies performed in Australia and New Zealand on hazardous air pollutants, which was published in 1999 (EPA Victoria, 1999). In addition to this, a recent document provides monitoring for various air toxics including benzene in Perth (DEP Western Australia, 2000). The studies outlined in these documents are summarised in Table 15.9. All figures have been converted to ppb.

Comparison of modelled urban atmospheric PEC to monitored data

The level of 3.5 ppb for a model urban centre derived above is within the range of results reported through monitoring studies in Australian urban centres.

15.3 Indoor air concentrations

15.3.1 Homes

In general, benzene concentrations in the home are higher than the corresponding outdoor level. Table 15.10 summarises the results of several studies comparing average airborne concentrations of benzene in homes with corresponding outdoor levels.

Table 15.10: Summary of studies comparing average airborne concentrations of benzene in homes with corresponding outdoor levels

Location	Region or area	Benzene (ppb)		Ratio	Reference
		Indoors	Outdoors		
Alaska, USA	Remote	6.2	2.5	2.5	Goldstein et al. (1992), as cited in Wallace (1996)
Antwerp, Belgium,	Urban	9.4	4.4	2.1	Cocheo et al. (2000)
Arizona, USA	State-wide	0.5	0.4	1.3	Robertson et al. (1999)
Athens, Greece	Suburban	10.1	20.7	0.5	Cocheo et al. (2000)
Bristol, England	Urban	2.5	1.6	1.6	Brown & Crump (1996)
California, Maryland and New Jersey, USA	State-wide	3.1	1.8	1.7	Wallace (1996)
California, USA	Rural	1.1	0.4	2.8	Sheldon et al. (1991), as cited in Wallace (1996)
Copenhagen, Denmark	Urban	4.5	3.1	1.5	Cocheo et al. (2000)
Erfurt, Germany	Urban	1.2	0.9	1.4	Schneider et al. (1999)
Hamilton, Canada	Urban	1.7	1.2	1.4	HAQI (1997)
Hannover, Germany	Urban	1.0	2.9	0.3	Levsen et al. (1996)
	Semi-rural	0.7	0.3	2.3	
Hertfordshire, England	Semi-rural	3.7	2.2	1.7	Brown & Crump (1996)
Huddersfield, England	Suburban	0.5	0.4	1.4	Kingham et al. (2000)
Melbourne, Australia	Suburban	1.0	0.6	1.7	Brown (2000)
Mumbai, India	Urban	11.6	13.5	0.9	Srivastava et al. (2000)
Munich, Germany	Urban	0.8	0.8	1.0	Gebefügi et al. (1995)
Murcia, Spain	Urban	12.3	11.7	1.1	Cocheo et al. (2000)
Nancy, France	Urban	3.3	1.4	2.4	Gonzalez-Flesca et al. (1999)
Padua, Italy	Urban	7.0	8.0	0.9	Cocheo et al. (2000)
Rouen, France	Urban	9.5	4.7	2.0	Cocheo et al. (2000)
Rotterdam, Holland	Urban	2.0	0.9	2.2	Lebret et al. (1986)
Windsor, Canada	Urban	1.0	0.8	1.3	Bell et al. (1994)

The predominant finding of higher benzene levels indoors than outdoors is attributed to specific sources located within or in the immediate vicinity of the

home and/or the inability of these emissions to escape (Wallace, 1989). These include environmental tobacco smoke (ETS), heating and cooking systems, cooking fumes, evaporation from products and materials used in the home, and drift of vapours from attached garages or external sources of benzene.

Longitudinal studies of individual homes have shown that contributions from domestic sources are overlaid on those from outdoor air, which generally account for most of the seasonal and diurnal variations in indoor benzene levels (Gebefügi et al, 1995; Lioy et al, 1991; Thomas et al, 1993). However, indoor air levels also depend on the proximity to road traffic and the degree of ventilation, which in turn is dependent on diurnal, seasonal and climatic conditions (Levsen et al, 1996; Gilli et al, 1996; Cocheo et al, 2000). Ambient air concentrations are lowest during the night. However, if houses are closed up at night, either for safety reasons or in colder climates, especially during winter, benzene levels acquired during the day are unable to escape. Therefore, the total exposure over 24 h is increased relative to outdoor levels. During summer, and in warmer climates, when ventilation is greater, indoor concentrations are similar to outdoor concentrations.

In addition to ventilation patterns, the types of furnishing materials may influence the amount of benzene retained in the home. A study of benzene exposure in European cities found that, while urban pollution levels increased from north to south, the ratio of indoor to outdoor air levels were lower in southern European urban areas than in the north (Cocheo et al, 2000). One explanation proposed by the authors is that volatile organic pollutants, including benzene, from both indoor and outdoor sources may be trapped in absorbent fabrics and other materials, resulting in higher residual levels in houses that have more carpets, wood, linoleum and soft furnishings, as do houses in cold climates.

In the absence of localised sources inside the home (such as proximity to an attached garage, room with smokers), there are no significant differences in benzene concentrations between kitchens, living rooms and bedrooms, height above floor level, or older versus newer apartments (Schneider et al, 1999).

Approximately 85% of ETS comprises sidestream smoke emitted by the smouldering end of a cigarette (cigar or pipe, as the case may be), with lesser contributions from the mainstream smoke that active smokers directly inhale and exhale. The emission factor for sidestream smoke is in the range of 300-500 μg benzene per cigarette, with little variability between brands (Daisey et al, 1998; Miller et al, 1998). In two studies conducted in 200-300 homes with and without smokers in Germany and USA respectively, the average increase in benzene levels in homes with one or more smokers was 4.0 $\mu g/m^3$ or 1.2 ppb (Wallace, 1996). Surprisingly, an international study of exposure to tobacco smoke reported that fixed-site measurements in an unspecified number of homes in Sydney found slightly higher indoor air concentrations of benzene (median: 1.1 ppb) in non-smoking houses than in those where cigarettes, pipes or cigars were smoked within the communal areas (median: 0.9 ppb) (Phillips et al, 1998).

Herbal cigarettes, marijuana, incense sticks and mosquito coils produce benzene emissions similar to cigarettes, about 0.4-0.5 mg/g material burnt (Löfroth et al, 1991). However, the frequency of burning such articles is much less than for tobacco cigarettes and their contribution to indoor benzene levels is expected to be minimal.

Combustion of wood produces approximately 400 mg benzene for every kg dry wood burned or 114 μg benzene per kJ heat produced compared to 9 μg/kJ for

kerosene and 2 μg/kJ for LPG. Per-meal emissions from unflued cookstoves, such as a caravan or enclosed barbecue, are estimated to be 36 μg.h/m^3 (LPG), 124-268 μg.h/m^3 (kerosene), 316 μg.h/m^3 (charcoal) and 1220 μg.h/m^3 (wood) (Zhang & Smith, 1996). In actively burning fires, most of the combustion products go up the chimney. However, even well constructed open fireplaces are subject to downdrafts and smoke drift, especially when the fire is low. In addition, high outdoor benzene levels in smoke produced by wood fires can re-enter homes, particularly in areas subject to temperature inversions during winter.

Volatile emissions from cooking oils have been studied by Pellizzari et al. (1995) and Shields et al. (1995) who found that emissions of benzene in wok cooking vapours were higher from unrefined rapeseed oil than from peanut, soybean and canola oils (2.4 compared to 0.2, 0.5 and 0.7 ng/mL respectively in one experiment). Wok cooking is generally carried out at very high temperatures (275-280°C). Reducing the temperature of cooking from 275°C to 185°C reduced benzene emissions by sevenfold (Shields et al, 1995). However, given the average use of cooking oil in the home (about 50 mL per day), this source would not contribute measurably to benzene levels in the indoor atmosphere.

Some petroleum-based domestic products such as oil-based paints, particleboard and adhesives may contain very low concentrations of benzene as an impurity. The contribution to indoor air levels of benzene from these sources has not been measured, but is expected to be low. In New Zealand, Stevenson & Narsey (1999) found that redecorating activities increased the indoor concentrations of toluene and xylenes, but not benzene. Brown (2000) did not identify significant sources of benzene release in new and renovated buildings in Melbourne.

Indoor air levels of benzene are significantly increased in houses with an attached garage (Brown, 2000; Gebefügi et al, 1995; Levsen et al, 1996; Thomas et al, 1993). Sources of benzene in garages include vapours from cars and lawn mowers and stored petrol or other solvents. In New Zealand, the highest indoor air level for any site investigated (16.6 ppb or 20 times typical ambient air levels) was in a home with smoker occupants and an internal double garage housing two cars (Stevenson & Narsey, 1999). Sampling of 52 private flats in Munich revealed one unusually high indoor concentration (>31 ppb, or >37 times the outdoor concentration) which was attributed to infiltration from a garage (Gebefügi et al, 1995). Benzene concentrations in four attached garages in the USA ranged from 1-61 ppb; the mass transfer from sources in the garage to living areas in three of these homes ranged from 730-26,000 μg/h (Thomas et al, 1993). In Alaska, where petrol contains 5% benzene compared to ≤1.5% in other US states, garages were found to have benzene air levels ranging from 19-350 ppb (Gordian & Guay, 1995). Removal of a car, lawn mower, petrol canister and solvents from a garage in Hannover, Germany, reduced the benzene concentration in the garage from 25 ppb to 0.4 ppb (Levsen et al, 1996).

The measured indoor air levels shown in Table 15.10 refer to one Australian and a number of overseas localities which are difficult to compare, let alone extrapolate to the Australian continent as a whole, because of differences in a number of factors such as ambient benzene levels, climate, construction methods, heating systems, ventilation practices, smoking occupancy and the prevalence of homes with attached garages. It is nonetheless reasonable to assume that the average indoor to outdoor ratio in urban environments in Australia is close to the median of the available Australian and overseas data excluding remote, rural and semi-rural regions and areas, or 1.4 (range: 0.3-2.4).

The PEC_{air} in the model Australian urban environment described in section 15.2.2 is 3.4 ppb. Therefore, the estimated indoor air level taken forward for public exposure assessment is 1.4 x 3.5 = 4.8 ppb.

15.3.2 Non-residential buildings

Measurements of benzene in offices, shops, classrooms, hotels, bars, restaurants, theatres and other non-residential indoor environments are limited compared to homes. Available literature data giving indoor to outdoor concentration ratios are presented in Table 15.11. The highest ratios were due to specific sources such as petrol vapours and engine exhaust at a motocross event or tobacco smoke in bingo halls and taverns. Of these, emissions from indoor use of motor vehicles are not expected to contribute measurably to average indoor air levels of benzene, whereas tobacco smoke may have a significant impact, depending on smoking policies and occupancy, volume of space, rate of ventilation, heating, air-conditioning and air cleaning systems.

Table 15.11: Benzene air concentrations in non-residential buildings

		Ppb			
Location	**Building or venue**	**Indoors**	**Outdoors**	**Ratio**	**Reference**
California, USA	Portable classrooms	0.5-0.6	0.5-0.6	1	SUSD (1999)
Manila, Philippines	Office	0.4	1.1	0.4	Reverente et al. (1996)
	Restaurant	5.3	14	0.4	
	Shopping centre	1.2	3.9	0.3	
Melbourne, Australia	Gymnasium	0.6-1.9	0.8	0.8-2.4	Brown (2000)
Mumbai, India	Offices, smoking	15.1	9.3	1.6	Srivastava et al. (2000)
	Research library	10.7	11.4	0.9	
Sydney, Australia	Entertainment centre (motocross event)	209-334	2.3*	90-150	Angove et al. (2000)
Washington DC, USA	Library of Congress	2.1	1.9	1.1	NIOSH (1996)
Windsor, Canada	Offices, non-smoking	1.0	0.8	1.3	Bell et al. (1994)
	Hotel rooms	1.2	0.8	1.5	
	Bingo halls	6.4	0.8	8.0	
	Taverns	10.7	0.8	13	

* Sample taken inside building prior to the motocross event.

Other Australian data include a study conducted in Hobart, Tasmania, which found indoor air levels of benzene ranging from 0.9-7.8 ppb in eleven office buildings in the central business district (Mesaros, 1998). However, these buildings were selected for investigation because a very high number of their occupants suffered from typical sick building syndrome symptoms.

In the absence of sufficiently representative measured data, it is reasonable to assume that the concentration of airborne benzene in non-residential buildings in Australia is similar to the estimated indoor air concentration in Australian homes, except in restaurants, bars and the like with high levels of ETS.

As benzene and nicotine are both found in the vapour phase of cigarette emissions, nicotine concentrations can be used as an indicator of relative benzene exposures. A recent review of ETS exposure quoted typical nicotine air levels of 1-3 $\mu g/m^3$ in residences, 3-8 $\mu g/m^3$ in restaurants, and 10-40 $\mu g/m^3$ in bars (Hammond, 1999). As such, the additive effect of ETS in restaurants and bars where smoking is

permitted could be higher than in the homes of smokers by a factor of 2.7 (8/3) and 13 (40/3) respectively. The indoor air concentration of benzene in Australian homes is estimated at 4.8 ppb (Section 15.3.1) and the average additive effect of ETS in homes with smokers at 1.2 ppb (Wallace, 1996). Therefore, the estimated benzene levels in restaurants and bars with smoking occupancy are 8.0 and 21 ppb respectively. These are higher than the measured values shown in Table 15.11, but are considered reasonable worst case estimates.

15.3.3 Motor vehicles and other means of transportation

Several studies conducted in a number of locations have consistently found that road users including drivers and passengers in all kinds of vehicle are exposed to higher levels of benzene than background air quality data may suggest (Taylor & Fergusson, 1997).

In parked vehicles, the excess benzene concentration is due to evaporative emissions from the vehicle itself. Among others, these depend on the benzene content and vapour pressure of the fuel, the fuel system, engine temperature, ambient weather conditions, and the degree of enclosure of the passenger cabin. In vehicles in traffic, however, part of the excess is due to the intake of exhaust fumes from the tailpipes of preceding vehicles. As such, it depends not only on the above factors, but also on traffic level, flow, and mix in terms of cars with or without catalytic converters, trucks, buses and motorcycles. Generally, the excess would be minimal in new cars with fuel injection travelling at high speed in light traffic on a windy day with vents, windows and sunroof tightly closed and the air-conditioning on. Conversely, it would be maximal in old, carburetted, poorly maintained cars that travel slowly in congested traffic on a still day with vents and windows open and fans on. High benzene levels may also build up in vehicles left in undercover car parks or transiting tunnels and during refuelling at petrol stations (Duffy & Nelson, 1997; Taylor & Fergusson, 1997).

Table 15.12 summarises the available Australian and New Zealand studies that have measured the concentration of benzene inside moving vehicles during city commutes.

Table 15.12: Summary of Australian and New Zealand studies of in-vehicle benzene air concentrations during city commutes

Location	Conditions	No. of commutes	Mean benzene level (ppb)	Reference
Private cars				
Auckland	Catalyst-equipped car, summer	2	12	Stevenson & Narsey (1999)
	Catalyst-equipped car, winter	2	20	
Melbourne	Post-1986 car	1	13	Torre et al. (1998)
	Post-1986 cars	5	10	Torre et al. (2000)
Sydney	Pre-1986 car	4	48	Duffy & Nelson (1997)
	Post-1986 cars	8	22	
Public transport				
Melbourne	Tram	1	7.7	Torre et al. (1998)
	Train	5	1.6	Torre et al. (2000)
Sydney	Diesel bus, air-conditioned	3	5.9	Duffy & Nelson (1997)
	Diesel bus, not air-conditioned	4	8.8	

Average air levels were approximately 15 ppb and 48 ppb in cars with and without catalytic converters respectively. Weighting the latter according to the estimated number of post-1985 vehicles in the model urban environment described in Section 15.2.1 gives an estimated average benzene concentration in motorcars of about 28 ppb. This is higher than levels measured in the USA where the car fleet is newer and petrol contains less benzene than in Australia, but within the range reported from Europe and Korea (Jo & Park, 1999; Rodes et al, 1998; Taylor & Fergusson, 1997).

In trams and buses, benzene levels averaged 7.5 ppb. The lowest level was found in trains, indicating that rail commuters are unlikely to be exposed to benzene levels exceeding those in ambient air.

In Melbourne, personal monitoring of two individuals commuting by foot or bicycle showed benzene exposure levels of 4.4 and 12.2 ppb respectively, compared to a concentration of 6.0 ppb at a roadside point along their route (Torre et al, 1998). Overseas data reviewed by Taylor & Fergusson (1997) suggest that pedestrians and cyclists typically are exposed to two times ambient air levels. This would equate to an average roadside concentration of 6.8 ppb in the model Australian urban environment described in Section 15.2.2, where the predicted atmospheric concentration of benzene is 3.4 ppb.

15.4 Concentrations in water and soil

15.4.1 Water

Predicted environmental concentration (local PEC)

Some release to wastewater may be expected to occur during benzene production. Within the sewage treatment plant (STP), 86% of benzene will be removed in the following manner, assuming benzene is inherently biodegradable and based on the SIMPLETREAT tables provided in TGD (EC, 1996): 70% in air, 1% in sludge and 15% by degradation. This leaves 14% available in the water column on release to receiving waters. This level of removal is supported by a report in IUCLID where benzene levels in the influent and effluent from a municipal STP in The Netherlands in 1989-1991 averaged 3.9 and <0.5 μg/L respectively. This suggests removal of >87%.

By far the highest producer of benzene (excluding incidental production through petrol combustion in motor vehicles) is the petroleum industry. Table 7.3 shows that in Australia in the order of 440 kilotonnes per annum benzene is either extracted, manufactured or imported. NPI data for the 1998-99 reporting year show that only two refineries reported releases to water, namely 1100 kg from the Mobil refinery in South Australia and 120 kg from the BP refinery in Queensland. The calculated benzene production at the Mobil refinery in South Australia is 82 kt per annum (Table 15.1). As such, the release of 1100 kg (which was derived through a combination of measured data, engineering controls and emission estimation) suggests a release of 0.0013%

Using the maximum reported release of 1100 kg per annum and assuming release to a sewage treatment plant with a daily outflow of 250 ML and production on 300 days per annum, the $PEC_{local\text{-}surfacewater}$ can be estimated as follows: Production = 82,000 t; days of operation per annum = 300; daily production = 273 t; daily release

to STP = 273,000 kg x 0.000013 = 3.7 kg; removal from STP = 3.15 kg; concentration in STP = 2 μg/L; $PEC_{local\text{-}surfacewater}$ = 0.2 μg/L (dilution of 10:1).

A continental PEC will not be considered for this assessment, as there are insufficient data to estimate the full extent of incidental benzene production and release around the country. However, the continental PEC would be expected to be lower than that derived for local conditions.

Comparison with measured data

Due to its high volatility and low residence time in water, benzene would not be expected to be detected at significant levels in surface waters. The 1996 Australian drinking water guidelines state that benzene has not been detected in Australian drinking water (NHMRC, 1996).

International data provided in IPCS (1993) are summarised in Table 15.13.

Table 15.13: International data on benzene levels in water (IPCS, 1993)

Source	Country	Concentration (μg/L)	Comments
Rainwater	UK	87.2	Appears high for unknown reason(s)
	Germany	0.1-0.5	
Surface water	USA	0.004-0.9	Downriver chemical plant outfall
	USA (13 locations)	1-13	Both upstream and downstream near industrial outfall
	USA (Potomac River)	<2	Detection limit 2 μg/L
	Switzerland (Lake Zurich)	0.03	
	UK (80 water bodies)	7.2	Average of 61of 154 samples above the detection limit of 0.1 μg/L
	Netherlands (Rhine River)	<0.1	Sampling in 1979
	Germany	<0.1-1	Occasionally up to 200 μg/L
Sea water	Gulf of Mexico	0.005-0.015	Unpolluted waters, sampling in 1977
	USA (Brazos River estuary)	0.004-0.2	Flows into Gulf of Mexico
	Atlantic Ocean	0.06×10^{-3}	Open sea
	Baltic Sea	$0.1\text{-}4.6 \times 10^{-3}$	Open sea
Ground water	USA	1.6	63 private wells, 3.2% of samples contained benzene
	Germany	0.02-0.005	
	USA	30-300	Contaminated well water
	Netherlands	0.005-0.03	Unpolluted areas

Compared to these overseas river water measurements, the calculation for an Australian PEC in surface water may be slightly less than expected. However, it is considered realistic for the purposes of this assessment.

Benzene was detected in 37 of 987 bottom sediments in Japan at levels of 0.5-30 μg/kg. Lake Pontchartrain in USA showed sediment levels of 8-21 μg/kg in 1985. Between 1980-82, benzene was detected in 9% of sediment samples taken from 335 observation sites in USA, the median level being <5 μg/kg (IPCS, 1993).

15.4.2 Soil

NPI data show that the highest reported benzene release to soil is 45 kg per annum from a bulk petroleum storage facility. The top five sites where benzene release to land was reported total <80 kg per annum throughout the country. While it is not possible to predict a concentration in soil that would have any meaning, the NPI data indicate that benzene release to soil is likely to be marginal and not result in significant soil contamination.

Some exposure may result though application of sewage sludge, although the full extent of this is not known in Australia. However, it is understood that Sydney Water sends >90% of their sludge (possibly up to 200,000 tonnes per annum) to beneficial use. Some goes to agricultural use and is soil incorporated, while some goes to compost and horticulture where surface application may occur. Measurements of benzene in sewage sludge or its subsequent soil concentration after application to land could not be identified. Additionally, as described above, only in the order of 1% benzene released to a STP is expected to bind to sludge, so sludge application to land is unlikely to provide a high exposure route.

Most measured data on benzene in soil were obtained to determine the extent of direct contamination by spillage or leakage. In soils in the vicinity of five industrial facilities using or producing benzene in USA, benzene levels ranged from <2 to 191 μg/kg, whereas the concentrations in unpolluted soils in The Netherlands were less than those found in ground water, that is <0.005-0.03 μg/L (IPCS, 1993).

15.5 Summary

Table 15.14 summarises the predicted environmental benzene concentrations in air and water, based on the assessment findings discussed above.

Table 15.14: Approximate predicted concentrations of benzene in air and water

			Benzene concentration	
Environment	**Source type**	**Description**	**μg/m^3**	**ppb**
Outdoor air	Point*	Petrol refineries	17.6	5.5
		Petrol terminals	13.4	4.2
		Coal coking plants	91	28.4
		Coal gas by-product plants	860	270
		Coal tar distilleries	25	7.8
		Alumina refineries	19	6.1
		Chemical industry	6.5	2.0
		Fossil fuel burning power plants	13	3.9
		Landfills	0.44	0.14
	Diffuse	Urban atmosphere	11	3.4
		Urban roadside air	22	6.8
Indoor air	Diffuse	Homes and other buildings	16	4.8
		Restaurants, smoking	26	8.0
		Bars, smoking	67	21
		Cars with catalytic converters	48	15
		Cars without catalytic converters	154	48
		Buses and trams	24	7.5
Surface water	Point	Treated oil refinery wastewater	200	0.20

* Maximum predicted concentrations at 100 m from source.

16. Public Exposure

16.1 Direct exposure

Active smoking

In Australia, 23.8% of the adult population are smokers; 27.4% are ex-smokers and 48.9% have never smoked (ABS, 2000b). In a survey conducted in Victoria in 1996-97, the self-reported number of factory-made cigarettes smoked per day averaged 17.8 in regular smokers aged 16 years and over (Trotter et al, 1998).

The quantity of benzene emitted in mainstream cigarette smoke varies from 0.4-104 μg/cigarette; it is proportional to the tar level and is not reduced by cigarette filters (Smith et al, 1997). In 26 brands on the UK market, the yields ranged from 3-60 μg/cigarette, with over half the brands yielding 45-55 μg (Darrall et al, 1998). Based on a value of 50 μg/cigarette, the daily benzene intake of an average smoker (17.8 cigarettes per day) is increased by 0.89 mg per day. For an adult person with a respiration rate of 22 m^3/day, this corresponds to the continuous inhalation of ambient air containing 12.5 ppb of benzene.

Petrol

In Australia, the benzene content of petrol can range from 1-5% v/v. Members of the public may be exposed directly to petrol used as fuel in private vehicles, lawn mowers, outboard engines etc. Although skin contact can occur from accidental drips or spills or the use of petrol for cleaning purposes, it is likely to be infrequent and of short duration. Direct exposure to benzene vapours occurs in garages and undercover parking stations, during petrol pumping at self-service petrol stations and inside parked cars with hot-soaked engines. Reported air levels in these locations vary widely depending on local circumstances, but are typically in the order of 10-100 ppb (Duffy & Nelson, 1996; Leung & Harrison, 1998; Nordlinder & Ljungkvist, 1992; Nordlinder & Ramnäs, 1987; Thomas et al, 1993) and are consistent with Australian data reported in Section 17.1.1.

Other consumer products

Paints, primers, paint strippers, lubricants, abrasives, model and hobby glues and other consumer products may contain organic solvents which in turn may contain very low concentrations of benzene as an impurity (Rastogi, 1993). The contribution to public exposure to benzene from these sources has not been quantified, but is expected to be very low.

16.2 Indirect exposure via the environment

Air

Although benzene is not persistent, it is ubiquitous in ambient air because of its widespread sources of release.

In Australia, measured levels of benzene in air range up to 77 ppb for outdoor urban environments (Table 15.10), 25 ppb in residential buildings (Brown, 2000) and 343 ppb inside petrol-fuelled vehicles (Duffy & Nelson, 1997).

The average atmospheric concentration of benzene in a model Australian urban environment is estimated at 3.4 ppb, with indoor/in-vehicle air levels ranging from 4.9-48 ppb (Table 15.15). These levels would be higher in homes, workplaces and vehicles with smoking occupancy. The contribution of ETS to indoor benzene levels is poorly documented, but is likely to vary considerably depending on local circumstances. In two large studies in Germany and USA, the average increase in benzene levels was 1.2 ppb in homes with one or more smokers (Wallace, 1996).

Drinking water

Contamination of surface water and groundwater can result from removal of benzene from the air in rain, leakage of underground storage tanks, and leaching of oil well and landfill sites.

A number of US studies have reported benzene at levels in the order of 5 ng/L in surface and well waters (Wallace, 1996). In Canada, benzene (detection limit 1 μg/L) was found in 50-60% of drinking water samples from 30 treatment facilities in a national survey, although in provincial monitoring programs it has rarely been detected at concentrations >1 μg/L (Government of Canada, 1993). In Germany, drinking water has been found to contain 18-45 ng/L benzene (GDCh, 1988).

To date, benzene has not been detected in drinking water in Australia (NHMRC, 1996).

Soil

Benzene has been detected in soil as a result of direct contamination by spillage or leakage, at concentrations ≤191 μg/kg (IPCS, 1993). The contribution from traffic emissions is likely to be negligible, because of the rapid volatilisation of benzene to air. Therefore, exposure of young children to benzene by ingestion of soil not directly contaminated by petrol is likely to be negligible.

Food

As discussed in Section 8.2.5, benzene does not accumulate in the food chain. However, the chemical may enter foods as a result of equilibration with surrounding media or through migration from cooking utensils and food packaging materials.

There are early reports of benzene being found in butter (0.5 ng/g), irradiated beef (19 ng/g), rum (120 ng/mL), eggs (500-1900 ng/g), fish (3-88 ng/g) and clams and oysters (220-260 ng/g) (IARC, 1982a; IPCS, 1993). More recent studies reviewed by Wallace (1996) found insignificant amounts in most of 107 foods (including raw eggs). Exceptions included preserved jams and sauces, shelled peanuts and fried eggs, which contained 5-38 ng/g benzene. A 1993 survey in the UK found low levels of benzene (1-18 ng/g) in carcass meat, offal, meat products, poultry, fish and nuts but not in dairy products, bread, cereals, sugar, vegetables, fruit or beverages (MAFF, 1995).

Benzene was, however, detected at low concentrations (27-56 ng/g) in the peel of three (apple, kiwi fruit and orange) of 24 fruit and vegetable samples purchased from local shops in Poland (Górna-Binkul et al, 1996); it was suggested by the authors that the likely source was absorption of benzene from the air by lipophilic components of the peel. The chemical was also found in olive oil produced from fruit stored in a shed housing harvesting machinery (Biedermann et al, 1996). Parking a small grass mower in the shed caused air levels within the shed to

increase from 0.1 to a maximum of 1.9 ppb in 3 h. The average benzene concentrations in oil obtained from olives stored in such sheds at three different locations were 3, 19 and 40 ng/g.

The mean airborne benzene levels in two petrol station kiosks in UK were 3.4 and 27 ppb (CONCAWE, 1998b). A survey of fatty foods sold from petrol stations or roadside stalls in the UK found benzene present in less than half of 114 packages of butter, margarine, lard and bacon (MAFF, 1996). Mean benzene levels were generally in the order of 10-20 ng/g. The pattern of distribution throughout the food samples indicated that the contamination was the result of either road transport or diffusion from the air and that penetration of products protected by impermeable packaging was negligible. Of 221 food products purchased at petrol service stations in Germany, only one (ice cream) contained benzene in detectable amounts (8 ng/g) (Eikmann et al, 1992). A recent, comprehensive review of studies conducted on hydrocarbon contamination of foods sold at petrol stations and shops situated near busy roads concluded that the levels of hydrocarbons (including benzene) in foods from these outlets was comparable to those found in food sold from shops remote from hydrocarbon sources (CONCAWE, 2000).

Benzene has been found in non-stick and thermoset polyester cookware, expanded polystyrene food packaging articles, polyvinyl chloride water bottles exposed to sunlight, containers made from contaminated recycled polyethylene terephthalate bottles, and in microwave heat susceptors packaged with foods such as pizzas and chips (Fayad et al, 1997; Gramshaw and Vandenburg, 1995; Jickells et al, 1990; Komolprasert et al, 1997; McNeal & Hollifield, 1993). However, in no case was migration considered likely to result in concentrations in foods exceeding 10 ng/g.

The daily dietary intake of benzene in the UK population is estimated at 0.5-2.4 μg/day (MAFF, 1995). There is no indication in the published or unpublished literature that food is an important source of exposure to benzene, accounting for at most 2.5% of total daily intake (CONCAWE, 2000).

16.3 Exposure assessment

Method and assumptions

It is widely agreed that inhalation is the predominant pathway for benzene exposure in humans, with <1-3% of total intake apportioned to skin contact and ingestion of food and water (Government of Canada, 1993; IPCS, 1993; Wallace, 1996; WHO, 2000). It has also been shown that the main microenvironments contributing to inhalation exposure are the home, the workplace, road use and tobacco smoke, while infrequent activities of short duration such as car refuelling have little impact on total intake (Leung & Harrison, 1998; MacIntosh et al, 1995). In consequence, this assessment of public exposure to benzene will disregard non-inhalation exposure and focus on the key environments of the home, the workplace and the road, and on active and passive smoking. In addition, although benzene has been detected in human breast milk (Section 9.2.2), quantitative data are not available and intake via this route is not included for the purposes of this report.

Exposures at home, at work and on the road are assumed to occur within the setting of the model urban environment described in Section 15.2. The corresponding air concentrations are those estimated in Section 15.3 and summarised in Table 15.15. Daily and cumulative exposures are estimated for four different age groups comprising 6 years of childhood, 14 years of education, a working life of 40 years

and 18 years of retirement (ABS, 2000b) and for lifestyles identified as non-ETS exposed, passive smoking and, in adults, active smoking. The estimated time spent in relevant environments by each age group is given in Table 16.1, based on data quoted in Langley et al. (1996). All age groups are assumed to spend 20 h/day in residential and other buildings. Non-ETS exposed persons are assumed to avoid all exposure to ETS and all schools and means of public transportation are assumed to be smoke-free. ETS exposure is assumed to entail an increase in indoor/in-vehicle air levels of benzene of 1.2 ppb, as described in Section 16.2. Finally, it is assumed that active smokers consume 17.8 cigarettes a day, equivalent to the inhalation of 12.5 ppb benzene for 24 h/day from the age of 21 and are exposed to ETS throughout.

Table 16.1: Public exposure scenarios

	Age group (years)			
Environment	**0-6**	**7-20**	**21-60**	**61-78**
Outdoors	4 h/day	4 h/day	4 h/day	4 h/day
Homes	19½ h/day	13 h/day for 190 days/year 19 h/day for 175 days/year	11 h/day for 225 days/year 19 h/day for 140 days/year	19 h/day
Schools	-	5½ h/day for 190 days/year	-	-
Workplaces	-	-	8 h/day for 225 days/year	-
Car transport	½ h/day	½ h/day for 190 days/year	1 h/day	-
Other road use*	-	1 h/day	-	1 h/day

* Walking along urban roads and bus, tram and bicycle riding.

Results

Table 16.2 summarises the estimated daily and cumulative exposures per age and lifestyle group calculated in accordance with the above assumptions.

Table 16.2: Estimated 24 h benzene exposures (in ppb) and cumulative exposures at the top of each age bracket (in ppb-years, rounded to the nearest 5) of the general population in a model Australian urban environment

	Non-ETS exposed		Passive smokers		Active smokers	
Age group	**Ppb**	**Ppb-years**	**Ppb**	**Ppb-years**	**Ppb**	**Ppb-years**
0-6 years	5.1	30	6.0	35	-	-
7-20 years	4.9	100	5.7	115	-	-
21-60 years	5.5	320	6.5	375	19.0	760
61-78 years	4.7	405	5.6	485	18.1	1086

Based on the assessed cumulative exposures, the lifetime weighted 24-h exposure is approximately 5.2 ppb in non-ETS exposed persons, 6.1 ppb in passive smokers and 15.2 ppb in active smokers. The daily intake in a 10-year old child with a bodyweight of 30 kg and a respiratory volume of 15 m^3/day is 8.0 µg/kg/day in the absence and 9.3 µg/kg/day in the presence of ETS exposure. In adult males aged 21-60 years with a bodyweight of 70 kg and a respiratory volume of 22 m^3/day, the

estimated intake is 5.6 μg/kg/day in non-ETS exposed persons, 6.6 μg/kg/day in passive and 19 μg/kg/day in active smokers. In adult females aged 21-60 with a bodyweight of 60 kg and a respiratory volume of 22 m^3/day, the estimated intake is 6.6 μg/kg/day in non-ETS exposed persons, 7.7 μg/kg/day in passive and 22 μg/kg/day in active smokers.

In terms of apportionment, indoor air, road use and outdoor air account for 73, 16 and 11% respectively of the estimated lifetime exposure to benzene in non-ETS exposed persons. In ETS exposed persons, passive smoking accounts for 15% of lifetime exposure. In smokers, 6% of lifetime exposure is attributable to ETS and 60% to the inhalation of mainstream smoke.

Population groups likely to be exposed to extreme benzene levels

The estimates given in Table 16.2 refer to a cross-section of the population in the model urban environment described in Section 15.2. Individual exposure levels may, of course, be vastly different, depending primarily on where people live and work, what car they drive, how much time they spend outdoors, on the road, or in smoky environments, and, in active smokers, on the quantity and type of tobacco they consume.

As a rule, exposures would be considerably below average in people who live in semi-rural or rural areas, work outdoors and spend little time on the road. Conversely, they would be well above average for a person who lives in a house with an attached garage, spends several hours a day commuting in a pre-1986 car, and works in an office or shop in a heavily trafficked street.

Comparison with overseas monitoring data and exposure estimates

Extensive monitoring studies conducted in 1980-87 by USEPA in about 800 subjects representing a cross-section of the populations of California, Maryland and New Jersey found a global average personal exposure level of 4.7 ppb (Wallace, 1996). In subsequent, smaller US studies, the average level was 1.6 ppb in a rural community in California and 7.4 ppb in a remote township in Alaska (Goldstein et al, 1992; Sheldon et al, 1991; both as cited in Wallace, 1996). In a mixed urban and rural subset of the Californian studies referred to above, the 24 h exposure level averaged 3.7 ppb in non-ETS exposed persons and 4.6 ppb in passive smokers (Miller et al, 1998). In Singapore, the measured personal exposure level in 20 persons with no known occupational exposure to benzene averaged 7 ppb (Foo, 1991). In Turin, Italy, the mean personal exposure level was 21.9 ppb in non-ETS exposed subjects and 28.6 ppb in passive smokers (Gilli et al, 1996). In a small study of students, homemakers and pensioners in Birmingham, UK, the measured personal exposure levels averaged 4.7 ppb during the day and 3.4 ppb during the night (Leung & Harrison, 1998). In nine council road and park workers in Nancy, France, who were monitored continuously from Monday morning to Friday evening, the mean personal exposure was 790 ppb-hours, corresponding to an average of about 7.6 ppb (Gonzalez-Flesca et al, 1999). In 100 office workers from Milan, Italy, the mean 24 h personal exposure amounted to 6.6 ppb (Carrer et al, 2000). In a large study in continental Europe, the annual average personal exposure level was 4.4 ppb, based on more than 1500 samples collected from 50 non-smokers in each of six cities (Cocheo et al, 2000). It was lowest in the northernmost city of Copenhagen (2.0 ppb) and highest in the southernmost cities of Athens (5.8 ppb) and Murcia (7.2 ppb). A study conducted in 1990-91 of 113 persons representing a cross-section of the adult Western German population found a mean

personal exposure level of 4.2 ppb, with 95% of the sample population exposed to ≤9.9 ppb (Hoffmann et al, 2000).

In Canada, public exposure in the general environment has been assessed under the Priority Substances Program (Government of Canada, 1993). The estimated intake by 5-11-year old, non-ETS exposed children was 2.7 μg/kg/day, compared to 4.0 μg/kg/day in passive smokers. In 20-70-year old adults, the estimated intake was 2.4 μg/kg/day in non-ETS exposed persons, 3.3 μg/kg/day in passive and 29.3 μg/kg/day in active smokers. Benzene intake in adults in UK has been estimated at 1-12 μg/kg/day depending on lifestyle and geographical factors (IEH, 1999), whereas CONCAWE (1999) arrived at an estimated airborne intake in non-smokers which was equivalent to approximately 2-4 and 7-8 μg/kg/day in urban and inner city environments respectively.

Ultimately, benzene intake in non-smokers derives primarily from vehicle and petrol emissions. The estimated Australian intake is higher than the measured data from California, Maryland and New Jersey in USA and the estimated intake in Canada, where the car fleet is newer and petrol contains less benzene than in Australia. However, it falls within the range measured in Alaska, Singapore and several European countries, where the age of the car fleet and/or the composition of petrol are more relevant to the Australian scenario.

As such, the estimated lifetime 24-h exposure level of 5.2-6.1 ppb in non-smokers in the Australian model urban environment is considered sufficiently realistic to serve as a point of reference for the assessment of public health risks from exposure to benzene in the general environment.

16.4 Summary and conclusions

Inhaled tobacco smoke contains about 50 μg benzene per cigarette, corresponding to a daily intake of 0.89 mg benzene by the average smoker (17.8 cigarettes/day). Non-smoking members of the general public are exposed to benzene through the inhalation of indoor, in-vehicle and outdoor air contaminated with the chemical through releases that predominantly derive from petrol evaporation, vehicle exhaust and tobacco smoke. Skin contact with benzene-containing products such as petrol and ingestion of contaminated water or food contribute minimally to human intake. There is no evidence that industrial emissions are a significant source of public exposure.

The 24 h average lifetime exposure in the Australian urban population is estimated at 5.2 ppb in non-ETS exposed persons. It is approximately one-fifth higher in passive smokers exposed to ETS at home, at work and in their cars (6.1 ppb) and almost three times as high (15.2 ppb) in the average smoker. The average 24 h exposure of the overall population of the model urban centre described in Section 15.2 can be estimated as follows:

Twenty-four per cent of the adult Australian population smokes (ABS, 2000b). At the 1996 census, about 71% of the population of Canberra was aged 18 years and over and the average household size was 2.7. Therefore, in the model city population of 300,000, there would be 300,000 x 0.24 x 0.71 = approximately 51,000 active adult smokers. In a survey of 887 active smokers in Victoria in 1997, 28% reported that they always smoked outside (Trotter & Mullins, 1998). Therefore, 51,000 x (1 − 0.28) = about 37,000 smokers would smoke indoors and thus potentially expose other people to their sidestream smoke. Given the average household size of 2.7, each indoor smoker is likely to expose about 1.7 other people

to his or her sidestream smoke, corresponding to 1.7 x 37,000 = approximately 63,000 ETS-exposed people. As such, the population of the model urban environment is composed of 186,000 non-ETS exposed inhabitants, 63,000 ETS-exposed inhabitants and 51,000 active smokers, which gives a fraction of 62, 21 and 17% respectively. Based on the average 24 h lifetime exposure levels mentioned above, this results in an average exposure level for the population as a whole of 0.62 x 5.2 + 0.21 x 6.1 + 0.17 x 15.2 = 7.1 ppb. Of this, 54% can be attributed to ETS-free indoor air, 22% to active smoking, 11% to road use, 8% to other outdoor activities and 5% to passive smoking.

The above exposure estimate is crude, but may nevertheless give guidance to the relative importance of the various sources that contribute to public exposure to benzene.

17. Occupational Exposure

Workers in the petroleum, steel, chemical and associated industries may be exposed to benzene during bulk manufacture, use, storage or distribution of the chemical or products that contain the chemical, such as crude oil, petrol, BTX and coal tar (see Section 7). Exposure may also occur in laboratories where benzene is used for research or analysis. In addition, the contamination of workplace environments with petrol vapours, engine exhaust or tobacco smoke may result in benzene exposures that exceed the population average, for example, in vehicle mechanics, professional drivers and hospitality workers.

Inhalation is the predominant route of occupational exposure to benzene, with the possible exception of workers having prolonged skin contact with petrol, such as mechanics during work on vehicle fuel systems (Laitinen et al, 1994). Incidental dermal contact may occur as a consequence of spills or leaks and the occasional use of petrol to clean tools or car parts, but would be infrequent and is unlikely to result in significant absorption because of the rapid evaporation of benzene applied to the skin (OECD, 2000). Pipetting and siphoning of benzene-containing liquids by mouth may result in ingestion of the chemical, but nowadays would be very rare.

Occupational exposure to benzene often occurs outdoors, where daily breathing zone levels may vary 10-fold depending on wind speed and other weather conditions (Kromhout et al, 1993).

17.1 Petroleum industry

The petroleum industry comprises an upstream segment involved in getting oil and gas out of the ground and a downstream segment involved in the refining, distribution and marketing of petroleum products.

Information on the number of petroleum industry workers in jobs with the potential for exposure to benzene was not available from applicants. However, it is known that there were about 6400 upstream employees in 1999 (APPEA, 2000). Petroleum refining is estimated to employ over 3000 people (DISR, 1999). As there are more than 40 terminals (Glass et al, 1998) and 8000 petrol stations (AIP, 2000), the number of workers engaged in petrol distribution and marketing is probably in the order of 20,000-30,000.

17.1.1 Petroleum production and refining

The production of crude oil and its refining to petrol and other end products comprise a series of continuous, fully enclosed processes which take place in naturally ventilated, open-air facilities. As such, the principal sources of exposure are fugitive emissions, waste streams, transfers resulting in vapour displacement, and situations where there is a need to break open or enter the system, such as sampling, cleaning and maintenance. All other things being equal, the potential for exposure is proportional to the concentration of benzene in the process stream. This is about 0.1% in crude oil, 0.5-2% in straight run gasoline, Avgas and cracked gasoline, 1-5% in blended petrol, and 4-8% in reformate (Section 7.2.1).

Australian exposure data have been culled from the retrospective exposure assessment for benzene conducted by Health Watch in the course of the nested

case-control study reviewed in Section 11.6.2 (Glass et al, 1998, 2000). This assessment primarily used local monitoring data from the participating petroleum companies to arrive at exposure estimates for a range of job categories and tasks. The available monitoring data were checked for correctness, completeness and consistency and analysed across sites by standard statistical methods. Personal samples taken over <180 min were excluded, except in the case of fitters whose overall exposure was calculated as the mean of a reasonable cross-section of short-term tasks that a fitter may perform. Non-detectable results were assigned a value of half the detection limit. For some job categories, exposure levels were normalised to a benzene concentration of 0.1% in crude oil and 3% in petrol.

Relevant summary statistics (arithmetic mean and range) that relate to current processes and technologies are shown in Table 17.1[9].

Table 17.1: Measured personal benzene exposure levels in the Australian petroleum industry (adapted from Glass et al, 1998)

Job category or task	Sample count	Benzene exposure levels in ppm (TWA)			
		Mean	Minimum	Maximum	$UTL_{95\%,95\%}$*
Crude oil production					
Fitter	12	0.04	<0.01	0.09	1.6
Operator	43	0.05	<0.01	0.53	0.6
Refining					
Catalytic cracking operator	295	0.16	0.02	5.52	0.5
Crude distillation operator	404	0.11	0.00	4.63	0.5
Crude storage tank cleaning†§	13	8.01	0.14	28.21	350
Fitters	369	0.62	0.01	39.00	4.8
Gas testing of storage tanks†§	12	9.08	0.09	58.21	620
Instrument fitter	42	0.48	0.01	10.60	1.7
Laboratory worker	65	0.15	0.01	1.13	1.3
Petrol blending	11	0.42	0.11	2.19	4.0
Plant-wide operator	25	0.08	0.01	0.31	0.7
Reformer operator	263	0.69	0.01	54.05	2.6
Separator cleaning	14	0.12	0.05	0.33	0.5
Slop tank cleaning*†	46	75.15	0.05	1043.89	2000
Sour water treatment	28	0.06	0.05	0.22	0.2
Tank farm workers	104	0.12	0.01	2.30	0.9
Distribution					
Drum filling§	24	1.55	<0.01	6.15	38
Fitter	13	0.67	0.02	5.80	23
Road tanker bottom loading§	31	0.55	<0.01	7.85	8.7

* Calculated for a lognormal distribution from the mean and standard deviation of the logtransformed data included in the Health Watch report.
† Respiratory protective equipment would have been worn.
§ Normalised to a concentration of 0.1% benzene in crude oil and 3% in petrol.

Table 17.1 above and some of the tables below also give the upper tolerance limit of the distribution's 95th percentile ($UTL_{95\%,95\%}$), which is a measure of the true maximum exposure level in the population the samples were drawn from. It is 95%

[9] Glass et al. (1998) tested their data for normality and, as expected, found that they generally showed a lognormal distribution; however, the arithmetic mean is considered the best measure of average long-term exposure.

certain that 95% of all members of a given exposure group are not exposed to levels that exceed the $UTL_{95\%,95\%}$.

Additional Australian monitoring data are included in a report on crude oil published by the European petroleum industry association, CONCAWE (1998a). These data, which are reproduced in Table 17.2, were taken from a petroleum company database covering the 1991-96 period. Some of them may have been included in the Health Watch statistics presented in Table 17.1.

Table 17.2: Personal monitoring at an Australian crude oil production unit (CONCAWE, 1998a)

		Measured exposure levels (ppm)			
Job category or task	Sample count	Duration (min)	Mean	Minimum	Maximum
Full-shift exposure					
Crude tanker loading	1	720	<0.01	-	-
Maintenance worker	7	600	0.03	<0.01	0.09
Platform operator	6	720	0.02	<0.01	0.06
Shutdown technician	4	720	<0.01	-	-
Stabilisation plant operator	25	720	0.03	<0.01	0.19
Storage tank cleaner	21	480	0.09	<0.01	0.34
Storage tank maintenance worker	3	480	0.01	<0.01	0.09
Short-term exposure					
Ballast tank cleaning, inside*	18	50-263	4.90	<0.03	34.10
Ballast tank cleaning, outside*	7	67-310	2.82	0.01	5.89
Crude tank cleaning*	7	158-378	0.31	<0.01	0.93
Inlet separator cleaning	2	104	<0.02	-	-
Manhole opening	2	21	0.62	0.12	1.12
Pipeline monitoring	8	50-200	0.34	<0.01	1.9
Plant equipment steaming	2	48-88	<0.01	-	-

* Respiratory protective equipment was worn.

The available Australian exposure data are in agreement with a number of Canadian, European or US surveys which generally report long-term exposure levels <0.1 ppm in crude oil production personnel and <1 ppm in petroleum refinery workers (CONCAWE, 1997, 1998a; Verma et al, 2000).

17.1.2 Petrol distribution and marketing

The delivery of petrol involves a series of transfers between stationary and mobile tanks, interrupted by storage periods that vary from hours to months. The main sources of release are tank breathing, vapour displacement during tank loading operations, and evaporation of minor spills. There is also the potential for exposure from vapour-filled pipework and tanks that are broken open or entered into during cleaning or maintenance operations.

The Health Watch exposure assessment described above provides some data on the exposure of workers at distribution terminals (Table 17.1). There are no Australian monitoring data for road tanker drivers and shipping tanker crew.

Overseas documentation on the exposure of workers involved in the distribution of petroleum products is limited. In Europe, exposure levels in drum fillers averaged 8.4 ppm (TWA_8) in a 1987 industry survey (CONCAWE, 1997). A US study from the 1970s found exposure levels <0.5 ppm in 126/128 pipeline maintenance workers, with levels ≤1.5 ppm in the remaining two (Domask, 1978, as cited in Weaver et al, 1983). In drivers of bottom-loaded road tankers, average short-term exposure levels during unloading ranged from 0.43-1.56 ppm benzene, with average full-shift exposure levels from 0.04-0.37 ppm (CONCAWE, 1997; Kawai et al, 1991; Weaver et al, 1983). Studies conducted in Europe in the 1980s and early 1990s found average TWA_8 levels ranging from 0.07-2.0 ppm in jetty workers and ship or barge crew involved in closed or open petrol loading operations (CONCAWE, 1997).

Data on Australian petrol station workers have been published by AIP (1996). Limited measurements in 1982 showed benzene exposure levels <1 ppm (TWA_8). This was followed up in 1993 with a study of 10 high volume petrol stations in Adelaide, Brisbane, Melbourne, Perth and Sydney, half of them self-service. Personnel monitored included attendants working in the pump area as well as managers, office and workshop staff. Area samplers were placed adjacent to the pumps. Non-detectable results were assigned a value of 0.707 times the detection limit. The results shown in Table 17.3 include the minimum variance unbiased estimate of the arithmetic mean (estimated arithmetic mean) and $UTL_{95\%,95\%}$ calculated from individual measurements by means of a commercially available spreadsheet (Mulhausen & Damiano, 1998).[10]

Table 17.3: Personal and area monitoring at 10 Australian petrol stations (benzene levels in ppm (TWA_8))

	Sample count	Estimated arithmetic mean	Range	$UTL_{95\%,95\%}$
Full-service stations				
• Attendants	25	0.21	0.03-0.61	0.86
• Other staff	18	0.09	0.02-0.62	0.59
• Area	40	0.15	0.02-0.60	0.69
Self-service stations				
• Attendants	9	0.08	0.05-0.12	0.22
• Other staff	9	0.05	0.03-0.09	0.15
• Area	43	0.06	0.03-0.13	0.14

The exposure levels of attendants and the area levels were significantly higher at full service than at self-service stations ($p < 0.05$). The difference in area levels may be due to attendants having a higher spill rate than motorists (AIP, 1996).

Overseas studies of exposure levels in petrol station attendants have been summarised by CONCAWE (1997) and Lagorio et al. (1993, 1997). The exposure levels shown in Table 17.3 are comparable to those in USA (0.03-0.31 ppm) and Europe (0.04-0.80) and lower than in India (1.16-1.44 ppm).

[10] The minimum variance unbiased estimate is the preferred point estimate of the arithmetic mean of a lognormal distribution (Mulhausen & Damiano, 1998).

17.1.3 Petroleum and petrol cleaning operations

Emergency crew and contractors may be exposed to evaporative emissions from petroleum or petrol spills, or from residues in leaking fuel storage tanks. In workers cleaning a beach after a major crude oil spill, full-shift personal exposure levels were <0.1 ppm benzene in 343/350 samples, with levels ranging from 0.16-0.81 ppm in the remaining seven (NIOSH, 1991, as cited in CONCAWE, 1998a). In contractors engaged to remove or repair leaking underground petrol tanks, benzene exposure levels ranged up to 18.8 ppm during short-term tasks, but remained ≤1 ppm for the shift as a whole (Kramer, 1989; Shamsky & Samimi, 1987).

17.1.4 Conclusions

In conclusion, mean TWA_8 exposure levels in the Australian petroleum industry are expected to be <0.1 ppm in crude oil production workers and <0.7 ppm in refinery and downstream distribution workers. A mean exposure of 1.55 ppm has been recorded for petrol drumming, but this activity is rarely undertaken and is not expected to result in long-term exposures ≥0.7 ppm. Higher individual full shift exposure levels have been measured in reformer operators (up to 54 ppm) and in distribution terminal workers (up to 7.9 ppm), but are not expected to be of regular or frequent occurrence. Although high to very high breathing zone levels have been measured during tank cleaning operations, actual exposures are likely to be much lower because of the routine deployment of respiratory protective equipment in confined spaces.

17.2 Steel and coal tar distillation industries

Workers in the steel industry may be exposed to benzene contained in coke oven gas and its by-products, BTX and coal tar (Section 7.2.2).

Monitoring data were obtained from the steelworks at Port Kembla, New South Wales, and Whyalla, South Australia, which recover BTX and coal tar from the gas. Data were not available for coke works that burn the oven gas as is, or from Koppers Coal Tar Products, who process coal tar to various end products.

17.2.1 Coke ovens

Coke oven workers are exposed to gas emissions through oven door leaks and when the doors are opened to remove coke and recharge the ovens. Information was not available on the number of workers employed at Australian coke ovens. Personal monitoring data collected in 1995-97 were provided for a number of cab drivers (30), oventop hands (6) and door adjusters (2) at Whyalla steelworks. The results are shown in Table 17.4.

In the 1980s, the average exposure level was 0.3 ppm (TWA_8) in British coke oven workers, with individual exposures at 27 coke works ranging from <0.2-8 ppm (Hurley et al, 1991). In a more recent study in Polish workers, the average level was 0.14 ppm (TWA_8), with a range from 0.02-0.42 ppm (Bienik, 1998). These findings are in overall agreement with the levels at Whyalla.

Table 17.4: Personal monitoring of coke oven and by-product workers at the Whyalla and Port Kembla steelworks (benzene levels in ppm (TWA))

Site and job category	Sample count	Duration (min)	Estimated arithmetic mean*	Range	$UTL_{95\%,95\%}$*
Whyalla					
Coke ovens	38	365-445	0.12	<0.01-1.04	0.74
By-product plant	27	350-420	0.72	<0.01-11.10	8.20
Port Kembla					
BTX (gas) operator	30	405-485	0.12	<0.01-0.26	0.25
BTX (liquid) operator	30	295-485	0.35	0.02-8.10	3.86
Tar operator	26	395-485	0.16	<0.01-1.04	1.02
Maintenance worker	53	No data	0.48	<0.01-4.50	4.57

* Calculated according to Mulhausen & Damiano (1998), with non-detectable results assigned a value of 0.7 times the detection limit.

17.2.2 Coal gas by-product plants

A coal gas by-product plant is designed to recover BTX from coal gasses and condense tar from gas wash oil and flushing liquor, as described in Section 7.2.2. BTX systems, which are fully enclosed, process streams containing up to 80% benzene. Tar systems are semi-enclosed, but handle streams with a much lower content of benzene (0.00003% in flushing liquor and <0.2% in the end product). Within the plant, the main sources of benzene exposure are fugitive emissions, evaporation from decanters and sumps, and maintenance work on vessels and pipework. At Whyalla, the final BTX product is re-injected into the gas system and burnt as fuel. At Port Kembla, it is piped to storage tanks for subsequent transport to Huntsman Chemical Company by road tanker (see below). The potential for exposure during loading is low, as the tankers are bottom-loaded and equipped with vapour return systems. The maximum number of exposed BTX and tar workers is in the order of 30-50 persons per site.

The results of personal monitoring conducted at Whyalla in 1995-97 and Port Kembla in 1997-99 are shown in Table 17.4 above.

At a US integrated steelworks, mean exposure levels in the by-product plant were estimated at 10.5 ppm over the 1957-1977 period (Hancock & Moffitt, 1984). However, the mean of 897 personal monitoring samples collected in 1978-83 by the American Iron and Steel Institute was only 1.35 ppm (TWA_8), with 25% of measurements in the 1-5 ppm range (Runion & Scott, 1985). In the 1980s, the average exposure level was 1.3 ppm (TWA_8) in British BTX workers, with individual exposures at 27 plants ranging from <0.2-12 ppm (Hurley et al, 1991). In a recent study in a Polish plant, the average level (TWA_8) was 0.63 ppm in BTX (gas) operators and 1.04 ppm in BTX (liquid) operators, with a range from 0.05-3.04 ppm (Bienik, 1998). The exposures measured in Australian workers fall within the ranges reported from Poland and UK.

17.2.3 Coal tar distillation

Crude coal tar from the steelworks at Port Kembla and Whyalla is transported to Koppers Coal Tar Products in Newcastle, where it is separated into various fractions in a series of fully enclosed, outdoor systems (see Section 7.2.2). The distillery employs 65 people, including fitters, laboratory chemists and operators. The crude tar and distillation products contain 0.11-0.16% and 0-4% benzene

respectively. The potential for exposure is limited to releases during unloading of the crude tar, fugitive emissions, and situations where there is a need to access the enclosed system, for example, to dip tanks and scrubber systems, collect and analyse samples, or carry out cleaning and maintenance.

Workers at Koppers are not routinely monitored for exposure to benzene. Overseas data on the exposure of coal tar distillation workers to benzene could not be identified. However, the coal tar and petroleum industries handle process streams with similar benzene content in similar, enclosed systems. By analogy, therefore, exposure levels among coal tar distillation workers are probably of the same order of magnitude as in petroleum refinery workers, that is, generally <0.7 ppm.

17.2.4 Conclusions

Based on the available data, mean benzene exposure levels are expected to be ≤0.7 ppm (TWA_8) in the steel and coal tar distillation industries. Higher individual full shift exposure levels have been measured in by-product plant operators (up to 11 ppm), but are not expected to be of regular or frequent occurrence. Mandatory changes have since been introduced to personal protective equipment requirements.

17.3 Chemical industry

17.3.1 Ethane and naphtha (gas oil) cracking

The Qenos petrochemical plants in Altona and Botany Bay produce a by-product known as pyrolysis gasoline which may contain from 6-36% benzene (Section 7.2.3). The pyrolysis gasoline stream is produced and contained in a fully enclosed, outdoor system. At the Altona plant, it is transferred in closed pipework to a neighbouring petroleum refinery, which stores the pyrolysis gasoline in floating-roof tanks and eventually ships it overseas. At the Botany plant, it is stored in a floating-roof tank until piped to Port Botany and shipped overseas for further processing. In consequence, the potential for exposure is limited to fugitive emissions at pumps and valves and to sampling and maintenance requiring access to the closed system. Potentially exposed workers comprise a maximum of 85 operators, supervisors and maintenance personnel per site.

According to a summary of personal monitoring data provided by Qenos, long-term full-shift exposure levels in a total of 14 job categories at the Altona and Botany pyrolysis plants did not exceed 0.10 ppm benzene in 1998-99, based on 7-11 samples per similar exposure group. This is in agreement with Tsai et al. (1983) who reported a mean exposure level of 0.11 ppm in 32 samples collected in the ethylene unit of an integrated US refinery in 1978-1983. Furthermore, at the Altona site, full-shift personal exposure levels were consistently <0.07 ppm in 32 fitters and operators involved in a turn-around operation in 1998 and <0.05 ppm in 9 maintenance contractors who were sampled in 2000. Qenos also provided personal monitoring data from a small number of contract surveyors and wharf operators involved in a shore to ship transfer at Port Botany of pyrolysis gasoline containing about 35% benzene. Full-shift exposure levels ranged from 0.1-0.4 ppm, which is comparable to the levels measured in workers engaged in ship to shore transfer of benzene feedstock (Table 17.5).

17.3.2 Bulk distribution

The predominant end user of benzene feedstock is Huntsman Chemical Company in Melbourne, with an approximate annual consumption of 20 kt BTX (see above) and 80 kt 95-99% pure benzene imported through Terminals Pty Ltd on Melbourne's waterfront (Section 7.2.3). About 0.05% of the imported quantity is diverted to the Qenos butadiene rubber plant in Altona, Victoria. There are no other users of bulk benzene in Australia.

BTX is transported from Port Kembla to Melbourne by road. The potential for exposure is low, as the product is transferred via dedicated, closed lines and transport takes place in bottom-loaded road tankers equipped with vapour return systems. Loading and unloading last 30-40 min each and the volume transported is equivalent to 2-3 trucks/day. The number of potentially exposed workers would be <10.

Benzene is delivered to Terminals by ship about 30 times a year. There is the potential for exposure from drips and spills when making and breaking the line connections for ship to shore transfer. A pump onboard the ship moves the cargo into one of six nitrogen-blanketed benzene storage tanks, which are vented to air through a carbon bed system, minimising the potential for exposure to displaced vapours. The time taken to connect, disconnect and pig the lines is <60 min. The transfer itself lasts from 12-26 h depending on the size of the cargo. The transfer requires a manning level of 4-5 operators. There is limited potential for exposure during subsequent transfer to Huntsman, as the chemical is transported in dedicated, bottom-loaded road tankers equipped with a vapour return system. Dedicated pipelines are used for loading and unloading, which last 25-30 min each.

The results of personal monitoring conducted at Terminals in 1997-1999 and at the Huntsman styrene plant in 1997 are shown in Table 17.5.

Table 17.5: Personal monitoring during road tanker loading/unloading of benzene feedstock and ship to shore transfer (benzene levels in ppm (TWA))

Process or task	Sample count	Duration (min)	Estimated arithmetic mean*	Range	$UTL_{95\%,95\%}$*
Terminals					
Road tanker loading	8	Full-shift	0.11	0.10-0.20	0.24
	6	Short-term	0.51	0.10-1.00	14.28
Ship to shore transfer	12	Full-shift	0.25	0.10-5.60	3.36
	12	Short-term	0.76	0.10-11.60	25.37
Huntsman					
Road tanker loading/ unloading	4	465-690	0.14	0.03-0.20	10.36

* Calculated according to Mulhausen & Damiano (1998).

The data indicate that short-term exposures during road tanker loading rarely exceed 1 ppm, whereas ship to shore transfer of bulk benzene may result in short-term exposures ≤12 ppm. Measured full-shift exposures are consistently <0.25 ppm in the case of road tanker loading/unloading. Wharf operators may occasionally be exposed to higher full-shift levels, but their average exposure over the year would remain <0.5 ppm as Terminals only receive about 30 benzene shipments/year. Wharf operators also wear a full face respirator mask with an organic canister while disconnecting hoses and hence personal exposure levels will be lower.

In 1978-83, the mean exposure level among 426 chemical industry workers loading unspecified road tankers with benzene was 0.42 ppm (Runion & Scott, 1985). There are no published data on the exposure of shipping tanker crew or wharf operators during the unloading of bulk benzene.

17.3.3 Butadiene rubber manufacture

The Qenos elastomer plant in Altona uses pure benzene as a component of a solvent employed in the manufacture of butadiene rubber in a fully enclosed batch process, as described in Section 7.2.3. There are approximately 20 operators, maintenance and laboratory workers in the rubber plant. These are potentially exposed to benzene released through fugitive emissions or when the system is opened for sampling or maintenance.

According to a summary of personal monitoring data provided by Qenos, long-term breathing zone levels at the elastomer plant in 1998-99 ranged from 0.01-0.51 ppm across a total of 11 job categories, based on 25-30 full-shift samples per similar exposure group. The highest breathing zone levels were observed in workers involved in the collection of samples of the rubber-solvent mixture and in the cleaning of a strainer system that separates rubber particles from the recycled solvent. However, these tasks require the use of respiratory protective equipment and actual exposures are therefore likely to be <0.5 ppm in all cases.

Most published data on benzene exposure in the rubber industry refer to outdated processes and workplace control measures. However, the Qenos findings are in reasonable agreement with a recent assessment of chemical exposures at US styrene-butadiene rubber plants which reported current benzene exposure levels in the order of 0.5-0.7 ppm (Macaluso et al, 1996).

17.3.4 Styrene and phenol manufacture

At Huntsman, the styrene and phenol plants convert large quantities of BTX and benzene to various intermediates in a series of interconnected, fully enclosed, outdoor systems. The processes are described in Section 7.2.3. The principal sources of exposure are fugitive emissions and maintenance operations such as the periodic replacement or regeneration of catalysts. There is little potential for exposure from transfers between systems, as these take place in closed pipework and all benzene holding and storage tanks are connected to an organic vapour recovery system. The potential exposure of quality assurance personnel is limited through the use of closed sampling systems and fume cupboards. There are approximately 100 workers in the styrene and phenol plants and about 30 laboratory and other service staff who may be exposed to benzene.

Full-shift personal monitoring data collected during routine operations in the styrene and phenol plants in 1994-98 and in the main quality control laboratory in 2000 are shown in Table 17.6.

Table 17.6: Personal monitoring in the Huntsman styrene and phenol plants and main quality control laboratory (benzene levels in ppm (TWA))

Site and job category	Sample count	Duration (min)	Estimated arithmetic mean*	Range	$UTL_{95\%,95\%}$*
Styrene plant					
Outside operator	28	420-685	0.16	<0.01-0.82	1.21
Plant engineer	4	340-545	0.39	0.05-1.52	-
Technical process leader	4	540-660	0.05	0.01-0.10	-
Phenol plant					
Day co-ordinator	3	330-435	0.07	<0.01-0.10	-
Outside operator	13	320-700	0.10	0.05-0.21	0.27
Main laboratory					
Technician	5	560-600	0.03	0.01-0.04	0.23

* Calculated according to Mulhausen & Damiano (1998), with non-detectable results assigned a value of 0.7 times the detection limit.

Short-term or instantaneous monitoring of workers engaged in maintenance tasks in the styrene and phenol plants in 1990-99 showed breathing zone levels from 0.2-4200 ppm benzene. The median result was 1.9 ppm in the styrene plant (83 readings) and <0.5 ppm in the phenol plant (199 readings). In a few instances, short-term breathing zone levels ranging up to 6.25 ppm have been recorded in workers collecting or disposing of quality control samples. In almost all cases, respiratory protection was worn where benzene levels in the presence of other hydrocarbons were unknown or exceeded 3 ppm and where benzene in the absence of other hydrocarbons was present in unknown concentrations or exceeded 1 ppm.

Personal monitoring (11 samples) of maintenance workers during turnaround of the phenol plant in 1994 indicated 6 to 12-hour TWA exposures of 0.02 to 0.38 ppm. Static monitoring (3.5 – 18 hour sampling) of benzene in the phenol plant conducted over three months in 1992-93 indicated that of 353 results, 161 were < 0.5 ppm, 104 were 0.5 – 1.0 ppm, 88 were > 1.0 ppm. The geometric mean was 0.73 ppm. Of the 88 results > 1.0 ppm, 25 were due to abnormal plant conditions which the plant personnel were aware of at the time. The company stated it has made some improvements in preventative maintenance and operating procedures since 1993.

There is limited documentation on the exposure of styrene and phenol workers to benzene. Based on 620 samples collected in 1990-94, average full-shift benzene exposure levels at three German styrene/phenol plants varied from 0.10-0.60 ppm, depending on job category (OECD, 2000). These findings are in reasonable agreement with those reported by Huntsman.

17.3.5 Conclusions

Based on the above, mean benzene exposure levels are expected to be <0.5 ppm (TWA_8) in the chemical industry. Higher individual full shift exposure levels have been measured in workers involved in ship to shore transfer of benzene feedstock (up to 5.6 ppm) and in chemical plant engineers (up to 1.5 ppm), but are not expected to be of regular or frequent occurrence. Although breathing zone levels up to 4200 ppm benzene have been recorded during short-term maintenance tasks, actual exposures would have been much lower because of the routine deployment of respiratory protective equipment in such situations.

17.4 Laboratory use for research or analysis

In telephone interviews conducted for this assessment with 55 laboratories identified as having purchased reagent grade benzene within recent years, 38 stated that they currently use benzene for research or analysis, 5 stated they no longer use the chemical, while a further 7 claimed not to be using it and 5 laboratories could not be contacted. The survey identified up to 620 potentially exposed laboratory staff with a median value of 4 (range: 1-116) staff per laboratory. These numbers do not include undergraduate students in university teaching laboratories but do include post-graduate students. The typical amounts used at any one time were as follows:

Volume (mL)	Percentage of laboratories
≤5	29
6-100	42
101-1000	24
>1000	5

Although the potential for exposure is expected to vary considerably depending on circumstances such as the quantity of benzene used, the opportunity for evaporation, the size of the laboratory and the air shift rate, the survey revealed that, in general, benzene is currently used in a manner likely to minimise the risk of exposure. This is achieved by confining the use of benzene to fume cupboards (95% of laboratories), providing other exhaust ventilation (29% of laboratories), the limited amount and duration of use, and appropriate procedures for the disposal of contaminated materials. However, some laboratories reported procedures that may increase the risk of exposure. These include the practice of decanting benzene from large bottles into measuring cylinders or beakers where splashing or spills are likely to occur; the disposal of contaminated pipette tips into open general purpose waste bins; and the storage of small quantities of benzene on work benches or shelves in containers that are permeable to benzene.

As relevant monitoring data were not identified, the Estimation and Assessment of Substance Exposure (EASE, 1997) model (version 2.0) was used to predict exposure levels. Based on the survey information, pure benzene was assumed to be handled directly in a non-dispersive manner, at room temperature and in the presence of local exhaust ventilation. According to this inherently conservative model, exposure levels of 10-20 ppm may be expected. As benzene typically is used for <1h/week, the corresponding long-term average would be 0.25-0.50 ppm. This is higher than the mean exposure levels measured in industrial laboratory workers, which range from 0.03 ppm in the chemical industry (Table 17.6) to 0.15 ppm in petroleum refineries (Table 17.1).

17.5 Contaminated workplace environments

17.5.1 Petrol vapours and vehicle exhaust

Occupational exposure to petrol vapours and vehicle exhaust fumes may occur in vehicle mechanics, professional users of petrol-fuelled implements such as gardeners and loggers, and people who work on or in the immediate vicinity of busy roads, such as professional drivers, road labourers, staff at fast food drive-in outlets, toll collectors and traffic wardens. Detailed information on the number of workers in such jobs was not collected. However, there were in the order of

150,000 vehicle tradespersons, 60,000 amenity horticultural tradespersons, 10,000 forestry and logging and 200,000 road transport workers in the Australian labour force in 1996 (ABS, 1996).

Australian monitoring data were not available for any of the above occupations. However, the Australian and New Zealand studies reviewed in Section 15.3.3 indicate that professional city drivers would be exposed to full-shift breathing zone levels of approximately 7.5 ppb in non-petrol fuelled vehicles such as buses, taxis and trucks, 15 ppb in post-1986 and 48 ppb in pre-1986 petrol-fuelled cars and vans. Furthermore, the exposure of roadside workers is likely to be similar to that of pedestrians and cyclists, that is, approximately 7 ppb (TWA_8) in the model Australian urban environment described in Section 15.2.

Overseas monitoring data are available for bus drivers, loggers, traffic wardens and vehicle mechanics, as shown in Table 17.7.

Table 17.7: Personal monitoring of bus drivers, loggers, traffic wardens and vehicle mechanics (full-shift benzene levels in ppm (TWA))

Occupation	No. of workers	Arithmetic mean	Range	Reference
Bus drivers	59	0.03	0.01-0.05	Rossi et al. (1999)
Loggers*	22	0.15	0.02-0.74	Nilsson et al. (1987)
Traffic wardens	20	0.02	<0.01-0.03	Fustinoni et al. (1995)
Vehicle mechanics	54	0.17	0.01-1.70	Foo (1991)
	65	0.15	<0.01-2.89	Javelaud et al. (1998)
	70†	0.14	0.01-1.77	Muzyka et al. (1998)

* Loggers using two-stroke chainsaws in sparse to thick pine forest stands at an ambient temperature of –16 to 8°C and wind speeds of 0-4 m/s.
† Workers in a garage servicing buses running on a particular diesel blend containing 2.1% benzene.

With regard to bus drivers and traffic wardens, the exposure levels shown in Table 17.7 are 3-4 times higher than those estimated above. However, they were recorded in Bologna and Milan in Italy, where traffic congestion in narrow streets is likely to result in in-vehicle and roadside benzene air concentrations that are substantially higher than in urban environments in Australia.

With regard to vehicle mechanics, the findings summarised in Table 17.7 are consistent with an average exposure level <0.2 ppm (TWA_8). This is in accordance with the personal exposure levels measured in car repair shops in Germany in 1996-97 (OECD, 2000). Tasks that require the fuel system to be broken open may result in short-term breathing zone levels ≤15 ppm benzene and may also entail a component of dermal exposure (Laitinen et al, 1994; Nordlinder & Ramnäs, 1987).

Although the concentration of benzene in jet fuel is very low, static air monitoring has shown benzene levels ≤1.3 ppm in a freshly drained commercial aircraft tank and ≤15 ppm in military aircraft tanks equipped with explosion suppression foams (Carlton & Smith, 2000; Yeung et al, 1997). However, entry into such tanks would be subject to confined space regulations and entrants would wear suitable respiratory protective equipment.

17.5.2 Environmental tobacco smoke

Occupational exposure to benzene contained in ETS may occur in waiters and other staff in restaurants and bars. Detailed information on the number of workers in such

jobs was not collected, however, there are about 150,000 employees in the Australian clubs and pubs, taverns and bars industries (ABS, 2000b).

Monitoring data for these occupations could not be identified. However, based on the indoor air levels in restaurants and bars with smoking occupancy assessed in Section 15.3.2, personal exposure levels are estimated at 8-21 ppb benzene (TWA_8).

17.5.3 Conclusions

Based on overseas studies, benzene exposure in professional users of petrol-fuelled implements and vehicle mechanics is estimated at <0.2 ppm (TWA_8). Occupational exposure to benzene in air contaminated with vehicle exhaust or ETS is estimated at 7.5-48 ppb (TWA_8) in professional city drivers, 7 ppb (TWA_8) in roadside workers and 8-21 ppb (TWA_8) in hospitality staff.

17.6 Aluminium industry

There are no published studies of benzene exposure in the aluminium industry and epidemiological studies do not indicate an excess incidence of benzene-related diseases. However, data reported to NPI show that one aluminium refinery is emitting benzene to the atmosphere in a quantity corresponding to 0.001% of alumina production (Section 15.1.3). Moreover, it has been hypothesised that benzene could form from coal tar pitch used at smelter facilities in the preparation of carbon anodes and smelting pot bases. In consequence, the industry was asked to provide any data in its possession that could clarify the potential for occupational exposure to benzene at aluminium refineries and smelters.

There is no routine personal or area monitoring for benzene in the aluminium industry. Some refineries reported that benzene had been determined at normal background levels in the course of broader studies of the release of volatile organic chemicals. Three smelter operations reported *ad hoc* monitoring projects in the anode manufacturing and baking departments and/or during the relining of smelting pot bases. In one case, benzene was not detected. The results obtained in the other two cases are shown in Table 17.8.

Table 17.8: Personal monitoring during anode manufacturing and pot construction at two Australian aluminium smelter facilities (benzene levels in ppm (TWA))

Process or task	Sample count	Duration (min)	Estimated arithmetic mean*	Range	$UTL_{95\%,95\%}$*
Smelter A					
Anode baking	11	300-585	0.94×10^{-3}	0.16×10^{-3} - 9.39×10^{-3}	14.53×10^{-3}
Anode manufacturing	12	360-645	0.75×10^{-3}	0.01×10^{-3} - 2.70×10^{-3}	18.26×10^{-3}
Smelter B					
Potlining cathode rodding	21	240-360	0.06	0.31×10^{-3} - 0.43	1.06
Potlining slotting/ sidewalling	10	330-415	4.03×10^{-3}	$<0.01 \times 10^{-3}$ - 8.99×10^{-3}	0.32

* Calculated according to Mulhausen & Damiano (1998).

Although liquor burning at aluminium refineries may result in the release of benzene, there is no evidence that this results in any measurable occupational exposure to the chemical.

Monitoring data collected at two aluminium smelters show that the preparation of anodes and smelting pots produce levels consistently <0.01 ppm benzene. At one smelter, cathode rodding was associated with exposures that averaged 0.06 ppm but ranged up to 0.43 ppm. However, a subsequent biological monitoring study found no increase in benzene breath levels in workers exposed to potlining fumes (Brown, 1996). Overall, these findings indicate that coal tar pitch is unlikely to be a significant source of occupational exposure to benzene.

17.7 Summary

The estimated average benzene exposure levels across various Australian industries and industry sectors discussed above can be summed up as follows:

- Long-term occupational exposures are estimated to range from 7-48 ppb benzene in people who work in roadside, indoor or in-vehicle environments contaminated with vehicle exhaust fumes or tobacco smoke;
- Workers in the upstream petroleum industry are generally exposed to benzene levels <0.1 ppm (TWA_8);
- Vehicle mechanics and professional users of petrol-fuelled implements are estimated to be exposed to benzene levels <0.2 ppm (TWA_8);
- Workers in the chemical industry are generally exposed to benzene levels <0.5 ppm (TWA_8) with some maintenance workers in phenol manufacture potentially exposed to levels of < 0.7 ppm (TWA_8), as indicated by static monitoring;
- Laboratory workers using benzene for research or analysis are estimated to be exposed to benzene levels from 0.25-0.50 ppm (TWA_8);
- Workers in the downstream petroleum industry are generally exposed to benzene levels <0.7 ppm (TWA_8);
- Workers in the coal tar distillation industry are estimated to be exposed to benzene levels <0.7 ppm (TWA_8);
- In the steel industry, coke oven and coal gas by-product workers are generally exposed to benzene levels ≤0.7 ppm (TWA_8);
- Occasional short-term exposures to benzene levels ranging from 10-20 ppm may occur in wharf operators involved in ship to shore transfer of benzene feedstock, in laboratory workers using pure benzene for research or analysis, and in vehicle mechanics with tasks that require fuel systems to be broken open; and
- Higher breathing zone levels may occur during maintenance tasks, particularly in confined spaces, but are generally short-term and limited to work situations that require the use of respiratory protective equipment.

18. Risk Characterisation

This section integrates the information on the biological effects of benzene presented in Sections 8-13 with the environmental, public and occupational exposure estimates developed in Sections 15-17, in an overall estimation of the incidence and severity of the adverse effects the chemical may cause on the environment and people of Australia. This process provides the basis for identifying areas of concern and evaluating risk management strategies, including the setting of ambient air quality and occupational exposure standards.

There is very little documentation on adverse human health effects in people exposed to benzene at non-occupational levels. Therefore, the risks to human health are first assessed with regard to occupational exposures and then extrapolated to the general public.

18.1 Environmental risks

Benzene is a volatile and water-soluble chemical. Its major release in Australia is expected to be to the atmosphere, predominantly through fumes released during petrol combustion in motor vehicles. Direct release to the aquatic compartment is expected to be minor by comparison and removal through degradation and volatilisation from sewage treatment plants is likely to greatly reduce the amounts of benzene reaching receiving waters. Monitoring data from around the world confirm the widespread transport of this chemical with substantial detections obtained in air and surface waters overseas. No surface water monitoring data is available in Australia , while international results show that where benzene was detected in surface waters, it was generally less than 10 μg/L.

18.1.1 Atmospheric risk

Limited experimental data on environmental organisms exposed through the gas phase are available. Exposure to benzene in the vapour phase exhibited toxic action on the grain weevil, but the concentration of benzene was not reported. At concentrations >50 mg/m^3 (>15.5 ppm), lethal effects may be expected in plants. It appears plants recover from sublethal effects, and plants are not expected to be exposed to concentrations at the level reported above, so a minimal risk to terrestrial plants is predicted when exposed through the gas phase.

Abiotic effects can also be assessed. While direct photolysis is not considered to be a significant removal process, the atmospheric half-life is relatively short (expected to be <20 days) due to reaction with photochemically produced hydroxyl radicals. The chemical contains no halogenated substituents and due to its short residence time is not expected to have a potential effect on stratospheric ozone.

Webster et al. (1998) state that transport times to the Arctic can be measured in weeks. With a half-life of up to 3 weeks for benzene, it can be expected that the chemical could undergo significant transport in the atmosphere and may migrate to the poles. No measurements appear to be available from these regions.

For chemicals to be considered persistent organic pollutants (POPs), they need to meet certain criteria with respect to persistence, bioaccumulation and the potential for long-range transport. Benzene meets the criteria for persistence in air (half-life

>2 days) and, therefore, possibly the criterion for long-range transport. Half-lives in soil and sediments need to be >6 months. There are no measurements in this area so no conclusions can be drawn, although benzene is not likely to remain associated with soil for extended periods as it is likely to be mobile (relatively low log K_{oc}), and is also expected to be removed significantly through volatilisation. Benzene fails the criteria of persistence in water for >2 months. While a small number of bioaccumulation results indicate a BCF >5000, the vast majority of results are much less than this and benzene also fails the bioaccumulation criterion of BCF>5000. Therefore, benzene cannot be considered a POP.

18.1.2 Aquatic risk

PEC/PNEC ratios for the aquatic compartment can be calculated using the worst case local scenario, in this instance, the PEC_{local} of 0.2 µg/L (see Section 15.4.1), and the derived PNEC of 17 µg/L (Section 8.3.2).

The ratio of PEC/PNEC has been calculated for local and continental compartments as follows: $PEC/PNEC_{local} = 0.01$.

In order to predict a low potential for an environmental hazard, the PEC/PNEC ratio must be <1. The PNEC has been conservatively determined by taking the lowest chronic effect from a large data source and applying a further safety factor of 10.

In determining the PEC/PNEC ratio, the PEC may be slightly underestimated as more than one major benzene emitting plant may be found in a local situation. However, surface waters in heavily industrialised areas of USA and UK show detections of benzene <10 µg/L, which would still result in a PEC/PNEC <1.

International measurements of benzene in sediments show a maximum concentration of 20.4 µg/kg (dry weight) in surface sediment. However, no benthic tests are available from which to conduct a meaningful risk assessment for sediments. It is reasonable to assume that benzene associated with the sediments is in fact adsorbed and so not bioavailable. If this were not the case, the chemical would be expected to volatilise. Based on this, the hazard to benthic organisms is anticipated to be low. However, anaerobic degradation studies indicate that benzene may be relatively resistant to biodegradation under the conditions expected in sediments.

This evidence supports a conclusion of a low expected risk to the aquatic environment. This assessment has not taken into account the environmental effects of large accidental releases of benzene.

18.1.3 Terrestrial risk

While a PEC was not determined (Section 15.4.2), measured data from contaminated sites overseas could be used as an approximation. Benzene in soil is usually the result of direct contamination through spillage or leakage, with the highest level found being 191 µg/kg in the US. Soil concentrations in the Netherlands are reported as less than those found in ground water (<0.005-0.03 µg/L).

The only soil dwelling terrestrial organism for which tests were available was the earthworm, and the only test performed in this regard was a contact test from benzene application to filter paper where an LC_{50} of 98 $\mu g/cm^2$ was determined. As such, a PNEC was not able to be determined.

However, for an industry to pollute soil surface at the reported LC_{50} concentration, it would be required to release around 100 kg of the chemical over a 1 ha area at the lethal concentration (10 kg to cover a 100 m^2 area) at any one time. The highest reported annual release to land in the NPI database is 45 kg from a petroleum bulk storage facility. While it is not known what area this applies to, it is highly unlikely that enough chemical would be released at any one time to cause an adverse impact on terrestrial organisms.

Therefore, a low risk to the terrestrial environment is expected.

18.2 Occupational health risks

Occupational exposure to benzene is predominantly by inhalation and may occur in workers in the petroleum, steel, chemical and associated industries, in laboratories where benzene is used for research or analysis, and in workplace environments contaminated with petrol vapours, engine exhaust or tobacco smoke (Section 17). Dermal absorption may be of significance for workers who have prolonged skin contact with petrol, such as mechanics during work on vehicle fuel systems.

18.2.1 Acute effects

For acute occupational effects, the risk characterisation process considers likely exposure patterns to assess whether single exposures are high enough to indicate a health concern.

Acute inhalation of benzene vapours has dose-dependent CNS depressant or anaesthetic effects, with clinical signs such as dizziness, headache and vertigo at levels of 250-3000 ppm, leading to drowsiness, tremor, delirium and loss of consciousness at 700-3000 ppm. Benzene vapours have been observed to cause skin, eye and respiratory tract irritation in workers exposed to concentrations >33 ppm. Exposure to 25 ppm benzene for 8 h is not associated with any adverse signs or symptoms.

As discussed in Section 17, occasional short-term exposures to benzene levels ranging from 10-20 ppm cannot be excluded in wharf operators involved in ship to shore transfer of benzene feedstock, laboratory workers who use pure benzene for research or analysis, or vehicle mechanics with tasks that require fuel systems to be broken open. There is also the potential for short-term air levels ranging up to several thousand ppm benzene during maintenance work in confined spaces such as tanks used for storage of benzene or benzene-containing products in the petroleum, coal gas, coal tar and chemical industries. However, these industries have procedures that prescribe the use of suitable controls (including eye, respiratory and skin protection) in all such situations. As such, Australian workers are unlikely to be effectively exposed to benzene vapours at concentrations exceeding 25 ppm.

Therefore, occupational exposure to benzene is not expected to pose any appreciable risk of acute health effects, given the control measures already in place.

18.2.2 Effects from repeated exposure

The most significant adverse effects from chronic exposure to benzene are haematotoxicity, genotoxicity and carcinogenicity. Human health hazards for

which a causative association with benzene exposure is established are bone marrow depression and leukaemia, in particular AML. There is also some evidence of an association between benzene exposure and the risk for lymphoma, particularly NHL and MM, but a dose-time-response relationship cannot be determined from the available data. In experimental animals, benzene has also been found to have reproductive effects and cause mammary gland and skin tumours at high exposure levels. However, the available epidemiological data indicate that these effects are only weakly associated with benzene exposure in humans and there is no evidence of a dose-response relationship. For these reasons, the critical health effects from repeated exposure to benzene are considered to be bone marrow depression and leukaemia. Whereas bone marrow depression is likely to have a threshold mechanism of action, leukaemia is considered a non-threshold effect.

Bone marrow depression

No data are available to determine an inhalation NOAEL based on bone marrow depression, although human studies with various limitations indicate that it is likely to be >0.5 ppm (TWA_8) in healthy workers. As discussed in Sections 11.4.4 and 13.2.3, based on current human data, 7.6 ppm (TWA_8) is considered the best estimate for a LOAEL which may be close to the point of departure for the onset of haematological effects. This LOAEL is derived from a study in 44 workers with long-term exposure to benzene where the only haematological abnormality in the lowest exposure group (n = 11; median exposure (TWA_8) = 7.6 ppm; range 1-20 ppm) was a modest decrease (16%) in ALC.

Long-term occupational exposure levels in Australia are assessed to be ≤0.7 ppm (TWA_8) (Section 17). An appropriate NOAEL is not available. However, since the observed haematological effects at the human LOAEL were minimal and unlikely to give rise to clinical signs in otherwise healthy people, the LOAEL is expected to be close to the NOAEL, justifying a risk characterisation based on the margin of exposure between the LOAEL and the estimated human exposure. This margin of exposure is >10 in all workers, which is likely to imply a low to negligible risk for bone marrow depression.

Leukaemia

Data from the most recent follow-up of the Pliofilm cohort indicate that the risk for leukaemia is significantly elevated at cumulative exposures >50 ppm-years, corresponding to a long-term occupational exposure level >1.25 ppm benzene over a working life of 40 years. This is higher than any of the assessed long-term exposure levels in Australian workers, which range from 7 ppb to 0.7 ppm. However, although exposures from 7 ppb to 0.7 ppm indicate a margin of exposure from 1.8-180, this is difficult to interpret as the human dose-response analysis derives from a single cohort with insufficient statistical power to rule out the possibility of some increase in leukaemia risk in individuals exposed to benzene levels <1.25 ppm over an entire working life.

In the absence of evidence to the contrary, genotoxic carcinogens such as benzene are assumed to have a non-threshold mechanism of action. As a ‘safe’or ‘no effect’ level therefore cannot be identified, quantitative risk estimation is often used to express the cancer risk (probability) in numerical terms. The quantitative estimate is derived from the slope of a modelled dose-response curve fitting the available data points for the carcinogenicity end point and then extrapolated downwards to (x,y) = (0,0) to predict the risk at lower exposure levels.

The available quantitative risk estimates based on the most recent follow-up of the Pliofilm cohort are summarised in Table 18.1.

Table 18.1: Estimates of human leukaemia risk from occupational exposure to a cumulative benzene dose of 45 ppm-years, based on dose-response data from the most recent follow-up of the Pliofilm cohort

Mathematical model	Exposure estimate	Additional lifetime leukaemia deaths per 1000 workers*	Reference
Linear	Crump & Allen (1984, unpublished)	5	Crump (1994)
	Paustenbach et al. (1992)	3	
Nonlinear (AUC-dependent)†	Crump & Allen (1984, unpublished)	5	Crump (1994)
	Paustenbach et al. (1992)	3	
Nonlinear (intensity-dependent)	Crump & Allen (1984, unpublished)	5	Crump (1994)
	Paustenbach et al. (1992)	0.04	
Proportional hazards regression	Crump & Allen (1984, unpublished)	0.3	Paxton et al. (1994b)
	Paustenbach et al. (1992)	0.5	
	Rinsky et al. (1981, 1987)	1	

* Rounded to one significant figure
† AUC = area under the curve = cumulative exposure

The estimated number of additional lifetime deaths from leukaemia assumes a cumulative occupational exposure of 45 ppm-years for which estimates are available from both Crump (1994) and Paxton et al. (1994b). Because 45 ppm-years is mid-range in the cumulative exposure distributions in the Pliofilm cohort for all three sets of exposure estimates, the estimations did not require extrapolation below the range of observation. Furthermore, 45 ppm-years correspond to an average exposure of 1.125 ppm, which is reasonably close to the assessed maximum average long-term exposure level in Australian workers of 0.7 ppm.

The mathematical dose-response models listed in the table are statistical in nature and make no assumptions about the mechanisms of carcinogenesis, other than the absence of a threshold level. Although 3 out of 9 risk estimates are 1-2 orders of magnitude lower than the others, the majority lie in the range from 1-5 additional deaths, irrespective of the choice of model and exposure estimate. It is therefore prudent to base the quantitative characterisation of the risk for leukaemia on an incidence of 5 additional lifetime leukaemia deaths per 1000 workers exposed to a cumulative benzene dose of 45 ppm-years.

To predict the risk at other occupational exposure levels, it can be assumed to be directly proportional to the cumulative exposure, with exposure spread evenly across the entire working life (40 years). The results of this estimation are shown below for the current national exposure standard and a range of assessed occupational exposures:

- At the current national exposure standard of 5 ppm (Section 19.2.1), the estimated additional lifetime risk for leukaemia is 22/1000 workers.
- The assessed maximum long-term benzene exposure level is 0.7 ppm in the downstream petroleum, steel and coal tar distillation industries. At an exposure level averaging 0.7 ppm over 40 years, the estimated additional lifetime risk for leukaemia is 3/1000 workers.
- The assessed maximum benzene exposure level is 0.5 ppm in the chemical industry and in laboratories using benzene for research and analysis. At an exposure level averaging 0.5 ppm over 40 years, the estimated additional lifetime risk for leukaemia is 2/1000 workers.
- The assessed maximum benzene exposure level is 0.2 ppm in vehicle mechanics and professional users of petrol-fuelled implements. At an exposure level averaging 0.2 ppm over 40 years, the estimated additional lifetime risk for leukaemia is 1/1000 workers.
- The assessed maximum benzene exposure level is 0.1 ppm in the upstream petroleum industry. At an exposure level averaging 0.1 ppm over 40 years, the estimated additional lifetime risk for leukaemia is 0.4/1000 workers.
- The assessed maximum benzene exposure level is 48 ppb in people who work in roadside, indoor or in-vehicle environments contaminated with vehicle exhaust fumes or tobacco smoke. At an exposure level averaging 48 ppb over 40 years, the estimated additional lifetime risk for leukaemia is 0.2/1000 workers.

In comparison, the lifetime risk (0-78 years, males and females combined) for leukaemia from any cause is 1 in 118, or 8.5/1000 population, based on 1996 incidence figures for Australia (AIHW, 1999).

18.2.3 Uncertainties involved

The risk characterisation for acute effects and bone marrow depression involves uncertainties due to limitations in the amount and/or quality of relevant animal and human data. In the case of bone marrow depression, further uncertainties arise from the lack of an appropriate NOAEL and the use of data from a single ethnic group. Additional uncertainties are inherent in the assessment of benzene exposure levels among Australian workers.

Large uncertainties are involved in the characterisation of the risk for benzene-induced leukaemia. These arise in part from deficiencies in the critical study itself (the Pliofilm cohort), such as its limited size and the assumptions and approximations made to assess exposure levels in the absence of personal monitoring data. They also arise from the lack of sufficient data to validate the mathematical models used to estimate the risk at different levels of cumulative exposure. Furthermore, in extrapolating the estimated risk at 45 ppm-years to a 40-year occupational exposure at average exposure levels from 48 ppb to 0.7 ppm, risk has been assumed to be directly proportional to cumulative exposure (average exposure level multiplied with the duration of exposure), although it is unknown whether a low continuous exposure over a long period of time is equivalent in terms of cancer risk to a shorter exposure to higher benzene levels. There are also uncertainties associated with the assessment of benzene exposure levels among Australian workers.

18.2.4 Areas of concern

The above risk characterisation does not give cause for concern about acute health effects from occupational exposure to benzene, given the control measures which are already in place in Australia.

With regard to chronic exposure to benzene, the risk characterisation suggests that there is little cause for concern about bone marrow depression in Australian workers. However, given the uncertainties involved in the risk characterisation and the likely intra-individual variation in susceptibility to benzene-induced haematotoxicity, it cannot be excluded that cases may occur at the exposure levels encountered in the downstream petroleum, coal gas by-product, coal tar distillation and chemical industries. However, such cases are expected to be mild and, therefore, reversible upon cessation of exposure.

With regard to leukaemia, there are reasons for concern in all workers with repeated occupational exposure to benzene, as no threshold has been established for the genotoxic and carcinogenic effects of the chemical. Based on the available quantitative risk estimates, the magnitude of the concern can be ranked approximately as follows: downstream petroleum industry, coke oven, coal gas by-product and coal tar distillation workers > workers in the chemical industry and in laboratories using benzene for research or analysis > vehicle mechanics and professional users of petrol-fuelled implements > workers in the upstream petroleum industry > workers in roadside, indoor or in-vehicle environments contaminated with vehicle exhaust fumes or tobacco smoke.

18.3 Public health risks

The public are exposed to benzene through the inhalation of indoor, in-vehicle and outdoor air contaminated with the chemical through releases that predominantly derive from vehicle exhaust, petrol evaporation and tobacco smoke. The 24-h average lifetime exposure in the Australian urban population is estimated at 5.2 ppb. It is one-fifth higher in passive smokers exposed to ETS at home, at work and in their cars (6.1 ppb) and almost three times as high (15.2 ppb) in the average smoker (Section 16). Other significant sources of benzene exposure are extended travel in automobiles and from attached garages with access directly from the garage into the house.

The critical effects for public health risk characterisation are the same as those for repeated occupational exposure, that is, bone marrow depression and leukaemia.

18.3.1 Bone marrow depression

As described in Section 18.2.2, a LOAEL of 7.6 ppm (TWA_8) for haematological effects was identified in workers. An occupational exposure level of 7.6 ppm can be considered equivalent to a 24 h, 7-day-a-week level of 7.6 x 8/24 x 5/7 = 1.8 ppm, or 1800 ppb. This is 320 - 375 times higher than the estimated average exposure in any non-ETS-exposed age group in the population in the Australian urban model used in this assessment (Table 16.2). However, the general population is expected to include subpopulations that are more susceptible to benzene-induced bone marrow depression, for example, those with specific genetic polymorphisms (Section 12.4.2) that are expressed with a greater frequency within certain ethnic groups, or lifestyle factors, such as alcohol consumption, that may act as additional risk factors. Even so, in considering the margins of exposure and the type of effects

seen at the LOAEL, the risk for bone marrow depression from environmental exposure to benzene at the assessed levels is likely to be low.

18.3.2 Leukaemia

As mentioned in Section 18.2.2, data from the most recent follow-up of the Pliofilm cohort indicate that the risk for leukaemia is significantly elevated at cumulative exposures >50 ppm-years, corresponding to a long-term occupational exposure level >1.25 ppm benzene over a working life of 40 years. In terms of cumulative dose, occupational exposure to 1.25 ppm over an entire working life is equivalent to lifelong continuous exposure to a benzene level of approximately 130 ppb[10]. This is 25 times higher than the estimated average lifetime exposure of an individual living in an urban area of an Australian city (5.2 ppb; Section 16). However, this margin of exposure is difficult to interpret as the human dose-response analysis derives from a single occupational cohort study with insufficient statistical power to rule out the possibility of some increase in leukaemia risk at cumulative exposure levels which correspond to continuous exposure levels <130 ppb over a lifetime.

The additional risk for leukaemia attributable to environmental exposure to benzene can be predicted by low-dose extrapolation of the quantitative estimates used above to characterise the excess lifetime leukaemia risk associated with occupational exposure to the chemical (Table 18.2). Crump (1994) and USEPA (1998a) both predict the number of additional leukaemia deaths at two lifetime exposure levels, namely 1 ppm and 1 ppb, however, only the latter is relevant for exposure to benzene in the general environment.

Table 18.2: Predicted human leukaemia risk from continuous lifetime exposure to 1 ppb benzene, based on the occupational risk estimates shown in Table 18.1 (from Crump (1994) and USEPA (1998a))

Mathematical model	Exposure estimate	Additional lifetime leukaemia deaths per 100,000 population*	Data source
Linear	Crump & Allen (1984, unpublished)	2	Crump (1994)
	Paustenbach et al. (1992)	2	
Nonlinear (AUC-dependent)†	Crump & Allen (1984, unpublished)	2	Crump (1994)
	Paustenbach et al. (1992)	1	
Nonlinear (intensity-dependent)	Crump & Allen (1984, unpublished)	2	Crump (1994)
	Paustenbach et al. (1992)	0.00002	
Proportional hazards regression	Crump & Allen (1984, unpublished)	0.2	Paxton et al. (1994b)
	Paustenbach et al. (1992)	0.4	
	Rinsky et al. (1981, 1987)	0.9	

† AUC = area under the curve = cumulative exposure
* Rounded to one significant figure

[10] A working life is assumed to comprise 8 h/day x 225 days/year x 40 years = 72,000 hours. A lifetime is assumed to comprise 24 h/day x 365 days/year x 78 years = 683,280 hours. Therefore, in terms of cumulative dose, an average occupational exposure of 1.25 ppm over 40 years is equivalent to a 24-h average lifetime exposure of 1.25 x (72,000/683,280) = 0.132 ppm, or approximately 130 ppb.

With the exception of one outlier which is 4-5 orders of magnitude lower than the remaining predictions, the risks shown in the table do not differ by more than one order of magnitude, irrespective of the choice of model and exposure estimate. It is therefore reasonable to base the risk characterisation on the most conservative prediction, that is, a lifetime leukaemia risk equivalent to 2 additional deaths per 100,000 population at 1 ppb.

By extrapolation, the lifetime leukaemia risk equivalent for increasing exposure levels can be calculated as follows:

1 ppb ~ 2 additional deaths/ 100,000 population

2 ppb ~ 4 additional deaths/ 100,000 population

5 ppb ~ 10 additional deaths/ 100,000 population

10 ppb ~20 additional deaths/ 100,000 population

20 ppb ~ 40 additional deaths/ 100,000 population

The estimated average lifetime exposure of an individual living in an urban area of an Australian city is 5.2 ppb. The predicted excess lifetime risk of leukaemia is therefore 1/10,000, or 1.2% of the lifetime risk of contracting leukaemia of any cause (1 in 118, or 85/10,000 population, based on 1996 incidence figures for Australia (AIHW, 1999)).

18.3.3 Uncertainties involved

Substantial uncertainties are involved in the above public health risk characterisations. These derive in part from the database limitations and lack of a validated risk estimation model. Moreover, the prediction of leukaemia risk at low environmental exposure levels by extrapolation from high occupational exposures may overestimate the risk, since the efficiency of DNA repair systems at low exposure levels may be increased. In addition, uncertainties are inherent in the assumptions and approximations made in order to estimate the likely exposure to benzene in the Australian urban population. The model assumes, for example, a relatively high population density, with associated high levels of benzene due to car use. At the same time, there is an assumption that each of the households in the model population will have a lawn which they mow on a weekly basis. The indoor levels, which contribute significantly to overall benzene exposure, are derived from the outdoor levels by the application of a standard ratio. The ratio was estimated by using ratios found in a range of overseas cities, and making conservative assumptions to extrapolate these findings to the Australian situation. This may result in an overestimation of indoor levels both from conservatively high outdoor levels, and also from a ratio which may overestimate the indoor levels.

18.3.4 Conclusions

Notwithstanding the considerable uncertainties involved in the public health risk characterisation, the findings set out above can be considered indicative of the risks to the public based on estimates of benzene levels in urban air. However, the general population will include subpopulations with a greater exposure to benzene and hence at greater risk. These subpopulations include smokers, those frequently using petrol powered devices (e.g. private motor vehicles, lawn mowers, outboard engines) and individuals who live or work in areas with a high traffic density or near to industrial sources of benzene emission. As such, adverse health effects

from benzene-induced bone marrow toxicity are not expected to constitute a significant public health risk.

With regard to leukaemia, no safe level of exposure has been established. USEPA (1989) and the Dutch Ministry of Housing, Physical Planning and the Environment (OECD, 1995) have provided guidance which effectively states that an additional lifetime cancer risk of 1/1,000,000 can be considered negligible, while an additional lifetime cancer risk of 1/10,000 is the maximum permissible risk to an individual for any single substance under consideration. At the assessed public exposure level in a model Australian urban environment, that is, at 5.2 ppb, the excess lifetime risk of benzene-induced leukaemia is estimated to be 1/10,000, or 1.2% of the lifetime risk of contracting leukaemia of any cause. This is a conservative estimate, based on the conservative nature of the quantitative risk prediction at low environmental exposure levels, and also based on the conservative exposure assessment. The risk could be estimated more accurately following collection of monitoring data which better represents the Australian benzene levels, including both indoor and outdoor levels.

18.4 Risk assessments by other national or international bodies

Government of Canada

Benzene has been assessed for environmental and public health effects (Government of Canada, 1993). It is considered to be of low risk to the environment, but is characterised as a non-threshold human carcinogen and as such meets the Canadian criteria for being considered a chemical that constitutes or may constitute a danger to human life or health. For AML, the $TD_{0.05}$ (the lower confidence limit of the benchmark dose that corresponds to a 5% increase in mortality) is calculated at 4.6 ppm, based on an early report on the Pliofilm cohort (Rinsky et al, 1987), the exposure estimates by Crump & Allen (1984, unpublished) and a linear-quadratic mathematical model. With an estimated average ambient air concentration of 1.4 ppb, the exposure level is about 3000 times lower than the $TD_{0.05}$. Under the Canadian criteria, the priority for further analysis of options to reduce exposure was therefore considered to be high.

UK Department of the Environment, Transport and the Regions

The Department's Expert Panel on Air Quality Standards took the view that although benzene is genotoxic and as such may be presumed to be a non-threshold carcinogen, the risks become smaller as the cumulative exposure of an individual is reduced and that, for all practical purposes, there is a concentration at which the risks are exceedingly small and unlikely to be detectable by any practicable method (DoE, 1994). Based on the available epidemiological studies, the Panel determined that the risk of leukaemia in workers was not detectable when average exposures over a working lifetime were around 0.5 ppm. Allowing for a 100-fold uncertainty factor to account for the difference between working lifetime and chronological life and for interindividual differences in sensitivity, the Panel concluded that an ambient air level of 5 ppb (as a running annual average) presented an exceedingly small risk to the health of the general public.

The Department subsequently commissioned the UK Medical Research Council's Institute for Environment and Health to prepare a more detailed evaluation of the possible adverse health effects resulting from exposure of the UK general population to benzene. This assessment found that the major health risk associated

with low-level benzene exposure is leukaemia, particularly ANLL, and that there is no evidence to suggest that continuous exposure to environmental levels of benzene manifests as any other adverse health effect. Based on the work by Schnatter et al. (1996b) discussed in Section 11.6.1, the lowest level of exposure at which an increased incidence of ANLL was reliably detected among occupationally exposed workers was estimated to be in the range of 10-25 ppm. The general population exposure levels were estimated to range from 1.19-12.80 ppb, or three orders of magnitude less than the occupational lowest observed effect level. As such, the assessment concluded that any risk of leukaemia to the general population is likely to be exceedingly small and probably not detectable using current methodology (IEH, 1999).

US Environmental Protection Agency

The critical public health effects considered by USEPA are haematotoxicity, immunotoxicity and leukaemia.

The risk for haemato- and immunotoxicity was assessed on the basis of an occupational study that identified a concentration of 7.6 ppm as a LOAEL for a reduction in ALC (Rothman et al, 1996a, 1996b; see Section 11.4.4). This value was multiplied by 10/20 x 5/7 to correct for differences between occupational and non-occupational respiratory volumes and a 5-day work week and divided by an uncertainty factor of 1000 to account for human variability, lack of an appropriate NOAEL, subacute exposure (<10% of a lifetime) and database deficiencies. The final result was a human chronic air concentration, considered to be safe, equal to 3 ppb (USEPA, 1998c).

The estimated additional lifetime risk for leukaemia due to continuous exposure to benzene is 26/1000 at 1 ppm (USEPA, 1985). This estimate was originally developed by Crump & Allen (1984, unpublished) as the geometric mean of a set of risk estimates determined separately for each of three occupational cohorts (the CMA, Dow Chemical and Pliofilm cohorts described in Section 11.6.1) and adjusted for the difference between working lifetime and chronological life. Since the mathematical model is linear, the predicted risk at 1 ppb is equal to the unit risk divided by 1000, that is, 26/1,000,000 population. The public health risk for leukaemia was reassessed in 1998 (USEPA, 1998a). USEPA concluded that the evidence made available since 1985 was insufficient to reject a linear dose-response curve for benzene and that more recent unit risk estimates based on a linear model ranged from 7-25/1000 and thus were close to the 1985 USEPA estimate.

However, in accordance with the proposed 1996 draft USEPA guidelines for carcinogen risk assessment, the USEPA (1998a) update includes an alternative risk characterisation based on a margin of exposure approach, in which exposure levels in the general population are compared to the lowest cumulative dose for which there is evidence of a carcinogenic effect ('the point of departure'). The point of departure is taken to be 40 ppm-years of occupational exposure, equivalent to a lifetime (76 years) environmental exposure of 120 ppb. Using the value of 4.7 ppb reported by Wallace (1996) as an example of a reasonable estimate of long-term average exposures in the general population, the margin of exposure for the general public equals 120/4.7, or about 26, which must then be interpreted in light of the uncertainty about the slope of the dose-response curve below the point of departure, the nature and magnitude of higher short-term exposures, the extent of individual

differences in sensitivity, and the modes of action of benzene and its metabolites (USEPA, 1998a).

World Health Organization (WHO) programs

Benzene risk assessments have been carried out by the International Agency for Research on Cancer (IARC), under the International Programme on Chemical Safety (IPCS), and in the course of the development of WHO drinking water and air quality guidelines.

In general, IARC does not provide risk estimates; however, an exception has been made for two chemicals classified as carcinogenic to man: benzidine and benzene (IARC, 1982b). Based on preliminary data from the Pliofilm cohort (Rinsky et al, 1981) and supported by other published evidence, IARC concluded that a working lifetime exposure to 100 ppm of benzene would be likely to result in 140-170 cases of leukaemia per 1000 exposed workers.

The IPCS (1993) assessment concluded that occupational exposure to benzene may lead to bone marrow depression and myelogenous leukaemia. With regard to the former, IPCS estimated very approximately that exposure to high benzene levels (50-100 ppm) for one year would most likely produce bone marrow toxicity in a large percentage of workers and aplastic anaemia in some cases, whereas little effect would be expected at lower doses. In contrast, exposure to low doses (1-10 ppm) for 10 years was roughly estimated to have the potential to result in a 1-5% incidence of bone marrow depression. With regard to carcinogenicity, IPCS concluded that a TWA of 1 ppm over a 40-year working period has not been statistically associated with any increase in deaths from leukaemia, although it emphasised that the epidemiological evidence was not capable of distinguishing between a small increase in mortality and a no-risk situation. IPCS also cautioned that since benzene is a human carcinogen, exposures should be limited to the lowest technically feasible level.

The drinking water quality guideline for benzene (10 μg/L) is stated to be derived from a rounded estimate of the inhalation exposure level associated with a low level excess risk of leukaemia (1/100,000), as calculated by the application of a linear extrapolation model to unspecified occupational data (WHO, 1984).

According to the most recent air quality guideline document, the predicted excess lifetime risk for leukaemia is 6/1,000,000 at a 24-h environmental exposure level of 1 $\mu g/m^3$ (19/1,000,000 at 1 ppb), based on the geometric mean of four occupational risk estimates selected from Crump (1994). This corresponds to an excess lifetime risk of 1/10,000 at a 24-h benzene exposure level of 5.3 ppb, 1/100,000 at 0.53 ppb and 1/1,000,000 at 0.053 ppb (WHO, 2000).

Organisation for Economic Cooperation and Development

An OECD Screening Information Assessment Report for benzene has been drafted, but is not available in final form at this time (OECD, 2000).

19. Risk Management

This section discusses currently employed measures to reduce the likelihood of adverse human health effects from exposure to benzene. The information reviewed was obtained from applicants, site visits and the open literature.

19.1 Environmental and public health controls

Several countries around the world have introduced regulations that aim to limit the exposure of the general public to benzene. Examples include standards for the maximum annual average concentration of benzene in ambient air in Japan (1 ppb), The Netherlands (3.1 ppb) and UK (5 ppb); maximum acceptable concentrations of benzene in drinking water ranging from 1 μg/L in the European Union to 10 μg/L in Japan and New Zealand; and limits on the concentration of benzene in petrol in Canada, Japan and New Zealand (all at a maximum of 1% v/v).

In Australia, the industrial use and discharge to the environment of benzene are controlled by State and Territory regulations pertaining to dangerous goods and protection of the environment that are enforced by means of a system of conditional permits, licenses and warrants.

Among other conditions, such permits may set limits relating to the emissions of benzene to the environment. As described in Section 7.2, systems employed to reduce industrial emissions of benzene to air include vapour return and recovery systems, carbon bed filters, fume-scrubbing systems and the burning of off-gases. Systems employed to reduce emissions to water include the treatment of effluents by steam stripping, in water/oil separators and/or in biological treatment plants.

Benzene is also one of 36 chemicals included in the National Pollutant Inventory, which was established in 1998 as a joint Commonwealth, State and Territory initiative, and must be reported if the quantity used or handled per site exceeds 10 t per annum (EA, 1999b).

Australian water quality guidelines for fresh and marine waters were established in 1992 and suggested a maximum benzene concentration of 300 μg/L. These guidelines are currently under revision, with the previous benzene limit likely to be replaced by freshwater and marine trigger levels equal to 230 μg/L and 170 μg/L respectively (ANZECC, 2000). These trigger levels are similar to the lowest NOEC of 170 μg/L for chronic exposure in aquatic organisms (Section 8.3.1). The current Australian drinking water guidelines propose a limit of 1 μg/L, based on a consideration of health effects in relation to the limit of determination, to provide guidance in the unlikely event of contamination and because benzene has been detected in drinking water overseas (NHMRC, 1996).

The Australian Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) lists benzene in Schedule 7, except for preparations containing ≤15 mL/L (1.5% v/v) or petrol containing ≤50 mL/L (5% v/v) of benzene (Australian Health Ministers' Advisory Council, 1999). Schedule 7 poisons must not be possessed, sold or supplied for domestic purposes. Furthermore, benzene is listed with a recommended condition in Part 2 of Appendix J that it is 'not to be available except to authorised or licensed persons'. The labelling requirements for benzene include the warning statement 'Vapour is harmful to health on prolonged exposure' and the

safety directions 'Avoid contact with eyes', 'Avoid contact with skin' and 'Use only in well ventilated area'. The recommended first aid instructions are: 'If poisoning occurs, contact a doctor or Poisons Information Centre'; 'If swallowed, do NOT induce vomiting; give a glass of water'; 'If skin contact occurs, remove contaminated clothing and wash skin thoroughly'; and 'Remove from contaminated area; apply artificial respiration if not breathing'. This schedule has been adopted by all jurisdictions and any use of preparations or petrol containing more than 1.5% or 5% benzene respectively requires the permission of the relevant state or territory health authority.

A voluntary Australian Standard limits the benzene content of petrol for motor vehicles to a maximum of 5% v/v (Standards Australia, 1990). In Western Australia, the *Environmental Protection (Diesel and Petrol) Regulations 1999* stipulate a maximum level of benzene in petrol of 2.0% v/v, with a further reduction to 1.0% v/v from 1 January 2001. In Queensland, the *Environmental Protection Amendment Regulation (No. 3) 2000*, which came into effect on 14 July 2000, prohibits the distribution of petrol with a benzene content exceeding 3.5% v/v (as an average over 6 months or over 6 consecutive batches). At the national level, the *Fuel Quality Standards Act 2000* enables the Commonwealth to make mandatory quality standards for fuel supplied in Australia. Among others, these will include a maximum content of benzene in petrol of 1% v/v from January 1 2006 (EA, 2000b).

The Victorian EPA has proposed setting an intervention level of 0.075 mg/m^3 (1 h average; 23.3 ppb) for benzene under their *State Environment Protection Policy (The Air Quality Management* (EPA, 2000). Intervention levels will be used to determine whether air quality within a local area or neighbourhood is acceptable. These are risk-based numbers designed for the protection of human health. The proposed intervention level for benzene is based upon the Effects Screening Level (ESL) set by the Texas Natural Resources Conservation Commission (TNRCC), which was set to protect against the carcinogenic effects of benzene.

In 1999 the Commonwealth Government established the *Living Cities* - Air Toxics Program (http://www.environment.gov.au/epg/airtoxics/). Under this program the Commonwealth is committed to supporting the development of national management strategies to address air toxics, including benzene. In February 2001, the National Environment Protection Council (NEPC) established a working group to scope an air toxics National Environment Protection Measure (NEPM). NEPMs are broad framework-setting statutory instruments outlining agreed national objectives for protecting or managing particular aspects of the environment. NEPC will consider commencing the development of an Air Toxics NEPM in June 2001. Subject to appropriate approvals from NEPC, the Air Toxics NEPM could be finalised by December 2002. The NEPM development process provides for extensive public consultation.

19.2 Occupational health and safety controls

19.2.1 Regulatory controls

Exposure standard

Table 19.1 summarises the current occupational exposure standards in Australia and several overseas countries, including 8-h TWA and short-term exposure limits

and the presence or absence of a skin notation to indicate that absorption through the skin may be a significant source of exposure.

In Australia, the current national exposure standard for benzene is 5 ppm (16 mg/m^3) expressed as an 8-h TWA airborne concentration, Carcinogen Category 1 (NOHSC, 1995a). This standard was established in 1990 and has been adopted by all States and Territories. Category 1 includes substances that are known to cause cancer in humans. There is no short-term exposure limit and no skin notation.

The current exposure standard is close to the level at which there is statistically significant evidence of haematotoxicity in humans (7.6 ppm (TWA_8)) and it is higher than the level at which there is statistically significant evidence of an increased risk for leukaemia (1.25 ppm (TWA_8)). Furthermore, benzene is a genotoxic carcinogen for which no safe level of exposure has been established.

The recommendations made by the NOHSC Exposure Standards Working Group were based on a review of the then available information about exposure levels associated with haematological, carcinogenic and genotoxic effects in animals and humans (NOHSC, 1996a). The Working Group did not agree with the exposure limit of 10 ppm recommended at the time by the American Conference of Governmental Industrial Hygienists (ACGIH), as cases of leukaemia could be identified with exposure of less than 10 ppm in some human studies. Nor did it support the US Occupational Safety and Health Administration (OSHA) limit of 1 ppm, which it considered to be based on inadequate epidemiological studies.

ACGIH first recommended an 8-h TWA threshold limit value for benzene of 35 ppm in 1948. The recommended limit was lowered to 25 ppm in 1952, 10 ppm in 1974 and 0.5 ppm in 1997. The basis for the currently recommended limit was the re-analysis of the Pliofilm cohort by Schnatter et al. (1996b) (Table 11.6 in Section 11.6.1) which was interpreted to suggest that at a TWA of 0.5 ppm, the odds of death from leukaemia due to occupational exposure to benzene would be indistinguishable from the odds of death from leukaemia for a worker who is not exposed to benzene (ACGIH, 2000).

A US federal standard of 10 ppm was established in 1970. Based on the reports by Infante et al. (1977) and Ott et al. (1978) of an excess mortality from leukaemia in rubber and chemical workers exposed to benzene, OSHA lowered the statutory limit to 1 ppm in 1978. This action was challenged by the US petroleum industry, and the US Supreme Court barred OSHA from upholding the lower limit on the grounds that it had not demonstrated that the new limit would achieve a substantial health risk reduction (Nicholson & Landrigan, 1989). OSHA subsequently developed a quantitative risk estimate for benzene-induced leukaemia and reimposed the 1 ppm limit in 1987.

Table 19.1: National occupational exposure standards for benzene (from ACGIH (2000) and EC (2000))

Country*	Exposure limit (ppm)		Skin notation‡
	8-h TWA	STEL†	
Australia	5	-	-
European Union			
• Belgium§	10	-	-
• Denmark§	5	-	+
• Finland§	5	10	+
• France§	5	-	-
• Germany§	1**	4	+
• Ireland§	5	-	-
• The Netherlands	1	-	+
• Sweden	0.5	3	+
• United Kingdom§	5	-	-
European Union as of 26/06/03	1	Optional	Optional
USA			
• ACGIH	0.5	2.5	+
• NIOSH	0.1	1	-
• OSHA	1	5	-

* ACGIH = American Conference of Governmental Industrial Hygienists (recommended limits); NIOSH = National Institute of Occupational Safety and Health (recommended limits); OSHA = Occupational Safety and Health Administration (statutory limits)
† STEL = short-term (15-min) exposure limit
‡ A skin notation indicates that absorption through the skin may be a significant source of exposure.
§ According to EC (2000), Council Directive 97/42/EC (27 June 1997, amending Directive 90/394/EEC) requires these European Union member states to implement a regulation in their national legislation which reduces the exposure limit to 1 ppm as of 26 June 2003
** In Germany, the exposure limit is 2.5 ppm for coking plants, tank farms and work on benzene- or petrol-conducting plant in the chemical and petroleum industries (OECD, 2000)

Atmospheric monitoring

The use of Category 1 carcinogens, such as benzene, should be controlled to the highest practicable standard. According to the NOHSC *Exposure Standards for Atmospheric Contaminants in the Occupational Environment* (NOHSC, 1995a), routine monitoring of the workplace is essential for indication of control performance.

Health surveillance

Following an amendment in 1995, Schedule 3 of the *NOHSC National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994b) lists benzene as a hazardous substance for which health surveillance is required where there is a significant health risk to workers from exposure to the substance. For benzene, the health surveillance must include demography, occupational and medical history and health advice, a baseline blood sample for haematological profile, and records of personal exposure (NOHSC, 1995b). A specific health surveillance guideline for benzene is available (NOHSC, 1996b).

Scheduled carcinogenic substances

The NOHSC *Model Regulations for the Control of Scheduled Substances* (NOHSC, 1995c) lists benzene as a Schedule 2 notifiable carcinogenic substance

when used as a feedstock containing more than 50% v/v of the chemical. Requirements of the regulation include:

- notification to the relevant public authority of any proposed use of benzene and the quantity to be used per annum;
- a work assessment including an assessment of potential exposure, to be carried out prior to its use;
- the keeping of records of employees likely to be exposed;
- the reporting of exposure incidents to the relevant public authority; and
- advising employees of any accidental exposure.

Employers who use any scheduled carcinogenic substance in a laboratory for research or analysis must make a separate notification to the relevant authority.

Control of major hazard facilities

Because of its Australian Dangerous Goods Class and Packaging Group status, benzene must be taken into account when determining whether a site is a major hazard facility under the NOHSC *National Standard for the Control of Major Hazard Facilities* (NOHSC, 1996c). For flammable liquids in Packaging Group II, such as benzene, the threshold quantity is 50,000 t. The purpose of this standard is to prevent, and minimise the effects of, major accidents and near misses by requiring the person in control of the facility to:

- identify and assess all hazards and implement control measures to reduce the likelihood and effects of a major accident;
- provide information to the relevant public authority and the community, including other closely located facilities, regarding the nature of hazards of a major hazard facility and emergency procedures in the event of a major accident;
- report and investigate major accidents and near misses, and take appropriate corrective action; and
- record and discuss the lessons learnt and the analysis of major accidents and near misses with employees and employee representatives.

State and Territory regulations

The States and Territories have included the NOHSC model regulations and standards referred to above in relevant workplace health and safety or dangerous goods legislation. Benzene is a flammable liquid and should be stored and handled in accordance with relevant state and territory dangerous goods legislation. In addition, some jurisdictions have regulations prescribing an upper limit on the concentration of benzene in paint, including New South Wales and the Northern Territory where the limit is 1% in all paints, and Western Australia where it is 1.5% in spray painting materials. However, benzene is no longer used in paints and painting materials.

19.2.2 Current control measures

Workplace control measures

Workplace control measures include isolation by distance or full containment in enclosed systems, engineering controls, safe work practices and personal protective equipment (PPE).

Petroleum industry

The petroleum industry has developed a guideline for the protection of employees from exposure to benzene (AIP, 1994). The guideline emphasises the importance of full containment and engineering controls such as closed sampling systems in reducing exposure to benzene, particularly in locations where streams containing >5% benzene are handled. It also recommends that areas where airborne benzene levels may exceed 1 ppm have restricted access and be clearly marked.

The recommended PPE includes suitable gloves and boots; a full apron and sleeves where contact with any liquids containing benzene is foreseeable; eye protection when handling products or streams containing >5% benzene; and suitable respiratory protection in all situations where benzene levels are expected or known to approach or exceed 1 ppm.

In the distribution sector, engineering controls include floating-roof tanks with electronic gauging systems, vapour return and recovery systems, and the use of bottom-loaded rail and road tankers fitted with dry break couplings, capacitance probes and earthing points. Workers are required to wear gloves during loading and unloading.

Steel and associated industries

At the Port Kembla steelworks, the coal gas by-product plant and BTX road tanker loading bay are fenced off and access restricted to inducted personnel and contractors. An emission control system under completion involves equipping all tank vents in the by-product plant with a sealpot arrangement to minimise releases to air from tank breathing.

At the Whyalla steelworks, personnel must wear gloves, protective clothing, waterproof boots and vapour cartridge masks to access the coke ovens and by-product plant for inspection and maintenance.

At Koppers coal tar distillery, control measures include full enclosure, fume scrubbing and written operating procedures specifying safe work practices and PPE for each task where there is the potential for exposure to hazardous chemicals.

Chemical industry

In the chemical industry, the main control measures are full containment in enclosed systems and engineering controls such as closed sampling systems. In addition, skin and eye protection is used where contact with liquids containing benzene may occur and suitable respiratory protection is worn in situations where airborne levels of benzene are likely to exceed a limit which varies from 1-3 ppm depending on company policy and the limit of detection of 'instantaneous' measuring equipment. Engineering controls applied to minimise emissions during transport and storage of BTX and feedstock benzene include the use of bottom-loaded road tankers fitted with dry break couplings, capacitance probes and

earthing points; closed dipping or electronic gauging of tanks; and vapour return and recovery systems to prevent releases to the atmosphere from vapour displacement and tank breathing.

Industrial and research laboratories

The main control measure in laboratories is the confinement of all handling procedures to a fume cupboard.

Contaminated workplace environments

Ventilation is the predominant control measure in workplaces such as garages and bars contaminated with petrol vapours, engine exhaust fumes or tobacco smoke from indoor sources. Air purification systems may be used in environments contaminated with vehicle exhaust from outdoor sources, such as vehicle cabins, tollbooths and drive-in outlets.

Emergency procedures

All applicants in the petroleum, chemical and steel and associated industries that manufacture, use or handle benzene have comprehensive written emergency response plans setting out how workers and emergency services should deal with on-site leaks, spills, releases, fires and explosions. These sites routinely handle or use a number of hazardous chemicals in large quantities and have on-site emergency squads which can be activated via alarm buttons posted throughout the site. Workers are trained to respond to emergencies by sounding the alarm, informing a manager and then promptly evacuating the incident area.

Separate exposure and first-aid procedures for emergencies involving benzene are in use in the petroleum industry (AIP, 1994). The exposure procedures include the following instructions:

- remove affected person immediately from contaminated area;
- urgently seek medical advice;
- give artificial respiration with oxygen if required;
- in the event of benzene being swallowed advise the hospital that a lavage of the stomach will be required.

Advice for doctors comprises the following:

- aspiration can take place. Aspiration after ingestion may cause chemical pneumonitis;
- acute exposure to high concentrations of benzene by inhalation may kill by depression of the CNS leading to unconsciousness and death or by fatal acardiac arrhythmia;
- keep victim under close observation and treat symptomatically as indicated by the patient's condition.

Hazard communication

Labels

Under the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994c) and the corresponding State and Territory legislation, suppliers or employers shall ensure that all containers of hazardous

substances used at work are appropriately labelled in accordance with the NOHSC *Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994a).

In accordance with the current version of the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999b), labels for containers of benzene should contain the following risk and safety phrases:

R11	**Highly flammable**	**S45**	**In case of accident or if you feel unwell, seek medical advice immediately (show the label whenever possible)**
R45(1)	**May cause cancer (Category 1)**		
R48/23/24/25	**Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed**	**S53**	**Avoid exposure – obtain special instructions before use**

However, Section 14.3 lists additional risk phrases that will apply as a result of this assessment.

Labelling with risk phrase R45(1) is required for all mixtures containing ≥0.1% benzene. Risk phrase R48/23/24/25 applies to liquid mixtures containing ≥10% and gaseous mixtures containing ≥5% benzene. Liquid mixtures containing ≥1% but <10% and gaseous mixtures containing ≥0.5% but <5% benzene must be labelled with risk phrase R48/20/21/22 ('Harmful: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed'). Liquid mixtures containing <1% and gaseous mixtures containing <0.5% benzene are not required to be labelled with risk phrase R48.

Labels for containers of reagent grade benzene for laboratory use were not available for assessment. One applicant provided a label from a cardboard box used to transport individual containers of the chemical. This label was found to meet all relevant requirements in the ADG Code (Section 19.3).

Bulk storage vessels and tanks must be labelled according to the appropriate State or Territory dangerous goods regulation, generally with an affixed hazard sign or placard similar to the one required for road tankers under the ADG Code. As a minimum, dedicated benzene lines and pipes must be labelled with the name of the chemical.

Material Safety Data Sheets

Material safety data sheets (MSDS) are the primary source of information for workers involved in the handling of chemicals. Under the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994c) and the corresponding State and Territory legislation, suppliers of a hazardous chemical for use at work are obliged to provide a current MSDS to their customers. Employers must ensure that an MSDS is readily accessible to employees with potential for exposure to the chemical.

A total of five MSDS for BTX, feedstock and analytical grade benzene were available for assessment against the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994b). No major deficiencies were identified.

A sample MSDS prepared in accordance with the findings of this assessment and the NOHSC *National Code of Practice for the Preparation of Material Safety Data*

Sheets (NOHSC, 1994b) is provided at Appendix 1. Although it refers to analytical grade benzene for laboratory use, some of the information may also be appropriate for other benzene-containing substances. The sample is for guidance purposes only, as manufacturers and importers are responsible for preparing their own MSDS and ensuring that the information is accurate and up-to-date.

Education and training

All applicants in the petroleum, chemical and steel and associated industries that manufacture, use or handle benzene have specific induction and refresher training for employees and contractors in benzene hazards and safety procedures. Workers potentially exposed to benzene are also trained in the proper use and maintenance of respiratory protective equipment.

19.3 National transport regulation (ADG Code)

Under the ADG Code, benzene (UN Number 1114) is classified in Class 3, flammable liquids, and assigned Packaging Group II (FORS, 1998).

According to the Code, road and rail tankers carrying benzene in bulk must be placarded with class 3 ('flammable liquid') and an emergency information panel containing additional information such as the proper shipping name of the chemical ('benzene'), its UN Number, Hazchem Code and the name and telephone number of the consignor of the goods. The Hazchem Code for benzene, 3WE, reflects the initial emergency response recommended in case of fire, leakage or spillage. The number '3' indicates that foam should be used for firefighting. The letter 'W' means that there is a risk of violent reaction or explosion; that emergency personnel should wear full protective clothing (breathing apparatus, protective gloves, appropriate boots and a chemical splash suit); and that any spillage should be contained so as to prevent the chemical from entering drains or water courses. The letter 'E' denotes that evacuation of people from the neighbourhood of an incident should be considered.

The ADG Code also contains provisions for the marking of packages containing benzene with its UN Number, shipping name, class label and the name and address in Australia of the consignor of the goods.

20. Discussion and Conclusions

Benzene is ubiquitous in the environment, with numerous sources of entry including bush fires, crop residue and forest management burning, petrol combustion and evaporation, wood fires, tobacco smoking and emissions and waste streams from various industries. In a modelled Australian urban environment, 85% of benzene in outdoor air is due to petrol engine exhaust fumes, with industry and petrol service station emissions accounting for 13% and solid fuel combustion for 2% of the total.

Based on 1998-99 data, industrial use of benzene in Australia is estimated at 535 kt per year. All of the benzene introduced by the petroleum industry (82% of total consumption) was retained as a component in petrol. Benzene feedstock for the synthesis of other organic chemicals accounted for 18% of total consumption and less than 1% was either burned as fuel or utilised as solvent or reagent. In consequence, future developments in petrol demand and composition will have a major impact on the introduction of benzene. Thus, the annual use of benzene as a component in petrol is predicted to increase from 434 kt in 1998 to 461 kt in 2007 if the average concentration of benzene in petrol is maintained at 2.6-3.3% v/v, whereas a nationwide standard limiting benzene content to a maximum of 1% would reduce the quantity to 176 kt in 2007.

20.1 Environmental exposure and risks

Benzene may be considered biodegradable and in environmental terms is soluble in water. Its removal from aqueous systems occurs significantly as a result of volatilisation and, at equilibrium, over 99% of the chemical would be expected to partition to the atmosphere where it will break down primarily through reaction with hydroxyl radicals. Concentrations likely to occur in aquatic systems are expected to be far lower than of concern, and this expectation is supported by reported international monitoring data. A low aquatic risk is therefore predicted.

Additionally, the short atmospheric lifetime of benzene indicates concentrations will not occur at levels harmful to the atmosphere. While widespread transport within the troposphere is possible, the chemical is not expected to reach the stratosphere and therefore would not have an influence on global warming or ozone depletion.

Due to the low expected exposure to the terrestrial compartment, a low environmental risk is predicted to terrestrial organisms.

As such, the findings in this assessment have not identified any significant risk to the environment resulting from exposure to benzene.

20.2 Health effects

Benzene is well absorbed by the inhalation and oral routes in all animal species tested. It is also absorbed through the skin, although in practice skin contact is unlikely to result in significant absorption because of the rapid evaporation of the chemical. In humans, the absorption of benzene by the inhalation route is maximal within minutes of exposure and subsequently declines to a constant level, with 20-80% of the inhaled dose being retained after short-term exposure to air levels in the

order of 1-100 ppm. In the body, benzene accumulates in lipid-rich tissues such as the brain. It also reaches the liver, where it is first metabolised by CYP2E1-mediated hydroxylation of the aromatic ring. Subsequent oxidations take place in several organs and result in a series of polyphenols and, to a lesser extent, cleavage of the ring, with a variety of metabolites occurring in the urine within 2 h of exposure. Benzene is also eliminated unchanged with exhaled air, particularly at higher exposure levels that saturate the enzymes which convert it to water-soluble metabolites.

Benzene is not highly acutely toxic to experimental animals. By repeated exposure, the main manifestations of benzene toxicity are CNS depression, immunosuppression, bone marrow depression, degenerative lesions of the gonads, and foetal growth retardation. Benzene also causes damage to genetic material (DNA, chromosomes); increases the incidence of lymphoma in mice strains where this is a common spontaneous tumour type; and induces malignant tumours in the mouth, nasal cavities, lung alveoli, Harderian, Zymbal, preputial and mammary glands, and the ovary. However, a valid animal model for benzene-induced leukaemia has not been identified.

The reported lethal dose in humans is 20,000 ppm by inhalation for 5-10 min, or 125 mg/kg by ingestion. The most significant acute effects are irritation of the skin, eyes and respiratory system at benzene vapour concentrations >33 ppm and progressive CNS depression at concentrations ≥250 ppm.

It is well documented through epidemiological studies that the critical human health effects from repeated exposure to benzene are bone marrow depression and leukaemia, specifically AML (ANLL). There are also observations showing an association between long-term benzene exposure and the risk for lymphoma (NHL, MM), although the evidence is not as conclusive as it is for leukaemia. Furthermore, structural and numerical chromosome aberrations have been detected in peripheral blood cells of workers exposed to high benzene levels.

For bone marrow depression, a NOAEL has not been determined, but occupational studies with various limitations indicate that it is likely to be >0.5 ppm. Based on current human data, 7.6 ppm (TWA_8) is considered the best estimate for a LOAEL which may be close to the threshold for blood count changes in otherwise healthy workers. No threshold has been established for the genotoxic and carcinogenic effects of benzene. However, the available epidemiological evidence shows that the risk of leukaemia increases with exposure and is significantly elevated at cumulative exposures >50 ppm-years, corresponding to >1.25 ppm (TWA_8) over a working life of 40 years.

Experimental studies indicate that the toxic effects of benzene on the bone marrow are due to several secondary metabolites that are formed locally in relatively high concentrations. These metabolites act in an additive or synergistic manner to disrupt a range of mechanisms that regulate blood stem cell division and maturation and cause other cell damage through a combination of genetic and non-genetic changes. Damaged cells are usually eliminated, but may on occasion possess activated oncogenes or have lost tumour suppressor genes, which could enable them to proliferate as clonal lines of leukaemic cells.

The kinetics and metabolism of benzene are similar in men and women. There are no data on age-related differences. However, there is likely to be substantial variations in the sensitivity of an individual to the toxic effects of the chemical as both genetic polymorphisms and lifestyle factors may modulate the expression or

activity of several enzymes involved in the biotransformation of benzene to toxic metabolites.

20.3 Public exposure and health risks

The benzene content in consumer products is nil or negligible, except for tobacco smoke and petrol. Mainstream tobacco smoke contains about 50 (range: 3-60) μg benzene per cigarette, corresponding to an intake of 0.89 mg benzene/day in the average smoker (17.8 cigarettes/day). This is equivalent to the intake from continuous inhalation of ambient air containing 12.5 ppb of the chemical. Although the general public may have skin contact with petrol and inhale petrol vapours, for example, during refuelling at self-service stations, such exposures would be infrequent and of short duration and thus have little impact on total intake. Benzene has been detected in human breast milk, however, there are no good data on secretion levels of benzene in breast milk. Benzene has not been detected in Australian drinking water and overseas studies have, in general, found benzene levels in food to be very low. As such, the predominant pathway for public exposure to benzene is the inhalation of ambient air in the microenvironments where people spend most of their time, that is, the home, the school or workplace, outdoors and in vehicles on the road.

For the purposes of this assessment, an estimated benzene level in outdoor urban air was predicted based upon releases to the atmospheric air column above a model city with a population of 300,000 and a population density approaching 2500 people/km^2 (similar to Sydney). This gave a value of 3.4 ppb, which is comparable to individual results measured in various Australian urban centres. Based on a review of the available Australian and overseas data, the concentration of benzene in indoor air in urban homes and non-residential buildings such as schools and offices was estimated at 1.4 times the outdoor air concentration, or 4.8 ppb. Average benzene exposures resulting from urban transport activities, such as the use of cars (15-48 ppb) and buses and trams (7.5 ppb), were also estimated. Contamination with ETS was estimated to augment exposures in indoor/in-vehicle microenvironments by 1.2 ppb. Overall exposure levels were then calculated by factoring in the length of time various age groups within the general population are likely to spend in the relevant microenvironments.

The results of this exercise indicate that the lifetime weighted 24-h exposure to benzene in the general urban population is 5.2 ppb in non-ETS exposed persons, 6.1 ppb in passive smokers exposed to ETS at home, at work and in their cars and 15.2 ppb in the average smoker.

Benzene emissions to the urban atmosphere will tend to reduce over time as older petrol vehicles with higher emissions are removed from the fleet (EA, 2000c). Under the *Fuel Quality Standards Act 2000*, the Commonwealth will establish national standards prescribing a range of characteristics for petrol and diesel. These will include a maximum content of benzene in petrol of 1% (v/v) from January 1 2006 and standards for other relevant quality parameters such as the content of aromatics, which are predicted to result in a further reduction in benzene emissions (EA, 2000c). As 85% of benzene in both outdoor and indoor air in the model used for this assessment is due to petrol engine exhaust fumes, renewal of the car fleet and implementation of the proposed fuel quality standards would clearly lead to a downward revision of the estimated levels of exposure among the general population.

Based upon the occupational LOAEL for bone marrow depression corrected for the difference between working hours and chronological time and on the conservative nature of the assessed exposure, adverse health effects from benzene-induced haematotoxicity are not expected to present a significant public health risk.

Based upon low-dose extrapolation of relevant quantitative risk estimates, the excess lifetime risk of benzene-induced leukaemia is 2/100,000 population per 1 ppb benzene in the air breathed. In the Australian model city mentioned above, the average exposure was estimated at 5.2 ppb corresponding to an excess lifetime risk of benzene-induced leukaemia in the order of 1/10,000, which is at the maximum permissible level for any single carcinogenic substance according to Dutch and US authorities. This estimate, however, is based on substantial uncertainties in the exposure assessment. Personal and ambient exposure monitoring of representative samples of the Australian population would enable validation of this estimate. Further indoor/outdoor air monitoring data, including in rural areas, would also be helpful in refining the assessment of benzene-associated risks to human health.

Nevertheless, as benzene is an established human carcinogen for which no safe level of exposure has been established, any increase in public exposure should be avoided and, where practicable, measures should be taken to reduce exposure. Given the extensive contribution of vehicle exhaust to ambient benzene levels, reductions in total benzene emitted by vehicles would be effective in lowering public exposure to benzene. A reduction in benzene levels in petrol from 3% to 1%, as proposed in the Fuel Quality Standards, will likely result in significantly lowering benzene exposure in the general population. Measures to reduce environmental tobacco smoke in enclosed public areas should also be continued. A range of lifestyle choices can also be made by individuals to reduce their exposure to benzene, including minimising the time spent in a vehicle in heavy traffic, avoidance of ETS and managing air flow in the home to minimise indoor benzene levels. As high indoor levels of benzene have been found in houses with direct access from attached garages, indoor levels may be reduced by ensuring that doorways are adequately sealed and fitted with automatic door closers.

In one study in the UK, mean benzene levels in fatty foods sold at petrol stations and roadside stalls were in the order of 10 to 20 ng/g. Other studies in both Germany and the UK did not detect elevated levels. The possibility of elevated benzene levels in food sold at sites with elevated ambient benzene levels should be referred to ANZFA and to State Health Departments for their consideration.

Australia, unlike a number of other countries, does not have an ambient air standard for benzene. It is important that such a standard be set, so that the results of monitoring studies can be considered and action taken where appropriate.

The development of a National Environment Protection Measure (NEPM) which sets ambient standards for air toxics is to be considered by the National Environment Protection Council (NEPC) in June 2001. Should the development of the NEPM proceed, it is expected that it will be completed in December 2002.

20.4 Occupational exposure and health risks

Inhalation of vapours is the predominant route of occupational exposure to benzene, although significant skin absorption may occur in workers having prolonged skin contact with benzene or benzene-containing liquids such as petrol.

Exposure occurs principally in the petroleum, chemical, coal gas, coal tar and associated industries which use, produce, store, distribute or otherwise handle high volume streams or products containing benzene at concentrations that range from 0.1% in crude oil to 99% in chemical feedstock. In these industries, exposure is generally controlled by full containment in enclosed systems located in naturally ventilated, outdoor facilities. As such, the main sources of exposure are fugitive emissions from pumps and seals, transfers between closed systems resulting in drips, spills and/or vapour displacement, and sampling, dipping, cleaning and maintenance operations that require open access to the closed system. Various engineering controls are also in place, as are safe work practices and the use of PPE in situations where air levels above 1-2 ppm benzene or skin contact with the chemical may occur.

The number of potentially exposed workers in these industries is not known with certainty, but is probably in the order of 25,000-35,000 in the petroleum industry and 700-800 in the chemical, coal gas and coal tar industries. It is estimated that current long-term occupational exposure levels are <0.1 ppm in the upstream petroleum industry; <0.5 ppm in the chemical industry (except < 0.7 ppm for maintenance workers in phenol plants); <0.7 ppm in the down-stream petroleum industry; and ≤0.7 ppm in coke oven, coal gas by-product and coal tar workers in the steel and associated industries. Higher individual full shift exposure levels have been measured in reformer operators (up to 54 ppm), distribution terminal workers (up to 7.9 ppm), workers involved in ship to shore transfer of benzene feedstock (up to 5.6 ppm), chemical plant engineers (up to 1.5 ppm) and coal gas by-product plant operators (up to 11 ppm), but are not expected to be of regular or frequent occurrence. The highest short-term exposure reported is 12 ppm during ship to shore transfer of benzene feedstock.

Furthermore, it is estimated that around 600 workers are potentially exposed to benzene vapour in laboratories where minor quantities of the chemical are used for research or analytical purposes. The predominant control measure in these workplaces is the confinement of all handling procedures to a fume cupboard. Based on an inherently conservative model, the average long-term occupational exposure level in these workers is predicted to range from 0.25-0.5 ppm, although short-term exposures may reach 10-20 ppm.

Finally, workplace environments may contain benzene air concentrations that exceed those of normal ambient or indoor air because of contamination with petrol vapours, engine exhaust or tobacco smoke. There are no control measures in these workplaces that specifically target benzene, however, ventilation and air purification systems in use for other reasons may also reduce the concentration of benzene in the workers' breathing zone.

Occupations potentially exposed to petrol vapours and engine exhaust include in the order of 100,000-400,000 vehicle mechanics, professional users of petrol-fuelled implements such as gardeners and loggers, and people who work in the immediate vicinity of busy roads, such as professional drivers, road labourers, staff at fast-food outlets, toll collectors and traffic wardens. Overseas data indicate that vehicle mechanics and professional users of petrol-fuelled implements have long-term occupational exposure levels <0.2 ppm benzene. Based on a modelled Australian urban environment, exposure levels are estimated at 7-48 ppb in the case of professions whose workplace environment is on or near heavily trafficked roads. In the case of vehicle mechanics, tasks that require the fuel system to be broken

open may entail short-term exposure levels ≤15 ppm benzene, in addition to skin contact with the chemical.

Occupations potentially exposed to environmental tobacco smoke include up to 150,000 workers in the clubs and pubs, taverns and bars industries, whose long-term occupational exposure levels are estimated at 8-21 ppb.

The impact of fuel quality standards on ambient benzene levels has been discussed in Section 20.3 above. A mandatory reduction of the concentration of benzene in petrol from current levels to 1% is likely to result in a substantial reduction in the average occupational exposure levels in workers exposed to petrol fumes, such as petrol distribution workers and vehicle mechanics. The reduction in exposure is likely to be approximately proportional to the reduction in benzene content, that is, 2- to 3-fold. The predicted reduction in vehicle emissions of benzene would also reduce the occupational exposure of professional drivers and similar road or roadside workers to the chemical. As non-benzene aromatics in fuel can cause around 70-80% of the exhaust benzene formed and some also forms from other hydrocarbons, it is difficult to quantify the extent of the reduction in occupational exposure.

The banning of smoking in all enclosed public areas would reduce benzene exposure in the hospitality industry to background levels.

The occupational risk characterisation does not give cause for concern about acute health effects from exposure to benzene.

With regard to chronic exposure to benzene, it cannot be excluded that cases of mild bone marrow depression may occur at the exposure levels encountered in the downstream petroleum, coal gas by-product, coal tar distillation and chemical industries. However, such cases would be picked up by the prescribed health surveillance and are expected to be reversible upon cessation of exposure.

There is concern about leukaemia in all workers with repeated occupational exposure to benzene. Although no threshold has been established for the genotoxic and carcinogenic effects of benzene, the risk for leukaemia is proportional to cumulative exposure. Therefore, exposure should be controlled to the highest practicable standard.

In workplaces where benzene or benzene-containing streams are contained in fully enclosed systems, particular attention should be paid to engineering control techniques and operating procedures aimed at reducing fugitive emissions from pumps and seals as well as drips, spills and vapour displacement during transfers between closed systems, and to improvements which eliminate the need to break open the closed system for the purposes of sampling, dipping and cleaning. In workplaces where benzene or benzene-containing products are not contained in closed systems, such as laboratories and car repair shops, particular attention should be paid to safe work practices and the availability of good local exhaust ventilation. Where skin contact with benzene or petrol may occur, workers should wear appropriate PPE, including benzene-resistant gloves.

As benzene is flammable and classified as a dangerous good, it should be stored, handled, labelled and transported in accordance with state and territory dangerous goods legislation.

The current national occupational exposure standard of 5 ppm (TWA_8) should be revised as 5 ppm is close to the human LOAEL for bone marrow toxicity (7.6 ppm)

and higher than the level at which there is statistically significant evidence of an increased risk for leukaemia (1.25 ppm). Furthermore, the exposure data collected from Australian workplaces for this assessment indicate that it is technically feasible to keep exposures below 0.5 ppm (TWA_8).

The NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999b) classifies benzene as flammable (R11), a carcinogen in Category 1 (R45) and toxic by prolonged exposure through inhalation, in contact with skin and if swallowed (R48/23/24/25). This classification has been adopted from the European Union where it has remained unchanged since 1967 (although currently under review). In accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999a), this assessment has reviewed the classification of benzene, and amended the above classification to include the following risk phrases: 'Irritating to eyes, respiratory system and skin' (R36/37/38) and 'Possible risk of irreversible effects' (R40, that is, a mutagenic substance in Category 3). Additional safety phrases are not considered warranted, given the prescribed labelling of all mixtures containing ≥0.1% benzene with S53: 'Avoid exposure – obtain special instructions before use'.

20.5 Data gaps

For the purposes of risk assessment, the most significant data gaps are as follows:

- epidemiological data permitting the establishment of an appropriate human NOAEL for bone marrow depression;
- epidemiological data on the additional risk for leukaemia at benzene exposure levels that fall within the range experienced by the general population;
- data on whether various subpopulations are more susceptible to adverse health effects of benzene exposure; and
- personal and ambient monitoring data on the benzene exposure of a representative cross-section of the Australian population.

In addition, further research is needed to determine if benzene is a germ cell mutagen and could cause reproductive effects and contribute to the risk for breast cancer in humans.

21. Recommendations

Preamble

This section provides the recommendations arising from the assessment of benzene. The critical issues, summarised below, have been taken into consideration in formulating these recommendations:

- benzene is a known human genotoxic carcinogen for which there is no known safe level of exposure;
- occupational exposure to benzene occurs during its manufacture and through the use of benzene-containing products, particularly petroleum products;
- the main sources of public exposure to benzene are the use of petrol and diesel fuelled equipment, smoking and releases from industrial processes;
- the best available LOAEL value for non-carcinogenic effects in humans is 7.6 ppm for haematotoxicity;
- a statistically significant increased risk for leukaemia has been measured at and above 1.25 ppm (TWA_8) in occupational studies;
- the estimated excess lifetime risk of benzene-induced leukaemia is 2/100,000 population per 1 ppb benzene in air;
- the current Australian exposure standard is set at 5 ppm (16 mg/m^3) TWA_8; and
- best practice must be implemented to minimise occupational and public exposure to benzene.

Recommendation 1: NOHSC occupational hazard classification

Benzene is currently listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999b) with the following classification:

R11	**Highly flammable**
R45	**May cause cancer, Carcinogen Category 1**
R48/23/24/25	**Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed**

This assessment has amended the above classification to include the following:

R36/37/38	**Irritating to eyes, respiratory system and skin**
R40	**Possible risks of irreversible effects, Mutagen Category 3**

Recommended safety phrases include:

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label whenever possible).

S53 Avoid exposure – obtain special instructions before use.

It is recommended that suppliers and employers take note of this amendment and that it be taken up in the NOHSC *List of Designated Hazardous Substances* as soon as possible.

Based on the cut-off concentration levels tabulated in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999b), risk phrase R36/37/38 applies to liquid mixtures containing ≥20% and gaseous mixtures containing ≥5% benzene, whereas R40 applies to all mixtures containing ≥1% benzene.

MSDS, labels and training materials should be amended accordingly and where appropriate provide relevant information about the irritant and mutagenic effects of benzene.

Recommendation 2: National occupational exposure standard

It is recommended that NOHSC lower the occupational exposure standard for benzene with urgency. Given benzene is a human carcinogen and noting that exposures below 0.5 ppm (TWA_8) for the majority of workers, NICNAS recommends that the standard be lowered to 0.5 ppm (TWA_8).

However, industry has raised concerns that lowering the standard below 1 ppm (TWA_8) is not practicable. The NOHSC exposure standard setting process requires a statutory period of public comment, where practicability data may be submitted. Therefore, it is recommended that NOHSC consult on a proposal to lower the exposure standard to 0.5 ppm (TWA_8), including seeking information on practicability. In the interim, it is recommended that companies immediately adopt 1 ppm (TWA_8) as an internal standard.

Recommendation 3: Workplace control measures

a) ***Engineering controls and safe work practices:*** It is recommended that benzene is eliminated or substituted with less hazardous chemicals in industrial processes wherever practicable. In workplaces where this is not practicable, employers should strive for further improvements in current workplace control measures and utilise best available technology to minimise worker exposure to benzene, wherever this is technically and economically feasible.

- In workplaces where benzene or benzene-containing streams are contained in fully enclosed systems, particular attention should be paid to engineering control techniques and operating procedures aimed at reducing fugitive emissions from pumps and seals as well as drips, spills and vapour displacement during transfers between closed systems;
- Improvements that eliminate the need to break open the closed system for the purposes of sampling, dipping, maintenance and cleaning;
- In workplaces where benzene or benzene-containing products are not contained in closed systems, such as laboratories and car repair shops, particular attention should be paid to safe work practices and the availability of good local exhaust ventilation;
- Where skin contact with benzene or petrol may occur, workers should wear appropriate personal protective equipment, including benzene-resistant gloves; and

- In workplace environments contaminated due to petrol vapour, vehicle exhaust or tobacco smoke, measures should be implemented to reduce exposure levels as low as possible, such as good local exhaust ventilation and air purification system.

b) ***Exposure monitoring:***

- Personal monitoring should be conducted where a workplace assessment indicates that there is a significant risk to health.
- Health surveillance should be conducted in accordance with the 1995 amendment to Schedule 3 of the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994b) listing benzene as a hazardous substance for which health surveillance is required where there is a significant health risk to workers from exposure to the chemical (NOHSC, 1995b). The health surveillance must include a baseline blood sample for haematological profile, as further detailed in the specific health surveillance guideline for benzene (NOHSC, 1996b).
- It is recommended that the State and Territory occupational health and safety authorities review the compliance of larger workplaces in the petroleum, chemical (benzene-related), coal gas by-product and coal tar industries with the requirements for personal exposure monitoring and health surveillance. State and Territory authorities should also review compliance of workplaces with respect to scheduled carcinogenic substances, major hazard facilities and dangerous good legislation.
- Given the very large number of small workplaces with the potential for occupational exposure to benzene contained in petrol, it is recommended that relevant industry organisations such as the Australian Institute of Petroleum and the Institute of Automotive Mechanical Engineers develop programs aimed at improving control measures in petrol stations and car repair shops. NICNAS will prepare a Safety Information Sheet for benzene, aimed primarily at workers, which can be distributed to workplaces. It is recommended that industry and State jurisdictions distribute this information widely.

Recommendation 4: Public health recommendations

a) Public health authorities should update their advice on how to minimise exposure to benzene. For example, indoor air levels of benzene in houses with attached garages may be reduced by ensuring that internal garage doorways are adequately sealed and fitted with automatic door closures.

b) Public health authorities should continue to seek measures to reduce environmental tobacco smoke in enclosed public areas.

c) This assessment report will be forwarded to the Australia New Zealand Food Authority and to State Health Departments for their consideration with respect to levels of benzene in foods sold in areas with high ambient benzene levels, such as roadside shops or stalls or petrol stations.

d) To more accurately estimate the risk to the public, personal and ambient air monitoring data should be collected. The data should focus on those scenarios where the greatest uncertainties exist, such as indoor air levels, urban air levels and air levels near potential industrial point sources. The Department of Environmental

Protection (Western Australia) in association with the University of Western Australia, Murdoch University, CSIRO, EPA Victoria, NSW EPA, SA EPA, Flinders University, Monash University and NSW Health is currently conducting an air monitoring study for the Living Cities – Air Toxics Program, which will provide some of these data.

e) It is recommended that an ambient air standard for benzene be set. The findings of this report will be provided to the National Environment Protection Council so that they may be taken into account when considering benzene in the context of developing a National Environment Protection Measure (NEPM) for air toxics.

f) It is recommended that government authorities improve public health cancer registers to include information on occupation and workplace factors and identification of leukaemia type.

22. Secondary Notification

Under Section 65 of the *Industrial Chemicals (notification and Assessment) Act 1989*, secondary notification of benzene may be required where an introducer of the chemical becomes aware of circumstances that may warrant a reassessment of its hazards and risks. Specific circumstances include:

- the function or use of benzene has increased, or is likely to change, significantly;
- the amount of benzene introduced into Australia has increased, or is likely to increase, significantly;
- the method of manufacture of the chemical in Australia has changed, or is likely to change, in a way that may result in an increased risk of adverse health effects or adverse environmental effects; and
- additional information has become available to the introducers as to the adverse health effects or adverse environmental effects of the chemical.

The Director (Chemicals Notification and Assessment) must be notified within 28 days of the manufacturer/importer becoming aware of any of the above or other circumstances prescribed under Section 65 of the Act.

Appendix 1

Sample Material Safety Data Sheet for Benzene

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Benzene is classified as Hazardous according to the National Occupational Health and Safety Commission's *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(1999)].

Company details

Company name

Address

State Postcode

Telephone number | Emergency telephone number

Facsimile number | Telex number

Identification

Product name
Benzene

Other names
Benzol; cyclohexatriene

Manufacturer's product code

UN number
1144

Dangerous goods class and subsidiary risk
Class 3

Hazchem code
3WE

Poisons Schedule number
Schedule 7

Use
Laboratory reagent

Physical description and properties

Appearance
Colourless liquid

Boiling point
80.1°C

Melting point
5.5°C

Vapour pressure
12.6 kPa at 25°C

Specific gravity
0.87 at 25°C (water = 1)

Flashpoint
-11°C (closed cup)

Flammability limits
1.4-7.9%

Solubility in water
1.8 g/L at 25°C

Other properties

Odour: Sweet, aromatic
Odour threshold: 0.8-160 ppm (average: 2 ppm)
Vapour density: 2.8 (relative to air = 1)
Autoignition temperature: 560°C

Ingredients

Chemical entity	CAS Number	Proportion
Benzene	71-43-2	

Health hazard information

HEALTH EFFECTS

Acute:

Inhalation: Irritating to respiratory system (including mouth, nose and throat) at 30-60 ppm. Dizziness and headache progressing to drowsiness and unconsciousness at 250-3000 ppm. Inhalation at 20,000 ppm may be fatal within 5-10 minutes.

Skin: Liquid and vapour irritating to skin. Readily absorbed through skin.

Eye: Liquid and vapour cause eye irritation.

Swallowed: The lowest reported fatal dose is 10 mL. Smaller doses can cause dizziness and headache progressing to drowsiness and unconsciousness. Severe lung damage can occur if drawn into the lungs (aspirated) during swallowing or vomiting.

Chronic:

Skin: No evidence of sensitisation in animals or humans.

Inhalation: Prolonged or repeated exposure can cause decreased bone marrow production of blood cells, severe blood disorders and leukaemia (cancer of the white blood cells). Damage to the chromosomes of white blood cells has been observed at high concentrations.

Other information:

Benzene is secreted in breast milk.

Risk phrases:

R11 Highly flammable

R45 May cause cancer (Carcinogen Category 1

R48/23/24/25 Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed

R36/37/38 Irritating to eyes, respiratory system and skin

R40 Possible risk of irreversible effects (Mutagen Category 3)

FIRST AID

Inhalation: Remove from exposure to fresh air immediately. If breathing with difficulty, give oxygen. If not breathing, clear airways and apply artificial respiration. Call a doctor.

Skin: Remove contaminated clothing and wash skin thoroughly. Seek medical attention if irritation develops.

Eye: Irrigate immediately with copious quantities of water for at least 15 minutes. Seek medical attention.

Swallowed: Do not give anything by mouth if victim is losing consciousness, unconscious or convulsing. Do not induce vomiting, give a glass of water. If breathing with difficulty, give oxygen. If not breathing, clear airways and apply artificial respiration. Call a doctor.

FIRST AID FACILITIES

An emergency safety shower and eye wash station should be available in the immediate work area.

ADVICE TO DOCTOR

Treatment is symptomatic and supportive. No specific antidote.

Precautions for use

EXPOSURE STANDARD

Australian Exposure Standard: 5 ppm (16 mg/m^3)TWA,
Carcinogen Category 1 (known human carcinogen).

ENGINEERING CONTROLS

Control airborne concentrations well below the exposure standard.

Use only in flameproof fume cupboard or with good local exhaust ventilation.

Cover working surfaces with an absorbent material backed by plastic and replace at regular intervals and immediately a spillage occurs.

Do not decant into plastic containers that may be permeable to benzene, or from large bottles into measuring cylinders or beakers as splashing or spills may occur.

PERSONAL PROTECTION

Wear long-sleeved protective clothing, safety glasses and benzene resistant gloves in accordance with manufacturer's recommendation. A respirator with full-face protection may be required where engineering controls are inadequate, such as in the case of spills.

FLAMMABILITY

Highly flammable. Vapour may form explosive mixtures with air.

Avoid all sources of ignition.

Vapour is heavier than air and may travel along the ground to a source of ignition and flash back.

Safe handling information

STORAGE AND TRANSPORT

Regulated dangerous goods in Class 3, Packaging Group III.

Store in screw-capped, unbreakable, chemically resistant container in locked flammable liquids/poisons cupboard in a ventilated storage room.

SPILLS AND DISPOSAL

Small spills can be cleaned up with absorbent material. Wear appropriate personal protective equipment to prevent skin and eye contamination and, if necessary, a respirator with full-face protection. Collect contaminated material in sealable containers for disposal in accordance with all Local, State and Federal regulations at an approved waste disposal facility.

Do not allow the substance to enter drains and waterways.

In the case of large spills, evacuate the area, shut off all possible sources of ignition and follow institutional emergency procedures.

Prior to disposal, keep contaminated pipette tips in ventilated fume cupboard until completely dry.

Dispose of at an approved waste disposal facility in accordance with all Local, State and Federal regulations.

FIRE/EXPLOSION HAZARD

Highly flammable. Vapour may form explosive mixtures with air.

Carbon monoxide may be released in a fire involving benzene.

Fire fighters should wear self-contained breathing apparatus and complete protective clothing. For fires, foam, carbon dioxide or dry chemical extinguishing media may be used.

Other information

ANIMAL TOXICITY DATA

Acute (inhalation) LD_{50}(4h): 13,700 ppm (rat).
Acute (oral) LD_{50}: 810-9900 mg/kg (rat).
Acute (dermal) LD_{50}: >8200 mg/kg (rabbit).

Repeated dose toxicity: Benzene has been shown to cause central nervous system depression, immunosuppression and bone marrow depression in mice and rats.

Fertility effects: High doses can have toxic effects on the testes and ovaries in mice. Data on reproductive capacity are inconclusive.

Developmental toxicity: In rats and mice exposed to 100-500 ppm during pregnancy, benzene causes foetal growth retardation. There is no evidence of birth defects.

Lactation effects: No data.

Genetic toxicity: Benzene is positive in several test systems.

Carcinogenicity: Benzene has been shown to induce malignant tumours in several organs in rats and mice.

ENVIRONMENTAL DATA

Acute (mg/L):

Selenastrum capricornutum (alga)	72-h EC_{50}	29
Daphnia magna	48-h EC_{50}	>100
Ceriodaphnia dubia (water flea)	24-h EC_{50}	18.4
Scylla serata (crab species)	96-h LC_{50}	3.7-7.7
Pimephales promelas (fathead minnow)	96-h LC_{50}	12.6-24.6
Poecilia reticulata (guppy)	96-h LC_{50}	28.6
Oncorhynchus mykiss (rainbow trout)	96-h LC_{50}	5.3-9.2
Morone saxatilis (striped bass)	96-h LC_{50}	5.8

Chronic:

The lowest recorded no observed effect concentration is 0.17 mg/L in *Cancer magister* (Dungeness crab).

FURTHER INFORMATION

National Health and Medical Research Council: Guidelines for laboratory personnel working with carcinogenic or highly toxic chemicals (Australian Government Publishing Service, 1990).

Guidelines for Health Surveillance: Benzene (NOHSC, 1996).

National Industrial Chemicals Notification and Assessment Scheme: Full Public Report – Priority Existing Chemical No. XX – Benzene (NICNAS, 2001).

Contact point

Contact name	Telephone number

Position title

Address

State Postcode Country

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Benzene

Toxicological overview

Key Points

Kinetics and metabolism

- The primary route of entry is via inhalation. Dermal absorption is poor.
- Reactive metabolites such as benzene oxide have been implicated in the mechanism(s) of benzene toxicity.

Health effects of acute exposure

- Acute exposure may resemble solvent intoxication, clinically manifest as drowsiness, dizziness, delirium, loss of consciousness, respiratory arrest and death.

Health effects of chronic exposure

- Two, well documented adverse health effects of chronic benzene exposure are anaemia and leukaemia.
- Benzene is a known human carcinogen and clastogen but is not considered to be a reproductive toxicant.

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2007
Version 2

Toxicological Overview

Summary of Health Effects

Acute exposure to relatively high concentrations of benzene (benzol) may result in CNS disturbances consistent with solvent exposure, *viz*., drowsiness, dizziness, headache, tremor, delirium, ataxia, loss of consciousness, respiratory arrest and death [1].

A characteristic effect of chronic benzene exposure is aplastic anaemia, resulting from suppression of bone marrow tissue [2]. Benzene is a known human carcinogen, with a substantial number of case reports and epidemiological studies providing evidence of a causal relationship between occupational (chronic) exposure and various types of leukaemia [3]. It is currently hypothesised that the carcinogenic effects of benzene are predominantly mediated via metabolites such as benzene oxide [4]. It is mutagenic and a genotoxic carcinogen. The assumption is made that there is no threshold to such effects and that any exposure results in some increase in risk, albeit this may be very small.

Kinetics and metabolism

Benzene is well absorbed by inhalation [11]: systemic absorption of benzene from the lungs is greatest over the first few minutes of exposure (70 – 80% of inhaled dose) and decreases thereafter to approximately 50% of the inhaled dose after one hour [12].

Dermal absorption of benzene is generally considered to be poor. When applied as a discrete droplet, 99.9% of an applied dose may vaporise from the skin surface before being absorbed [13]. However, excessive dermal contamination may make a substantial contribution to the daily intake and so skin contact with benzene should be avoided [14].

Systemic absorption of benzene after ingestion is likely to be high, especially when dissolved in water and case reports of poisoning indicate that benzene is extensively absorbed by the oral route [11].

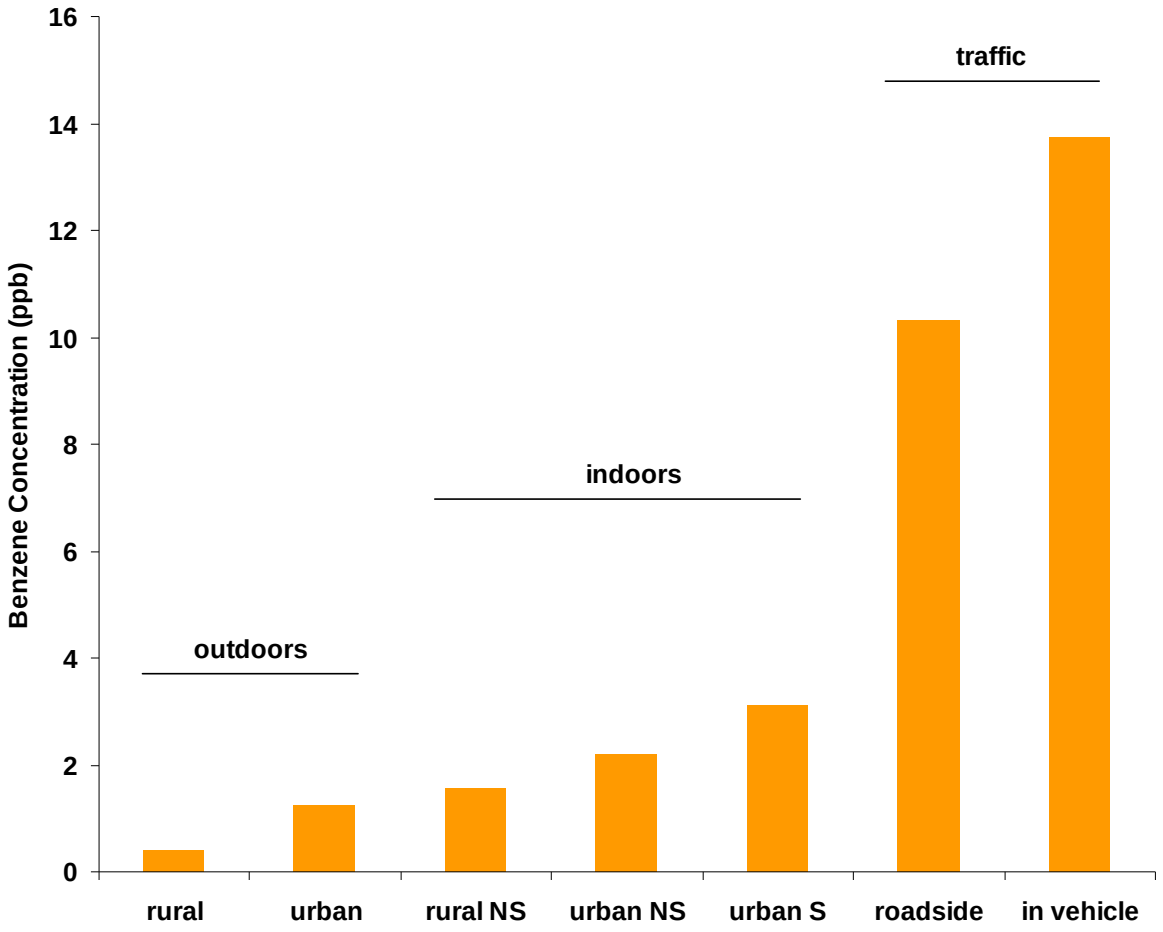
The metabolism of benzene has been widely studied in human and animal models due to its putative role in carcinogenesis (metabolic activation) [15, 16]. The metabolic pathways implicated in benzene toxicity are broadly comparable across species. However, there are significant species-specific differences in the extent to which benzene is metabolised by each pathway [17]. Furthermore, the proportion of benzene metabolised by each pathway appears to be dose-dependent, with hydroquinone and phenyl conjugation pathways predominating at lower (<10ppm) and higher (>10ppm) concentrations, respectively ([18, 19] as reviewed by [1]).

Two enzymes have been particularly implicated in the mechanism of benzene toxicity: cytochrome P450 2E1 (CYP2E1) and quinone oxidoreductase (NQ01). Absence of CYP2E1 leads to a reduction of the cytotoxic and genotoxic effects of benzene in transgenic mice [20]. Conversely, susceptibility to benzene toxicity is augmented in human individuals and animals lacking NQ01 [21, 22]. Although the actual metabolite that is responsible for the carcinogenic effects of benzene has not been definitively identified [16], there is evidence that this is mediated by benzene oxide, a reactive metabolite of CYP2E1 [4] which is sufficiently stable ($t_{½}$ ~ 7 – 9 minutes) to ensure distribution throughout the body.

Sources and route of human exposure

Benzene is a ubiquitous air pollutant which can vary widely in concentration according to location (Figure 1). Historically, road traffic vehicles have represented the major source of benzene in the UK and accounted for ~ 60% of total emissions in 1990 [5]. Legislation to decrease the benzene content of engine fuels to less than 1% [6] and the compulsory introduction of catalytic converters on vehicle exhausts have significantly reduced this source of pollution; in 2004, road vehicles accounted for less than 20% of UK benzene emissions [5]. Domestic sources currently contribute the greatest proportion (33%) of benzene emissions, principally through the combustion of fuels for cooking and heating and the operation of garden appliances such as lawn mowers and patio heaters [5] .

Figure 1: Approximate concentrations of benzene in ambient air; data from [7]. "ppb" = parts per billion; "NS" = non-smoker; "S" = smoker.



Contributions to the daily intake of benzene from food and water are relatively low (less than 1½ to 2% of total; Table 1), although contaminated groundwater may potentially represent a significant source of benzene exposure [8]. For the majority of the population, smoking and propinquity to road traffic are the predominant factors affecting daily exposure (Table 1). Ambient air concentrations of benzene within dwellings tend to be around twice as high as comparable outdoor concentrations (Figure 1) and smoking indoors can make a significant contribution to the concentration of benzene [9].

Table 1: Current estimated (average) daily intake of benzene in adults; data from [10].

Source		Total Benzene (µg day^{-1})
Diet	Food	1.5
	Water	< 0.1
Air	Rural (non smoker)	73
	Urban (non-smoker)	92
Smoking	20 cigarettes per day	400

Health Effects of Acute / Single Exposure

Human Data

General toxicity

Benzene is not generally regarded as an acutely toxic material and there are correspondingly few reports pertaining to the (human) health effects of a single exposure. In general, acute exposure to concentrations of benzene in excess of 500 ppm may illicit signs and symptoms consistent with solvent intoxication (Table 2). Overt signs of exposure have previously been referred to as "benzol jag", characterised by euphoria, unsteady gait and confusion [23]. Recovery from an acute exposure is dose-dependent, with breathlessness, nervous irritability and unsteadiness in gait persisting in severe cases for two to three weeks [24].

Table 2: Summary of acute benzene vapour toxicity [11].

Concentration		Duration of exposure (min)	Effect(s)
ppm	mg m^{-3}		
25	80	480	No observable effect
50-150	160-480	300	Headache, lassitude, weakness
500	1,600	60	Symptoms of illness
1500	4,800	60	Serious symptoms
3000	9,600	30	Endurable
7500	24,000	30	Dangerous to life
19000-20000	60,800-64,000	5-10	Fatal

Inhalation

The commonly quoted "lethal dose" of benzene (20,000 ppm) is an estimate based on a review of a single case report following 5 – 10 minutes' exposure [25]. Fatal exposures have been associated with asphyxiation, respiratory arrest, central nervous system depression and possibly cardiac arrhythmias [26]. Death may be due to CNS depression, asphyxiation or respiratory or circulatory arrest. It has been observed that aspiration of benzene directly onto the lungs causes "immediate pulmonary oedema and haemorrhage at the site of contact with the pulmonary tissue" [24].

Benzene is irritating to the nose and respiratory tract at "high" concentrations [24].

Ingestion

The single, acute lethal dose of benzene in humans is estimated to be 125 mg kg^{-1}, equivalent to 10 ml per 70 kg man^{-1}. Signs of intoxication following ingestion include staggered gait, vomiting, shallow and rapid pulse, somnolence, delirium, pnuemonitis, central nervous system depression, coma and death [11].

Dermal / ocular exposure

Whilst benzene is poorly absorbed through the skin, prolonged or excessive contact may cause signs consistent with the defatting (delipidising) effects of organic solvents, *viz.*, erythema, vesiculation and dermatitis [27].

Benzene vapour may cause a smarting effect on the eyes at high concentrations. Eye contamination with droplets of benzene may cause a moderate burning sensation with only slight, transient injury to the epithelial cells [28].

Delayed effects following an acute exposure

Most cases of acute benzene intoxication resolve spontaneously or with supportive care in the absence of long-term sequelae [29].

Health Effects of Chronic / Repeated Exposure

Human Data

General toxicity

The adverse health effects of chronic benzene exposure have been extensively documented and primarily relate to impairment of the haemopoietic system [23-34], with bone marrow depression leading to aplastic anaemia being the most common clinical manifestation (occurring in approximately 1% of individuals exposed to > 100 ppm benzene) [11, 30]. Other effects on the haemopoietic system include leukopenia, agranulocytosis, anaemia, pancytopenia and myelodysplastic syndrome [31]. Benzene is also established as being leukaemogenic in humans [3, 32-38]. Exposures to 1 ppm benzene for 40 working years has been considered not to be associated with any increase in leukaemia or any other haematological abnormality [11, 39]. However, since benzene is a genotoxic carcinogen (see below) the assumption is made that there is no threshold for its effect. I.e. any exposure is associated with some increase in risk, although this may be very small [4].

Susceptibility to benzene toxicity has been related to genetic polymorphisms [40-45]. For example, an excess of CYP2E1 [46-48] or deficiency of quinone oxidoreductase (NQO) [49-51] may enhance benzene toxicity. Indeed, one study has suggested that chronic benzene exposure to less than 1 ppm may induce haematotoxic effects in such genetically susceptible populations, although it was recognised that more data were required before any definite conclusions could be drawn [52].

Other factors that influence the toxicity of benzene include the systemic distribution rates of metabolites and consequent events within bone marrow tissue such as secondary metabolic activation, induction of apoptosis, altered differentiation of early progenitor cells and depletion of the stem cell pool [30].

In addition to effects on the haematopoietic system, benzene exposures have been implicated in neurological disorders [53], immune dysfunction [11] and cancer [3]. Further, detailed information on the general toxicity of benzene can be obtained from a number of comprehensive reviews [11, 26, 36, 54].

Genotoxicity

Benzene is a human clastogen [3]: chronic exposure results in consistent structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells which may be observed for at least five years after cessation of (occupational) exposure [11].

Although the actual metabolite that is responsible for the carcinogenic effects of benzene has not been definitively identified [16], there is evidence that it is mediated by benzene oxide, a metabolite of CYP2E1 which is sufficiently stable ($t_{½}$ ~ 7 – 9 minutes) to ensure distribution throughout the body [9].

Carcinogenicity

Benzene is classified by the IARC as Group 1 human carcinogen [3] and its role as a leukaemogen has been clearly established through a number of epidemiological studies (Table 3).

Table 3: Empirical summary of studies pertaining to chronic (occupational) benzene exposure and putative disease outcomes. Putative disease outcome = disease state associated/correlated with occupational benzene exposure. Data derived from IPCS [11].

Study Type¶	"n"	Putative disease outcome	Original Reference
Case	44	Leukaemia	[55]
	63	Leukaemia, multiple myeloma, malignant lymphoma	[56]
	94	Hodgkin's disease	[57]
	1	Subacute granulocytic leukaemia	[58]
	6	Haemocytoblastic leukaemia	[59]
	1	Acute myelogenous leukaemia	[60]
Epidemiology	28,500	Aplastic anaemia, acute leukaemia	[61]
		Myeloid and monocytic leukaemia	[62]
		Leukaemia, multiple myeloma	[63]
	956	Leukaemia, acute myelogenous leukaemia	[64]
	3636	Leukaemia	[65]
	259	Lymphatic and haemopoietic neoplasms	[66]
	2013	Aplastic anaemia, leukaemia	[67]
	28,460	Acute and chronic leukaemia	[68]
	391	Leukaemia	[69]
		Leukaemia	[70]

Reproductive and developmental toxicity

Benzene diffuses across the placenta and is considered to be fetotoxic in the presence of maternal toxicity [27]. Benzene is not considered to be a teratogen and there is currently no evidence that it causes reproductive effects in humans [71].

Animal Data

Animal studies are largely in accordance with the known toxicity of benzene in humans. However, the carcinogenic effects of benzene in animals is primarily associated with epithelial tissue rather than leukaemia [11].

General toxicity

Numerous repeated dose studies in animals have shown that benzene produces bone marrow depression leading to a range of haematological effects including reduction in haematocrit, decreased haemoglobin and a reduction in RBC (red blood cell), WBC (white

blood cell) and platelet counts. Decreased proliferation of B and T lymphocytes and resistance to infection has also been demonstrated [11].

Genotoxicity

Although assays for the ability of benzene to induce gene mutations in bacteria were negative, benzene has consistently given positive results from *in-vitro* assays for clastogenicity [72]. Thus, benzene clearly has mutagenic potential. There is *in-vivo* evidence to demonstrate that benzene is both clastogenic and induces gene mutations in animals. For example, in studies with transgenic mice (using lac1 as a reporter gene), benzene induced gene mutations in the lung and spleen [11]. Benzene is clearly an *in-vivo* mutagen.

Carcinogenicity

Benzene has been shown to produce several types of neoplasms in both rats and mice after either oral dosing or inhalation exposure. These include various types of epithelial neoplasms and a few lymphomas and leukaemias [11].

Reproductive Toxicity

A number of studies have been carried out to investigate the effect of exposure to benzene during pregnancy. None has detected any teratogenic potential even at exposure levels that produced some evidence of toxicity in the maternal animals. Some adverse effects on the foetus (reduced birth weight and/or minor skeletal variants) were seen at relatively high exposure levels (~ 150 mg m^{-3} and above). Some haematopoietic changes were observed in offspring from adults exposed to lower levels (16 mg m^{-3} and above) [11].

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This document will be reviewed not later than 3 years or sooner if substantive evidence becomes available.