TOXICOLOGICAL PROFILE FOR HEXACHLOROBENZENE

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

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DISCLAIMER

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UPDATE STATEMENT

A Toxicological Profile for Hexachlorobenzene, Draft for Public Comment was released in June 2013. 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 these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The 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.

Each profile includes the following:

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

(B) 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. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

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

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find 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:

Chapter 1	**How Can (Chemical X) Affect Children?**
Chapter 1	**How Can Families Reduce the Risk of Exposure to (Chemical X)?**
Section 3.7	**Children's Susceptibility**
Section 6.6	**Exposures of Children**

Other Sections of Interest:

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

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional material is available online at www.atsdr.cdc.gov:

Case Studies in Environmental Medicine—Case Studies are self-instructional publications designed to increase primary care provider's knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients.

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, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: (202) 245-0625 or 1-800-CDC-INFO (800-232-4636).

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.

Clinical Resources

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 Environmental 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 hexachlorobenzene. The panel consisted of the following members:

1. Eric P. Hoffman, Ph.D., Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC;

2. Lucio Costa, Ph.D., Professor, Department of Environmental Health, University of Washington, Seattle, Washington;

3. Shane Que Hee, Ph.D., UCLA School of Public Health, Los Angeles, California.

These experts collectively have knowledge of hexachlorobenzene'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 FOR HEXACHLOROBENZENE

This Public Health Statement summarizes the Division of Toxicology and Human Health Science's findings on hexachlorobenzene, tells you about it, the effects of exposure, and describes what you can do to limit that exposure.

The U.S. Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are sites targeted for long-term federal clean-up activities. U.S. EPA has found hexachlorobenzene in at least 113 of the 1,699 current or former NPL sites. The total number of NPL sites evaluated for hexachlorobenzene is not known. But the possibility remains that as more sites are evaluated, the sites at which hexachlorobenzene is found may increase. This information is important because these future sites may be sources of exposure, and exposure to hexachlorobenzene may be harmful.

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

WHAT IS HEXACHLOROBENZENE?

Hexachlorobenzene is a white, crystalline solid at room temperature. Hexachlorobenzene is not currently manufactured as a commercial end product in the United States, and evidence indicates that it has not been commercially produced since the late 1970s. Hexachlorobenzene was used as a fungicide in the United States until 1984, when the last registered use of the compound as a pesticide was voluntarily cancelled. Small amounts of hexachlorobenzene can still be used in chemical laboratories for research purposes.

More information on the chemical and physical properties as well as the production and uses of hexachlorobenzene is presented in Chapters 4 and 5.

WHERE IS HEXACHLOROBENZENE FOUND?

Hexachlorobenzene can mostly be found in treated and background soils, sediments, and oceans. It can also be found in air, surface water, and groundwater due to use and disposal of hexachlorobenzene products and as a byproduct of other processes. Hexachlorobenzene can accumulate up through the food chain and has been detected in breast milk.

Hexachlorobenzene is usually detected in air at low levels (typically in the picogram to nanogram per cubic meter [pg/m^3 to ng/m^3] range). Incineration of chlorinated materials is also a source of hexachlorobenzene. Hexachlorobenzene is very slow to break down in air and is subject to long-range transport in the atmosphere.

Hexachlorobenzene has been detected in groundwater, drinking water, and surface water. Levels are typically in the low parts per billion (ppb) to parts per trillion (ppt) range. Hexachlorobenzene is very slow to break down in water. It tends to adsorb to suspended particles and sediment in the water column and is highly bioaccumulated by aquatic organisms like fish.

Hexachlorobenzene has been detected in soil and sediment samples both in agricultural areas where it was formerly used and in urban soils near production and waste disposal sites. The levels of hexachlorobenzene can vary greatly in soil and sediment. Hexachlorobenzene levels as high as 53,000 ppb were observed in soil from a contaminated area. Hexachlorobenzene has low mobility in soil and may evaporate from soil surfaces. It is very persistent and takes many years to break down. It is slowly degraded by soil microorganisms.

Hexachlorobenzene has been detected in fish and food products. The Food and Drug Administration (FDA) Total Diet Study market basket surveys have found trace levels (<1 ppb) of hexachlorobenzene in many store-purchased food items. Fish from contaminated areas can contain levels of hexachlorobenzene greater than 100 ppb. The main exposure pathway for the general public to hexachlorobenzene is from the ingestion of food, typically only low-level exposure. Exposure to higher levels may occur through the contamination of food, such as consumption of fish caught in contaminated areas, and the ingestion of contaminated breast milk for infants.

More information on levels of hexachlorobenzene found in the environment is presented in Chapter 6.

Although hexachlorobenzene is not currently manufactured, it is formed as a waste product in the production of several chlorinated hydrocarbons, such as tetrachloroethylene and trichloroethylene, and is a contaminant in some pesticides, such as pentachloronitrobenzene and pentachlorophenol. Small amounts of hexachlorobenzene can also be produced during combustion processes, such as burning municipal waste. Hexachlorobenzene may also be produced as a byproduct in waste streams of chlor-alkali plants and wood preserving plants.

HOW MIGHT I BE EXPOSED TO HEXACHLOROBENZENE?

The primary route of exposure to hexachlorobenzene for the general population of the United States is from food. You may be exposed to very low levels of hexachlorobenzene through ingestion of contaminated foods, such as fatty fish. Young children may be exposed to hexachlorobenzene by playing in contaminated soil. Children who play in and on contaminated soil may have a higher potential for exposure through the skin and through inhalation of contaminated dust. Children who eat contaminated soil may ingest hexachlorobenzene. Hexachlorobenzene can also accumulate in breast milk. Breastfed babies may be exposed to hexachlorobenzene through their mother's breast milk. Other sources of exposure may include contact with water or air contaminated with hexachlorobenzene. Hexachlorobenzene has been found in ambient water samples at concentrations of less than 0.1 parts per trillion (ppt) and ambient air samples at concentration ranges of 0.1 pg/m^3 to 1.5 ng/m^3. Workers involved in the production of chlorinated hydrocarbons, which releases hexachlorobenzene as a byproduct, may also be exposed.

Additional information on levels in the environment and potential for human exposure is presented in Chapter 6.

HOW CAN HEXACHLOROBENZENE ENTER AND LEAVE MY BODY?

Hexachlorobenzene can enter your body from the air, water, or contaminated food or soil. If you breathe air containing hexachlorobenzene, some of it will be absorbed through your lungs. Some of the hexachlorobenzene in contaminated drinking water, food, soil, or breast milk will be rapidly absorbed through the digestive tract. This is the most likely route of significant exposure to hexachlorobenzene.

Hexachlorobenzene rapidly spreads through your blood to many tissues, especially fat, where it can remain for years. Hexachlorobenzene easily moves from blood of pregnant mothers, across the placenta to the unborn child and into breast milk of a nursing mother, resulting in exposure to her baby. Because hexachlorobenzene accumulates in fat, levels in babies (particularly breast-fed babies) may be higher than those in the mothers.

Most hexachlorobenzene leaves your body as hexachlorobenzene in the feces. Some hexachlorobenzene is converted to other chemicals in your body and some of these chemicals leave your body in the urine.

HOW CAN HEXACHLOROBENZENE AFFECT MY HEALTH?

Exposure to very high levels of hexachlorobenzene for short periods caused effects on the nervous system such as weakness, tremors, and convulsions; skin sores; liver effects such as porphyria, which is a decrease in the production of the heme (iron-protein) portion of red blood cell hemoglobin that carries oxygen to cells; and thyroid effects such as decreased thyroid hormones. These types of effects were seen in some people in Turkey who were exposed to high levels of hexachlorobenzene in bread made from grain that had been treated with the chemical as a pesticide.

Long-term exposure to hexachlorobenzene can cause effects similar to those from short-term exposure. Because hexachlorobenzene accumulates in fat (including breast tissue) where it can remain for long periods, long-term exposure can result in a build-up of hexachlorobenzene in the body. Therefore, long-term exposure may be more serious than acute or short-term exposure.

Studies in animals suggest that eating foods with hexachlorobenzene for months or years can cause cancer of the liver, kidney, and thyroid.

The U.S. Department of Health and Human Services (DHHS) considers hexachlorobenzene as reasonably anticipated to be a human carcinogen. The U.S. EPA says that hexachlorobenzene is a probable human carcinogen. The International Agency for Research on Cancer (IARC) says that hexachlorobenzene is possibly carcinogenic to humans.

See Chapters 2 and 3 for more information on hexachlorobenzene health effects.

HOW CAN HEXACHLOROBENZENE AFFECT CHILDREN?

This section discusses potential health effects of hexachlorobenzene exposure in humans from when they're first conceived to 18 years of age, and how you might protect against such effects.

Infants and young children appeared to be especially sensitive to the effects of hexachlorobenzene in the Turkish grain poisoning epidemic. Breastfed infants of mothers known to have eaten bread contaminated with high levels of hexachlorobenzene developed a skin disorder known as pembe yara or "pink sore". Other symptoms were weakness and convulsions. Many of the sickened infants died from this disease. Young children beyond 2 years of age did not get pink sore, but they did develop skin, nervous system, and bone abnormalities later in life.

Although hexachlorobenzene has been banned in the United States since 1966 and globally under the Stockholm Convention since 2004, it is ubiquitous in the environment. This environmental lingering exists because of its past production and use as an organic synthesis compound, and former applications as fungicides and pesticides. However, it is unlikely that hexachlorobenzene will be detected in soil samples in the United States at or near levels that caused the epidemic in Turkey during the 1950's when it was added as a fungicide to wheat seedlings. For more information on the releases, occurrences, and movements of this substance, see Chapters 5 and 6.

One study found higher levels of hexachlorobenzene in the milk of breastfed babies who had ear infections than in milk of breastfed babies without ear infections. We do not know if hexachlorobenzene increased susceptibility to infection in these babies.

Young animals exposed to hexachlorobenzene before and soon after birth are especially sensitive to hexachlorobenzene. Liver lesions developed during adulthood in rats treated with combined pre- and postnatal exposure. Effects on the nervous system and immune function occurred at lower doses in the young developing animals than in adults. Animal studies also showed that hexachlorobenzene has effects on various endocrine organs, including the thyroid gland (hypothyroidism), parathyroid gland (hyperparathyroidism), adrenal gland, and ovaries. These tissues produce hormones that are important for normal growth and development of the organism.

Higher levels of hexachlorobenzene were found in the fat of boys with a specific type of birth defect, undescended testis, than in the fat of boys without this defect; however, we do not know if hexachlorobenzene caused the birth defect. Some studies evaluated possible associations between

maternal serum hexachlorobenzene levels and developmental end points such as birth size (weight and/or length) or preterm birth, recurrent miscarriage, postnatal growth, postnatal neurodevelopment, sexual maturation, undescended testes, hypospadias (a congenital defect in which the urethra exits the penis on the underside rather than the tip), and indicators of postnatal thyroid function. Although most studies found no associations between maternal serum hexachlorobenzene levels and developmental effects, there were reports of associations between levels of hexachlorobenzene in maternal or umbilical cord blood and birth weight, postnatal growth, and hypospadias. There is some indication that hexachlorobenzene in the blood of young boys and girls may cause changes in blood sex hormone levels.

HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO HEXACHLOROBENZENE?

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

The primary way that most people are exposed to hexachlorobenzene is through food. Fatty food may be higher in hexachlorobenzene than less fatty food. Additionally, when hexachlorobenzene is present in food, more may be absorbed when the food is fatty than when the food is less fatty. Therefore, eating less fatty food may reduce the risk of exposure to hexachlorobenzene.

Exposure to contaminated drinking water should be limited. Hexachlorobenzene has been detected in some drinking water supplies. For bottled water, consumers should contact the bottler with specific questions on potential contaminants.

If you live near an industrial site where hexachlorobenzene was produced or is produced as an unintentional byproduct, or if you live near a hazardous waste site where it has been discarded, there may be high levels of hexachlorobenzene in the water and soil. Substituting cleaner sources of water and limiting contact with soil (for example, through use of a dense ground cover or thick lawn) would reduce family exposure to hexachlorobenzene. Produce grown in hexachlorobenzene-contaminated soil should not be eaten. By paying careful attention to dust and dirt control in the home (air filters, frequent cleaning), you can reduce family exposure to hexachlorobenzene-contaminated dirt. Some children eat a lot of dirt. You should prevent your children from eating dirt. You should discourage your children from putting objects in their mouths. Make sure that they wash their hands frequently and before eating. Discourage your children from putting their hands in their mouths or from other hand-to-mouth activity.

Check labels for hexachlorobenzene. In the past, some technical-grade pesticides or solvents when produced were found to contain trace amounts of hexachlorobenzene as an impurity. However, levels of hexachlorobenzene would be expected to be much lower than those causing health problems.

ARE THERE MEDICAL TESTS TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO HEXACHLOROBENZENE?

Blood, breast milk, urine, and feces may be tested to determine if you have ever been exposed to hexachlorobenzene. These tests are not usually available at a doctor's office because they require special equipment found at county, state, university, and independent analytical laboratories. Because hexachlorobenzene can deposit and remain in human fat for several years, tests for hexachlorobenzene and its breakdown products (metabolites) can tell you only that you have been exposed to hexachlorobenzene, but not when or to how much. Furthermore, the detection of hexachlorobenzene or its metabolites cannot predict the kind of health effects that might develop from that exposure. Blood, urine, and feces can also be monitored for porphyrins. High porphyrin levels indicate slowed formation of heme, which is a major effect of hexachlorobenzene in the body. The production of persistent purple urine is diagnostic. The usefulness of this test as a sign of hexachlorobenzene exposure is limited, because there are many other potential causes of high porphyrin levels.

For more information on the different substances formed by hexachlorobenzene and tests to detect these substances in the body, see Chapters 3 and 7.

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

Regulations and recommendations can be expressed 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 usually based on levels that affect

animals; levels are then adjusted to help protect humans. Sometimes these not-to-exceed levels differ among federal organizations. Different organizations use different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or emphasize some factors over others, depending on their mission.

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 issued the regulation or recommendation.

The U.S. EPA has determined that exposures to hexachlorobenzene in drinking water of adults or children (10 years old or younger) at concentrations less than or equal to 0.05 milligrams per liter (mg/L) for up to 10 days or adults at less than or equal to 0.03 mg/L for a lifetime (assuming 100% of hexachlorobenzene exposure is from drinking water) are not expected to cause any adverse noncancer effects.

WHERE CAN I GET MORE INFORMATION?

If you have any 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 provide publically available information regarding medical specialists with expertise and experience recognizing, evaluating, treating, and managing patients exposed to hazardous substances.

- Call the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636) or

- Write to:

  Agency for Toxic Substances and Disease Registry
  Division of Toxicology and Human Health Sciences
  1600 Clifton Road NE
  Mailstop F-57
  Atlanta, GA 30329-4027

Toxicological profiles and other information are available on ATSDR's web site: http://www.atsdr.cdc.gov.

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO HEXACHLOROBENZENE IN THE UNITED STATES

Hexachlorobenzene (HCB) is a fully chlorinated benzene industrial chemical that is practically insoluble in water, but is very soluble in fat, oils, and organic solvents. Hexachlorobenzene is one of the most persistent environmental pollutants, and bioaccumulates in the environment, in animals, and in humans. It is not currently manufactured as a commercial product in the United States, and virtually all commercial production ended in the late 1970s. Small amounts can be imported for research use. However, some hexachlorobenzene is produced as a by-product or impurity in the manufacture of chlorinated solvents and other chlorinated compounds (e.g., chloranil, chlorophenols, tetrachloroethylene, trichlorobenzenes, trichloroethylene, trichlorotoluenes, and vinyl chloride), including several pesticides (pentachloronitrobenzene, chlorothalonil, Dacthal®, picloram, pentachlorophenol, atrazine, and simazine). It is estimated that 3,500–11,500 kg of hexachlorobenzene were inadvertently produced in the manufacture of chlorinated solvents in 1984. There is no current commercial manufacturing of hexachlorobenzene in the United States, although hexachlorobenzene was used as a fungicide on the seeds of onions, sorghum, wheat, and other grains until 1984, when its registration as a pesticide was voluntarily canceled. Hexachlorobenzene had also been used in the production of pyrotechnic and ordinance materials for the military and in the production of synthetic rubber. Hexachlorobenzene is one of the "dirty dozen" chemicals that have been outlawed by the Stockholm Convention, which was convened in 2001 to assess selected persistent organic pollutants.

The general population is not likely to be exposed to large amounts of hexachlorobenzene, but some exposure is likely, as many studies have detected small amounts in food and air samples. Fish and meat products seem to have the highest frequency of detection (see Tables 6-5 and 6-6) and infant exposure may result from breast milk containing hexachlorobenzene. Traces of hexachlorobenzene have been found in almost all people tested for hexachlorobenzene or its metabolites. These amounts of hexachlorobenzene are most likely the result of consumption of low levels in food, with an estimated yearly uptake of 68, 22, and 5 μg for adults, toddlers, and infants, respectively. Other sources of exposure may include contact with contaminated soil and air. Hexachlorobenzene levels as high as 53,000 ppb were observed in soil from a contaminated area. Ambient air samples have usually been reported to range from 0.1 pg/m^3 to 1.5 ng/m^3. Hexachlorobenzene has a very low solubility in water, so exposure by water is not likely to be significant; ambient water samples have usually been below 0.1 parts per trillion (ppt). If released to water, hexachlorobenzene will adsorb to suspended solids and partition to sediment.

Children are expected to be exposed to hexachlorobenzene by the same routes as adults. Young children may be particularly at risk by playing in hexachlorobenzene soil-contaminated areas. Additionally, if hexachlorobenzene is present in their mothers, unborn children may be exposed through the placenta and nursing children may be exposed to hexachlorobenzene present in milk. Human milk samples from the general population found hexachlorobenzene concentrations in the range of 11–70 ng/g fat. Children who play in and on contaminated soil may have a higher potential for exposure through the skin and through inhalation of contaminated dust. Children who eat contaminated soil may ingest hexachlorobenzene.

See Chapter 6 for more detailed information regarding concentrations of hexachlorobenzene in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

Hexachlorobenzene is a toxic organochlorine that has been shown to cause death, systemic (e.g., liver, skin, bone, and thyroid), neurological, developmental, endocrine, and immunological toxicity in humans and animals. Animal studies have demonstrated that hexachlorobenzene causes reproductive toxicity and increases the risk for cancer formation. The most notable targets of hexachlorobenzene toxicity are the liver, ovary, and central nervous system.

A limited number of occupational studies have associated inhalation of hexachlorobenzene with liver effects (strongly with increased porphyrins and weakly with hepatocellular carcinoma), immunological effects (decreased neutrophil activity, increased immunoglobulins, and susceptibility to infection), and renal effects (microproteinuria). Most data for the inhalation effects of hexachlorobenzene in humans were presented by studies of workers from an organochlorobenzene factory and the residents of a nearby rural town (Flix, Spain). Exposure to hexachlorobenzene (primarily airborne) pollution has been linked with elevated blood levels of hexachlorobenzene and hepatic effects (increased porphyrins and hepatic enzymes), thyroid effects (decreased thyroxine levels; weakly with hypothyroidism, goiter, and thyroid cancer), and impaired development of locomotor skills in infants.

Striking epidemiological evidence was found in studies of a population orally exposed to hexachlorobenzene in southeast Anatolia, Turkey. In the 1950s, widespread ingestion of bread made from grain that had been treated with hexachlorobenzene as a pesticide caused an epidemic in this region. The ingested dose of hexachlorobenzene was estimated to be in the range of 0.05–0.2 g/day, equivalent to 0.7–

2.9 mg/kg/day for an average 70 kg person. An extremely high (95%) rate of mortality occurred in infants under 2 years of age who had been breast fed by mothers who had ingested the contaminated bread. Poisoned infants displayed a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (annular erythema). The infant deaths were primarily associated with cardiorespiratory failure secondary to this disease. Other clinical symptoms in these infants included weakness and convulsions. A disease called *kara yara* or “black sore” was observed most frequently in children between the ages of 6 and 15 years, although some younger children and adults were also affected. It appeared after approximately 6 months of exposure; symptoms included photosensitivity, skin fragility (causing ulcers and scarring), hyperpigmentation, and hirsutism (growth of hair in unusual amounts and locations). There was a 10% mortality rate among *kara yara* patients. These skin lesions were diagnosed as porphyria cutanea tarda, a specific type of vesiculobullous porphyria. The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the generation of porphyrins, which may cause tissue damage, especially in the skin. The human studies and supporting animal studies have clearly demonstrated that hexachlorobenzene causes porphyria. It is worth mentioning that results of some recent studies indicate that alterations of porphyrin metabolism and excretion may be important characteristics of autistic spectrum disorders. However, there is no information to indicate that autistic spectrum disorders may be linked to hexachlorobenzene exposure.

Other symptoms diagnosed in this hexachlorobenzene-exposed population were: loss of appetite, weakness, arthritis (a swelling and spindling of the fingers, but with little pain), hepatomegaly, enlarged thyroid, and inability to perform simple, everyday activities such as handling eating utensils, rising from a squat, and climbing stairs. Clinical findings persisted in most subjects, including high porphyria, dermal lesions, multiple neurological effects, skeletomuscular effects, enlarged liver, and enlarged thyroid. In adult pregnant women who had been exposed as children, suggestive (but not conclusive) evidence of elevated incidences of miscarriages and stillbirths was found. Similar irreversibility has been seen in animal studies: developmentally exposed rats exhibited a significantly increased startle response as adults and acutely exposed rats still exhibited porphyria more than 500 days after exposure.

No studies were located regarding health effects in humans or animals following dermal exposure to hexachlorobenzene. However, an acute study in rats suggested that hexachlorobenzene can be absorbed across the skin.

The primary targets of toxicity for hexachlorobenzene are the liver, reproductive end points, developmental end points, and carcinogenesis; these are discussed below. The reader is referred to

Section 3.2, Discussion of Health Effects by Route of Exposure, for more detailed information and discussions of additional effects.

Hepatic Effects. The most consistently identified effect following exposure of humans or animals to hexachlorobenzene is porphyria. The porphyrias are a class of inherited and/or acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the accumulation of porphyrins (see Section 3.5, Mechanisms of Action). The build-up of high levels of porphyrins in the body is known to cause liver (including cirrhosis, siderosis [accumulation of iron], focal necrosis, hyperplasia), and kidney (renal failure) damage. Additionally, phototoxicity occurs as porphyrins accumulated in the skin are activated by sunlight to generate reactive oxygen species, causing tissue damage. As a result, skin lesions occur most commonly on areas exposed to sunlight, such as the hands and face. Available data also suggest that porphyrins may activate the immune system. Porphyria, diagnosed by the presence of high levels of porphyrins in the blood, feces, or urine, has been detected following exposures to hexachlorobenzene in workers, in the residents of Flix, Spain (primarily inhalation exposures resulting from a nearby organochlorine factory), and in the Turkish epidemic (oral exposure with contaminated grain). Exposed patients from the Turkish epidemic also exhibited hepatomegaly.

Several studies in rats and mice have observed porphyria, but no clear relationship has been established between porphyria and other hepatic changes observed in animals, such as peribiliary lymphocytosis and fibrosis, hepatomegaly, increased liver weight, enzyme induction, and degenerative pathological changes.

Reproductive Effects. Possible associations between serum hexachlorobenzene and selected reproductive outcomes in humans have been evaluated by a number of investigators. However, these studies provide no clear evidence of hexachlorobenzene-related effects on reproductive outcomes in the study groups.

The reproductive performance of rats has been adversely affected at oral doses as low as 16 mg/kg/day of hexachlorobenzene (decreased fertility, increased numbers of stillborn pups). Distribution studies have identified the ovaries as a site of hexachlorobenzene accumulation, and intermediate-duration administration of hexachlorobenzene at oral doses as low as 0.01 mg/kg/day have been shown to cause ovarian lesions in adult female Cynomolgus monkeys. At higher doses, studies in Cynomolgus monkeys, Rhesus monkeys, and rats have reported changes in organ weight, degenerative changes, and disruptions in steroidogenesis (estrogen and progesterone).

Developmental Effects. Human and animal studies have demonstrated that hexachlorobenzene crosses the placenta to accumulate in fetal tissues and is transferred in breast milk.

The poisoning epidemic in Anatolia, Turkey, demonstrated that hexachlorobenzene is a developmental toxin. Children exposed under 2 years of age were the most susceptible (95% mortality, skin lesions). However, children under 15 were also more susceptible than adults, and exhibited both immediate (10% mortality, skin lesions) and persistent (dermal, neurological, skeletomuscular, hepatic, and thyroid effects) symptoms. Exposure for adults was estimated at 0.05–0.2 g/day of hexachlorobenzene (in bread made from contaminated grain) between 1955 and 1959.

Other human studies investigating developmental toxicity, although limited by small study size and low levels of hexachlorobenzene exposure, have found suggestive evidence of an increased risk of undescended testis and impaired development of locomotor skills in newborn babies. One study found a significantly lower lifetime proportion of male offspring from women reporting hexachlorobenzene exposure at the peak of the Turkish epidemic (1955–1957) compared to women exposed at a later date.

Some studies evaluated possible associations between maternal serum hexachlorobenzene levels and developmental end points such as birth size (weight and/or length) or preterm birth, recurrent miscarriage, postnatal growth, postnatal neurodevelopment, sexual maturation, cryptorchidism, hypospadias, and indicators of postnatal thyroid function. Although most studies found no significant association between maternal serum hexachlorobenzene levels and risk of developmental effects, there were reports of significant associations between maternal or cord blood hexachlorobenzene and birth weight, postnatal growth, and hypospadias. Some studies that assessed serum hexachlorobenzene levels in young boys and girls reported significant effects on markers of sexual development.

Animal studies have verified that hexachlorobenzene impairs neurological development and reduces neonatal viability and growth. Although an increased risk of undescended testis has not been observed in animals, the occurrence of cleft palate, renal agenesis, and minor skeletal abnormalities in mice are consistent with a possible teratogenic role for hexachlorobenzene.

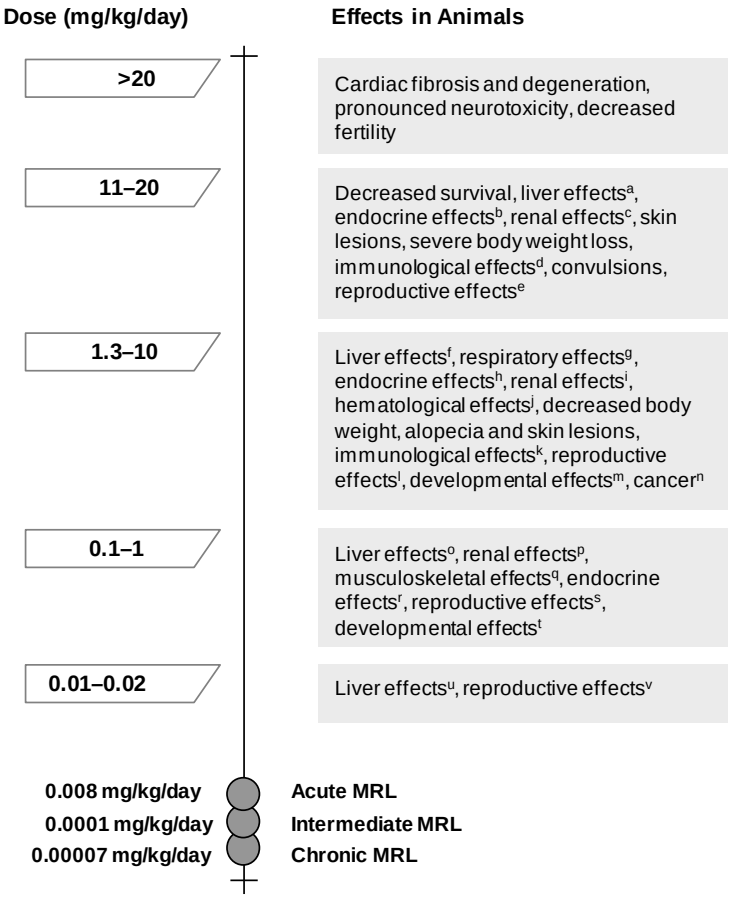
Cancer. Among the general population, case-control studies have generally found no association between hexachlorobenzene levels in blood or tissues and incidence of breast or other cancers. Data from men exposed to hexachlorobenzene by inhalation (occupationally or as a result of nearby air pollution from an organochlorine factory in Flix, Spain) provide weak evidence for an association between

hexachlorobenzene exposure and cancer of the liver, thyroid, and brain. Because hexachlorobenzene produces porphyria, it is noteworthy that several human studies have associated porphyria with the increased incidence of liver cancer.

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumor formation. The evidence of carcinogenicity is strongest in the liver; hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumors (hepatoma in mice and rats; hemagniohepatoma and bile duct adenoma in rats), and malignant tumors (hepatocarcinoma in rats, mice, and hamsters; bile duct adenocarcinoma in rats). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas and renal cell carcinomas (in rats, mice, and hamsters); lymphosarcomas (in rats, mice, and hamsters); adrenal hyperplasia and pheochromocytoma (in rats); parathyroid adenomas (in rats); and hemangioendothelioma and thyroid tumors (in hamsters). In the Twelfth Report on Carcinogens, the NTP classified hexachlorobenzene as *reasonably anticipated to be a human carcinogen*. The EPA classified hexachlorobenzene as a *probable human carcinogen*, Group B2, on the basis that oral administration of hexachlorobenzene has been shown to induce tumors in the liver, thyroid, and kidney in three rodent species. IARC has classified hexachlorobenzene as *possibly carcinogenic to humans* (Group 2B), based on inadequate evidence in humans and sufficient evidence in experimental animals for carcinogenicity. The American Conference of Governmental Industrial Hygienists classified hexachlorobenzene as an A3 carcinogen (*confirmed animal carcinogen of unknown relevance to humans*). For more information, see Sections 3.2.1.7 and 3.2.2.7, Cancer.

Health effects of hexachlorobenzene ingestion in laboratory animals and the dose ranges at which these effects occur are shown in Figure 2-1. An estimate of an oral dose posing minimal risk to humans (MRL) is also presented in this figure. An MRL is an estimate of the daily human exposure that is likely to be safe over a certain period of exposure. MRLs are not intended to define clean-up or action levels, but are intended only to serve as a screening tool to help public health professionals decide where to look more closely. Therefore, MRLs are set at levels well below where effects have been observed.

Figure 2-1. Health Effects for Ingesting Hexachlorobenzene



[a]Hyperplasia, fibrosis, and necrosis
[b]Large decrease in thyroid weight, decreased serum thyroxine and triiodothyronine
[c]Increased renal porphyrin, nephrosis
[d]Increased spleen weight, increased serum IgM autoantibodies, decreased pulmonary natural killer cell activity, decreased resistance to infection
[e]Increased abnormal sperm, decreased sperm production
[f]Porphyria, fatty degeneration, increased serum liver enzymes
[g]Increased macrophages, focal areas of fibrosis in lung, hypertrophy and proliferation of endothelial cells of pulmonary venules
[h]Increased adrenal weight, increased thyroid weight, hyperplasia of adrenal cortex, increased serum parathyroid hormone
[i]Increased kidney weight, increased urinary lactate dehydrogenase and alkaline phosphatase
[j]Decreased hemoglobin and hematocrit
[k]Thymic cortical atrophy, increased serum 1,25-dihydroxy vitamin D3 and parathyroid hormone,
[l]Increased length of menstrual cycle, decreased ovulatory estradiol, blocked ovulation, degeneration of ovarian epithelium, decreased number of primary follicles, ovarian follicular cysts
[m]Decreases in pup viability, weight gain, efficiency in operant behavior, increased pup hyperactivity
[n]Hepatocarcinoma, tumors in parathyroid and adrenal
[o]Increased liver weight, mitochondrial swelling, hepatocellular hypertrophy and vacuolation
[p]Increased urinary lactate dehydrogenase
[q]Increased bone density, minimal incisor degeneration
[r]Increased serum 1,25-dihydroxy vitamin D3, decreased serum corticosterone
[s]Degenerative changes in ovarian epithelium, changes in serum progesterone levels
[t]Decreased delayed-type hypersensitivity response in offspring
[u]Increased peribiliary lymphocytosis and fibrosis
[v]Mitochondrial degeneration in developing ovarian follicles

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for hexachlorobenzene. 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 1990a), 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

Although limited evidence identified organs (liver, kidneys) and systems (endocrine, neurological and immune) that may be targets of hexachlorobenzene toxicity following inhalation exposures in humans and animals, these data are qualitatively and quantitatively inadequate for use in developing acute, intermediate, or chronic inhalation MRLs for hexachlorobenzene.

Oral MRLs

- An MRL of 0.008 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to hexachlorobenzene.

This MRL is based on a critical evaluation of a developmental study (Goldey and Taylor 1992) that observed a lowest-observed-adverse-effect level (LOAEL) of 2.5 mg/kg/day for hyperactivity in rat

offspring. An uncertainty factor of 300 was used (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

Human data have shown that the developing central nervous system is a target of hexachlorobenzene toxicity. Many breast-fed infants of mothers who ingested hexachlorobenzene-contaminated bread during an epidemic of hexachlorobenzene poisoning in Turkey between 1955 and 1959 showed symptoms of *pembe yara*, which included weakness, convulsions, and annular erythema prior to death (Cripps et al. 1984; Peters et al. 1982, 1987). In children exposed (at an average age of 7 years) to hexachlorobenzene-contaminated grain, neurological effects persisted into adulthood (weakness, paresthesia, sensory shading, myotonia, and cogwheeling [irregular jerkiness of movement due to increased muscle tone as seen in Parkinson's disease]) (Cripps et al. 1984; Peters et al. 1982). Additionally, a preliminary report of infants and their mothers from Flix, Spain (where the highest blood levels of hexachlorobenzene have been recorded) found an association between prenatal exposure to hexachlorobenzene and impaired development of locomotor skills (Sala et al. 1999a).

Toxicology experiments have identified the same targets in developing animals. A developmental study in rats (Goldey and Taylor 1992) provided the most appropriate data from which to derive an acute-duration oral MRL for hexachlorobenzene. Virgin female rats were administered 2.5 or 25 mg/kg/day hexachlorobenzene by gavage for 4 days, 2 weeks prior to mating with unexposed males, and the developmental neurotoxicity of hexachlorobenzene was assessed in offspring using a battery of tests (Goldey and Taylor 1992). Pups treated with either dose reoriented themselves significantly more quickly in a negative geotaxis test (on postnatal days 6 and 8), required less time in an olfactory discrimination test on postnatal days 9–11, and demonstrated increased exploratory activity in a motor activity test (on postnatal days 19 and 20). No significant effects on learning (swim T-maze) or motor activity (measured in older offspring on postnatal days 40 and 50, respectively) were detected. Hexachlorobenzene-exposed offspring at the 25 mg/kg/day dose level exhibited significantly altered acoustic startle responses (decreased at 23 days of age and increased at 90 days of age compared to controls). Thus, the study identified a LOAEL of 2.5 mg/kg/day for hyperactivity in the rat offspring, and this was the most sensitive LOAEL identified for the acute toxicity of hexachlorobenzene.

Other animal studies have demonstrated that exposure to hexachlorobenzene can produce neurological effects. In a two-generation study of rats exposed prenatally through adulthood, an oral dose level of 1.3 mg/kg/day resulted in decreased operant learning ability ("post-reinforcement pause" and "index of curvature") at postnatal day 150 assessment (Lilienthal et al. 1996). Moreover, repeated oral exposure to

hexachlorobenzene in the range of 50–427 mg/kg/day has also been shown to interfere with the function of the nervous system, inducing mild reduction in conduction velocity of the sciatic nerve and denervation (fibrillations, chronic repetitive discharges) (Sufit et al. 1986), hyperexcitability, tremors, muscle fasciculations, clonic convulsions, ataxia, lethargy, and paralysis in adult rats (Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Koss et al. 1978; Nikolaev et al. 1986; Ockner and Schmid 1961); convulsions, tremors, and progressive weakness in litters of female rats (Cripps 1990); tremors in adult C57B1/6J mice (Hahn et al. 1988); dysrhythmic electroencephalogram in adult Beagle dogs (Sundlof et al. 1981); severe tremors and muscular weakness in adult Rhesus monkeys (Knauf and Hobson 1979); hypoactivity, lethargy, and ataxia in infant Rhesus monkeys (Iatropoulos et al. 1978); and tremors, panting, and unsteady gait in adult SPF pigs (Den Tonkelaar et al. 1978).

- An MRL of 0.0001 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to hexachlorobenzene.

This MRL is based on a LOAEL of 0.01 mg/kg/day for minimal ovarian effects in monkeys (Bourque et al. 1995). An uncertainty factor of 90 was used (3 for extrapolation from monkeys to humans, 10 for human variability, and 3 for use of a minimal LOAEL). Ultrastructural studies of ovaries collected from monkeys (Babineau et al. 1991; Bourque et al. 1995; Jarrell et al. 1993) provide the most appropriate data for use deriving an intermediate-duration oral MRL for hexachlorobenzene. Female Cynomolgus monkeys were fed doses of 0.01–10 mg/kg/day of hexachlorobenzene in glucose in gelatin capsules for 90 days; the studies focused exclusively on end points relevant to reproductive toxicity (ovarian function and histopathology). The LOAEL of 0.01 mg/kg/day for reproductive effects is the most sensitive LOAEL for the intermediate toxicity of hexachlorobenzene (Bourque et al. 1995).

Two early studies (Babineau et al. 1991; Jarrell et al. 1993) used doses of 0.1, 1, and 10 mg/kg/day of hexachlorobenzene. In all treated animals, hexachlorobenzene decreased the total number of oocytes and primary follicles, caused oocyte necrosis and follicular degeneration, and induced histopathological changes in the ovarian epithelium (cell stratification; decreased nuclear membrane distinction; increased density and granularity of oocyte nuclei; and increased numbers of aggregated lysosomes, vesicles, and vacuoles). The severity was dose-dependent.

The Bourque et al. (1995) follow-up study extended the observation of ultrastructural effects in the ovary to 0.01 mg/kg/day. At this dose, mitochondria in developing follicles were condensed and deformed. At higher doses (up to 10 mg/kg/day), the mitochondria were progressively more damaged and other changes were noted, such as indentation of nuclear membranes and abnormal accumulation of lipid in the

cytoplasm of follicular cells. Because these effects were not associated with changes in oocyte fertility (measured *in vitro*), they were considered minimally adverse. Thus, these studies identify a LOAEL of 0.01 mg/kg/day for minimal reproductive effects in the treated monkeys.

Other monkey studies have also observed evidence of ovarian effects. In female Cynomolgus monkeys given capsules with at least 0.1 mg/kg/day of hexachlorobenzene for 90 days, a dose-related decrease in serum progesterone levels during the luteal (but not follicular and periovulatory) phase of the menstrual cycle, lengthening of the menstrual cycle, ultrastructural changes in surface epithelium of the ovary (indicative of cellular degeneration), and changes in ovary surface epithelial cell shape (length to width ratio) were detected (Foster et al. 1992a; Sims et al. 1991). In female Rhesus monkeys, gavage doses of at least 64 (but not lower doses up to 32) mg/kg/day of hexachlorobenzene for 60 days induced degenerative changes of the ovarian follicle, stroma, and germinal epithelium (Iatropoulos et al. 1976), and suggestive evidence of unusual steroidogenic activity (depressed serum potassium) was seen in Rhesus monkeys given 128 mg/kg/day for at least 60 days (Knauf and Hobson 1979).

Rat studies have confirmed the reproductive toxicity of hexachlorobenzene in the ovary. Increased serum progesterone levels and elevated ovarian weights were observed in superovulated female Sprague-Dawley rats orally administered ≥1 mg/kg/day hexachlorobenzene by gavage for 21 days (Foster et al. 1992b).

Super-ovulated (but not normal cycling) female Sprague-Dawley rats gavaged with 50 mg/kg/day of hexachlorobenzene for 5 days exhibited significant elevation of serum levels of progesterone (Foster et al. 1993). In a subsequent study with ovariectomized female Sprague-Dawley rats, gavage doses of at least 1 mg/kg/day for 30 days significantly decreased circulating corticosterone and cortisol levels, without affecting levels of circulating aldosterone and progesterone levels or adrenal gland weight (Foster et al. 1995a). The investigators concluded that hexachlorobenzene exposure induces alterations in steroidogenesis of cells of the adrenal cortex inner zone.

Although clear evidence of reproductive toxicity has not been observed in humans, suggestive data indicated that hexachlorobenzene may increase the risk for spontaneous abortion (miscarriages and stillbirths) and endometriosis. Therefore, the intermediate-duration oral MRL is considered relevant to human health.

- An MRL of 0.00007 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to hexachlorobenzene.

This MRL was based on a critical evaluation of a multigenerational study (Arnold et al. 1985), which observed a LOAEL of 0.022 mg/kg/day for hepatic effects in F_1 male rats. An uncertainty factor of 300 was used (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

Although studies designed to detect liver histopathology have not been conducted in humans, epidemiological studies have detected hepatic effects as indicated by increased porphyrin and enzyme levels in people exposed for chronic durations to hexachlorobenzene (Herrero et al. 1999; Sala et al. 1999b; Selden et al. 1999). Analysis of the available human and animal chronic oral toxicity data for hexachlorobenzene indicated that a 130-week study in rats reported by Arnold et al. (1985) provided the most appropriate data for use in the development of an oral chronic MRL for hexachlorobenzene. Groups of male and female weanling Sprague-Dawley rats were administered diets containing hexachlorobenzene at 0, 0.32, 1.6, 8, or 40 ppm (estimated doses of 0, 0.022, 0.11, 0.55, or 2.8 mg/kg/day for males and 0, 0.026, 0.13, 0.64, or 3.2 mg/kg/day for females) for 3 months prior to mating and through weaning of F_1 offspring, when F_0 parental rats were sacrificed. The F_1 offspring were continued on their parents' diet from weaning throughout the remainder of their lives (up to 130 weeks; estimated postweaning doses of 0, 0.028, 0.14, 0.69, and 3.4 mg/kg/day, respectively, for F_1 males and 0, 0.03, 0.16, 0.78, and 3.9 mg/kg/day, respectively, for the F_1 females). Statistically significant increases in the incidences of peribiliary lymphocytosis and fibrosis were observed in the livers of F_1 male rats at doses $\geq$0.022 mg/kg/day. Significant dose-dependent trends were also found for hepatic basophilic chromogenesis at $\geq$0.55 mg/kg/day in the F_1 males and 0.64 mg/kg/day in the F_1 females. The high-dose group exhibited increased pup mortality (in both genders) and severe chronic nephrosis (in males only).

The increased incidences of peribiliary lymphocytosis and fibrosis in treated males were considered to represent a minimal effect. These are common spontaneous lesions in aging rats and occurred in approximately 30% of controls in this study. For peribiliary fibrosis, incidence was increased in all treated groups (statistically significant in the 0.022 and 2.8 mg/kg/day groups), but there was no clear evidence of a dose-response (13/48, 23/48, 21/48, 21/49, and 23/49, respectively, in the control, 0.022, 0.11, 0.55, and 2.8 mg/kg/day groups). For peribiliary lymphocytosis, the incidence was increased in all treated groups (statistically significant in the 0.022, 0.11, and 2.8 mg/kg/day groups), and the trend was also statistically significant (16/48, 27/48, 26/48, 21/49, and 32/49, respectively, in the control, 0.022, 0.11, 0.55, and 2.8 mg/kg/day groups). Because incidences of these lesions in the control and treated females were similar to the control males (ranging from 6/49 to 14/49), the incidence levels in control males do not appear unusually low. Overall, these findings suggest that hexachlorobenzene produced a

minimal hepatic effect in male rats at the lowest doses administered by increasing the incidence of age-related hepatic lesions. The LOAEL of 0.022 mg/kg/day reported in this study for hepatic effects in the F_1 males is the most sensitive LOAEL for the characteristic chronic toxicity of hexachlorobenzene, liver toxicity. However, the study did not identify a no-observed-adverse-effect level (NOAEL).

Numerous animal studies have clearly demonstrated that the liver is a major target organ of hexachlorobenzene exposure. Short-term experiments in rats have observed increased liver weight, increased hepatic porphyrins, liver histopathology (cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes), and increased serum cholesterol (Krishnan et al. 1991; Lecavalier et al. 1994; Rajamanickam and Padmanaban 1974; Richter et al. 1981). Intermediate- and chronic-duration experiments in rats have observed these and other signs, including additional liver histopathology (degeneration, hypertrophic hepatocytes with eosinophilic cytoplasm with thready basophilic structures, as well as inflammatory cell infiltrates), decreased liver retinoid levels, and elevated liver enzymes (Andrews et al. 1989, 1990; Den Besten et al. 1993; Elder and Urquhart 1986; Kennedy and Wigfield 1990; Koss et al. 1978, 1983; Kuiper-Goodman et al. 1977; Ockner and Schmid 1961; Smith and Cabral 1980; van Raaij et al. 1993b; Wolfe and Pepperl 2005). Similar effects have been observed in intermediate-duration experiments in Rhesus monkeys (Iatropoulos et al. 1976; Knauf and Hobson 1979), beagle dogs (Sundlof et al. 1981), and pigs (Den Tonkelaar et al. 1978).

2. RELEVANCE TO PUBLIC HEALTH

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3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of hexachlorobenzene. 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 hexachlorobenzene are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

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

3.2.1 Inhalation Exposure

3.2.1.1 Death

No studies were located regarding death in humans or animals following inhalation exposure to hexachlorobenzene.

3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, dermal, or ocular effects in humans or animals following inhalation exposure to hexachlorobenzene.

Hepatic Effects. It is well known that ingestion of hexachlorobenzene can produce porphyria (see Section 3.2.2.2), but there are few data to suggest a similar effect from inhalation exposure. There was a small, but statistically significant, increase (in comparison to matched controls) in total urinary porphyrins in a group of nine workers exposed for 1–19 years to hexachlorobenzene and other organochlorine

compounds (including octachlorostyrene, another porphyrinogenic substance [Smith et al. 1986d]) while using hexachloroethane (thermal byproducts of which include hexachlorobenzene and octachlorostyrene) as an aluminum degassing agent at aluminum smelters (Selden et al. 1999). The prevalence of porphyria-related symptoms did not differ between groups. Serum levels of hexachlorobenzene were positively correlated to urine porphyrin levels, but urine porphyrin levels also correlated, to a similar degree, with serum octachlorostyrene levels. Therefore, this study cannot ascertain whether the preclinical porphyria observed in aluminum smelter workers is due to hexachlorobenzene, octachlorostyrene, or both chemicals.

End points of hepatic toxicity have been investigated in residents of Flix, Spain, a small village in Catalonia; a nearby electrochemical factory that manufactures organochlorines has been implicated in the village's high environmental levels of hexachlorobenzene (35 ng/m^3 as a 24-hour average in air). Analysis of blood hexachlorobenzene and urinary porphyrins in 604 residents of Flix, including 185 factory workers, showed that blood hexachlorobenzene levels were roughly 5-fold higher in factory workers (93.4±223.3 ng/mL) than in non-factory workers (16.9±17.1 ng/mL). However, there were no cases of clinical porphyria cutanea tarda in either group, no evidence that preclinical porphyria was more prevalent in the factory workers than in other residents, and no association between urinary porphyrin levels and blood hexachlorobenzene levels (Herrero et al. 1999; Sala et al. 1999b). A subsequent study verified the 5-fold increase in 75 factory workers, and detected a positive, statistically significant correlation between blood hexachlorobenzene levels and serum γ-glutamyltransferase (Sala et al. 2001a). However, no correlation was seen for serum levels of either aspartate or alanine aminotransferase.

Sunyer et al. (2002) determined the urinary porphyrin profile among the most highly hexachlorobenzene-exposed residents of Flix (n=241). Concentrations of coproporphyrins I and III decreased with increasing hexachlorobenzene concentration ($p<0.05$) independent of age, alcohol consumption, smoking, or exposure to other organochlorine compounds. No significant association was found between excretion of uroporphyrin I, uroporphyrin III, or heptaporphyrin and blood hexachlorobenzene concentration. Sunyer et al. (2008) determined the urinary porphyrin profile among 68 neonates from Flix and surrounding towns who provided urine samples on the third day of life for porphyrin assessment; urine and blood samples were successfully analyzed from 52 of these children at 4 years of age as well. In the 4-year-old children, quantitative porphyrin excretion was within normal values, but total porphyrins, coproporphyrin I, and coproporphyrin III (adjusted to creatinine excretion) increased with increasing levels of hexachlorobenzene independent of breastfeeding and of organochlorine and porphyrin levels at

birth. The increase of urinary coproporphyrins was considered suggestive of an incipient toxic effect on the hepatic heme pathway.

Ozalla et al. (2002) collected maternal serum samples and cord blood and neonatal urine samples on day 3 after birth from full-term singleton neonates born in Flix and compared them with samples from unexposed mothers and neonates from neighboring villages. Detectable hexachlorobenzene concentrations were found in all samples of fetal cord blood and maternal serum, but the exposed population from Flix was statistically higher ($p<0.05$). There was no positive relationship between urinary porphyrin excretion and hexachlorobenzene levels in maternal serum or cord blood, but heptaporphyrin isomer III was detected significantly ($p<0.012$) more in the exposed group compared with the unexposed group, and neonates in the highest tertile of hexachlorobenzene had lower levels of coproporphyrin I ($p<0.05$).

The NOAEL for liver effects in the Flix residents is shown in Table 3-1 and Figure 3-1. In 52 workers employed for 1–25 years at a chemical plant where hexachlorobenzene was the primary byproduct, compared with controls drawn from a local blood bank (Queiroz et al. 1998a), serum aspartate and alanine transaminase levels were increased but no correlation was detected between serum levels of transaminase and blood hexachlorobenzene. The available human data, while suggestive, have not conclusively shown hepatic effects due to inhaled hexachlorobenzene.

No studies were located regarding the hepatic effects of inhaled hexachlorobenzene in animals.

Renal Effects. Based on analysis of blood samples from 608 individuals, workers (n=189) at an electrochemical factory near Flix, Spain did not have a significantly increased risk of high serum creatinine, a marker for glomerular disease, in comparison to other Flix residents (n=419) despite hexachlorobenzene blood levels that were approximately 5-fold higher (Sala et al. 1999b). However, the power of this study to find an effect was limited by the small sample size (only nine individuals with high serum creatinine were found in the study population). "Marked changes in kidney functions" including microproteinuria were observed in Czechoslovakian workers with high blood hexachlorobenzene levels following occupational inhalation exposure to hexachlorobenzene, originally at 2.1–10.8 mg/m^3 and then at 0.012–0.022 mg/m^3, from 1983 to 1990 (Richter et al. 1994).

No studies were located regarding renal effects of inhaled hexachlorobenzene in animals.

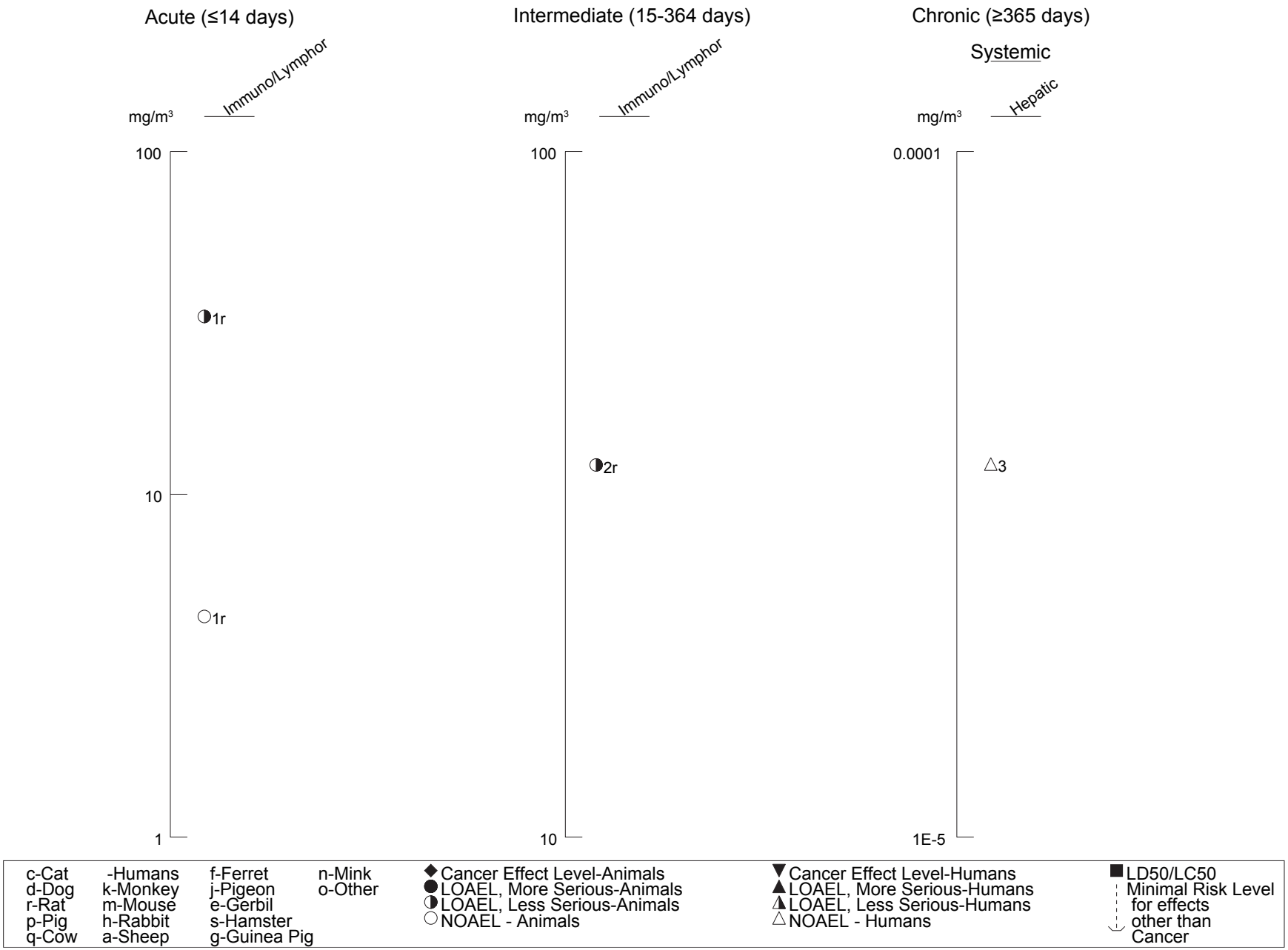
Table 3-1 Levels of Significant Exposure to Hexachlorobenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Immuno/ Lymphoret								
1	Rat (Sprague-Dawley)	1-4 d 4 hr/d		4.4 M	33 M (slight impairment of pulmonary immune defenses)		Sherwood et al. 1989	
INTERMEDIATE EXPOSURE								
Immuno/ Lymphoret								
2	Rat	4 wk 4 d/wk 4 hr/d			35 M (slight impairment of pulmonary immune defense)		Sherwood et al. 1989	
CHRONIC EXPOSURE								
Systemic								
3	Human	40 yr (Occup)	Hepatic	0.000035			Herrero et al. 1999 HCB	

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

d = day(s); hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Occup = occupational; wk = week(s); yr = year(s)

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



Endocrine Effects. Although oral exposure to hexachlorobenzene has been clearly associated with thyroid effects, only limited information is available regarding the endocrine effects of hexachlorobenzene following inhalation exposure. A study of residents (192–558, depending on end point) of Flix, Spain, where a nearby electrochemical factory had resulted in high air and blood levels of hexachlorobenzene, detected statistically significant correlations between increased blood hexachlorobenzene levels and decreased levels of total thyroxine (T4); serum levels of thyroid stimulating hormone (TSH) were not affected (Sala et al. 2001a). Similarly, an earlier study did not detect changes in serum levels of TSH in 189 workers at the factory in comparison to 419 other Flix residents despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). Both studies were limited by small sample size, as only 10 cases of elevated TSH were observed in each of the study populations. A larger survey found that the prevalences of goiter and hypothyroidism were similar in ever exposed workers (n=507) and nonexposed residents (n=1,293) (Sala et al. 1999b). Ribas-Fitó et al. (2003b) assessed possible associations between prenatal exposure to organochlorine compounds, including hexachlorobenzene, and thyroid status in newborns (a total of 98 mother-infant pairs) born during 1997–1999 in the Flix area. Concentrations of TSH were measured in plasma from the newborns 3 days after birth. TSH concentrations were all within the normal range. There was no significant association between blood TSH and hexachlorobenzene levels.

Álvarez-Pedrerol and coworkers assessed possible associations between cord serum organochlorine levels (including hexachlorobenzene) and thyroid status in 387 newborns exposed prenatally (Álvarez-Pedrerol et al. 2008a) and between serum organochlorine levels and thyroid status in 259 children 4 years of age (Álvarez-Pedrerol et al. 2008b). The newborns and 4-year-old children were selected from the general population on the Spanish island of Menorca, which is not in the vicinity of chemical facilities that produce organochlorine compounds. Although measurable hexachlorobenzene levels were observed in the newborns and the 4-year-old children, there were no significant associations between cord blood hexachlorobenzene levels and serum TSH levels in the newborns or serum hexachlorobenzene and serum thyroid hormones (free T4, total triiodothyronine [T3], TSH) in the 4-year-old children.

No studies were located regarding endocrine effects of inhaled hexachlorobenzene in animals.

3.2.1.3 Immunological and Lymphoreticular Effects

Occupational studies indicate that inhaled hexachlorobenzene may cause physiological changes in immune parameters, but these effects are not clearly toxic. Queiroz et al. (1997, 1998a, 1998b) studied a group of Brazilian workers (n=51–66) employed for up to 25 years at a chemical plant whose primary waste product was hexachlorobenzene. Although it is not clear from the report, workers were presumably exposed to hexachlorobenzene by inhalation, although dermal exposure was possible as well. Findings in blood samples of exposed workers compared to controls selected from a local blood bank were significant decreases in neutrophil chemotaxis, impaired neutrophil cytolytic activity (but not phagocytic activity) and significant increases in serum immunoglobulins (IgG and IgM, but not IgA). Blood hexachlorobenzene levels were elevated in exposed workers, but were not correlated with changes in immunological parameters. Serum immunoglobulins (IgG, IgA, IgM, and IgE, but not IgD) were also increased in Czechoslovakian workers with high blood hexachlorobenzene levels who had been exposed to hexachlorobenzene in the workplace air from 1983 to 1990, originally at 2.1–10.8 mg/m^3 and then at 0.012–0.022 mg/m^3 (Richter et al. 1994). Immunological parameters and serum organochlorine levels were measured in a group of 141 German medical patients presenting with variety of acute symptoms (mainly lack of concentration, exhaustion, and common cold) who had been occupationally exposed (as teachers, construction workers, and telecommunication technicians) for at least 6 months to multiple organochlorines (Daniel et al. 2001). A strong, statistically significant, association was detected between high blood levels of hexachlorobenzene and decreased levels of interferon-γ blood (IFN-γ) levels. Moreover, patients with low overall organochlorine levels had elevated IFN-γ levels. The authors note that IFN-γ is important for increasing the secretion of immunoglobulins by plasma cells, and speculate that decreased IFN-γ might increase susceptibility to infection. It is not clear whether hexachlorobenzene exposure is responsible for the observed effects, because significant cross-correlations were detected between hexachlorobenzene levels and several polycyclic biphenyl compounds.

The animal data provide weak support for an immunological effect of inhaled hexachlorobenzene. Observations in male rats exposed to 33–35 mg/m^3 of hexachlorobenzene aerosol for durations ranging from 4 hours to 4 weeks (4 days/week, 4 hours/day) included a slight decrease in pulmonary macrophage bactericidal activity to inhaled *Klebesiae pneumoniae*, a slight increase in phagocytic activity of alveolar (but not peritoneal) macrophages *in vitro*, and altered lymphocyte mitogenesis induced by T-cell (phytohemagglutinin [PHA]) and B-cell (*Salmonella typhimurium* lipopolysaccharide) mitogens in lung-associated and mesenteric lymph nodes *in vitro* (Sherwood et al. 1989). No effects were found at 4.4 mg/m^3 (1-day exposure only). The authors concluded that exposure to hexachlorobenzene at about

35 mg/m^3 resulted in slight changes to humoral and pulmonary cellular defenses. However, the reported changes were only marginally different from controls, the magnitude of the reported effects did not generally increase with exposure duration, and some of the results were contradictory (e.g., there was a significant increase in PHA-induced mitogenesis in lung-associated lymph nodes, but a significant decrease in PHA-induced mitogenesis in mesenteric lymph nodes, in rats exposed to hexachlorobenzene for 4 weeks). The NOAEL and LOAEL values from this experiment are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

No clear evidence of neurological effects following inhalation exposure to hexachlorobenzene is available. The prevalence of Parkinson's disease was not significantly increased in workers at an electrochemical factory near Flix, Spain (4/507) compared with other Flix residents (4/1,293) despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). The small number of cases is a limitation of this study.

No studies were located regarding the neurological effects of inhaled hexachlorobenzene in animals.

Neurodevelopmental effects are discussed in Section 3.2.1.6 (Developmental Effects).

3.2.1.5 Reproductive Effects

No studies were located regarding the reproductive effects of inhaled hexachlorobenzene in humans or animals.

3.2.1.6 Developmental Effects

Developmental effects (spontaneous abortions, low birth weight, and congenital malformations) occurred with a similar prevalence among females who ever worked at an electrochemical factory near Flix, Spain (n=46–60 for the different end points), a village with unusually high atmospheric levels of hexachlorobenzene), and female residents who had never worked at the factory (n=719–936), despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). The small number of women factory workers (n=46–60 for the different end points) is a limitation of this study. A possible association between prenatal exposure to hexachlorobenzene and alterations in anthropometric measures (prematurity, small length for gestational age, crown-heel length) was examined among 70 infants whose

mothers lived in Flix or in neighboring towns (Ribas-Fitó et al. 2002). Cord serum levels of hexachlorobenzene and other polychlorinated hydrocarbons were measured in newborns born between 1997 and 1999. The 50th percentile concentration of hexachlorobenzene was 1.13 ng/mL. Cord serum hexachlorobenzene levels >1.48 ng/mL were significantly ($p<0.05$) correlated with a smaller crown-heel length and small length for gestational age. No significant associations between hexachlorobenzene concentrations and birth weight, head circumference, or prematurity were found. The study was limited by small sample size, as <15 infants were born premature and/or had a small weight or small length for gestational age.

The effect of prenatal exposure to hexachlorobenzene on social behavior was examined in two cohorts of 4-year-old children for whom cord serum organochlorines (including hexachlorobenzene) had been measured at birth (Ribas-Fitó et al. 2007). One cohort (n=70) was from the Ribera d'Ebre area of Spain, which encompasses the village of Flix where unusually high atmospheric levels of hexachlorobenzene (as high as 35 μg/m^3) in the proximity of an electrochemical factory have been measured. The other cohort (n=405) was from the Spanish island of Menorca, which is not in the vicinity of organochlorine-producing facilities. The ranges of hexachlorobenzene concentrations in cord blood were 0.17–5.77 ng/mL (median of 1.13 ng/mL) for the cohort from the Flix, Spain area, and 0.14–9.82 ng/mL (median of 0.68 ng/mL) for the cohort from Menorca. Behavioral assessments were conducted at 4 years of age using several tests of social competence. For both cohorts, subjects with hexachlorobenzene levels ≥1.5 ng/mL at birth had statistically significant increased risk of having poor social competence (adjusted relative risk of 4.04; 95% confidence interval [CI] 1.79, 9.58) and attention deficit hyperactivity disorder (adjusted relative risk of 2.71; 95% CI 1.05, 6.96). Adjustments for exposure to other organochlorine compounds such as polychlorinated biphenyls (PCBs), *p,p'*-DDE, and *p,p'*-DDT did not change the results, and no significant associations were seen between serum levels of these organochlorine compounds at 4 years of age and measures of social behavior.

A preliminary report (Sala et al. 1999a), based on 63 cases, detected a statistically significant association between prenatal hexachlorobenzene exposure and impaired development of locomotor skills in newborn babies in Flix, compared with those of nearby villages.

Álvarez-Pedrerol et al. (2008b) did not find an association between cord serum hexachlorobenzene levels and plasma thyrotropin concentration in 387 newborns (3 days old) born on the Spanish island of Menorca. Several organochlorines in addition to hexachlorobenzene were measured in the cord serum of the newborns, including seven PCB congeners, beta-hexachlorocyclohexane, *p,p'*-DDE, and *p,p'*-DDT. Only five children had serum thyrotropin levels above the detection limit (10 mU/L).

No studies were located regarding the developmental effects of inhaled hexachlorobenzene in animals.

3.2.1.7 Cancer

In comparison to the surrounding Province of Tarragona, the incidences of thyroid cancer and soft-tissue sarcoma were significantly increased, and brain tumors marginally increased, for the years 1980–1989 in male residents of Flix, Spain, where a nearby organochlorine factory had produced high levels of hexachlorobenzene in the ambient air for decades (40 measurements in 1989–1992 averaged 35 ng/m^3, and the researchers suspected concentrations had been higher in years past) (Grimalt et al. 1994). Tumor incidences were not elevated in female Flix residents, but exposures of the females (few of whom worked at the factory) may have been considerably lower than those of the males (many of whom, including all those with tumors, worked at the factory). The findings in males were based on very small numbers of observed cases (2–4 for the various tumor types), and were not duplicated in a companion analysis of cancer mortality reported in the same paper. Therefore, this study was not conclusive. Hepatocellular carcinoma was diagnosed in 1985 in a 65-year-old male who had been exposed to airborne hexachlorobenzene and, to a lesser extent, other organochlorine compounds (e.g., chlorinated benzenes, chlorophenols, dioxins, and dibenzofurans) at an aluminum smelter from 1967 to 1973 while using hexachloroethane as an aluminum degassing agent (Selden et al. 1989). This finding is suggestive, but does not provide rigorous evidence for an association between tumor development and inhalation exposure to hexachlorobenzene. No other data were located specifically associating cancer with exposure to hexachlorobenzene, but several studies have investigated an apparent association between porphyria and subsequent development of liver cancer in humans. These data are relevant because hexachlorobenzene is porphyrogenic in humans. However, factors such as cancer evolution times, liver pathology (hepatitis viral infection, fibrosis, or cirrhosis), and age were found to be better predictors of subsequent tumor development than porphyrin status in these studies (Axelson 1986; Keczkes and Barker 1976; Salata et al. 1985; Siersema et al. 1992; Topi et al. 1980; Waddington 1972).

Animal data regarding the carcinogenicity of hexachlorobenzene via the inhalation route were not located, although there is evidence that hexachlorobenzene produces liver tumors in animals by the oral route (see Section 3.2.2.7).

3.2.2 Oral Exposure

3.2.2.1 Death

Evidence of human lethality following oral exposure to hexachlorobenzene is derived mainly from epidemiologic data from 1955 to 1959. An estimated 3,000–4,000 people ingested bread prepared from grain treated with fungicides composed of 10% hexachlorobenzene, at an estimated dose of 2 kg/1,000 kg wheat. There was an extremely high rate of mortality in breast fed children (under 2 years of age) of mothers known to have ingested this bread. All children born to porphyric mothers during that epidemic died (Gocmen et al. 1989; Peters et al. 1982) and an estimated 1,000–2,000 infants died due to a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (blistering and epidermolysis and annular erythema) (Cripps et al. 1984; Peters et al. 1982, 1987). Although a 10% rate of mortality in exposed adults has been reported, it is not clear how that figure relates to the expected mortality rates for comparable control cohorts (Peters et al. 1982, 1987). No quantitation of exposure (dose and duration) was presented in any of these clinical reports. However, an estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963). Analysis of human milk from exposed women and unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). Sala et al. (1999a) found higher hexachlorobenzene levels in capillary blood of infants in Flix and nearby villages than in cord blood or maternal blood; levels were higher in breastfed infants than bottle-fed infants. A report on an epidemiological study in New South Wales, Australia, which produced hexachlorobenzene concentrations ranging from trace amounts to 8.2 ppm in human body fat and ≤0.41 ppb in whole blood, found no adverse health effects or mortality associated with these levels of body burden (Brady and Siyali 1972; Siyali 1972).

One death was observed among 10 rats given a single dose of 600 mg/kg by gavage in corn oil (Lecavalier et al. 1994), but it is not clear from the report that the death was due to hexachlorobenzene. Lethal levels in animal studies are progressively lower as exposure duration is increased. Lethal levels ranged from 19 to 205 mg/kg/day in most (the exception is discussed below) intermediate-duration feeding studies (Cantoni et al. 1990; Cuomo et al. 1991; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Ockner and Schmid 1961; Smith et al. 1987) and from 6 to 16 mg/kg/day in chronic oral studies (Cabral et al. 1977, 1979; Gralla et al. 1977). Death in these studies was closely associated with occurrence of neurological symptoms (e.g., tremors, paresis, weakness, convulsions) and, in some cases,

weight loss (Cabral et al. 1977; Cuomo et al. 1991; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Gralla et al. 1977; Ockner and Schmid 1961).

Aside from duration of exposure, other factors that appear to influence susceptibility to hexachlorobenzene-induced mortality include species, strain, sex, age, pregnancy status, diet, nutritional status (fasting versus normal diet), and dosing protocol (including the vehicle used and the method of exposure). A direct comparison of multiple species was performed by De Matteis et al. (1961), who treated rats, mice, guinea pigs, and rabbits with 5,000 ppm of hexachlorobenzene in the diet, providing estimated doses of 526, 976, 385, and 161 mg/kg/day, respectively. Guinea pigs, with the lowest estimated daily dose, and mice, with the highest dose, were the most severely affected of the species tested, with severe neurological effects and death occurring as soon as 8–10 days after the start of exposure. Rats and rabbits also developed neurological symptoms and died, but only after ≥8 weeks of exposure. The severe effects in guinea pigs despite the low dose suggest that this species may be especially sensitive to hexachlorobenzene; however, additional supporting data are lacking. Although death was reported in both male and female animals in various studies, studies that included both sexes generally reported a higher incidence of mortality in females than in males treated with the same doses (Gralla et al. 1977; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Pregnant females in particular seem to be especially susceptible to hexachlorobenzene-induced mortality. Two pregnant rats fed hexachlorobenzene during gestation were much more severely affected than the nonpregnant rats in the De Matteis et al. (1961) study, with one dying before giving birth and the other dying 4 weeks after giving birth. Grant et al. (1977) observed death of pregnant dams at doses as low as 15.7 mg/kg/day in an intermediate-duration reproduction study, which is considerably lower than the lethal dose range to nonpregnant animals in other intermediate-duration studies (19–205 mg/kg/day).

Toxicity of hexachlorobenzene is enhanced by use of an oil vehicle in animal studies. This was demonstrated by Kennedy and Wigfield (1990), who fed female Wistar rats a diet to which hexachlorobenzene was added either in corn oil or as crystalline chemical. At 1,000 ppm (estimated dose of 129 mg/kg/day), 2 deaths occurred within 23 days of the start of exposure in the corn oil group and the remaining 27 animals were removed from the study 2 days later due to obvious ill health. No deaths occurred in the crystalline chemical group (n=27) receiving the same estimated dose for 56 days. The increased toxicity of hexachlorobenzene fed with corn oil appeared to be related to increased accumulation of the chemical in the body (measured in liver, kidney, and spleen) under these conditions.

Reliable LOAEL values for mortality in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The available data in humans and laboratory animals indicate that the liver, and specifically, the heme biosynthesis pathway, is the major systemic target of hexachlorobenzene toxicity. Human data have also shown effects on other systemic targets, including the skin, bone, and thyroid. These effects were less common than inhibition of heme biosynthesis in exposed people. No information was located regarding respiratory, cardiovascular, gastrointestinal, hematological, renal, ocular, or body weight effects in humans following oral exposure to hexachlorobenzene. However, animal data are available for these systemic effects and suggest that the blood, lungs, and kidneys may be additional systemic targets of hexachlorobenzene.

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

Respiratory Effects. No studies were located regarding respiratory effects of oral hexachlorobenzene exposure in humans.

Animal studies have shown that ingested hexachlorobenzene can produce pathological effects in the lungs. The most widely reported lesions were hypertrophy and proliferation of the lining endothelial cells of the pulmonary venules and intra-alveolar accumulation of foamy-looking macrophages. The foamy appearance of macrophages is a result of increased lipid content (Goldstein et al. 1978). These lesions, typically occurring together, were found in six different strains of rats and both sexes, at doses as low as 5.9–46 mg/kg/day in intermediate-duration feeding studies (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997). They were seen at doses as low as 0.4 mg/kg/day, in rats that received both pre- and postnatal exposure (Vos et al. 1979a, 1983). (The Vos studies are included as developmental toxicity studies in Table 3-2 and Figure 3-2). Michielsen et al. (1997) hypothesized an immunomodulated etiology for these lesions, but the lesions occurred to a similar extent in five rat strains (Wistar, Lewis, Brown Norway; athymic and euthymic WAG/Rij) with very different responses to immunomodulating agents and did not correlate with observed immune changes, providing no support for this hypothesis (Michielsen et al. 1997, 1999, 2001).

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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	Mouse (NS)	8-10 d (F)				976 F (death)	de Matteis et al. 1961 HCB	
2	Gn Pig (NS)	8-10 d (F)				385 F (death)	de Matteis et al. 1961 HCB	
Systemic								
3	Rat (Wistar)	1 or 2 wk 5 d/wk 1 x/d (G)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 1991	
4	Rat (Wistar)	1, 2, 3, or 4 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 2000a HCB	
5	Rat (Sprague-Dawley)	2 wk 5 d/wk 1 x/d (GO)	Renal		100 M (increased kidney weight, degenerative and regenerative foci in tubules, accumulation of protein droplets in tubular cells)		Bouthillier et al. 1991	
6	Rat (Sprague-Dawley)	5 d 1 x/d (GO)	Endocr		50 F (decreased serum thyroxine)		Foster et al. 1993	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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 (CD)	1 wk (F)	Hepatic	5 F	16 F (increased hepatic ALA-S activity)		Goldstein et al. 1978	
			Bd Wt	159 F				
8	Rat (Wistar)	1 d (F)	Hepatic		128 F (increased highly carboxylated porphyrins in liver)		Kennedy and Wigfield 1990	
9	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1 x/d (G)	Bd Wt	40 F		80 F (loss of body weight by pregnant rats)	Khera 1974	
10	Rat (Sprague-Dawley)	1 d 2 x/d (G)	Hepatic	700 F	1400 F (increased ornithine decarboxylase activity in the liver)		Kitchin and Brown 1989	
11	Rat (Wistar)	7 d 1 x/d (GW)	Hepatic	250 F	500 F (decreased hepatic URO-D activity)		Kleiman de Pisarev et al. 1990	
			Endocr		250 F (decreased serum T4 levels)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
12	Rat (Wistar)	1 wk (GO)	Endocr		1000 F (18% decreased serum T4; 70% decreased serum T3)		Kleiman de Pisarev et al. 1995	
			Bd Wt	1000 F				
13	Rat (Sprague-Dawley)	2-16 d 1 x/d (GO)	Hepatic		25 F (increased urinary porphyrins; increased hepatic porphyrin content)		Krishnan et al. 1991	
14	Rat (Sprague-Dawley)	12 d 1x/d (GO)	Hepatic		50 F (increased hepatic and urinary uroporphyrins)		Krishnan et al. 1992	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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 (Sprague-Dawley)	once (GO)	Resp	600 F			Lecavalier et al. 1994	
			Cardio	600 F				
			Gastro	600 F				
			Hemato	600 F				
			Musc/skel	600 F				
			Hepatic		400 F (16-18% increased liver weight; cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes)			
			Renal	600 F				
			Endocr		400 F (reduced follicle size and colloidal density, increased epithelial height in the thyroid; reduced cortical and medullary volume in thymus)			
			Dermal	600 F				
			Ocular	600 F				
			Bd Wt	600 F				
16	Rat (Sprague-Dawley)	6 d 1 x/d (GO)	Hepatic		10 M (increased liver weight)		Mehendale et al. 1975	
			Bd Wt	25 M				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
17	Rat (Brown Norway)	7 or 21d (F)	Hepatic		50.8 F (31% increased liver weight)		Michielsen et al. 2001	
			Dermal		50.8 F (skin lesions after 10 days of treatment)			
			Bd Wt	50.8 F				
18	Rat (Brown Norway)	6, 14, or 21 d (F)	Hepatic		50.8 F 33% increased relative liver weight)		Michielsen et al. 2002	
			Dermal		50.8 F (head and neck skin lesions)			
			Bd Wt	50.8 F				
19	Rat (NS)	5 d 1 x/d (GO)	Bd Wt	221 M			Simon et al. 1979	
20	Rat (Wistar)	2 wk 3 d/wk 1 x/d (IP)	Endocr	484 M	740 M (decreased serum total and free T4)		van Raaij et al. 1993a	
			Bd Wt	997 M				
21	Mouse (CD-1)	Gd 7-16 (GO)	Hepatic		100 F (increased dam liver weight)		Courtney et al. 1976	
			Bd Wt	100 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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	Gn Pig (NS)	10 d (F)	Hepatic		385 F (fatty changes in liver)		de Matteis et al. 1961 HCB	
			Renal		385 F (increased blood ammonia levels)			
Immuno/ Lymphoret								
23	Rat (Brown Norway)	7 or 21d (F)			50.8 F (eosinophilic lung inflammation)		Michielsen et al. 2001	
24	Rat (Brown Norway)	6, 14, or 21 d (F)			50.8 F (increases in weights of spleen and lymph nodes, increased in vitro tracheal reactivity to arecoline, eosinophilic lung inflammation)		Michielsen et al. 2002	
Neurological								
25	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1 x/d (G)		40 F		80 F (hyperesthesia, tremors, and convulsions in pregnant rats)	Khera 1974	
26	Rat (Brown Norway)	7 or 21d (F)			50.8 F (tremors starting at treatment day 14)		Michielsen et al. 2001	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
27	Mouse (NS)	8-10 d (F)				976 F (marked weakness, hyperexcitability, tremors, clonic contractions)	de Matteis et al. 1961 HCB	
28	Gn Pig (NS)	8-10 d (F)				385 F (marked weakness, hyperexcitability, tremors, convulsions)	de Matteis et al. 1961 HCB	
Reproductive								
29	Rat (Sprague-Dawley)	5 d 1 x/d (GO)			50 F (increased serum progesterone)		Foster et al. 1993	
30	Rat (NS)	5 d 1 x/d (GO)		70 M	221 M (decreased male impregnation of females)		Simon et al. 1979	
Developmental								
31	Rat (Sprague-Dawley)	4 d 1 x/d (GO)			2.5[b] F hyperactivity in young pups)		Goldey and Taylor 1992	
32	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1 x/d (G)		20 F	40 F (increased incidence of skeletal variations)		Khera 1974	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
33	Mouse (CD-1)	Gd 7-16 (GO)				100 (increased incidence of abnormal fetuses per litter)	Courtney et al. 1976	
INTERMEDIATE EXPOSURE								
Death								
34	Rat (Wistar)	80 d (F)				205 F (16/25 died)	Cantoni et al. 1990	
35	Rat (Wistar)	80 d (F)				205 F (60/90 died)	Cuomo et al. 1991 HCB	
36	Rat (Wistar)	13 wk (F)				19 F (4/9 died)	den Besten et al. 1993	
37	Rat (Sprague-Dawley)	4 gen (F)				15.7 F (1/20 dams died)	Grant et al. 1977	
38	Rat (Wistar)	Up to 56 d (F)				129 F (2/29 died)	Kennedy and Wigfield 1990	
39	Rat (Sherman)	4 mo (F)				50 M (14/20 died) 56.5 F (2/10 died)	Kimbrough and Linder 1974	
40	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)				50 F (5/19 died)	Koss et al. 1978	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Rat (COBS)	15 wk (F)				32 F (increased mortality in females)	Kuiper-Goodman et al. 1977	
42	Rat (Sprague-Dawley)	56 d (F)				172 M (13/33 died within 1 month)	Ockner and Schmid 1961	
43	Hamster (Golden Syrian)	6, 18, or 28 wk (F)				19 M (2/11 and 8/18 died during 18 and 28 weeks of treatment, respectively)	Smith et al. 1987 HCB	
44	Rabbit (NS)	12 wk (F)				161 F (4/4 died)	de Matteis et al. 1961 HCB	
45	Pig (SPF)	90 d (F)				50 M (5/5 died)	Den Tonkelaar et al. 1978	
Systemic								
46	Monkey (Cynomolgus)	90 d 1 x/d (C)	Hemato	10 F			Foster et al. 1995a HCB	
			Bd Wt	10 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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	Monkey (Rhesus)	60 d 1 x/d (G)	Resp	128 F			Iatropoulos et al. 1976	
			Cardio	128 F				
			Gastro	128 F				
			Musc/skel	128 F				
			Hepatic		8 F (hepatocellular hypertrophy, cloudy swelling)			
			Renal		8 F (vacuolization of proximal renal tubules)			
			Endocr	128 F				
			Ocular	128 F				
48	Monkey (Cynomolgus)	90 d 1 x/d (C)	Hepatic	0.1 F	1 F (hepatocellular vacuolation, intrahepatic cholestasis)		Jarrell et al. 1993	
			Endocr	1 F	10 F (increased adrenal weight)			
			Bd Wt	10 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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	Monkey (Rhesus)	60 d 1 x/d (GW)	Hemato	128 F			Knauf and Hobson 1979	
			Hepatic	64 F	128 F (increased blood urea nitrogen)			
			Renal	64 F	128 F (increased serum AST)			
			Bd Wt		8 F (unspecified weight loss)			
50	Rat (Wistar)	30 d 1 x/d (GW)	Hepatic		1000 F (increased liver weight)		Alvarez et al. 2000 HCB	
			Bd Wt	1000 F				
51	Rat (Fischer 344)	5 wk 5 d/wk 1 x/day (GO)	Hepatic	0.1 M	1 M (increased liver weight)		Andrews et al. 1988	
			Renal	25 M				
			Endocr		10 M (increased serum 1,25-dihydroxy- vitamin D3 and parathyroid hormone)			
			Bd Wt		0.1 M (9 % depressed body weight)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
52	Rat (Fischer 344)	15 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.1 M	1 M (increased femur density)		Andrews et al. 1989	
			Hepatic	1 M	10 M (increased liver weight)			
			Renal	1 M	10 M (increased kidney weight; increased urinary LDH and alkaline phosphatase)			
			Endocr	0.1 M	1 M (increased serum 1,25-dihydroxy vitamin D3)			
			Bd Wt	25 M				
53	Rat (Fischer 344)	15 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.1 M	1 M (increased femur density and cortical area)		Andrews et al. 1990	
			Renal	0.1 M	1 M (increased urinary LDH)			
			Endocr	1 M	10 M (increased serum parathyroid hormone)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
54	Rat (Sprague-Dawley)	3 mo prior to mating through weaning of F1 pups (F)	Hemato	3.4 M 3.9 F			Arnold et al. 1985	
			Hepatic	0.14 M 3.9 F	0.69 M (increased liver weight)			
			Renal	3.4 M 3.9 F				
			Bd Wt	3.4 M 3.9 F				
55	Rat (Wistar)	3 or 4 wk 5 d/wk 1 x/d (G)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 1991	
56	Rat (Wistar)	1, 2, 3, or 4 wk 5 d/wk 1 x/d	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 2000a HCB	
57	Rat (Wistar)	1 or 7 wk	Hepatic		1000 F (increased urinary porphyrin excretion)		Billi de Catabbi et al. 2000a HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
58	Rat (Wistar)	1, 2, 4, or 4 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria changes in hepatic sphingolipid levels)		Billie de Catabbi et al. 2000b	
59	Rat (Chbb)	1, 2, 4, or 4 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria changes in hepatic sphingolipid levels)		Billie de Catabbi et al. 2000b	
60	Rat (Sprague-Dawley)	7 wk 5 d/wk 1 x/d (GO)	Renal		50 M (increased kidney weight, glucosuria, proteinuria, degenerative and regenerative foci in tubules, accumulation of protein droplets in tubular cells)		Bouthillier et al. 1991	
61	Rat (Wistar)	80 d (F)	Hepatic		205 F (increases in liver weight, hepatic porphyrins, lipid peroxidation; decreased hepatic URO-D)		Cantoni et al. 1990	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
62	Rat (Wistar)	Up to 28 d 1x/d (GO)	Endocr		4 M (decreased free and total T4, increased TSH in serum; increased thyroid weight, histopathological thyroid lesions)		Chalouati et al. 2013 HCB	
			Bd Wt	16 M				
63	Rat (Wistar)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria)		Cochon et al. 2001 HCB	
64	Rat (Chbb)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria)		Cochon et al. 2001 HCB	
65	Rat (Wistar)	80 d (F)	Hepatic		205 F (porphyria, increased liver weight, fatty degeneration)		Cuomo et al. 1991 HCB	
			Dermal		205 F (photosensitive skin lesions)			
			Bd Wt	205 F				
66	Rat (NS)	NS (F)	Hepatic		526 (porphyria)		de Matteis et al. 1961 HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
67	Rat (Wistar)	13 wk (F)	Hepatic		9.5 F (increased liver weight, increased urinary and liver porphyrins)		den Besten et al. 1993	
			Renal	9.5 F	19 F (increased kidney weight, basophilic renal tubules, protein casts)			
			Endocr	9.5 F	19 F adrenal: increased weight, cortical hypertrophy and hyperplasia; thyroid: decreased serum T4 and T3)			
			Dermal	9.5 F	19 F (skin lesions)			
			Bd Wt	9.5 F		19 F (severe weight loss in 4/9 rats)		
68	Rat (Wistar)	107 d (F)	Hepatic		308 F (marked decrease in hepatic URO-D activity, massive increase in hepatic porphyrin content)		Elder and Urquhart 1986	
69	Rat (Brown Norway)	21 d (F)	Dermal		50.8 F (skin lesions)		Ezendam et al. 2004a	
			Bd Wt	50.8 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
70	Rat (Wistar)	4 wk 5 d/wk 1 x/d (GW)	Renal		1000 F (porphyria and lipid peroxidation in cortex)		Fernandez-Tome et al. 2000 HCB	
71	Rat (Sprague-Dawley)	21 d 1 x/d (GO)	Bd Wt	100 F			Foster et al. 1992b	
72	Rat (Sprague-Dawley)	30 d 1 x/d (GO)	Endocr		1 F (decreased serum corticosterone)		Foster et al. 1995b	
			Bd Wt	100 F				
73	Rat (CD)	4 mo (F)	Resp	4 F	12 F (hypertrophy and proliferation of endothelial cells, increased macrophages)		Goldstein et al. 1978	
			Cardio	36 F				
			Hepatic	4 F	12 F (increased urinary and hepatic porphyrins, enlarged hepatocytes)			
			Endocr	36 F				
			Dermal	12 F	36 F (small sores)			
			Bd Wt	36 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
74	Rat (Fischer- 344)	5 wk (GO)	Hepatic		28 M (liver enlargement, porphyria, increased enzyme expression, increased protooncogene expression)		Gustafson et al. 2000 HCB	
75	Rat (Sprague-Dawley)	4 wks 1 x/d (GO)	Endocr	0.16 M	4 M (decreased plasma total T4)		Hadjab et al. 2004 HCB	
76	Rat (Wistar)	56 d (F)	Hepatic		12.9 F (increased highly carboxylated porphyrins in liver)		Kennedy and Wigfield 1990	
			Renal		12.9 F (increased highly carboxylated porphyrins in kidney)			
			Bd Wt	12.9 F		129 F (large decrease in body weight gain)		

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
77	Rat (Sherman)	4 mo (F)	Resp		10 M (increased macrophages, focal areas of fibrosis)	56.5 F (extensive intra-alveolar hemorrhage, inflammation, and edema, accompanied by an increase in lung weight)	Kimbrough and Linder 1974	
					11.3 F (increased macrophages, focal areas of fibrosis)			
			Cardio	10 M		50 M (fibrosis, degeneration)		
				11.3 F		56.5 F (fibrosis, degeneration)		
			Hemato		10 M (decreased hemoglobin and hematocrit)			
					11.3 F (decreased hemoglobin and hematocrit)			
			Hepatic		10 M (increased liver weight, enlarged hepatocytes)	50 M (necrosis, fibrosis)		
						56.5 F (necrosis, fibrosis)		
					11.3 F (increased liver weight, enlarged hepatocytes)			
			Renal	10 M	50 M (increased kidney weight)			
				11.3 F	56.5 F (increased kidney weight)			
			Endocr		10 M (hyperplasia of the adrenal cortex)			
					11.3 F (hyperplasia of the adrenal cortex)			
			Dermal	10 M	50 M (skin eruptions)			
				11.3 F	56.5 F (skin eruptions)			
			Bd Wt	10 M	50 M (decreased body weight gain in males)			
				112.9 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
78	Rat (Fischer- 344)	5 wk (F)	Hepatic		4.5 M (increased liver weight, centrilobular hypertrophy, foci of GST-P)		Kishima et al. 2000 HCB	
79	Rat (Sprague-Dawley)	1 gen (F)	Resp		5.9 F (intraalveolar foamy histiocytes, hypertrophy and proliferation of endothelial cells of pulmonary venules in dams)		Kitchin et al. 1982	
			Bd Wt	13.7 F				
80	Rat (Wistar)	4 wk 1 x/d (GW)	Endocr		1000 F (decreased serum T4, increased T4 metabolism, increased serum TSH, increased thyroid iodine uptake)		Kleiman de Pisarev et al. 1989	
			Bd Wt	1000 F				
81	Rat (Wistar)	30 d 1 x/d (GW)	Endocr	125 F	500 F (decreased serum T4 level)		Kleiman de Pisarev et al. 1989	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
82	Rat (Wistar)	8 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 F (increased liver weight, decreased URO-D activity, increased hepatic and urinary porphyrins, increased dehalogenation of T4)		Kleiman de Pisarev et al. 1990	
			Endocr		1000 F (decreased serum T4, increased serum TSH)			
			Bd Wt	1000 F				
83	Rat (Wistar)	4 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 F (increased liver weight)		Kleiman de Pisarev et al. 1995	
			Endocr		1000 F (decreased serum T4; increased serum TSH)			
			Bd Wt	1000 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
84	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)	Resp	50 F			Koss et al. 1978	
			Cardio	50 F				
			Hepatic		50 F (increased liver weight, massive increased liver and urine porphyrins)			
			Renal		50 F (increased kidney weight)			
			Endocr		50 F (increased adrenal weight)			
			Dermal		50 F (rough appearance of fur, hair loss, skin lesions)			
			Bd Wt	50 F				
85	Rat (Sprague-Dawley)	3-6 wk 5 d/wk 1 x/d (GO)	Hepatic		50 F (increased urinary and hepatic porphyrins)		Krishnan et al. 1991	
86	Rat (Sprague-Dawley)	6 wk 5 d/wk 1 x/d (GO)	Hepatic		50 F (increased hepatic and urinary porphyrins)		Krishnan et al. 1992	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
87	Rat (COBS)	15 wk (F)	Hemato	8 F	32 F (decreased red blood cell count, hematocrit, and hemoglobin; leukocytosis in females)		Kuiper-Goodman et al. 1977	
			Musc/skel	32				
			Hepatic	0.5	2 (slight basophilic clumping without hepatocyte enlargement)			
			Renal	8	32 (increased kidney weight)			
			Dermal	8	32 (alopecia)			
			Bd Wt	8 M	32 M (13% decreased terminal body weight)			
88	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.3 F	1 F (minimal incisor degeneration)		Long et al. 2004	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
89	Rat (Wistar)	30 d (F)	Resp		46 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997 HCB	
			Hepatic		46 F (increased liver weight, hepatocyte hypertrophy with cytoplasmic inclusions)			
			Renal	92 F				
			Dermal		46 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)			
			Bd Wt	92 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
90	Rat (Lewis)	29 d (F)	Resp		17 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997 HCB	
			Hepatic		17 F (increased liver weight, hepatocyte hypertrophy with cytoplasmic inclusions)			
			Renal	51 F				
			Dermal		17 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)			
			Bd Wt	51 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
91	Rat (Brown Norway)	28 d (F)	Resp		17 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997 HCB	
			Hepatic		17 F (increased liver weight, hepatocyte hypertrophy with cytoplasmic inclusions)			
			Renal	102 F				
			Dermal		17 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)			
			Bd Wt	51 F		102 F (sudden weight loss)		
92	Rat (Brown Norway)	4 wk (F)	Resp	50.8 F			Michielsen et al. 2000 HCB	
			Hepatic		50.8 F (increased liver weight)			
			Dermal		50.8 F (dermal lesions-hyperplasia, deep venules with activated endothelium, inflammation)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
93	Rat (Brown Norway)	7 or 21d (F)	Hepatic		50.8 F (86% increased relative liver weight)		Michielsen et al. 2001	
			Dermal		50.8 F (skin lesions after 10-14 days of treatment)			
			Bd Wt	50.8 F				
94	Rat (Brown Norway)	6, 14, or 21 d (F)	Hepatic		50.8 F (78% increased relative liver weight)		Michielsen et al. 2002	
			Dermal		50.8 F (head and neck skin lesions)			
			Bd Wt	50.8 F				
95	Rat (Wistar)	60 d (F)	Hepatic		427 M (increased serum ALT, bilirubin, and glutamate dehydrogenase)		Nikolaev et al. 1986	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
96	Rat (Sprague-Dawley)	90d 5d/wk 1x/d (GO)	Resp	1 F	3 F (chronic pulmonary inflammation)		NTP 2002 HCB	
			Hepatic	3 F	10 F (hepatocellular hypertrophy)			
			Dermal	10 F	25 F (skin inflammation and ulceration)			
			Bd Wt	25 F				
97	Rat (Sprague-Dawley)	56 d (F)	Hemato	172 M			Ockner and Schmid 1961	
			Musc/skel		172 M (porphyrin accumulation in cortex of long bones)			
			Hepatic		172 M (porphyria, hepatomegaly, hepatocellular degeneration)			
98	Rat (Wistar)	13 weeks (F)	Dermal		15.4 F (skin lesions)		Schielen et al. 1995a	
			Bd Wt	30.8 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
99	Rat (Wistar)	3 wk (F)	Dermal		92 M (skin lesions) 103 F (skin lesions)		Schielen et al. 1995b	
			Bd Wt	92 M 103 F				
100	Rat (Porton-Wistar)	112 d (F)	Hepatic	10 F			Smith et al. 1979	
101	Rat (Agus)	112 d (F)	Hepatic		10 F (increased hepatic porphyrin content, decreased hepatic URO-D activity, increased hepatic ALA-S activity)		Smith et al. 1979	
102	Rat (Fischer 344)	15 wk (F)	Hepatic		20 M (increased liver weight and hepatocellular hypertrophy) 22.6 F (increases in liver weight, hepatocellular hypertrophy, and hepatic porphyrin)		Smith et al. 1985	
			Renal		22.6 F (increased renal porphyrin level)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
103	Rat (Wistar)	60 d 1 x/d (GW)	Hepatic		1000 F (decreased URO-D activity in liver)		Sopena de Kracoff et al. 1994	
			Endocr		1000 F decreased serum T4, increased serum TSH			
104	Rat (Fisher CD)	6 wk (F)	Hepatic			226 F (severe porphyria)	Sweeney et al. 1986	
105	Rat (Wistar)	6 wk (F)	Hepatic	13.8 M	41.5 M (increased liver weight)		van Loveren et al. 1990 HCB	
106	Rat (Wistar)	4 wk 3 d/wk 1 x/d (GW)	Endocr		997 M (reduced serum total and free TT4 and FT4 levels; increased serum TSH)		van Raaij et al. 1993a	
			Bd Wt	997 M				
107	Rat (Wistar)	4 wk 3 d/wk 1 x/d (GW)	Hepatic		1000 M (increased liver weight)		van Raaij et al. 1993b	
			Endocr		1000 M (decreased serum T4 level)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
108	Rat (Wistar)	gestation + lactation + 2 wks post-weaning (F)	Hepatic	5.1	15.4 (increased liver weight)		Vos et al. 1979a	
			Endocr	5.1	15.4 (increased adrenal gland weight)			
109	Rat (Wistar)	3 wk (F)	Hemato		46 M (increased extramedullary hematopoiesis in spleen; neutrophilia)		Vos et al. 1979b	
			Hepatic		46 M (increased liver weight, liver cell hypertrophy and cytoplasmic hyalinization)			
			Renal	184 M				
			Endocr	184 M				
			Bd Wt	184 M				
110	Rat (BD VI)	5 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 decreased URO-D activity in males and females; increased hepatic and urinary porphyrins in females)		Wainstok de Calmanovici et al. 1991	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
111	Rat (Sprague-Dawley)	2 gen (GO)	Hepatic	2.5	12.5 25-39% increased relative liver weight in males and females, hepatocellular degeneration and fatty changes in males)		Wolfe and Pepperl 2005 HCB	
			Renal	2.5 M 12.5 F	12.5 M (22% increased relative kidney weight)			
			Bd Wt	12.5				
112	Mouse (BALB/c)	6 wk (F)	Resp	30 M			Loose et al. 1977	
			Hepatic		30 M (increased liver weight, hepatocellular hypertrophy)			
			Bd Wt	30 M				
113	Mouse (C57BL/ 10ScSn)	7 wk (F)	Hepatic		36 M (increased hepatic porphyrin levels)		Vincent et al. 1989	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
114	Hamster (Golden Syrian)	6, 18, or 28 wk (F)	Hepatic			19 M (2.5 to 2.7-fold increased liver weight; extensive hepatocellular hypertrophy and necrosis)	Smith et al. 1987 HCB	
			Endocr			19 M (3-fold decreased thyroid weight; 2.5 to 2.7-fold depressed serum T3)		
			Bd Wt		19 M (18-22% lower terminal body weight)			
115	Dog (Beagle)	21 d 1 or 2 x/d (C)	Resp	100 F			Sundlof et al. 1981	
			Cardio	100 F				
			Hemato	100 F				
			Hepatic		50 F (fatty changes in liver, swollen hepatocytes and hepatomegaly)			
			Renal	100 F				
			Endocr	100 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
116	Rabbit (NS)	12 wk (F)	Hemato	161 F			de Matteis et al. 1961 HCB	
			Musc/skel			161 F (necrosis, degeneration, and focal calcification in muscle, increased porphyrins in bone)		
			Hepatic			161 F (increased urinary and hepatic porphyrins, fatty change, necrosis)		
			Bd Wt			161 F (weight loss)		
117	Pig (SPF)	90 d (F)	Hemato	50 M			Den Tonkelaar et al. 1978	
			Hepatic	0.05 M	0.5 M (hepatocellular hypertrophy)			
			Renal	0.5 M	5 M (increased kidney weight)	50 M (degeneration of proximal tubules and loop of Henle)		
			Endocr	0.5 M	5 M (increased thyroid weight)			
			Bd Wt	5 M		50 M (seriously depressed growth)		

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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								
118	Monkey (Rhesus)	60 d 1 x/d (G)			8 F (thymic cortical atrophy)		Iatropoulos et al. 1976	
119	Rat (Brown Norway)	21 d (F)			50.8 F (increased weight of spleen and auricular lymph nodes, increased serum IgE and IgM, increased numbers of splenic T-cells and auricular lymph node B-cells, histopathologic lesions in lung and spleen)		Ezendam et al. 2004a	
120	Rat (Wistar)	56 d (F)		12.9 F	129 F (88% increased spleen weight; increased highly carboxylated porphyrins in spleen)		Kennedy and Wigfield 1990	
121	Rat (Sherman)	4 mo (F)		10 M 11.3 F	50 M (increased white blood cell count, increased spleen weight) 56.5 F (increased white blood cell count, increased spleen weight)		Kimbrough and Linder 1974	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
122	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)			50 F (increased spleen weight)		Koss et al. 1978	
123	Rat (COBS)	15 wk (F)		8	32 (increased spleen weight in males and females, increased lymphocyte count and congestive splenomegaly in females)		Kuiper-Goodman et al. 1977	
124	Rat (Wistar)	30 d (F)			46 F (increased spleen weight, “high endothelial” venules in popliteal lymph node, increased serum IgM)		Michielsen et al. 1997 HCB	
125	Rat (Lewis)	29 d (F)			17 F (“high endothelial” venules in popliteal lymph node)		Michielsen et al. 1997 HCB	
126	Rat (Brown Norway)	28 d (F)			17 F (increased spleen weight, “high endothelial” venules in popliteal lymph node; increased serum IgM autoantibodies)		Michielsen et al. 1997 HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
127	Rat (Brown Norway)	4 wk (F)			50.8 F (increased spleen weight)		Michielsen et al. 2000 HCB	
128	Rat (Brown Norway)	7 or 21d (F)			50.8 F (increased spleen weight)		Michielsen et al. 2001	
129	Rat (Brown Norway)	6, 14, or 21 d (F)			50.8 F (increases in weights of spleen and lymph nodes, increased in vitro tracheal reactivity to arecoline, eosinophilic and granulomatous lung inflammation)		Michielsen et al. 2002	
130	Rat (Sprague-Dawley)	90d 5d/wk 1x/d (GO)		3 F	10 F (splenic lymphoid hyperplasia)		NTP 2002 HCB	
131	Rat (Wistar)	3 wk (F)			46 M (increased IgM autoantibodies)		Schielen et al. 1993	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
132	Rat (Wistar)	13 weeks (F)			15.4 F (increases in spleen weight, total serum IgM, serum IgM autoantibodies)		Schielen et al. 1995a	
133	Rat (Wistar)	3 wk (F)			92 M (increased spleen and lymph node weight, altered size distribution of splenocytes, selective activation of splenic B-1 cells) 103 F (increased spleen and lymph node weight, altered size distribution of splenocytes, selective activation of splenic B-1 cells)		Schielen et al. 1995b	
134	Rat (Wistar)	6 wk (F)			13.8 M (decreased NK cell activity in lung)		van Loveren et al. 1990 HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
135	Rat (Wistar)	3 wk (F)			46 M (increased weight and proliferation of high-endothelial venules in lymph nodes, enlarged white pulp [marginal zones and follicles] in spleen, increased neutrophil count)		Vos et al. 1979b	
136	Mouse (Balb/C, nude)	6 wk (F)				30.1 M (increased susceptibility to hepatitis infection) 32.6 F (increased susceptibility to hepatitis infection)	Carthew et al. 1990	
137	Mouse (BALB/c)	6 wk (F)			30 M (immunosuppression, decreased antibody production)		Loose et al. 1977	
138	Mouse (Balb/c)	3 or 6 wk (F)			30 M (increased susceptibility to bacterial endotoxin and protozoan infection)		Loose et al. 1978	
139	Mouse (BALB/c)	18 wk (F)		30 M			Loose et al. 1981	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
140	Mouse (BALB/c)	18 wk (F)			0.9 M (increased susceptibility to tumor challenge in vivo, decreased killing of tumor cells in vitro, reduced spleen cell cytocidal activity)		Loose et al. 1981	
141	Mouse (C57BL/6)	40 wk (F)			30 M (decreased graft-host activity)		Silkworth and Loose 1981 HCB	
142	Pig (SPF)	90 d (F)		5 M	50 M (atrophy of lymph nodes)		Den Tonkelaar et al. 1978	
Neurological								
143	Monkey (Rhesus)	60 d 1 x/d (G)		128 F			Iatropoulos et al. 1976	
144	Monkey (Rhesus)	60 d 1 x/d (GW)		32 F		64 F (severe tremors, muscular weakness)	Knauf and Hobson 1979	
145	Rat (Wistar)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)			1000 F (altered phospholipid levels in brain)		Cochon et al. 2001 HCB	
146	Rat (Chhb)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)			1000 F (altered phospholipid levels in brain)		Cochon et al. 2001 HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
147	Rat (Wistar)	80 d (F)				205 F (severe neurotoxicity)	Cuomo et al. 1991 HCB	
148	Rat (NS)	NS (F)				526 (tremors, paresis)	de Matteis et al. 1961 HCB	
149	Rat (CD)	4 mo (F)		12 F		36 F (excessive irritability)	Goldstein et al. 1978	
150	Rat (Sprague-Dawley)	4 gen (F)		7.3 F		14.6 F (convulsions in dams)	Grant et al. 1977 HCB	
151	Rat (Sprague-Dawley)	4 wks 1 x/d (GO)		0.16 M	4 M (significantly increased auditory threshold in the 2-16 kHz sound frequency range)		Hadjab et al. 2004 HCB	
152	Rat (Wistar)	56 d (F)		12.9 F		129 F (lethargy, tremor, convulsions)	Kennedy and Wigfield 1990	
153	Rat (Sherman)	4 mo (F)		10 M 11.3 F		50 M (tremor, hyperexcitability) 56.5 F (tremor, hyperexcitability)	Kimbrough and Linder 1974	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
154	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)				50 F (muscle fasciculations, tremors)	Koss et al. 1978	
155	Rat (COBS)	15 wk (F)		8		32 (tremors, ataxia, hind limb paralysis)	Kuiper-Goodman et al. 1977	
156	Rat (Brown Norway)	7 or 21d (F)			50.8 F (tremors beginning on treatment day 14)		Michielsen et al. 2001	
157	Rat (Wistar)	60 d (F)				427 M (clonic convulsions, tremors, hyper-excitability)	Nikolaev et al. 1986	
158	Rat (Sprague-Dawley)	56 d (F)				172 M (ataxia, tremor, paralysis)	Ockner and Schmid 1961	
159	Rat (Sprague-Dawley)	20 wk (F)			69 M (decreased nerve conduction velocity)		Sufit et al. 1986	
160	Mouse (C57BL/6J)	9, 15, 17 wk (F)				39 F (tremor)	Hahn et al. 1988	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
161	Dog (Beagle)	21 d 1 or 2 x/d (C)				50 F (dysrhythmic electroencephalogram)	Sundlof et al. 1981	
162	Rabbit (NS)	12 wk (F)				161 F (tremors, paresis)	de Matteis et al. 1961 HCB	
163	Pig (SPF)	90 d (F)		5 M		50 M (tremors, unsteady gait)	Den Tonkelaar et al. 1978	
Reproductive								
164	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.1 F (cellular degeneration of ovarian surface epithelium)		Babineau et al. 1991	
165	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.01[c] F (mitochondrial degeneration in developing ovarian follicles)		Bourque et al. 1995 HCB	
166	Monkey (Cynomolgus)	13 wk 1 x/d (C)		0.1 F	1 F (decreased serum progesterone levels during the luteal phase of menstruation)		Foster et al. 1992a	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
167	Monkey (Cynomolgus)	90 d 1 x/d (C)		1 F	10 F (increased length of menstrual cycle, decreased ovulatory levels of estradiol)		Foster et al. 1995a HCB	
168	Monkey (Rhesus)	60 d 1 x/d (G)			8 F (degeneration of the germinal epithelium, reduced number of primary follicles, and multiple follicular cysts in the ovaries)		Iatropoulos et al. 1976	
169	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.1 F (degenerative lesions in oocytes)		Jarrell et al. 1993	
170	Monkey (Rhesus)	24 d 1 x/d (GW)				4 F (blocked ovulation)	Muller et al. 1978 HCB	
171	Monkey (Cynomolgus)	12 wk 7 d/wk 1 x/d (C)			0.1 F (altered morphology of ovary surface epithelium cells)	1 F (necrosis of ovary surface epithelium cells, denuding of ovary)	Sims et al. 1991	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
172	Rat (Wistar)	30 d 1 x/d (GW)			1000 F (increased estrus duration, altered hormone levels, reduced ovulation, degenerative ovarian lesions)		Alvarez et al. 2000 HCB	
173	Rat (Sprague-Dawley)	3 mo prior to mating through weaning of F1 pups (F)		3.4[d] O			Arnold et al. 1985	
174	Rat (Sprague-Dawley)	21 d 1 x/d (GO)			1 F (increased serum progesterone levels)		Foster et al. 1992b	
175	Rat (Sprague-Dawley)	4 gen (F)		13.8[d] O		27.6[d] O (decreased fertility; increased number of stillborns)	Grant et al. 1977 HCB	
176	Rat (COBS)	15 wk (F)		32			Kuiper-Goodman et al. 1977	
177	Rat (Sprague-Dawley)	90d 5d/wk 1x/d (GO)		10 F	25 F (mammary gland hyperplasia)		NTP 2002 HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
178	Rat (Wistar)	3 wk (F)		184 M			Vos et al. 1979b	
179	Rat (Sprague-Dawley)	2 gen (GO)		2.5	12.5 M (8% increased number of F0 males with abnormal sperm; 25% decrease in total sperm/cauda of F0 males)		Wolfe and Pepperl 2005 HCB	
Developmental								
180	Monkey (Rhesus)	60 d (G)				64 F (67% pup mortality; hematoma and bilateral hemorrhagic pneumonia, congested lungs; ataxia)	Bailey et al. 1980	
181	Monkey (Rhesus)	22-60 d 1 x/d (GW)				64 (2/3 infants died after exhibiting lethargy, ataxia, and listlessness)	Iatropoulos et al. 1978 HCB	
182	Rat (Sprague-Dawley)	3 mo prior to mating through weaning of F1 pups (F)				3.4[d] O (decreased postpartum day 4 pup viability)	Arnold et al. 1985	
183	Rat (Sprague-Dawley)	4 gen (F)		3.4	6.9 (decreased pup weight gain)	13.8 (decreased pup viability)	Grant et al. 1977	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
184	Rat (Sprague-Dawley)	1 gen (F)			5.9 (decreased pup weight gain)	7.8 (decreased neonatal survival)	Kitchin et al. 1982	
185	Rat (Wistar)	2 gen (F)		0.6 M	1.3 M (reduced efficiency of pups in operant behavior task)		Lilienthal et al. 1996 HCB	
186	Rat (Wistar)	gestation + lactation + 2 wks post-weaning (F)			5.1 F (decreased resistance to infection, increased IgG response to tetanus toxoid, and proliferation of high endothelial venules in lymph nodes in pups)		Vos et al. 1979a	
187	Rat (Wistar)	gestation + lactation + 2 wks or 7 mo post-weaning (F)			0.4 F (increased IgG and IgM response to tetanus toxoid, increased delayed-type hypersensitivity reaction to ovalbumin, and accumulation of foamy macrophages in the lung in offspring)	10.3 (high pup mortality)	Vos et al. 1983 HCB	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
188	Rat (Sprague-Dawley)	2 gen (GO)		2.5		12.5 (20-25% decreased postpartum F1c pup body weight, 100% F1c pup mortality by PND 9)	Wolfe and Pepperl 2005 HCB	
189	Mouse (BALB/c)	Gd 0-18 (F)			0.5 (depressed delayed-type hypersensitivity response in offspring)		Barnett et al. 1987 HCB	
190	Pig (SPF)	90 d (F)		5 M	50 M (retarded development of the testes)		Den Tonkelaar et al. 1978	
Cancer								
191	Rat (Sprague-Dawley)	90 d (F)				10 (CEL: renal adenomas, hepatocarcinomas, lymphosarcomas)	Ertürk et al. 1986	
CHRONIC EXPOSURE								
Death								
192	Rat (Wistar)	75 wk (F)				8.4 F (1/6 died)	Smith and Cabral 1980	
193	Mouse (Swiss)	120 wk (F)				24 (decreased survival)	Cabral et al. 1979	
194	Hamster (Syrian)	lifespan (F)				16 (decreased lifespan)	Cabral et al. 1977	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
195	Dog (Beagle)	1 yr 1 x/d (C)				11 F (2/6 females died)	Gralla et al. 1977	
Systemic								
196	Rat (Sprague-Dawley)	130 wk and via mothers during gestation and lactation (F)	Hemato	2.8 M 3.2 F			Arnold et al. 1985	
			Hepatic	0.13 F	0.022[e] M (increased peribiliary lymphocytosis and fibrosis in the liver of F1 adults at terminal sacrifice)			
					0.64 F (dose-related increases in the incidence and/or severity of hepatic centrilobular basophilic chromogenesis in F1 females)			
			Renal	0.55 M 3.2 F	2.8 M (increased incidences of severe chronic nephrosis)			
			Bd Wt	2.8 M 3.2 F				
197	Rat (Sprague-Dawley)	1 yr (F)	Hepatic	0.069 M 0.08 F	0.34 M (mitochondrial swelling and elongation)		Mollenhauer et al. 1975	
					0.4 F (mitochondrial swelling and elongation)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
198	Rat (Agus)	90 wk (F)	Hepatic		7 F (porphyria)		Smith and Cabral 1980	
			Dermal		7 F (alopecia)			
			Bd Wt		7 F (decreased body weight gain)			

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
199	Rat (Fischer 344)	90 wk (F)	Hepatic		15.8 M (decreased URO-D activity, increased hepatic porphyrin levels, hepatocyte hypertrophy, fatty degeneration, bile duct hyperplasia)		Smith et al. 1985	
					18.3 F (decreased URO-D activity, increased hepatic porphyrin levels, hepatocyte hypertrophy, fatty degeneration, bile duct hyperplasia)			
			Renal		15.8 M (increased kidney weight and renal porphyrin levels, nephrosis)			
					18.3 F			
			Endocr	15.8 M				
				18.3 F				
			Bd Wt		15.8 M (decreased body weight)			
					18.3 F (decreased body weight)			
200	Rat (Fischer- 344)	65 wk (F)	Hepatic		18.3 F (increased liver weight; biliary hyperplasia; increased liver porphyrins; induction of microsomal enzymes and glutathione S-transferase)		Smith et al. 1993	
			Bd Wt	18.3 F				

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
201	Mouse (C57BL/ 10ScSn)	18 mo (F)	Hepatic		17.2 M (hepatocyte hypertrophy)		Smith et al. 1989	
			Bd Wt		17.2 M (decreased body weight)			
202	Hamster (Syrian)	lifespan (F)	Bd Wt			16 M (marked decrease in body weight gain)	Cabral et al. 1977	
203	Dog (Beagle)	1 yr 1 x/d (C)	Cardio	11		110 (arteriopathy)	Gralla et al. 1977	
			Gastro	1		11 (diarrhea)		
			Hemato	1	11 (neutrophilia)	110 (anemia)		
			Hepatic	1		11 (hepatomegaly, bile duct hyperplasia, pericholangitis, periportal fibrosis, increased serum alkaline phosphatase and AST)		
			Bd Wt	1		11 (body weight loss)		
Immuno/ Lymphoret								
204	Rat (Sprague-Dawley)	130 wk and via mothers during gestation and lactation (F)			0.022 M (peribiliary lymphocytosis)		Arnold et al. 1985	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
205	Dog (Beagle)	1 yr 1 x/d (C)			0.1 (increased severity of nodular hyperplasia of the gastric lymphoid tissue)		Gralla et al. 1977	
Neurological								
206	Rat (Agus)	90 wk (F)		7 F			Smith and Cabral 1980	
207	Rat	2 yr (F)			9.1 (slight decrease in nerve conduction velocity)		Sufit et al. 1986	
208	Mouse (Swiss)	120 wk (F)				24 (tremors, convulsions)	Cabral et al. 1979	
Reproductive								
209	Dog (Beagle)	1 yr 1 x/d (C)			110 M (slight testicular degeneration)		Gralla et al. 1977	
Developmental								
210	Rat (Fischer 344)	90 wk (F)			15.8 M (40% increased testicular weight)		Smith et al. 1985	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Cancer								
211	Rat (Sprague-Dawley)	130 wk and via mothers during gestation and lactation (F)				2.8 M (CEL: parathyroid adenoma, adrenal pheochromocytoma)	Arnold et al. 1985	
						3.2 F (CEL: neoplastic liver nodules, adrenal pheochromocytoma)		
212	Rat (Sprague-Dawley)	104 wk (F)				5.2 M (CEL: hepatoma, bile-duct adenoma, hepatocarcinoma, renal adenoma)	Ertürk et al. 1986	
						6 F (CEL: hepatoma, bile-duct adenoma, hepatocarcinoma, renal adenoma)		
213	Rat (Wistar)	75 wk (F)				4.5 F (CEL: liver cell tumors)	Smith and Cabral 1980	
214	Rat (Agus)	90 wk (F)				7 F (CEL: liver-cell tumors)	Smith and Cabral 1980	
215	Rat (Fischer 344)	90 wk (F)				15.8 M (CEL: hepatocarcinoma)	Smith et al. 1985	
						18.3 F (CEL: hepatocarcinoma)		
216	Rat (Fischer- 344)	65 wk (F)				18.3 F (CEL: liver tumors)	Smith et al. 1993	

Table 3-2 Levels of Significant Exposure to Hexachlorobenzene - 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
217	Mouse (Swiss)	120 wk (F)				12 (CEL: liver tumors)	Cabral et al. 1979	
218	Mouse (C57BL/ 10ScSn)	18 mo (F)				17.2 M (CEL: hepatocellular carcinoma in iron-pretreated mice)	Smith et al. 1989	
219	Hamster (Syrian)	lifespan (F)				4 (CEL: hepatoma, liver hemangioendothelioma, thyroid alveolar adenoma)	Cabral et al. 1977	

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

b Used to derive an acute oral minimal risk level (MRL) of 0.008 mg/kg/day; dose divided by an uncertainty factor of 300 (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

c Used to derive an intermediate oral MRL of 0.0001 mg/kg/day; dose divided by an uncertainty factor of 100 (3 for extrapolation from monkeys to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

d This value represents the most sensitive gender-independent dose because both male and female animals were exposed to hexachlorobenzene, and the observed LOAEL or NOAEL for the effect could not be attributed to a particular gender.

e Used to derive a chronic oral MRL of 0.00007 mg/kg/day; dose divided by an uncertainty factor of 300 (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

ALA-S = delta-aminolevulinic acid synthetase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; FT4 = free thyroxine; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gen = generation(s); Gn pig = guinea pig; (GO) = gavage in oil; GST-P = glutathione S-transferase; (GW) = gavage in water; Hemato = hematological; IgE = immunoglobulin E; IgG = immunoglobulin G; IgM = immunoglobulin M; Immuno/Lymphoret = immunological/lymphoreticular; LDH = lactose dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; PND = post-natal day; Resp = respiratory; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; TT4 = total thyroxine; URO-D = uroporphyrinogen decarboxylase; wk = week(s); x = time(s); yr = year(s)

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

Acute (≤14 days)

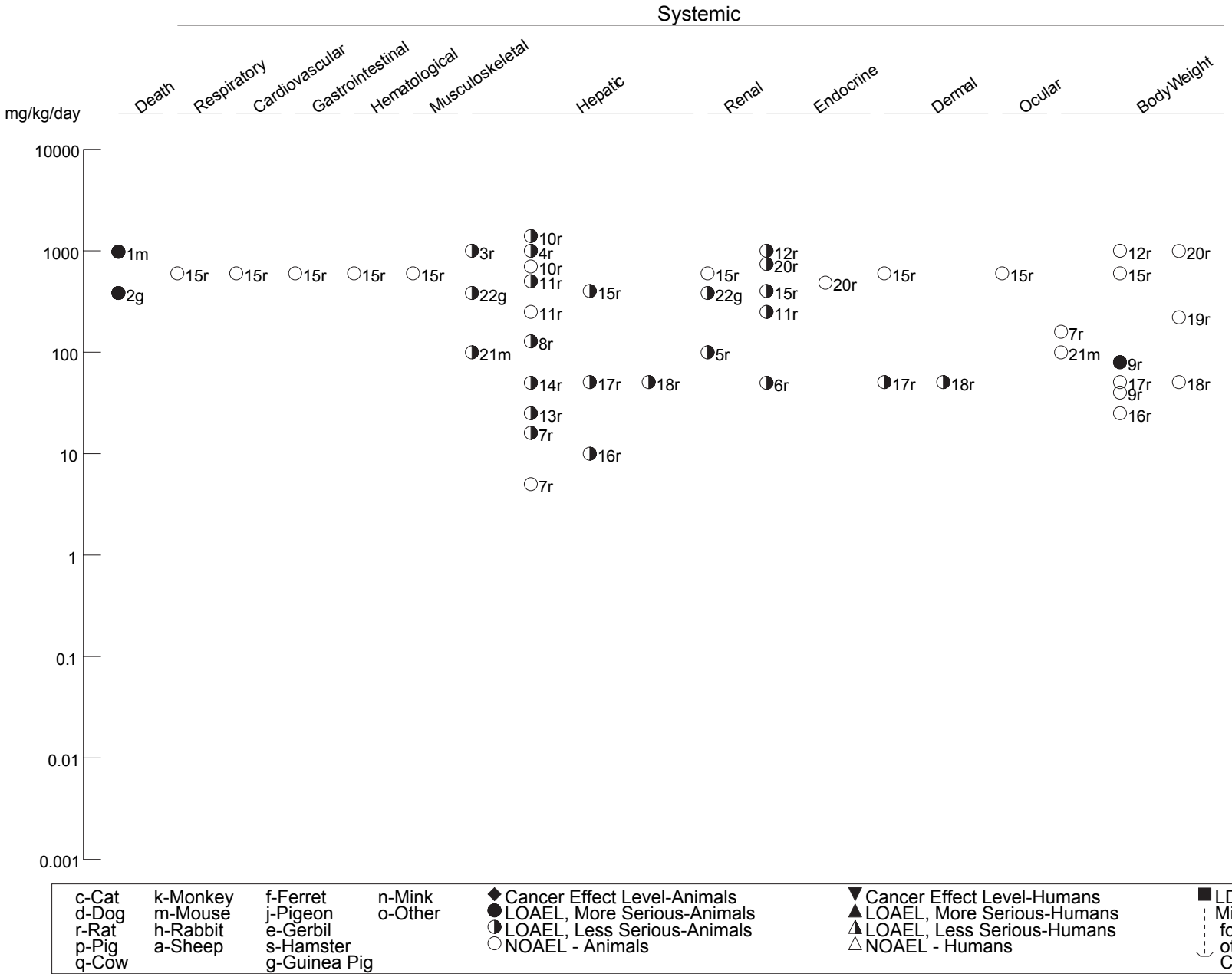


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Acute (≤14 days)

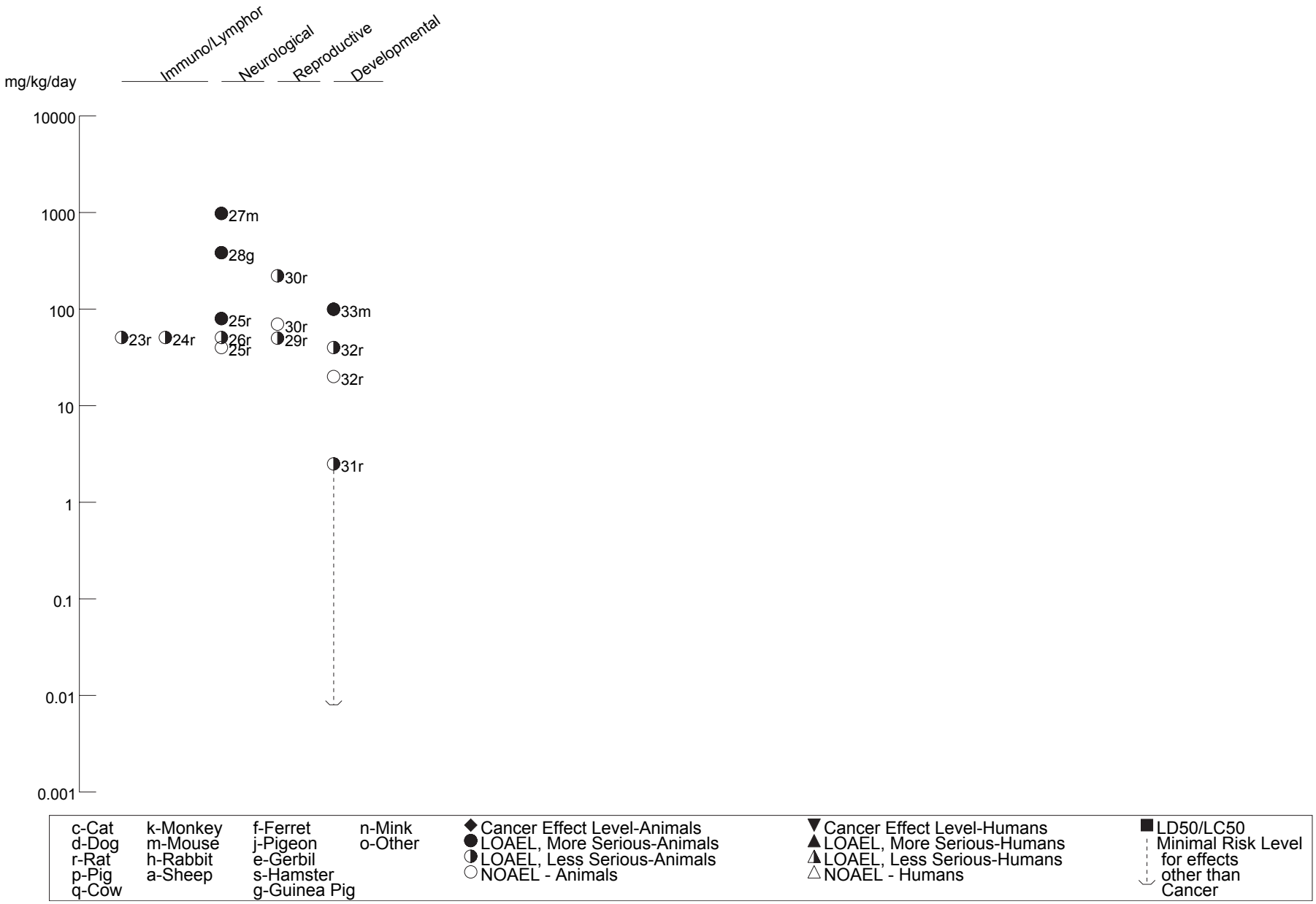


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

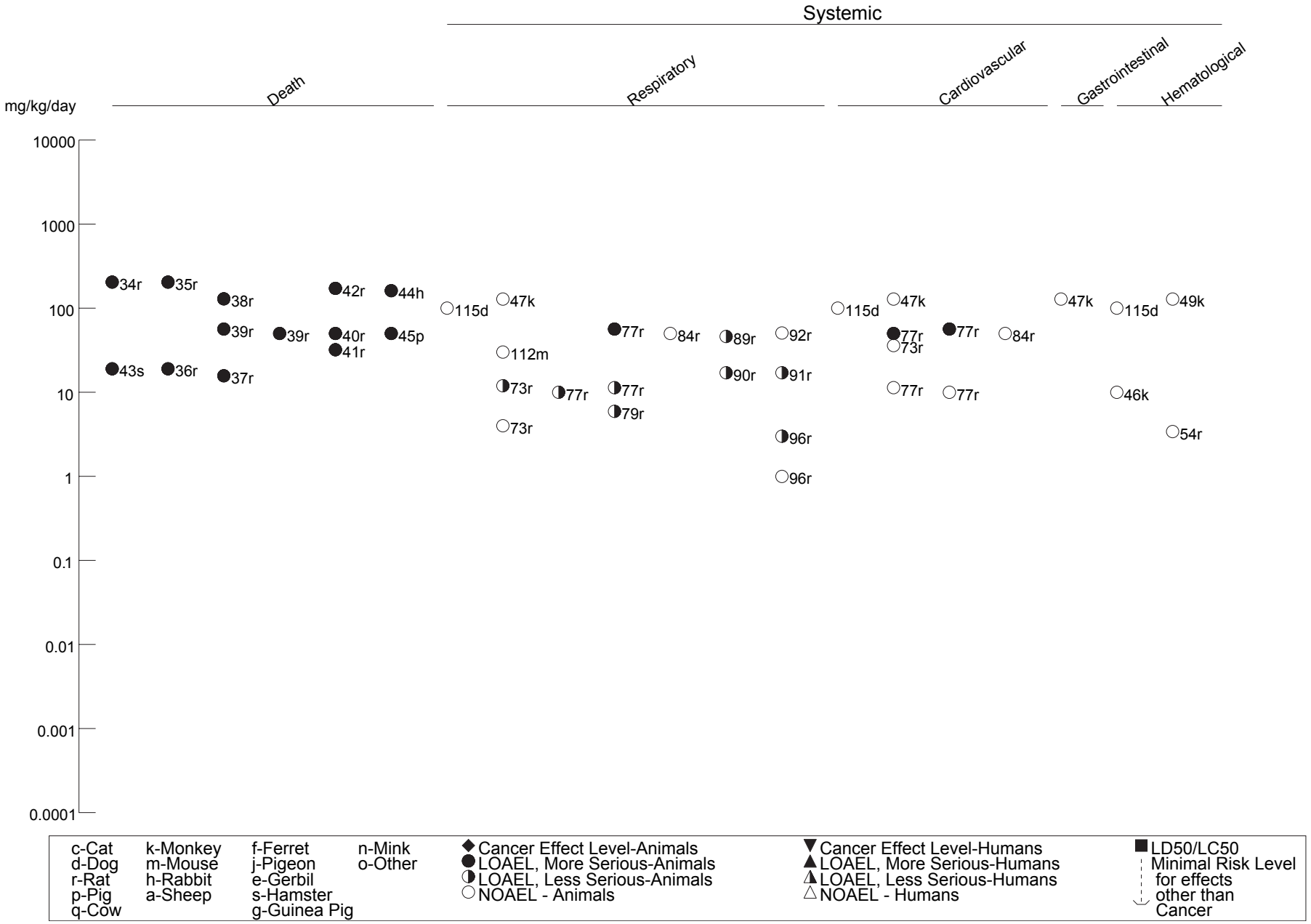


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

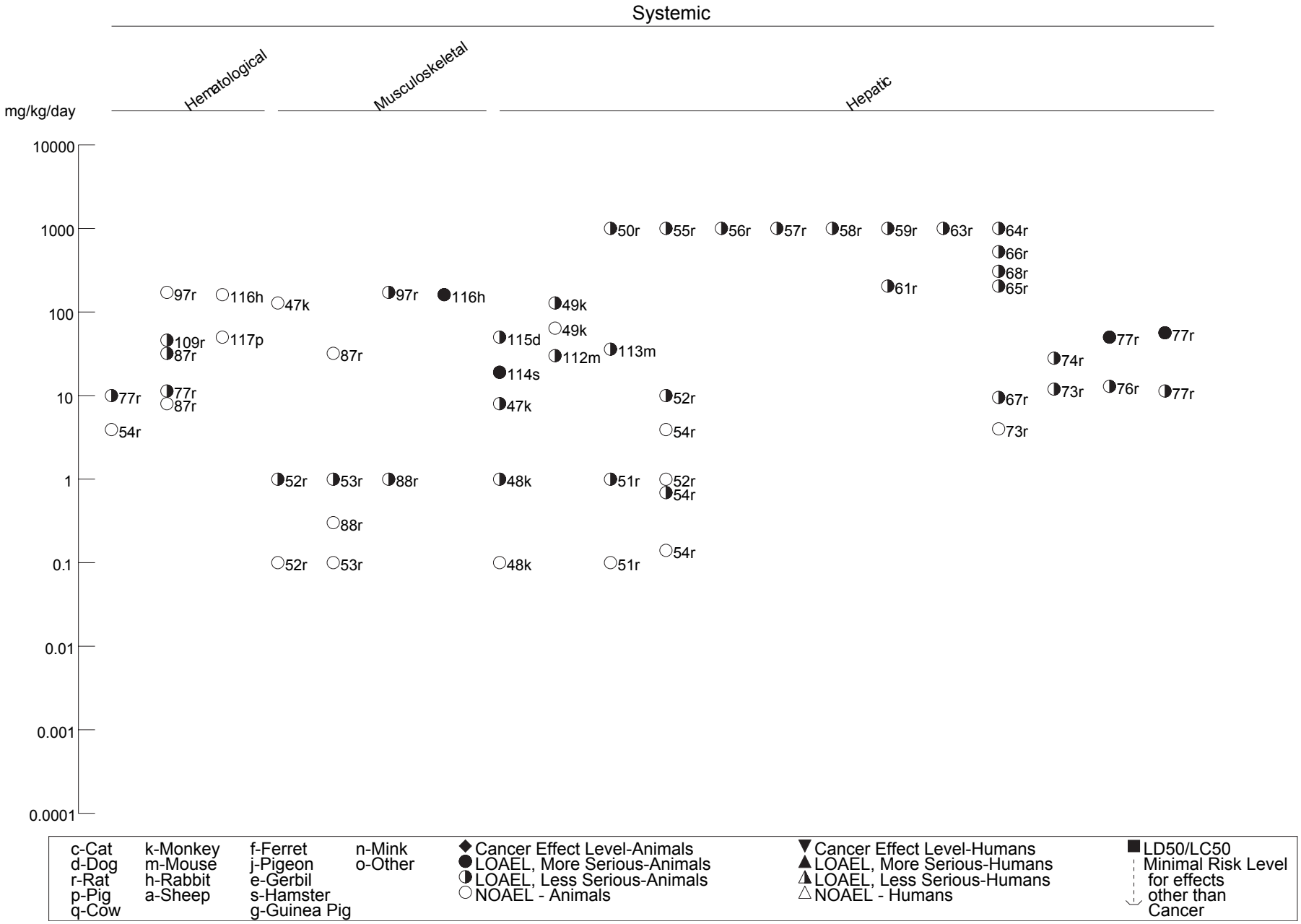


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

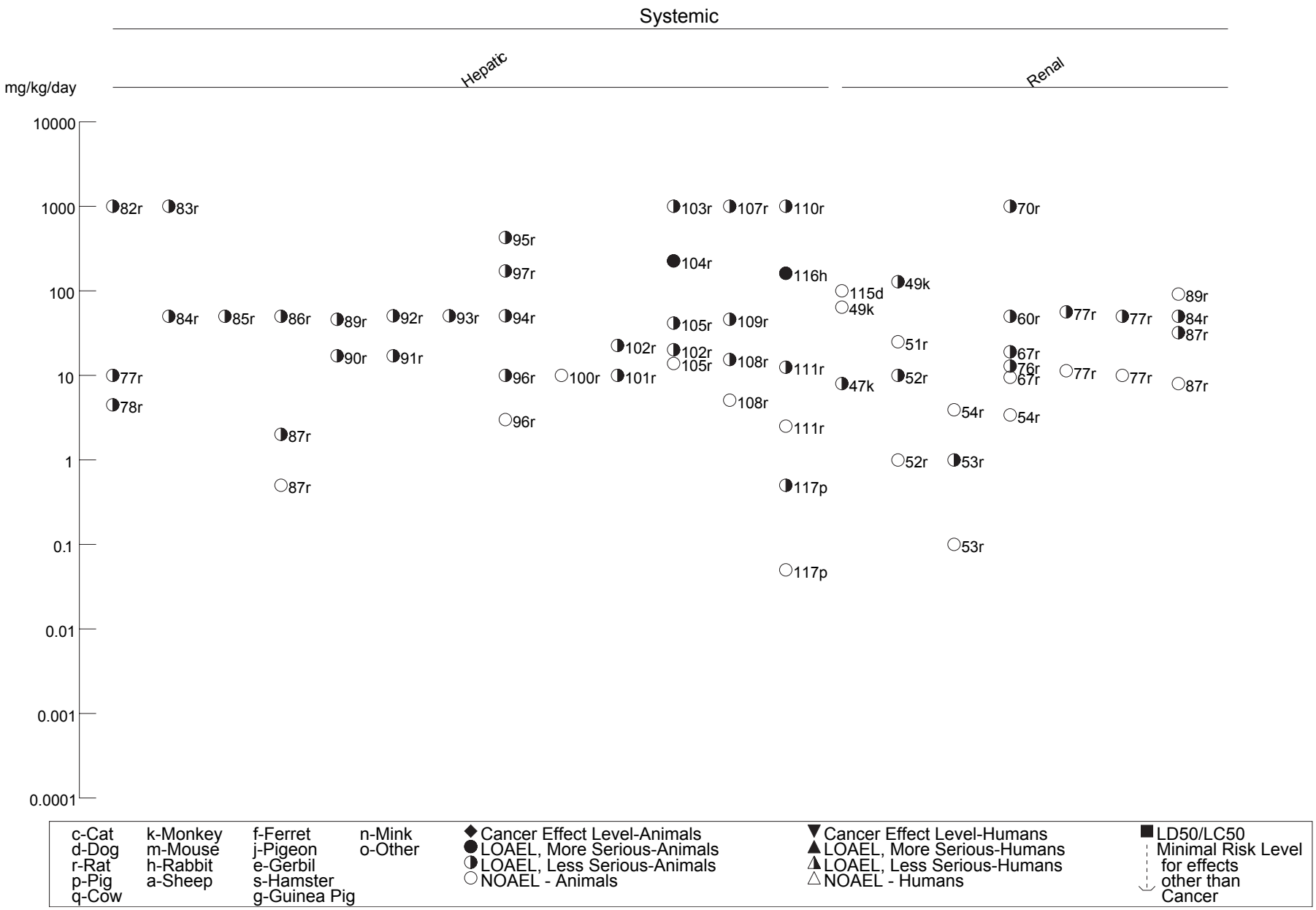


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

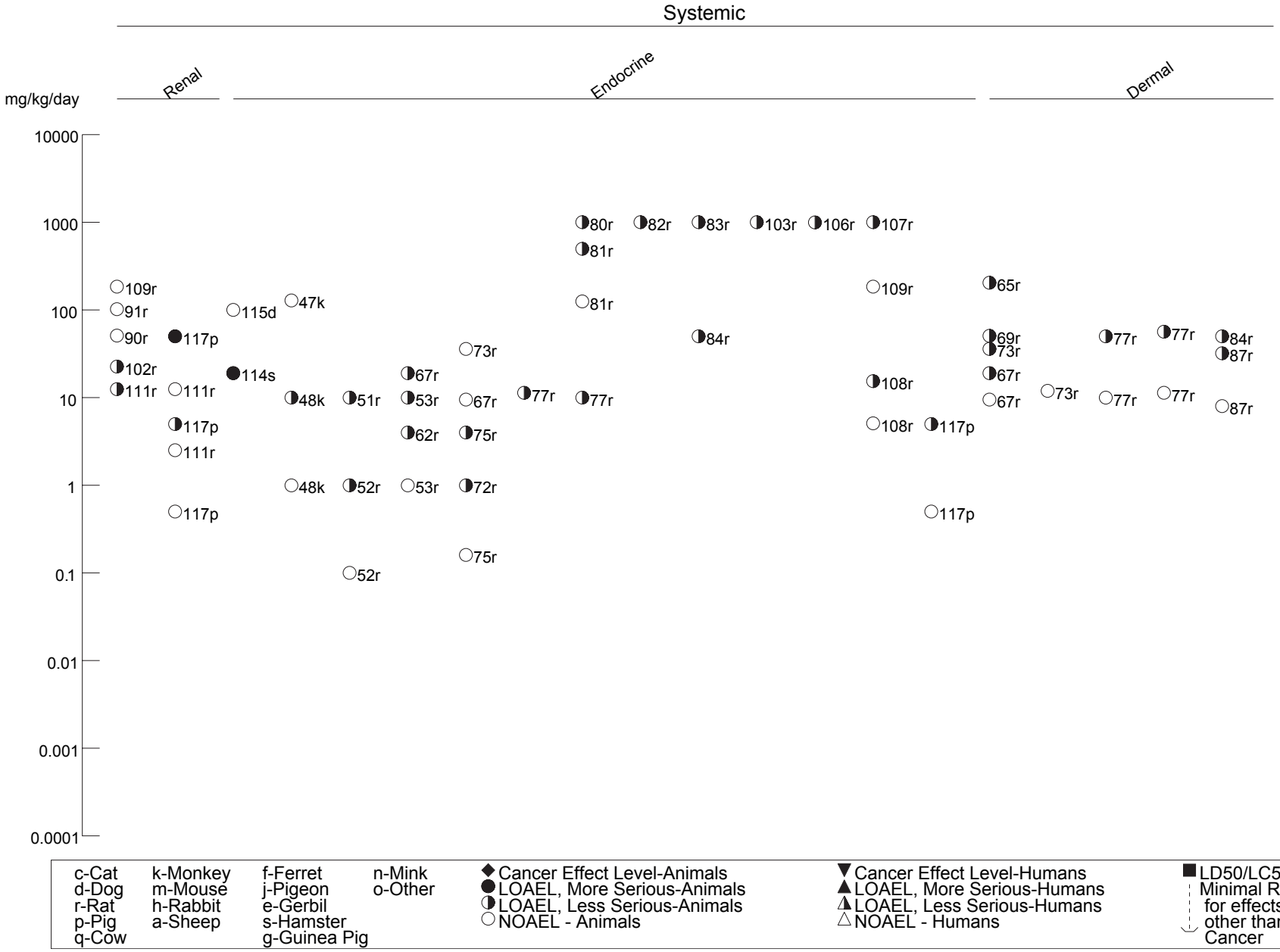


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

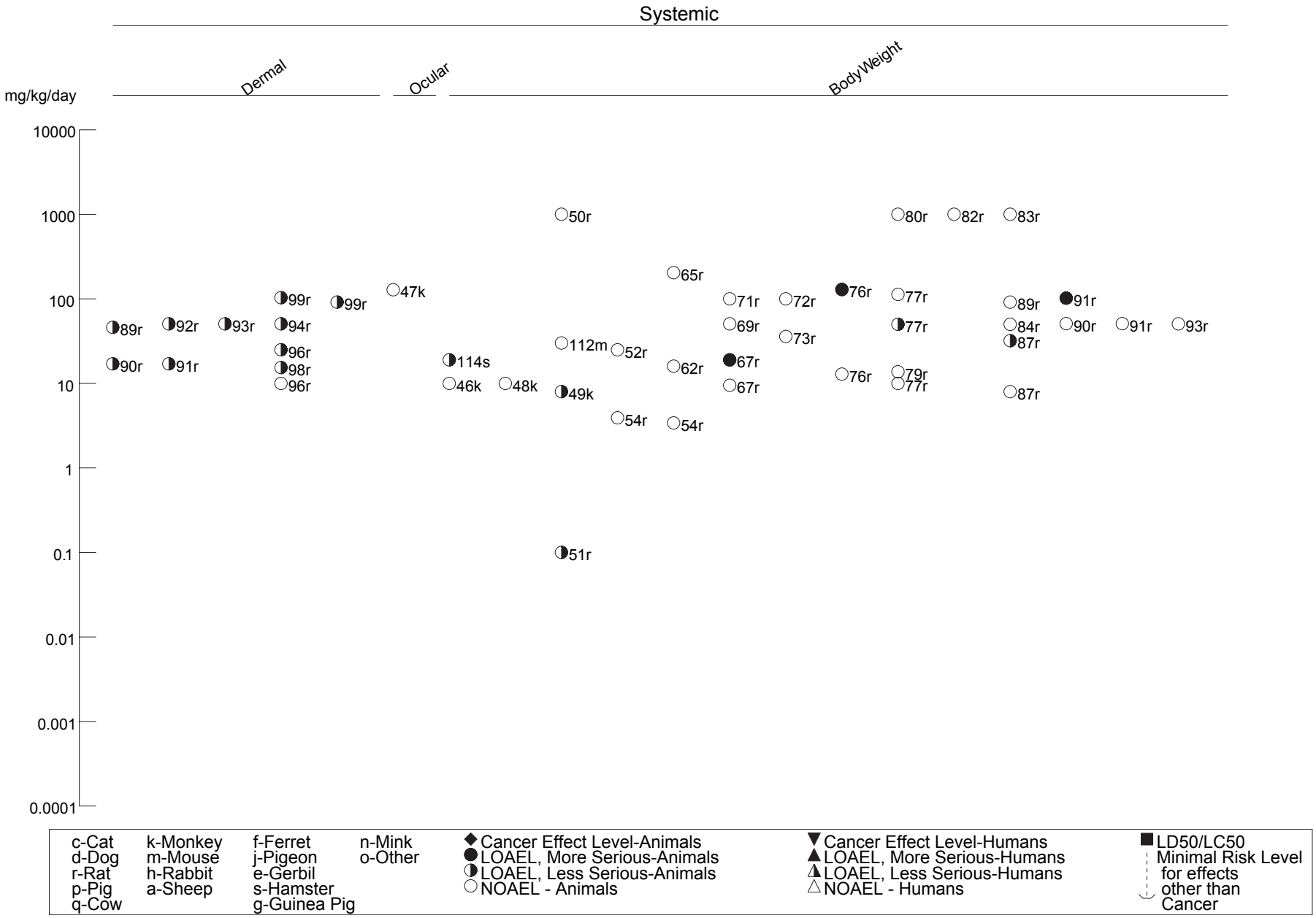


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

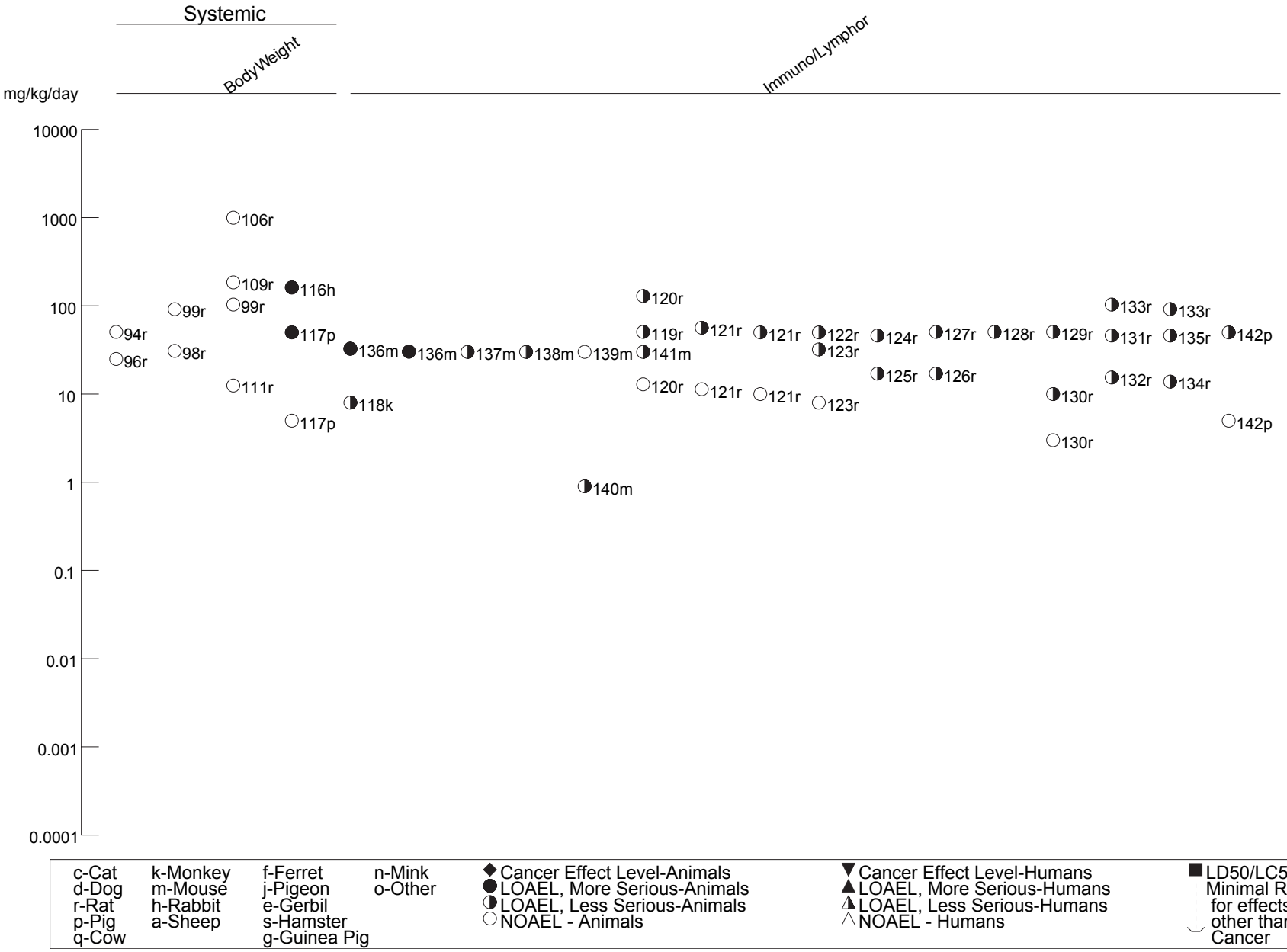


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

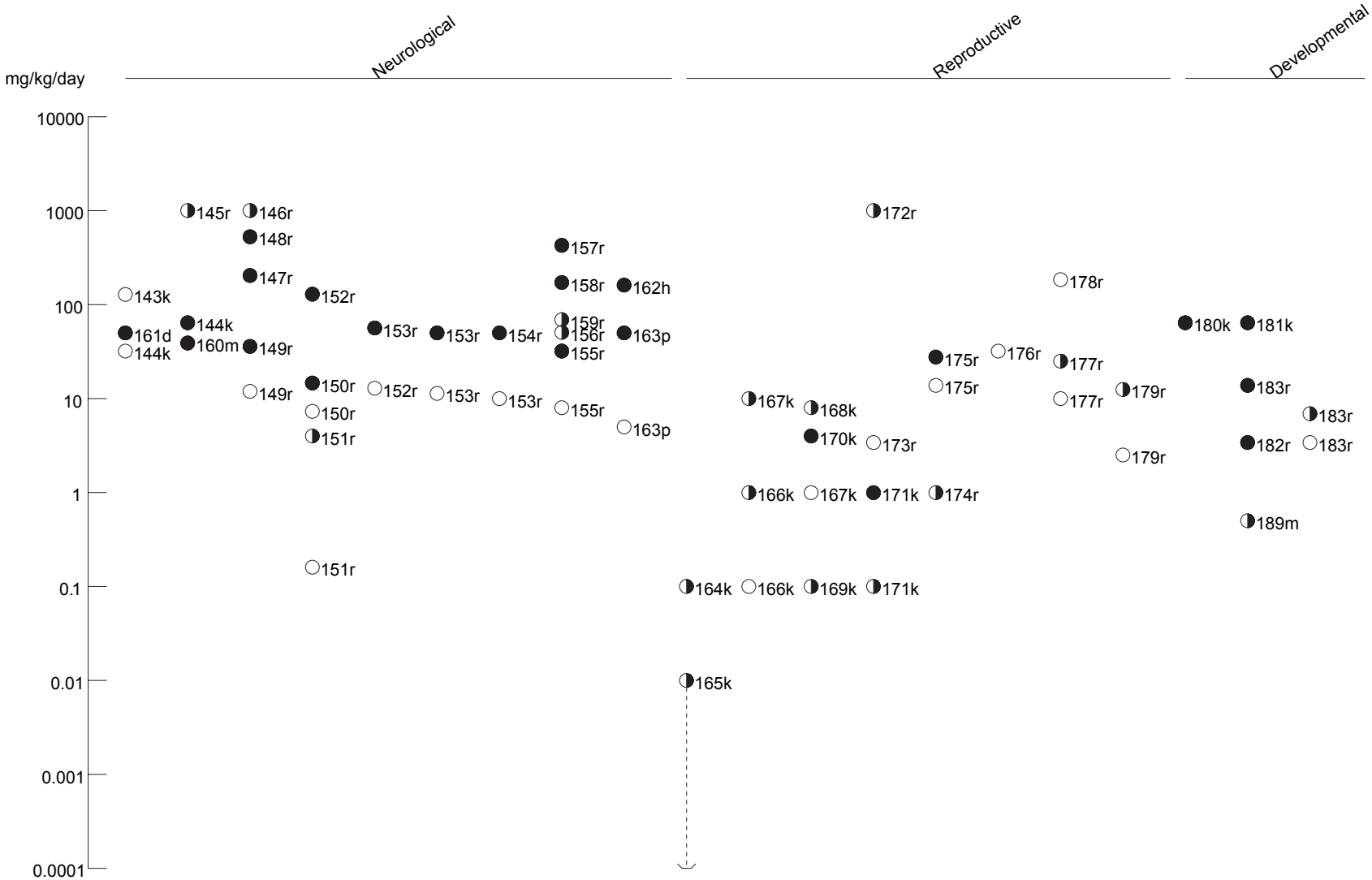
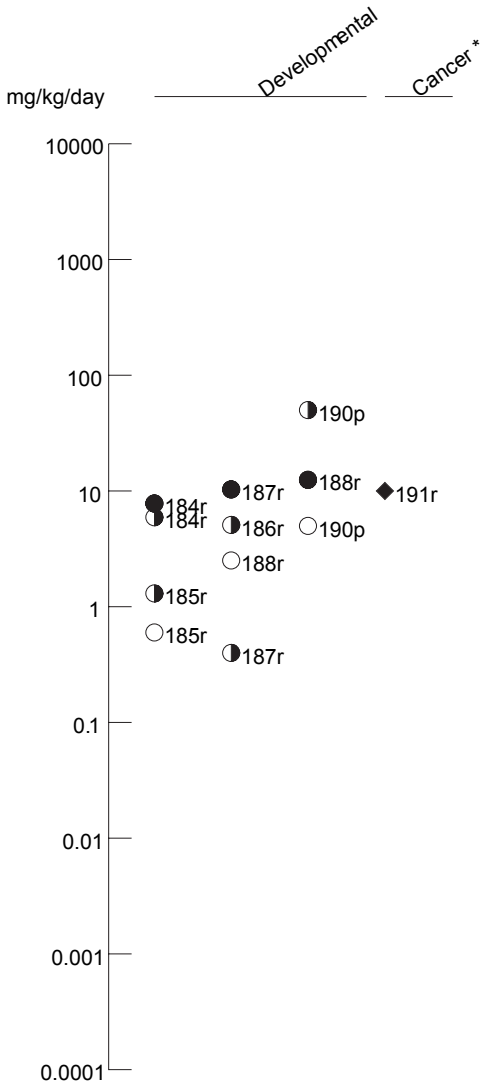


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

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

◆ 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 Hexachlorobenzene - Oral *(Continued)*

Chronic (≥365 days)

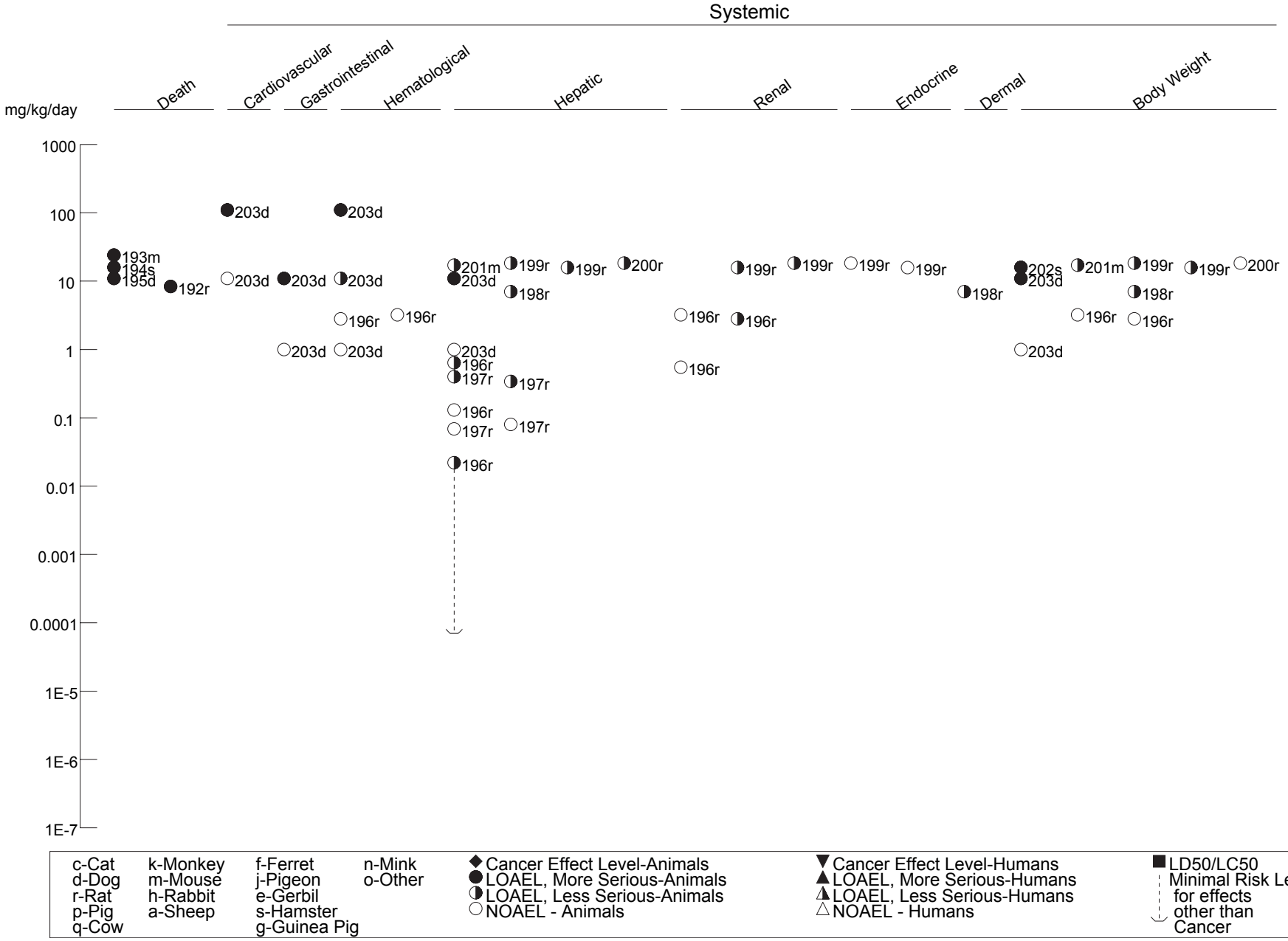
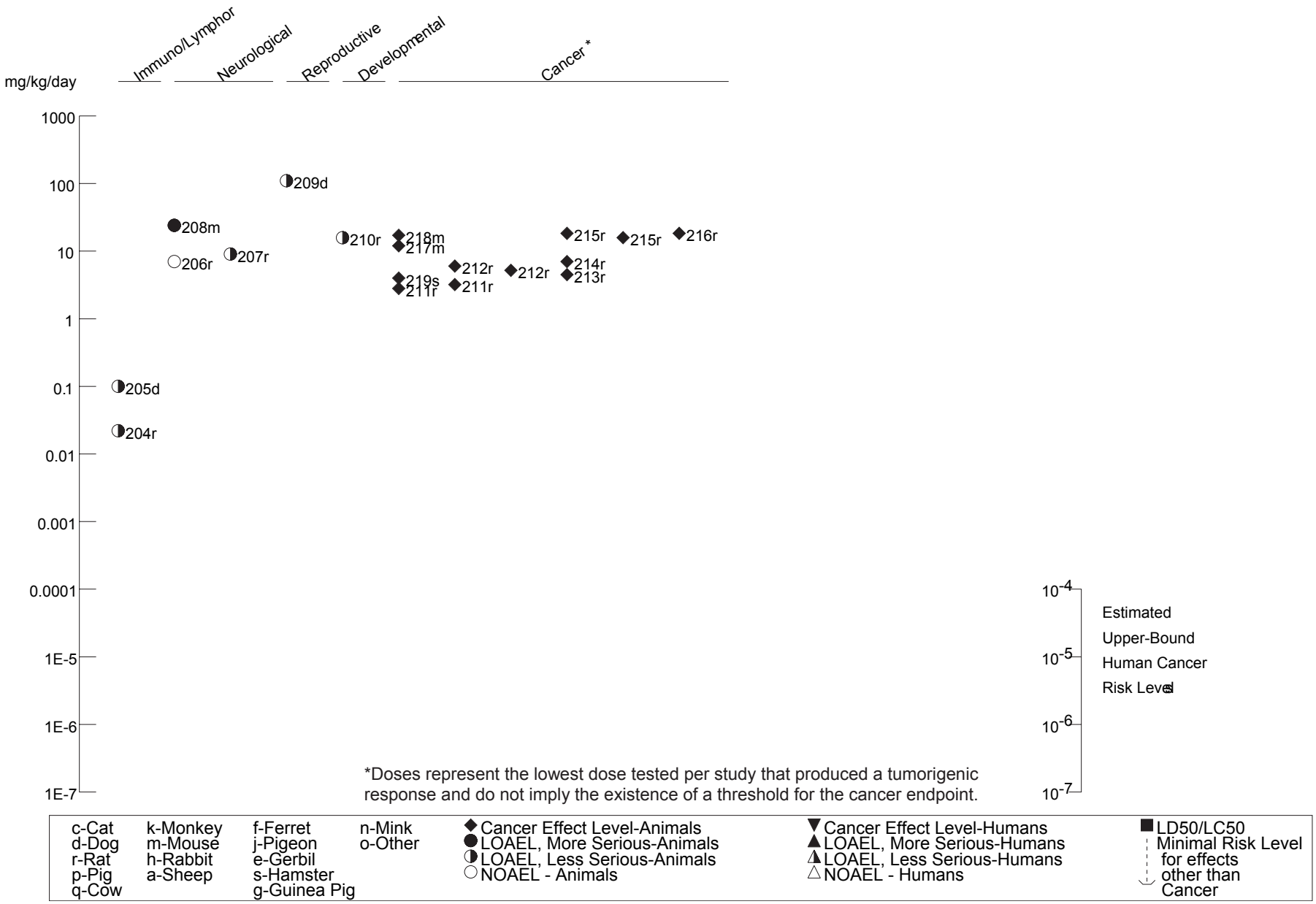


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

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

NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.05$) increased incidences of chronic pulmonary inflammation were noted at hexachlorobenzene doses ≥3 mg/kg/day (incidences of 3/10, 1/10, 1/10, 2/10, 7/10, 8/10, 10/10, and 10/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively). More severe pulmonary effects have also been observed in rats at higher doses. In addition to macrophage accumulation and focal areas of interstitial fibrosis, which they observed in male and female rats ingesting ≥10–11 mg hexachlorobenzene/kg/day from the diet for 4 months, Kimbrough and Linder (1974) also observed extensive intra-alveolar hemorrhage, inflammation, and edema, accompanied by an increase in lung weight, in females at doses ≥56.5 mg/kg/day. Adverse effects on respiratory function, granulomatous lung inflammation, and airway hyperresponsiveness have been associated with exposure of rats to hexachlorobenzene via the diet at concentrations resulting in estimated daily doses of approximately 50 mg/kg/day (Ezendam et al. 2004a; Michielsen et al. 2001, 2002); however, these effects are most likely attributable to hexachlorobenzene-induced effects on the immune system (see Section 3.2.2.3). Only limited pulmonary histopathology data are available for other species, and pulmonary lesions were not seen in available studies on monkeys, dogs, or mice (Iatropoulos et al. 1976; Loose et al. 1977; Sundlof et al. 1981).

Cardiovascular Effects. No studies were located regarding cardiovascular effects of oral hexachlorobenzene exposure in humans.

There have been a few reports of cardiovascular lesions in animals exposed to hexachlorobenzene. Gralla et al. (1977) described an arteriopathy affecting multiple organs in dogs treated with 110 mg/kg/day of hexachlorobenzene for 1 year. The lesion was characterized by inflammation of small arteries and arterioles, with focal proliferative endartitis, fibrinoid necrosis, and thrombosis, and occasionally involved fibrosis and inflammation adjacent to the arterioles in the heart and the liver. Although features of the lesion suggested a hypersensitivity reaction, an immune etiology was not supported by serum electrophoretic data. The arteritis was seen in 4 of 12 beagle dogs treated with 110 mg/kg/day, a dose that produced weight loss, mortality, and other frank toxic effects, but was not seen in dogs treated with lower doses. It is not known if these effects were produced by a direct effect of hexachlorobenzene or were secondary to general poor health of the dogs in this study. However, similar observations in the heart were made by Kimbrough and Linder (1974) in rats. These researchers found fibrosis and degeneration of muscle fibers in the heart of rats receiving hexachlorobenzene from the diet for 4 months at doses ≥50 mg/kg/day. Degenerated tissue was infiltrated by inflammatory cells. Other studies that included

pathological examination of cardiovascular tissues in dogs, rats, and monkeys did not find treatment-related lesions (Goldstein et al. 1978; Iatropoulos et al. 1976; Sundlof et al. 1981).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects of oral hexachlorobenzene exposure in humans.

Gastrointestinal effects have not been commonly reported in animal studies of hexachlorobenzene. Dogs given ≥11 mg/kg/day by capsule for 1 year experienced intermittent episodes of diarrhea (Gralla et al. 1977). Necropsy revealed necrotic and inflammatory lesions of the omentum and abdominal serosa, but there were apparently no findings in the stomach, small intestines, or large intestines. Pathological examination of female pigs exposed to 0.025 or 0.5 mg/kg/day for 212 days throughout mating, gestation, and lactation showed gastrointestinal lesions ranging from catarrhal exudation to mild ulceration, but microscopic signs of gastritis were also found in some control group animals, suggesting that the observed lesions were not an effect of hexachlorobenzene (Hansen et al. 1979). Gastrointestinal lesions were not observed in female Rhesus monkeys given oral hexachlorobenzene at doses of up to 128 mg/kg/day for 60 days (Iatropoulos et al. 1976).

Hematological Effects. No data were located on the hematological effects of hexachlorobenzene in humans.

Limited animal data suggest that hexachlorobenzene can produce anemia and leukocytosis. Decreases in hemoglobin, hematocrit, and/or red blood cell count were reported in rats administered hexachlorobenzene. in the diet for approximately 4 months at concentrations resulting in estimated doses of 10–32 mg/kg/day (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Female rats were much more sensitive than male rats in these studies (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Other studies in rats did not find changes in these parameters, but used lower doses (Arnold et al. 1985), a much shorter exposure period (Lecavalier et al. 1994), or only the less sensitive male rats (Ockner and Schmid 1961). Other reported findings in rats consistent with the hypothesis that hexachlorobenzene can produce anemia were increased extramedullary hematopoiesis in the spleen, which was seen at doses as low as 46 mg/kg/day after dietary exposure for 3 weeks (Vos et al. 1979b), and reduced medullary area in the femur, which was found at doses ≥10 mg/kg/day in a 15-week study (Andrews et al. 1990). Limited data are available regarding hematological effects in other species. Anemia was observed in dogs exposed to 110 mg/kg/day for 1 year (Gralla et al. 1977), but not in dogs exposed to 100 mg/kg/day for only 3 weeks (Sundlof et al. 1981), in rabbits exposed to 161 mg/kg/day for 12 weeks (De Matteis et al.

1961), in pigs exposed to 50 mg/kg/day for 13 weeks (Den Tonkelaar et al. 1978), or in monkeys exposed to 128 mg/kg/day for 60 days (Knauf and Hobson 1979) or 10 mg/kg/day for 90 days (Foster et al. 1995a).

Many of the same studies that reported anemia and related findings also reported neutrophilia and/or leukocytosis. In rats, the white blood cell increases were found at the same or higher doses than the red cell changes (≥32 mg/kg/day in 3–16 week feeding studies), and there appeared to be less of a disparity in response between males and females (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Vos et al. 1979b). In dogs, afebrile neutrophilia was observed at 11 mg/kg/day, while anemia was found only at the high dose of 110 mg/kg/day (Gralla et al. 1977). Negative studies in these species used lower doses (Arnold et al. 1985) or a much shorter exposure period (Lecavalier et al. 1994; Sundlof et al. 1981). Although values remained within normal ranges, Den Tonkelaar et al. (1978) identified a tendency towards leukocytosis and relative neutrophilia in male pigs treated with dietary doses as low as 0.05 mg/kg/day for 90 days. However, studies in monkeys given doses up to 10 mg/kg/day for 90 days (Foster et al. 1995a) or doses up to 128 mg/kg/day for 60 days (Knauf and Hobson 1979) were negative.

Musculoskeletal Effects. Hexachlorobenzene has been associated with painless arthritis (swelling of the joints distinct from rheumatoid arthritis), osteoporosis, and small distinctive hands in patients exposed to the chemical from consumption of bread prepared from contaminated grain. Although there was severe shortening of digits due to osteoporosis in the bones of the hands (phalangeal, carpal, and metacarpal), particularly at the ends, no limitation of movement was reported. Painless arthritic changes were also reported in the patients (Cripps et al. 1984; Peters et al. 1982, 1987).

Effects on both bone and muscle have been reported in animal studies of hexachlorobenzene. Detailed studies of bone effects were conducted by Andrews et al. (1989, 1990) in male rats treated by gavage in corn oil for up to 15 weeks. These researchers found significant, dose-related increases in femur density (osteosclerosis) at doses of 1 mg/kg/day and above, and identified a NOAEL of 0.1 mg/kg/day for this effect. Femur length, weight, volume, and cross-sectional area were not consistently altered, indicating no effect on the rate of bone growth. The other dose-related changes in bone were an increase in cortical area at 1 mg/kg/day and above and a corresponding decrease in medullary area at 10 mg/kg/day and above. Other pertinent findings were decreases in serum alkaline phosphatase and increases in serum 1,25-dihydroxy-vitamin D3 and parathyroid hormone (hyperparathyroidism). The joint findings of osteosclerosis, increased cortical and reduced medullary area without a change in the rate of bone growth, and decreased serum alkaline phosphatase are consistent with a mechanism involving reduced resorption

of bone. The increases in serum 1,25-dihydroxy-vitamin D3 and parathyroid hormone, both of which are involved in calcium regulation and bone resorption, suggest that the hypothesized effect on bone resorption is probably secondary to hyperparathyroidism. The decrease in medullary area (bone marrow cavity) that ultimately results from these changes may, in turn, contribute to hematological and immune effects associated with hexachlorobenzene.

Studies conducted at higher doses that produced marked interference with heme metabolism found substantial accumulation of porphyrins in bone cortex, but not marrow. This was observed in rats ingesting 172 mg hexachlorobenzene/kg/day from the diet for 56 days (Ockner and Schmid 1961) and rabbits ingesting 161 mg/kg/day from the diet for 84 days (De Matteis et al. 1961). No bone (or liver or kidney) accumulation of porphyrins was observed by Andrews et al. (1989), but the highest dose in this study was only 25 mg/kg/day.

Skeletal muscle lesions have been reported in animals exposed to hexachlorobenzene, but only with repeated exposure to high doses. Rabbits ingesting 161 mg hexachlorobenzene/kg/day from the diet for 12 weeks were observed to have necrosis, degeneration, and focal calcification in skeletal muscle (De Matteis et al. 1961). Skeletal muscle lesions were not found in rats receiving up to 32 mg/kg/day from the diet for 15 weeks (Kuiper-Goodman et al. 1977).

Degenerative lesions of maxillary incisors were noted in female Sprague-Dawley rats administered hexachlorobenzene at doses ranging from 1 to 25 mg/kg/day, but not at doses ≤0.3 mg/kg/day (Long et al. 2004). Incidences and severity of the degenerative lesions increased with increasing dose.

Hepatic Effects. The major evidence that oral exposure to hexachlorobenzene by humans can result in hepatopathology is derived from studies of an outbreak of porphyria in Turkey attributed to the consumption of bread prepared from hexachlorobenzene-contaminated grain from 1955 to 1959 (Cam and Nigogosyan 1963; Cripps et al. 1984; Peters et al. 1982, 1987). These adverse hepatic effects were mainly characterized by porphyria. The appearance of abnormal levels of porphyrin precursors in the urine suggests that hexachlorobenzene disturbed the body's porphyrin metabolism in the liver, which caused histopathologic changes in the liver. Uroporphyrin and δ-aminolevulinic acid (d-ALA) synthase increased in the urine, and uroporphyrin and coproporphyrin increased in the stool of patients who had ingested hexachlorobenzene-contaminated bread (Cripps et al. 1984; Peters et al. 1982, 1987). No quantitation of exposure (dose and duration) was presented in any of these clinical reports. However, an

estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963).

Studies in animals have confirmed that the liver is an important target organ for hexachlorobenzene following ingestion. Hepatic effects associated with oral exposure to hexachlorobenzene in animal studies include disruption of heme synthesis (culminating in porphyria), induction of microsomal enzymes, hepatocellular hypertrophy, hepatomegaly, and cellular damage.

Disruption of hepatic heme synthesis by hexachlorobenzene has been well studied in rats (see below). Hexachlorobenzene inhibits the activity of hepatic uroporphyrinogen decarboxylase, an enzyme in the heme biosynthesis pathway, leading to build up of heme precursors (porphyrins) in the liver and other tissues and their excessive excretion in the urine (porphyria). The activity of other enzymes in the heme biosynthesis pathway may also be altered; in particular, an increase in the activity of δ-aminolevulinic acid synthetase has been reported in some studies (see Section 3.5, Mechanisms of Action). This pattern of effects is very similar to what is seen in human porphyria cutanea tarda.

Following acute exposure, the lowest dose reported to induce outright porphyria in an animal study was 25 mg/kg/day in an 8-day study in female rats in which hexachlorobenzene was administered by gavage in corn oil; the rats were monitored for urinary and hepatic porphyrins after 35–50 days (Krishnan et al. 1991). Goldstein et al. (1978) observed a statistically significant increase in hepatic δ-aminolevulinic acid synthestase activity in female rats ingesting ≥16 mg/kg/day of hexachlorobenzene from the diet for 1 week, but there was little or no effect on hepatic porphyrin levels at that time, possibly because of insufficient latency time for the effect to develop. (Time course studies have shown that there may be a delay of approximately 4 weeks between treatment with hexachlorobenzene and development of porphyria [Billi de Catabbi et al. 2000a; Krishnan et al. 1991; Mylchreest and Charbonneau 1997], although there may be little or no delay if the hexachlorobenzene is administered in a form that is readily absorbed [e.g., predissolved in corn oil] at high doses [Kennedy and Wigfield 1990].) There was no increase in hepatic δ-aminolevulinic acid synthetase activity at 5 mg/kg/day in the Goldstein et al. (1978) study. The dose level at which hexachlorobenzene will produce porphyria depends on the exposure protocol. When hexachlorobenzene was administered to female rats by gavage for 7 days in an aqueous suspension rather than oil, no effect on hepatic uroporphyrinogen decarboxylase (monitored at 7 days) was observed even at 250 mg/kg/day, and only doses of 500 mg/kg/day or higher were effective (Kleiman de Pisarev et al. 1990), reflecting the fact that hexachlorobenzene administered in water is only minimally absorbed from the gastrointestinal tract, and also perhaps, the short latency time. Even at

1,000 mg/kg/day in this study, uroporphyrinogen decarboxylase activity was decreased only 25%, while δ-aminolevulinic acid synthetase activity and liver porphyrin levels were unchanged from controls. Billi de Catabbi et al. (2000a) observed that acute (5-day) exposure above a threshold (1 g/kg) caused porphyria lasting at least as long as the 20-week observation period.

Hexachlorobenzene doses as low as 5–51 mg/kg/day have been reported to produce porphyrinogenic effects, such as increased liver weight, inhibition of hepatic uroporphyrinogen decarboxylase, accumulation of porphyrins in liver, excretion of porphyrins in urine, and increased hepatic δ-aminolevulinic acid synthetase activity, in female rats exposed for intermediate durations (Den Besten et al. 1993; Goldstein et al. 1978; Kennedy and Wigfield 1990; Kuiper-Goodman et al. 1977; Michielsen et al. 2001, 2002; Smith et al. 1979, 1985; Wolfe and Pepperl 2005). One of these studies, Goldstein et al. (1978), identified a NOAEL of 4 mg/kg/day for increases in liver porphyrins and δ-aminolevulinic acid synthestase activity after a 4-month dietary exposure. In male rats, there was little or no evidence of porphyria at doses up to 25 mg/kg/day (Andrews et al. 1989; Kuiper-Goodman et al. 1977; Smith et al. 1985), but mild changes were noted at 32–50 mg/kg/day (Krishnan et al. 1991; Kuiper-Goodman et al. 1977) and severe porphyria was observed at 172 mg/kg/day (Ockner and Schmid 1961). The reduced sensitivity of male rats in comparison to females may be related to differences in metabolism of hexachlorobenzene between the sexes in this species, particularly with regard to glutathione conjugation (D'Amour and Charbonneau 1992; Richter et al. 1981; Rizzardini and Smith 1982), which have been linked to the presence of estradiol in the females (Legault et al. 1997). Among female rats, strain-related differences in sensitivity have also been reported (Billi de Catabbi et al. 2000a; Michielsen et al. 1997), possibly related, at least in one case, to differences in nonheme iron content of the liver between strains (Smith et al. 1979). In other species, there was no evidence of porphyria in female monkeys treated with up to 10 mg/kg/day by capsule for 3 months (Jarrell et al. 1993) or in male pigs receiving up to 50 mg/kg/day from the diet for 3 months (Den Tonkelaar et al. 1978); these results appear to reflect species differences, but may be influenced by the lack of oil vehicle to enhance absorption.

In studies of chronic exposure duration, hexachlorobenzene doses of 7–18 mg/kg/day from the feed produced complete inhibition of uroporphyrinogen decarboxylase and high levels of porphyrins in the liver and urine in both male and female rats (Smith and Cabral 1980; Smith et al. 1985, 1993). Although uroporphyrinogen decarboxylase activity was completely inhibited in both sexes, liver accumulation of porphyrins was 5-fold higher in females than in males (Smith et al. 1985). Chronic rat studies that employed lower dose levels did not monitor porphyrin levels (Arnold et al. 1985; Mollenhauer et al. 1975). Male mice ingesting slightly higher doses of hexachlorobenzene (17 mg/kg/day) showed only a

modest transitory increase in hepatic porphyrin levels after 6 months of treatment, which was not found at subsequent sacrifices at 12 and 18 months (Smith et al. 1989). There was no evidence of porphyrin accumulation in the liver or other tissues of dogs treated with hexachlorobenzene doses as high as 110 mg/kg/day for 1 year (Gralla et al. 1977).

As in human porphyria cutanea tarda, porphyria in rats produced by hexachlorobenzene typically occurs along with other effects on the liver, such as induction of microsomal enzymes, increased liver weight, hepatocellular hypertrophy, cytoplasmic vacuolation, fatty degeneration, and biliary hyperplasia (Cuomo et al. 1991; Den Besten et al. 1993; Kimbrough and Linder 1974; Koss et al. 1978; Kuiper-Goodman et al. 1977; Michielsen et al. 1997, 2001, 2002; Smith et al. 1985, 1993; Sweeney et al. 1986; Vos et al. 1979b). The relationship between porphyria and these other hepatic effects is uncertain. In some instances, effects on liver weight, enzymes, and/or histopathology occurred in rats at lower doses than porphyria or in the absence of porphyria (e.g., Arnold et al. 1985; Gustafson et al. 2000; Kishima et al. 2000; Mehendale et al. 1975; Mollenhauer et al. 1975; Michielsen et al. 2000). Liver lesions have also been observed in species, such as monkeys, dogs, and pigs, where there is no evidence of a porphyrinogenic effect (Den Tonkelaar et al. 1978; Gralla et al. 1977; Iatropoulos et al. 1976; Jarrell et al. 1993). Kishima et al. (2000) reported that the hepatotoxicity of hexachlorobenzene was increased by an energy-restricted diet. NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.001$) increased incidences of hepatocellular hypertrophy were noted at hexachlorobenzene doses ≥10 mg/kg/day (incidences of 0/10, 0/10, 0/10, 0/10, 0/10, 0/10, 9/10, and 10/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively). The most sensitive hepatic end points following acute, intermediate, and chronic exposure, respectively, were increased liver weight and induction of microsomal enzymes in male rats exposed to 10 mg/kg/day for 6 days (Mehendale et al. 1975), hepatocellular hypertrophy in male pigs exposed to 0.5 mg/kg/day for 90 days (Den Tonkelaar et al. 1978), and peribiliary lymphocytosis and fibrosis in F_1 adult male rats exposed to hexachlorobenzene via their mothers during gestation and lactation followed by direct ingestion of hexachlorobenzene at 0.022 mg/kg/day from the diet for their postweaning lifetime (Arnold et al. 1985; listed as a developmental effect in Table 3-2 and the basis for the chronic-duration oral MRL of 0.00007 mg/kg/day, as described in the footnote to Table 3-2 and in Appendix A).

Renal Effects. No studies were found regarding renal effects in humans following oral exposure to hexachlorobenzene.

Animal studies have demonstrated that the kidney is a target for hexachlorobenzene. Renal effects that have been widely reported in animal studies are increased kidney weight, accumulation of porphyrins in association with disruption of heme metabolism (as in the liver), and direct and indirect evidence of renal tissue damage. Increases in kidney weight have been observed in many studies, primarily those involving ≥7 weeks of exposure (Andrews et al. 1989; Bouthillier et al. 1991; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Kimbrough and Linder 1974; Koss et al. 1978; Kuiper-Goodman et al. 1977; Smith et al. 1985; Wolfe and Pepperl 2005). Most studies shorter than 7 weeks in duration did not find increases in kidney weight, even using doses as high as 100 mg/kg/day (Andrews et al. 1988; Richter et al. 1981; Sundlof et al. 1981; Vos et al. 1979b). This includes interim sacrifices in longer duration studies that did eventually show increases in kidney weight (Andrews et al. 1989). However, Bouthillier et al. (1991) observed increased kidney weight in male Sprague-Dawley rats administered hexachlorobenzene by gavage at 100 mg/kg/day, 5 days/week for 2 weeks. Multiple-dose feeding studies of 12–16 weeks identified LOAEL values of 19–56.5 mg/kg/day and NOAEL values of 5–11.3 mg/kg/day for increased kidney weight in male and female rats (Den Besten et al. 1993; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Increased kidney weight was observed at doses much lower than 100 mg/kg/day in pigs treated for 90 days (Den Tonkelaar et al. 1978), rats administered hexachlorobenzene by gavage in oil rather than in the diet (Andrews et al. 1989, 1990), and rats receiving hexachlorobenzene from the diet for 1 year instead of 12–16 weeks (Smith et al. 1985). The lowest LOAEL and NOAEL for increased kidney weight in any study were 5 and 0.5 mg/kg/day, respectively, for hexachlorobenzene-treated pigs (Den Tonkelaar et al. 1978). It has been proposed that increased kidney weight in animals exposed to hexachlorobenzene may result from induction of renal microsomal enzymes (Bouthillier et al. 1991). Renal pathology (described below) may have been a contributing factor in some studies.

Among the available studies, the most sensitive indication of renal pathology due to hexachlorobenzene was increased urine enzyme levels. Male rats treated with ≥1 mg/kg/day of hexachlorobenzene by gavage in oil for 15 weeks had increased concentrations of alkaline phosphatase and/or lactate dehydrogenase in the urine, an effect that was not found at 0.1 mg/kg/day (Andrews et al. 1989, 1990). These effect levels correspond with those for changes in kidney weight in the same studies. Increased urinary levels of these enzymes suggest the occurrence of either glomerular damage allowing the enzymes to leak into the urine from the serum or tubular cell damage where the enzymes are released directly from the damaged cells into the urine. There was an apparent increase in calcium excretion in a higher dose group that could be interpreted to indicate impaired reabsorption of calcium by the distal tubules, which would support the hypothesis that damaged tubular cells were responsible for the observed enzymuria.

Studies that included histopathological examination of the kidneys provided more direct evidence of damage to renal tubule cells. Bouthillier et al. (1991) observed degenerative and regenerative foci of epithelial cells in the proximal tubules and accumulation of protein droplets in proximal tubular cells in male rats treated with 50 mg/kg/day for 7 weeks or 100 mg/kg/day for 2 weeks. These lesions were accompanied by glucosuria, proteinuria, and an 11-fold increase in $\alpha_{2\mu}$-globulin levels. The nature of the effects in males and near-absence of effects in females (just glucosuria and an increase in urinary γ-glutamyl transpeptidase were found) led the researchers to suggest that hexachlorobenzene produces a male rat specific protein droplet nephropathy, as is seen with some other chlorinated benzenes. Support for a gender-specific nephrotoxic effect in rats includes findings of mild to severe nephrosis in 12/12 surviving male rats ingesting 15.8 mg hexachlorobenzene/kg/day from the diet for 90 weeks, but 0/10 surviving male controls and only 1/10 surviving treated females (Smith et al. 1985), and significantly increased incidences of severe chronic nephrosis in male (but not female) rats exposed to hexachlorobenzene via their mothers during gestation and lactation followed by direct ingestion of hexachlorobenzene from their diet for a lifetime at a concentration resulting in an estimated dose of 2.8 mg/kg/day (Arnold et al. 1985).

Renal lesions, however, have been reported in female rats treated with hexachlorobenzene. Basophilic renal tubules and protein casts were seen in female rats exposed to 19 mg/kg/day for 13 weeks (Den Besten et al. 1993). Renal accumulation of porphyrins has been reported in female rats receiving hexachlorobenzene from the diet at doses as low as 12.9 mg/kg/day for 56 days (Kennedy and Wigfield 1990). Renal effects have also been noted in other animal species. Female monkeys treated with ≥8 mg/kg/day for 60 days developed vacuolization of proximal tubules, and at 128 mg/kg/day, thickening of the basement membranes, glomerular hyperemia, and increased blood urea nitrogen (BUN) were also found (Iatropoulos et al. 1976; Knauf and Hobson 1979). Male pigs exposed to 50 mg/kg/day in the diet all died prior to scheduled sacrifice and were found upon necropsy to have degeneration of the proximal tubules and loop of Henle (Den Tonkelaar et al. 1978). Blood ammonia levels were increased 3-fold in female guinea pigs receiving hexachlorobenzene from the diet at 385 mg/kg/day (De Matteis et al. 1961). There is also a brief paper by Ertürk et al. (1986), lacking detail regarding experimental methods or results, that reports marked renal lesions, including severe hyperemia, necrotic tubular degeneration, hemorrhage, and nephritis in male and female rats, mice, and hamsters given hexachlorobenzene in the diet for 90 days at doses as low as 17.2 mg/kg/day (although the effects were most severe in male rats, they were seen in all groups). The occurrence of renal effects in female rats and in other species of laboratory animal shows that the nephrotoxic effects of hexachlorobenzene are not limited to the $\alpha_{2\mu}$-

globulin nephropathy demonstrated by Bouthillier et al. (1991), and suggests that this chemical may produce renal effects by multiple mechanisms.

Renal accumulation of porphyrins has been related to disruption of heme metabolism and lipid peroxidation. Female rats treated with 1,000 mg/kg/day of hexachlorobenzene by gavage in aqueous Tween 20 had a significant decrease in uroporphyrinogen decarboxylase (URO-D) activity in the renal cortex after 3 weeks of exposure and a subsequent increase in porphyrin levels in the renal cortex, but not the renal medulla or papilla, after 4 weeks of exposure (Fernandez-Tome et al. 2000). Lipid peroxidation, indicated by measurement of conjugated dienes and malondialdehyde (MDA), was significantly increased throughout most of the exposure period in the renal cortex, but was not increased at all in the renal medulla or papilla. Based on these findings, the researchers suggested that disruption of heme metabolism and accumulation of porphyrins in the renal cortex are secondary to lipid peroxidation produced by hexachlorobenzene in this tissue. Distribution of porphyria in kidney is consistent with fact that enzymes of heme metabolism are localized in the renal cortex; the occurrence of lipid peroxidation in the renal cortex is consistent with its relative susceptibility to oxidative stress, compared to the papilla or medulla. The use of such a high dose in this study reflects the fact that hexachlorobenzene is not well absorbed from water. Renal accumulation of porphyrins was observed at doses as low as 13–23 mg/kg/day in feeding studies in female rats (Kennedy and Wigfield 1990; Smith et al. 1985), particularly when hexachlorobenzene was added to the diet in an oil vehicle (Kennedy and Wigfield 1990). No experimental evidence was found for renal accumulation of porphyrins in male rats receiving hexachlorobenzene from the diet or by gavage in oil at doses up to 25 mg/kg/day for 15 weeks (Andrews et al. 1989; Smith et al. 1985).

Despite the numerous data supporting an effect of hexachlorobenzene on the kidney, it should be noted that several well-conducted investigations of kidney histopathology failed to find any treatment-related lesions in either male or female rats, even with high exposures (up to 50–100 mg/kg/day for 4 months) that, based on the database as a whole, would have been expected to produce tissue damage (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Michielsen et al. 1997; Vos et al. 1979b).

Endocrine Effects. Human data suggest that hexachlorobenzene adversely affects the endocrine system; the thyroid is a target organ. Two follow-up studies conducted 25 (Peters et al. 1982) and 20–30 years (Cripps et al. 1984) after patients (n=161–225) were exposed as children to bread contaminated with hexachlorobenzene in Southeast Turkey detected thyromegaly in 60% of women and 25% of men (Cripps et al. 1984; Peters et al. 1982). The background incidence for this area was 5%. Additionally,

hirsutism and small stature were observed in 47 and 44%, respectively, of the study population. However, a study of serum hormone and organochlorine levels in a cohort of 110 Swedish or Latvian men who consumed Baltic sea fish (Hagmar et al. 2001) found no age-adjusted correlation between hexachlorobenzene levels and any of the measured serum hormones (follicle-stimulating hormone, luteinizing hormone, prolactin, plasma thyrotropin, free and total T3, free and total T4, and free testosterone). The authors considered the fish to be a diet high in persistent organohalogens, but did not quantitate intake or report on hexachlorobenzene levels in fat, which would likely have been a more relevant measure of long-term exposure.

Several more recent studies focused on possible associations between serum hexachlorobenzene and circulating thyroid hormone levels. It should be noted that other organochlorine compounds and residues were detected in test samples as well. Among 342 adult men who were partners of, and/or patients at, an infertility clinic in Boston, Massachusetts, a statistically significant ($p<0.05$) decline in total T3 levels was associated with an interquartile range increase in serum hexachlorobenzene levels (Meeker et al. 2007).

In a population-based survey that included 193 children (mean age 6.5 years) living in an area of Brazil where high levels of organochlorine pesticides were present in soil, water, and local food, a significant trend ($p<0.01$) was observed for increasing serum T3 with increasing serum hexachlorobenzene levels; no significant association was found between serum T4 or TSH and serum hexachlorobenzene (Freire et al. 2012).

In a population-based survey of 303 men and 305 women living in the same areas of Brazil, a significant ($p<0.05$) positive association was noted between serum hexachlorobenzene levels and free T4 among the women (but not the men); no significant association was observed between serum total T3, TSH, or anti-thyroperoxidase and serum hexachlorobenzene level among men or women (Freire et al. 2013).

Within a group of 105 pregnant women in Korea who supplied serum samples at delivery, a slight, but significant ($p<0.05$) negative association was found between serum free T4 and serum hexachlorobenzene levels; there was no significant association between serum hexachlorobenzene and serum free T3, total T3, total T4, or TSH (Kim et al. 2013).

Significant negative associations were reported between plasma hexachlorobenzene concentrations and total free T4 in groups of 623 Inuit adults (Dallaire et al. 2009a) and 149 pregnant women in a region of southwest Quebec, Canada (Takser et al. 2005), and cord blood hexachlorobenzene and free T3 and T4

from 198 births among subjects in the Netherlands (Maervoet et al. 2007). Croes et al. (2014a, 2014b) reported a significant (p=0.02) positive association between serum hexachlorobenzene and serum TSH (OR 1.03; 95% CI 1.01, 1.05) in a group of 606 adolescents in the Flemish Environment and Health Study (FLEHS II).

Within a group of 334 pregnant women from Salinas Valley, California, serum hexachlorobenzene was not significantly associated with T4 levels after adjusting for serum PCBs (Chevrier et al. 2008). In studies of Akwesasne Mohawk subjects, no significant associations were found between serum hexachlorobenzene and T4 levels among children <17 years of age (Schell et al. 2004, 2008) or levels of anti-thyroid peroxidase antibodies in adults aged 17–20 years (Schell et al. 2009). Among 410 neonates from remote coastal regions in Quebec, Canada, cord blood hexachlorobenzene levels did not adversely affect cord blood thyroid hormone levels (Dallaire et al. 2008). No significant associations were found between hexachlorobenzene and thyroid hormone levels in serum from 204 pregnant Inuit women, cord blood from 108 newborns, or serum from 175 infants 7 months of age (Dallaire et al. 2009b).

In a large-scale study of 2,046 male and female adults (age 20–75 years) from a polluted town in East Slovakia located near a former PCB-producing chemical factory, and two control populations located in adjacent towns, statistically significant positive correlations were found between elevated hexachlorobenzene levels and thyroid volume ($p<0.01$), TSH levels ($p<0.001$), and thyroperoxidase antibody levels ($p<0.01$) (Langer et al. 2007). An earlier study using smaller numbers of subjects reported similar results (Langer et al. 2003). However, elevated levels of PCBs were also found in the subjects living in the polluted area and a determination could not be made as to whether the thyroid effects were due to hexachlorobenzene exposure, PCB exposure, or both.

Animal studies have demonstrated that hexachlorobenzene has multiple endocrine effects; the most striking are the induction of hypothyroidism and hyperparathyroidism in rats. Limited evidence suggests that hexachlorobenzene also affects serum retinoid levels, the adrenal gland and serum levels of corticosterone and cortisol (see below). Moreover, studies have shown that hexachlorobenzene affects serum levels of estrogen and progesterone (discussed in Section 3.2.2.5, Reproductive Effects).

Multiple studies have demonstrated that serum T4 levels decrease rapidly in rats following gavage treatment with hexachlorobenzene (Den Tonkelaar et al. 1978; Foster et al. 1993; Kleiman de Pisarev et al. 1989, 1990, 1995; Smith et al. 1986b; Sopena de Kracoff et al. 1994; van Raaij et al. 1993a, 1993b). Effects on serum TSH levels (both increases and decreases) are delayed and appear secondary to

decreases in T4. The most sensitive acute study observed statistically significant decreases in T4 levels in female rats at doses as low as 50 mg/kg/day for 5 days (Foster et al. 1993). A time-course in female Wistar rats gavaged with 1,000 mg/kg/day of hexachlorobenzene in corn oil found that serum T4 levels rapidly decreased, reaching a steady-state after 8 days, approximately 75% below controls. In contrast, serum TSH levels reached a steady state after 30 days, at 80% below controls (Sopena de Kracoff et al. 1994). Similarly, experiments in female Wistar rats gavaged with 1,000 mg/kg/day for at least 8 weeks observed significantly decreased serum T4 and protein-bound iodine and elevated TSH levels and thyroid weight (Kleiman de Pisarev et al. 1989, 1990, 1995). Hadjab et al. (2004) reported significantly decreased plasma total T4 levels in male Sprague-Dawley rats (as compared to pre-exposure levels) exposed to hexachlorobenzene at gavage doses of 4 or 16 mg/kg/day for 4 weeks; no alterations in plasma T4 were observed at a dose level of 0.16 mg/kg/day.

Smith et al. (1987) reported significantly enlarged thyroid glands and depressed serum T3 (but not T4) in hamsters receiving hexachlorobenzene from the diet at 47.4 mg/kg/day for 6 weeks or 19.0 mg/kg/day for 18 weeks or 28 weeks; serum T3 levels were as much as 3-fold lower than that of controls. Thyroid weight increased significantly, and correlated with histopathological observations of large and irregularly shaped follicles in the thyroid. Decreased T3 and T4 levels were observed in female Wistar rats ingesting hexachlorobenzene from the feed at doses up to 19.0 mg/kg/day for 13 weeks (Den Besten et al. (1993), and significantly increased thyroid weight was noted in male Landvarken pigs fed 5 mg/kg/day of hexachlorobenzene for 12 weeks (Den Tonkelaar et al. 1978); these effects were observed in the absence of histopathological signs of thyroid lesions.

Chalouati et al. (2013) reported significantly decreased plasma free and total T4 levels and significantly increased plasma TSH in male Wistar rats administered hexachlorobenzene by gavage (in olive oil) at 4 or 16 mg/kg/day for 28 days; in addition, the high-dose group exhibited significantly decreased plasma free T3 and significantly increased mean relative thyroid gland weight (40% greater than that of controls). The study authors reported histological changes in the thyroid glands from hexachlorobenzene-treated rats (closed follicles and increased number of follicles at 4 mg/kg/day and hyperplasia and hypertrophy of follicular cells and appreciable loss of colloid mass at 16 mg/kg/day); however, incidence data were not presented in the study report. In a time-course portion of the study, hexachlorobenzene-treated rats exhibited significantly decreased plasma free and total T4 and free T3 and significantly increased plasma TSH at days 21 and 28, but no significant differences from controls were observed at day 14. In a recovery portion of the study, decreased plasma free and total T4 and T3 levels, increased plasma TSH,

and increased mean relative thyroid weight that were observed following 28 days of hexachlorobenzene dosing at 16 mg/kg/day persisted after 28 days of vehicle-only treatment.

Increased hepatic metabolism and hepatic excretion (into bile) appear to be important to the mode-of-action for the thyroid effects of hexachlorobenzene. Kleiman de Pisarev et al. (1989, 1990, 1995) observed that gavage administration of 1,000 mg/kg/day of hexachlorobenzene in corn oil to female Wistar rats for 28–30 days not only significantly decreased serum T4 levels, but also increased both the metabolism (deiodination) and fecal excretion of T4. In male Wistar (WAG/MBL) rats orally dosed with 120 mg/kg/day of hexachlorobenzene 3 times/week for 4 weeks, levels of T4 glucuronide increased but levels of serum T4 and nonconjugated T4 decreased (van Raaij et al. 1993b). Hepatic T4 UDP-glucuronyltransferase (UDPGT) activity was increased while T4 iodothyronine deiodinase activity was decreased. Bile flow and T4 excretion were increased. However, serum T3 levels were unaffected. Taken together, these data indicate that hexachlorobenzene induces liver activity that results in decreased serum levels of T4. Treatment of female Sprague-Dawley rats with 50 mg/kg/day of hexachlorobenzene by gavage for 5 days significantly decreased serum T4 levels and free thyroxine index without affecting T3 uptake (Foster et al. 1993). However, following gonadotropin pretreatment to induce superovulation, 3-day treatment with 50 mg/kg/day of hexachlorobenzene significantly decreased both serum T4 levels and serum T3 uptake. The authors concluded that the acute induction of hypothyroidism was augmented by ovulation (Foster et al. 1993). Gavage treatment of inbred male Wistar (WAG/RIJ) rats with 27 mg/kg/day pentachlorophenol was more effective than 780 mg/kg/day of hexachlorobenzene at reducing free and total T4 levels (van Raaij et al. 1993a). The authors concluded that metabolites of hexachlorobenzene, rather than hexachlorobenzene itself, may be involved in the reduction of serum thyroid hormones.

Andrews et al. (1988, 1989, 1990) conducted detailed studies into hexachlorobenzene-induced hyperparathyroidism and osteoporosis in male Fischer 344 rats. Gavage treatment of rats with doses as low as 1.0 mg/kg for 5 weeks, 5 days/week, significantly increased serum levels of vitamin D_3 and urinary levels of phosphorous. Treatment with at least 10 mg/kg significantly increased serum levels of PTH, increased urinary levels of calcium, and decreased serum levels of alkaline phosphatase (an enzyme important in bone mineralization). The combination of high PTH and vitamin D_3 is expected to cause calcium resorption from bone and calcium conservation by the kidneys, and this is consistent with the adverse skeletal effects seen by authors at 1.0 mg/kg (see Section 3.2.2.2, Musculoskeletal Effects).

Animal studies show that hexachlorobenzene affects the adrenal gland (weight, histopathology, and hormone levels). Hyperplasia of the adrenal cortex was observed in male and female Sherman rats receiving hexachlorobenzene from the diet at concentrations resulting in estimated doses ≥10 mg/kg/day; adrenal gland weight was significantly increased at doses ≥50 mg/kg/day for 4 months (Kimbrough and Linder 1974). Adrenal gland weight was also significantly increased in Wistar rat pups exposed to hexachlorobenzene via their mothers at a maternal dietary concentration resulting in an estimated dose of 25.6 mg/kg/day during gestation and lactation and for 2 weeks postweaning directly from the diet (Vos et al. 1979a). In female Wistar rats receiving hexachlorobenzene from the diet at 9.5 or 19.0 mg/kg/day for 13 weeks (Den Besten et al. 1993), adrenal gland weight (69%) was significantly increased in the high-dose group. Histopathology (reported only for the high-dose group) revealed adrenal cortex hypertrophy, hyperplasia, occasional hemorrhaging, cortical cell vacuolation, and inflammatory cell infiltrates. In female ovariectomized Sprague-Dawley rats gavaged for 30 days with 1, 10, or 100 mg/kg/day of hexachlorobenzene, corticosterone was decreased at all doses, but serum cortisol levels were only decreased by 100 mg/kg/day (Foster et al. 1995a). Moreover, no effect was seen on serum progesterone and aldosterone levels or adrenal weight. Koss et al. (1978) detected statistically significant increases in relative adrenal weight (up to 43%) in female Wistar rats that were treated with 50 mg/kg hexachlorobenzene every other day by gavage for 9–15 weeks; this effect had reversed after a 38-week posttreatment period. In another experiment, single adult female monkeys received 0, 8, 32, or 64 mg/kg and two monkeys received 128 mg/kg of hexachlorobenzene by gavage in methylcellulose daily for 60 days (Iatropoulos et al. 1976). One of the monkeys given 128 mg/kg exhibited moderate adrenal medullary hyperplasia and the monkey given 64 mg/kg/day exhibited slight hyperplasia of the adrenal zona fasciculata; however, these findings are not conclusive due to the small numbers of animals used.

Limited animal evidence suggests that hexachlorobenzene affects retinoid levels. In blood samples taken from 101 polar bears from Svalbard, Norway, statistically significant correlations were observed between higher blood levels of hexachlorobenzene and both lower levels of retinol and a lower ratio of total T4 to free T3 (Skaare et al. 2001). In female Wistar rats receiving hexachlorobenzene from the diet at 19 (but not 9.5) mg/kg/day for 13 weeks (Den Besten et al. 1993), significant increases were seen in adrenal gland weight (69%) and both liver retinol and retinyl palmitate levels. Plasma retinol levels were not affected at 1 week, but were significantly increased at 13 weeks by the high dose.

Dermal Effects. Studies of humans exposed to hexachlorobenzene in bread prepared from contaminated grain in Turkey demonstrated that hexachlorobenzene can produce skin lesions following oral exposure. It is well known that ingestion of hexachlorobenzene can produce porphyria (see

Section 3.2.2.2, Hepatic Effects). The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the generation of porphyrins. One of the most serious symptoms of porphyria is photosensitivity; porphyrins accumulated in the skin absorb radiation (maximally at 400–410 nm) and then generate reactive oxygen species, causing tissue damage (Lim and Cohen 1999; Meola and Lim 1993; Sandberg et al. 1982). Skin lesions occur most commonly on areas exposed to sunlight, such as the hands and face. Porphyria cutanea tarda, a specific type of vesiculo-bullous porphyria, was widespread in southeast Anatolia, Turkey in the late 1950s (approximately 1955–1959). The disease was traced to ingestion of bread made from seed grain that had been treated with hexachlorobenzene as a fungicide (Cam and Nigogosyan 1963). The ingested dose of hexachlorobenzene by exposed persons was estimated to be in the range of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) during the episode (Cam and Nigogosyan 1963). Symptoms of what was called *kara yara* or "black sore" appeared after approximately 6 months of exposure (Gocmen et al. 1989). The disease was observed most frequently in children between the ages of 6 and 15 years, although some younger children and adults were also affected (Cam and Nigogosyan 1963; Dogramaci 1964). The initial lesions resembled comedones (blackhead acne) and milia (small whitish epidermal cysts caused by hair follicle and sweat gland obstruction) with photosensitivity and the development of erythema on sun-exposed areas; moreover, the skin was sensitive to minor trauma. These lesions progressed to include large bullous lesions that ulcerated and healed leaving severe mutilating scars, hyperpigmentation that was most prominent on exposed areas but usually affected the entire skin, and hypertrichosis (hirsutism) that occurred principally on the forehead, cheeks, arms, and legs but occasionally involved the whole body (Gocmen et al. 1989). Infants who had been breast fed by mothers who had ingested the contaminated bread displayed a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (annular erythema) (Peters et al. 1966, 1982). The medical history of people who were exposed to hexachlorobenzene in the Turkish poisoning episode was followed for up to 30 years (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). A total of 252 patients who had become porphyric during the Turkish epidemic were studied over a 10-year period from 1977 to 1987 (Gocmen et al. 1989). Subjects had an average age of 7.6 years at onset of symptoms and an average age at follow-up of 35.7 years. Thirty years after onset, clinical findings persisted in most subjects, including severe scarring (83.7%), hyperpigmentation (65%), hypertrichosis (44.8%), pinched face appearance with perioral scarring (40.1%), and fragile skin (33.7%). Dermal lesions were more prominent in sun-exposed areas of the skin.

Studies into the mode of action of porphyria (unrelated to hexachlorobenzene-exposure) have suggested that the dermal toxicity of porphyrins is exacerbated by involvement of the immune system; following

irradiation, porphyrins may activate the complement system and stimulate mast cells and neutrophils to damage nearby tissues (Lim and Cohen 1999; Meola and Lim 1993). Additionally, an increased risk of porphyria cutanea tarda has been associated with human immunodeficiency virus (HIV) infection (Drobacheff et al. 1998; Egger et al. 2002).

Although several animal studies have demonstrated that oral exposure to hexachlorobenzene (for at least 4 weeks) results in dermal lesions and causes immunological effects with dermal lesions, none established a causal relationship (Koss et al. 1978; Michielsen et al. 1997, 2000; NTP 2002; Schielen et al. 1995a; Torinuki et al. 1981). Additionally, it is unclear whether porphyrin-induced phototoxicity occurs in rats; the combination of hexachlorobenzene and sunlight exposure induced dermal lesions in rats similar to those reported in people (Torinuki et al. 1981). However, hexachlorobenzene-treated rats have exhibited skin lesions without detectable dermal porphyrin accumulation (assayed with fluorescence microscopy) (Michielesen et al. 1997). Dermal lesions in rats following hexachlorobenzene exposure have been seen most frequently on the head, neck, and shoulders (similar to humans) although the rats' exposure to sunlight was presumably limited by experimental design and body fur (Koss et al. 1978; Michielsen et al. 1997, 2000). These data seem to suggest that additional modes-of-action are important.

Dermal effects were noted in a study of female Wistar rats that were gavaged with 50 mg/kg hexachlorobenzene in olive oil every other day for 9–15 weeks, followed by a 38-week observation period (Koss et al. 1978). The fur had a roughened appearance during treatment, and round, ulcerous lesions on the head, ears, throat, and shoulders, with diameters of 2–20 mm, were observed in 10% of treated animals after 4 weeks and 50% of animals after 9 weeks. These lesions resolved 12–16 weeks after discontinuation of treatments. At 15 weeks only, spleen weight was significantly increased. A subsequent study, using lower doses and more sensitive end points, observed strain specificity in rats fed hexachlorobenzene for 4 weeks (Michielsen et al. 1997). Brown Norway and Lewis rats received estimated doses of 17 or 51 mg/kg/day from the diet, while Wistar rats received estimated doses of 46 or 92 mg/kg/day from the diet. Dose-related increased incidence of macroscopic skin lesions were noted in all dose groups of each mouse strain compared to strain-specific controls. In Brown Norway rats, skin lesions were very severe, and their incidence was correlated with signs of immunomodulation (increased IgG, IgE, and IgM levels, spleen weight, and lung inflammation). In Lewis rats, skin lesions were moderate, and correlated less strongly with evidence of immune effects. In Wistar rats, skin lesions and immune effects were considered minor. Grossly, the lesions were found in the head and neck region and ranged from redness only to large exudating sores with crusts. Histopathology of both lesional and non-lesional skin observed epidermal hyperplasia, deep dermal venules with activated endothelial cells, and deep inflammatory cell

infiltrates. A subsequent experiment (Michielsen et al. 2000) verified that a dose of 50.8 mg/kg/day from the feed of female Brown Norway rats for 4 weeks induced skin lesions in the head and neck areas, the severity of which increased with time. Likewise, female Wistar rats receiving hexachlorobenzene from the diet at 15 or 30 mg/kg/day for 13 weeks exhibited increased incidence of wounds appearing by weeks 5–7 on the face, neck, shoulders, and behind the ears (Schielen et al. 1995a). The incidence (but not severity) increased with increasing dose. Treatment also induced porphyria and increased serum levels of IgM, IgA, and autoantigen-specific IgM. Torinuki et al. (1981) treated rats for 2 months with hexachlorobenzene with repeated exposure to sunlight. In addition to porphyria, gross pathology of the skin observed erythema, erosion, crusting, skin thickening, and scarring. Histopathology found acanthosis (abnormal dermal thickening), vacuolization of Malpighian cells, subepidermal vesicles, blood vessel dilation, and perivascular cell infiltration of lymphocytes, histiocytes, and mast cells. Hexachlorobenzene-induced skin lesions of the head and neck have been reported in other rat studies as well (Ezendam et al. 2004a; Michielsen et al. 2001, 2002). NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.001$) increased incidences of chronic pulmonary inflammation were noted at the highest dose level (incidences of 0/10, 0/10, 0/9, 0/10, 0/10, 0/9, 0/10, and 9/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively). Ulcerated skin was observed only among 7 of the 10 high-dose rats. In addition to lesions, hexachlorobenzene has induced dermal effects that are not clearly toxic. In female Wistar rats, skin cytochrome P450-dependent 7-ethoxyresorufin-O-deethylase was induced after 60 or 70 days (but not 10 days) of hexachlorobenzene ingestion at 50 mg/kg/day (Goerz et al. 1978, 1994). No dermal lesions were observed in female Agus or Wistar rats ingesting 7 or 5 mg/kg/day of hexachlorobenzene, respectively, from the diet for 90 weeks, although treated animals “possessed less hair than controls” (Smith and Cabral 1980).

Ocular Effects. No studies were found regarding adverse ocular effects in humans following oral exposure to hexachlorobenzene.

Only one relevant animal study was identified. In adult female Rhesus monkeys treated by gavage with hexachlorobenzene in methylcellulose at doses up to 128 mg/kg/day for 60 days, gross pathology and histopathology of the eyes did not detect any adverse effects (Iatropoulos et al. 1976).

Body Weight Effects. No studies were located regarding body weight effects in humans following oral exposure to hexachlorobenzene.

Decreases in body weight following oral exposure of animals to hexachlorobenzene were not observed following acute exposure and were observed in intermediate- and chronic-duration experiments only in the presence of other adverse effects such as mortality, clinical evidence of weakness or lethargy, increased incidences of tumors, or liver toxicity (see below and Section 3.3.2). The available data suggest that body weight loss may be secondary to organ-specific toxicity.

Body weight was not affected in rats or mice orally exposed to hexachlorobenzene for acute durations at doses ranging from 25 to 1,000 mg/kg/day (Courtney et al. 1976; Goldstein et al. 1978; Kleiman de Pisarev et al. 1995; Lecavalier et al. 1994; Mehendale et al. 1975; Michielsen et al. 2001, 2002; Simon et al. 1979; van Raaji et al. 1993a). Similarly, many intermediate-duration experiments did not detect significant changes in body weight in Wistar rats receiving hexachlorobenzene by gavage or from the diet at up to 1,000 mg/kg/day for 4 weeks (Alvarez et al. 2000; Kleiman de Pisarev et al. 1995; Michielsen et al. 1997; Schielen et al. 1993, 1995b; Vos et al. 1979b), in Brown Norway rats receiving approximately 51 mg/kg/day for 3–4 weeks (Ezendam et al. 2004a; Michielsen et al. 1997, 2000), in ovariectomized adult female Sprague-Dawley rats gavaged with doses up to 100 mg/kg/day of hexachlorobenzene in corn oil for 30 days (Foster et al. 1995b), in female CD rats receiving up to 36 mg/kg/day from the diet for 4 months (Goldstein et al. 1978), in male BALB/c mice receiving 30 mg/kg/day of hexachlorobenzene from the diet for 6 weeks (Loose et al. 1977), or in male or female Sprague-Dawley rats administered hexachlorobenzene by daily gavage for 90 days at doses up to 25 mg/kg/day (NTP 2002). Intermediate-duration experiments that did report statistically significant weight loss in rats (Kuiper-Goodman et al. 1977; Smith and Cabral 1980; Smith et al. 1985) or mice (Cabral et al. 1979; Shirai et al. 1978) usually considered it to be slight-to-moderate. Although one study reported statistically significant decreases in body weight (ca. 9% less than controls) in male Fisher 344 rats at doses as low as 0.1 mg/kg of hexachlorobenzene by gavage in corn oil 5 days/week for 5 weeks (Andrews et al. 1988), subsequent studies using the same protocols in 15-week experiments revealed no signs of effects on body weight (Andrews et al. 1989, 1990). Oral intermediate-dosing studies that observed hexachlorobenzene-induced mortality also found significant weight loss. In a 13-week feeding study, four of nine female Wistar rats receiving the high dose of 19 mg/kg/day of hexachlorobenzene in corn oil for 13 weeks were euthanized during the study due to severe weight loss and distress, while no body weight effects were seen in surviving or lower dose animals (Den Besten et al. 1993). Smith et al. (1987) reported 18–22% depressed terminal body weight in male Syrian golden hamsters ingesting hexachlorobenzene from the diet at 19 mg/kg/day for 18 or 28 weeks. Among Syrian golden hamsters ingesting 16 mg/kg/day (but not lower doses up to 8 mg/kg/day) hexachlorobenzene from the diet for life, males exhibited “marked weight reduction” and

significantly increased mortality (Cabral et al. 1977). Similar mortality was observed in treated female hamsters in the absence of corresponding weight loss.

Similar findings have also been demonstrated in dogs, monkeys, and pigs. Weight loss was observed in 4 of 12 beagle dogs fed capsules of hexachlorobenzene at 11 mg/kg/day for up to 12 months (Gralla et al. 1977). Similarly, unspecified weight loss was reported beginning at 4 weeks in adult female Rhesus monkeys given hexachlorobenzene by daily gavage (in aqueous methylcellulose) for 60 days at doses as low as 8 mg/kg/day; renal and neurological effects were also reported (Knauf and Hobson 1979). All male SPF pigs fed 50 mg/kg/day (but not lower doses up to 5 mg/kg/day) for 90 days exhibited depressed growth (beginning at week 4) prior to death between weeks 8 and 12 (Den Tonkelaar et al. 1978). In contrast, experiments in which female Cynomolgus monkeys were dosed with hexachlorobenzene in gelatin capsules at doses up to 10 mg/kg/day for 90 days did not detect changes in body weight (Foster et al. 1992a, 1995a; Jarrell et al. 1993).

In fetal and immature pups, hexachlorobenzene-induced changes in body weight were observed only in the presence of maternal toxicity. No decreases in maternal or pup body weight were observed at doses as high as 100 mg/kg/day on gestation days 7–16 in CD-1 mice or as long as *in utero* and lifetime exposure of Sprague-Dawley rats to approximately 2–3 mg/kg/day (Arnold et al. 1985; Courtney et al. 1976; Goldey and Taylor 1992; Lilienthal et al. 1996; Taylor and Goldey 1990; Vos et al. 1979a, 1983). In Wistar rats, maternal and fetal body weight were decreased by gavage doses of at least 80 mg/kg/day of hexachlorobenzene in corn oil on gestation days 6–21 (Khera 1974); however, slight changes in the dosing protocols (fewer treatment days, use of aqueous gum tragacanth instead of corn oil) prevented weight loss. Likewise, Sprague-Dawley rat pup weight and viability were significantly reduced at birth, 5 days, and 24 days, following lifetime dietary exposure of both male and female parent rats to at least 6.9 mg/kg/day (Grant et al. 1977). Maternal signs reported by these two studies (Grant et al. 1977; Khera 1974) included mortality, convulsions, and reduced fertility.

Metabolic Effects. Several groups of investigators evaluated possible associations between serum organochlorine pesticide levels (including hexachlorobenzene) and risk or prevalence of diabetes (Burns et al. 2014; Codru et al. 2007; Cox et al. 2007; Gasull et al. 2012; Glynn et al. 2003; Lee et al. 2010; 2011; Son et al. 2010; Wu et al. 2013). Study limitations include the use of mostly small group sizes, lack of quantification of exposure to hexachlorobenzene, and/or presence of other organochlorine compounds in the serum.

Within a group of 205 women in areas of Sweden where organochlorine-contaminated fish were readily available, Glynn et al. (2003) reported a significant difference (p=0.008) in mean hexachlorobenzene serum levels among 7 women with reported diabetes (85 ng/g lipid; 95% CI 66, 109) and 198 women without diabetes (60 ng/g lipid; 95% CI 58, 63).

Codru et al. (2007) reported a significant association between serum hexachlorobenzene and evidence of diabetes (serum fasting glucose values >125 mg/dL and/or use of antidiabetic medication) (OR 4.8: 95% CI 1.7, 13.9) in a cross-sectional study of 352 adults from a Native American (Mohawk) population.

Wu et al. (2013) reported a significant positive association between plasma hexachlorobenzene level and incident type 2 diabetes (OR 3.59; 95% CI 1.49, 8.64; third tertile versus first tertile) in a group of 1,095 women who were free of diabetes at blood draw in 1989–1990 and who participated in two case-control studies in the Nurses' Health Study. The study included 48 cases of diabetes and 1,047 controls.

In a cohort study of 318 boys with serum organochlorine pesticide measurements at entry into the Russian Children's Study (8–9 years of prepubertal age at baseline; 10–13 years of pubertal age at follow up), a significant association was reported between baseline serum hexachlorobenzene (third tertile versus first tertile) and risk of insulin resistance at follow up (OR 4.37; 95% CI 1.44, 13.28) (Burns et al. (2014).

In a community-based health interview survey of 886 subjects in Catalonia, Spain (Gasull et al. 2012), prevalence of prediabetes (202 cases) and diabetes(143 cases) increased across quartiles of serum hexachlorobenzene levels; comparing the highest quartile with the lowest quartile, ORs were 2.1 (95% CI 1.0, 4.4) for prediabetes and 3.2 (95% CI 1.3, 8.1) for diabetes. Both groups exhibited significant trends for increasing incidence with increasing quartile of serum hexachlorobenzene.

In another community-based health survey of 40 cases of type 2 diabetes and 40 controls in Uljin County, South Korea (Son et al. 2010), comparison of the highest tertile of serum hexachlorobenzene with the lowest tertile resulted in an OR of 6.1 (95% CI 1.0, 36.6) for prevalence of type 2 diabetes; the trend for increasing prevalence with increasing serum hexachlorobenzene was statistically significant (p=0.03).

In a sample of 1,303 Mexican-Americans from the Hispanic Health and Nutrition Examination Survey who were 20–74 years of age and resided in the southwestern United States from 1982 to 1984, no significant association was found between serum hexachlorobenzene and prevalence of self-reported diabetes (Cox et al. 2007). There were no significant associations between serum hexachlorobenzene and

prevalence of type 2 diabetes in a nested case-control study of 90 diabetes cases and 90 controls (Lee et al. 2010) or in a cross-sectional study of 725 subjects living in Uppsala, Sweden (70 years of age and free of diabetes at baseline; 36 cases of diabetes diagnosed during 5-year follow up) (Lee et al. 2011).

3.2.2.3 Immunological and Lymphoreticular Effects

Although epidemiological studies have suggested that consumption by mothers of fish high in organochlorines may affect the immune system of their infants, oral exposure of people to hexachlorobenzene has not been clearly associated with immunological effects. The levels of hexachlorobenzene, dieldrin, and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in milk samples collected shortly after the birth of Canadian Inuit infants correlated with increased risk of otitis media in their first year of life, but not with other serum immune parameters (immunoglobulins, cytokines, lymphocyte activation markers) (Dewailly et al. 2000). A similar study of immune parameters in umbilical blood of Canadian mothers with high or low consumption of fish from the Lower-North-Shore of the St. Lawrence River detected a statistically significant association only between decreased lymphocyte secretion of cytokine IL-10 and increased levels of hexachlorobenzene, *p,p'*-DDE, and mercury (Belles-Isles et al. 2000). However, evidence suggests that hexachlorobenzene may indirectly affect the immune system by inducing porphyria (see Section 3.2.2.2 Hepatic Effects). Mode-of-action studies of individuals with inherited and acquired porphyria (unrelated to hexachlorobenzene) have found that irradiated porphyrins may activate the complement system and stimulate mast cells and neutrophils to damage nearby tissues (Lim and Cohen 1999; Meola and Lim 1993). Additionally, an increased risk of porhyria cutanea tarda has been associated with HIV infection (reviewed Drobacheff et al. 1998; Egger et al. 2002).

In a birth cohort of 965 women in Denmark, Hansen et al. (2014) evaluated possible associations between maternal serum persistent organochlorine pollutants (POPs), including hexachlorobenzene, and risk of asthma in offspring followed through 20 years of age. After dividing the study group by tertiles of serum hexachlorobenzene levels (0.07–0.45, >0.45–0.63, >0.63–2.45 ng/mL), comparison of the first and third tertile suggested a significant association between maternal serum hexachlorobenzene level and reported offspring asthma medication use (hazard ratio 1.92; 95% CI 1.15, 3.21).

Gascon et al. (2014) evaluated possible associations between maternal serum DDE, hexachlorobenzene, and PCB levels in 405 participants of the INMA-Menorca birth cohort (Spain) and reported increased prevalence of wheeze, chest infections, atopy, and asthma among the offspring from birth to 14 years of age. At 4 years of age, 275 children provided serum samples for measurement of cytokines and

biomarkers of inflammation (IL2, IL8, IL10, and TNFα). Increasing maternal serum hexachlorobenzene was significantly associated with increasing postnatal serum IL10 (β 0.22; 95 CI 0.02, 0.41), but not with IL6, IL8, or TNFα. Maternal hexachlorobenzene serum level was also significantly associated with wheeze (relative risk [RR] 1.58; 95% CI 1.04, 2.41) and chest infections (RR 1.89; 95% CI 1.10, 3.25) at 10 years of age.

The effects of oral exposure to hexachlorobenzene to the immune system of animals appear to be species- and strain- (Michielsen et al. 1997) dependent, with immunosuppression observed in mice (see below), monkeys (Iatropoulos et al. 1976) and bears (Bernhoft et al. 2000), and at least a partial stimulation of the immune system in rats (see below) and dogs (Gralla et al. 1977). Additionally, a number of animal studies have observed inflammation and immune cell infiltration in tissues such as the liver (Arnold et al. 1985; Ertürk et al. 1986), respiratory tract (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997, 1999, 2001; Vos et al. 1979a, 1983), and skin (Koss et al. 1978; Michielsen et al. 1997, 2000; Schielen et al. 1993, 1995b; Torinuki et al. 1981) following oral exposure to hexachlorobenzene. The lowest dose to cause an immune response was 0.022 mg/kg/day, which induced peribiliary lymphocytosis in F_1 Sprague-Dawley rats exposed via the diet for life after having been exposed via their mothers during gestation and lactation (Arnold et al. 1985). Because the mode-of-action is unclear, it is not known if these immune effects are secondary following toxicity to target organs or if they are involved in the etiology of disease in these organs. Mode-of-action studies in rats have suggested that the immune effects of hexachlorobenzene may be secondary to the accumulation of porphyrins produced by the liver in the spleen or other organs of immunological importance (Kennedy and Wigfield 1990; Kuiper-Goodman et al. 1977) or by the metabolic products of hexachlorobenzene (Schielen et al. 1995a).

Immunosuppression has been observed in male mice following oral exposure to hexachlorobenzene. Hexachlorobenzene doses as low as 0.9 mg/kg/day, received from the diet, resulted in significantly reduced spleen cell cytocidal activity after 6 weeks of exposure and increased susceptibility to tumor challenge *in vivo* and decreased killing of tumor cells *in vitro* after exposure for 18 weeks (Loose et al. 1981). Ingestion of 18 mg/kg/day decreased survival time by as much as 46% following ascites tumor cell challenge (Loose et al. 1981), and ingestion of 30 mg/kg/day decreased resistance to bacterial endotoxin (*Salmonella typhosa* lipopolysaccharide [LPS]) and to malarial infection (*Plasmodium berghei*) (Loose et al. 1978). Feeding at the same dose (30 mg/kg/day) for 6 weeks reduced primary and secondary plaque-forming cell responses to sheep red blood cells, reduced serum IgG, IgM, and IgA levels, increased spleen weight (Loose et al. 1977), increased susceptibility to infection by hepatis (but not

cytomegalovirus or pneumonia) virus (Carthew et al. 1990), and reduced ability of spleen cells to lyse P388 tumor cells (Silkworth and Loose 1981). Feeding of 30 mg/kg/day for 37 (but not 13 or fewer) weeks of treatment decreased graft-versus-host response (measured by injecting harvested spleen cells into neonatal BDF1 mice) (Silkworth and Loose 1981). In addition to the effects seen in adult male mice, similar effects were observed in a developmental study. Pups of pregnant BALB/c mice fed doses as low as 0.5 mg/kg/day on gestation days 1–18 exhibited a marked, significant decrease in delayed type hypersensitivity (DTH) response when tested on postnatal day 45 (Barnett et al. 1987). Analyses of collected spleen cells found that 5 (but not 0.5) mg/kg/day decreased mixed lymphocyte response and decreased B cell numbers; neither dose affected spleen blastogenesis induced by T- or B-cell mitogens.

In contrast to the immunosuppression observed in mice exposed to hexachlorobenzene, studies in rats have generally found stimulation of the immune system, as indicated by such effects as increased spleen and lymph node weights, increased neutrophil counts, and increased serum immunoglobulins. In feeding studies with male rats, exposure to at least 46 mg/kg/day of hexachlorobenzene for 3 weeks caused increased neutrophil counts, elevated popliteal lymph node weight with a corresponding proliferation of popliteal lymph node high-endothelial venules (Vos et al. 1979b), and spleen histopathology consisting of extramedullary hematopoiesis, enlarged marginal zones and follicles, and increased macrophage density in the marginal zones (Schielen et al. 1993; Vos et al. 1979b). In Wistar and Brown Norway rats ingesting doses of approximately 50–129 mg/kg/day for 3 weeks, immunological effects observed included increased spleen and lymph node (auricular, popliteal, inguinal, and/or mediastinal, but not parathymic) weight (Ezendam et al. 2004a, 2004b; Kennedy and Wigfield 1990; Koss et al. 1978; Michielsen et al. 2001, 2002; Schielen et al. 1995b; Vos et al. 1979a, 1979b); increased basophil, monocyte, and total leukocyte counts (Kennedy and Wigfield 1990; Koss et al. 1978; Schielen et al. 1995b; Vos et al. 1979a, 1979b); increased splenic (but not lymph nodal) cell size; selectively activated B cell (but not T cell) subpopulations (Schielen et al. 1995b); increased numbers of splenic T-cells and auricular lymph node B-cells (Ezendam et al. 2004a); increased splenic and/or serum levels of total IgM and/or IgE (Ezendam et al. 2004a, 2004b; Schielen et al. 1993, 1995b); increased serum levels of specific IgM (anti-phosphatidylcholine IgM, and anti-single stranded DNA IgM) without increases in IgM against foreign antigens (tetanus toxoid, sheep erythrocytes, and bovine serum albumin) (Schielen et al. 1993, 1995b); increased CD25 expression in mesenteric lymph nodes (Ezendam et al. 2004b); hyperplasia of the B-cell compartments and increased extramedullary hemopoiesis in the spleen (Ezendam et al. 2004b); and histopathologic evidence of inflammatory lesions in the lung (Ezendam et al. 2004a, 2004b; Michielsen et al. 2002). The reported changes in B cell populations and IgM levels strongly suggest an autoimmune response. Remarkably, no changes were seen immune function tests for thymus-independent

(*Escherichia coli* lipopolysaccharides) and thymus-dependent (tetanus toxoid) antibody responses, cell-mediated immunity (rejection of skin transplants, resistance to *Listeria monocytogenes* infection, or delayed-type hypersensitivity to *Mycobacterium tuberculin*), phagocytic competence (clearance of carbon particles), the mitogenic response of peripheral blood lymphocytes (to pokeweed mitogen, concanavalin A, and phytohemagglutinin), or susceptibility to *E. coli* endotoxin in Wistar rats at doses up to 184 mg/kg/day for 3 weeks (Vos et al. 1979b). Feeding of male Wistar rats with doses as low as 13.8 mg/kg/day for 6 weeks reduced natural killer cell activity (Van Loveren et al. 1990). Atrophy of the thymus was observed in male rats ingesting 184 mg/kg/day for 3 weeks (Vos et al. 1979b) and in female Wistar rats ingesting 15 mg/kg/day for 13 weeks (Schielen et al. 1995a).

Two developmental studies in rats by the same laboratory reported hexachlorobenzene effects on humoral and cellular immunity (Vos et al. 1979a, 1983). The first study reported that hexachlorobenzene exposure strongly enhanced humoral immunity (antibody response to tetanus toxoid), but slightly depressed cellular immunity (as evaluated by susceptibility to infection, skin graft rejection time, and response to mitogens) in pups whose mothers ingested estimated 15.4 or 25.6 mg/kg/day of hexachlorobenzene from early pregnancy and continuing through gestation and lactation; after weaning pups were fed the same doses for an additional 2 weeks prior to testing (Vos et al. 1979a). In both treatment groups, resistance to infection (*L. monocytogenes* and *Trichinella spiralis*) was reduced, the IgG response to tetanus toxoid was significantly increased, and histopathology found proliferation of high-endothelial venules in the paracortex of the lymph nodes. At 25.6 mg/kg/day, the IgG response to *Trichinella* was increased, increases were seen in blood levels of eosinophils, basophils, IgM and IgG, and histopathology found accumulation of foamy macrophages in the lung and increased extramedullary hematopoiesis in the spleen. Neither dose affected rejection of skin transplant, passive cutaneous anaphylaxis, IgM response to LPS, mitogenic responsiveness of lymphocytes, clearance of carbon particles, or clearance of *L. monocytogenes*. However, the follow-up study found that hexachlorobenzene stimulated both humoral and cell-mediated immunity in the Wistar rat pups whose mothers ingested an estimated 0.4, 2.1, or 10.3 mg/kg/day of hexachlorobenzene from early pregnancy through lactation; weaning pups were exposed to the same dietary concentrations as their mothers for up to 7 months (Vos et al. 1983). Cytotoxicity of spleen cells (to injected lymphoma cells) was not affected by treatment. Treatment with at least 0.4 mg/kg/day significantly increased the IgG and IgM response to tetanus toxoid and delayed-type hypersensitivity reaction to ovalbumin, and induced accumulation of foamy macrophages in the lungs. At 2.1 mg/kg/day and above, increased popliteal lymph node weight with a corresponding increase in lymph node cellular proliferation were observed, and cell proliferation was also detected in the endothelial cells lining pulmonary capillaries and venules. At 10.3 mg/kg/day, increases were observed

in spleen, lung, and mesenteric lymph node weight; serum IgM (but not IgG) levels; the relative number of basophils in the blood; and extramedullary hematopoiesis of the spleen. These effects demonstrated stimulation of both humoral and cell-mediated immunity.

A strain-dependent correlation between immunological and dermal effects was observed in female rats fed diets containing hexachlorobenzene (Michielsen et al. 1997). For 28 days, Wistar rats consumed approximately 46 or 92 mg hexachlorobenzene/kg/day; Lewis rats ingested approximately 17 or 51 mg hexachlorobenzene/kg/day; and Brown Norway rats ingested approximately 17, 51, or 102 mg/kg/day. Brown Norway rats were the most sensitive to hexachlorobenzene exposure, and correlations were observed between the incidence and severity of immune responses and dermal lesions. In contrast, Wistar rats were the most resistant, and correlations were not apparent, while some correlations were seen in Lewis rats. Hexachlorobenzene induced skin lesions in all treatment groups, most severe in Brown Norway rats and least severe in Wistar rats, characterized by epidermal hyperplasia, inflammatory infiltrate in the dermis, and activation (due to hypertrophy and proliferation) of endothelial cells in dermal vessels. Relative spleen weights were significantly increased in a dose-related fashion in all three strains while popliteal lymph node weight was increased in the high-dose Lewis and Brown Norway rats, but not in Wistar rats. All strains showed increases in IgM, but only Brown Norway rats exhibited increases in serum IgE and IgG. However, lung pathology was not strain dependent; histopathology observed lung lesions consisting of venules lined with unusually plump endothelial cells and surrounded by large perivascular infiltrate and accumulation of alveolar macrophages. The authors concluded that the inflammatory responses in the skin and lungs were of different etiologies, and speculated an involvement of the immune system in the observed dermal lesions.

NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.01$) increased incidences of splenic lymphoid hyperplasia were noted at hexachlorobenzene doses ≥10 mg/kg/day (incidences of 0/10, 0/10, 0/10, 0/10, 0/10, 0/10, 6/10, and 8/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively).

Studies of the immunological effects of hexachlorobenzene in nonrodents observed evidence of immunosuppression in monkeys and bears, and stimulation of the immune system in dogs. In female Rhesus monkeys, gavage with hexachlorobenzene in methylcellulose at doses as low as 8 mg/kg for 60 days caused thymic cortical atrophy, consisting of a reduction or absence of individual lobules, increased numbers of thymic corpuscles, and medullar hyperplasia or reticular cells, macrophages, plasma

cells, and lymphocytes. However, the use of only one or two monkeys in this study diminishes the reliability of these data (Iatropoulos et al. 1976). A significant correlation between hexachlorobenzene levels and decreased IgG was observed in an analysis of sera from 56 polar bears in Svalbard, Norway (Bernhoft et al. 2000). Because similar correlations were also observed for three polychlorinated biphenyl congeners (99, 194, and 206), this effect cannot be clearly attributed to hexachlorobenzene. Although no immunologically-related gross pathology or histopathology were observed in a 21-day oral study in female dogs at doses as high as 150 mg/kg/day (Sundlof et al. 1981), nodular hyperplasia of gastric lymphoid tissue was found in all beagle dogs given hexachlorobenzene in gelatin capsules daily for 12 months at doses as low as 10 mg/kg/day and an increased incidence of infebrile neutrophilia (increased numbers of blood neutrophils without fever) was observed at 10 and 110 mg/kg/day (Gralla et al. 1977).

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

The evidence of neurotoxicity in humans following oral exposure to hexachlorobenzene was provided by studies of people in southeast Turkey who consumed contaminated bread in the late 1950s. The ingested dose of hexachlorobenzene by exposed persons was estimated to be in the range of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) during the episode (Cam and Nigogosyan 1963).

Neurological symptoms included loss of appetite, tremors, convulsions, and “weakness that often made it impossible to eat with a knife and fork, rise from a squat, or climb stairs” (Gocmen et al. 1989; Peters et al. 1982). Follow-up studies of 25 (Peters et al. 1982) and 30 years (Cripps et al. 1984) included 161 and 204 patients, respectively. They found that neurological symptoms persisted in adults who had been exposed as children, and included weakness (62–66%), paresthesia (spontaneous tingling or burning sensations, 55%), sensory shading (graded sensory loss that diminishes upon testing more proximally and is indicative of polyneuropathy, 61–63%), myotonia (delayed muscle relaxation after an initial contraction, 38–50%), and cogwheeling (irregular jerkiness of movement due to increased muscle tone as seen in Parkinson’s disease, 29–41%). During the grain poisoning epidemic, there was an extremely high (95%) rate of mortality in infants under 2 years of age, who had been breast fed by mothers who had ingested the contaminated bread; these children exhibited convulsions, tremors, and progressive weakness prior to death (Cripps 1990; Peters et al. 1966). Analysis of human milk from exposed women and

unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). A study investigating the potential effects of consuming fish from the Great Lakes was unable to correlate hexachlorobenzene levels in umbilical blood or breast milk with infant intelligence test results (Darvill et al. 2000).

Multiple studies have shown that hexachlorobenzene induces serious neurological effects such as convulsions, tremors (intermittent and constant), lethargy, and progressive weakness in rats, mice, rabbits, pigs, monkeys, and quail (Cabral et al. 1979; Cripps 1990; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Grant et al. 1977; Hahn et al. 1988; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Khera 1974; Knauf and Hobson 1979; Ockner and Schmid 1961; others). In several studies, these effects were only seen prior to death or in treatment groups with significant mortality. The lowest dose to cause such serious effects was 14.6 mg/kg/day in Sprague-Dawley rats as part of a multigeneration study, with convulsions preceding death (Grant et al. 1977). Mice receiving 39 mg/kg/day of hexachlorobenzene from the diet for up to 17 weeks exhibited severe tremors prior to death (Hahn et al. 1988). Male SPF pigs fed diets containing hexachlorobenzene at concentrations resulting in ingestion of approximately 50 mg/kg/day for 90 days exhibited tremors, panting, and unsteady gait without histopathology (Den Tonkelaar et al. 1978). Adult female Rhesus monkeys given oral doses of hexachlorobenzene for 60 days suffered severe tremors and muscular weakness at doses as low as 64 mg/kg/day and marked lethargy and weakness were observed at 128 mg/kg/day (Knauf and Hobson 1979). Two of three infant Rhesus monkeys whose mothers were treated with 64 mg/kg/day for up to 60 days during lactation displayed hypoactivity, lethargy, and ataxia, and subsequently died (Iatropoulos et al. 1978).

Other studies have investigated less overt neurological effects resulting from oral exposure to hexachlorobenzene. Electrophysiological changes (dysrhythmic electroencephalogram) in the central nervous system were demonstrated in dogs receiving doses of ≥50 mg/kg/day for 21 days (Sundlof et al. 1981). Another functional experiment observed axonal effects in the sciatic nerve (fibrillations, repetitive or pseudomyotonic discharges, and mild slowing of conduction velocities) in rats ingesting hexachlorobenzene at 69 mg/kg/day of hexachlorobenzene for 20 weeks or at least 9.1 mg/kg/day for 2 years (Sufit et al. 1986). In male Wistar rats gavaged with 317 mg/kg/day for 4 weeks, significant decreases were observed in the rate of T4 uptake into cerebrospinal fluid and brain tissue (van Raaij et al. 1994). Hadjab et al. (2004) reported significantly increased auditory threshold in the 2–16 kHz frequency range in male Sprague-Dawley rats administered hexachlorobenzene by gavage for 4 weeks at 4 or 16 mg/kg/day. Gavage treatment of Chbb THOM and Wistar rats with 1,000 mg/kg/day of hexachlorobenzene in a

water-Tween suspension for up to 28 days induced changes in brain phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositiol, phosphatidylserine, and sphingomyelin) without affecting brain porphyrin levels (Billi de Catabbi et al. 2000b; Cochon et al. 2001). Because these effects were seen prior to the onset of porphyrin accumulation in the liver, the authors concluded that the effects of hexachlorobenzene on phospholipids in the brain were different from its effects on phospholipids of the liver or Harderian gland, which reportedly occur following porphyrin accumulation.

One of two developmental studies in rats that investigated the neurobehavioral effects of hexachlorobenzene observed hyperactivity. Two weeks prior to mating, female Sprague-Dawley rats were gavaged for 4 days with 2.5 or 25 mg/kg/day (Goldey and Taylor 1992; Taylor and Goldey 1990). In the first 3 weeks postnatally, both treatment groups of pups exhibited a significantly increased level of hyperactivity compared to controls. Specifically, treated pups reoriented themselves significantly more quickly in a negative geotaxis test, required less time in an olfactory discrimination test, and demonstrated increased exploratory activity in a motor activity test (postnatal days 15–20). No significant effects on learning (swim T-maze) or motor activity (measured in older offspring on postnatal days 40 and 50, respectively) were detected. Pups in the high-dose group exhibited decreased acoustic startle response (ASR) on postnatal day 23 and increased ASR on postnatal day 90. The LOAEL of 2.5 mg/kg/day for increased hyperactivity in the offspring was used to calculate an acute-duration oral MRL as described in Table 3-2 and in Appendix A.

In the other developmental study, groups of female Wistar rats received hexachlorobenzene from the diet at estimated doses of 0.3, 0.6, or 1.3 mg/kg/day for 90 days prior to mating and through gestation and lactation; offspring were maintained on the same diet until postnatal day 150 (Lilienthal et al. 1996). In pups from the high-dose group (1.3 mg/kg/day), significant decreases were seen in operant learning ("post-reinforcement pause" and "index of curvature") on postnatal day 150. However, because the rats were exposed both developmentally and as adults, the developmental significance of changes in operant learning is unclear. No changes were seen in an open field activity test (a measure of early locomotor skills) on day 21 or an active avoidance learning test on day 90.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species for intermediate duration category are shown in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

Epidemiological studies suggest that hexachlorobenzene may cause spontaneous abortion in women. In Southeastern Turkey, consumption of bread made from grain treated with hexachlorobenzene resulted in widespread poisoning between 1955 and 1959. Although no quantitation of exposure was presented in any of these clinical reports, an estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963). A follow-up study conducted between 1977 and 1981 identified 42 porphyric mothers who had been exposed as children, with 188 pregnancies (Peters et al. 1982, 1987). Of these, 15 were fetal deaths (13 miscarriages and 2 stillbirths), and 31 produced children who died in the first several years of life. Similarly, another follow-up study conducted 20–30 years after initial exposure identified 57 porphyric mothers, who had a total of 276 pregnancies (Gocmen et al. 1989). Of these, 23 were fetal deaths, and 54 produced children who died in the first several years of life. Porphyric mothers had an average of 0.51 ppm hexachlorobenzene in their breast milk, compared to 0.07 ppm in unexposed controls (Gocmen et al. 1989). The degree to which these two exposed populations overlap and the expected frequencies of adverse pregnancy outcomes for the unexposed cohorts were unclear. Surviving offspring of porphyric mothers were clinically normal, and had urine and stool porphyrin levels similar to control children.

A subsequent retrospective study, conducted 40 years after initial exposure, compared three groups of 42 women (controls from outside the exposed area and women from the hexachlorobenzene-exposed region either with or without a diagnosis of porphyria cutanea tarda) (Jarrell et al. 1998). The incidence of women with blood levels of hexachlorobenzene exceeding 1 ng/mL was greater in women with porphyria cutanea tarda or women living in the contaminated region than country-wide controls and correlated (across exposure-groups) with an increased risk of spontaneous abortion. Notably, blood levels did not correlate with the number of pregnancies, sex ratio of born children, or onset of menopause. Statistically significant increases in the levels of inhibin (a hormone secreted by ovarian granulosa cells to decrease the release of follicle-stimulating hormone [FSH] from the pituitary) were observed in women diagnosed with porphyria cutanea tarda. Because no exposure-related differences were seen for FSH or estradiol, the biological significance is unclear, but ovarian effects would be consistent with animal studies (see below).

Studies of other populations with exposure to multiple organochlorines did not find significant differences in blood hexachlorobenzene levels between controls and cases of spontaneous abortion in Italy (Leoni et al. 1986, 1989) or Germany (Gerhard et al. 1998). Average maternal blood levels of hexachlorobenzene

were 1.6 and 0.679 ng/mL, respectively. Similarly, no changes in reproductive outcomes in Xixin, China, were detected following the cessation of agricultural uses of hexachlorobenzene (Huang et al. 1989).

Possible associations between serum hexachlorobenzene and selected reproductive outcomes have been evaluated by a number of investigators. However, these studies provide no clear evidence of hexachlorobenzene-related effects on reproductive outcomes in the study groups. Akkina et al. (2004) found no significant association between serum hexachlorobenzene and age at onset of menopause in a group of 219 Hispanic women residing in the United States. The authors noted that organochlorine serum levels measured at the time of sampling may not be representative of exposure levels at the time of menopause. Cooney et al. (2010) reported an odds ratio (OR) of 6.6 (95% CI 1.0, 42.8) for endometriosis in a group of women with hexachlorobenzene levels >0.04 ng/g serum (adjusted for total serum lipids, smoking, and other pesticides) compared to a referent group with hexachlorobenzene levels <0.02 ng/g serum (adjusted similarly). Thirty-two of the 84 women evaluated in this study were diagnosed with endometriosis. There were 14 endometriosis cases among 27 women with aromatic fungicide (including hexachlorobenzene) levels >0.04 mg/g serum and 6 cases among 26 women in the referent group with aromatic fungicide (including hexachlorobenzene) levels <0.02 mg/g serum. Upon grouping the serum organochlorine pesticides tertiles by chemical structure and odds of an endometriosis diagnosis and adjusting for total serum lipids and smoking, the aromatic fungicides group (which included hexachlorobenzene) with serum levels >0.04 ng/g serum exhibited an OR of 5.3 (95% CI 1.2, 23.6). Significant ORs were not found within the groupings of chlorinated insecticides (included β-benzene hexachloride and dichloro-diphenyl-dichloroethylene) or cyclodiene insecticides (included aldrin, mirex, and trans-nonachlor). The study is limited by small numbers of subjects and lack of quantification of hexachlorobenzene exposure levels. In another case-control study of 80 cases of endometriosis and 78 controls in Rome, Italy, no significant association was found between risk of endometriosis and serum hexachlorobenzene levels (Porpora et al. 2009).

No significant associations were observed between serum hexachlorobenzene and serum testosterone levels among 257 adult male and 436 adult female Native Americans (Mohawks) (Goncharov et al. 2009) or among 341 adult men from a fertility clinic (Ferguson et al. 2012). In a cross-sectional study, Freire et al. (2014) evaluated possible associations between serum organochlorine levels (including hexachlorobenzene) and serum hormone levels among 304 men and 300 women in a rural area of Brazil heavily contaminated with organochlorine pesticides. There were no significant associations between serum hexachlorobenzene and serum testosterone levels in the men or serum estradiol, progesterone,

prolactin, luteinizing hormone (LH), or FSH in premenopausal women; however, a slight but significant ($p<0.05$) negative association was found for serum LH among 77 peri- and postmenopausal women.

The ovaries are a sensitive target organ for hexachlorobenzene. Distribution studies have identified the ovaries as a site of hexachlorobenzene accumulation (Foster et al. 1993; Sitarska et al. 1995; others). Studies have reported changes in organ weight; histological (light microscopy) and ultrastructural (electron microscopy) degenerative changes; and altered serum levels of gonadal hormones (estrogen and progesterone). Investigations into the mode-of-action generally found disruptions in steroidogenesis.

The acute data for ovarian effects are limited (Foster et al. 1993). Simon et al. (1979) reported decreased reproductive performance, as indicated by decreased impregnation of female rats by male rats administered hexachlorobenzene by gavage at 221 mg/kg/day for 5 days prior to mating; there was no effect on fertility index (number of pregnant females/number of females impregnated). Serum progesterone levels were increased in female Sprague-Dawley rats that were superovulated prior to gavage treatment with 50 mg/kg/day of hexachlorobenzene in corn oil for 5 days but not in normally-cycling rats similarly treated (Foster et al. 1993).

In a 90-day assay in adult female Cynomolgus monkeys, ovarian toxicity was noted at the lowest oral dose tested, 0.01 mg/kg/day of hexachlorobenzene (Bourque et al. 1995). Ultrastructural analyses of developing ova detected mitochondrial changes, which increased in frequency and severity with dose. Swelling of the cristae resulting in abnormal intracristae spaces was seen at 0.01 mg/kg/day; mitochondrial matrices became more coarsely granular and exhibited occasional irregular morphology at 0.1 mg/kg/day; and mitochondria had “electron-lucent” matrices and reduced membrane integrity at 10 mg/kg/day. A similar increase in frequency and severity was observed for lesions in follicular cells: abnormal nuclei were seen in “a few cells” at 0.01 mg/kg/day, while nuclear membrane infolding was clearly apparent at 0.1 mg/kg/day. Abnormal spaces between follicular cells were observed at 1 mg/kg/day, and follicular cells exhibited abnormal lipid accumulation and deeply folded and indented nuclear membranes at 10 mg/kg/day. Thecal cells exhibited deformed nuclei only at 10 mg/kg/day. Since mitochondrial changes may represent nonspecific cell injury, the specific mode of action of degenerative ovarian changes remains unclear. The LOAEL of 0.01 mg/kg/day from this study was used to calculate an intermediate-duration oral MRL as described in Table 3-2 and in Appendix A.

Supporting data are provided by previous 90-day studies in female Cynomolgus monkeys that had dose-related degenerative changes in oocytes and ovaries at all doses tested, 0.1–10 mg/kg/day (Babineau et al.

1991; Foster et al. 1992a, 1995a; Jarrell et al. 1993; Sims et al. 1991). Increasing in frequency and severity, oocyte effects progressed from increased numbers of lysosomal elements and irregularly arranged thecal layer cells to altered oocyte morphology (shape irregularities, increased granularity and density, less distinct membrane), cytoplasmic vacuolation, lysomal aggregation, and pyknosis of follicular granulosa cells. Similarly, ovarian changes progressed from cellular hypertrophy, increasing columnarization of normally cuboidal cells, and small lipid inclusions, to cellular necrosis and separation of epithelium from connective tissue, stratification and elongation of epithelial cells, and increased numbers of lysosomes, vesicles, and lipid inclusions. Additionally, luteal phase progesterone levels were reduced at 1 mg/kg/day, ovulatory surge estrogen levels were decreased at 10 mg/kg/day, and menstrual cycle length variability was increased at 10 mg/kg/day (Foster et al. 1992a, 1995a). Remarkably, doses up to 10 mg/kg/day did not affect fertility (measured in oocytes by *in vitro* tests), serum inhibin levels, or numbers or size of oocytes, follicles, or corpora lutea.

Earlier studies in female Rhesus monkeys observed similar lesions. In female Rhesus monkeys given hexachlorobenzene for 60 days, ovarian effects seen at 8 mg/kg/day included cortical degeneration, reduced numbers of primary follicles with a concurrent increase in relative corpora lutea volume, multiple follicular cysts, and a thickening of the ovarian germinal epithelium with cells exhibiting a columnar appearance progressing to pseudostratification (Iatropoulos et al. 1976). These effects increased in incidence and severity with dose. At 32 mg/kg/day and above, epithelial nuclei were pyknotic (condensed) and karyorrhectic (fragmented) and at 128 mg/kg/day, ovarian cortices were predominated by dense stroma. These ovarian changes were similar to those normally seen in menopause, and indicate that the corpora lutea were not producing steroids. A subsequent study in female Rhesus monkeys found that serum cholesterol was increased by gavage doses of 8 mg/kg/day of hexachlorobenzene in methyl cellulose for 60 days (Knauf and Hobson 1979); this effect may be secondary to changes in ovarian steroidogenic activity. These findings are supported by a study in which hexachlorobenzene blocked ovulation (estrogen and progesterone remained low, LH and FSH continued to climb, and menstruation was delayed) in one of four female Rhesus monkeys gavaged with 4 mg/kg/day of hexachlorobenzene in aqueous methyl cellulose for up to 78 days (Muller et al. 1978). A lower-dose study found no changes in serum levels of estrogen, progesterone, FSH, or LH in female Rhesus monkeys fed 0.03 mg/kg/day of hexachlorobenzene in monkey chow for 11 months (Rozman et al. 1978). The difference between this study and the one in Cynomolgus monkeys that observed changes in estrogen and progesterone levels (Foster et al. 1992a, 1995a) may reflect strain specificity or differences in absorption following disparate methods of oral exposure.

No evidence of histopathology was found in the ovaries of female beagle dogs given hexachlorobenzene in gelatin capsules with corn oil at doses up to 100 mg/kg/day for 21 days; data for other reproductive organs were not reported (Sundlof et al. 1981).

Although rat studies have not investigated doses as low as those used in monkey studies, they have identified histological evidence of degeneration and ultrastructural changes in the ovaries of animals treated with hexachlorobenzene. Gavage treatment of female Wistar rats with 1,000 mg/kg/day of hexachlorobenzene for 30 days caused degenerative lesions (increased numbers of atresic follicles, inflammatory infiltration of primary follicles, stratification and proliferation of ovarian surface epithelial cells, and irregular nuclei in epithelial cells) and changes in hormone and hormone receptor levels (decreased serum estradiol and prolactin, increased FSH, decreased estrogen receptor levels) (Alvarez et al. 2000). Similarly, a 21-day study detected increased serum progesterone levels in female Sprague-Dawley rats gavaged with at least 1 mg/kg/day of hexachlorobenzene (Foster et al. 1992b). In treated animals, the number of ova produced per rat decreased significantly and the length of estrus increased significantly. Another study in female Sprague-Dawley rats observed suggestive evidence of ovarian lesions (increased prominence of Golgi complexes, smooth endoplasmic reticulum, and free polysomes) at 10 (but not 1 or 100) mg/kg/day (MacPhee et al. 1993). The differences observed between rats and monkeys for changes in progesterone levels may be related to differences in their cycle lengths.

To investigate the contributions of adrenal steroidogenesis, adult female Sprague-Dawley rats were ovariectomized prior to treatment (Foster et al. 1995a). Gavage treatment for 30 days at doses as low as 1 mg/kg/day decreased serum corticosterone, while serum cortisol was decreased only at 100 mg/kg/day; no effects were seen on aldosterone or progesterone levels. The authors concluded that hexachlorobenzene induced alterations in steroidogenesis of cells of the inner zone of the adrenal cortex.

NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.001$) increased incidences of mammary gland hyperplasia were noted at the highest dose level (incidences of 2/10, 2/10, 1/10, 0/10, 2/10, 2/10, 3/10, and 10/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively).

Some intermediate-duration experiments have demonstrated that hexachlorobenzene adversely affects reproductive performance. In a multigenerational study in which male and female Sprague-Dawley rats received hexachlorobenzene from the diet at doses ranging from approximately 0.9 to 63 mg/kg/day

through premating and two series of mating, gestation, and lactation for up to four generations, statistically significant decreases in fertility and increases in the number of stillborns were observed at approximately 28 mg/kg/day (parental males) and 31 mg/kg/day (parental females) and average litter size was decreased at doses ≥14 mg/kg/day (parental males) and ≥16 mg/kg/day (parental females) (Grant et al. 1977). No reproductive toxicity was observed, either in female Sprague-Dawley rats receiving hexachlorobenzene from the diet at doses up to 13.7 mg/kg/day continuously from 96 days prior to first mating through gestation of two successive litters (Kitchin et al. 1982) or in female Cynomolgus monkeys given 10 mg/kg/day orally for 90 days (Jarrell et al. 1993). No reproductive effects were observed in a study in which both male and female Sprague-Dawley rats received hexachlorobenzene from the diet at estimated doses up to 3.4 mg/kg/day (males) and 3.9 mg/kg/day (females) from 3 months prior to mating through weaning of the F_1 offspring (Arnold et al. 1985).

In male Fischer 344/N rats ingesting hexachlorobenzene in arachis oil at approximately 16 mg/kg/day for 90 weeks, testicular weight was significantly increased and testicular interstitial cell tumors were more severe (although incidence was not affected) compared to controls (Smith et al. 1985). Slight testicular degeneration, with numerous spermatogonic giant cells and incomplete complement of spermatogonia in the seminiferous tubules, was observed in two of six male beagle dogs given 110 mg/kg/day of hexachlorobenzene in gelatin capsules with corn oil for 12 months (Gralla et al. 1977). Additionally, retarded sexual maturation of the testes was observed in male SPF pigs fed 50 (but not lower doses up to 5 mg/kg/day of hexachlorobenzene for 90 days (Den Tonkelaar et al. 1978).

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

3.2.2.6 Developmental Effects

Human and animal studies have demonstrated that hexachlorobenzene crosses the placenta to accumulate in fetal tissues; additionally, hexachlorobenzene is concentrated in milk and can be transferred to the suckling neonate (for more information, see Sections 3.4.1 and 3.4.2).

Oral exposure to hexachlorobenzene has been associated with serious developmental toxicity in a study of a poisoning epidemic (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1966, 1982, 1987). In southeast Anatolia, Turkey, ingestion of an estimated 0.7–2.9 mg/kg/day of hexachlorobenzene between 1955 and 1959 (in bread made from grain treated with hexachlorobenzene as a fungicide) resulted in

dramatic developmental effects, including a 95% mortality rate in infants under 2 years of age who had been breast fed by exposed mothers (Peters et al. 1966). These infants were diagnosed with a condition known as *pembe yara* or "pink sore" because of associated skin lesions consisting of blistering, epidermolysis, and annular erythema. The cause of death in these infants was cardiorespiratory failure; weakness and convulsions were also seen frequently. Older children, between the ages of 6 and 15 years, exhibited a condition known as *kara yara* or “black sore” more frequently than younger children or adults. The symptoms of this disease began with photosensitivity and progressed within 6 months to include hyperpigmentation, dermal fragility (resulting in ulcerating lesions and severe mutilating scars), and hirsutism (Cam and Nigogosyan 1963; Dogramaci 1964; Gocmen et al. 1989). Mortality was 10% among *kara yara* patients. These skin lesions (pink sore and black sore) have been diagnosed as porphyria cutanea tarda, a specific type of vesiculobullous porphyria. The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the accumulation of porphyrins, which may cause tissue damage, especially in the skin (for more information, see Sections 3.2.2.2, Hepatic Effects and Dermal Effects, and 3.5.2, Mechanisms of Toxicity). Similar dermal lesions, but no increase in mortality incidence, were reported for exposed adults, who also exhibited neurological disorders (weakness and diminished muscle control).

Follow-up studies have found persistent symptoms of developmental toxicity in a cohort of 252 adults (162 men and 90 women) who had been exposed as children in the poisoning epidemic (average age of the cohort at the time of exposure was 7.6 years) (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982). Short stature was seen in 42.1% of the patients, considered striking in comparison to their unexposed siblings. Additionally, 66.6% of the exposed patients exhibited osteoporosis of bones in the hands, associated with distinctive small hands, painless swelling, and spindling of fingers. Osteoporosis and osteosclerosis have been observed in adult hexachlorobenzene-exposed rats (see Section 3.2.2.2, Musculoskeletal Effects). In addition to the profound weakness and decreased muscle control observed in exposed adults (see Section 3.2.2.4, Neurological Effects), this cohort also presented paresthesia (spontaneous tingling or burning sensations, in 53.6% of patients) and graded sensory loss indicative of polyneuropathy (in 60.6% of patients).

Two follow-up investigations have found potential reproductive effects in women exposed as children to hexachlorobenzene in the Turkish epidemic (Gocmen et al. 1989; Jarrell et al. 1998). A study conducted 20–30 years after initial exposure identified 57 porphyric mothers with 23 fetal deaths and 54 children who died in the first several years of life from a total of 276 pregnancies (Gocmen et al. 1989). Porphyric mothers had an average of 0.51 ppm hexachlorobenzene in their breast milk, compared to 0.07 ppm in

unexposed controls. The results of this study were inconclusive because no information was provided regarding the expected incidence of fetal deaths and newborn-death in this population. Another study found a statistically significant increased risk of abortion among a subset of exposed women who exhibited porphyria cutanea tarda and had blood levels of hexachlorobenzene above 1.0 ng/mL (Jarrell et al. 1998). A more recent report by Jarrell and coworkers (Jarrell et al. 2002) found a significantly lower lifetime proportion of male offspring from women reporting hexachlorobenzene exposure at the peak of the Turkish epidemic (1955–1957) compared to women exposed at a later date.

A German case-control study found that adipose hexachlorobenzene levels in 18 male patients who underwent orchidopexy to correct unilateral or bilateral undescended testis (mean age 4.2 years) were 3-fold higher compared to a group of 30 male control patients (mean age 3.5 years); this difference was highly significant statistically (Hosie et al. 2000). These results were inconclusive because a similar correlation was also observed for heptachloroepoxide (but not for other organochlorines measured). This study is also limited by its small study size and lack of age-adjustment.

Although epidemiological studies have suggested that consumption by mothers of fish high in organochlorines may affect the immune system of their infants, perinatal exposure to hexachlorobenzene has not been clearly associated with immunological effects in these populations. The levels of hexachlorobenzene, dieldrin, and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in milk samples collected shortly after the birth of Canadian Inuit infants correlated with increased risk of otitis media in their first year of life, but not with other serum immune parameters (immunoglobulins, cytokines, lymphocyte activation markers) (Dewailly et al. 2000). A similar study of immune parameters in umbilical blood of Canadian mothers with high or low consumption of fish from the Lower-North-Shore of the St. Lawrence River detected a statistically significant association only between decreased lymphocyte secretion of cytokine IL-10 and increased levels of hexachlorobenzene, *p,p'*-DDE, and mercury (Belles-Isles et al. 2000).

Several groups of investigators have evaluated possible associations between levels of hexachlorobenzene in maternal blood or breast milk, cord blood, or children's blood and developmental end points such as birth size (weight and/or length) or preterm birth (Basterrechea et al. 2014; Eggesbø et al. 2009; Fenster et al. 2006; Gladen et al. 2003; Guo et al. 2014; Lopez-Espinosa et al. 2011; Sagiv et al. 2007; Szyrwińska and Lulek 2007; Torres-Arreola et al. 2003; Vafeiadi et al. 2014), recurrent miscarriage (Sugiura-Ogasawara et al. 2003), postnatal growth (Burns et al. 2012; Cupul-Uicab et al. 2013; Mendez et al. 2011; Smink et al. 2008; Valvi et al. 2014), postnatal neurodevelopment (Cheslack-Postava et al. 2013; Darvill

et al. 2000; Forns et al. 2012; Sioen et al. 2013; Strøm et al. 2014), sexual maturation (Croes et al. 2014a, 2014b; Denham et al. 2005; Lam et al. 2014; Schell and Gallo 2010), cryptorchidism (Pierik et al. 2007), hypospadias (Giordano et al. 2010; Rignell-Hydbom et al. 2012), and indicators of postnatal thyroid function (Freire et al. 2011; Julvez et al. 2011). Limitations of these studies include lack of quantifiable exposure to hexachlorobenzene, small numbers of subjects, and/or the presence of measureable levels of other organochlorine compounds. Although most studies found little evidence of significant associations between hexachlorobenzene levels and these developmental end points, significant associations were reported in several studies as discussed below.

Vafeiadi et al. (2014) reported that birth weight was negatively associated with increasing levels of maternal serum hexachlorobenzene (β -154.3 g; 95% CI -300.8, -7.9) in a population-based survey of 1,117 pregnant women and their children in Heraklion, Crete, Greece.

Lopez-Espinosa et al. (2011) reported a marginally significant (p=0.047) decrease of 0.39 cm in birth length for each 10-fold increase in umbilical cord hexachlorobenzene level in a birth cohort of 494 mothers and their newborns in Valencia, Spain (born 2003–2006). In another Spanish birth cohort study of 1,285 infants, Valvi et al. (2014) reported significant positive associations between maternal serum hexachlorobenzene level and rapid postnatal growth during the first 6 months (RR 1.44; 95% CI 1.04, 1.99) and overweight at 14 months of age (RR 1.45; 95% CI 1.10, 1.92) when comparing the highest tertile (maternal serum hexachlorobenzene >73 ng/g lipid) with the lowest tertile (≤22.6 ng/g lipid).

A case-control study of 80 hypospadia cases and 80 controls from two hospitals in Rome, Italy, reported significantly ($p<0.05$) increased risk of hypospadias with each increase of 10 pg/g hexachlorobenzene in the maternal serum (OR 1.26; 95% CI 1.04, 1.52) (Giordano et al. 2010). Another case-control study of 237 hypospadia cases and 237 controls in Sweden reported increased risk of hypospadias among those mothers with serum hexachlorobenzene levels >0.26 ng/mL compared to those mothers with lower serum hexachlorobenzene levels (OR 1.65; 95%CI 1.02, 2.69) (Rignell-Hydbom et al. (2012).

Croes et al. (2014a, 2014b) reported a significant (p=0.02) negative association between serum hexachlorobenzene and reaching menarche at 14–15 years of age (OR 0.35; 95% CI 0.15, 0.84) in a group of 282 girls in FLEHS II; among 324 boys in the same study, serum hexachlorobenzene was significantly positively associated with total serum testosterone (OR 1.04; 95% CI 1.01, 1.07; p=0.004), the ratio of testosterone to estradiol (OR 1.05; 95% CI 1.01, 1.08; p=0.007), and reaching of the adult stage of

testosterone (OR 1.29; 95% CI 1.01, 1.65; p=0.04), and borderline significantly positively correlated with age at pubic hair development (OR 1.77; 95% CI 1.00, 3.14; p=0.052).

In a prospective cohort study of 350 prepubertal Russian boys (8–9 years of age) who were monitored yearly for serum organochlorine levels (including hexachlorobenzene), higher serum hexachlorobenzene levels were associated with later mean age of reaching puberty as determined by testicular volume pubic hair growth; however, there were no significant associations after adjusting for baseline body mass index (BMI) categories and height (Lam et al. 2014).

Acute-duration developmental studies have verified that hexachlorobenzene impaired neurological development at doses as low as 2.5 mg/kg/day in rats (Goldey and Taylor 1992) and produced teratogenic abnormalities at doses as low as 40 mg/kg/day (Courtney et al. 1976; Khera 1974). Intermediate-duration developmental studies in rats include reports of immunodevelopmental effects at 0.5 mg/kg/day (Barnett et al. 1987); neurodevelopmental effects at 1.3 mg/kg/day (Lilienthal et al. 1996); and reduced neonatal viability and growth, and organ weight changes at approximately 6–14 mg/kg/day (Grant et al. 1977; Kitchin et al. 1982). In Rhesus monkey pups, death (accompanied by neurological effects, lung edema, and liver damage) resulted from nursing for 15–38 days from female monkeys exposed to 64 mg/kg/day of hexachlorobenzene (Bailey et al. 1980; Iatropoulos et al. 1978). In a multigenerational reproductive study (chronic-duration exposure) in Sprague-Dawley rats, hexachlorobenzene induced decreased viability of neonatal pups of parental rats administered hexachlorobenzene in the diet for 3 months prior to mating and throughout mating and gestation at estimated doses of 3.4 mg/kg/day (males) and 3.9 mg/kg/day (females) (Arnold et al. 1985).

The most sensitive acute-duration study evaluated neurodevelopmental end points and detected evidence of hyperactivity in Sprague-Dawley rat pups (Goldey and Taylor 1992; Taylor and Goldey 1990). This study was considered acute because virgin female Sprague-Dawley rats were gavaged for 4 days with 2.5 or 25 mg/kg/day of hexachlorobenzene 2 weeks prior to mating. Compared to controls, pups from both treatment groups reoriented themselves significantly more quickly in a negative geotaxis test, required less time in an olfactory discrimination test (postnatal days 6, 8, and 10), and demonstrated increased exploratory activity in a motor activity test (postnatal days 15–20). Pups exposed to 25 mg/kg/day exhibited decreased acoustic startle response (ASR) on postnatal day 23 and increased ASR on postnatal day 90. The LOAEL of 2.5 mg/kg/day from this study has been used to calculate an acute oral MRL of 0.008 mg/kg/day as described in the footnote to Table 3-2 and in Appendix A.

Other acute-duration studies have used higher doses to investigate traditional end points of developmental toxicity. An acute single-dose study found an increase in the overall incidence of fetal abnormalities (but not any specific abnormality) in the fetuses of pregnant female CD-1 mice gavaged with 100 mg/kg/day of hexachlorobenzene on gestation days 7–16; cleft palate and renal agenesis were the most common anomalies noted (Courtney et al. 1976). Three acute (3–10 days) and one intermediate (15 days) developmental toxicity experiments in pregnant Wistar rats observed increases in the incidences of sternal variations and the 14th rib formation at ≥40 mg/kg/day (Khera 1974). At ≥80 mg/kg/day, decreased fetal and maternal body weights were seen with other maternal effects.

The most sensitive intermediate-duration study evaluated immunodevelopmental toxicity. Pups of pregnant BALB/c mice fed doses as low as 0.5 mg/kg/day on gestation days 1–18 exhibited a marked, significant decrease in delayed type hypersensitivity (DTH) response when tested on postnatal day 45 (Barnett et al. 1987). Splenic effects (decreased B cell numbers and decreased mixed lymphocyte responses) were seen at 5 mg/kg/day. Studies in Wistar rats have also demonstrated immunodevelopmental toxicity. In pups born to dams exposed through gestation and lactation to hexachlorobenzene and continued on the same diets, doses ≥0.4 mg/kg/day resulted in increased immune responses (IgG and IgM responses to tetanus toxin, delayed-type reaction to ovalbumin, and pulmonary accumulation of foamy macrophages) (Vos et al. 1983). Doses ≥2.1 mg/kg/day caused lymph node endothelial proliferation and increased weights of liver and popliteal lymph nodes. Effects in the high-dose group (10.3 mg/kg/day) included increased pup mortality, increased weights of spleen, lung, adrenals, and mesenteric lymph nodes, increased basophils (without an overall increase in leukocytes) and serum IgM (but not IgG), and histopathologic evidence of hepatocellular hypertrophy and necrosis. In a similarly-designed study (Vos et al. 1979a), F_1 rats from the group receiving 25.6 mg/kg/day of hexachlorobenzene from the diet exhibited significantly increases in liver and adrenal weights, blood levels of eosinophils and basophils, serum IgM and IgG levels, slight cytoplasmic hyalinization of parenchymal cells in the liver, accumulation of foamy macrophages in the lung, increased extramedullary hematopoiesis in the spleen, and increased IgG response to *Trichinella*. In 15.4 and 25.6 mg/kg/day dose groups, effects included proliferation of high-endothelial venules in the paracortex of the lymph nodes, decreased resistance to *Listeria* and *Trichinella* infection, and increased IgG response to tetanus toxoid. Similar developmental effects were observed in an intermediate-duration reproductive study in which female Sprague-Dawley rats were fed hexachlorobenzene for 96 days prior to mating through two rounds of breeding (Kitchin et al. 1982); in both F_{1a} and F_{1b} pups, decreased body weight and decreased survival were observed at dietary concentrations resulting in doses ≥5.9 and 7.8 mg/kg/day, respectively.

The only animal developmental study in non-rodents used Rhesus monkey mothers (Bailey et al. 1980; Iatropoulos et al. 1978). Only one of three infant monkeys (between 21 and 118 days of age) nursing from mothers fed 64 mg/kg/day of hexachlorobenzene by daily gavage survived; the durations of dosing were 15 and 38 days (for the mortalities) and 60 days (for the survivor). Although the mothers were asymptomatic, both infant mortalities exhibited neurological effects (listlessness, lethargy, depression, and ataxia) and lung edema prior to death. Microscopic findings included mild hepatocellular hypertrophy in the infant that survived, and hepatic fatty changes, slight renal proximal tubule vacuolation, and mild cerebral gliosis in one or both infants that died.

A four-generation assay found increased liver weight and hepatic aniline hydroxylase activity at hexachlorobenzene dietary concentrations resulting in doses of approximately 3–4 mg/kg/day (F_{1a} and F_{3a} animals), consistently decreased pup weight at ≥6.9 mg/kg/day (all pup generations), and decreased pup viability at doses ≥13.8 mg/kg/day (F_{1a} and F_{1b} animals) (Grant et al. 1977). Arnold et al. (1985) fed hexachlorobenzene to male and female Sprague-Dawley rats from 3 months prior to mating through weaning; pups were continued on the same diet for their entire lifetime. The high-dose group (estimated doses of 2.8 and 3.2 mg/kg/day for F_0 parental males and females, respectively) exhibited decreased pup survival. When examined as adults (week 130), treatment-related effects in F_1 males included peribiliary lymphocytosis and fibrosis at an estimated dose of 0.022 mg/kg/day and hepatic basophilic chromogenesis at ≥0.55 mg/kg/day. The LOAEL of 0.022 mg/kg/day served as the basis for a chronic-duration oral MRL of 0.00007 mg/kg/day as described in the footnote to Table 3-2 and in Appendix A.

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

3.2.2.7 Cancer

The Department of Human Health Services (DHHS) considers the evidence for the carcinogenicity of hexachlorobenzene in experimental animals sufficient, and this chemical is reasonably anticipated to be a carcinogen in humans (NTP 2014). A cancer assessment for hexachlorobenzene is available on Integrated Risk Information System (IRIS 2003) in which the chemical is assigned to U.S. EPA cancer weight-of-evidence Group B2, probable human carcinogen, on the basis that oral administration of hexachlorobenzene has been shown to induce tumors in the liver, thyroid, and kidney in three rodent species. IRIS (2003) presents an oral slope factor of 1.6 per (mg/kg)/day and an inhalation unit risk of 0.00046 per

($\mu g/m^3$) for hexachlorobenzene based on hepatocellular carcinoma in orally exposed female rats (Ertürk et al. 1986).

No evidence of cancer was reported in a 25-year follow-up study of 161 people (Peters et al. 1982) or a 20–30-year follow-up study of 204 people (Cripps et al. 1984) who consumed hexachlorobenzene-contaminated grain in Turkey from 1955 to 1959. However, these two studies did not examine patients for internal cancer and were not designed to detect increases in cancer incidence. These follow-up studies (Cripps et al. 1984; Peters et al. 1982) did detect porphyria in some adults (17/204 and 33/161, respectively) who had been exposed as children to hexachlorobenzene (see Section 3.2.2.2, Hepatic Effects). This is relevant to cancer formation in humans because other epidemiology studies (unrelated to hexachlorobenzene) have found statistically significant associations between porphyria and increased risk of liver cancer. Fracanzani et al. (2001) reported that the presence of porphyria conferred a 5-fold increased risk of liver cancer. Linet et al. (1999) reported that porphyria cutanea tarda and acute intermittent porphyria were associated, respectively, with 20- and 70-fold increases in liver cancer and 3-fold increases in lung cancer. However, porphyria and liver cancer in the general population share common etiologies, so the association could possibly be causal (Axelson 1986; Salata et al. 1985; Topi et al. 1980; Waddington 1972).

Other available epidemiology studies that have assessed possible associations between hexachloro-benzene and cancer end points collectively do not support an association between hexachlorobenzene exposure and increased cancer incidence. However, limitations of these studies (including small study sizes, similar tissue hexachlorobenzene levels between cancer and control groups, and potentially confounding effects of other organochlorines) preclude definitive conclusions regarding the carcinogenicity hazard of hexachlorobenzene in humans.

Most case-control studies (ranging in size from 20 to >300 subjects per group) investigating organochlorine levels in serum or breast tissue samples surgically removed from groups of patients with breast cancer or benign breast tumors and serum or adipose tissue samples from subjects without diagnosed breast tumors were unable to detect statistically significant associations between hexachloro-benzene levels and breast cancer (Dorgan et al. 1999; Falck et al. 1992; Guttes et al. 1998; Høyer et al. 2001; Itoh et al. 2009; Iwasaki et al. 2008; Liljegren et al. 1998; López-Carillo et al. 2002; McCready et al. 2004; Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhammaa et al. 1990; Pavuk et al. 2003; Waliszewski et al. 2003; Zheng et al. 1999). Evidence of a possible association between serum hexachlorobenzene and breast cancer is provided by reports of significantly higher mean serum

hexachlorobenzene levels in breast cancer patients relative to controls with benign breast tumors or without evidence of breast tumors (Charlier et al. 2003, 2004; Dewailly et al. 1994). However, the numbers of breast cancer cases were small (n=≤50 for each study), which limits interpretation of the results.

No significant association was found between serum hexachlorobenzene and risk of testicular germ cell carcinoma in a population-based, case-control study of 18–44-year-old male residents of three Washington State counties (246 cancer cases and 630 controls) (Biggs et al. 2008). In a study of testicular germ cell tumor cases identified in the Norwegian Cancer Registry, no significant association was found between prediagnostic serum hexachlorobenzene (from blood samples taken between 1972 and 1978) and risk of testicular germ cell tumors among 49 cases diagnosed after blood samples had been taken; 51 control subjects were matched by region of residence, blood draw year, and age at blood draw (Purdue et al. 2009). Hardell et al. (2003, 2006b) found no significant association between serum hexachlorobenzene and testicular cancer among 58 cases and 61 age-matched controls in Stockholm, Sweden. The study authors noted that among case and control mothers who gave blood samples, serum hexachlorobenzene was significantly higher in the mothers of the men with testicular cancer compared to the mothers of the controls. Two case-control studies found no significant association between plasma hexachlorobenzene and prostate cancer (Aronson et al. 2010; Sawada et al. 2010). Hardell et al. (2006a) reported a significant association between hexachlorobenzene adipose tissue concentrations and risk of prostate cancer (OR=9.84; 95% CI 1.99, 48.5) among 26 prostate cancer cases with prostate-specific antigen (PSA) >16 ng/mL compared to 10 control subjects.

No significant associations were observed between blood hexachlorobenzene levels and risk of pancreatic cancer among 108 cases in the San Francisco Bay area (Hoppin et al. 2000), risk of endometrial cancer among 154 cases in Sweden (Weiderpass et al. 2000), risk of colorectal cancer among 132 cases in Barcelona, Spain (Howsam et al. 2004), or risk of non-Hodgkin's lymphoma (NHL) among 74 cases in Washington County, Maryland (Cantor et al. 2003). No significant association was observed between hexachlorobenzene bone marrow levels and risk of leukemia among 13 German leukemia patients (Scheele et al. 1996), hexachlorobenzene adipose tissue levels and risk of Ewing's sarcoma of the bone among 4 male Swedish patients (Hardell et al. 1997), or hexachlorobenzene adipose tissue levels and risk of NHL among 175 cases in the U.S. EPA National Human Adipose Tissue Survey (Quintana et al. 2004) or 256 cases enrolled in the Danish Cancer Registry (Bräuner et al. 2012).

In a population-based, case-control study in British Columbia, Canada, Spinelli et al. (2007) reported a significant association between plasma hexachlorobenzene and risk of NHL among 138 cases with hexachlorobenzene levels >22.78 ng/g compared to 83 cases with hexachlorobenzene levels ≤11.45 ng/g (OR=1.94; 95% CI 1.25, 3.03). Björnforth et al. (2007) reported a significant association between hexachlorobenzene adipose tissue levels and risk of pancreatic cancer among 21 cases (OR=53.0; 95% CI 4.64, 605) compared with controls.

Oral exposure of rats, mice, and hamsters to hexachlorobenzene has induced tumors in the liver ("liver cell" tumor, hepatocellular carcinoma, hepatoma, hemangiohepatoma, hemangioendothelioma, bile duct tumor) (see below). Individual studies have also reported statistically significant increases in the incidences of kidney (renal cell adenoma), thyroid (alveolar adenoma), parathyroid (adenoma), and adrenal gland (pheochromocytoma) tumors, as well as the induction of lymphosarcoma (non-Hodgkin's lymphoma) (Arnold et al. 1985; Ertürk et al. 1986). Female rats (Ertürk et al. 1986; Pereira et al. 1982; Smith and Cabral 1980) and mice (Cabral et al. 1979) appear to be more susceptible than males to the hepatocarcinogenic effects of hexachlorobenzene and limited evidence suggests that males are more susceptible to renal cancer (Ertürk et al. 1986). The cause of these gender specificities is unclear.

Chronic oral exposure to hexachlorobenzene induces liver tumors in rats, with female rats appearing more susceptible than males. Ertürk et al. (1986) fed hexachlorobenzene to Sprague-Dawley rats at dietary concentrations resulting in estimated doses of 5.2 or 10.3 mg/kg/day (males) and 6.0 or 12.0 mg/kg/day (females) for up to 2 years, with 9 interim sacrifices. Hexachlorobenzene induced statistically significant increases in the incidences of hepatoma, hepatocarcinoma, and renal carcinoma in both genders. In the liver, degenerative lesions were seen after 2–3 weeks, preneoplastic changes were detected after 200 days, and hepatocarcinomas were detected beginning at 300 days. Hepatomas, hemangiohepatomas, and hepatocellular carcinomas were significantly more common in females than in males. Bile duct adenomas (statistically significant increased incidence) and bile duct adenocarcinomas (not significant) were seen only in treated females. In contrast, renal adenomas and renal cell carcinomas were more frequent in males. Smith and Cabral (1980) detected liver cell tumors in all (14/14) female Agus rats and in a majority of female Wistar rats (4/6) fed hexachlorobenzene for 90 weeks at concentrations resulting in estimated doses of 7 and 4.5 mg/kg/day, respectively. Moreover, in those treated Wistar rats without tumors (2/6), evidence of preneoplastic changes (hepatocellular hypertrophy) was observed. Similar results were seen in a subsequent study (Smith et al. 1985). In Fischer 344/N rats fed hexachlorobenzene at dietary concentrations resulting in estimated doses of 15.8 mg/kg/day (males) and 18.3 mg/kg/day (females) for 90 weeks, all surviving females had multiple liver tumors and at least 50% exhibited

hepatocellular carcinomas. In contrast, only 16% of males exhibited liver tumors, which were smaller and limited to one per animal. Liver tumors stained heavily for gamma-glutamyl transpeptidase. In female (but not male) F_1 Sprague-Dawley rats with lifetime dietary exposure to hexachlorobenzene at a concentration resulting in an estimated dose of 3.2 mg/kg/day, the incidence of liver neoplastic nodules was significantly increased compared to controls (Arnold et al. 1985).

Hexachlorobenzene also induced liver tumors in mice and hamsters, but gender specificity was apparent only in mice. Syrian golden hamsters were fed 0, 4, 8, or 16 mg/kg/day of hexachlorobenzene in the diet for life (Cabral et al. 1977). All treatment groups exhibited statistically significant increases in incidences of hepatomas and hemangioendotheliomas (liver and spleen), with a slightly higher incidence in males than in females. Thyroid tumors were not seen in control groups, but were found in all treatment groups except low dose males; however, the increased incidence was significant only for males at 16 mg/kg/day. Outbred Swiss mice were fed 6, 12, or 25 mg/kg/day of hexachlorobenzene for up to 120 weeks (Cabral et al. 1979). Statistically significant increased incidence of liver cell tumors was observed at 12 and 25 mg/kg/day (but not 6 mg/kg/day) for both genders. Liver tumor incidence was significantly more common in females than in males treated with 25 mg/kg/day. Tumor multiplicity and size increased with increasing dose, while the latency period decreased.

A brief paper by Ertürk et al. (1986) reported the induction of liver and other tumor types in Sprague-Dawley rats, Swiss mice, and Syrian golden hamsters after only 90 days of exposure to hexachlorobenzene in the diet at 0, 100, or 200 ppm (mice) and 0, 200, or 400 ppm (rats and hamsters). However, the report is limited by its lack of methodology and quantitative results. Liver effects included hepatomas, metaplasia, and stromal activation. Some support for the findings of a rapid onset of liver tumor formation is found in intermediate oral dosing (10 days to 24 weeks) experiments with hexachlorobenzene that induced hepatocellular hypertrophy in rats (Den Besten et al. 1993; Smith et al. 1985), mice (Shirai et al. 1978), pigs (Den Tonkelaar et al. 1978), dogs (Sundlof et al. 1981), and Rhesus monkeys (Iatropoulos et al. 1976; Knauf and Hobson 1979). However, other intermediate-duration studies (13 weeks to 1 year) in rats did not detect neoplasia or preneoplastic effects in the liver or other organs (Goldstein et al. 1978; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Mollenhauer et al. 1975; Smith et al. 1979, 1985, 1986a).

Three studies in rats provide limited evidence that hexachlorobenzene is a promoter but not an initiator of liver cancer. Treatment of intact Sprague-Dawley rats with 100 ppm of hexachlorobenzene in the food for 45 days (estimated dose of 9.1 mg/kg/day) did not induce hepatic foci positive for gamma-glutamyl

transpeptidase, but foci were induced following liver initiation by partial hepatectomy with and without diethylnitrosoamine (a known liver tumor promoter) (Pereira et al. 1982). This finding suggests that hexachlorobenzene may act as a promoter at doses insufficient to initiate tumors. Abdo et al. (2013) administered diethylnitrosamine to male F344 rats by intraperitoneal injection at 200 mg/kg, followed by a 2-week period without treatment and subsequent administration of hexachlorobenzene in the diet for 6 weeks at 0, 70, or 350 ppm; a partial hepatectomy was performed at week 3. The low- and high-dose groups of hexachlorobenzene-treated rats exhibited significant increases in numbers of hepatic GST-P positive foci (approximately 2- and 5-fold, respectively, greater than that of controls) and areas of hepatic GST-P positive foci (approximately 2.3- and 4.2-fold, respectively, greater than that of controls). These results support the findings of Pereira et al. (1982). No evidence of altered foci were detected in male Fisher 344 rats pretreated with partial hepatectomy and treated with a single gavage dose of 5,000 mg/kg hexachlorobenzene followed by liver tumor promotion with carbon tetrachloride and cholic acid for 12 weeks (Tsuda et al. 1993).

Reported incidences of other tumor types have also increased following oral exposure to hexachlorobenzene. A group of five adult female Rhesus monkeys were dosed by gavage with hexachlorobenzene in methylcellulose daily for 60 days (Iatropoulos et al. 1976). Single monkeys received 0, 8, 32, or 64 mg/kg and two monkeys received 128 mg/kg. In one of the high dose monkeys (but not in the others), a benign mammary fibroadenoma was detected. This evidence is inconclusive, because of the low statistical power of the study. The other monkey given 128 mg/kg exhibited moderate adrenal medullary hyperplasia and the monkey given 64 mg/kg exhibited slight hyperplasia of the adrenal zona fasciculata; these findings support observations made in rats. In F_1 Sprague-Dawley rats exposed to hexachlorobenzene via their mothers during gestation and lactation (estimated maternal dose of 3.9 mg/kg/day) and directly from the feed for their lifetime (approximately 130 weeks postweaning; estimated dose 2.8 mg/kg/day for males and 3.2 mg/kg/day for females), statistically significant increases in adrenal pheochromocytomas were seen in both males and females, while a significantly increased incidence of parathyroid adenomas was observed only in females (Arnold et al. 1985). The biological significance of the adrenal effect is also supported by observations of adrenal gland cortical hyperplasia in female Wistar rats at doses as low as 9.5 mg/kg/day for 13 weeks (Den Besten et al. 1993) and in male and female Sherman rats exposed to hexachlorobenzene in the diet at doses at low as 10 mg/kg/day for 4 months (Kimbrough and Linder 1974). No other studies have reported any parathyroid histopathology caused by oral exposure to hexachlorobenzene, but parathyroid effects (changes in hormone levels) have been observed in male Fischer 344 rats (Andrews et al. 1988, 1989, 1990). The observations made during the 90-day study in which Sprague-Dawley rats, Swiss mice, and Syrian golden hamsters were administered

hexachlorobenzene in the food have not been corroborated (Ertürk et al. 1986). Renal damage with metaplastic regenerative changes was observed in treated animals; renal tumors were "most frequent in rats" and "more frequent in males." Lymphosarcomas (detected in the thymus, spleen, and lymph nodes) were apparently common in all treatment groups, with frequent lymphohematopoietic hyperplasia and lymphocytic infiltrations. These lesions occurred 2–4-fold more frequently in female mice than in male mice.

Randi and coworkers (García et al. 2010; Peña et al. 2012; Pontillo et al. 2011; Randi et al. 2006) performed a series of experiments designed to elucidate possible mechanisms of hexachlorobenzene mammary tumor co-carcinogenicity observed in rats (Randi et al. 2006). The investigators found that hexachlorobenzene: (1) induced cell proliferation in the MCF-7 breast cancer cell line in an estrogen receptor (ER) alpha-dependent manner; (2) induced migration in the MDA-MB-231 breast cancer cell line; and (3) increased cellular sarcoma/human growth factor receptor1 (cSrc/HER1) and ER alpha signaling pathways. The results suggest that alterations in the estrogenic microenvironment may influence the biological behavior of mammary gland or breast tumors.

The CEL (i.e., lowest dose that produced a tumorigenic response for each species), the duration category of exposure to hexachlorobenzene, and the estimated upper-bound risk levels from 10^{-4} to 10^{-7} are shown in Table 3-2 and plotted in Figure 3-2.

3.2.3 Dermal Exposure

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

3.2.3.1 Death
3.2.3.2 Systemic Effects
3.2.3.3 Immunological and Lymphoreticular Effects
3.2.3.4 Neurological Effects
3.2.3.5 Reproductive Effects
3.2.3.6 Developmental Effects
3.2.3.7 Cancer

3.3 GENOTOXICITY

Collectively, the results of available studies do not indicate that hexachlorobenzene acts as a genotoxic agent, although the database of information is limited.

An increased incidence of micronuclei was observed in the peripheral lymphocytes of 41 chemical workers in San Paulo, Brazil, who had been exposed to a mixture of chlorinated solvents that included hexachlorobenzene, as well as carbon tetrachloride and perchloroethylene (da Silva Augusto et al. 1997). The usefulness of this study is limited by the confounding effect of exposure to multiple chemicals.

No studies were located regarding genotoxic effects in animals following inhalation exposure to hexachlorobenzene.

No studies were located regarding the genotoxic effects of hexachlorobenzene in humans following oral exposure.

In vivo studies in rats revealed the lack of significant genotoxic activity in mammals following oral exposures to hexachlorobenzene. Negative results were observed in two dominant lethal mutation assays in which rats were exposed orally at doses ranging from 60 to 221 mg/kg (Khera 1974; Simon et al. 1979). No evidence of genotoxicity was observed in mouse liver, lung, kidney, spleen, or bone marrow after oral dosing (Sasaki et al. 1997). An oral exposure study to test the DNA induction potential of hexachlorobenzene in Wistar rats provided equivocal evidence that hexachlorobenzene reacts directly with DNA (Gopalaswamy and Nair 1992). Male rats were untreated or pretreated with phenobarbital (0.1% sodium phenobarbital in drinking water for 2 weeks) and then administered 25 mg/kg (specific activity 14.0 mCi/mmole) hexachlorobenzene in 0.1 mL refined peanut oil for 24 hours. The animals were sacrificed and DNA obtained from liver extracts. Upon analysis, hexachlorobenzene was observed to be bound to DNA (2.23±0.27 pmoles/mg DNA for phenobarbital untreated animals and 3.56±0.18 pmoles/mg DNA for phenobarbital pretreated animals). The comparative values for lindane in the same study were 5.82±0.31 and 6.90±0.14 pmoles/mg DNA, respectively. No hexachlorobenzene untreated control values were provided in the study report. However, there is evidence that phenobarbital is mutagenic *in vitro* in several test systems (Jackson et al. 1993). Other studies have likewise failed to observed gene mutations or unscheduled DNA repair in microbial assays (Gopalaswamy and Nair 1992; Haworth et al. 1983; Siekel et al. 1991).

No studies were located regarding genotoxic effects in humans or animals following dermal exposure to hexachlorobenzene.

Hexachlorobenzene did not produce chromosomal aberrations in human peripheral lymphocytes *in vitro* (Siekel et al. 1991). However, hexachlorobenzene produced weak positive results in assays for DNA fragmentation and micronuclei formation in primary cultures of human hepatocytes (Canonero et al. 1997). Treatment with hexachlorobenzene induced only minimal formation of DNA adducts in cultured human Hep G2 hepatoma cells (Dubois et al. 1997).

The micronucleus assay, but not the DNA fragmentation assay, was positive in cultured rat hepatocytes (Canonero et al. 1997). The researchers concluded that hexachlorobenzene is a weak genotoxic carcinogen and that negative responses in standard genotoxicity assays were due to limitations in the ability of exogenous metabolic activation systems to duplicate the complex interactions of the intact liver cell. Hexachlorobenzene was also positive in an assay for replicative DNA synthesis in mouse hepatocytes (Miyagawa et al. 1995). Treatment with hexachlorobenzene induced only minimal formation of DNA adducts in fetal hepatocytes from rats and quail (Dubois et al. 1997).

Hexachlorobenzene tested negative or ambiguous in reverse mutation assays in *S. typhimurium* (Gopalaswamy and Aiyar 1986; Gopalaswamy and Nair 1992; Haworth et al. 1983; Siekel et al. 1991) and *E. coli* (Siekel et al. 1991) with and without metabolic activation, although an assay for reverse mutation in the yeast *Saccharomyces cerevisiae* was positive (Guerzoni et al. 1976). Hexachlorobenzene also tested negative in a DNA repair assay in *E. coli* (Siekel et al. 1991).

Data from a study in male Long Evans rats suggested that the metabolism of hexachlorobenzene to pentachlorobenzene and other more polar metabolites proceed either through a free-radical mechanism or by initial formation of an arene oxide. These reactive intermediates may form covalent bonds with cellular constituents (such as protein amino acids or DNA nucleic acids) leading to irreversible cell damage (Lui and Sweeney 1975). Several other studies have also found evidence of binding to cellular proteins by reactive electrophilic metabolites of hexachlorobenzene formed by cytochrome P-450 system (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985).

Key *in vivo* genotoxicity studies are presented in Table 3-3 and *in vitro* genotoxicity studies are presented in Table 3-4.

Table 3-3. Genotoxicity of Hexachlorobenzene *In Vivo*

End point	Species (test system)	Exposure route	Results	Reference
Mammalian systems:				
Dominant lethals	Rat	Oral	–	Khera 1974
	Rat	Oral	–	Simon et al. 1979
DNA binding	Rat (Wistar)	Oral	±	Gopalaswamy and Nair 1992

– = negative result; ± = weakly positive; DNA = deoxyribonucleic acid

Table 3-4. Genotoxicity of Hexachlorobenzene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms: Reverse mutation				
Salmonella typhimurium	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium	Gene mutation	±	–	Gopalaswamy and Nair 1992
S. typhimurium	Gene mutation	–	–	Siekel et al. 1991
Escherichia coli	Gene mutation	–	–	Siekel et al. 1991
DNA Repair Assays				
E. coli	DNA repair	–	–	Siekel et al. 1991
Eukaryotic cells:				
Human peripheral blood lymphocytes	Chromosomal aberration	–	–	Siekel et al. 1991
Mammalian system:				
Rat (Wistar)	DNA binding	±	–	Gopalaswamy and Nair 1992

– = negative result; ± = weakly positive; DNA = deoxyribonucleic acid

3.4 TOXICOKINETICS

In humans, inhalation accounts for an unknown, but probably low, amount of exposure due to the low vapor pressure of hexachlorobenzene ($1.1x10^{-5}$ mmHg at 25 °C; see Table 4-2). Current information indicates that human absorption of inhaled hexachlorobenzene is poor; approximately two orders of magnitude less than the exposure estimate for the oral route (Arnot et al. 2010; Burns et al. 1974; Burton and Bennett 1987; Currier et al. 1980). Other data of absorption following inhalation exposure come from studies of occupational exposures (Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994; Selden et al. 1997), people living in the area of Flix, Spain, who have been exposed to airborne hexachlorobenzene from an organochlorine factory (Carrizo et al. 2008; Grimalt et al. 1994; Herrero et al. 1999; Ozalla et al. 2002; Ribas-Fitó et al. 2003a, 2003b, 2005, 2007; Sala et al. 1999b; Sunyer et al. 2002, 2008; To-Figueras et al. 1997), and people living in the area of Menorca, Spain, a rural county with no known source of high levels of atmospheric hexachlorobenzene (Carrizo et al. 2008). Based on information from an epidemic resulting from ingestion of hexachlorobenzene-contaminated bread in Turkey, ingested hexachlorobenzene is moderately absorbed from the gastrointestinal tract (Albro and Thomas 1974; Cam and Nigogosyan 1963; Gocmen et al. 1989; Peters et al. 1982). However, most of the hexachlorobenzene body burden in the U.S. population derives from dietary intake of fatty foods (Arnot et al. 2010; Burton and Bennett 1987). Schlummer et al. (1998) estimated that 85.4% of ingested hexachlorobenzene will be absorbed when the blood contains no hexachlorobenzene, and that this percentage will be reduced by 0.2% for each ng of hexachlorobenzene per g lipid in blood, and hypothesized a “fat-flush” theory of hexachlorobenzene absorption: temporary increases in lipid content in the gut dilute hexachlorobenzene concentrations and increase the diffusion gradient from the gut into the lymph and blood. Data from animal studies indicate that the gastrointestinal absorption of hexachlorobenzene is quite variable, depending upon the solvent vehicle used for administration, ranging from 6% when administered in aqueous solution to 82% when administered with squalene in cottonseed oil (Albro and Thomas 1974), olive oil (Freeman et al. 1989; Goldey et al. 1990; Knauf and Hobson 1979; Koss and Koransky 1975; Mehendale et al. 1975; Sundlof et al. 1982; Villeneuve and Hierlihy 1975), or peanut oil (Ingebrigtsen and Nafstad 1983; Ingebrigtsen et al. 1981). The lymphatic system has been shown to play an important role in the gastrointestinal uptake of hexachlorobenzene in animals (Iatropoulos et al. 1975). Although no empirical data are available on the dermal absorption of hexachlorobenzene in humans, data from a rat study were used to develop a compartment model for application to a 70-kg worker. Using these data, the dermal absorption constant for hexachlorobenzene was calculated as $1.4x10^{-3}$ per hour (Koizumi 1991).

Information on distribution in people following inhalation exposure to hexachlorobenzene is limited (Ataniyazova et al. 2001; Sala et al. 1999c, 2001b) and no information is available on the distribution of inhaled hexachlorobenzene in animals. However, orally absorbed hexachlorobenzene distributes widely in mammalian tissue, rapidly partitioning to blood, liver, breast milk, adipose tissue, endocrine organs, bone marrow, and ovarian follicular fluid (Ellenhorn and Barceloux 1988; Foster et al. 1995a; Ingebrigtsen 1986; Ingebrigtsen and Nafstad 1983; Knauf and Hobson 1979; Wickstrom et al. 1983), and preferentially distributing to adipose tissue or organs with high fat content (Burton and Bennett 1987; Cohn et al. 1978; Koss and Koransky 1975; Lecavalier et al. 1994; Mehendale et al. 1975; Robinson et al. 1990; Teufel et al. 1990; van Raaij et al. 1993a; Verschueren 1983). Animal studies of oral dosing have showed that levels of hexachlorobenzene increase in a dose-dependent manner in all tissues up to 100 mg/kg/day (Foster et al. 1995a; Jarrell et al. 1993; Sundlof et al. 1982). Hexachlorobenzene body burden is readily transferred from pregnant mother to the fetus through the placenta in animals (Courtney and Andrews 1985; Courtney et al. 1979; Cripps 1990; Goldey et al. 1990; Nakashima and Ikegari 2000; Nakashima et al. 1997, 1999; Villeneuve and Hierlihy 1975; Villeneuve et al. 1974a). Additionally, hexachlorobenzene is concentrated in the milk and can be transferred to the suckling neonate (Bailey et al. 1980; Cripps 1990; Goldey et al. 1990; Nakashima and Ikegari 2000; Nakashima et al. 1997, 1999). Evidence from animal studies indicates that protein-poor diets may promote the preferential partitioning of ingested hexachlorobenzene to fatty tissue (Rodrigues et al. 1991). In a survey of the U.S. population, it was found that concentration of hexachlorobenzene tended to increase with increasing age, a testimony to the propensity of hexachlorobenzene to bioaccumulate in mammalian tissue (Robinson et al. 1990). In a group of 350 German children, blood hexachlorobenzene levels (and levels of other organochlorines) correlated strongly with the length of breast-feeding (Karmaus et al. 2001). Weak associations were seen between decreased blood hexachlorobenzene levels and increased child body mass index (above 18 kg/m^2), and between increased hexachlorobenzene levels and both maternal age at birth (36–45-year-old group only) and late birth order (3rd or later, with spacing between children of at least 4 years). These data suggest that increased size may dilute hexachlorobenzene in the body, and that levels of hexachlorobenzene in mothers may increase with age. No correlations were seen for mothers who smoked during pregnancy, or the age and gender of the child.

Hexachlorobenzene is slowly metabolized in mammals, and the majority of hexachlorobenzene is excreted unchanged (in feces). Reductive dechlorination of hexachlorobenzene—catalyzed by enzymes located in the microsomal fraction of liver, lung, kidney, and intestine—appears to be an important pathway for the metabolism of hexachlorobenzene (Ingebrigtsen et al. 1986). It has been suggested that epoxide formation also occurs in this metabolism (Lui et al. 1976). Pentachlorophenol has been identified

in human liver preparations incubated with hexachlorobenzene (Koss et al. 1986). In animals, hexachlorobenzene is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system, conjugated with glutathione to yield glutathione conjugates excreted in the bile, or reductively dechlorinated to form pentachlorobenzene. Other metabolites include less chlorinated benzenes, pentachlorothiophenol, chlorophenols, S-conjugated phenols, and S-conjugated benzenes. Pentachlorophenol is subsequently converted to tetrachlorohydroquinone (Hahn et al. 1988, 1989; Linko et al. 1986; Mehendale et al. 1975; Rozman et al. 1977a). The feces contain mostly unchanged parent compound, and about 1% pentachlorobenzene and traces of pentachlorophenol after oral hexachlorobenzene exposure in mammals, while urinary excretion consists of mostly the metabolites, pentachlorobenzene, 2,4,5-trichlorophenol, *N*-acetyl-*S*(pentachlorophenyl)cysteine (a mercapturic acid), mercaptotetrachlorothioanisole, and tetrachlorobenzene, 2,3,5,6-tetrachlorobenzene-1,4-diol; and unchanged parent compound (Koss et al. 1978; Mehendale et al. 1975; Rizzardini and Smith 1982; Rozman et al. 1978). Pentachlorothiophenol, pentachlorophenol, methylthiopentachlorobenzene, 1,4-bis-(methylthio)-2,3,5,6-tetrachlorobenzene, chlorophenols, S-conjugated phenols and benzenes, and less chlorinated benzenes have also been identified in the liver following oral exposure in animals (D'Amour and Charbonneau 1992; Engst et al. 1976; Ingebrigtsen et al. 1981, 1986; Jansson and Bergman 1978; Koss et al. 1976, 1979; Lui and Sweeney 1975; Renner 1988; Richter et al. 1981; Stewart and Smith 1986; To-Figueras et al. 1992; van Ommen et al. 1985, 1989; Yang et al. 1978). Sex differences in the metabolism of hexachlorobenzene in the adult animals have been reported. Urinary excretion of pentachlorophenol, 2,3,5,6-tetrachlorobenzene-1,4-diol, and pentachlorothiophenol was greater in females than in males in this study (Rizzardini and Smith 1982).

To-Figueras et al. (2000) observed a high correlation between fecal and blood levels of hexachlorobenzene in 53 people highly exposed to airborne hexachlorobenzene. No information is available on the excretion of hexachlorobenzene following inhalation exposure in animals or following dermal exposure in humans or animals. In humans, ingested hexachlorobenzene is excreted in the urine mainly as its metabolites, pentachlorophenol and pentachlorothiophenol (To-Figueras et al. 1992). In animals, the excretion of hexachlorobenzene appears to be quite variable, depending upon the solvent vehicle used (Albro and Thomas 1974; Rozman et al. 1977a). Based on decreasing concentrations in the liver, the biological half-life of hexachlorobenzene has been estimated to be 8 days at the start of the elimination phase, 10 weeks after 3 months, and 12 months after 1.5 years (Koss et al. 1983), suggesting differential release of hexachlorobenzene from tissue stores, perhaps as a function of lipophilicity. Ingested hexachlorobenzene is excreted predominantly in the feces, mainly as unchanged parent compound, and to a lesser extent in the urine, as its metabolites (pentachlorophenol, pentachlorothiophenol, pentachloro-

benzene) (Mehendale et al. 1975). Approximately 99% of unchanged ingested hexachlorobenzene was excreted in the feces; 50% of urinary excretion was pentachlorophenol, 25% was pentachlorobenzene, and 25% was unchanged hexachlorobenzene in Rhesus monkeys treated with 0.03 mg/kg/day in the diet for 15 months (Rozman et al. 1977a). Based on animal studies, the urinary excretion of hexachlorobenzene exhibits sex- and age-specific differences; the excretion of pentachlorothiophenol increases with sexual maturity in female rats and slightly decreases in male rats (To-Figueras et al. 1991). Biliary excretion was not an important excretory pathway in rats given a single hexachlorobenzene dose of 10 mg/kg by gavage in peanut oil, accounting for <4% of the administered dose (Ingebrigtsen et al. 1981).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Limited data show that hexachlorobenzene can be absorbed through the respiratory tract in humans, although no information is available as to the rate and extent of respiratory tract absorption of hexachlorobenzene in either humans or animals.

Spanish researchers have studied a population with long-term exposure to high levels of hexachlorobenzene in air (Grimalt et al. 1994; Herrero et al. 1999; Ozalla et al. 2002; Sala et al. 1999b; Ribas-Fitó et al. 2003a, 2003b, 2005, 2007; Sunyer et al. 2002, 2008; To-Figueras et al. 1997). The rural Spanish village of Flix contains an organochlorine factory that has been producing volatile chlorinated solvents for decades, and no other large industrial facilities. Following complaints of odor, approximately 40 air samples were collected in July and November of 1989 and May and October of 1992 at diverse sites in the village. As a control, five air samples were collected in the city of Barcelona. Average air levels of hexachlorobenzene in Flix (35 ng/m^3) were over 100-fold higher than in Barcelona (0.3 ng/m^3), while other organochlorines were found at similar or lower concentrations in Flix than in Barcelona. Corresponding to the high air levels, it was found that residents of Flix had unusually high serum levels of hexachlorobenzene (mean of 39.8 ng/mL based on a total number of 604 tested) in comparison to populations in Barcelona (mean=4.13 ng/mL, n=100), the United States (mean=0.19 ng/mL, n=370), Croatia (mean=1.00, n=15), and Germany (mean=1.12, n=6). Serum levels of other organochlorines in Flix residents were much lower than hexachlorobenzene levels and did not differ from other populations. Among Flix residents, serum hexachlorobenzene levels were several fold higher in factory workers (mean=93.4 ng/mL, n=185) than other residents (mean=16.9 ng/mL, n=419). Factory workers were presumably exposed to much higher air levels of hexachlorobenzene than other village residents, and some may have had dermal exposure as well. It is noteworthy that mean serum hexachlorobenzene levels

in Flix residents who did not work at the factory (and therefore, can be assumed to have had no direct dermal exposure to hexachlorobenzene) were still 4-fold higher than Barcelona residents. However, the difference was not entirely due to inhalation exposure. In addition to working at the factory, other variables associated with serum hexachlorobenzene levels were age and consumption of local fish. Among women (very few of whom had ever worked at the factory), the geometric mean serum hexachlorobenzene level was 14.9 ng/mL in those that did not eat local fish (176/180) and 18.2 ng/mL in those that did (only 4/180). Therefore, indirect exposure to hexachlorobenzene via consumption of contaminated fish may have contributed slightly to serum hexachlorobenzene levels in nonfactory worker Flix residents, but was not a major factor. Studies of the rural Spanish village of Flix show that exposure to high levels of hexachlorobenzene in air leads to high levels of hexachlorobenzene in serum, and that a significant portion of hexachlorobenzene uptake in this situation can be attributed to inhalation and absorption across the respiratory tract.

Studies of workers with occupational exposure to hexachlorobenzene, where exposure was probably primarily by inhalation, but may have involved dermal contact as well, also show increased serum levels of hexachlorobenzene in the exposed workers. Selden et al. (1997) found significantly higher serum hexachlorobenzene levels in 29 hazardous waste incineration workers (63 ng/g lipid) than in 60 matched controls (35 ng/g lipid). The exposed workers also had significantly increased serum hexachlorobenzene levels compared with their own historical samples given before the start of employment (0.40 ng/g plasma vs. 0.27 ng/g plasma). Airborne hexachlorobenzene levels in different locations in the plant ranged from 0.066 to 11 ng/m^3. Queiroz et al. (1997, 1998a, 1998b) observed that each of the 51 workers on leave from a closed chemical plant had blood hexachlorobenzene levels >0.1 μg/dL (mean=4.4 μg/dL), while controls chosen from blood donors at the local blood bank to be similar in age and race to the exposed group all had blood hexachlorobenzene levels lower than the limit of detection (0.02 μg/dL). The plant produced carbon tetrachloride and tetrachloroethylene; hexachlorobenzene was generated as a byproduct of the production process as a solid residue. Richter et al. (1994) documented high serum hexachlorobenzene levels in workers exposed to 2.1–10.8 mg/m^3 of hexachlorobenzene in air, which persisted even after air concentrations of hexachlorobenzene were reduced to 0.012–0.022 mg/m^3. Although dermal exposure cannot be ruled out in these studies, uptake of hexachlorobenzene across the respiratory tract is likely to have contributed significantly to hexachlorobenzene body burden in all of these studies.

No studies were located regarding inhalation exposure to hexachlorobenzene in animals.

3.4.1.2 Oral Exposure

Widespread occurrence of porphyria cutanea tarda in southeast Anatolia in Turkey in the late 1950s was shown to be due to ingestion of bread made from grain that had been treated with hexachlorobenzene (Cam and Nigogosyan 1963). The ingested dose of hexachlorobenzene was estimated to be in the range of 0.05–0.2 g/day, or 0.7–2.9 mg/kg/day for a 70-kg person. The occurrence of systemic health effects following ingestion of hexachlorobenzene demonstrates that this chemical can be absorbed via the gastrointestinal tract in humans, and in amounts sufficient to produce serious health effects. Follow-up studies conducted between 1977 and 1981 found that hexachlorobenzene levels in 56 samples of human milk obtained from porphyric mothers averaged 0.51 ppm (standard deviation [SD]=0.75 ppm, highest value=2.8 ppm), while levels in women from families without porphyria or outside the affected area had an average level of 0.07 ppm (SD=0.07 ppm) (Peters et al. 1982). Therefore, even 20 years after exposure, there was a large difference in breast milk hexachlorobenzene concentrations between people from families that had consumed the contaminated grain and those that did not.

One study was located in which gastrointestinal absorption of hexachlorobenzene in humans was quantified. Schlummer et al. (1998) used a mass-balance approach to estimate absorption of hexachlorobenzene ingested at low concentrations in the diet in seven volunteers (four males and three females) ranging in age from 24 to 81 years. Hexachlorobenzene was measured in the food (uniform meals of varying portion sizes) ingested by volunteers over a 3-day period (using duplicate portions) and in the corresponding feces (first and last meals identified using iron capsules to produce black feces). Similar experiments were then conducted in which the volunteers chose their own foods. Whole blood samples were collected 3 weeks after the last mass balance experiment. Percent net absorption was calculated as the difference between ingested and excreted hexachlorobenzene, divided by the ingested amount. When fed a standardized meal, the percent absorption was a relatively uniform 70–82% in the four young adults tested (one female and three males ranging in age from 24 to 36 years). It decreased to 1% in a 53-year-old male volunteer and further to -56 and -210% in 76- and 81-year-old female volunteers, respectively. The negative values in the elderly volunteers indicate net excretion, rather than absorption, in these individuals. Similar results were reportedly obtained when volunteers chose their own meals.

Blood levels of hexachlorobenzene (expressed as ng/g blood lipid) also varied with age, ranging from 65 to 82 in the young adult volunteers and increasing to 230, 680, and 1,420 in the 53-, 76-, and 81-year-old volunteers, respectively (Schlummer et al. 1998). A trend for decreasing net absorption ([ingested – excreted in feces]/ingested) with increasing blood levels was observed in the volunteers, and a linear

regression was fit to these data. The calculated regression equation was (R^2=0.98):net absorption hexachlorobenzene = 0.8538–0.0021 x (ng hexachlorobenzene/g blood lipid). This equation predicts nearly complete absorption of ingested hexachlorobenzene (approximately 85%) at low blood concentrations and that net absorption decreases by approximately 0.2% for each ng increase in hexachlorobenzene per g lipid in blood.

Gastrointestinal absorption of hexachlorobenzene has been well studied in laboratory animals. Ingebrigtsen and Nafstad (1983) demonstrated that gastrointestinal absorption of hexachlorobenzene in an oil vehicle is rapid. Male Wistar rats were given a single gavage dose of 0.4 mg/kg of radiolabeled hexachlorobenzene dissolved in peanut oil and examined by whole body autoradiography at various time intervals starting 2 hours after dosing. A considerable amount of radiolabeled material was absorbed and distributed throughout the body within 2 hours of dosing, and peak levels were reached within 24 hours. Rapid absorption was also shown in beagle dogs that had hexachlorobenzene levels in blood monitored during and after 7 days of daily oral dosing with 10 or 100 mg/kg of hexachlorobenzene in corn oil by capsule (Sundlof et al. 1982). Peak blood concentrations occurred 3 hours after dosing in low-dose dogs, and after a somewhat longer (unspecified) interval in high-dose dogs. Blood concentrations continued to increase over several days after the last dose was administered in both groups, possibly due to continued absorption from the intestines during that time. This finding suggests that while the bulk of an oral dose of hexachlorobenzene in oil is absorbed rapidly in a few hours, absorption of residual quantities can continue for a period of days.

Hexachlorobenzene administered by gavage in aqueous methylcellulose suspension is also absorbed from the gut within a few hours (Iatropoulos et al. 1975). Sprague-Dawley rats given single gavage doses of 0.15 mg (approximately 0.6 mg/kg) of radiolabeled hexachlorobenzene in 1% methylcellulose solution were sacrificed at intervals between 1 and 48 hours after dosing for determination of tissue radioactivity levels. The ingested material was absorbed by the walls of the stomach and duodenum within 1 hour of dosing, and by the jejunum and ileum within 3 hours. Peak levels in the duodenum and jejuno-ileum occurred 3 hours after dosing. The majority of ingested hexachlorobenzene was absorbed from these regions of the small intestine by the lymphatic system and deposited in the fat, bypassing portal circulation to the liver, systemic circulation, and the excretory organs.

Although absorption of hexachlorobenzene from aqueous suspension occurred in a similar time frame as absorption from an oil vehicle in the studies described above, other studies have shown that the extent of absorption from aqueous suspension is much less (as would be expected based on a water solubility of

0.006 mg/L at 20–25 °C; see Table 4-2). Koss and Koransky (1975) measured absorption of radiolabeled hexachlorobenzene in female Wistar rats following gavage administration of the chemical in olive oil at doses of 20, 60, and 180 mg/kg, and in aqueous suspension (6% gum arabic in water) at doses of 16, 120, and 970 mg/kg. Two days after dosing in olive oil, 73–88% of the administered radioactivity was recovered in the body, while 1% was recovered in the gut contents, 18–26% in the feces, and 0.4–0.6% in the urine in the different dose groups. This finding suggests oral absorption of about 80% of ingested hexachlorobenzene, regardless of dose, when administered in oil. When administered in aqueous suspension, however, absorption was much lower and appeared to depend on dose. At the low dose of 16 mg/kg, roughly 20% of administered radioactivity was recovered in body tissues 3 days after dosing in aqueous suspension, compared with 0.4% in the gut contents, 74% in the feces, and 0.4% in the urine. At the higher doses, only 2–5% of the administered hexachlorobenzene was absorbed from the aqueous suspension.

Other studies determined absorption of hexachlorobenzene from oil vehicles to be similar to that reported by Koss and Koransky (1975). Albro and Thomas (1974) gave male CD rats single gavage doses of 12 or 30 mg/kg of hexachlorobenzene in cottonseed oil. They found that after 96 hours, 72% (high dose) to 82% (low dose) of the dose had not been excreted in the feces. No hexachlorobenzene was detected in the bile or urine, and only about 3% of the dose was present in the intestinal tissue and contents (primarily the former), and an associated *in vitro* experiment showed that fecal bacteria do not metabolize hexachlorobenzene; this suggests that the "removed" 72–82% had been absorbed into the body. Ingebrigtsen et al. (1981) used bile duct cannulated rats to monitor biliary excretion of radiolabeled hexachlorobenzene after gavage dosing with 10 mg/kg in peanut oil. A total of 3.6% of the administered radioactivity was recovered in the bile within 48 hours, while bile flow remained steady, showing that biliary excretion is only a minor pathway for hexachlorobenzene. After 96 hours, approximately 25% of the administered radioactivity was recovered in the feces and 2% in the urine. These data again suggest oral absorption of somewhere near 80% of the ingested dose for hexachlorobenzene administered in oil.

The studies described above showed that absorption of hexachlorobenzene in the gut is much more extensive from oil vehicles than from aqueous vehicles. Zabick and Schemmel (1980) demonstrated that a high fat diet also enhances absorption of hexachlorobenzene, in comparison to a high carbohydrate diet. Groups of 6 female Osbourne-Mendel rats were fed either a high fat diet or one of two high carbohydrate diets (one using corn starch, the other using sucrose) supplemented with 32 mg/kg/day of hexachlorobenzene for 6, 12, or 18 days. The high fat diet resulted in higher carcass fat content (data not shown; cited to Shier and Schemmel 1975) and greatly increased concentrations of hexachlorobenzene in

perirenal fat, liver, and gastrocnemius. At the same time, concentrations of hexachlorobenzene in the feces were much lower with the high fat diet, suggesting that the high fat diet facilitated absorption of hexachlorobenzene from the gut, thereby leading to the increase in tissue levels. The data from all of these studies showing enhanced absorption of hexachlorobenzene when administered in oil or a high fat diet are consistent with the "fat-flush" hypothesis for hexachlorobenzene absorption proposed by Schlummer et al. (1998) based on the human data.

3.4.1.3 Dermal Exposure

No studies were located regarding absorption in humans following dermal exposure to hexachlorobenzene.

Evidence from rats suggests that hexachlorobenzene can be absorbed across the skin. Using radiolabelled hexachlorobenzene, Koizumi (1991) conducted a mass-balance study of dermal absorption in male Fisher 344 rats. A dermal dose of approximately 2.5 mg/kg of 14C-hexachlorobenzene dissolved in tetrachloroethylene was applied to a 4 cm^2 clipped area on the back under occlusion. The rats, 3 per group, were transferred to metabolic cages and sacrificed after 6, 24, or 72 hours. Cumulative absorption of hexachlorobenzene (the sum recovered from the urine, feces, liver, carcass, skin not directly contaminated, and subcutaneous tissue) increased with duration of exposure from 1.05% of the applied dose after 6 hours to 2.67% after 24 hours and 9.71% after 72 hours. A one-compartment linear pharmacokinetic model was used to calculate an absorption constant of 1.40×10^{-3} per hour.

A modeling exercise conducted by Koizumi (1991) suggests that dermal absorption of hexachlorobenzene can contribute significantly to body burden in exposed workers. Assuming the rate constant in rats applies to man and a biological half-life ranging from 100 to 730 days, a three-compartment linear pharmacokinetic model developed based on the rat data and scaled up to a 70-kg man showed that hexachlorobenzene blood levels will increase with duration of exposure and that dermal doses as low as 2.56–18.2 mg (which could result from contamination small enough to go unnoticed) could, over a period of years, lead to hexachlorobenzene blood levels in the vicinity of 200 ppb, regarded by some researchers (Currier et al. 1980) as the upper safe limit in humans.

Koizumi (1991) also collected data showing that washing can decrease the absorption of dermally-contacted hexachlorobenzene by a significant degree. In the rats, washing the test area with soap 6 hours

after application of hexachlorobenzene removed 34% of the applied dose and reduced the cumulative amount absorbed after 72 hours by 50% (from 9.71 to 4.90%).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Information regarding the distribution of hexachlorobenzene in humans exposed via inhalation derives mainly from studies of people employed by, or living near, an organochlorine-producing electrochemical factory in the area of Flix, Spain, where high levels of airborne hexachlorobenzene were detected. Mean serum hexachlorobenzene levels as high as 26–37 ng/mL have been reported in groups of local inhabitants (Grimalt et al. 1994; Sala et al. 1999c). A mean serum hexachlorobenzene level of 54.6 ng/mL was noted for a group of males who worked at the factory (Sala et al. 1999b). Among inhabitants of the area, relatively high levels of hexachlorobenzene have been measured in maternal blood, umbilical cord blood, and breast milk samples of people living nearby. Sala et al. (2001a) compared two populations; for a group of 31 pairs of mothers and infants from Flix, the respective geometric means of hexachlorobenzene maternal and cord blood levels were 3.98 ng/mL (range 0.50–20.78 ng/mL) and 1.40 ng/mL (range 0.30–5.77 ng/mL). For subjects from villages nearby to Flix, the respective geometric means of hexachlorobenzene maternal and cord blood levels were 2.51 ng/mL (range 0.36–7.46 ng/mL) and 0.85 ng/mL (range 0.13–2.45 ng/mL). A statistically significant correlation between maternal and umbilical cord blood was detected. Several other reports include measurements of elevated hexachlorobenzene levels in serum and/or cord blood from inhabitants of the area surrounding Flix (Ribas-Fitó et al. 2003a, 2003b, 2007; Sunyer et al. 2002, 2008; To-Figueras et al. 2000). Ribas-Fitó et al. (2005) reported a statistically significant ($p>0.05$) correlation between breastfeeding and serum hexachlorobenzene levels in 1-year-old children living in Flix. Hexachlorobenzene and other organochlorines (*p,p'*-DDE, PCBs) were measured in the colostrum of mothers and cord blood serum of infants in 92 infant-mother pairs within the first 3 days of delivery. Hexachlorobenzene levels were measured in breast milk at 3 weeks, and serum blood hexachlorobenzene concentration was measured again at 13 months after delivery. Hexachlorobenzene concentrations in breastfed and formula-fed children at 13 months post-delivery were 4.26 and 2.13 ng/mL, respectively. However, the correlation between breastfeeding and hexachlorobenzene levels in children did not remain statistically significant after adjustment for parity, maternal age, maternal body mass index, and residence time in Flix.

In a study on serum hexachlorobenzene levels in 4-year-old children in Ribera d'Ebra county, Spain, a location exposed to high levels of atmospheric hexachlorobenzene and Menorca, Spain, a rural county

with no known source of hexachlorobenzene (Carrizo et al. 2008), breastfed children had statistically higher concentrations of hexachlorobenzene than formula-fed children ($p<0.01$ in the high-exposure population and $p<0.0001$ in the low-exposure population). Mean concentrations of hexachlorobenzene in breastfed versus nonbreastfed children from Ribera d'Ebra were 1.5 ng/mL (range 0.30–0.58 ng/L) and 0.99 ng/mL (range 0.17–2.05 ng/L), respectively, and mean concentrations in breastfed versus nonbreastfed children from Menorca were 0.47 ng/mL (range 0.067–2.1 ng/L) and 0.23 ng/mL (range 0.11–0.46 ng/L), respectively. In the Menorca population, there was a statistically significant correlation ($p<0.05$) between serum hexachlorobenzene concentration in children and maternal body mass index. Maternal feeding was the primary route of exposure of children in both populations.

The Aral Sea in Uzbekistan is a putative source of airborne exposure to hexachlorobenzene, metals, and other pollutants because its water levels are decreasing, resulting in sediment-to-wind dissemination over the surrounding area. Ataniyazova et al. (2001) analyzed 18 maternal blood samples, 28 umbilical cord blood samples, and 41 milk samples collected from mothers and infants within 200 kilometers of the southern border of the Aral Sea in Uzbekistan. The respective mean concentrations of hexachlorobenzene in maternal and cord blood were 167 ng/L (range 72–9,920 ng/L) and 70 ng/L (range 25–1,300 ng/L) and in 93% of milk samples, with a mean of 28 ng/g fat (range 10–109 ng/L).

No studies were located regarding distribution of hexachlorobenzene following inhalation in animals.

3.4.2.2 Oral Exposure

Hexachlorobenzene rapidly distributes throughout the body following absorption. A whole-body autoradiography experiment in rats showed that hexachlorobenzene was extensively distributed throughout the body within 2 hours of receiving a single oral dose of 0.4 mg/kg in peanut oil (Ingebrigtsen and Nafstad 1983). In another experiment, radiolabeled hexachlorobenzene was found in multiple internal tissues in rats 3 hours after the rats received a single oral dose of about 0.6 mg/kg in aqueous methylcellulose (Iatropoulos et al. 1975).

Although hexachlorobenzene is widely distributed in the body, it is not evenly distributed. Due to its lipophilic nature, hexachlorobenzene distributes preferentially to fat, and to a lesser extent, other lipid-rich tissues. In the Iatropoulos et al. (1975) study, radiolabel occurred at the highest levels and was most persistent in the mesenteric lymph node and adipose tissue. Levels were much lower and declined rapidly in the lung, liver, and kidney. The researchers interpreted these findings to indicate that a significant

fraction of the gastrointestinally absorbed hexachlorobenzene is transported via the lymphatic system to the fat, bypassing portal circulation to the liver and systemic circulation to the excretory organs.

Numerous studies have shown preferential distribution of hexachlorobenzene to adipose tissue. In the rat autoradiography study mentioned above, Ingebrigtsen and Nafstad (1983) found peak levels of radioactivity in the fat to be roughly 60-fold higher than peak levels in the blood and 30-fold higher than peak levels in the brain and liver. Besides the fat, other tissues found to contain relatively high concentrations of radioactivity included the skin, bone marrow, Harderian gland, nasal mucosa, preputial gland, and intestinal tract. Koss and Koransky (1975) found similar results in rats given single gavage doses of 20–180 mg/kg of radiolabeled hexachlorobenzene in oil, with radioactivity in fat two days after administration being about 60-fold higher than in blood, 30-fold higher than in liver, and 5-fold higher than in skin. Levels in brain and kidney were intermediate between liver and blood, while levels in muscle were lower. Lecavalier et al. (1994) also found approximately 30-fold higher concentrations of hexachlorobenzene in the fat than in the liver 14 days after a single gavage dose of 400 or 600 mg/kg of hexachlorobenzene in corn oil. Brain and kidney concentrations were slightly (within a factor of 2) lower than liver concentrations, while serum levels were an order of magnitude lower than liver concentrations. In a repeated dose study, ovariectomized adult female rats treated by gavage in oil with 1, 10, or 100 mg/kg/day of hexachlorobenzene for 30 days had hexachlorobenzene concentrations in fat that were 30-fold higher than levels in liver, which were in turn 20-fold higher than levels in serum. Levels in the adrenals were roughly 20-fold greater than levels in liver (Foster et al. 1995a). A study in which rats were dosed with 50 mg/kg of hexachlorobenzene by gavage in oil every other day for 15 weeks also showed hexachlorobenzene concentrations in the fat to be 30–60 times greater than concentrations in the liver, brain, kidney, and blood throughout dosing and a subsequent 38-week observation period (Koss et al. 1978).

Preferential distribution to fat and high lipid tissues has also been demonstrated in other animal species. Dogs given seven consecutive daily doses of 10 or 100 mg/kg/day of hexachlorobenzene in corn oil by capsule had peak hexachlorobenzene concentrations in fat that were 30-fold greater than peak liver levels (Sundlof et al. 1982). Other tissues with relatively high levels (5- to 10-fold greater than liver) were the skin, adrenal, and thyroid. Levels in the kidney, heart, brain, spleen, pancreas, and muscle were similar to levels in the liver. Cynomolgus monkeys treated with 0.1, 1, or 10 mg/kg/day of hexachlorobenzene in gelatin capsules for 90 days had hexachlorobenzene concentrations in fat that exceeded liver concentrations by 10–15-fold (Jarrell et al. 1993). Kidney and brain hexachlorobenzene levels were lower than in the liver, while serum and follicular fluid levels were much lower still (Foster et al. 1995b;

Jarrell et al. 1993). In Rhesus monkeys given oral doses of 8–128 mg/kg/day of hexachlorobenzene by gavage in aqueous methyl cellulose for 60 days, the highest concentrations of hexachlorobenzene were found in the body fat and bone marrow, with considerably lower concentrations in the adrenals, liver, kidney, brain, ovaries, muscle, and serum (Knauf and Hobson 1979).

The animal studies showed that levels of hexachlorobenzene increased in a dose-dependent manner in all tissues, at least at doses up to around 100 mg/kg/day. The study by Foster et al. (1995a) found dose-dependent increases in tissue levels of hexachlorobenzene in rats treated with 1, 10, or 100 mg/kg/day, the study by Sundlof et al. (1982) found dose-dependent tissue levels in dogs treated with 10 or 100 mg/kg/day, and the study by Jarrell et al. (1993) found dose-dependent tissue levels in monkeys treated with 0.1, 1, or 10 mg/kg/day. In the fat, the increase in hexachlorobenzene concentration was directly proportional to dose at doses up to 100 mg/kg/day in dogs (Sundlof et al. 1982), 10 mg/kg/day in rats (Foster et al. 1995a), and 1 mg/kg/day in monkeys (Jarrell et al. 1993). Lecavalier et al. (1994) found no difference in tissue levels of hexachlorobenzene following dosing with 400 or 600 mg/kg in rats, showing that dose-dependence is lost at high doses. Knauf and Hobson (1979) found no clear relationship between tissue levels of hexachlorobenzene and dose in Rhesus monkeys given between 8 and 128 mg/kg. However, their highly variable results may have been due to very small group sizes (one or two monkeys per dose) and, they speculated, variation in the amount of body fat in the monkeys used. Although Koss and Koransky (1975) tested multiple dose levels, their results were not presented in sufficient detail to assess dose-dependence of tissue levels.

The effect of repetitive weight gain and weight loss on the metabolism of hexachlorobenzene in mice was investigated by Jandacek et al. (2005). Radiolabeled hexachlorobenzene ([^{14}C]-hexachlorobenzene) was administered 1 time via gavage in groups of eight C57BL/6 mice at a dose of 0.7 µCi/day. The groups were then given alternating diets high in fat or of reduced calorie over 5-day intervals to simulate a “yo-yo diet”. Higher hexachlorobenzene concentrations in the brain, kidney, and adipose tissue were associated with loss of body fat, as was an increase in plasma hexachlorobenzene with prolonged caloric restriction. Hexachlorobenzene levels in the epididymal fat pad remained constant during caloric restriction. Weight regains resulted in a statistically significant (significance level not reported) increase in liver lipids and hexachlorobenzene concentrations.

In humans, data regarding tissue concentrations of hexachlorobenzene are limited to autopsy cases and easily sampled tissues and fluids, such as breast milk and blood serum. Two studies were located in which both breast adipose/milk and blood serum were collected from the same individuals and analyzed

for hexachlorobenzene. In a study of 36 Connecticut women between 50 and 80 years of age, hexachlorobenzene was detected in the breast adipose of all 36 women (median concentration=17.7 ng/g fat), but was not detected in the serum of any of these women (quantitation limit=0.6 ng/g) (Archibeque-Engle et al. 1997). In a study of seven pregnant/lactating New York women (each with a differing interval between collection of blood and milk samples), hexachlorobenzene was detected in the serum (0.03–0.29 ng/g), but at lower concentrations than in the milk (0.21–0.74 ng/g) (Greizerstein et al. 1999). The difference between serum and milk levels can be attributed in part to the differing lipid content of these fluids. Lipid-normalized concentrations of hexachlorobenzene were 8–48.4 ng/g lipid in serum and 11–22.5 ng/g lipid in milk. Three other studies were located that looked at both breast adipose/milk and serum levels of hexachlorobenzene in the same populations, although not necessarily in the same individuals. In Veracruz, Mexico, a group of 65 volunteer mothers in the hospital for delivery had average hexachlorobenzene blood serum concentrations of 1.1 ng/g, and a group of 60 volunteer mothers in the hospital for Cesarian delivery (extent of overlap with blood volunteers unknown) had average hexachlorobenzene concentrations in milk fat and adipose tissue of 25 and 58 ng/g fat, respectively, 30 days after delivery (Waliszewski et al. 1999a, 1999b). Similarly, a group of six women from a city in northern Germany had a mean serum hexachlorobenzene level of 1.0 ng/g, while breast milk samples from seven women from northern Germany in the same year (overlap with blood donors not known) showed a mean hexachlorobenzene concentration of about 70 ng/g fat (Petzold et al. 1999). Ntow et al. (2008) evaluated organochlorine levels (including hexachlorobenzene) in serum samples from 115 subjects (56 males and 59 women) and breast milk samples from 109 women (45 of whom also provided serum) among vegetable farmers in Ghana. Mean levels of hexachlorobenzene in pooled serum samples and pooled milk samples were 5.3 and 4.9 ng/g lipid, respectively. Collectively, these results indicate that hexachlorobenzene is readily stored in fat, which may result in long-term health implications.

In studies of 199 full-term healthy neonates in Fulda and Dusseldorf, Germany, the median hexachlorobenzene cord blood concentration from the 95th percentile by rank of hexachlorobenzene concentration in both locations was 0.5 µg/L in 1998 (Lackmann 2002). This concentration was approximately 90% less than levels measured in cord blood of neonates from the same localities sampled in 1994 and 1995 (Lackmann et al. 1996).

An association between breastfeeding and serum hexachlorobenzene levels in 6-week-old infants was examined by Lackmann (2004). Blood samples from 25 breast- and bottle-fed infants born in Germany were analyzed for hexachlorobenzenes, PCBs, and *p,p'*-DDE. Mean hexachlorobenzene levels were significantly different ($p<0.0001$) for breastfed and bottle-fed infants (0.13 and 0.04 µg/L, respectively).

Confounding factors such as gestational age, age of the mother, and smoking behavior of the parents did not alter significance levels. Lackmann (2006) reported significantly ($p<0.0001$) different mean serum hexachlorobenzene levels among breast- and bottle-fed infants 6 months of age as well (0.43 and 0.073 µg/L, respectively).

Link et al. (2005) found that 10-year-old children in Baden-Wuerttemberg, Germany, who had been breastfed as babies had statistically significantly ($p<0.0001$) higher concentrations of hexachlorobenzene and other organochlorines (*p,p'*-DDE, PCBs, gamma-hexachlorocyclohexane, dioxins, furans) than formula-fed children. Blood serum levels of hexachlorobenzene and other constituents were measured in fourth grade primary school children between 1996 and 2003; 1,614 blood samples were analyzed individually and 2,372 blood samples were pooled (30–70 individual samples were typically pooled per analysis) and analyzed during this time period. Mean concentrations of serum hexachlorobenzene levels in the individual blood samples (computed on a yearly basis) were 0.21, 0.14, 0.12, and 0.08 µg/L in breastfed children and 0.017, 0.12, 0.09, and 0.07 µg/L in nonbreastfed children in 1996/1997, 1998/1999, 2000/2001, and 2002/2003, respectively. In individually analyzed samples, boys had a statistically significantly ($p<0.0001$) higher blood concentration of hexachlorobenzene than girls after adjusting for influencing factors such as age, weight, concentration of triglycerides and cholesterol, duration of breastfeeding, investigation area, and year. There was also a positive association ($p=0.0004$) between serum triglycerides and hexachlorobenzene concentration in individual samples after adjusting for influencing factors. Hexachlorobenzene showed a statistically significant ($p<0.0001$) decrease in concentration during the time period investigated.

Hexachlorobenzene concentrations in breast milk of women from Tromsø and Oslo, Norway collected between 2000 and 2002 were low, averaging 18 ng/g (Polder et al. 2008). Breast milk samples were collected between 2000 and 2002, as part of the third World Health Organization (WHO)-coordinated exposure study, from 29 women living in Norway and analyzed for hexachlorobenzene, hexachlorocyclohexane, chlordanes, trans-nonachlor, PCBs, *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE, mirex, toxaphenes, polybrominated diphenylethers, and hexabromocyclodecanes. There were no significant differences in concentrations between the locations studied. Compared to data published by Johansen et al. (1994), hexachlorobenzene levels in breast milk in Norway have declined 56% since 1991. Declining hexachlorobenzene levels in breast milk have been observed in other regions as well. For example, Klinčić et al. (2014) reported a nearly 9-fold decrease in hexachlorobenzene levels in breast milk from 2006 to 2009/2010 in Zagreb, Croatia. Zietz et al. (2008) reported a 43% reduction in breast milk hexachlorobenzene levels in northern Germany in 2006 compared to levels measured in 1999. Mikeš et

al. (2012) observed >10-fold decrease in hexachlorobenzene concentrations in breast milk samples collected in the Czech Republic between 1994 and 2009.

Levels of hexachlorobenzene and other organochlorines in follicular fluid were measured by De Felip et al. (2004). Follicular fluid specimens from 12 women undergoing *in vitro* fertilization at a fertility clinic in Rome, Italy were obtained, and the specimens were pooled into two samples (six specimens each) for analysis of hexachlorobenzene, PCBs, dioxins, furans, and *p,p'*-DDT and metabolites. Hexachlorobenzene concentrations in the follicular fluid of the two pooled samples were 0.021 and 0.022 ng/g wet weight (69 and 73 ng/g lipid basis).

An association between hexachlorobenzene in breast milk and age of mother and number of children was investigated by Ennaceur et al. (2007). Breast milk samples were obtained from 87 lactating mothers in Tunisia between September 2002 and February 2003 and analyzed for hexachlorobenzene, hexachlorocyclohexane (beta and gamma), dieldrin, DDT, DDD, and DDE (*o,p'*- and *p,p'*). All subjects tested contained hexachlorobenzene residues in their breast milk; the mean concentration was 0.260 mg/kg milk fat (range 0.003–3.127 mg/kg milk fat). No significant relationship between number of childbirths and concentration of hexachlorobenzene was found, and although a relationship between increasing hexachlorobenzene levels and mothers' age was observed, there was no statistical significance ($p>0.05$). Ntow et al. (2008) evaluated relationships between maternal age and levels of DDTs, hexachlorocyclohexanes, hexachlorobenzene, and dieldrin in breast milk samples from 51 farmers in Ghana. For hexachlorobenzene, breast milk levels increased nonsignificantly ($p=0.067$) with increasing age (range 20–40 years).

Other studies have found hexachlorobenzene in human blood (e.g., Alvarado-Hernandez et al. 2013; Arrebola et al. 2012; Becker et al. 2008; Chovancová et al. 2004; Croes et al. 2014a, 2014b; Porta et al. 2012; Rutten et al. 1988; Sala et al. 1999b; Schlummer et al. 1998; Siyali 1972), liver (Dewailly et al. 1999; Weistrand and Noren 1998), bone marrow (Bucholski et al. 1996; Scheele et al. 1995), brain (Dewailly et al. 1999), fat (e.g., Ansari et al. 1986; Arrebola et al. 2012; Dewailly et al. 1999; Lordo et al. 1996; Malarvannan et al. 2013; Robinson et al. 1990; Scheele et al. 1995; Siyali 1972; Teufel et al. 1990; Weistrand and Noren 1998), and breast milk (e.g., Behrooz et al. 2009; Çok et al. 2011; Colles et al. 2008; Craan and Haines 1998; Czaja et al. 1997; Devanathan et al. 2009; Ennaceur and Driss 2013; Ennaceur et al. 2007, 2008; Erdoğrul et al. 2004; Fujii et al. 2012; Gladen et al. 1999; Gocmen et al. 1989; Greizerstein et al. 1999; Guerranti et al. 2011; Haraguchi et al. 2009; Johansen et al. 1994; Johnson-Restrepo et al. 2007; Klinčić et al. 2014; Lunden and Noren 1998; Malarvannan et al. 2009;

Mannetje et al. 2013; Mikeš et al. 2012; Nasir et al. 1998; Newsome and Ryan 1999; Ntow et al. 2008; Petzold et al. 1999; Polder et al. 2008; Scheele et al. 1995; Shen et al. 2007; Szyrwińska and Lulek 2007; Tsydenova et al. 2006; Waliszewski et al. 1999a, 1999b; Weisenberg 1986; Wickstrom et al. 1983; Zhou et al. 2011, 2012; Zietz et al. 2008).

In human plasma, close to half of the hexachlorobenzene present is found in the lipoprotein depleted fraction (containing primarily albumin), while the rest is split between the high density (≈20%), low density (≈20%), and very low density (≈10%) lipoprotein fractions (Noren et al. 1999). Mean serum hexachlorobenzene levels have been declining in recent years. For example, the FLEHS reported a mean serum hexachlorobenzene level of 21 ng/g lipid for the years 2003–2004 (FLEHS I), whereas a mean serum hexachlorobenzene level of 8.34 ng/g lipid was noted for the years 2008–2009 (FLEHS II) (Croes et al. 2014a, 2014b).

Schlummer et al. (1998) found that blood levels of hexachlorobenzene (expressed as ng/g blood lipid) varied with age in a group of seven volunteers, ranging from 65 to 82 in four young adult volunteers and increasing to 230, 680, and 1,420 in the 53-, 76-, and 81-year-old volunteers, respectively. This result shows that hexachlorobenzene accumulates in people as they age. Tissue build up occurs because people are continually exposed to hexachlorobenzene in the environment and excretion is slow. In their rat experiments, Koss and Koransky (1975) observed an estimated elimination half-time of 8–10 days for hexachlorobenzene in the fat and other tissues following administration of a single gavage dose of 20–180 mg/kg. However, this finding was based on only a 14-day observation period. Koss et al. (1978) monitored tissue hexachlorobenzene levels 14 and 38 weeks after a 15-week dosing period (50 mg/kg every other day) in rats. Although the researchers could not produce an estimate of biological half-life, they found that the rate of elimination decreased over time and speculated that elimination of hexachlorobenzene might continue for years. This issue is discussed in more detail in Section 3.4.4 on Elimination and Excretion.

Human studies have shown that hexachlorobenzene can pass from the mother to the neonate through the placenta. For example, in a study of 160 full-term neonates in Germany, hexachlorobenzene was found at an average concentration of 2.03 µg/L in 1984/1985 and 0.61 µg/L in 1994/1995 in neonatal blood obtained from an unblocked peripheral vein within the first 12 hours of life before the first oral feeding (Lackmann et al. 1996, 1999). Ando et al. (1985) found hexachlorobenzene in maternal blood, placenta, and neonatal cord blood in 36 pregnant Japanese women and their babies. There was a statistically significant correlation between the concentration of hexachlorobenzene in the placenta and in cord blood.

Waliszewski et al. (1999c) reported a statistically significant correlation (R=0.87) between levels of hexachlorobenzene in maternal blood serum (mean=1 μg/L, detected in 100% of samples) and umbilical cord serum (mean=0.8 μg/L, detected in 98% of samples) in a group of 65 volunteer mothers in Veracruz, Mexico. Hexachlorobenzene was also found in the cord blood of all 63 births (geometric mean [GM]=1 μg/L) analyzed in the village of Flix, Spain (Sala et al. 1999a), in all 656 births (GM=0.04 μg/L) analyzed in Quebec, Canada (Rhainds et al. 1999), and at a mean concentration of 0.54 μg/L among 92.8% of 1,438 cord blood samples from deliveries in Shanghai, China (Cao et al. 2011). Alvarado-Hernandez et al. (2013) reported higher concentrations of hexachlorobenzene in umbilical cord plasma than in maternal plasma (median concentrations of 137 and 58 ng/g lipid, respectively) among mother infant pairs living in a rural agricultural area of Mexico.

Studies in laboratory animals support the findings in humans that hexachlorobenzene is transferred from pregnant mother to the fetus through the placenta. Hexachlorobenzene was found in the fetus and placenta of pregnant mice treated with 50 mg/kg/day of hexachlorobenzene by gavage in corn oil on days 7–11 of gestation and examined 24 hours after the last dose (Courtney et al. 1976). Follow-up studies by these researchers demonstrated that fetal (whole body) and placental hexachlorobenzene concentrations increased with dose, with the duration of dosing, and with the day of dosing (dosing later in gestation leads to higher levels) in mice, and that results in rats were similar to those in mice (Courtney and Andrews 1985; Courtney et al. 1979). Other studies showing transfer of maternal hexachlorobenzene to the fetus in rats were reported by Nakashima et al. (1997) and Cripps (1990). Nakashima et al. (1997) observed that lactational transfer of hexachlorobenzene was increased by feeding nursing rats high fat diets. Goldey et al. (1990) measured maternal and fetal tissue levels of hexachlorobenzene in rats given a total dose of 11 mg/kg over a 3-day period 3 weeks prior to breeding. They found that fetal blood and liver concentrations were slightly lower than maternal blood concentrations, while fetal brain levels were about half of the maternal blood levels. Villeneuve and co-workers measured hexachlorobenzene levels in fetal tissues after administering hexachlorobenzene at a series of dose levels. The fetuses of pregnant rats treated with 5–120 mg/kg/day of hexachlorobenzene by gavage in corn oil on days 6–16 of gestation and sacrificed for cesarian section on day 22 of gestation showed dose-related increases in hexachlorobenzene concentration in whole fetus, fetal liver, and fetal brain (Villeneuve and Hierlihy 1975). The concentration of hexachlorobenzene in fetal liver was about 20–40% of the concentration in maternal liver. The concentration in fetal brain was about half that in fetal liver. A similar study in rabbits also demonstrated dose-dependent placental transfer of hexachlorobenzene, although in this species, fetal liver concentrations of hexachlorobenzene were 2–3-fold higher than maternal liver concentrations (Villeneuve

et al. 1974a). Fetal brain levels, however, were much lower than fetal liver levels and were also less than maternal brain concentrations in rabbits.

The occurrence of hexachlorobenzene in breast milk of humans is well documented in many populations, as noted above. Many of the same animal studies that investigated placental transfer of hexachlorobenzene also studied lactational transfer from mothers to their offspring (Courtney and Andrews 1985; Cripps 1990; Goldey et al. 1990; Nakashima et al. 1997). These data are discussed in Section 3.4.4 on Elimination and Excretion.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution of hexachlorobenzene following dermal exposure in humans.

Male Fischer 344 rats that received a single dermal dose of approximately 2.5 mg/kg ^{14}C-hexachlorobenzene dissolved in tetrachloroethylene applied to a 4 cm^2 clipped area on the back absorbed only 9.7% of the dose; 90.3% of the applied dose remained unabsorbed on the skin after 72 hours. The concentration of hexachlorobenzene in the liver and blood increased steadily after dermal application. Washing decreased mean hexachlorobenzene concentrations in blood from 263 to 0.128 μg/g and in the liver from 0.68 to 0.556 μg/g liver at 72 hours. The authors developed a compartment model based on the data collected, for application to a 70-kg worker (Koizumi 1991).

3.4.3 Metabolism

3.4.3.1 Inhalation Exposure

Pentachlorophenol may be a metabolite of hexachlorobenzene, as measured in 4-year-old children exposed to high levels of atmospheric hexachlorobenzene (Carrizo et al. 2008). Serum hexachlorobenzene, pentachlorobenzene, and pentachlorophenol were measured in populations of preschoolers (age 4 years) from the town of Flix, Spain, where there are high atmospheric concentrations of hexachlorobenzene, and from Menorca, in the Balearic Islands, a rural area not exposed to any known source of hexachlorobenzene. Neither area has any known source of pentachlorophenol exposure. Both hexachlorobenzene and pentachlorophenol levels in Flix children were higher than the Menorca population. The correlation between hexachlorobenzene and pentachlorophenol levels in Flix children

suggests a precursor-metabolite relationship. No other studies were located regarding metabolism in humans or animals after inhalation exposure to hexachlorobenzene.

3.4.3.2 Oral Exposure

Hexachlorobenzene is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms) (Den Besten et al. 1994; Mehmood et al. 1996; Schielen et al. 1995a), conjugated with glutathione to yield a glutathione conjugate and ultimately pentachlorothiophenol, or reductively dechlorinated to form pentachlorobenzene (Ingebrigtsen et al. 1986; Koss et al. 1979; Renner 1988). Other metabolites include less chlorinated benzenes, chlorophenols, S-conjugated phenols, and benzenes (Den Besten et al. 1994; Koss et al. 1986). Pentachlorophenol is subsequently converted to tetrachlorohydroquinone (Mehmood et al. 1996; van Ommen et al. 1985).

Pentachlorobenzene and pentachlorophenol were identified as the major metabolites of 14C-labeled hexachlorobenzene (0.03 mg/kg/day) administered in the diet to Rhesus monkeys for 15 months (Rozman et al. 1977a). In the urine, approximately 50% of the excreted radiolabel was pentachlorophenol, 25% was pentachlorobenzene, and the remaining 25% consisted of unidentified metabolites and unchanged hexachlorobenzene. Of the excreted radioactivity in the feces, 99% was unchanged hexachlorobenzene, with <1% pentachlorobenzene and trace amounts of pentachlorophenol. A subsequent report of a similar study in Rhesus monkeys found that fecal excretion consisted of 99% unchanged parent compound, about 1% pentachlorobenzene, and traces of pentachlorophenol (Rozman et al. 1978). Urinary metabolites consisted of 50–75% pentachlorophenol. The remainder of radioactivity (25–50%) was composed of pentachlorobenzene, hexachlorobenzene, and tetrachlorobenzene. Only unchanged parent compound was found in the plasma, and the red blood cells contained 95% unchanged parent compound and 5% pentachlorophenol.

A similar metabolic pattern was observed in the rat. Extraction and analysis of fecal radioactivity, which accounted for 16% of the administered dose, 7 days after gavage administration of 5 mg/kg of ^{14}C-labeled hexachlorobenzene in arachis oil to rats did not reveal the presence of metabolites. Urine, which contained 0.85% of the administered radiolabel, contained 2,4,5-trichlorophenol, pentachlorophenol, and several unidentified chlorinated benzenes (Mehendale et al. 1975). Gas/liquid chromatography-mass spectrometry identified 20% of the radioactivity as parental hexachlorobenzene together with the metabolites pentachlorothiophenol and pentachlorophenol in isolated perfused rat (male Wistar) liver treated with ^{14}C-hexachlorobenzene diluted with unlabeled hexachlorobenzene to yield a concentration of

0.1 mg hexachlorobenzene/mL perfusate. Most of the radioactivity was found in the perfusate and in the liver; unchanged hexachlorobenzene was responsible for most of the radioactivity. Traces of pentachlorothiophenol and pentachlorophenol were identified in the perfusate and the liver, respectively (Ingebrigtsen et al. 1981, 1986). A study reported that 98% of biliary radioactivity, which constituted 3.6% of the administered dose 48 hours after administration, was in the form of metabolites; 25% of this radioactivity was glutathione-conjugated pentachlorophenol (Ingebrigtsen et al. 1981). No sulfur-containing metabolites of hexachlorobenzene were found in the bile. However, a study of the metabolic fate of hexachlorobenzene (particularly as it relates to transformation of hexachlorobenzene into any methylthio- and methylsulfonyl-metabolites) in male Wistar rats identified methylthiopentachlorobenzene and 1,4-bis-(methylthio)-2,3,5,6-tetrachlorobenzene as metabolites of hexachlorobenzene (Jansson and Bergman 1978). These compounds were excreted to a greater extent than the corresponding monosubstituted metabolites. One methylthiotetrachlorobenzene was also found. Pentachlorophenol was the only detectable metabolite in blood, liver, urine, or feces of female Wistar rats 38 weeks after the end of 15-week gavage exposure to 50 mg/kg/day of hexachlorobenzene (Koss et al. 1978).

In other rat studies, N-acetyl-S(pentachlorophenyl)cysteine (PCTP-NAC) was the most abundant urinary product in female Wistar rats administered dietary hexachlorobenzene in 4% corn oil for 13 weeks resulting in doses of 7.5 or 15 mg/kg/day (Den Besten et al. 1994) or treated twice a week for 35 weeks by gavage with 50 mg/kg in olive oil (Rietjens et al. 1995). Other rats in the Den Besten et al. (1994) study were similarly administered dietary levels of 0.03 or 0.13% pentachlorobenzene to provide a basis for comparison. Pentachlorophenol and tetrachlorohydroquinone were common urinary metabolites of both hexachlorobenzene and pentachlorobenzene. Mercaptotetrachlorothioanisole (MTCTA), which was excreted as a glucuronide, was also detected in the urine of rats given hexachlorobenzene. Pentachlorophenol, pentachlorothiophenol, 2,3,4,6- and 2,3,5,6-tetrachlorophenol, and pentachlorobenzene were identified as metabolites of hexachlorobenzene in another study (Richter et al. 1981). Significant sex-related differences were observed, with higher amounts of pentachlorothiophenol observed in the livers of female rats. This was accompanied by a slower decrease in hepatic levels of hexachlorobenzene in the female rat liver compared to the male liver. Sex differences in the metabolism of hexachlorobenzene in the adult rat have also been observed. After 10 weeks of treatment, the urinary excretion of pentachlorophenol, 2,3,5,6-tetrachlorobenzene-1,4-diol, and pentachlorothiophenol was greater in females than in males in this study (Rizzardini and Smith 1982). Sex-related differences in biotransformation of hexachlorobenzene could account for differences observed in porphyrinogenic activity of hexachlorobenzene in male and female rats (D'Amour and Charbonneau 1992; Richter et al. 1981; Rizzardini and Smith 1982).

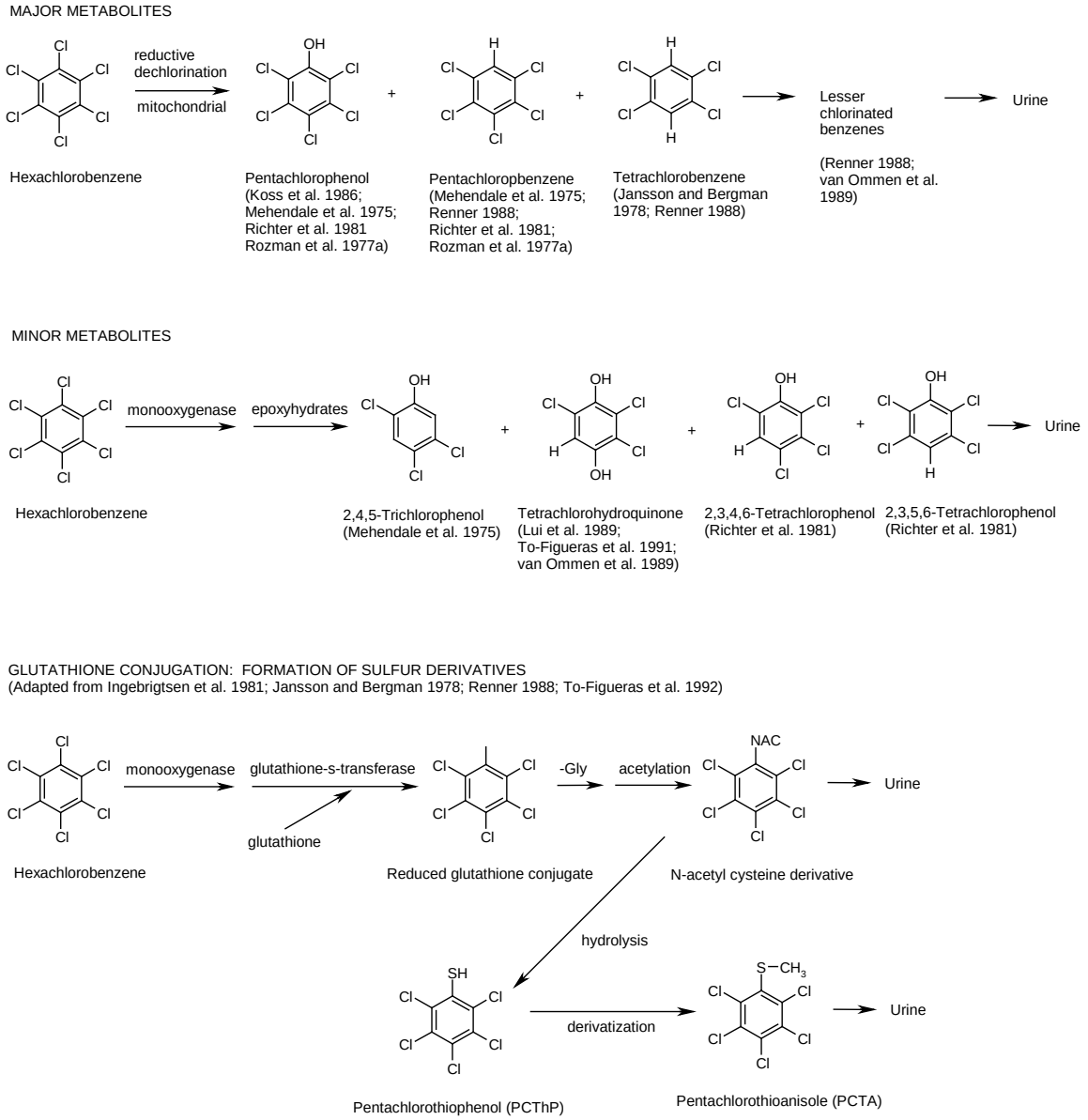
Two alternatives have been proposed for the mechanism of conversion of hexachlorobenzene to pentachlorophenol: formation of an epoxide or free radical attack on the carbon-chlorine bond. Substitution of adjacent carbons with chlorine argues against a mechanism involving an epoxide; either cleavage of the bond by free radical attack followed by hydroxylation, or conjugation with glutathione seems more plausible. However, if the presence of o- and p-hydroxy derivatives of pentachlorophenol could be confirmed, there would be a strong possibility that epoxides are intermediates in the dechlorination or hydroxylation of hexachlorobenzene or both (Lui et al. 1976). *In vitro* studies with perfused rat livers demonstrated that 14C-labeled hexachlorobenzene was converted to acidic conjugates (45%), while 5% was converted to sulfate or glucuronic acid conjugates (Ingebrigtsen et al. 1986). There is evidence that mammalian metabolism of hexachlorobenzene to pentachlorophenol is mediated by the hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms; others) (Den Besten et al. 1994; Mehmood et al. 1996; Schielen et al. 1995a).

Several other studies in laboratory animals also identified the following mammalian biotransformation products of hexachlorobenzene: pentachlorophenol (Ingebrigtsen et al. 1981; Koss et al. 1976, 1979; Lui and Sweeney 1975; van Ommen et al. 1985; Yang et al. 1978); pentachlorothiophenol (D'Amour and Charbonneau 1992; To-Figueras et al. 1992); less chlorinated benzenes, chlorophenols, S-conjugated phenols and benzenes (Engst et al. 1976; Koss et al. 1979; Renner 1988; Stewart and Smith 1986); and tetrachlorohydroquinone and tetrachlorocatechol (Koss et al. 1976, 1979; Lui et al. 1976; Mehmood et al. 1996; van Ommen et al. 1985, 1989). The various pathways and metabolites of hexachlorobenzene are depicted in Figure 3-3.

There is some evidence to indicate that metabolism of hexachlorobenzene to pentachlorophenol is accelerated in rats fed fish oil, in comparison to lard or soybean oil (Umegaki and Ikegami 1998). Rats fed fish oil had significant increases in liver cytochrome P-450, blood pentachlorophenol and pentachlorophenol:hexachlorobenzene ratio, and urinary excretion of pentachlorophenol, while levels of hexachlorobenzene in the feces were unchanged (indicating no difference in absorption between groups).

Limited human data are consistent with results from animal studies. Portions (0.5 g) of abdominal subcutaneous adipose tissue obtained as histological samples during surgery of patients and urine from these patients in Germany were extracted with benzene for hexachlorobenzene and its metabolites (Koss et al. 1986). Hexachlorobenzene was detected in the adipose tissue of the patients, while only pentachlorophenol was detected in the urine. A correlation was found between levels of hexachlorobenzene

Figure 3-3. Metabolism and Urinary Metabolites of Hexachlorobenzene



found in adipose tissue and urinary pentachlorophenol. However, it is possible that the urinary pentachlorophenol originated from other chlorinated hydrocarbons such as pentachlorobenzene or pentachloronitrobenzene. Human cytochrome P-450 3A4 expressed in the yeast *Saccharomyces cerevisiae* metabolized hexachlorobenzene to pentachlorophenol, which was further transformed to tetrachlorohydroquinone, in both *in vitro* and *in vivo* experiments (Mehmood et al. 1996).

3.4.3.3 Dermal Exposure

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

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

To-Figueras et al. (2000) observed a high correlation between fecal and blood levels of hexachlorobenzene in a group of 25 men and 28 women from Flix, Spain. This population was highly exposed to airborne hexachlorobenzene from a nearby chlorinated solvent factory. The geometric mean of hexachlorobenzene in blood was 163 µg/5.4 L (30.2 µg/L). Estimated fecal excretion of unchanged hexachlorobenzene was 10.4 µg/day, 4–6.4% of the estimated total blood level. No unchanged hexachloro benzene was detected in urine; urinary excretion of metabolites was 5.1 µg/day, 1.8–3.1% of the estimated total blood level.

No studies were located regarding excretion of hexachlorobenzene in animals following inhalation exposure.

3.4.4.2 Oral Exposure

Elimination of absorbed hexachlorobenzene is slow and occurs primarily via the feces, with smaller amounts being excreted in the urine. Hexachlorobenzene eliminated in the feces is predominantly unchanged parent compound, although small amounts of various metabolites have also been found. Conversely, hexachlorobenzene in the urine is almost all in the form of metabolites.
Both biliary and intestinal excretion contribute to fecal excretion of hexachlorobenzene. Bile duct cannulated rats given hexachlorobenzene by gavage excreted 3.6% of the administered dose in the bile within 48 hours (Ingebrigtsen et al. 1981). Although one report suggested that biliary excretion was more

important than intestinal excretion (Sundlof et al. 1982), other studies have shown that biliary excretion is a minor contributor to fecal excretion. Rozman et al. (1981) estimated biliary excretion to account for about 10% of fecal excretion in rats and monkeys treated with oral hexachlorobenzene. Intestinal excretion was responsible for the bulk of the fecal excretion in this study. Feeding with aliphatic hydrocarbons (mineral oil, hexadecane) enhanced fecal excretion in both rats and monkeys, with a corresponding decrease in blood and adipose hexachlorobenzene concentrations, primarily due to increased elimination of hexachlorobenzene in the large intestine (Rozman et al. 1981). In contrast to aliphatic hydrocarbons, cholestyramine, which interferes with enterohepatic recycling, had no effect on fecal excretion of hexachlorobenzene (confirming the minor role of biliary excretion for this chemical), and sesame oil produced only a very small increase in fecal hexachlorobenzene excretion (possibly by increasing gastrointestinal absorption). Richter and Schafer (1981) showed that addition of hydrocarbons (light liquid paraffin and squalane) to the perfusion medium enhanced elimination of unchanged hexachlorobenzene in perfused intestine into the lumen of the jejunum, ileum, and colon. The researches hypothesized that the hydrocarbons, which are not significantly absorbed, act as a lipophilic compartment in the gut lumen, shifting the equilibrium between gut wall and lumen in favor of the lumen for hydrophilic substances such as hexachlorobenzene. This is consistent with the fat-flush hypothesis of gastrointestinal absorption proposed by Schlummer et al. (1998). After fat-flush enhanced lipid absorption in the duodenum and jejunum is complete, the diffusion gradient is reversed in subsequent portions of the intestines.

In lactating mothers, breast milk is also an important route of excretion for hexachlorobenzene. Hexachlorobenzene has been detected in human breast milk in numerous studies spanning virtually all regions of the world (e.g., Behrooz et al. 2009; Çok et al. 2011; Colles et al. 2008; Craan and Haines 1998; Czaja et al. 1997; Devanathan et al. 2009; Ennaceur and Driss 2013; Ennaceur et al. 2007, 2008; Erdoğrul et al. 2004; Fujii et al. 2012; Gladen et al. 1999; Gocmen et al. 1989; Greizerstein et al. 1999; Guerranti et al. 2011; Haraguchi et al. 2009; Johansen et al. 1994; Johnson-Restrepo et al. 2007; Klinčić et al. 2014; Lunden and Noren 1998; Malarvannan et al. 2009; Mannetje et al. 2013; Mikeš et al. 2012; Nasir et al. 1998; Newsome and Ryan 1999; Ntow et al. 2008; Petzold et al. 1999; Polder et al. 2008; Scheele et al. 1995; Shen et al. 2007; Szyrwińska and Lulek 2007; Tsydenova et al. 2006; Waliszewski et al. 1999a, 1999b; Weisenberg 1986; Wickstrom et al. 1983; Zhou et al. 2011, 2012; Zietz et al. 2008). Several animal studies have quantified lactational transfer of hexachlorobenzene from mothers to their offspring (Courtney and Andrews 1985; Cripps 1990; Goldey et al. 1990; Nakashima et al. 1997). These studies confirm the importance of breast milk as a route of elimination in the mother and as a source of exposure in neonates.

Studies that monitored elimination of hexachlorobenzene for an extended period of time noted that the rate of elimination decreases over time (Koss et al. 1978, 1983; Sundlof et al. 1982; Yang et al. 1978). In rats treated with hexachlorobenzene every other day for 6 weeks, the elimination half-time was estimated as a relatively rapid 8 days soon after exposure stopped, a much slower 10 weeks 3 months later, and a very slow 12 months after 1.5 years, suggesting that elimination of hexachlorobenzene could continue for years (Koss et al. 1978, 1983). Yang et al. (1978) and Sundlof et al. (1982) both applied 3-compartment pharmacokinetic models to their data on dogs and monkeys, respectively. Sundlof et al. (1982) obtained elimination half-time estimates ranging from 6 weeks to 3 years in the three dogs modeled. Yang et al. (1978) calculated elimination rate constants corresponding to half-times of 91–114 days in two monkeys, but also performed additional modeling exercises that suggested elimination half times as long as 250 years. Freeman et al. (1989) used a physiologically based pharmacokinetic (PBPK) model of hexachlorobenzene in the rat to show that approximately 75% of the decline in fat concentrations over time is due to growth (i.e., dilution), with only 25% due to excretion. Thus, the growth of animals during experiments may affect the apparent half-life of hexachlorobenzene. A PBPK model developed by Yesair et al. (1986) predicted a half-life of 215 days for hexachlorobenzene in a growing human female exposed to doses of 0.5–32 mg/kg/day for 15 weeks at 15 years of age.

3.4.4.3 Dermal Exposure

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

3.4.4.4 Other Routes of Exposure

No studies were located regarding excretion in humans after other routes of exposure to hexachlorobenzene.

The major portion of injected hexachlorobenzene is eliminated unchanged in feces, while a smaller fraction, composed of metabolites, is eliminated in urine. Yang et al. (1978) administered a single intravenous dose of hexachlorobenzene to monkeys; 1 year after intravenous injection, only 28.2% of the administered dose had been excreted in the feces, 90% unchanged, and in urine, 1.6% of the total dose was excreted as metabolites (no unchanged compound detected). The researchers attributed slow elimination of hexachlorobenzene to long-term storage of the chemical in the fat. Unchanged hexachlorobenzene and metabolites were detected in bile. Yang et al. (1978) administered a single intravenous dose

of hexachlorobenzene to rats; within 48 hours, only 1% appeared in the feces, and 0.2% in the urine. Following a single intraperitoneal injection of radiolabeled hexachlorobenzene (4 mg/kg) in rats, approximately 34% of the administered radioactivity was recovered in the feces over the following 14 days, 80% of which was unchanged parent compound (Koss and Koransky 1975). By contrast, only 5% of the administered radioactivity was recovered in the urine, and only 4% of that was unchanged parent compound. A 14-day recovery period was used due to the slow elimination of hexachlorobenzene from the body. Neither Koss and Koransky (1975) nor Yang et al. (1978) detected hexachlorobenzene elimination in air expired by treated animals. Yang et al. (1978) calculated an initial half-life of 91–114 days, and subsequent half-lives as long as 250 years. Hexachlorobenzene and/or its metabolites were found in the bile after intravenous injection of hexachlorobenzene in monkeys, suggesting the possibility of biliary excretion (Yang et al. 1978).

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.

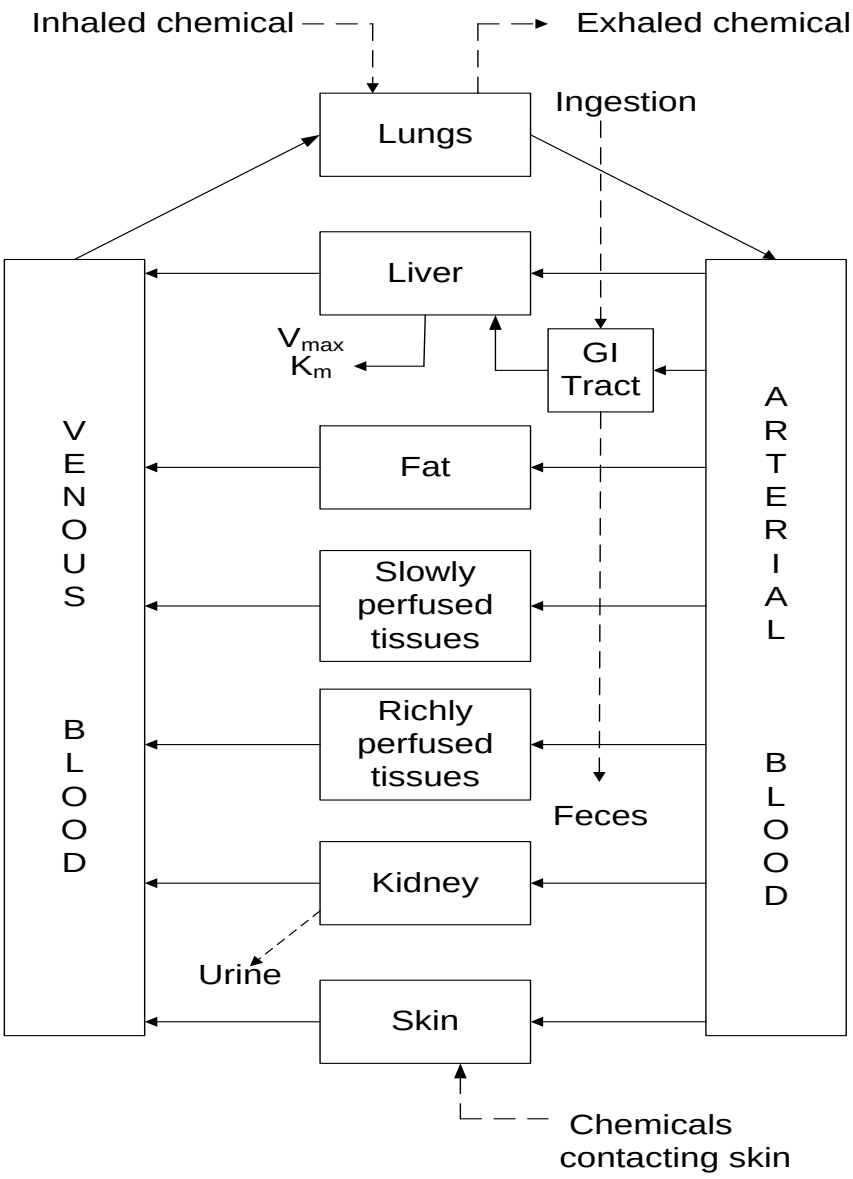
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in 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 hexachlorobenzene 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.

3.4.5.1. Summary of PBPK Models

PBPK models for hexachlorobenzene have been developed by Yesair et al. (1986), Freeman et al. (1989), and Lu et al. (2006). The Yesair et al. (1986) model describes the absorption, distribution, and elimination of ingested hexachlorobenzene in growing rats and humans. The Freeman et al. (1989) model describes distribution and excretion of intravenously injected hexachlorobenzene in growing rats. The Lu

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

et al. (2006) model describes the distribution and excretion of intravenously injected and orally administered hexachlorobenzene in rats, and the effect of partial hepatectomy treatment on hexachlorobenzene distribution in the liver, muscle, and blood.

3.4.5.2 Hexachlorobenzene PBPK Model Comparison

The Yesair et al. (1986) and Freeman et al. (1989) models are similar attempts to describe distribution and clearance of hexachlorobenzene. Both models included numerous tissue compartments and allowed for growth over time. The Yesair et al. (1986) model went further than the Freeman et al. (1989) model by including oral exposure, fetal and breast milk compartments, and metabolism and tissue sequestration of hexachlorobenzene, and by modeling humans as well as rats. Both rat models were validated using experimental data. Compared to the Yesair et al. (1986) and Freeman et al. (1989) models, Lu et al. (2006) updates the PBPK model in rats by including erythrocyte binding and exsorption (passive diffusion from blood to digestive tract) processes. Additionally, the Lu et al. (2006) model simulates the effect of a partial hepatectomy on hexachlorobenzene distribution.

3.4.5.3 Discussion of Models

The Yesair Model

Risk assessment. The Yesair model is not adequate for use in risk assessment. Although both rat and human models were developed and the rat model was validated with experimental data, the human model was not validated. Interspecies and interroute extrapolations were not attempted with this model.

Description of the model. The Yesair model was initially developed to simulate oral exposure to hexachlorobenzene in growing male and female rats, and was then expanded to include pregnancy and offspring in the female model. A human model was produced by using the same model structure with human physiological parameter values. The model includes compartments for intestinal lumen, systemic circulation, feces, liver, metabolites, kidney, urine, brain, richly perfused tissues, poorly perfused tissues, breast milk, fetus, and lactating offspring. Parameters used in the model included body and organ weights and growth rates, blood-flow rates, empirical clearance factors, reaction-rate constants, distribution ratios, and capacity limits. The forms of the growth characteristics and the parameter values were obtained from the literature, experimental data from Kuiper-Goodman et al. (1977), and empirical considerations. Both free and sequestered forms of hexachlorobenzene were estimated in each compartment, and only freely available material was allowed to leave the compartment (except for the systemic circulation and breast

milk compartments). When the rat models were adapted for humans, the appropriate human physiological values were substituted for the rat values.

Validation of the model. The rat model was compared to data from Courtney et al. (1979), Iatropoulos et al. (1975), Koss and Koransky (1975), Koss et al. (1978), and Kuiper-Goodman et al. (1977). In general, the model approximated the observed results reasonably well in all tissues. The human model was not validated.

Target tissues. Using the rat model, correlations were obtained between predicted yield of metabolites and experimentally observed liver toxicity (porphyria and increased liver weight reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), between estimated concentrations of hexachlorobenzene in the brain and observed central nervous system effects (tremors and other signs reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), and between estimated extent of lactational transfer of hexachlorobenzene and observed offspring mortality (data from Kitchen et al. 1982). The model predicts little transfer of hexachlorobenzene to the fetus during gestation and extensive mobilization of hexachlorobenzene to the offspring during lactation. The data are consistent with this model. A half-life of 215 days was predicted for hexachlorobenzene in a growing human female exposed to doses of 0.5–32 mg/kg/day for 15 weeks at 15 years of age, suggesting that approximately 4 years (7 half-lives) are required to establish equilibrium between intake and excretion. Further simulations showed that doubling or halving the administered dose resulted in doubling or halving, respectively, of the tissue concentrations after 3–5 years.

Species extrapolation. Although Yesair et al. (1986) developed both rat and human models, the human model was not validated and species extrapolation was not attempted.

Interroute extrapolation. Interroute extrapolation was not attempted in this model.

The Freeman Model

Risk assessment. The Freeman model is not adequate for use in risk assessment. The model was developed to simulate intravenous injection of hexachlorobenzene in growing rats. Interspecies and interroute extrapolations were not attempted.

Description of the model. The model includes compartments for plasma, gastrointestinal tract, colon, feces, liver, lung, kidney, urine, brain, heart, spleen, skin, muscle, and fat. Parameters used in the model include organ weights and blood flow fractions obtained from the literature and tissue:serum partition coefficients derived from experimental studies by Scheufler and Rozman (1984a, 1984b). The model was designed to accommodate differential growth of tissue/organ weights as a function of total body weight. Metabolism was assumed to be zero based on experimental data (attributed to Rozman and colleagues) suggesting little metabolism of hexachlorobenzene in the rat.

Validation of the model. The model predictions were compared to data from Scheufler and Rozman (1984a, 1984b). In general, the model approximated the observed results reasonably well in the compartments examined: blood, liver, fat, urine, and feces.

Target tissues. Levels in liver and fat were well-predicted by this model. An interesting prediction is that approximately 75% of the decline in fat concentrations over time is due to growth (i.e., dilution), with only 25% due to excretion. Thus, the growth of animals during experiments may affect the apparent half-life of hexachlorobenzene.

Species extrapolation. Species extrapolation was not attempted in this model.

Interroute extrapolation. Interroute extrapolation was not attempted in this model.

The Lu Model

Risk Assessment. The Lu model is not adequate for use in risk assessment. Interspecies and interroute extrapolations were not attempted.

Description of the model. Lu et al. (2006) developed a PBPK model for both intravenous injection and gavage (single and repeated dose) of hexachlorobenzene in the rat that incorporated erythrocyte binding, exsorption processes, and pathophysiological conditions following partial hepatectomy. The model includes compartments for liver, blood (plasma and erythrocytes), fat, rapidly and slowly perfused muscle tissues, and upper and lower gastrointestinal lumen. Parameters used in the model included body weights, growth rates, tissue and organ volumes, blood-flow and plasma-flow rates, and tissue:plasma coefficients derived from Koss et al. (1978), and rate constants of metabolism, exsorption, reabsorption,

and fecal excretion. The forms of the growth characteristics and the parameter values were obtained from the literature and from a time-course pharmacokinetics bioassay.

Validation of the model. The model (single oral dose only) was compared to data from Koss and Koransky (1975). Model predictions agreed with the data when the exsorption rate constant (an adjustable parameter) was set at 0.02 L/hour, but underpredicted the second and third time points when the value was 0.045 L/hour.

Sensitivity analysis. Sensitivity analysis was determined for liver concentration, fat and liver volume fractions, tissue partition coefficients, and the adjustable parameters (metabolism, exsorption, resorption, fecal excretion) following a single oral dose. The liver partition coefficient had the largest effect on the liver concentration. Fat volume fraction and partition coefficients had moderate effects. Other parameters had little or no effect.

Target tissues. The intravenous injection model traced liver and plasma concentrations well, and simulations matched data better than previous models (Freeman et al. 1989; Roth et al. 1993; Yesair et al. 1986) that did not consider erythrocyte binding. Single and repeated gavage model simulations for fat, liver, and blood hexachlorobenzene concentrations were in good agreement with experimental data reported by Yamaguchi et al. (1986) and Koss et al. (1978) and with the time-course pharmacokinetics bioassay. Estimates of hexachlorobenzene metabolism and excretion via feces were generally in agreement with experimental data. Simulations with partial hepatectomy and hexachlorobenzene treatment were good for late time points, but overpredicted early time points.

Species extrapolation. Species extrapolation was not attempted in this model.

Interroute extrapolation. Interroute extrapolation was not attempted in this model.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Results of a study of human subjects indicate that absorption of ingested hexachlorobenzene decreases with increasing blood hexachlorobenzene levels and that absorption from the gut likely includes mechanisms in addition to passive diffusion (Schlummer et al. 1998). Circulation is the primary mechanism for inter-tissue distribution; hexachlorobenzene distributes preferentially to fat due to its

lipophilic nature. Refer to Section 3.4 (Toxicokinetics) for a detailed discussion of absorption, distribution, metabolism, and elimination and excretion following exposure to hexachlorobenzene. Human and animal studies suggest that breast milk is enriched with hexachlorobenzene, relative to blood, and that blood levels actually drop in lactating mothers (Greizerstein et al. 1999; Petzold et al. 1999; Nakashima et al. 1997, 1999; Nakashima and Ikegari 2000; Waliszewski et al. 1999a, 1999b). This is probably due to the lipophilicity of hexachlorobenzene.

Hexachlorobenzene is slowly metabolized by hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms) (Den Besten et al. 1994; Mehmood et al. 1996; Schielen et al. 1995a); by conjugation with glutathione, glucuonide, and sulfate; and by reductive dechlorination (Ingebrigtsen et al. 1986; Koss et al. 1979; Renner 1988). Two alternatives have been proposed for the mechanism of conversion of hexachlorobenzene to pentachlorophenol: formation of an epoxide or free radical attack on the carbon-chlorine bond. Substitution of adjacent carbons with chlorine argues against a mechanism involving an epoxide; either cleavage of the bond by free radical attack followed by hydroxylation, or conjugation with glutathione seems more plausible. However, if the presence of o- and p-hydroxy derivatives of pentachlorophenol could be confirmed, there would be a strong possibility that epoxides are intermediates in the dechlorination or hydroxylation of hexachlorobenzene or both (Lui et al. 1976).

Following metabolism to more polar metabolites, hexachlorobenzene is excreted in urine (Ingebrigtsen et al. 1981, 1986; Koss and Koransky 1975; Koss et al. 1986; Lui and Sweeney 1975; Rozman et al. 1977a; Scheufler and Rozman 1984a, 1984b; To-Figueras et al. 1992; van Ommen et al. 1985; Yang et al. 1978).

Hexachlorobenzene eliminated in the feces is predominantly unchanged parent compound, although small amounts of various metabolites have also been found (Koss and Koransky 1975; Yang et al. 1978). Fecal elimination is primarily the product of fecal excretion, although biliary excretion (from the liver) is also important (Ingebrigtsen et al. 1981; Richter and Schafer 1981; Rozman et al. 1981; Yang et al. 1978).

For nursing mothers, excretion of unchanged hexachlorobenzene into milk may represent a significant, and even the primary, route of excretion (Courtney and Andrews 1985; Craan and Haines 1998; Cripps 1990; Czaja et al. 1997; Gladen et al. 1999; Gocmen et al. 1989; Goldey et al. 1990; Lunden and Noren 1998; Nakashima et al. 1997; Newsome and Ryan 1999; Scheele et al. 1995; Weisenberg 1986; Wickstrom et al. 1983; and many others).

Refer to Section 3.4 (Toxicokinetics) for detailed information regarding absorption (Section 3.4.1), distribution (Section 3.4.2), metabolism (Section 3.4.3), and excretion (Section 3.4.4) following exposure to hexachlorobenzene.

3.5.2 Mechanisms of Toxicity

Mechanistic data for hexachlorobenzene focus mainly on hexachlorobenzene-induced porphyria and associated effects as detailed below. Limited data are available regarding mechanisms of hexachlorobenzene-induced endocrine and immunological effects. Limited information was located regarding possible mechanisms of hexachlorobenzene-induced neurotoxic effects.

Hexachlorobenzene induces porphyria characterized by increased d-ALA synthase (the enzyme that controls the rate of porphyrin production) activity and decreased uroporphyrinogen decarboxylase (the enzyme that converts uroporphyrinogen III to coproporphyrinogen III) activity (Dowdle et al. 1967; Rajamanickam et al. 1972; Smith and de Matteis 1990). Uroporphyrinogen III is the first cyclic tetrapyrrole in the pathway of heme biosynthesis. This is the reduced colorless precursor of uroporphyrin III (hexahydro-uroporphyrin) and will give rise to the corresponding porphyrin on reoxidation. Uroporphyrinogen decarboxylase (a cytosolic enzyme) converts uroporphyrinogen III to coproporphyrinogen III by the stepwise decarboxylation of the four acetic acid side chains to leave methyl residues, but the corresponding porphyrin (uroporphyrin III) cannot be decarboxylated and will not be metabolized further. Thus, the accumulation of uroporphyrins in the liver may be due to a deficiency of the decarboxylation of uroporphyrinogen III catalyzed by uroporphyrinogen decarboxylase. This hypothesis led to the proposal that in certain porphyrias where uroporphyrin accumulates (uroporphyrias), the mechanism responsible may be an accelerated oxidation of uroporphyrinogen, causing an "oxidative escape" of this intermediate from the pathway of heme biosynthesis (Meola and Lim 1993; Smith and de Matteis 1990). A marked inhibition of uroporphyrinogen decarboxylase has been widely reported to occur prior to the manifestation of the typical porphyrinogenic effects of hexachlorobenzene (Blekkenhorst et al. 1976; Elder et al. 1976; Smith and Francis 1987; Smith et al. 1986a; Sopena et al. 2008). A study conducted with Osteogenic Disorder Shionogi (ODS) rats provided evidence that chemically-induced porphyria seems to be mediated by inhibition of CYP1A2-catalyzed uroporphyrinogen oxidation (Sinclair et al. 1995).

In vitro studies with hexachlorobenzene have demonstrated that this chemical does not exert a direct action on uroporphyrinogen decarboxylase (Rios de Molina et al. 1980). The major hexachlorobenzene

metabolites (tetrachlorohydroquinone, pentachlorophenol, pentachlorothiophenol, pentachlorothioanisole) had no influence on the porphyrin pathway as indicated by alteration in total hepatic porphyrin levels and urinary levels of d-ALA, porphobilinogen, or uroporphyrins (Goldstein et al. 1977, 1978; Kimbrough and Linder 1978; Lui et al. 1976; Smith and Francis 1987; Wainstok de Calmanovici and San Martin de Viale 1980). However, pentachlorophenol and tetrachlorohydroquinone appear to be capable of altering porphyrin metabolism in *in vitro* systems containing d-ALA (Goerz et al. 1978; Goldstein et al. 1977). Furthermore, co-administration of pentachlorophenol and tetrachlorohydroquinone with hexachlorobenzene increased the severity of the resultant porphyria (Debets et al. 1980a), indicating a probable role for these metabolites in porphyria induction. It has been suggested that changes in K^+ permeability mediated by lipid peroxidation and mitochondrial dysfunction may be contributing factors in hexachlorobenzene-induced hepatotoxicity based on the results of a study in rats in which mitochondrial lipid peroxidation was found to have increased proportionally with a 100-fold increase in hepatic porphyrin content (Feldman and Bacon 1989; Masini et el. 1988). Porphyrin uptake in the mitochondria of iron-supplemented rats was inhibited by pentachlorophenol (hexachlorobenzene metabolite), indicating that peroxidative reactions in the mitochondrial membranes may be responsible for changes in membrane permeability (Masini et al. 1988).

It is unlikely, however, that the hexachlorobenzene metabolites, pentachlorobenzene and pentachlorophenol, are by themselves porphyrinogenic agents, and P-450 induction may not correlate with porphyria development. In one study, rats fed diets containing hexachlorobenzene or its metabolites (pentachlorobenzene and pentachlorophenol) exhibited increases in hepatic cytochrome P-450, but the metabolites had no effect on urinary porphyrin excretion, while hexachlorobenzene produced a high level of urinary porphyrins (Vos et al. 1988). In other studies, the major hexachlorobenzene metabolites (tetrachlorohydroquinone, pentachlorophenol, pentachlorothiophenol, pentachlorothioanisole) had no influence on the porphyrin pathway as indicated by alteration in total hepatic porphyrin levels and urinary levels of d-ALA, porphobilinogen, or uroporphyrins (Goldstein et al. 1977, 1978; Kimbrough and Linder 1978; Smith and Francis 1987; Wainstok de Calmanovici and San Martin de Viale 1980). However, pentachlorophenol and tetrachlorohydroquinone appear to be capable of altering porphyrin metabolism in *in vitro* systems containing d-ALA (Goerz et al. 1978; Goldstein et al. 1977). Furthermore, co-administration of pentachlorophenol and tetrachlorohydroquinone with hexachlorobenzene increased the severity of hexachlorobenzene-induced porphyria (Debets et al. 1980a).

In a study with rats, it was proposed that the involvement of the histidine residue of the enzyme in substrate (hexachlorobenzene) binding may be the mechanism by which hexachlorobenzene exerts its

porphyrinogenic action *in vivo* (Billi de Catabbi et al. 1991). Another study in rats and mice concluded that hexachlorobenzene induces chronic porphyria by modifying sulfhydryl groups in porphyrinogen decarboxylase, the action restricted to the catalytic or substrate-binding sites (Elder and Urquhart 1986).

Hexachlorobenzene intake has been associated with an initial increase of coproporphyrinogen and subsequent increase in highly carboxylated porphyrins such as uroporphyrin and heptacarboxylic porphyrin in the urine and presence of isocoproporphyrin and smaller amounts of coproporphyrin in the feces. Fecal isocoproporphyrin results from increased pentacarboxylic porphyrinogen III, which is formed in the cytosol and competes with coproporphyrinogen III for coproporphyrinogen oxidase decarboxylation. Results from a series of *in vivo* and *in vitro* (liver assays) in rats indicate that hexachlorobenzene-induced porphyria involves an uncoupling of the enzyme coproporphyrinogen oxidase from the outer surface of the inner mitochondrial inner membrane in the liver, which may allow pentacarboxylic porphyrinogen III to compete with coproporphyrinogen III for the coproporphyrinogen oxidase catalytic site to produce isocoproporphyrin (Sopena et al. 2008).

Lelli et al. (2007) assessed the effects of hexachlorobenzene on adrenal synthesis and stimulation of plasma glucocorticoids, as well as kinetic parameters of its hepatic receptors in orally-treated rats. Hexachlorobenzene caused decreases in plasma corticosterone, number of hepatic glucocorticoid receptors (without modifying affinity), gluconeogenic enzyme phospoenolpyruvate-carboxylase activity, and adrenal corticosterone production. These results suggest that hexachlorobenzene may exert a hormonal effect by disrupting glucocorticoids, their hepatic receptors, and glucose synthesis via gluconeogenic enzyme phospoenolpyruvate-carboxylase regulation, thus modulating porphyria.

Iron (as iron dextran) has been shown to induce porphyria; therefore, iron may have a role in the pathogenesis of hexachlorobenzene-induced porphyria (Siersema et al. 1991; Smith and Francis 1983; Smith et al. 1986a), although co-administration of carbonyl iron did not have a significant effect on elevated hepatic and mitochondrial fraction porphyrin contents in rats following hexachlorobenzene treatment (Masini et al. 1988), but in other animal studies, exposure of mice to hexachlorobenzene and iron produced a dramatic increase (nearly 1,000-fold) in hepatic uroporphyrin levels (Vincent et al. 1989). The small number of animals used in this study limits the reliability of the conclusions of this study. Another investigator concluded that liver mitochondrial porphyrin uptake may involve the K^+ transmembrane gradient and further suggested that peroxidative reactions in the mitochondrial membranes may be responsible for changes in membrane permeability that affects K^+ permeability (Masini et al. 1988). Liver malonaldehyde levels increased while glucose-6-phosphate activity decreased

in rats administered intraperitoneal injections of 50 mg of hexachlorobenzene per day (total dose=300 mg) during a 42-day test period followed by treatment with intraperitoneal doses of iron (as ferrihydroxide-dextran complex), suggesting a close relationship between accumulation of porphyrins, iron overload, and free radical formation or lipid peroxidation (Visser et al. 1989).

Multiple studies indicate that non-heme iron potentiates the hepatocarcinogenic effects of hexachlorobenzene (Adjarov 1990; Elder and Urquhart 1986; Hahn et al. 1988; Smith and Francis 1983; Smith et al. 1989, 1993; Vincent et al. 1989). Experiments with rats and iron-loaded mice indicate that there may also be an association between the induction of uroporphyria and the development of liver tumors after the administration of polyhalogenated aromatic chemicals (Smith and De Matteis 1990). Hyperplastic nodules were observed in the liver lobes of 80% of female Fischer 344 rats pretreated with Imferon (iron-dextran) and then given dietary hexachlorobenzene in arachis oil for 65 weeks resulting in a dose of 5 or 10 mg/kg/day. There was a high incidence of fibrin throughout the liver with sinusoidal telangiectasis, and nodular peliosis hepatitis and hepatocellular necrosis. The study proposed a nongenotoxic mechanism for tumor induction by hexachlorobenzene, concluding that the formation of hepatomas and hemangiomas with elements of peliosis could be explained by the compensatory hyperplastic responses to hepatocellular injury or necrosis and by the simultaneous loss of hepatocellular cords. The study further concluded that the accumulation of iron in the liver would strongly potentiate the development of hepatic tumors (Carthew and Smith 1994).

Iron overload also greatly sensitized mice to the development of liver tumors. Mice given oral hexachlorobenzene doses preceded by subcutaneous administration of iron developed iron-excluded hyperplastic nodules (all treated animals) and hepatocellular carcinoma (most animals). Based on the results of this investigation, an alternate mechanism has been suggested for the hepatic toxicity of hexachlorobenzene that may involve the uncoupling of an induced cytochrome P-450 system releasing active oxygen species. Iron is seen as catalyzing the formation of the hydroxyl radical or perhaps forming reactive iron-oxygen complexes (Smith 1989).

Data from a study in male Long Evans rats suggested that the metabolism of hexachlorobenzene to pentachlorobenzene and other more polar metabolites proceeds either through a free-radical mechanism or by initial formation of an arene oxide. These reactive intermediates may form covalent bonds with cellular constituents (such as protein amino acids or DNA nucleic acids) leading to irreversible cell damage (Lui and Sweeney 1975). Several other studies have also found evidence of binding to cellular proteins by

reactive electrophilic metabolites of hexachlorobenzene formed by cytochrome P-450(Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985).

Hexachlorobenzene has been shown to affect the thyroid (see Section 3.2.2.2 [Endocrine Effects]). The effect of hexachlorobenzene on thyroxine appears to involve stimulation of dehalogenation of the hormone in the liver, rather than an effect on synthesis of the hormone in the thyroid (Kleiman de Pisarev et al. 1989, 1990). There is also some evidence that hexachlorobenzene may competitively inhibit binding of thyroxine to serum carrier proteins, further depressing circulating levels of the hormone (Foster et al. 1993; Van Den Berg 1990). Results from *in vitro* assays of hexachlorobenzene-treated rat thyroid cells led Chiappini and coworkers (Chiapini et al. 2009, 2013, 2014) to conclude that hexachlorobenzene induces apoptosis in thyroid cells via mechanisms that include involvement of transforming growth factor-beta (TGF-β1) in hexachlorobenzene-induced alterations of cytosolic and nuclear p27 protein and cyclin D1 protein levels.

Hexachlorobenzene effects on ovarian and adrenal hormones have been hypothesized to reflect alterations in steroidogenesis in these tissues, possibly as a consequence of lipid peroxidation of mitochondrial membranes (Foster et al. 1995a, 1995b). Ultrastructural lesions consistent with lipid peroxidation have been observed in mitochondria from the ovaries of monkeys treated with hexachlorobenzene (Bourque et al. 1995).

Mundy et al. (2010) demonstrated that hexachlorobenzene induced ethoxyresorufin O-deethylase (EROD) activity, CYP1A4 mRNA and CYP1A5 mRNA in chicken embryo hepatocyte primary cultures similar to induction elicited by 2,3,3',4,4'-pentachlorobiphenyl (PCB-105) and 2,3',4,4',5-pentachlorobiphenyl (PCB-118). A dioxin (tetrachlorodibenzo-*p*-dioxin [TCDD]) equivalent factor was determined to be 0.0001. These results suggest that hexachlorobenzene may act through pathways similar to those of TCDD.

Ezendam et al. (2004a) designed a study to assess possible mechanisms for hexachlorobenzene-induced immunopathology. Brown Norway rats were exposed to a control diet or a diet containing hexachlorobenzene at 450 mg/kg food for 21 days. Treatment with hexachlorobenzene resulted in skin lesions, increases in spleen and auricular lymph node weights, increased serum IgE and IgM against ssDNA levels, macrophage infiltration into spleen and lung, and infiltration of eosinophilic granulocytes into the lung. Cotreatment with cyclosporin A, known to decrease peripheral T-cell number and inhibit antigen-induced T-cell activation, greatly reduced or eliminated the hexachlorobenzene-induced immuno-

pathological effects, with the exception of macrophage infiltrations into the spleen and lung. Restimulation of spleen cells with the T-cell mitogen ConA and the macrophage activator, LPS, demonstrated that cyclosporin A inhibited T-cell activation, but not macrophage activation. These results indicate that T-cells and macrophages are involved in hexachlorobenzene immunotoxicity.

Results from *in vitro* exposure of mouse embryonic stem cells to hexachlorobenzene suggest that hexachlorobenzene interferes with neurite outgrowth of GABAergic, but not glutamatergic neuronal cells, presumably via induction of reactive oxygen species (ROS) production since the effect on GABAergic neuronal cells was repressed in the presence of an ROS scavenger (Addae et al. 2013). *In vitro* exposure of human peripheral blood lymphocytes to hexachlorobenzene resulted in increases in ROS formation, numbers of lymphocytes with reduced transmembrane mitochondrial potential, and caspase 3 activity, which were likely related to increased numbers of apoptotic lymphocytes (Michalowicz et al. (2013). Pontillo et al. (2013) demonstrated that *in vitro* exposure of a mouse breast cancer cell line to hexachlorobenzene resulted in enhanced MMP2 (metalloprotease 2) expression and cell invasion and that aryl hydrocarbon receptor (AhR), proto oncogene c-Src, and epidermal growth factor receptor 1 (HER1) pathways were likely involved in these effects.

The toxicogenomics in the Brown Norway rat resulting from subchronic exposure to hexachlorobenzene was investigated by Ezendam et al. (2004b). The rats were administered hexachlorobenzene up to 450 mg/kg in the diet for 4 weeks, and DNA microarray assays, blood and serum analysis, and pathology experiments were performed. Hexachlorobenzene induced expression of genes involved in systemic inflammatory response, oxidative and acute phase response, drug metabolism, hepatic porphyria, and the reproductive system. The study confirmed stimulatory effects of hexachlorobenzene on the immune system and induction of enzymes involved in drug metabolism, porphyria, and the reproductive system. Hexachlorobenzene induced gene expression of proinflammatory cytokines, antioxidants, acute phase responses, complement and mast cell markers, chemokines, and cell adhesion molecules.

Systemic inflammatory responses included increases in gene expression related to tumor necrosis, mast cell enzymes, chemokines, cell adhesion molecules, complement component, cytokine production, antioxidants, and pleiotropic cytokine. Acute-phase gene expression responses included heat shock proteins in spleen and mesenteric lymph node, matrix metalloproteinases and inhibitors in spleen, liver, and mesenteric lymph nodes, and transcript levels of haptoglobin, lipopolysaccharide-binding protein, orosomucoid, metallothionein, and ceruloplasmin. Gene expression increased for autoantibodies in the spleen, thymus, liver, and kidney, expression of T and B cells and major histocompatibility complex II,

and certain drug-metabolizing enzymes associated with estrogen metabolism. Gene expression of CYP1A1 was strongly upregulated in the liver, an effect associated with certain dioxins. Other effects observed included significantly increased body weights in both dose groups, increases in liver and spleen weights in both dose groups, and histopathological changes in the liver and spleen (described in detail in Michielsen et al. 1997). In the high-dose group, kidney weights were significantly increased and thymus weight was significantly decreased.

3.5.3 Animal-to-Human Extrapolations

Studies have investigated the adverse effects of hexachlorobenzene in rats, mice, hamsters, dogs, pigs, and monkeys following subchronic exposure and in rats, mice, and hamsters following chronic exposure. Substantial bodies of both of human data and animal data are available that demonstrate qualitative similarities between animals and humans for such end points as porphyria and dermal lesions. Overall, data in animal studies do not suggest species variations in the toxicokinetics of hexachlorobenzene except in carcinogenic responses. The cancer toxicity data suggest that species differences exist, as demonstrated by multi-tumor-type responses evident in hamsters and single-tumor-type responses observed in mice (Cabral et al. 1977, 1979; EPA 1980a; Ertürk et al. 1986; Lambrecht et al. 1983; Smith 1989; Smith et al. 1985).

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 1997b). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Hexachlorobenzene mediates multiple adverse effects through the neuroendocrine axis. Hormonal changes associated with exposure to hexachlorobenzene at dose levels ≥1 mg/kg/day include decreased serum thyroxine (hypothyroidism), increased serum parathyroid hormone (hyperparathyroidism), decreased corticosterone released from the adrenal gland, changes in estradiol and progesterone levels in females at certain times in the menstrual cycle, and hirsutism. The female hormone changes are coincident with ovarian lesions and changes in female reproductive cycles in the same studies. Reduced fertility in breeding trials with hexachlorobenzene may be secondary to the ovarian effects. These alterations are described in more detail in Section 3.2. The effect of hexachlorobenzene on thyroxine appears to involve stimulation of dehalogenation of the hormone in the liver, rather than an effect on synthesis of the hormone in the thyroid (Kleiman de Pisarev et al. 1989, 1990). There is also some evidence that hexachlorobenzene may competitively inhibit binding of thyroxine to serum carrier proteins, further depressing circulating levels of the hormone (Foster et al. 1993; Van Den Berg 1990). The effects on ovarian and adrenal hormones have been hypothesized to reflect alterations in steroidogenesis in these tissues, possibly as a consequence of lipid peroxidation of mitochondrial membranes (Foster et al. 1995a, 1995b). Ultrastructural lesions consistent with lipid peroxidation have been observed in mitochondria from the ovaries of monkeys treated with hexachlorobenzene (Bourque et al. 1995); similar lesions have also been observed in rats (Alvarez et al. 2000). Breast cancer is another end point believed to be influenced through the neuroendocrine axis. Studies available to date have found little or no evidence for an association between hexachlorobenzene and breast cancer in humans (Dorgan et al. 1999; Guttes et al. 1998; Liljegren et al. 1998; Mendonca et al. 1999; Moysich et al. 1998; Zheng et

al. 1999). Adipose hexachlorobenzene levels were increased in males with undescended testis compared to controls (Hosie et al. 2000); this adverse effect may be related to *in utero* changes in hormone levels.

The ability of hexachlorobenzene to interact with α-estrogen receptor, androgen receptor, progesterone receptor, and estrogen-related receptor was examined *in vitro* in yeast strains expressing β-galactosidase (Li et al. 2008). Hexachlorobenzene was found to be an antagonist for androgen and estrogen-related receptor; no response was found for the other receptors.

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 fetus/infant has an immature (developing) blood-brain

barrier that past literature has often described as being leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the blood-brain barrier, there are differences between fetuses/infants and adults which are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

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).

Infants and young children appeared to be especially sensitive to the effects of hexachlorobenzene in the Turkish grain poisoning epidemic. During the epidemic, there was an extremely high rate of mortality (close to 100% in some villages) in breast fed infants (under 2 years of age) of mothers known to have ingested the contaminated bread (Gocmen et al. 1989; Peters et al. 1982). This is in contrast to a 10% rate of mortality in exposed adults (Peters et al. 1982, 1987). Poisoned infants displayed a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (blistering and epidermolysis and annular erythema) (Cripps et al. 1984; Peters et al. 1982, 1987). The infant deaths were caused by respiratory and cardiovascular failure resulting from the disease, and sometimes followed tremors and convulsions (Peters et al. 1982). Pink sore was not seen in exposed adults. Infants in this study were likely exposed *in utero* via transplacental transfer and postnatally by lactational transfer (Cripps et al. 1984; Peters et al. 1982, 1987). No quantitation of exposure (dose and duration) was presented in any of these clinical reports. However, an estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963). Analysis of human milk from exposed women and unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987).

Based on 20–30-year follow-up studies (Cripps et al. 1984; Peters et al. 1982), patients who were young children (average age 7 years) during this exposure later developed numerous dermatologic, neurologic, and orthopedic abnormalities associated with the developmental toxicity of hexachlorobenzene. The reproductive histories of 42 females exposed to hexachlorobenzene as children or young adults were also studied. Of the 188 pregnancies in the 42 women that occurred in a 4-year period (1977–1981), there were 15 fetal deaths (13 miscarriages and 2 stillbirths) and 173 live births (Peters et al. 1982, 1987); however, the relevance of this study is limited because the numbers of expected miscarriages and stillbirths were not provided. These mothers had 0.51 ppm hexachlorobenzene in their breast milk compared to 0.07 ppm in unexposed controls (Gocmen et al. 1989). The fetal mortalities may be related

to the mobilization of hexachlorobenzene from the maternal fat pool and its subsequent exposure to the fetus through the placenta.

Based on partial evaluation of 63 of a planned 100 cases, Sala et al. (1999b) published a preliminary study, reporting that a significant association between prenatal hexachlorobenzene exposure and impaired development of locomotor skills had been detected in newborn babies in Flix, compared with those of nearby villages. A study of a less-exposed population in New York was unable to correlate hexachlorobenzene levels in umbilical blood or breast milk with infant intelligence test results (Darvill et al. 2000).

A German case-control study found that adipose hexachlorobenzene levels in 18 male patients who underwent orchidopexy to correct unilateral or bilateral undescended testis (mean age 4.2 years) were 3-fold higher compared to a group of 30 male control patients (mean age 3.5 years); this difference was highly significant (Hosie et al. 2000). A similar correlation was also observed for heptachloroepoxide (HCE), but not for other organochlorines. The weaknesses of this study are the small study size, the lack of age-adjustment between groups, and the potentially confounding effect of HCE.

Although there is only limited direct evidence that hexachlorobenzene crosses the placenta in humans (Ando et al. 1985), animal studies have shown that hexachlorobenzene crosses the placenta readily and accumulates in fetal tissues (Courtney and Andrews 1985; Courtney et al. 1979; Cripps 1990; Villeneuve and Hierlihy 1975; Villeneuve et al. 1974a). While numerous human studies have demonstrated the presence of hexachlorobenzene in breast milk, animal studies have shown in addition that hexachlorobenzene concentrates in the breast milk and is transferred to the suckling neonate in considerable amounts (Bailey et al. 1980; Cripps 1990; Goldey et al. 1990).

Animal studies have also confirmed that the developing organism is an especially sensitive target for hexachlorobenzene. Findings from laboratory animal single- and multi-generation reproductive toxicity studies conducted in rats exposed to hexachlorobenzene indicate that fertility, gestational viability, and lactational indices may be affected by hexachlorobenzene exposure (Grant et al. 1977; Kitchin et al. 1982). Studies on prenatally exposed animals have shown immune and neurological effects at lower doses in the young developing animals than in adults (Goldey and Taylor 1992; Vos et al. 1979a). In the study of Goldey and Taylor (1992), maternal dosing with hexachlorobenzene occurred prior to conception. The most sensitive end point in any study, and the basis for the chronic MRL, was liver lesions that developed during adulthood in rats treated with combined pre- and postnatal lifetime exposure (Arnold et al. 1985).

Animal studies have also shown that hexachlorobenzene mediates toxicity through the neuroendocrine axis, with multiple effects on the thyroid gland (hypothyroidism), parathyroid gland (hyperparathyroidism), adrenal gland, mammary gland, and ovaries (Alvarez et al. 2000; Andrews et al. 1988, 1990; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1992b, 1993, 1995a, 1995b; Kimbrough and Linder 1974; Kleiman de Pisarev et al. 1989, 1990; Peña et al. 2012). Because the hormones produced by these endocrine organs play a crucial role in growth and development of the organism, it is not surprising that hexachlorobenzene interferes with these processes. Neuroendocrine end points have not been studied in developing organisms.

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).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data for hexachlorobenzene from this report is discussed in Section 6.5. 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 hexachlorobenzene 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 hexachlorobenzene 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 Hexachlorobenzene

Human tissues and body fluids that have been analyzed for hexachlorobenzene to identify and quantify exposure include blood and serum (Ataniyazova et al. 2001; Cooney et al. 2010; Den Hond et al. 2011; Glynn et al. 2000; Hagmar et al. 2001; Karmaus et al. 2001; Rutten et al. 1988; Sala et al. 1999b, 2001b; Schettgen et al. 2011; Schlummer et al. 1998; Siyali 1972; Waliszewski et al. 2001; Weiderpass et al. 2000; and many others), liver (Dewailly et al. 1999; Weistrand and Noren 1998), bone marrow (Bucholski et al. 1996; Scheele et al. 1995), brain (Dewailly et al. 1999), fat (Ansari et al. 1986; Dewailly et al. 1999; Lordo et al. 1996; Robinson et al. 1990; Scheele et al. 1995; Siyali 1972; Szymczynski and Waliszewski 1981; Teufel et al. 1990; Weistrand and Noren 1998), semen (Szymczynski and Waliszewski 1981), follicular fluid (De Felip et al. 2004); the placenta (Poli et al. 1999), the umbilical cord (Burse et al. 2000; Darvill et al. 2000; Lackmann et al. 2002), and breast milk (Ataniyazova et al. 2001; Craan and Haines 1998; Czaja et al. 1997; Darvill et al. 2000; Dewailly et al. 2000; Ennaceur et al. 2007; Fitzgerald et al. 2001; Gladen et al. 1999; Gocmen et al. 1989; Huang et al. 1989; Lunden and Noren 1998; Newsome and Ryan 1999; Polder et al. 2008; Scheele et al. 1995; Weisenberg 1986; Wickstrom et al. 1983; and others).

Reliable methods are also available to measure hexachlorobenzene in feces (Albro and Thomas 1974; Koss and Koransky 1975; Schlummer et al. 1998) and urine. Trace amounts of unchanged hexachloro-

benzene have been detected in urine; however, urinary metabolites are more easily detected and quantified (Ingebrigtsen et al. 1981, 1986; Koss et al. 1976; Lui and Sweeney 1975; Rozman et al. 1977a; To-Figueras et al. 1992; van Ommen et al. 1985; Yang et al. 1978) as biomarkers for hexachlorobenzene exposure. Although urinary pentachlorophenol and tissue hexachlorobenzene correlated in 60 patients studied, it is possible that the urinary pentachlorophenol originated from other chlorinated hydrocarbons such as pentachlorobenzene, alpha-hexachlorocyclohexane, or pentachloronitrobenzene (Burton and Bennett 1987; Currier et al. 1980; Koss et al. 1986; To-Figueras et al. 1992).

Indirect biomarkers of hexachlorobenzene exposure include measurement of *gamma*-glutamyl transferase in blood, uroporphyrin and d-ALA in urine, and coproporphyrin in feces (Koss et al. 1986; To-Figueras et al. 1992). Because these biomarkers are not specific for hexachlorobenzene, their usefulness in monitoring exposed populations is limited.

Several studies have correlated hexachlorobenzene levels with different end points. In humans, hexachlorobenzene levels are correlated between feces and serum (To-Figueras et al. 2000), maternal and umbilical cord blood levels (Ataniyazova et al. 2001; Sala et al. 2001a; Waliszewski et al. 2000b), breast-feeding and serum levels in infants or small children (Abraham et al. 2000; Lackmann 2004; Ribas-Fitó et al. 2005), and the presence of other organochlorines in serum (Burse et al. 2000; Glynn et al. 2000; Hoppin et al. 2000; and others).

Sufficient data of air levels of hexachlorobenzene have not been available to determine quantitative biomarkers of inhalation exposure. However, hexachlorobenzene levels have been assayed in people of Flix, Spain, who were exposed to hexachlorobenzene from a nearby electrochemical plant that produced organochlorines (Ballester et al. 2000; Carrizo et al. 2008; Grimalt et al. 1994; Herrero et al. 1999; Ribas-Fitó et al. 2003a, 2003b, 2005, 2007; Sala et al. 1999a, 1999b, 2001a; Sunyer et al. 2002, 2008; To-Figueras et al. 1997, 2000). These studies found higher serum levels in factory workers compared to nonworkers, in male workers compared to females (presumably due to increased work-related exposure), in nonworkers who lived with factory workers compared to nonworkers who did not live with factory workers, and in people living near the factory compared to people living further away.

3.8.2 Biomarkers Used to Characterize Effects Caused by Hexachlorobenzene

Although not specific to hexachlorobenzene, porphyria is the primary biomarker of effect from human exposure to hexachlorobenzene. Disturbance of the heme biosynthesis pathway of the body's porphyrin

metabolism in the liver is the major action of hexachlorobenzene in short- or long-term exposure. Due to this disturbance, abnormal levels of porphyrin precursors are found in exposed individuals (see Section 3.5.2 [Mechanisms of Toxicity] for additional information regarding hexachlorobenzene-induced porphyria). In some cases, porphyria cutanea tarda, displayed as scarring or cutaneous annular erythema (a condition termed *pembe yara*, or pink sore), is present. Such exposed people also exhibited painless arthritis, osteoporosis, and small distinctive hands (Cripps et al. 1984; Peters et al. 1982, 1987). Increases in serum *gamma*-glutamyl transferase, uroporphyrin (red-tinged urine), and d-ALA in the urine, and uroporphyrin and coproporphyrin in the stool are also indicative of the effect of hexachlorobenzene. While low levels of hexachlorobenzene have been found in human tissues and body fluids, such reported low levels have not generally been associated with adverse health effects (Booth and McDowell 1975). Associations have been found between increased hexachlorobenzene levels and decreased interferon-γ (Daniel et al. 2001), decreased lymphocyte IL-10 secretion (Belles-Isles et al. 2000), ear infections in infants (Dewailly et al. 2000), undescended testis (Hosie et al. 2000), and locomotor skill impairment in newborns (Sala et al. 1999b).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Certain chemicals may modify the toxicity of hexachlorobenzene, which itself may modify the toxicity of other chemicals. Selected chemicals may interfere with the toxicity of hexachlorobenzene indirectly by influencing its metabolism through their actions on drug metabolizing enzymes. The duration and intensity of action of hexachlorobenzene are largely determined by the speed at which it is metabolized in the body by the liver microsomal cytochrome P-450 system. More than 200 drugs, insecticides, and other chemicals are known to induce the activity of liver microsomal drug-metabolizing enzymes. The characteristic biological actions of these chemicals are highly varied. Although there is no clear relationship between their actions and structures and their ability to induce enzymes, most of the inducers are lipid soluble at physiological pH. These inducers of the P-450 system include the following classes of drugs: hypnotics and sedatives (barbiturates, ethanol); anesthetic gases (methoxyflurane, halothane); central nervous system stimulants (amphetamine); anticonvulsants (diphenylhydantoin); tranquilizers (meprobamate); antipsychotics (triflupromazine); hypoglycemic agents (carbutamide); anti-inflammatory agents (phenylbutazone); muscle relaxants (orphenadrine); analgesics (aspirin, morphine); antihistaminics (diphenhydramine); alkaloids (nicotine); insecticides (chlordane, DDT, BHC, aldrin, dieldrin, heptachlorepoxide, pyrethrins); steroid hormones (testosterone, progesterone, cortisone); and carcinogenic polycyclic aromatic hydrocarbons (3-methylcholanthrene, 3,4-benzpyrene) (Klaassen et al. 1995; Williams and Burson 1985).

Hexachlorobenzene has been reported to increase the activity of aryl hydrocarbon hydroxylase and other enzymes associated with both the 3-MC and phenobarbital-inducible isozymes of cytochrome P-450 in the rat (Goldstein et al. 1986); this could lead to accelerated biotransformation to the more toxic pentachlorophenol. The extent of toxicity mediated by this phenomenon is dependent on how rapidly the pentachlorophenol is hydrolyzed or conjugated to the less chlorinated benzenes which are much less toxic. In animal studies, pretreatment of rats with 3-methylcholanthrene or phenobarbital increased the metabolism and toxicity of hexachlorobenzene (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985; Vos et al. 1988). Pentachlorophenol (500 ppm) accelerated the onset of hexachlorobenzene-induced porphyria, indicated by an increase in urinary excretion of uroporphyrin and a decrease of porphyrin with 2 and 3 carboxylic groups in female rats fed diets containing 1,000 ppm hexachlorobenzene (Debets et al. 1980a). This increase occurred 3 weeks earlier in the hexachlorobenzene plus pentachlorophenol-treated animals than in animals treated with hexachlorobenzene alone. Intraperitoneal pretreatment with diethylstilbestrol followed by oral administration of hexachlorobenzene to male and female rats stimulated the excretion of hexachlorobenzene metabolites via urine and feces (Rizzardini and Smith 1982). A 2-fold increase in the accumulation of hexachlorobenzene in tissues also occurred (Villeneuve et al. 1977). An exacerbation in the increase of hepatic accumulation and urinary excretion of uroporphyrin occurred in rats given doses of hexachlorobenzene (25 mg/kg/day for 12 consecutive days) when hexachlorobenzene was co-administered with methyl isobutyl ketone. The authors speculated on the involvement of hepatic isozyme inhibition and porphyria induction by methyl isobutyl ketone in hexachlorobenzene porphyrinogenic action (Krishnan et al. 1992).

Similarly, prior or concurrent exposure to hexachlorobenzene and mixed function oxidase (MFO) enzyme-inhibiting substances (e.g., carbon monoxide; ethylisocyanide; SKF 525A, halogenated alkanes, such as CCl_4; alkenes, such as vinyl chloride; and allelic and acetylenic derivatives) may decrease the toxicity of hexachlorobenzene by decreasing the rate of the hydrolytic dealkylation and hydrolysis of both parent hexachlorobenzene (Williams and Burson 1985). Rats treated with hexachlorobenzene in combination with the cytochrome P450IIIA1 (CYP3A1) inhibitor, TAO, showed a marked reduction in hexachlorobenzene-induced immunomodulatory effects. These results suggest that the oxidative metabolites, pentachlorophenol and tetrachlorohydroquinone, are not likely to be implicated in the immunostimulatory effects of hexachlorobenzene (Schielen et al. 1995a). Similar conclusions were reached by the investigators of a 13-week rat study to assess the role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis (Den Besten et al. 1993) as well as in other animal studies in which pretreatment of rats with TAO decreased the metabolism of hexachloro-

benzene (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985; Vos et al. 1988).

Food deprivation has also been shown to increase susceptibility of animals to the toxicity of hexachlorobenzene. During 4 weeks of exposure to hexachlorobenzene at 40 and 200 mg/kg, food deprivation (50%) increased the ability of hexachlorobenzene to cause liver hypertrophy and induce microsomal enzyme activity. A 2-fold increase in the accumulation of hexachlorobenzene in tissues also occurred (Villeneuve et al. 1977). Since absorption was not measured, it is not clear whether these observations are due to metabolic changes in fat metabolism and release of hexachlorobenzene to target organs or to increased fractional absorption of hexachlorobenzene. These findings were validated by Kishima et al. (2000); 50 ppm hexachlorobenzene did not induce liver toxicity in liver-initiated Wistar rats when administered in a normal diet, but caused liver damage (decreased weight, foci of altered enzyme expression, hypertrophy) when administered in an energy-restricted diet which provided only 50% of the calories in the normal diet.

Results from other studies indicate that hexachlorobenzene has the potential to alter the toxicity of other chemicals. Co-treatment of hexachlorobenzene (400 μmol/kg) and 2,3,7,8-TCDD (10 or 30 μg/kg) in rats exacerbated both body weight loss and thymic atrophy caused by 2,3,7,8-TCDD, while hexachlorobenzene administered at doses as high as 3,000 μmol/kg did not cause any significant effects on these parameters (Li et al. 1989). Exposure to 4 mg/kg/day of hexachlorobenzene from 2 weeks prior to mating through lactation and partially through the placenta increased the LD_{50} value for malathion in 17–18-day-old Wistar rat pups by more than a factor of 2. In general, the inhibitory effect of malathion on cholinesterase activities was decreased by pretreatment with hexachlorobenzene. The authors attributed the increased resistance to intoxication by malathion and reduction of esterase inhibition in the pups to an increase in tissue carboxylesterase activity, presumably malathionase, and a decrease in malaxon formation (Mendoza and Shields 1976). In a study with female Sprague-Dawley rats administered single gavage doses of 400 or 600 mg/kg hexachlorobenzene in corn oil, or 10 or 12.5 mg/kg mercuric chloride, or a combination of 400 or 600 mg/kg hexachlorobenzene and 10 or 12.5 mg/kg mercuric chloride, hexachlorobenzene and mercuric chloride interacted additively with respect to lethality and endocrine, kidney, and liver toxicity. Although no deaths were reported in the 400 mg/kg hexachlorobenzene or 10 mg/kg mercuric chloride dose group animals, one death each was reported in the combined 400 mg/kg hexachlorobenzene plus 10 mg/kg mercuric chloride, and 600 mg/kg hexachlorobenzene plus 10 mg/kg mercuric chloride dose group animals; and two animals died in the 600 mg/kg hexachlorobenzene plus 12.5 mg/kg mercuric chloride dose group animals. Similarly, mild to moderate morphological changes

observed in the liver, thyroid, thymus, and bone marrow of rats exposed to hexachlorobenzene or hexachlorobenzene plus mercuric chloride; and in the kidneys of mercuric chloride- or mercuric chloride plus hexachlorobenzene-exposed rats were more severe in animals that received a combination of hexachlorobenzene and mercuric chloride (Lecavalier et al. 1994). The mechanism of these interactive effects are not known.

Iron overload aggravates hexachlorobenzene-induced porphyria and related hepatopathology. There was increased porphyrin excretion in female C57BL/6J mice (strains B6-Ah^{b} and B6-Ah^{d}) pretreated with iron and then fed diets containing 26 mg/kg/day of hexachlorobenzene for 9, 15, or 17 weeks as compared to rats given hexachlorobenzene alone. This is consistent with the proposition that the sustained induction of either P3-450 (the mouse CYP1A2 ortholog), or P1-450 (CYP1A1), or both may be a causative factor in the development of this disease. Furthermore, differential induction of the P3-450 (the mouse CYP1A2 ortholog) and P1-450 (CYP1A1) isozymes in B6-Ah^{b} responsive versus B6-Ah^{d} nonresponsive mice suggests that hexachlorobenzene may act through the Ah receptor (Hahn et al. 1988). Iron overload also caused a significantly depressed EROD (an estimate of CYP1A1 activity), in the livers of hexachlorobenzene-fed rats for 5 or 15 weeks while PROD (an estimate of CYP2B1 activity) and BROD (an estimate of CYP2B1 and other P-450 isozymes activity) were depressed in female Fischer 344 rats that received iron-dextran solution (50 mg/mL) by subcutaneous injection for 1 week and then were administered dietary hexachlorobenzene at a dose of 10 mg/kg/day in corn oil for 65 weeks (Smith et al. 1993).

The interactive effect of hexachlorobenzene with other substances in cancer induction has also been studied in animals. The toxicological effects of hexachlorobenzene exposure as a consequence of varying the dietary levels of vitamin A were evaluated in a single-generation lifetime study. There were no significant differences in hematological and pathological lesions in rats fed basal diets with either 0.1 or 10 times the vitamin A content of the control diet and animals fed similar diets which also included hexachlorobenzene (Arnold et al. 1985). Hyperplastic nodules were observed in the liver lobes of 80% of female Fischer 344 rats pretreated with Imferon (iron-dextran) then given dietary hexachlorobenzene at doses of 5 or 10 mg/kg/day in arachis oil for 65 weeks. There was a high incidence of fibrin throughout the liver with sinusoidal telangiectasis, and nodular peliosis hepatitis and hepatocellular necrosis. The study proposed a nongenotoxic mechanism for tumor induction by hexachlorobenzene, concluding that the formation of hepatomas and hemangiomas with elements of peliosis could be explained by the compensatory hyperplastic responses to hepatocellular injury or necrosis and by the simultaneous loss of

hepatocellular cords. The study further concluded that the accumulation of iron in the liver would strongly potentiate the development of hepatic tumors (Carthew and Smith 1994).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to hexachlorobenzene than will most persons exposed to the same level of hexachlorobenzene 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 hexachlorobenzene, or compromised function of organs affected by hexachlorobenzene. Populations who are at greater risk due to their unusually high exposure to hexachlorobenzene are discussed in Section 6.7, Populations with Potentially High Exposures.

Hexachlorobenzene has been shown to elevate porphyrin levels in humans following inhalation exposure (Herrero et al. 1999; Sala et al. 1999b; Selden et al. 1999) and to cause porphyria cutanea tarda (a specific disease resulting from elevated porphyrin levels) following oral exposure (Cam and Nigogosyan 1963; Cripps et al. 1984; Dogramaci 1964; Gocmen et al. 1989; Peters et al. 1982, 1987). Studies unrelated to hexachlorobenzene-exposure have associated the diagnosis of porphyria cutanea tarda with infections of HIV and hepatitis C virus (Drobacheff et al. 1998; Egger et al. 2002; Meola and Lim 1993). It is not known if, or the degree to which, these diseases contribute to or exacerbate one another; however, HIV and hepatitis C-infected individuals may have increased susceptibility to porphyria cutanea tarda following hexachlorobenzene exposure. Although no information was located regarding a possible role for certain genetic polymorphisms (e.g., polymorphisms for metabolic enzymes such as CYPs or enzymes involved in the porphyrin cascade) in hexachlorobenzene toxicity, such a role is theoretically possible.

Case studies of hexachlorobenzene poisoning in humans indicate that young children are more sensitive to hexachlorobenzene intoxication. Children (average age, 7 years) who had ingested hexachlorobenzene-contaminated bread during the epidemic of hexachlorobenzene poisoning in Turkey between 1955 and 1959 developed short stature, pinched faces, osteoporosis of bones of the hand, and painless arthritic changes. Some of the young children in this study were presumed to have been exposed *in utero* via transplacental transfer and postnatally by lactational transfer. The children who died were between the ages of 1 and 2 years, and died from a disease known as *pembe yara* or “pink sore” (Cripps et al. 1984; Peters et al. 1982, 1987). Analysis of human milk from exposed women and unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al.

1984; Gocmen et al. 1989; Peters et al. 1982, 1987). Refer to Section 3.7 (Children's Susceptibility) for more detailed information regarding age-related susceptibility to hexachlorobenzene toxicity.

In laboratory animals, reduced survival of suckling offspring of lactating mothers and fetuses of mothers exposed to hexachlorobenzene was also reported in several studies (Arnold et al. 1985; Grant et al. 1977; Kitchin et al. 1982). There is evidence that hexachlorobenzene is concentrated in milk of lactating monkeys exposed to hexachlorobenzene, suggesting that the risk of exposure to nursing infants may be greater than the risk to their mothers. Blood and tissue levels in the infants were higher than in mothers, and infants exhibited clinical symptoms of toxicity sooner than their mothers (Bailey et al. 1980).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to hexachlorobenzene. 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 hexachlorobenzene. 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 hexachlorobenzene:

Bebarta VS, Phillips SD. 2004. Fungicides. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1529-1532.

Craig SA. 1998. Herbicides and fungicides. In: Viccellio P, ed. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 415-423.

Leikin JB, Paloucek FP, eds. 2002. Poisoning and toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 647-648.

3.11.1 Reducing Peak Absorption Following Exposure

Decontamination should be initiated immediately following overexposure to hexachlorobenzene. For inhalation exposure, management commonly includes moving the exposed individual to fresh air, and then monitoring for respiratory distress. Supplemental oxygen should be applied if needed (Bebarta and Phillips 2004). For acute oral exposure to hexachlorobenzene, gastric lavage is most likely beneficial if administered within 1 hour or so of ingestion using activated charcoal (Bebarta and Phillips 2004; Leikin and Paloucek 2002). Following dermal exposure, contaminated clothing should be removed and exposed

skin should be washed copiously with soap and water. Please consult a physician or clinical toxicologist if you have been exposed to hexachlorobenzene.

3.11.2 Reducing Body Burden

Diuresis is not likely to be effective because of the high lipophilic nature of hexachlorobenzene. Exchange transfusion, peritoneal dialysis, hemodialysis, and hemoperfusion are not likely to be beneficial because of the rapidity with which hexachlorobenzene leaves the blood and locates in peripheral compartments, since this substance has an initial large volume of distribution. However, continued treatment with multi-dose activated charcoal or cholestyramine may be useful to enhance elimination (Cohn et al. 1978; Leikin and Paloucek 2002), although no differences in fecal excretion of hexachlorobenzene were observed when cholestyramine or sesame oil was administered orally to rats and Rhesus monkeys following ingestion of hexachlorobenzene. When mineral oil was added to the diet of Rhesus monkeys treated with hexachlorobenzene, a 6–9-fold increase in fecal excretion of hexachlorobenzene and its metabolites was observed. Continuous administration of mineral oil led to increased depletion of hexachlorobenzene from both the blood and adipose tissues. Administration of n-hexadecane enhanced fecal excretion of hexachlorobenzene about 5-fold in rats (Rozman et al. 1981). The barbiturates, which have been used to control poison-induced convulsions, may hasten metabolism and elimination of hexachlorobenzene (Smith 1991).

Hexachlorobenzene is known to be toxic to developing perinatal animals. The particularly potent effects seen in nursing infants following maternal exposure to hexachlorobenzene must be recognized when counseling hexachlorobenzene-exposed women of childbearing age. It is recommended that plans for pregnancy and contraception be included in the physician's clinical assessment, with consideration to specialty evaluation of residual hexachlorobenzene contamination.

Olestra fed to mice that were gavaged with radio labeled hexachlorobenzene (0.7 µCi/day) resulted in a 30-fold increase in the excretion of hexachlorobenzene and decreased hexachlorobenzene levels in the epididymal fat pad, brains, and liver, compared with mice undergoing caloric restriction without olestra (Jandacek et al. 2005). The authors suggest that the increased excretion was caused by olestra providing a lipophilic sink that interfered with the enterohepatic circulation of hexachlorobenzene, or else that olestra enhanced transport of hexachlorobenzene into the lumen.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Hexachlorobenzene appears to produce little central nervous system toxicity at low doses. At high doses, central nervous system depression can dominate the clinical profile (de Matteis et al. 1961). Thus, management of seizures with anticonvulsants is not likely to be needed except in high-dose acute intoxication management. Benzodiazepenes should be used for seizure control. Contact dermatitis can be treated symptomatically with antihistamines and topical or systemic steroids (Bebarta and Phillips 2004).

In Turkey, in 1956, prolonged oral exposure to hexachlorobenzene was associated with an outbreak of acquired porphyria cutanea tarda characterized by neurological, visceral, arthritic, cutaneous, and hepatic symptoms. The victims also exhibited bulbous, erythematous skin lesions, which progressed to atrophy, hyperpigmentation, hypertricosis (increased body hair), and ulcerations. Treatment for these conditions was primarily supportive. Anecdotal reports from Turkey indicated that chelating agents (disodium ethylenediaminetetraacetic acid [EDTA] and dimercaptopropanol [BAL]) administration over 3 months (1.5 g daily for 5 days intravenously followed by daily oral doses of 1–2 g) successfully reduced the symptoms of patients with hexachlorobenzene-induced porphyria (Peters 1956, 1993; Peters and Cripps 1985; Peters et al. 1957, 1966, 1986). However, this method of treating acute porphyria has not been validated.

The role of iron overload in the pathogenesis of hexachlorobenzene-induced porphyria has also been examined based on observations that 80% of patients with porphyria cutanea tarda have increased liver stores of iron and increased levels of uroporphyrin 1 (Smith and de Matteis 1990; Wainstok de Calmanovici et al. 1991). In these patients, phlebotomy often induces disease remission and a decrease in urinary porphyrin 1 excretion. It remains to be seen whether iron overload plays a permissive or etiologic role in patients exposed to porphyria-producing toxins, and whether phlebotomy has any role in the treatment of these patients.

In other reports, co-administration of S-adenosyl-L-methionine (SAM) via subcutaneous injection for the last 15 days of treatment with an oral dose of hexachlorobenzene (100 mg/kg/day) reversed some of the effects of hexachlorobenzene exposure (elevation of liver porphyrin content and liver weight). It has been suggested that the beneficial effects of SAM on hexachlorobenzene-induced toxicity may be related to effects on adenosine triphosphate availability (Cantoni et al. 1990; Cuomo et al. 1991).

A study conducted with Osteogenic Disorder Shionogi (ODS) rats provided evidence that large doses of ascorbic acid may inhibit chemically-induced uroporphyria in humans. This effect seems to be mediated by inhibition of CYP1A2-catalyzed uroporphyrinogen oxidation. However, a significant depletion of hepatic ascorbic acid may be required for any beneficial effect of ascorbic acid to be observed (Sinclair et al. 1995).

Injections of 20 mg/kg/day Cyclosporin A (CsA) reduced immunopathological response in Brown Norway rats when administered concurrently with 450 mg/kg hexachlorobenzene via the diet for 21 days (Ezendam et al. 2004b). T-cells were predominantly affected by CsA treatment.

3.12 ADEQUACY OF THE DATABASE

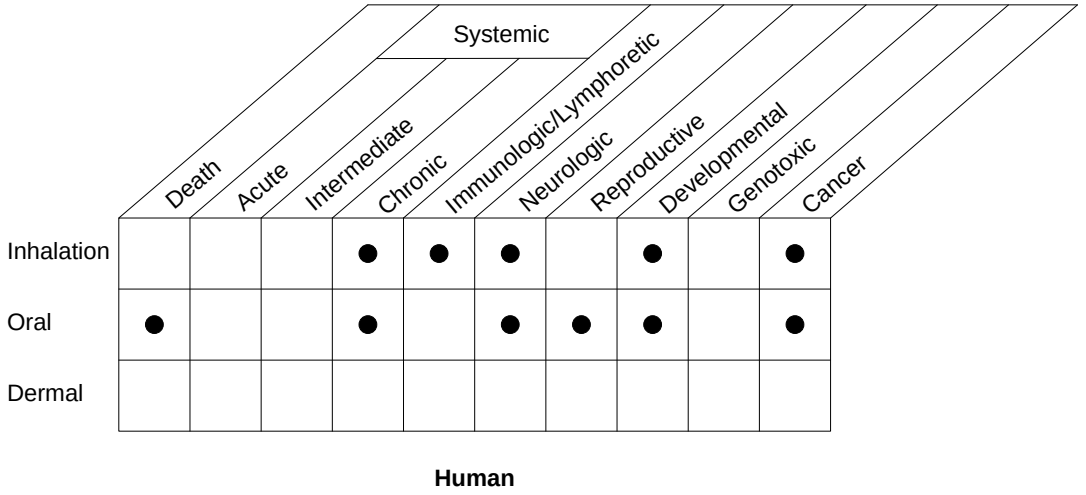
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of hexachlorobenzene is available. Where adequate information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

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.

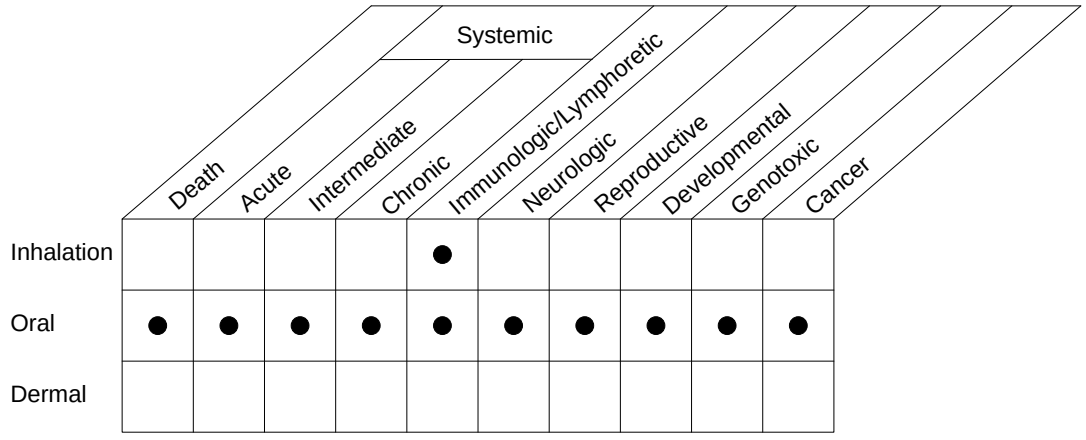
3.12.1 Existing Information on Health Effects of Hexachlorobenzene

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

Figure 3-5. Existing Information on Health Effects of Hexachlorobenzene



Human



Animal

● Existing Studies

Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-5, there are data available regarding a number of toxic end points in humans exposed to hexachlorobenzene by inhalation and ingestion, including death, chronic systemic toxicity, immunological, neurological, reproductive and developmental effects, and cancer. However, the extent of the data available is limited, particularly for inhalation exposure. No data were located regarding the toxicity of hexachlorobenzene by dermal exposure in humans. Hexachlorobenzene has been well studied in animals following oral exposure; the full range of end points has been assessed. However, only immunological effects have been studied in animals following inhalation exposure and no studies at all were located regarding toxicity of hexachlorobenzene in animals following dermal exposure.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No information was located regarding health effects in humans following acute-duration exposure to hexachlorobenzene by any route. The available animal data were sufficient to identify target organs of acute oral exposure. Extensive study of animals exposed by the oral route for acute durations has identified doses producing a wide range of health effects, including porphyria and other hepatic effects, renal tubular lesions, changes in thyroid and reproductive hormone levels, impaired male reproductive performance, developmental effects ranging from subtle neurobehavioral effects in neonates to fetotoxic and teratogenic effects, overt neurological effects, and lethality (Bouthillier et al. 1991; Courtney et al. 1976; De Matteis et al. 1961; Foster et al. 1993; Goldey and Taylor 1992; Goldstein et al. 1978; Kennedy and Wigfield 1990; Khera 1974; Mehendale et al. 1975; Simon et al. 1979). Most of the existing acute studies were conducted in rats, but mice and guinea pigs were also studied. Pharmacokinetic models for oral exposure in rats and humans are available (Freeman et al. 1989; Yesair et al. 1986), and provided good correlations between predicted yield of metabolites and experimentally observed liver toxicity (porphyria and increased liver weight reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), between estimated concentrations of hexachlorobenzene in the brain and observed central nervous system effects (tremors and other signs reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), and between estimated extent of lactational transfer of hexachlorobenzene and observed offspring mortality (data from Kitchen et al. 1982). The database was sufficient to support derivation of an acute oral MRL for hexachlorobenzene. Acute studies by inhalation exposure in animals were limited to a single study of immunological effects (Sherwood et al. 1989). No acute dermal studies

in animals were located. Although an acute study in rats suggested that hexachlorobenzene can be absorbed across the skin (Koizumi 1991), pharmacokinetic models for inter-route extrapolation are not available. Additional acute studies by the inhalation and dermal routes would identify acute effect levels for these routes of exposure.

Intermediate-Duration Exposure. A few case-control studies have found evidence of developmental toxicity in newborn humans (Belles-Isles et al. 2000; Hosie et al. 2000); no information was located regarding health effects in humans following intermediate-duration exposure to hexachlorobenzene by any route. Extensive study of animals exposed by the oral route for intermediate durations has identified doses producing a broad spectrum of health effects, including porphyria and other hepatic effects, renal tubular lesions, pulmonary lesions, cardiac lesions, anemia and leukocytosis, osteosclerotic changes in bone, necrotic lesions in muscle, skin lesions, thymic atrophy, splenomegaly and altered spleen morphology, lymph node histopathology, altered immunoglobulin levels, suppression of immune function, changes in the thyroid, parathyroid, and adrenal glands and associated hormone levels, ovarian and testicular lesions, alterations in female menstrual cycling and reproductive hormone levels, reduced fertility, developmental effects ranging from subtle neurobehavioral effects in neonates to fetotoxic effects and pup death, overt neurological effects, and lethality (Andrews et al. 1988, 1989, 1990; Babineau et al. 1991; Bourque et al. 1995; Bouthillier et al. 1991; Chalouati et al. 2013; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1992a, 1992b; Iatropoulos et al. 1976; Jarrell et al. 1993; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Knauf and Hobson 1979; Koss et al. 1978; Kuiper-Goodman et al. 1977; Lilienthal et al. 1996; Loose et al. 1978, 1981; NTP 2002; Ockner and Schmid 1961; Schielen et al. 1995a, 1995b; Smith et al. 1985; Vos et al. 1979a, 1979b, 1983; others). Most of the existing intermediate-duration studies were conducted in rats, but monkeys, mice, rabbits, dogs, and pigs were also studied. Pharmacokinetic models for oral exposure in rats and humans are available (Freeman et al. 1989; Yesair et al. 1986), and provided good correlations between predicted yield of metabolites and experimentally observed liver toxicity (porphyria and increased liver weight reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), between estimated concentrations of hexachlorobenzene in the brain and observed central nervous system effects (tremors and other signs reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), and between estimated extent of lactational transfer of hexachlorobenzene and observed offspring mortality (data from Kitchen et al. 1982). The database was sufficient to support derivation of an intermediate-duration oral MRL for hexachlorobenzene. Intermediate-duration studies by inhalation exposure in animals were limited to a single study of immunological effects (Sherwood et al. 1989). No intermediate-duration dermal studies in animals were located. Pharmacokinetic models for inter-route extrapolation are not available. Additional

intermediate-duration studies by the inhalation and dermal routes would identify intermediate-duration effect levels for these routes of exposure.

Chronic-Duration Exposure and Cancer. There are data available on humans chronically exposed to hexachlorobenzene by the inhalation and oral routes, but no quantitative exposure information. The inhalation data are very limited, but tentatively found effects on the liver and immune system of exposed individuals (Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994; Selden et al. 1999). The oral data much more clearly identified the liver, skin, bone, thyroid, and central nervous system as target tissues for hexachlorobenzene in chronically exposed people (Cam and Nigogosyan 1963; Cripps et al. 1984; Peters et al. 1982, 1987). Based on very limited data, the original investigators of the Turkey epidemic estimated that the daily average oral dose was 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) (Cam and Nigogosyan 1963). No information regarding chronic dermal exposure in humans was located. Chronic oral animal studies identified dose levels associated with systemic (cardiovascular, gastrointestinal, hematological, hepatic, renal, and dermal), immunological, overt neurological, and developmental effects, as well as death (Arnold et al. 1985; Cabral et al. 1977, 1979; Gralla et al. 1977; Mollenhauer et al. 1975; Smith and Cabral 1980; Smith et al. 1985, 1989, 1993; others). The database was sufficient to support derivation of a chronic oral MRL for hexachlorobenzene. No chronic animal studies were located using inhalation or dermal exposure. Pharmacokinetic models for inter-route extrapolation are not available. Additional chronic studies by the inhalation and dermal routes would identify chronic effect levels for these routes of exposure.

Data from people exposed to hexachlorobenzene by inhalation provide weak evidence for an association between exposure to hexachlorobenzene and cancer of the thyroid, brain, and liver (Grimalt et al. 1994; Selden et al. 1989), while very limited data from orally exposed people showed no increase in cancer risk (Cripps et al. 1984; Peters et al. 1982). One case-control study associated elevated adipose levels of hexachlorobenzene with increased risk of breast cancer (Dewailly et al. 1994), but other case-control studies have not found any relationship between body burdens of hexachlorobenzene and breast cancer (Dorgan et al. 1999; Falck et al. 1992; Guttes et al. 1998; Liljegren et al. 1998; Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhamaa et al. 1990; Zheng et al. 1999), bone sarcoma or leukemia (Hardell et al. 1997; Scheele et al. 1996). The available epidemiology reports taken together do not support an association between hexachlorobenzene exposure and increased cancer incidence, but their limitations (including small study sizes and potentially confounding effects of other organochlorines) preclude considering them evidence of noncarcinogenicity. Because hexachlorobenzene produces

porphyria, it is noteworthy that several human studies have associated porphyria with increased incidence of liver cancer (Axelson 1986; Fracanzani et al. 2001).

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumor formation. The evidence of carcinogenicity is strongest in the liver; hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumors (hepatoma in mice and rats; hemagniohepatoma and bile duct adenoma in rats), and malignant tumors (hepatocarcinoma in rats, mice, and hamsters; bile duct adenocarcinoma in rats) (Arnold et al. 1985; Cabral et al. 1979; Ertürk et al. 1986; Pereira et al. 1982; Smith and Cabral 1980; Smith et al. 1985). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas, and renal cell carcinomas (in rats, mice, and hamsters); lymphosarcomas (in rats, mice, and hamsters); adrenal hyperplasia and pheochromocytoma (in rats); parathyroid adenomas (in rats); and hemangioendothelioma and thyroid tumors (in hamsters) (Arnold et al. 1985; Den Besten et al. 1993; Ertürk et al. 1986; Kimbrough and Linder 1974). No animal cancer bioassays by inhalation or dermal exposure were located. Pharmacokinetic models for inter-route extrapolation are not available. Based on these findings in animals, hexachlorobenzene is considered a probable human carcinogen. Additional epidemiological studies of people with known hexachlorobenzene exposure would enable better assessment of the carcinogenic risk of this chemical to humans. Additional studies that focus on possible mechanisms of hexachlorobenzene toxicity and carcinogenicity are needed.

Genotoxicity. Human genotoxicity data for hexachlorobenzene are limited to a case study (route of exposure unknown) and *in vitro* studies in human cell lines. The frequency of micronuclei in peripheral lymphocyte was increased in 41 workers exposed to a mixture of chlorinated solvents that included hexachlorobenzene (da Silva Augusto et al. 1997). Hexachlorobenzene did not produce chromosomal aberrations in human peripheral lymphocytes *in vitro* (Siekel et al. 1991), but did produce weak positive results in assays for DNA fragmentation and micronuclei formation in primary cultures of human hepatocytes (Canonero et al. 1997) and minimal formation of DNA adducts in cultured human Hep G2 hepatoma cells (Dubois et al. 1997). No information on the genotoxicity of hexachlorobenzene in animals by inhalation or dermal exposure was located. Hexachlorobenzene did not cause gene mutations or unscheduled DNA repair in microbial assays (Gopalaswamy and Nair 1992; Haworth et al. 1983; Siekel et al. 1991), and did not produce dominant lethal mutations in orally-exposed rats (Khera 1974; Simon et al. 1979), or bind strongly to rat DNA *in vitro* or *in vivo* (Gopalaswamy and Nair 1992). However, hexachlorobenzene did produce DNA fragmentation in cultured rat hepatocytes (Canonero et

al. 1997) and DNA adducts in fetal hepatocytes from rats and quail (Dubois et al. 1997). Additional studies employing other *in vivo* and *in vitro* assays would be useful to determine the genotoxic potential of hexachlorobenzene.

Reproductive Toxicity. No data were located regarding reproductive effects in humans or animals with inhalation or dermal exposure. Several miscarriages and stillbirths were reported among people with previous oral exposure to hexachlorobenzene (Peters et al. 1982, 1987), but it is not clear that the rate of miscarriages was significantly higher than normal for this population. One study associated elevated hexachlorobenzene blood levels with increased risk for spontaneous abortion (Jarrell et al. 1998), but other studies did not (Gerhard et al. 1998; Leoni et al. 1986, 1989). No associations were found between serum reproductive hormone levels and serum hexachlorobenzene levels in men (Freire et al. 2014; Goncharov et al. 2009) or premenopausal women (Freire et al. 2014), although a slight, but significant, negative association between serum hexachlorobenzene and serum LH was noted among peri- and postmenopausal women (Freire et al. 2014). The cessation of agricultural uses of hexachlorobenzene in Xixin did not affect reproductive outcomes there (Huang et al. 1989). Animal studies using oral exposure have identified doses associated with reproductive effects, including ovarian lesions and hormonal and menstrual changes, in female rats and monkeys (Alvarez et al. 2000; Babineau et al. 1991; Bourque et al. 1995; Foster et al. 1992a, 1992b, 1993, 1995a; Iatropoulos et al. 1976; Jarrell et al. 1993; Muller et al. 1978; Sims et al. 1991), reduced fertility in rats (Grant et al. 1977), reduced mating index in male rats (Simon et al. 1979), testicular effects in rats and pigs (including increased weight, degenerative lesions, and retarded maturation) (Den Tonkelaar et al. 1978; Gralla et al. 1977; Smith et al. 1985), and mammary gland lesions in rats (NTP 2002). The intermediate-duration MRL for oral exposure is based on ovarian effects in monkeys. Reproductive effects have not been studied in animals by inhalation or dermal exposure, and pharmacokinetic models for inter-route extrapolation are not available. Epidemiological studies of people with known hexachlorobenzene exposure would be useful to establish whether these effects are also seen in people. Additional mechanistic studies to better understand the ovarian and hormonal changes might also help establish the relevance of these findings to humans.

Developmental Toxicity. No studies on the developmental effects of hexachlorobenzene in humans following dermal exposure or in laboratory animals following inhalation or dermal exposure were identified, and pharmacokinetic models for inter-route extrapolation are not available. Dramatic developmental toxicity (high mortality, skin lesions) was seen in infants whose mothers consumed bread contaminated with hexachlorobenzene (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982); this study clearly established hexachlorobenzene as a developmental toxicant. Other human studies have

found suggestive evidence linking hexachlorobenzene exposure with developmental toxicity, e.g., locomotor skill impairment associated with inhalation exposure (Sala et al. 1999b) and increased risk of undescended testis (route of exposure unknown) (Hosie et al. 2000), although an additional study found no correlation between hexachlorobenzene levels (in blood and milk) and infant intelligence test results (Darvill et al. 2000). Some studies evaluated possible associations between maternal serum hexachlorobenzene levels and developmental end points such as birth size (weight and/or length) or preterm birth (Basterrechea et al. 2014; Eggesbø et al. 2009; Fenster et al. 2006; Gladen et al. 2003; Guo et al. 2014; Lopez-Espinosa et al. 2011; Sagiv et al. 2007; Szyrwińska and Lulek 2007; Torres-Arreola et al. 2003; Vafeiadi et al. 2014), recurrent miscarriage (Sugiura-Ogasawara et al. 2003), postnatal growth (Burns et al. 2012; Cupul-Uicab et al. 2013; Mendez et al. 2011; Smink et al. 2008; Valvi et al. 2014), postnatal neurodevelopment (Cheslack-Postava et al. 2013; Darvill et al. 2000; Forns et al. 2012; Sioen et al. 2013; Strøm et al. 2014), sexual maturation (Croes et al. 2014a, 2014b; Denham et al. 2005; Lam et al. 2014; Schell and Gallo 2010), cryptorchidism (Pierik et al. 2007), hypospadias (Giordano et al. 2010; Rignell-Hydbom et al. 2012), and indicators of postnatal thyroid function (Julvez et al. 2011). Although most studies found no significant association between maternal serum hexachlorobenzene levels and risk of developmental effects, there were reports of significant associations between maternal or cord blood hexachlorobenzene and birth weight (Lopez-Espinosa et al. 2011; Vafeiadi et al. 2014), postnatal growth (Valvi et al. 2014), and hypospadias (Giordano et al. 2010; Rignell-Hydbom et al. 2012). Some studies that assessed serum hexachlorobenzene levels in young boys and girls reported significant effects on markers of sexual development (Croes et al. 2014a, 2014b; Lam et al. 2014).

Studies in orally exposed rats have demonstrated neurodevelopmental (Goldey and Taylor 1992; Lilienthal et al. 1996) and immunodevelopmental effects (Barnett et al. 1987; Vos et al. 1979a, 1983), reduced neonatal viability and growth (Grant et al. 1977; Kitchin et al. 1982; Vos et al. 1979a, 1983), and some evidence of teratogenic abnormalities (Courtney et al. 1976; Khera 1974). Hexachlorobenzene caused neurological, hepatic, and cardiovascular effects, as well as death, in lactionally exposed Rhesus monkey infants (Bailey et al. 1980; Iatropoulos et al. 1978). Additional studies that included an assessment of more sensitive end points, such as endocrine changes and neurological or immunological effects, and an investigation of different periods of developmental sensitivity (such as prenatal versus postnatal exposures) would contribute to a clearer understanding of the developmental toxicity of hexachlorobenzene.

Immunotoxicity. Studies on the immunotoxicity of hexachlorobenzene in humans following oral or dermal exposure are lacking. Occupational studies have associated inhalation exposure to

hexachlorobenzene with effects on immunological parameters (neutrophil chemotaxis and cytolytic activity, serum immunoglobulin and IFN-γ levels) (Daniel et al. 2001; Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994). Case-control studies have associated increased body burdens of hexachlorobenzene (putatively resulting from consumption of contaminated food) with alterations in markers of immune function and susceptibility to infection (Belles-Isles et al. 2000; Dewailly et al. 2000). Two studies reported significant associations between maternal serum hexachlorobenzene and risk of asthma in offspring (Gascon et al. 2014; Hansen et al. 2014).

No studies on the immunotoxicity of hexachlorobenzene in animals after dermal exposure were located. An intermediate-duration study found slight decreases in humoral and pulmonary cellular defenses of rats exposed to hexachlorobenzene via inhalation (Sherwood et al. 1989). Following oral exposure, immunosuppression has been observed in rats, mice, monkeys, and bears (Bernhoft et al. 2000; Carthew et al. 1990; Iatropoulos et al. 1976; Loose et al. 1977, 1981; Michielsen et al. 1997; Silkworth and Loose 1981; Van Loveren et al. 1990) and at least a partial stimulation of the immune system in rats and dogs (Gralla et al. 1977; Kennedy and Wigfield 1990; Koss et al. 1978; Schielen et al. 1993, 1995b; Vos et al. 1979a, 1979b). Additionally, a number of animal studies have observed inflammation and immune cell infiltration in tissues such as the liver (Arnold et al. 1985; Ertürk et al. 1986), respiratory tract (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997, 1999, 2001; Vos et al. 1979a, 1983), and skin (Koss et al. 1978; Michielsen et al. 1997, 2000; Schielen et al. 1993, 1995b; Torinuki et al. 1981) following oral exposure to hexachlorobenzene. NTP (2002) reported significantly increased incidences of splenic lymphoid hyperplasia in rats administered hexachlorobenzene by gavage for 90 days. Additional chronic-duration studies in humans in the workplace would identify effect levels for immunotoxicity.

Neurotoxicity. No information regarding neurotoxicity in humans following dermal exposure was located. Following ingestion of bread contaminated with hexachlorobenzene, observed neurological effects included profound weakness, loss of muscle control (inability to handle utensils, myotonia [delayed muscle relaxation after an initial contraction], and cogwheeling [irregular jerkiness of movement due to increased muscle tone as seen in Parkinson's disease]), paresthesia (spontaneous tingling or burning sensations), and sensory shading (graded sensory loss indicative of polyneuropathy) (Cam and Nigogosyan 1963; Gocmen et al. 1989; Peters et al. 1982, 1987). Inhalation data are limited to suggestive evidence linking inhalation exposure to hexachlorobenzene with impaired development of locomotor skills in newborn babies (Sala et al. 1999a). A case-control study did not associate umbilical blood or breast milk hexachlorobenzene levels with infant intelligence test results (Darvill et al. 2000). No studies

on the neurotoxicity of hexachlorobenzene in animals after inhalation or dermal exposure were located. Oral studies, primarily in rats but also in mice, rabbits, pigs, monkeys, and quail, have demonstrated serious neurological effects such as convulsions, tremors (intermittent and constant), lethargy, and progressive weakness (Cabral et al. 1979; Cripps 1990; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Grant et al. 1977; Hahn et al. 1988; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Khera 1974; Knauf and Hobson 1979; Ockner and Schmid 1961; others) as well as neurobehavioral effects in rats following developmental exposure (Goldey and Taylor 1992; Lilienthal et al. 1996; Taylor and Goldey 1990), changes in adult rat brain chemistry (Billi de Catabbi et al. 2000b; Cochon et al. 2001), and electrophysiological changes in the brains of adult dogs (Sufit et al. 1986; Sundlof et al. 1981). The acute-duration MRL for oral exposure is based on neurobehavioral changes in rats following developmental exposure (Goldey and Taylor 1992). Additional inhalation and dermal studies would identify the potential neurotoxicity of hexachlorobenzene by these routes of exposure.

Epidemiological and Human Dosimetry Studies. No information regarding the adverse effects of hexachlorobenzene in humans following dermal exposure is available, and no human dosimetry data are available. Health effects (death, systemic [e.g. liver, skin, bone, and thyroid], neurological, developmental, and endocrine) were identified in cohorts from a group of approximately 4,000 people orally exposed in Turkey to hexachlorobenzene (in contaminated bread) between 1955 and 1959 (Booth and McDowell 1975; Cam and Nigogosyan 1963; Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987; Selden et al. 1989). Related to inhalation exposure, health effects (systemic [hepatic, renal, and endocrine] and neurological) have been identified in residents of a rural town (Flix, Spain) with airborne hexachlorobenzene pollution attributed to a nearby organochlorine factory, workers from that factory, and other people with occupational exposure (Grimalt et al. 1994; Herrero et al. 1999; Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994; Sala et al. 1999a, 1999b, 2001a; To-Figueras et al. 1997). Multiple case-studies have investigated possible associations between body burdens of hexachlorobenzene levels (in blood, fat, urine, and feces) with multiple health effects (Belles-Isles et al. 2000; Darvill et al. 2000; Dewailly et al. 1994, 2000; Dorgan et al. 1999; Falck et al. 1992; Gerhard et al. 1998; Guttes et al. 1998; Hagmar et al. 2001; Hosie et al. 2000; Leoni et al. 1986, 1989; Liljegren et al. 1998; Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhamaa et al. 1990; Zheng et al. 1999; others). None of these human studies have provided reliable direct exposure data (dose and duration); therefore, no evidence of an exposure-response relationship has been possible. Further studies of populations with elevated exposures to hexachlorobenzene (e.g., occupational, consumption of fish from contaminated areas) would provide additional information useful in assessing dosimetry and health effects such as reproductive and developmental toxicity.

Biomarkers of Exposure and Effect.

Exposure. Hexachlorobenzene has been measured in human blood and serum, liver, bone marrow, brain, fat, semen, placenta, umbilical cord (and cord blood), breast milk, feces, and urine (Ataniyazova et al. 2001; Bucholski et al. 1996; Burse et al. 2000; Dewailly et al. 1999; Poli et al. 1999; Schlummer et al. 1998; Szymczynski and Waliszewski 1981; many others). Metabolites of hexachlorobenzene (including chlorophenols, pentachlorobenzene, alpha-hexachlorocyclohexane, or pentachloronitrobenzene) have been measured in blood, urine, and feces (Ingebrigtsen et al. 1981, 1986; Koss et al. 1976; Lui and Sweeney 1975; Rozman et al. 1977a; To-Figueras et al. 1992; van Ommen et al. 1985; Yang et al. 1978). Indirect biomarkers used to detect intermediate- and chronic-exposure to hexachlorobenzene exposure include measurement of *gamma*-glutamyl transferase in blood, uroporphyrin and d-ALA in urine, and coproporphyrin in feces (Koss et al. 1986; To-Figueras et al. 1992); because of their lack of specificity, the usefulness of these biomarkers is limited. Information regarding biomarkers of exposure to hexachlorobenzene appears adequate; it is uncertain whether additional biomarkers that are specific for hexachlorobenzene exposure could be developed. If so, they might be useful in the monitoring of people living near hazardous waste sites at which hexachlorobenzene has been detected.

Effect. Porphyria is the primary biomarker of effect from human acute, intermediate, and chronic exposure to hexachlorobenzene. Studies of an orally exposed population have diagnosed several unusual disease states of porphyria cutanea tarda, including dermal lesions (*pembe yara* or “pink sore” and *kara yara* or “black sore,” associated with photosensitivity, dermal fragility and scarring, hyperpigmentation and hirsutism) and small distinctive hands (shortened and spindled fingers with painless swelling and osteoporosis) (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). Increases in serum *gamma*-glutamyl transferase, uroporphyrin and d-ALA in the urine (red-tinged urine), and uroporphyrin and coproporphyrin in the stool are also indicative of the effect of hexachlorobenzene (Booth and McDowell 1975; others). Additional studies that identified alternative biomarkers would complement these existing biomarkers. Moreover, direct assessments of exposure would facilitate the identification of effect levels.

Absorption, Distribution, Metabolism, and Excretion. One study was located in which gastrointestinal absorption of hexachlorobenzene in humans was quantified (Schlummer et al. 1998). Information regarding absorption in humans following inhalation exposure is based on observations of toxicity (Grimalt et al. 1994; Herrero et al. 1999; Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994;

Sala et al. 1999b; Selden et al. 1997; To-Figueras et al. 1997). No experimental information regarding absorption in humans by dermal exposures was located; dermal data from a rat study were used to develop a compartment model for application to a 70-kg worker (Koizumi 1991). Only one dermal absorption study (Koizumi 1991) and no inhalation absorption studies in animals are available. Animal data suggest that oral absorption is rapid if dissolved in a lipid, but absorption from aqueous solution is low (Ingebrigtsen and Nafstad 1983; Koss and Koransky 1975). Additional information on the absorption of hexachlorobenzene, especially by the inhalation route, as well as data regarding enterohepatic circulation and gastrointestinal reabsorption would allow more accurate estimations of exposure and evaluation of route-specific differences in hexachlorobenzene toxicity.

No data on distribution in humans following inhalation or dermal exposure or in animals following inhalation exposure were available, but limited information on the distribution of hexachlorobenzene following oral exposure was located. Available data suggest that hexachlorobenzene is preferentially and rapidly distributed to tissues with high lipid content (Cripps 1990; Ingebrigtsen and Nafstad 1983; Jarrell et al. 1993; Mehendale et al. 1975; Verschueren 1983). Additional distribution studies would provide information regarding the tissue doses associated with adverse effects.

The metabolism of hexachlorobenzene has not been studied in humans. Studies in monkeys and rats indicate that hexachlorobenzene is metabolized to less chlorinated benzenes, chlorinated phenols, other minor metabolites, and glucuronide and glutathione conjugates (Ingebrigtsen and Nafstad 1983; Ingebrigtsen et al. 1981, 1986; Jansson and Bergman 1978; Koss et al. 1986; Rozman et al. 1977a). Because differences in metabolism may occur with differences in the route of exposure, it would be useful to have more data on inhalation and dermal metabolic studies as a comparison with the available oral studies.

No studies were located regarding excretion of hexachlorobenzene in animals or humans following inhalation or dermal exposure. Oral studies in animals indicate that the parent hexachlorobenzene is excreted primarily in feces, while metabolites were detected in urine (Albro and Thomas 1974; Ingebrigtsen et al. 1981; Mehendale et al. 1975; Rozman et al. 1977a, 1981; To-Figueras et al. 1991). Studies on excretion following inhalation and dermal exposure to hexachlorobenzene would be useful to determine if excretion patterns vary with different routes.

PBPK models for hexachlorobenzene have been developed by Yesair et al. (1986) and Freeman et al. (1989). The Yesair et al. (1986) model describes the absorption, distribution, and elimination of ingested

hexachlorobenzene in growing rats and humans. The Freeman et al. (1989) model describes distribution and excretion of intravenously injected hexachlorobenzene in growing rats. Additional PBPK models to extrapolate from high- to low-exposure and between routes of exposure would aid in risk analysis.

Comparative Toxicokinetics. Although no toxicokinetic information is available for humans following dermal or inhalation exposure, data from a rat study were used to develop a compartment model for application to a 70-kg worker (Koizumi 1991).

Overall, data in animal studies do not indicate the toxicokinetics of hexachlorobenzene are similar among species (Albro and Thomas 1974; Cripps 1990; Goldey et al. 1990; Iatropoulos et al. 1975; Koizumi 1991; Koss et al. 1986; Rozman et al. 1977a; others). Differences observed in absorption may be related to vehicle and route of administration (Iatropoulos et al. 1975; Ingebrigtsen and Nafstad 1983; Koss and Koransky 1975; Lecavalier et al. 1994; Sundlof et al. 1982; others), and no remarkable differences have been seen for distribution, metabolism, or excretion (Cripps 1990; Ingebrigtsen and Nafstad 1983; Jarrell et al. 1993; Mehendale et al. 1975; Verschueren 1983; others).

PBPK models for hexachlorobenzene have been developed for rats to describe the absorption, distribution, and elimination of ingested hexachlorobenzene, but were not considered appropriate for inter-species extrapolations (Freeman et al. 1989; Yesair et al. 1986). One of the models was adapted for human modeling by using the same model structure with human physiological parameter values (Yesair et al. 1986). Although no empirical data are available on the dermal absorption of hexachlorobenzene in humans, dermal data from a rat study were used to develop a compartment model for application to a 70-kg worker (Koizumi 1991).

Further development of a human toxicokinetic model would be valuable in assessing risks to human health from hexachlorobenzene exposure.

Methods for Reducing Toxic Effects. Although available poison-treatment recommendations provide some guidance for reducing the toxic effects of absorbed hexachlorobenzene through inhalation, oral, or dermal exposures, these recommendations are not specific to hexachlorobenzene. Recommendations to reduce peak absorption following exposure include such general procedures as moving the individual to fresh air following inhalation exposure, emesis and gastric lavage with activated charcoal following oral exposure, and removal of contaminated clothing and washing of the skin following dermal exposure. Little can be done to reduce body burden of hexachlorobenzene. Treatments

that interfere with the mechanism of action for toxicity or repair tissue damage have not been developed specifically for hexachlorobenzene. However, some general recommendations are available, such as use of diazepam or phenobarbital to control convulsions related to hexachlorobenzene exposure. Development of methods for reducing toxic effects targeted specifically to hexachlorobenzene would be useful, and additional studies into the mechanisms of action would support this goal.

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 data regarding children's susceptibility following dermal or inhalation exposure to hexachlorobenzene were identified. The available human data suggest that infants and young children are at increased risk from exposure to hexachlorobenzene compared to adults (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). Studies of an orally exposed population reported 95% mortality in exposed infants (under 2 years of age) associated with dermal lesions; adolescents (between the ages of 6 and 15 years) exhibited health effects (including 10% mortality and dermal lesions) more frequently than adults (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). These results were reported from studies conducted in Turkey 25 to 30 years after the epidemic where people were exposed to very high levels of hexachlorobenzene that was added as a fungicide to wheat seedlings. Other studies focused on children's health found suggestive evidence of neurological and immunological effects, but did not assess exposure (Belles-Isles et al. 2000; Darvill et al. 2000; Dewailly et al. 2000; Hosie et al. 2000; Sala et al. 1999). Although immunological effects have been seen in humans exposed as adults (Richter et al. 1994; Queiroz et al. 1997, 1998a, 1998b), neurological effects have not; therefore, children may be more susceptible than adults to the neurotoxicity of hexachlorobenzene.

No animal studies relevant to children's susceptibility following dermal or inhalation exposure were identified. Animal studies have confirmed that hexachlorobenzene is transferred to the developing organism through the placenta *in utero* and via lactation after birth, and that the developing animals exhibited signs of toxicity (such as reduced survival and anatomical abnormalities) not seen in parental animals at the same exposure levels (Arnold et al. 1985; Bailey et al. 1980; Courtney et al. 1979; Grant et al. 1977; Iatropoulos et al. 1978; Khera 1974; Kitchin et al. 1982; others). These experiments suggest that the ability of hexachlorobenzene to sensitively affect the developing organism may be related to its demonstrated capabilities to mediate toxicity through the neuroendocrine axis (Alvarez et al. 2000; Andrews et al. 1988, 1990; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1992b, 1993,

1995a, 1995b; Kimbrough and Linder 1974; Kleiman de Pisarev et al. 1989, 1990). However, endocrine end points have not been monitored in developing organisms. A developmental study that included assessment of endocrine end points along with other sensitive end points such as neurobehavioral and immune function would be useful to determine the role of endocrine changes with regard to these effects, and would identify critical levels of effect.

No data were located concerning whether pharmacokinetics of hexachlorobenzene in children are different from adults. A PBPK model (Yesair et al. 1986) has modeled fetal and breast milk compartments, in humans as well as rats, for oral exposure to hexachlorobenzene. Two PBPK models (Freeman et al. 1989; Yesair et al. 1986) have characterized the pharmacokinetics of hexachlorobenzene in growing rats, and have been validated using experimental data. No information regarding biomarkers of exposure and effect or potential interactions of hexachlorobenzene with other chemicals pertinent to children's susceptibility were identified. There are no pediatric-specific methods to reduce peak absorption of hexachlorobenzene following exposure, or to reduce body burden, or to interfere with mechanisms of action for hexachlorobenzene.

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

The following ongoing study pertaining to hexachlorobenzene was identified in the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2015):

Lawrence M. Schell, State University of New York at Albany, is assessing the effect of exposure to PCBs, other persistent organic pollutants (presumably including hexachlorobenzene), and lead on characteristics of the menstrual cycle among Mohawk women between 20 and 35 years of age and living in Akwesasne, which is adjacent to a federal and two state Superfund sites. A total of 180 women will be followed through one menstrual cycle with collection of blood, urine, and daily saliva samples to investigate the relationship of PCB congeners and other toxicants to gonadal function, pituitary function, and other characteristics of the menstrual cycle. The study is sponsored by the National Institute on Minority Health and Health Disparities.

4. CHEMICAL AND PHYSICAL INFORMATION

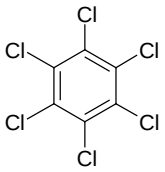
4.1 CHEMICAL IDENTITY

Hexachlorobenzene is a fully chlorinated hydrocarbon industrial chemical. Although hexachlorobenzene is not currently manufactured as a commercial end product in the United States, it is formed as a waste product in the production of several chlorinated hydrocarbons such as tetrachloroethylene and trichloroethylene, and is a contaminant in some pesticides such as pentachloronitrobenzene and pentachlorophenol. Its presence in the environment is also due to its previous use as a fungicide. Hexachlorobenzene is a very persistent environmental chemical due to its chemical stability and resistance to biodegradation. Information regarding the chemical identity of hexachlorobenzene is located in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Hexachloro¬benzene is a white, crystalline solid (Verschueren 2001) that is practically insoluble in water (Haynes and Lide 2010). When heated to decomposition, hexachlorobenzene emits highly toxic fumes of hydrochloric acid, other chlorinated compounds, carbon monoxide, and carbon dioxide (NTP 2014). Dimethyl formamide and hexachloro¬benzene react violently above 65 °C (HSBD 2012). Information regarding the physical and chemical properties of hexachloro¬benzene is located in Table 4-2.

Table 4-1. Chemical Identity of Hexachlorobenzene

Characteristics	Information[a]
Chemical name	Hexachlorobenzene[b]
Synonyms	Perchlorobenzene[b]; HCB[c]; pentachlorophenyl chloride
Trade names	AntiCarie; Ceku C. B.; No Bunt[c]
Chemical formula	C_6Cl_6[b]
Chemical structure	
Identification numbers:	
CAS Registry	118-74-1[c]
NIOSH RTECs	DA2975000
EPA Hazardous Waste	U127[d]
OHM/TADS	8100010
DOT/UN/NA/IMDG shipping	UN2729
HSDB	1724[e]
NCI	No data

[a]All information obtained from HSDB 2012, except where noted
[b]O'Neil et al. 2006
[c]Farm Chemicals Handbook 2001
[d]EPA 1999
[e]HSDB 2012

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

Table 4-2. Physical and Chemical Properties of Hexachlorobenzene

Property	Information	Reference
Molecular weight	284.78	O'Neil et al. 2006
Color	White	Verschueren 2001
Physical state	Crystalline solid	Verschueren 2001
Melting point	231 °C	O'Neil et al. 2006
Boiling point	325 °C 323–326 °C	Haynes and Lide 2010 O'Neil et al. 2006
Density at 23 °C	2.044	O'Neil et al. 2006
Odor	No data	
Odor threshold: Water Air	 No data No data	
Solubility: Water at 25 °C	 0.0062 mg/L 0.0047 mg/L	 Farmer et al. 1976
Water at 20 °C	0.006 mg/L	Yalkowsky 1992
Organic solvents	Insoluble in water, slightly soluble in ethanol, soluble in diethyl ether and chloroform, and very soluble in benzene	Verschueren 2001 Haynes and Lide 2010
Partition coefficients: Log octanol/water	 5.73	 Hansch et al. 1995
Log K_{oc}	6.08 5.22 3.59	EPA 1981 Kenaga and Goring 1978
Vapor pressure at 20 °C	1.09×10^{-5} mmHg	O'Neil et al. 2006
Henry's law constant	5.8×10^{-4} atm-m^3/mol	ten Hulscher et al. 1992
Hydroxyl radical constant at 25 °C	2.7×10^{-14} cm^3/molecule-second	Brubaker and Hites 1998
Autoignition temperature	No data	
Flashpoint	242 °C	O'Neil et al. 2006
Flammability limits	No data	
Conversion factors	1 ppm = 11.8 mg/m^3 1 mg/m^3 = 0.08 ppm	Verschueren 2001
Explosive limits	No data	

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

5.1 PRODUCTION

Hexachlorobenzene is not currently manufactured as a commercial end product in the United States, and evidence indicates that it has not been commercially produced since the late 1970s (Beyer 1996; EPA 1986b). However, limited amounts of hexachlorobenzene are imported for laboratory and research use. The compound can be produced commercially by reacting benzene with excess chlorine in the presence of ferric chloride at 150–200 °C. In addition, at least one former producer isolated hexachlorobenzene from distillation residues obtained as a by-product in the manufacture of tetrachloroethylene (IARC 1979). Hexachlorobenzene occurs as a byproduct or impurity in the manufacture of several chlorinated solvents (e.g., tetrachloroethylene, trichloroethylene); other chlorinated compounds (e.g., vinyl chloride, chloroanil); and several pesticides, including pentachloronitrobenzene (PCNB), tetrachloro-isophthalonitrile (chlorothalonil), 4-amino-3,5,6-trichloropicolinic acid (picloram), pentachlorophenol (only in Europe) (EPA 1986b; Liu et al. 2012; Tobin 1986), and dimethyltetrachloroterephthalate (DCPA or Dacthal®) (Verschueren 1996); and was also produced as a by-product during the production of atrazine, propazine, and simazine (IARC 1979). Hexachlorobenzene was also a contaminant in several chlorinated substances that had previously been used in large quantities but are no longer produced or used, such as the pesticides, mirex and lindane, and the solvent, carbon tetrachloride, which is only available in small quantities for research purposes. Hexachlorobenzene is also released in the environment due to ongoing use in developing countries and improper storage or disposal in developed countries (Dewailly et al. 1999).

In 1972, hexachlorobenzene produced as a by-product during the production of many other chlorinated chemicals was estimated to range from 1,123,500 kg (2,476,868 pounds) to 2,224,900 kg (4,905,015 pounds) (IARC 1979). Limited data indicate that hexachlorobenzene was produced at the Dover Chemical Company, Dover, Ohio and Hummel Chemical Company, South Plainfield, New Jersey, until 1977. It is estimated that 1,450 kg (3,200 pounds) of hexachlorobenzene (as an end-product) were produced in the United States in 1975, and that 3,500–11,500 kg (7,700–25,350 pounds) of hexachloro-benzene were inadvertently produced in the manufacture of chlorinated solvents in 1984 (EPA 1986b). No current estimates of hexachlorobenzene production are available

Table 5-1 lists the facilities in each state that manufacture or process hexachlorobenzene, the intended use, and the range of maximum amounts of hexachlorobenzene that are stored on-site. There are 65 facilities that produce, process, or use hexachlorobenzene in the United States. Current estimates for

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

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	4	0	9,999	7, 8, 12, 14
AR	1	10,000	99,999	1, 5, 9, 12
CA	2	10,000	99,999	1, 5, 13, 14
CO	1	100	999	2, 13
DE	1	0	99	1, 13
FL	1	100	999	1, 13
ID	1	0	99	14
IL	1	100	999	12
IN	2	0	999	1, 2, 5, 9, 12, 13, 14
KS	1	0	99	1, 13
KY	2	0	99,999	1, 3, 6, 12
LA	9	0	999,999	1, 2, 5, 7, 12, 13, 14
MI	1	0	99	2, 12, 13, 14
MN	1	0	99	14
MS	2	0	999	1, 5, 14
MT	1	0	99	1, 5
NY	3	0	9,999	1, 5, 12, 14
OH	5	0	99,999	12, 14
OR	4	0	999	1, 12, 13, 14
PA	2	0	999	12
SC	1	10,000	99,999	8
TN	1	0	99	1, 5
TX	14	0	99,999	1, 5, 6, 8, 12, 13, 14
UT	3	0	99,999	1, 12, 13, 14
WA	1	0	99	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. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacturing Aid
12. Ancillary/Other Uses
13. Manufacturing Impurity
14. Process Impurity

Source: TRI13 2014 (Data are from 2013)

the amounts of hexachlorobenzene stored on-site as a by-product or impurity range from 0 to 999,999 pounds per year (0–453,951 kg/year) (TRI13 2014). The data from the Toxics Release Inventory (TRI) listed in Table 5-1 should be used with caution, however, since only certain types of facilities were required to report (EPA 1995b). This is not an exhaustive list.

There are some indications that any process that produces dioxins or dibenzofurans (e.g., pulp and paper mills using chlorine for bleaching) will also yield other chlorinated organic compounds such as hexachlorobenzene (EPA 1992). In addition, hexachlorobenzene may be produced as a by-product in waste streams of chlor-alkali plants and wood preserving plants (Leger 1992), and in fly ash (Eicman et al. 1981) and flue gas effluents from municipal incineration (Oberg and Bergstrom 1985; Oehme et al. 1987; Tiernan et al. 1985). No estimates for the amount of hexachlorobenzene produced as a by-product via these sources was available.

5.2 IMPORT/EXPORT

Hexachlorobenzene imports of 2,440 kg (5,400 pounds) in 1977 and 17,300 kg (38,100 pounds) in 1982 were reported (HSDB 2012). No other recent data for import or export in the United States was located. However, the United Nations Environment Program conducted a survey of import/export data for persistent organic pollutants worldwide. Although this report contained no production data, it did contain worldwide import/export data. From 1990–1994, 158.45 tons of hexachlorobenzene were exported worldwide, while 2,258 tons of hexachlorobenzene were imported worldwide (UNEP 1996). It should be noted that U.S. import/export data are not included in this study.

5.3 USE

There are no current commercial uses of hexachlorobenzene as an end-product in the United States. However, hexachlorobenzene was used as a fungicide on the seeds of onions, sorghum, wheat, and other grains (IARC 1979) until 1984, when the last registered use of the compound as a pesticide was voluntarily cancelled. Hexachlorobenzene was also used in the production of pyrotechnic and ordinance materials for the military, the production of synthetic rubber (EPA 1986b), as a porosity controller in the manufacture of electrodes, a chemical intermediate in dye manufacturing, and a wood preservative (IARC 1979).

5.4 DISPOSAL

Hexachlorobenzene is listed as a toxic substance under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA) under Title III of the Superfund Amendments and Reauthorization Act (SARA) (EPA 1995b). Disposal of wastes containing hexachlorobenzene is controlled by a number of federal regulations. Land disposal restrictions (e.g., treatment standards) apply to wastes containing hexachlorobenzene. Incineration at high temperatures is a proposed disposal method, but incineration can lead to chlorinated products as toxic as hexachlorobenzene. Past disposal methods for industrial wastes containing hexachlorobenzene included incineration, disposal in landfills, discharge to municipal sewage treatment plants, and emission to the atmosphere (Clayton and Clayton 1981; EPA 1988a, 1989a). High temperature incineration (around 1,300 °C) with a retention time of approximately 0.25 seconds is the recommended disposal technique because it is reported to destroy more than 99% of the chemical (HSBC 2012). Lamb et al. (1994) reported that organic waste compounds including hexachlorobenzene are used to co-fire cement kilns. These authors reported that the destruction efficiency of hexachlorobenzene fed to a rotary kiln/afterburner incinerator was greater than 99.9999%. Landfill disposal of hexachlorobenzene can lead to migration of the compound via water and sublimation of the compound into the air. Calaminus et al. (1993) conducted pyrolysis experiments with hexachlorobenzene in an inert atmosphere of argon. These authors reported hexachlorobenzene was substantially pyrolyzed (70%) at temperatures of 1,100 °C for 20 seconds into elemental carbon (soot) and chlorine (Cl_2), but that other polychlorinated compounds (e.g., hexachlorohexane, hexachloro-1,3-cyclopentadiene, octachlorostyrene, octachloronaphthalene, octochloroacenaphthalene, and decachloronaphthoacenaphthalene) were also produced. Process wastes containing hexachlorobenzene from the production of chlorinated aliphatic hydrocarbons has an EPA-prescribed treatment standard before land disposal. These wastes must be treated to specified concentrations prior to land disposal at a hazardous waste facility (EPA 1995b). Deep well injection of hexachlorobenzene is also not recommended (HSBC 2012). A review of remediation and disposal for materials containing hexachlorobenzene is available (Tong and Yuan 2012).

The waste water treatment technology that most closely resembles incineration is wet air oxidation. It is specifically designed to destroy organics in waste waters and efficiently oxidizes organics in aqueous media by operating at relatively high temperatures and pressures. Furthermore, wet air oxidation is typically performed on waste waters that contain relatively high concentrations of organics (i.e., those that are at or near the 1% total organic carbon cutoff for waste water). Carbon adsorption has been specified

as part of the treatment train because hexachlorobenzene is believed to be adsorbable when present in low concentrations as might be expected in an effluent from either wet air or chemical oxidation (EPA 1985).

No other information was located on the past or present volumes of hexachlorobenzene disposed of by each disposal method.

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6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

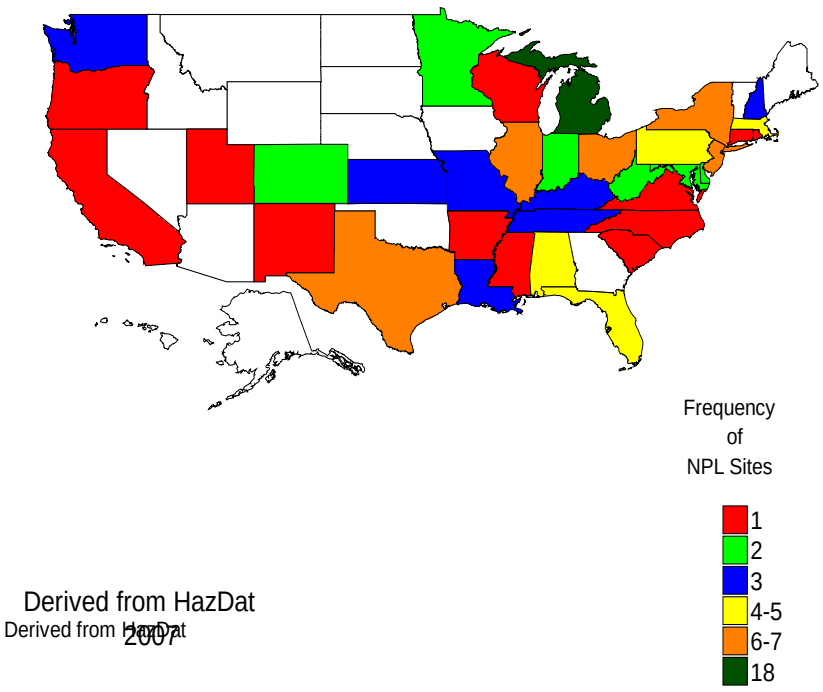
Hexachlorobenzene has been identified in at least 113 of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for hexachlorobenzene is not known. The frequency of these sites can be seen in Figure 6-1.

Hexachlorobenzene has not been manufactured in the United States (as an end-product) since its last registered use as a pesticide was voluntarily canceled in 1984. Small amounts of hexachlorobenzene are still released to the environment by industrial activities as fugitive and stack emissions, or in waste water. It is released as a by-product during the manufacture of several chlorinated solvents or as an impurity in several currently registered pesticides. Additional amounts of hexachlorobenzene are also formed during combustion processes such as incineration of municipal refuse (EPA 1986b) or through use of pyrotechnic mixtures (Karlsson et al. 1991). Bailey (2001) provided a detailed analysis of the global emissions of hexachlorobenzene during the 1990s from these various sources (see Section 6.2).

Hexachlorobenzene is among the most persistent environmental pollutants because of its chemical stability and resistance to degradation. If released to the atmosphere, hexachlorobenzene exists primarily in the vapor phase and degradation is extremely slow. Half-life estimations for hexachlorobenzene in the atmosphere are highly variable, ranging from 0.63 years in tropical/subtropical regions, to 1.94 years in temperate/boreal regions, to 6.28 years in polar regions (Wania and Mackay 1995). A global calculated half-life of 1.69 years was attained from a measured hydroxyl rate constant ($2.7x10^{-14}$ cm^3/molecule-second) (Brubaker and Hites 1998). Long-range global transport is possible from the temperate to the polar regions (Barber et al. 2005). Physical removal of hexachlorobenzene from the air may occur via washout by rainfall or snowfall, or via dry deposition. If released to water, hexachlorobenzene will partition from the water column into sediment and suspended particulate matter (EPA 1981).

In water, it is a persistent chemical not readily degraded by either abiotic or biotic processes. The half-life value of hexachlorobenzene is estimated to range from 2.7 to 5.7 years in surface water and from 5.3 to 11.4 years in groundwater (Howard et al. 1991). Volatilization of hexachlorobenzene from the water column will occur; however, the compound's strong adsorption to particulates and organic matter in water slow the rate of volatilization. If released to soil, hexachlorobenzene can volatilize from the soil surface, but will be strongly adsorbed to organic matter and is generally considered immobile with respect

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



to leaching (HSDB 2012). Its half-life value in soils is estimated to range from 3 to 6 years (Beck and Hansen 1974). Hexachlorobenzene bioaccumulates significantly in both terrestrial and aquatic food chains. Bioconcentration factors (BCFs) as high as 17,000,000 and 21,900 have been reported for lichens and fish, respectively (Muir et al. 1993; Veith et al. 1979). Hexachlorobenzene has been used as a referent compound for BCFs in fish (Adolfsson-Erici et al. 2012).

Temporal trends of hexachlorobenzene levels in the environment vary, depending on the media and study location, and the time period measured, but the average half-life from all of these studies is about 9 years. Estimates made of the present-day burden in the environment range between 20 and 60,000 million pounds and are dominated by the loadings in treated and background soils, sediments, and oceans (Barber et al. 2005).

Monitoring data for hexachlorobenzene levels are extensive in the Great Lakes region where production of chlorobenzenes was historically high. Atmospheric monitoring detected the compound at mean and median concentrations of 36.68 (0.03668 ng/m^3) and 30.94 (0.03094 ng/m^3) pg/m^3, respectively, from 56 air samples in Villeroy, Quebec in 1992 (Poissant et al. 1997). Hexachlorobenzene has also been detected in minute amounts (up to 0.174 ng/L [ppt]) in precipitation samples from the Great Lakes region (Chan et al. 1994) and in precipitation samples collected from Villeroy, Quebec in 1992 (0.04 ng/L) (Poissant et al. 1997). It was also detected in drinking water in three cities on Lake Ontario at a mean concentration of 0.1 ppt (Oliver and Nicol 1982a). Sediment samples (2 cm depth) collected from lakes (Allen-Gil et al. 1997) and landfills have also been contaminated with hexachlorobenzene (Yasuhara et al. 1999).

Concentrations of hexachlorobenzene have been reported for a variety of commercial fish species in the Great Lakes (Allen-Gil et al. 1997; Kuchlick and Baker 1998) with concentrations up to 17 ppb in raw fish fillets (Newsome and Andrews 1993; Zabik et al. 1995). In the National Pesticide Monitoring Program, concentrations as high as 700 ppb were reported in whole fish samples collected from contaminated areas (Schmitt et al. 1990). This chemical has also been detected in the fatty tissues and muscle of a wide variety of waterfowl (Foley 1992; Gebauer and Weseloh 1993; Swift et al. 1993), marine mammals (Becker et al. 1997; Langlois and Langis 1995), and mammals (Corsolini et al. 1999). In terrestrial ecosystems, hexachlorobenzene has been detected in lichens (Muir et al. 1993) and in caribou that graze primarily on lichens (Elkin and Bethke 1995). Concentrations of hexachlorobenzene in these fish and wild game species can be a source of hexachlorobenzene exposure to man.

Hexachlorobenzene residues have been detected in 76% of samples analyzed as part of the National Human Adipose Tissue Survey (FY82) (EPA 1986c). These hexachlorobenzene residues are most likely the result of consumption of low levels of hexachlorobenzene in food, with a calculated yearly intake of 68, 22, and 5 μg for adults, toddlers, and infants, respectively (EPA 1986b). Compared to this, exposure to hexachlorobenzene via inhalation or through drinking water is relatively low. Human exposure may also occur via dermal contact with contaminated soil or sediment or via ingestion of contaminated soil by children. In occupational settings, exposure occurs primarily via inhalation or dermal contact.

Due to extensive research conducted on hexachlorobenzene, the data reported herein do not encompass complete and thorough research for this chemical.

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 2005). 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 2005).

Additional releases of hexachlorobenzene to the environment occur from processes such as combustion of coal, cement, sewage sludge, or biomass; iron sintering; incineration of municipal, hazardous, or medical wastes; during the use of pyrotechnic mixtures; as a byproduct during the manufacture of several chlorinated solvents; or as an impurity in several currently registered pesticides (Bailey 2001; Barber et al. 2005). Quantitative information on releases of hexachlorobenzene to specific environmental media is discussed below.

6.2.1 Air

Estimated releases of 1,211 pounds (~0.55 metric tons) of hexachlorobenzene to the atmosphere from 65 domestic manufacturing and processing facilities in 2013, accounted for about 5.5% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-1.

Releases to the air from the production of chlorinated solvents, where hexachlorobenzene is a minor byproduct, have been estimated at 0.3 kg (0.7 pounds) annually and emissions from municipal refuse incineration have been estimated at 85–8,512 kg (187–1,870 pounds) annually (Bailey 2001). As incineration has emerged as a prevalent technology for reducing the bulk of hazardous and nonhazardous wastes, investigations have shown that even with careful controls it is virtually impossible to eliminate unwanted by-products (Products of Incomplete Combustion [PICs]) (Martens et al. 1998). Slight temperature differences on the surfaces of incinerator kiln and reactor components, or other reactions in flues, can lead to the formation of numerous chemical compounds. Where the original wastes contain organochlorines, one type of toxicant may be transformed into another (Dellinger et al. 1989). Hexachlorobenzene, for example, has been detected at concentrations ranging from 20 to 70 ppm in gases emitted by the thermal degradation of toxaphene (Lahaniatis et al. 1992). Since incineration of wastes is a growing global phenomenon, there are concerns that inadequate management attention is given to minimizing PICs. For hexachlorobenzene, and many other organochlorines that can be dispersed widely through atmospheric transport pathways, the virtual absence of data on PICs can lead to complications in estimating environmental releases and mass balances of hexachlorobenzene for regional areas or on a global scale (Lahaniatis et al. 1992).

Hexachlorobenzene emissions were reported as 0.94-3.8 g/year from siderurgies in Portugal and 0.013–1.7 mg/year from metal non-siderurgy plants (Antunes et al. 2012). Hexachlorobenzene was detected in the emissions from cement plants (0.98–60.5 ng/m^3) and from waste incinerators (3.21–2,500 ng/m^3) (Wegiel et al. 2011).

Nonpoint source dispersal of hexachlorobenzene historically has resulted from its use as a seed fungicide (Beyer 1996) and results from the use of a number of registered pesticides in which it is a contaminant. Seven major pesticides (chlorothalonil, DCPA or Dacthal®, pentachlorophenol or pentachlorophenol, picloram, PNCB or quintozene, atrazine, and simazine) in current use contain up to 0.3% hexachloro-

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

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	4	0	0	0	14	0	14	0	14
AR	1	0	0	0	0	0	0	0	0
CA	2	1	0	0	0	0	1	0	1
CO	1	0	0	0	0	0	0	0	0
DE	1	0	1	0	197	0	1	197	197
FL	1	0	0	0	0	0	0	0	0
ID	1	0	0	0	1	0	0	1	1
IL	1	0	0	0	1	0	0	1	1
IN	2	0	0	0	268	0	0	268	268
KS	1	0	0	1	0	0	1	0	1
KY	2	909	1	0	98	0	910	98	1,008
LA	9	122	22	0	40	0	184	0	184
MI	1	0	0	0	31	0	31	0	31
MN	1	0	0	0	0	0	0	0	0
MS	2	21	0	0	79	0	100	0	100
MT	1	10	0	0	0	0	10	0	10
NY	3	0	0	0	3	0	0	3	3
OH	5	3	0	6	25	0	3	31	34
OR	4	0	0	0	546	0	542	4	546
PA	2	1	0	0	9	0	1	9	10
SC	1	0	0	0	0	0	0	0	0
TN	1	0	0	0	159	0	159	0	159
TX	14	139	27	519	17,790	0	18,457	18	18,475
UT	3	5	0	0	943	0	948	0	948

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Hexachlorobenzene[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
WA	1	0	0	0	0	0	0	0	0
Total	65	1,211	51	526	20,203	0	21,362	629	21,991

[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 onsite 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: TRI13 2014 (Data are from 2013)

benzene as an impurity. Hexachlorobenzene was also an impurity in lindane; however, all of lindane's remaining uses were cancelled in 2006. When these pesticides are applied in sprays, they have the greatest potential for release into the air. Most of the pesticide, and the hexachlorobenzene impurities, end up on the top layer of the soil and can become airborne through volatilization of the vapor or adsorbed to soil particles. The hexachlorobenzene agriculturally applied through the use of these eight pesticides amounts to an estimated 1,270 kg/year (2,790 pounds/year); however, the total amount of hexachlorobenzene actually released into the air could not be estimated (Bailey 2001). At its peak, the amount of hexachlorobenzene emitted from treated soil to air may have been in the millions of pounds per year, which would have made it a significant source of hexachlorobenzene in the environment. While the amount of hexachlorobenzene being emitted from present day soil is much lower, only a small amount of re-emission of hexachlorobenzene from soil to air is required to maintain the current air concentrations given its persistence (Barber et al. 2005).

Other minor sources of hexachlorobenzene releases to the air come from the use of pyrotechnic mixtures that produce white obscurant screening smokes (Karlsson et al. 1991) and fireplace and woodstove combustion (Gullett et al. 2003). Screening smokes are used by the military to obscure vision and hide targets, and are used by civilian firefighters during fire training sessions. In a study of emissions from residential fireplace and woodstove combustion in the San Francisco Bay Region, mean emission factors of 13 ng/kg oak for woodstoves and 310, 380, and 990 ng/kg oak, pine, and artificial log, respectively, for fireplaces were measured (Gullett et al. 2003).

6.2.2 Water

Estimated releases of 51 pounds (~0.02 metric tons) of hexachlorobenzene to surface water from 65 domestic manufacturing and processing facilities in 2013, accounted for about 0.2% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). This estimate includes releases to waste water treatment and publicly owned treatment works (POTWs) (TRI13 2014). These releases are summarized in Table 6-1.

The principal release of hexachlorobenzene into water in the past has been through direct discharges from chemical solvent manufacturing facilities. Total production of chlorobenzenes in the United States has declined from more than 300,000 metric tons (300 million kg or 661 million pounds) in 1970 to about 200,000 metric tons (200 million kg or 441 million pounds) in 1980. The total amount of hexachlorobenzene released as a by-product in production of all chlorinated solvents has been estimated to range

from 70,343 to 241,311 kg/year (154,000–532,000 pounds/year) (EPA 1986b). Estimated hexachlorobenzene releases into water from these sources, however, were only 70 kg/year (154 pounds/year) (EPA 1986b).

6.2.3 Soil

Estimated releases of 20,203 pounds (~9.2 metric tons) of hexachlorobenzene to soils from 65 domestic manufacturing and processing facilities in 2013, accounted for about 97% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). An additional 526 pounds (~0.2 metric tons), constituting about 2.4% of the total environmental emissions, were released via underground injection (TRI13 2014). These releases are summarized in Table 6-1.

Historically, hexachlorobenzene was released to soils directly through its application as a fungicide on seed grains. Even after use of hexachlorobenzene as a pesticide ceased, an estimated 95% of hexachlorobenzene produced in the manufacture of chemical solvents was disposed of in land applications (EPA 1986b). Current releases to soils may occur through land disposal of hexachlorobenzene-containing wastes, from discharges from manufactured facilities, and from use of currently registered pesticides containing small amounts of hexachlorobenzene. Contamination of soil and sediment with hexachlorobenzene is frequently secondary to the discharge of contaminated water, from which the hexachlorobenzene is then adsorbed by the soil or sediment. Sediment samples (2 cm depth) were collected from four lakes (Feniak, Desperation, Schrader, and Elusive) from the foothills of the Brooks Range, Alaska. All lakes were oligotrophic, and the pH was neutral to slightly alkaline. The mean concentration among the six samples was 0.17 ng/g dry weight, derived from the concentrations of the sediments from Lakes Feniak (0.27 ng/g dry weight), Desperation (0.08 ng/g dry weight), Schrader (0.11 ng/g dry weight), and Elusive (0.21 ng/g dry weight) (Allen-Gil et al. 1997). Substantial levels of hexachlorobenzene, ranging from 0.5 to 460 ppb, have been detected in sediment cores sampled at 1 cm intervals to a sediment depth of 8 cm in Lake Ontario. The highest sediment contamination in Lake Ontario was found in sediments 1–2 cm in depth which correspond to sediments laid down from 1971 to 1976 during a period of high U.S. production of chlorobenzenes (Oliver and Nicol 1982a). Although no studies concerning the release of hexachlorobenzene from landfills have been located for the United States, Yasuhara et al. (1999) sampled leachates from 11 landfills in Japan. Leachates were sampled at the outlet of the leachate collecting pipe from open and controlled landfills. Hexachlorobenzene concentrations in the 11 leachate samples from 11 landfills ranged from not detected to 0.054 ng/L. Of the 11 sites, 6 are currently under reclamation and at least 3 sites were sampled 12–17 years after completion of reclamation. Site 2, which was sampled

after 14 years of reclamation, had no detectable levels of hexachlorobenzene. Leachates from sites 3 and 4, which were sampled after 17 and 12 years of reclamation, had hexachlorobenzene concentrations of 0.033 and 0.054 ng/L, respectively. Site 4 had the highest detection of hexachlorobenzene concentration. The median concentration among these sites was 0.03 ng/L.

The presence of hexachlorobenzene as an impurity in several registered pesticides appears to be a continuing source of exposure for the general population. The pesticides containing impurities of hexachlorobenzene include: picloram, PCNB or quintozene, chlorothalonil, DCPA or Dacthal®, pentachlorophenol or pentachlorophenol, atrazine, simazine, and lindane (Bailey 2001; Kutz et al. 1991). Picloram is a herbicide used in agriculture and silviculture to control broad-leaf weeds and conifers in grasses (MacBean 2010). PCNB is a fungicide used to control diseases of turf, ornamentals, cole crops, potatoes, cotton, and other agricultural and horticultural crops (EPA 2009a). In February 2009, several uses of PCNB were cancelled. These uses included application of PCNB on golf course roughs; residential sites including lawns, yards, and ornamental plants and gardens around homes and apartments; grounds around day care facilities; school yards; parks (except industrial parks); playgrounds; and athletic fields (except professional and college fields) (EPA 2009a). Chlorothalonil (sold under the trade name Bravo®) is a fungicide used on horticultural crops, golf courses, and residential turf, and as a biocide in paints and wood preservatives. Dacthal® is a pre-emergent herbicide widely used in agriculture and on lawns and turf grass (EPA 1986b). Pentachlorophenol is an insecticide and fungicide used to protect timber from fungal rot and wood-boring insects (MacBean 2010). Atrazine and simazine are selective herbicides used to control broadleaf and grassy weeds in corn and other crops (MacBean 2010). Lindane is an insecticide and fumigant that was used on a wide range of soil-dwelling and plant-eating insects and as a seed treatment for barley, corn, oats, rye, sorghum, and wheat. All uses of lindane were cancelled in 2006 and its only current use in the United States is in shampoos and lotions to treat lice and mites.

Although hexachlorobenzene impurities in Dacthal® were as much as 10% in the early 1970s, current levels of hexachlorobenzene contamination in all five pesticides are much lower. A registration standard was issued for picloram in 1985 that specified a maximum hexachlorobenzene content of 0.02%. By the terms of an EPA PCNB rebuttal presumption against registration in 1982, PCNB registrants agreed to reduce hexachlorobenzene contamination levels in PCNB to 0.5% by 1983 and to 0.1% by April 1988. A registration standard was issued by EPA for chlorothalonil in 1984 requiring that hexachlorobenzene contamination not exceed 0.05%. Since 1973, the maximum allowable hexachlorobenzene content of technical grade DCPA (Dacthal®) has been 0.3% (EPA 1986b).

The annual total emission of hexachlorobenzene in the United States during the 1990's was estimated to average 2,785 kg (Bailey 2001). These emissions are indirect or unintentional emissions from the manufacture and use of products that may contain varying amounts of hexachlorobenzene as a contaminant. This estimated total was calculated using emission subtotals of 1,270 kg for pesticide use, 0.3 kg for chlorinated solvent use, 399 kg from manufacturing processes, 156 kg from metal industries (using mean emission factors), and 960 kg from combustion sources (using mean emission factors).

Earthworms have been utilized as biosentinel for hexachlorobenzene soil presence (Vampre et al. 2010).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Hexachlorobenzene has a moderate vapor pressure ($1.09x10^{-5}$ mmHg) (O'Neil et al. 2006) and has a very low solubility (0.005815 mg/L) (Yalkowsky 1992) in water (see Table 4-2). If released to the atmosphere, hexachlorobenzene can exist in both the vapor phase and in association with particulates (Eisenreich et al. 1981); however, monitoring studies have demonstrated that the vapor phase generally predominates (Ballschmiter and Wittlinger 1991; Bidleman et al. 1989; Lane et al. 1992). Hexachlorobenzene concentrations in the vapor phase represented 92–100% of the total hexachlorobenzene concentration in air samples collected in a monitoring study conducted in Ontario, Canada (Lane et al. 1992). Although physical removal of hexachlorobenzene from the atmosphere is possible via both wet and dry deposition (Howard 1990), the compound is hydrophobic, and somewhat resistant to wet deposition scouring unless it becomes sorbed to airborne dust or cloud condensation nuclei. Its resistance to wet deposition tends to slow down its transfer across the equatorial areas between the northern and southern hemispheres. At high latitudes, the typically cold air conditions encourage dry deposition of aerosols (Ballschmiter and Wittlinger 1991; Lane et al. 1992; Wania and Mackay 1993). These factors lead to atmospheric pathways as a major transport mechanism for hexachlorobenzene. The net residence time of hexachlorobenzene in the atmosphere is significantly less than 1 year, and is based on physical translocation and not on chemical transformation (Ballschmiter and Wittlinger 1991). The atmospheric mechanisms can operate over large distances, perhaps on a hemispheric scale (Kelly et al. 1991). One study modeled the atmospheric residence time of hexachlorobenzene and calculated the transport distance (the distance over which 50% of the chemical is removed) to be 10^5 km (Barber et al. 2005). At higher latitudes, transfers and partitioning back to aqueous systems may be accentuated by the cold, dry air. Since these areas are not zones of hexachlorobenzene production or use, the presence of such contaminants has attracted considerable attention in research studies (Ballschmiter and Wittlinger 1991).

The Henry's law constant value for this compound ($5.8x10^{-4}$ atm-m^3/mol) (ten Hulscher et al. 1992) suggests that releases of hexachlorobenzene to surface water will volatilize at a moderate rate, and that volatilization can be a significant transfer mechanism (Thomas 1990). If released to water, adsorption of hexachlorobenzene to sediment or suspended particulate matter is also expected to be significant on the basis of the high organic carbon partition coefficient (K_{oc}) value (EPA 1981) (see Table 4-2). This tendency to partition to sediment and adsorb to suspended solids in the water column attenuates the rate of volatilization. Since hexachlorobenzene will adsorb strongly to soil particles and sediments, it may build up in the bottom sediments of large aquatic systems such as the Great Lakes. The concentration of hexachlorobenzene in Lake Ontario sediment is about 1 million times higher than in Lake Ontario water (Oliver and Nicol 1982a). In Lakes Superior, Michigan, and Huron, the very large sizes of the water bodies, considerable depths, and appreciable retention times have allowed much of the historical organochlorine burden to become immobilized in bottom sediments, with a concomitant reduction in the levels of hexachlorobenzene found in surface waters. In the upper Great Lakes, the vast majority of the ongoing mass balance inputs seem related to atmospheric deposition (Eisenreich et al. 1981). For other parts of the Great Lakes system, and especially the connecting waters and Lakes Erie and Ontario, mass balance studies can give different impressions. Detailed studies on Lake Erie suggest that well over half of the hexachlorobenzene inputs come from wet or dry atmospheric deposition processes (270 kg/year or 600 pounds/year) (Kelly et al. 1991). However, a significant portion (110 kg/year or 240 pounds/year) also comes from river pathways such as the highly polluted Detroit River via surface runoff and contaminated sediments resuspended during their passage through the connecting waters. Because of its strong adsorption to sediment, most of the hexachlorobenzene is transported with silt and sediment particles during floods, and very little is dissolved in the water. Quemerais et al. (1994) reported that 23% of the hexachlorobenzene in whole water samples collected from the St. Lawrence River was associated with the dissolved phase, while 77% was associated with the particulate phase. Although Rostad et al. (1988) did not quantify the percentage of hexachlorobenzene found in the dissolved versus the particulate phase, they did report that hexachlorobenzene was one of the organic compounds associated with suspended sediment particles in several river systems within the Mississippi River drainage area. In a 1999 study, Rostad et al. (1999) measured hexachlorobenzene concentrations in suspended sediment particles within the Mississippi River in the spring and summer of 1989 and 1990. Concentrations of hexachlorobenzene fluctuated between Winfield, Missouri and Belle Chasse, Louisiana during the spring and the summer; however, in both seasons, the concentration was higher at Chasse, Louisiana (1.9 ng/g in the spring; 2.1 ng/g in the summer) than at Winfield, Missouri (1.2 ng/g in the spring; 0.13 ng/g in the summer). Furthermore, Rostad et al. (1999) estimated annual transport of hexa-

chlorobenzene via suspended sediments to the Gulf of Mexico by averaging the St. Francisville, Louisiana and Belle Chasse, Louisiana daily loads, averaging the spring and summer values for hexachlorobenzene, and projecting annual transport. The Gulf of Mexico receives an estimated 145 kg/year hexachlorobenzene via suspended sediment particles of the Mississippi River (Rostad et al. 1999).

Because of its high sorption characteristics, hexachlorobenzene is expected to be immobile in soil and unlikely to leach into groundwater (Swann et al. 1983). At waste disposal sites, where bioremediation techniques are proposed to reduce the mass of carbon-containing contaminants, there is the potential for augmenting the leaching properties of hexachlorobenzene and other organochlorines. The lipid materials in bacterial cell membranes may lead to a repartitioning of hexachlorobenzene sorbed to soil colloids. This can lead to a phenomenon called facilitated transport where the mobility of hydrophobic pollutants such as hexachlorobenzene adsorbed to soils may be enhanced by biosorption on bacteria and move into aquifers along with the bioremedial bacterial cultures (Lindqvist and Enfield 1992). Except at NPL sites, however, this potential source of groundwater pollution would seem to be remote.

The Henry's law constant value suggests that hexachlorobenzene released to moist soil will volatilize at a moderate rate. Several studies have indicated that volatilization may be a significant mechanism for loss of hexachlorobenzene released to soils. Beall (1976) studied the persistence of aerially applied hexachlorobenzene (equivalent to 10 ppm in the top 5 cm of soil) in a simulated pasture maintained for 19 months in a greenhouse. Twenty hours postapplication, the top 2 cm of soil contained 5.6 ppm (air-dry basis). Hexachlorobenzene concentrations in the top 2 cm of soil found after 0.5, 1, 6.5, 12, and 19 months were 45.2, 24.4, 7.9, 4.7, and 3.4% of day 1 values, respectively. However, no significant change in the deeper 2–4 cm layer of soil which averaged hexachlorobenzene residues of 0.11 ppm was observed over the 19-month study. Concentrations of hexachlorobenzene in pasture grass on day 1 were 1,060 ppm, but 2 weeks postapplication only 15.6 ppm (1.5% of day 1 residues) was detected. Although hexachlorobenzene volatilized fairly rapidly from plant and soil surfaces, it could be persistent within the soil if treated surface soil were mixed into deeper soil layers by plowing. Nash and Gish (1989) studied the volatilization and dissipation of several halogenated pesticides from moist sandy loam soil under controlled conditions in micro-agroecosystem chambers maintained in a greenhouse for 154 days. As soil temperature increased from 5 to 35 °C, the percentage of originally applied hexachlorobenzene that was detected in the soil compartment decreased, while the percentage detected in the air increased suggesting that hexachlorobenzene volatilizes more rapidly with increased soil temperature. Reported soil/air partition coefficient values for hexachlorobenzene range from 5.0 to 7.3, signifying that, at equilibrium, soil will contain a much greater mass of hexachlorobenzene than air (Barber et al. 2005).

The high octanol/water partition coefficient (K_{ow}) value (Hansch et al. 1995) for hexachlorobenzene (see Table 4-2) suggests that bioconcentration and biomagnification of hexachlorobenzene are likely to occur to a significant degree. Veith et al. (1979) measured bioconcentration factor (BCF) values of 16,200 for fathead minnows, 21,900 for green sunfish, and 5,500 for rainbow trout exposed to hexachlorobenzene at 15 °C for 32 days. Oliver and Niimi (1983) studied bioconcentration in rainbow trout exposed to water containing 2 concentrations of hexachlorobenzene (0.32 and 8 ng/g [ppb]) for 119 and 105 days, respectively. The BCF values were 12,000 and 20,000 at the 0.32 and 8 ng/g (ppb) exposure levels, respectively. Chaisuksant et al. (1997) conducted a bioconcentration experiment using mosquito fish (*Gambusia affinis*) as well. The fish were exposed to three concentrations of eight chemicals, and the highest concentration used consisted of a mixture with each chemical present in a concentration equal to 1/20 of the LC_{50}. After 96 hours of exposure, the BCF of hexachlorobenzene in mosquito fish was 3,730. After an 8-week exposure period of carp (*Cyprinus carpio*) to concentrations of 0.5 and 0.05 μg/L of hexachlorobenzene, the BCFs were 11,000–27,000 and 6,000–30,000, respectively (NITE 2010).

In a model aquatic ecosystem to which hexachlorobenzene was introduced, the BCF averaged 740 for algae *(Oedogonium cardiacum)*, 1,500 for the snail *(Helisoma sp.)*, 910 for the daphnid *(Daphnia magna)*, 1,610 for the mosquitofish *(G. affinis)*, and 10,610 for the catfish *(Ictalurus punctatus)* (Isensee et al. 1976). The authors concluded that biomagnification was also occurring within the food chain because the catfish (highest trophic level species) accumulated over 10 times more hexachlorobenzene than the next highest trophic level (snails and mosquitofish), and these species accumulated 1.5–2 times more than the lowest food chain species, the daphnids (primary consumers) and the algae (primary producers). In studies of natural populations of white bass in Lake Erie, Russell et al. (1995) concluded that biomagnification of hexachlorobenzene did not occur. These authors did report biomagnification in Lake Erie fish populations was occurring for several other organic chemicals with log K_{ow} values greater than 6.1. Hexachlorobenzene bioaccumulation factors (BAFs) in aquatic fish species has been measured by Burkhard et al. (1997) in the Bayou d'Inde of the Calcasieu River system near Lake Charles, Louisiana. This field study resulted in log BAF values of 5.80 for blue crab (*Callinectes Sapidus*), 6.03 for mummichog fish (*Fundulus Heteroclitus*), 6.30 for Atlantic croaker (*Micropoganias undulatus*), and 6.68 for gulf menhaden (*Brevoortia Patronus*). The author further compared the measured values obtained to previously reported and predicted BAF values. A comparison of these data with that of Pereria et al. (1988) reveals a difference that was not considered significant by the author. Pereria et al. (1988) determined log BAF values of 4.03 for blue crab, 4.56 for Atlantic croaker, 4.12 for spotted sea trout *(Cynoscion nebulosis)*, and 4.61 for blue catfish (*Ictalurus furcatus*). Hexachlorobenzene was

shown to biomagnify in lake trout food webs in lakes in the northeastern United States using lake trout and other food web organisms collected between 1998 and 2001, as measured by trophic magnification factors (Houde et al. 2008).

Connell et al. (1988), using data derived from terrestrial laboratory microcosm studies with two oligochaete worms (*Limnodrilus hoffmeisteri* and *Tubifex*), suggest that interstitial water may be the source from which lipophilic compounds such as hexachlorobenzene in sediment are bioconcentrated by oligochaetes. The concentration factor was 0.54 for hexachlorobenzene during a 110-day exposure test. In a similar study of the earthworm *(Eisenia andrei)* raised in field-contaminated soil, Belfroid (1995) reported a biota-to-soil accumulation factor of 0.507 for hexachlorobenzene. These authors also noted an initial elimination half-life of 1.9 days followed by a period of slower elimination with a half-life of 47 days. In a terrestrial food web study conducted on the Niagara Peninsula of Ontario, Canada from 1987 to 1989, Hebert et al. (1994) reported concentrations of hexachlorobenzene increased from the lower trophic level species to higher trophic level predator species. Concentrations of hexachlorobenzene were not detected in soil or plant material; however, concentration ranges were 0.2–0.3 μg/kg (ppb) (wet weight) in earthworms, not detected to 1.0 μg/kg (ppb) in mammals, 2.0–2.4 μg/kg (ppb) in starlings, 1.8–2.5 μg/kg (ppb) in robins, and 2.1–5.1 μg/kg (ppb) in kestrels at the top of the food web.

Several agricultural species of plants have been shown to bioaccumulate hexachlorobenzene in their roots and in portions of the plant growing closest to the soil, such as low stems (Scheunert et al. 1983; Smelt and Leistra 1974). There were marked differences in the BCFs among the various plant species with higher residues associated with those species with the higher lipid content (Schroll et al. 1994; Smelt and Leistra 1974). The roots of the plants generally accumulate higher concentrations of soil-applied hexachlorobenzene than do the aerial parts of the plants. This has been demonstrated for hexachlorobenzene in sugar beets, carrots, turnips, wheat, and pasture grass (Scheunert et al. 1983; Smelt and Leistra 1974). The edible root portion of carrots accumulated the highest concentration of hexachlorobenzene (1,250 ppb with a plant/soil BCF of 19) for a human food source. The measured BCF for hexachlorobenzene was 210 and 470 in soy bean plants via root uptake from water containing 0.2 and 0.4 μg/L hexachlorobenzene, respectively (Kraaij and Connell 1997). Concentrations of hexachlorobenzene were also high in grass roots (810 ppb) and the lower (0–5 cm) part of the blade (220 ppb) (Smelt and Leistra 1974). It is assumed that hexachlorobenzene in soil is mobile mainly in the gas phase. Gaseous hexachlorobenzene can diffuse directly into the plant root or evaporated hexachlorobenzene can be taken up by plant foliage (Ecker and Horak 1994). Some studies have reported no marked translocation of the hexachlorobenzene from roots to shoots or vice versa (Schroll et al. 1994). Residues in the roots were associated only with

root uptake from the soil; those residues in the shoots were only from foliar uptake from the air. Studies by Ecker and Horak (1994), however, suggest that root uptake of hexachlorobenzene by oil pumpkins occurred and that the hexachlorobenzene was translocated into the shoots. These authors report that uptake of dissolved hexachlorobenzene from soil solution into the roots may not have been considered earlier as a source for the translocated compound. Pollutants entering the plant from contaminated soil via roots would be translocated in the plant by the xylem while gas- and particle-phase deposition onto leaves or uptake by the stomata would be translocated by the phloem (Simonich and Hites 1995). Concentrations of hexachlorobenzene in agricultural crops can be directly transferred to humans via direct consumption, while concentrations in grass and other forage crops can be indirectly transferred to humans via consumption of dairy products or meat from cattle grazing on contaminated pastures.

Lichen from Northwestern Ontario and South Central Ontario exhibited BCF values of $1.7x10^7$ and $8.8x10^6$, respectively, for hexachlorobenzene. These BCF values were calculated as the concentration of hexachlorobenzene in the lichen (ng/m^3 wet weight) compared to the concentration in the air (ng/m^3) (Muir et al. 1993). Furthermore, bioconcentration of hexachlorobenzene by lichen, a major forage food for caribou can transfer hexachlorobenzene to recreational hunters and natives peoples that consume caribou in their diets (Elkin and Bethke 1995).

6.3.2 Transformation and Degradation

6.3.2.1 Air

Photodegradation of hexachlorobenzene in its vapor phase, or as an adsorbable on silica gel, has been reported as not occurring when hexachlorobenzene was irradiated with ultraviolet radiation of wavelength 290 nm for 6 days (Parlar 1978); however, production of HCl and CO_2 was observed when hexachlorobenzene was irradiated at 230 nm (Parlar 1978). In the troposphere, hexachlorobenzene is probably photochemically stable, but degradation in the stratosphere by photo-dissociation by shorter-wavelength, higher energy-ultraviolet light may be a mechanism for atmospheric degradation in the stratosphere. Hexachlorobenzene, 20 μg, was degraded completely within 20 minutes when dipped onto a titanium dioxide film surface and irradiated with an ultraviolet radiation high-pressure mercury lamp with a wavelength of 365 nm, demonstrating how nano-titanium dioxide can increase the degradation of hexachlorobenzene under ultraviolet light radiation (Yu et al. 2007).

The photo-oxidation half-life (first-order kinetics) of hexachlorobenzene based on the vapor phase reaction with hydroxyl radicals in air was estimated to range from 156.4 days to 4.2 years by Howard et

al. (1991) and from 158 days to 4.3 years by Kwok and Atkinson (1995). Wania and Mackay (1995) estimated the degradation half-life (first-order kinetics) of hexachlorobenzene to be 0.63 years (230 days), 1.94 years (708 days), and 6.28 years (2,292 days) in air in tropical/subtropical regions, temperate/boreal regions, and polar regions, respectively. Brubaker and Hites (1998) measured a hydroxyl rate constant of $2.7x10^{-14}$ cm^3/molecule-second at 25 °C, corresponding to a calculated half-life of 1.69 years. Thus, atmospheric degradation is extremely slow and is not an efficient method of hexachlorobenzene removal.

6.3.2.2 Water

Hexachlorobenzene is a persistent compound and is not significantly degraded by either abiotic or biodegradation processes in water. It is resistant to the types of hydrolysis reactions that can help degrade other organochlorines or organophosphates, and it is not markedly subject to photolytic decay (Mill and Haag 1986). Biodegradation of organic priority pollutants in a waste water inoculum system amended with yeast extracts was studied by Tabak et al. (1981). Among the 57 environmental pollutants tested, hexachlorobenzene at concentrations of 5 and 10 ppm was among the more slowly biodegraded compounds tested. Biodegradation of hexachlorobenzene in waste water treatment systems is expected to be slow.

An aquatic ecosystem study conducted by Schauerte et al. (1982) shows that hexachlorobenzene will mainly absorb onto particulate matter in the water and then be transported to the bottom sediment. After 145 weeks, the study found a significant amount (10–20%) of hexachlorobenzene remaining in the upper sediment layers (0–10 cm). The half-life (first-order kinetics) of hexachlorobenzene was estimated to range from 2.7 to 5.7 years in surface water and from 5.3 to 11.4 years in groundwater based on unacclimated aqueous aerobic biodegradation (Howard et al. 1991).

Hirsch and Hutzinger (1989) conducted surface water photolysis test with hexachlorobenzene in a laboratory setting and found that this process may occur. A first order rate constant ($1.3x10^{-6}$/sec) corresponding to a half-life of 6.17 days for the photolysis of hexachlorobenzene in distilled water in a photochemical reactor equipped with mercury arc lamps was reported. Hexachlorobenzene in an acetonitrile:water mixture exposed to wavelengths of 290 nm for 8 hours resulted in a 33.5% loss of hexachlorobenzene. 1,2,3,4,5-Pentachlorobenzene (76.8%), 1,2,3,5-tetrachlorobenzene (1.2%), 1,2,4,5-tetrachlorobenzene (1.7%), and 1,2,4-trichlorobenzene (0.2%) were found as hexachlorobenzene transformation products (Choudhry et al. 1986). In another experiment, hexachlorobenzene in a water:acetonitrile solution was exposed to sunlight and resulted in a half-life of 70 days (Mill and Haag

1986). The studies above found photolysis of hexachlorobenzene a feasible loss process with half-lives ranging from 6.17 to 70 days.

Hydrolysis is not expected to be an important fate process. EPA (1987) observed zero hydrolysis after 13 days for pH values of 3, 7, and 11 at 85 °C.

Hexachlorobenzene can also be eliminated by ozone reactions. Roche and Prados (1995) conducted a study and compared the efficiencies of ozone and ozone-hydrogen peroxide systems in removing hexachlorobenzene from water treatment processes. The concentration of ozone during the experiments was 70 mg O_3/L. When ozone was applied, 11–14% of an initial concentration of 1.0 µg/L hexachlorobenzene was removed. This removal increased to 15–48% when hydrogen peroxide and ozone were applied together.

6.3.2.3 Sediment and Soil

Hexachlorobenzene is a persistent compound and is not significantly degraded in soils by either abiotic or biodegradation processes. In a year-long laboratory study, soil treated with 0.1, 1.0, and 10 ppm of hexachlorobenzene was stored under aerobic (sterile and nonsterile) conditions and under anaerobic nonsterile conditions in covered containers to retard hexachlorobenzene volatilization (Isensee et al. 1976). No loss in the soil-incorporated hexachlorobenzene was observed at any treatment concentration or under any storage condition. Beck and Hansen (1974) measured a half-life (first-order kinetics) of 3–6 years for hexachlorobenzene in soils. Anaerobic biological dechlorination of hexachlorobenzene has also been demonstrated in anaerobic sewage sludge (Fathepure et al. 1988). These authors reported that hexachlorobenzene was dechlorinated to tri- and dichlorobenzenes under anaerobic conditions when sewage sludge was maintained in serum bottles and incubated in the laboratory. Complete biotransformation of a 50 ppm inoculum occurred within 3 weeks. Two routes of dechlorination were observed. The major route was hexachlorobenzene → pentachlorobenzene → 1,2,3,5-tetrachlorobenzene → 1,3,5-trichlorobenzene; the minor route was hexachlorobenzene → pentachlorobenzene → 1,2,4,5-tetrachlorobenzene → 1,2,4-trichlorobenzene → dichlorobenzenes (i.e., 1,2-, 1,3-, and 1,4-dichlorobenzene). Yuan et al. (1999) also conducted an anaerobic biological dechlorination study using sewage sludge obtained from the Di-Hua Municipal Sewage Treatment Plant in Taipei, Japan. All experiments were performed using 25 mL serum bottles containing 9 mL of sewage sludge and various concentrations of hexachlorobenzene. After a 20-day incubation period, 98% of the 2 mg/L hexachlorobenzene remained, while addition of 1,2,3-trichlorobenzene adapted consortium accelerated dechlorination which occurred at a

calculated rate of 0.29 mg/L/day. At hexachlorobenzene concentrations of 2, 5, and 10 mg/L, complete dechlorination occurred within 6 days and at the 50 mg/L concentration, dechlorination occurred in 8 days. Optimal dechlorination occurred at a rate of 0.29 mg/L/day, pH of 7.0, and 30 °C. According to Yuan et al. (1999), dechlorination occurred via the following path: hexachlorobenzene → pentachlorobenzene → 1,2,3,4-tetrachlorobenzene + 1,2,3,5-tetrachlorobenzene → 1,2,4-trichlorobenzene + 1,2,3-trichlorobenzene + 1,3,5-trichlorobenzene → 1,2-dichlorobenzene + 1,4-dichlorobenzene. From this and other studies, it is clear that in a time frame of days to years, anaerobic biodegradation may remove hexachlorobenzene from soils.

Under aerobic conditions, Uhlik et al. (2014) reported detection of bacterial populations capable of deriving carbon from hexachlorobenzene. The bacteria populations were primarily Proteobacteria, including *Methylobacterium* and *Pseudomonas* collected from contaminated soils at a chemical factory in Neratovice, Czech Republic; however, the exact biodegradation pathways and degradation rates were not determined. Additionally, hexachlorobenzene, present at 100 mg/L in an aerobic sludge study to test biodegradation, reached 0% of its theoretical biological oxygen demand (BOD) in 2 weeks using an activated sludge inoculum at 30 mg/L and the Japanese Ministry of International Trade and Industry (MITI) test (NITE 2010); thus, aerobic degradation is not an important fate process. Contaminated soil from Klatovy-Luby, Hajek, and Neratovice, Czech Republic contained bacterial strain, *Stenotrophomonas sp*., capable of degrading hexachlorobenzene (Lovecka et al. 2014). A 34.9% decrease of the original concentration of hexachlorobenzene was detected in the sample of isolate H1D7.

Likewise, in areas of the Great Lakes region with a long history of hexachlorobenzene contaminated waste water discharges affecting aquatic sediments, the concentrations of hexachlorobenzene in the sediments can be significant (see Section 6.4.2). Susarla et al. (1997) examined the transformation of hexachlorobenzene in fresh water lake (Lake Kasumigaura, Japan) sediments under anaerobic conditions. Dechlorination occurred after a 4-day lag phase and was complete in 32 days. The calculated rate of dechlorination was 0.110/day. Hexachlorobenzene transformation pathway under sulfidogenic conditions resulted in hexachlorobenzene → pentachlorobenzene → 1,2,3,5-tetrachlorobenzene → 1,3,5-trichlorobenzene → 1,3-dichlorobenzene. Under methanogenic conditions the pathway was as follows: hexachlorobenzene → pentachlorobenzene → 1,2,3,4-tetrachlorobenzene → 1,2,4-trichlorobenzene → 1,4-dichlorobenzene. After almost a year, 98% of the hexachlorobenzene was dechlorinated to 1,3- and 1,4-dichlorobenzene (Susarla et al. 1997). In another experiment, dechlorination of hexachlorobenzene in an estuary sediment collected from the mouth of Tsurumi river occurred at a rate of 0.0256/day with a

half-life of 27.1 days (Masunaga et al. 1996). Thus, aquatic sediment degradation of hexachlorobenzene occurs in a month to a year.

6.3.2.4 Other Media

The explosion and collapse of the World Trade Center (WTC) on September 11, 2001 produced aerosols that dispersed into the environment. Two bulk samples of the dust that settled at indoor locations surrounding the WTC site contained mean hexachlorobenzene concentrations of 2.14 and 2.66 ng/g. Six bulk samples of dust from various outdoor locations around the WTC site contained an average hexachlorobenzene concentration of 1.3 ng/g (Offenberg et al. 2004).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to hexachlorobenzene depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of hexachlorobenzene 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 hexachlorobenzene 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 hexachlorobenzene in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Due to its long persistence, hexachlorobenzene is subject to long-range transport in the atmosphere and can be detected in nonsource air samples including rural and remote locations. Hexachlorobenzene measured in air in Villeroy, Quebec in 1992 had mean and median concentrations of 36.68 (0.03668 ng/m^3) and 30.94 pg/m^3 (0.03094 ng/m^3), respectively, from 56 air samples (Poissant et al. 1997). A meteorological station located in a semirural area outside Lancaster, England was the site of air samples. Four air samples per day (taken at 6-hour intervals) were taken for 7 days. The minimum, maximum, and mean concentrations of hexachlorobenzene in these samples were <28.8, 76.1, and 39.3 pg/m^3, respectively (Lee et al. 2000b). Air samples taken along a North-South Atlantic transect during five legs in transit from the island of Texel in the Netherlands to Walvis Bay, Namibia and Cape Town, South Africa contained hexachlorobenzene concentrations of 145, 96, not detected, <0.1, and 0.1 pg/m^3 for each leg of the trip, respectively (Booij et al. 2007). Huang et al. (2014) evaluated air and water concentrations

of hexachlorobenzene in samples collected from the Indian Ocean. Hexachlorobenzene concentrations in the air ranged from 2.2 to 17 pg/m^3 and the average concentration was 6.2 pg/m^3.

Eisenreich et al. (1981) reported atmospheric concentrations of hexachlorobenzene in the Great Lakes region ranging from 0.09 to 0.28 ng/m^3. Results of airborne samples collected between 1990 and 1993 from the Great Lakes region by the Integrated Atmospheric Deposition Network are provided by Hoff et al. (1996). The annual mean gas-phase and particulate-phase concentrations of hexachlorobenzene were 98 and 0.2 pg/m^3, respectively, in samples from Lake Superior near Eagle Harbor, Michigan, 120 and 0.1 pg/m^3, respectively, in samples from Lake Michigan near Sleeping Bear Dunes, Michigan, 80 and 0.2 pg/m^3, respectively, in samples from Lake Erie near Sturgeon Point, New York, and 130 and <0.1 pg/m^3, respectively, in samples from Lake Ontario near Point Petre, Ontario. From July 1988 to September 1989, 143 air samples were collected at Egbert, Ontario, Canada and were analyzed for PCB and organochlorine concentrations. Hexachlorobenzene was detected at concentrations ranging from a minimum of 0.04 pg/m^3 (0.00004 ng/m^3) to a maximum of 640 pg/m^3 (0.64 ng/m^3) (annual mean >54 pg/m^3 (0.054 ng/m^3) (Hoff et al. 1992).

The variability in hexachlorobenzene air concentrations has decreased as emissions from point sources have declined due to the banning of hexachlorobenzene from agricultural use in the 1970s. Regionally, levels of hexachlorobenzene in the atmosphere are becoming uniform (Barber et al. 2005). Studies of hexachlorobenzene concentrations at a number of sites within a region tend to show little variation. For example, a passive sampling survey in 2002 showed that hexachlorobenzene concentrations varied by less than a factor of 6 over the continent of Europe (Barber et al. 2005). Hexachlorobenzene air concentrations measured along a latitudinal transect of remote/rural sites from the south of the United Kingdom to the north of Norway during 1998 to 2000 indicated that the differences between highest and lowest concentrations were only a factor of about 3 (Meijer et al. 2003a). Hexachlorobenzene concentrations showed a relatively uniform global distribution during a study under the Global Atmospheric Passive Sampling Network, which employed passive samplers at 20 sites globally for a 3-month spring sampling period during 2009 (Koblizkova et al. 2012). Hexachlorobenzene concentrations ranged from 37 to 240 pg/m^3.

Hexachlorobenzene air concentrations have been measured in urban and rural areas in France. Atmospheric fallout from the urban area, Paris, and the rural area, La Ferté-sous-Jouarre, was collected in raw form as bulk precipitation. Hexachlorobenzene concentration in rural fallout measured in February–July 1992 and January –September 1993 ranged from 2.5 to 4.5 ng/L and from 0.3 to 4 ng/L, respectively.

For the same time periods, urban fallout measured 1.8–17 and 0.3–5.6 ng/L, respectively (Chevreuil et al. 1996). The mean concentrations of hexachlorobenzene in precipitation samples collected in the Great Lakes region from 1986 to 1991 ranged from 0.145 ng/L (ppt) at Sibley Park on Lake Superior, to 0.108 ng/L (ppt) at Pelee Island in Lake Erie, to 0.174 ng/L (ppt) at Wolfe Island in Lake Ontario (Chan et al. 1994). The mean and median concentrations of hexachlorobenzene from eight precipitation samples collected from Villeroy, Quebec in 1992 were 0.04 and 0.05 ng/L, respectively (Poissant et al. 1997). Precipitation samples collected between 1990 and 1993 from the Great Lakes region by the Integrated Atmospheric Deposition Network were analyzed. The annual mean concentration of hexachlorobenzene in these precipitation samples were 0.1 ng/L in samples from Lake Superior near Eagle Harbor, Michigan, 0.06 ng/L in samples from Lake Michigan near Sleeping Bear Dunes, Michigan, 0.04 ng/L in samples from Lake Erie near Sturgeon Point, New York, and 0.3 ng/L in samples from Lake Ontario near Point Petre, Ontario (Hoff et al. 1996). The concentrations of hexachlorobenzene in air, particulate matter, and rain from Galveston Bay, Texas were 87.3±103.3, 0.4±0.5, and 42.5 pg/m^3 (not detected–48.1 pg/m^3), respectively, between 1995 and 1996 (Park et al. 2001). The median levels of hexachlorobenzene in ambient air samples collected in Zagreb, Croatia in 1997 were 29 pg/m^3 (range, 0.5–49 pg/m^3) in the northern residential region of Ksaverska and 31 pg/m^3 (range, 15–61 pg/m^3) in the southern region near a landfill (Romaníc and Krauthacker 2000). The average concentration of hexachlorobenzene in air at Lake Malawi, in southeast Africa, from February 1997 to May 1998, was 11±7.5 pg/m^3 (Karlsson et al. 2000). Seasonal snowpack samples collected in spring 2003 from six national parks in the western United States contained mean hexachlorobenzene concentrations of 0.0065, 0.015, 0.017, and 0.035 ng/L for the four contiguous national parks, and 0.0055 and 0.0077 ng/L for the national parks in Alaska (Hageman et al. 2006). Average concentrations of hexachlorobenzene in the air samples collected from cities in China have been reported. Seasonal variation was evaluated and the concentrations of hexachlorobenzene detected were 91.2, 49.7, 85.4, and 420 pg/m^3 in Suzhou, 241, 22.6, 218, and 468 pg/m^3 in Wuxi, and 123, 46.5, 102, and 257 pg/m^3 Nantong for spring, summer, autumn, and winter, respectively (Zhang et al. 2013). In Beijing, China, average concentrations from samples of urban atmosphere were 200, 68, 180, and 400 pg/m^3 for spring, summer, autumn, and winter, respectively (Zhang et al. 2011).

Nonoccupational exposure to hexachlorobenzene for residents of two U.S. cities (Jacksonville, Florida and Springfield, Massachusetts) was studied over three seasons: summer 1986, spring 1987, and winter 1988 (Whitmore et al. 1994). The study focused primarily on inhalation exposures with primary environmental monitoring consisting of 24-hour indoor and outdoor air. For the Jacksonville, Florida, population, the estimated mean hexachlorobenzene concentration ranged from 0.3 to 1.3 ng/m^3 for indoor air and from not detected to 0.2 ng/m^3 for outdoor air. For the Springfield, Massachusetts population,

mean exposures were much less. The estimated hexachlorobenzene concentrations ranged from not detected to 0.1 ng/m^3 for indoor air and no detectable concentrations of hexachlorobenzene for outdoor air.

Extremely high concentrations of hexachlorobenzene in air have been detected in areas close to production and disposal sites in both outdoor and indoor air. Mann et al. (1974) measured hexachlorobenzene concentrations ranging from 70 to 23,296 ng/m^3 near chlorinated solvent and pesticide manufacturing facilities; air levels near a chemical waste landfill were as high as 16,000 ng/m^3 (EPA 1975b). Hexachlorobenzene has been detected at 11,000 ng/m^3 in flue gas effluents from a municipal refuse-fired steam boiler in Virginia, and at 9.5 ng/m^3 in flue gas effluents from a refuse-derived fuel fired power plant in Ohio (Tiernan et al. 1985). Air concentrations of hexachlorobenzene inside industrial plants can be as high as 150,000 ng/m^3 (Currier et al. 1980); air concentrations inside a pesticide production facility were measured at 22,000 ng/m^3 (Davis and Morgan 1986).

Due to the moderate volatility and extremely slow atmospheric degradation of hexachlorobenzene, evaporation for environmental reservoirs can sustain atmospheric levels of hexachlorobenzene within an order of magnitude of those resulting from primary emissions, which may explain why atmospheric concentrations have remained steady decades after its main primary emissions have been largely eliminated (Choi and Wania 2011).

6.4.2 Water

Drinking water in three cities in the Lake Ontario vicinity contained hexachlorobenzene ranging from 0.06 to 0.2 ppt (mean of 0.1 ppt), about the same as water from the lake (Oliver and Nicol 1982a).

Hexachlorobenzene was detected in ambient water samples from two of the Great Lakes and their tributary rivers. Mean concentrations of hexachlorobenzene in Lake Ontario, Lake Huron, and the Grand River were 0.06 ppt (range, 0.02–0.1 ppt), 0.04 ppt (range, 0.02–0.1 ppt), and 0.06 ppt (range, 0.02–0.1 ppt), respectively. In the Niagara River, concentrations of 0.02–17 ppt were detected with the highest value measured downstream of a waste disposal site (Oliver and Nicol 1982a). Widely varying measurements in this river may be attributed to the fact that measurements were near the analytical detection limit of 0.01 ppt. Hexachlorobenzene was detected in 42% of whole water samples (dissolved plus particulate phases) collected during 1991 in the St. Lawrence River and several of its tributaries. Hexachlorobenzene concentrations detected in the St. Lawrence River ranged from not detected to

0.09 ng/L (mean 0.01 ng/L [ppt]) (Quemerais et al. 1994). Hoff et al. (1996) obtained and presented water concentration data from a 1992 sampling study. Hexachlorobenzene concentrations in Lakes Superior, Michigan, Huron, Erie, and Ontario were 0.01, 0.014, 0.007, 0.014, and 0.045 ng/L, respectively. In remote European mountain lake waters, the concentrations of hexachlorobenzene were 8.4±11 pg/L at Redó, Spain, 4.0±1.8 pg/L at Gossenkölle, Austria, and 6.2±1.0 pg/L at Øvre Neådalsvatn, Norway (Vilanova et al. 2001).

The concentrations of hexachlorobenzene in microlayer and subsurface Mediterranean seawater off the coast of Alexandria, Egypt were on average, 27.3±17 and 12±6.9 ng/L, respectively (Abd-Allah 1999). Seawater samples taken along a North-South Atlantic transect during five legs in transit from the island of Texel in the Netherlands to Walvis Bay, Namibia and Cape Town, South Africa contained hexachlorobenzene concentrations of 9.0, 6.3, 1.9, 3.3, and 2.7 pg/L for each leg of the trip, respectively (Booij et al. 2007). Hexachlorobenzene has been detected in shallow groundwater in China at depths of 4–8 m. Monitoring wells and drinking wells sampled in the Taihu Lake region of China in 2011 had an average hexachlorobenzene concentration of 16.53 ng/L and a range of 3.05–65.24 ng/L (Wu et al. 2014).

A study was conducted from 1974 to 1975 to collect and analyze surface water samples from sites of known hexachlorobenzene contamination along the Mississippi River near an industrial area in Geismar, Louisiana (EPA 1976a). The maximum hexachlorobenzene concentration detected in water was 90.3 ppb. A concentration of 2 ppb has been measured in the Mississippi River near Baton Rouge, Louisiana (Laska et al. 1976).

Industrial waste water samples contained hexachlorobenzene levels as high as 300 ppb (EPA 1976b; Schmitt et al. 1990). Effluent concentrations of hexachlorobenzene from four Canadian plants in the Great Lakes region ranged from 0.001 to 0.002 ppb (0.0015 ppb mean) (Oliver and Nicol 1982a).

Hexachlorobenzene concentrations were measured in water at an uncontrolled hazardous waste site near Bayou Baton Rouge, Louisiana (Davis and Morgan 1986). Surface water samples collected from a containment pond used for disposal of wastes from both rubber production and manufacture of chlorinated organics at the site contained up to 8,100,000 ppb hexachlorobenzene.

6.4.3 Sediment and Soil

Mean concentrations of hexachlorobenzene in lake sediments in the Great Lakes ranged from 0.2 to 97 ppb with the highest values measured in Lake Ontario. Deeper sediment layers (1–2 cm) had even higher concentrations of hexachlorobenzene (460 ppb) than surface (0–1 cm) samples (270 ppb), with the peak value corresponding to deposition in the years 1971–1976 declining to 270 ppb in 1976–1980 (Oliver and Nicol 1982a). A study of hexachlorobenzene concentration in Lake Erie sediments revealed a decrease of roughly 80%, with lake-wide average measured concentrations of 5.7 and 1.6 ng/g dry weight in 1971 and 1997, respectively (Marvin et al. 2004). In 1992, 2 cm deep bed sediment samples were collected from the South Platte River at Henderson, Colorado and Cache La Poudre River near Greeley, Colorado. The sediment contained 1.5 and <1 μg/kg, dry weight, hexachlorobenzene, respectively. The authors concluded that this concentration was correlated to the hexachlorobenzene concentrations found in urban and agricultural lands in the South Platte River Basin (Tate and Heiny 1996). Outside of the United States, 12 sediment samples were collected in June 1993 near known discharges from municipalities and industries from Lake Ladoga, Russia. Hexachlorobenzene concentrations were 3.58 and 14.6 ng/g in 2 out of the 12 samples, and hexachlorobenzene was not detected in the remaining 10 samples (Ristola et al. 1996). In the levees of the Mississippi River near Baton Rouge, where the river water contained 2 ppb hexachlorobenzene, the soil contained 167 ppb (Laska et al. 1976).

Meijer et al. (2003b) studied hexachlorobenzene concentrations in background surface soils collected in 1998 from 191 locations around the world. The reported global mean background concentration was 0.68 ng/g dry weight. The lowest measured concentration, 0.010 ng/g dry weight, was found in samples from Bear Island in Norway, and the highest concentrations, 5.20 and 4.83 ng/g dry weight, were found in south Norway and Russia, respectively. This study also demonstrated how hexachlorobenzene concentration is directly correlated with soil organic matter (SOM) content. The highest hexachlorobenzene concentrations were measured in the northern hemisphere, in general, and in Europe, in particular. This corresponds with the location of soils with the highest SOM content, associated with forests, peat bogs, and grassland systems.

Yuan et al. (2014) evaluated hexachlorobenzene concentrations from soils collected in 2011 from the Tibetan Plateau. The soil samples were collected at locations with low soil organic carbon (SOC) content and >200 km from possible sources at high altitudes, where soil-air exchange processes are expected to greatly influence adsorption and re-volatilization of hexachlorobenzene. The average hexachlorobenzene concentration was 54.0 ng/kg. Hexachlorobenzene was detected in surface soil samples from agriculture

soil used to grow rice, wheat, corn, bean, cotton, and vegetables in central China. The average concentration of hexachlorobenzene detected was 3.01 ng/g soil, based on a range of concentrations from not detected to17.77 ng/g soil in 44 soil samples; the detection frequency was 86% (Zhou 2013). In Tianjin, China, hexachlorobenzene concentrations ranged from not detected to 1,924 µg/kg dry weight near the Tanggu Chemical Industrial District (Hou et al. 2013).

Hexachlorobenzene concentrations were measured in soil and sediment at several uncontrolled hazardous waste sites in several states (Davis and Morgan 1986). Hexachlorobenzene concentrations of up to 20,000 ppb were measured in soil at a scenic highway site near Bayou Baton Rouge, Louisiana, while concentrations in sediment of 39,500 ppb were measured from a bayou bank downstream of the site. Soil cores from a monitoring well (25–27 feet deep) were as high as 400,000 ppb, and as high as 90,000 ppb in soils collected at 40–41 feet deep (Davis and Morgan 1986). Soil and sediment collected from a disposal site near Sorrento, Louisiana contained 62,000 and 130,000 ppb hexachlorobenzene, respectively. Soil collected at a Crystal City, Texas pesticide disposal site was found to contain 20,000 ppb hexachlorobenzene. A maximum hexachlorobenzene concentration detected in soil at an industrial site of known contamination near Geismar, Louisiana was 53,130 ppb (Laseter et al. 1976).

Sediment concentrations of hexachlorobenzene vary widely from relatively unpolluted areas to those areas used extensively for disposal of hexachlorobenzene-containing wastes. Sediment hexachlorobenzene concentrations from San Luis Pass, located near industrial areas of West Galveston Bay, Texas ranged from 0.05 to 1.5 ppb (dry weight) with a mean of 0.49 ppb (Murray et al. 1981).

Hexachlorobenzene concentrations in marine sediment samples collected from an industrialized area of the harbor in Portland, Maine ranged from <0.03 to 0.37 ppb (mean 0.14 ppb) (Ray et al. 1983). Concentrations of hexachlorobenzene in sediment from the Niagara River watershed in the vicinity of several hazardous waste disposal areas ranged from 8,000 to 30,000 ppb (Elder et al. 1981). The average concentration of hexachlorobenzene in surficial sediments of the Kaohsiung coast (southwestern Taiwan), which receives wastewater from the largest industrial city in Taiwan (Kaohsiung City), ranged from 1.7 to 24.7 ng/g (Lee et al. 2000a). In Northern China, the average concentrations of hexachlorobenzene in surficial sediments of the Laizhou Bay ranged from below the limit of detection to 0.17 ng/g in marine sediment and from below the limit of detection to 14 ng/g in the adjacent river riverine sediment (Zhong et al. 2011). Concentrations in sediment from the Xiangjiang River in China ranged from 0.6 to 14.2 ng hexachlorobenzene/g sediment (Li et al. 2013). Surface sediments from Muroran Port, Japan were evaluated for hexachlorobenzene; concentrations ranged from 110 to 25,000 pg/g dry weight (Anezaki

and Nagahora 2014). Hexachlorobenzene was detected in Lenga Estuary, Chile at concentrations of 1–870 ng/g dry weight (Pozo et al. 2014).

6.4.4 Other Environmental Media

Concentrations of hexachlorobenzene have been detected in several species of fish and shellfish. Hexachlorobenzene concentrations were determined for several species of marine organisms collected from San Luis Pass near Galveston Bay, Texas (Murray et al. 1981). Mean concentrations of hexachlorobenzene at were 0.49 ppb wet weight for flounder (species unspecified), 0.65 ppb for longnose killifish *(Fundulus similis)*, 0.88 ppb for brown shrimp *(Penaeus aztecus)*, 9.6 ppb for blue crab *(Callinectes sapidus)*, and 0.71 ppb for the dwarf squid *(Lollingnucula brevis)*. Oysters *(Crassotrea virginica)* collected at the lower end of the Houston Ship Channel were found to contain hexachlorobenzene concentrations ranging from 0.31 to 1.41 ppb with a mean of 0.63±0.39 ppb (Murray et al. 1980). The Sheboygan River in Wisconsin is another area of concern for contamination of organochlorine pesticides in fish due to the existence of wetlands, urban and developed land, woodland, and agricultural land surrounding this river. In addition, the lower segment of the river has a history of shipping, industrial and municipal activities, and dredging, including the existence of a landfill designated as a federal superfund site. Schrank et al. (1997) collected white suckers (*Catostomus commersoni*) from two sites of the Sheboygan River; one site was 1 to 2 km from the mouth of the river and the other was 50 km from the mouth of the river, which served as the reference site. The fish collected from both sites contained less than detectable residues of hexachlorobenzene along with other organochlorine compounds, thus indicating a minimized risk of exposure to hexachlorobenzene from this river. Hexachlorobenzene was detected in whole fish composites collected from bass and carp from 13 river sites collected in the Mobile, Apalachicola-Flint-Chattahoochee, Savannah, and Pee Dee River Basins at mean concentrations of 0.66 and 2.48, 0.81 and 2.38, 1.03 and 2.73, and 0.37 and 0.43 ng/g wet weight, respectively (Hinck et al. 2008).

DeVault (1985) reported concentrations of hexachlorobenzene in whole fish composites collected from the Great Lakes during 1980 and 1981 ranged from <0.002 to 3.47 mg/kg (<2–347 ppb). Hexachlorobenzene concentrations in fresh water trout (4–6+ years old) from the Great Lakes region ranged from 8 to 127 ppb with the highest concentration found in a fish collected near the discharge of the Niagara river into Lake Ontario (Oliver and Nichol 1982a). In another study of Great Lakes fish species, Newsome and Andrews (1993) reported hexachlorobenzene concentrations in fish fillet composites ranged from 0.22 ng/g (ppb) in bullhead to 9.05 ng/g (ppb) wet weight in trout in lake areas with open

fisheries. Zabik et al. (1995) reported that skin-off processing and selected cooking methods reduced hexachlorobenzene residues in chinook salmon and carp harvested from the Great Lakes. Concentrations of hexachlorobenzene averaged 0.017 ppm (17 ppb) and 0.011 ppm (11 ppb) (wet weight) in raw and cooked salmon fillets, respectively, and averaged 0.005 ppm (5 ppb) and 0.003 ppm (3 ppb) in skin-on and skin-off fillets, respectively. The average percentage loss of hexachlorobenzene from chinook salmon fillets by baking, charbroiling, and canning was 40%. Losses of hexachlorobenzene residues from carp fillets were slightly greater than 40% (Zabik et al. 1995). Walleye, siscowet, carp, and whitefish were collected for organochlorine pesticides analysis from Lake Superior along the Apostle Islands region during 1991 and 1992. Walleye and carp had hexachlorobenzene concentrations below the limit of quantification, while siscowet and whitefish measured concentrations of 3.2 and 2.8 ng/g wet weight of tissue, respectively (Gerstenberger et al. 1997). Organisms sampled during the summer of 1994 from the Keweenaw Peninsula of Lake Superior contained measured hexachlorobenzene concentrations ranging from 0.8 to 1.8 ng/g wet weight in smelts, 3.0–4.3 ng/g wet weight in herrings, 4.7–8.4 ng/g wet weight in bloaters, 1.1–4.1 ng/g wet weight sculpins, <0.1–0.2 ng/g wet weight in mysis, 0.8–1.4 ng/g wet weight in limnocalanus, 0.8 ng/g wet weight in amphipods, and 1.7–3.1 ng/g wet weight in lake trout (Kuchklick and Baker 1998). Grayling and lake trout were collected from four lakes (Feniak, Desperation, Schrader, and Elusive) from the foothills of the Brooks Range, Alaska. All lakes were oligotrophic, and the pH was neutral to slightly alkaline. Fifty-six grayling liver samples and 39 grayling muscle samples were analyzed for hexachlorobenzene concentration, and mean and median values were derived. In the 56 grayling liver samples, the mean and median concentrations were 0.65 and 0.48 ng/g dry weight, respectively. The 39 grayling muscle samples had mean and median concentrations of 0.33 and 0.22 ng/g dry weight, respectively. In lake trout, the mean and median concentrations in 33 liver samples were 1.15 and 0.87 ng/g dry weight, respectively, and in 34 muscle samples were 0.46 and 0.26 ng/g dry weight, respectively (Allen-Gil et al. 1997). Hexachlorobenzene concentrations in sea organisms from the Barents Sea were as follows (units=ng/g lipid weight): copepods (13.5), euphausids (16.5), amphipods (19.5), polar cod (39±1.7), and cod (65±7.7) (Borgå et al. 2001). In 1991, the concentrations of hexachlorobenzene in amphipods, isopods, and sculpins from the Bothnian Bay (Baltic Sea) were 340 (n=3), 370 (n=5), and 37 (n=3) ng/g dry weight, respectively (Strandberg et al. 2000). In a study of a set of 136 fish from 14 remote lakes in eight western U.S. National Parks/Preserves sampled between 2003 and 2005, the mean muscle tissue concentration of hexachlorobenzene was 0.26 ng/g whole weight and the range was 0.01–1.3 ng/g whole weight (Ackerman et al. 2008).

The bioaccumulative tendencies of hexachlorobenzene have made it a candidate for monitoring in the U.S. Fish and Wildlife Service National Pesticide Monitoring Program (Schmitt et al. 1990) and the

National Study of Chemical Residues in Fish which was started in 1986 (NSCRF) (EPA 1992). Maximum hexachlorobenzene tissue concentrations (wet weight) detected in whole fish were 700, 130, 120, and 410 ppb in the 1976–1977, 1978–1979, 1980–1981, and 1984 sampling years, respectively. The geometric mean tissue concentration was 10 ppb for 1976–1977 and <10 ppb for all other sampling years (Schmitt et al. 1990). The highest hexachlorobenzene concentrations in the 1984 sampling period (410 ppb) were detected in whole fish from the Tombigbee River, Alabama in the vicinity of a pesticide production facility where concentrations during all sampling years had been the highest nationally. These national results from the ongoing study conducted by NSCRF show that hexachlorobenzene was detected at 46% of the 362 sites surveyed for fish tissue analysis. The mean hexachlorobenzene concentration for fish tissue samples analyzed in this program was 5.8 ppb. The five sites with the highest concentrations are listed in Table 6-2 (EPA 1992). The Freeport, Texas site is near a pesticide plant and the other four sites are close to a variety of chemical manufacturing plants. The Calcasieu River, Louisiana site is close to a Superfund site involving a variety of organic solvents (EPA 1992).

Hazardous waste dumping during the early 1940s and 1950s contaminated the Devil's Swamp, Louisiana with chlorinated hydrocarbons, which has greatly affected fish species. As fish is an important food source for the community surrounding this area, concentrations of contaminants are of great concern. Levels of hexachlorobenzene in various fish species collected from Devil's Swamp, Louisiana, was compared to those from a control site, Tunica's Swamp, Louisiana. Mean hexachlorobenzene concentrations calculated from concentrations of 13 different edible fish species tissues were 23.65 ng/g compared to 2.0 ng/g calculated from 10 different edible fish species from Tunica's Swamp (Tchounwou et al. 1998).

Hexachlorobenzene has been detected in tissues of various wildlife species throughout North America, but especially in wildlife indigenous to the Great Lakes region. Swift et al. (1993) reported mean concentrations of hexachlorobenzene of 0.02 ppm (20 ppb) wet weight (0.04 ppm [40 ppb] lipid weight basis) and 0 ppm wet weight (0.07 ppm [70 ppb] lipid weight basis) in mesenteric and subcutaneous fat and breast tissue, respectively, of common goldeneye waterfowl wintering in New York state. The detection limit in this study was 0.002 ppm. Gebauer and Weseloh (1993) reported that the geometric mean hexachlorobenzene concentrations of 0.4 and 0.9 μg/kg (ppb) in muscle tissue in mallard ducks using a contaminated sediment site, and sewage lagoon site, respectively, as a resting and feeding area were significantly greater than levels found in ducks using a natural marsh area. Foley (1992) reported hexachlorobenzene residues in muscle tissues of several species of ducks and geese collected in New

Table 6-2. Sites with the Five Highest Concentrations of Hexachlorobenzene in Fish

Whole-body hexachlorobenzene concentration (ppb; wet weight)	Type of sample (fish)	Location
913	Sea catfish	Brazos River, Freeport, Texas
202	Catfish	Bayou D'Inde, Sulfur, Louisiana
93.7	Carp	Mississippi River, St. Francisville, Louisiana
85.5	White sucker	Quinnipiac River, North Haven, Connecticut
75	Sea catfish	Calcasieu River, Moss Lake, Louisiana

[a]From the EPA 1992 National Study of Chemical Residues in Fish

York State in 1983–1984. Statewide residues were 64 ppb (wet weight) for buffleheads, 49 ppb for scaups, 26 ppb for mallards, 20 ppb for black ducks, 6 ppb for wood ducks, and 11 ppb for Canada geese. Hexachlorobenzene was detected at concentrations ranging from 0.002 to 0.022 mg/kg wet weight in liver samples of eight seabird species collected opportunistically from a fishery in the North Pacific Ocean in 1992 (Elliott 2005). Adult sea otters that had died along the coast of California were collected by the U.S. Fish and Wildlife Service and the California Department of Fish and Game. Hexachlorobenzene concentrations in liver, kidney, and brain tissues were 0.74–8, 0.28–2.6, and 0.28–0.74 ng/g wet weight, respectively (Nakata et al. 1998). The mean hexachlorobenzene concentration for 207 wild mink liver tissue samples, collected from 1991 to 1995 in the Northwest Territories, Canada from seven mink communities, ranged from 0.21 to 0.67 ng/g wet weight (Poole et al. 1998). Snail composite samples, without shells, were collected from two lakes (Feniak and Elusive) from the foothills of the Brooks Range, Alaska. All lakes were oligotrophic, and the pH was neutral to slightly alkaline. The mean concentration among these six samples was 0.15 ng/g dry weight with a median of 0.10 ng/g dry weight (Allen-Gil et al. 1997).

Hexachlorobenzene was detected in nestling bald eagle plasma collected in 2003 from four areas in southwestern British Columbia and one site in California at mean concentrations of 0.20, 0.26, 0.35, 0.31, and 0.08 μg/kg wet weight, for Central Fraser Valley, Lower Fraser Valley, Nanaimo/Crofton area, Barkley Sound, and Santa Catalina Island, respectively (Cesh et al. 2008).

Hexachlorobenzene has also been detected in the eggs of various wildlife species in the Great Lakes region and Canada. Yamashita et al. (1992) reported hexachlorobenzene residues ranges of 8–36 ng/g (ppb) and 18–26 ng/g (ppb) on a wet weight basis in the eggs of the double-crested cormorant and the Caspian tern, respectively, collected during 1988 from the Great Lakes region. Somers et al. (1993) reported geometric mean concentrations of 0.013 μg/g (13 ppb) (wet weight) of hexachlorobenzene in double-crested cormorant eggs collected in southern Alberta, Canada. Elliott and Martin (1994) reported mean hexachlorobenzene concentrations in sharp-shinned hawk eggs in south central Ontario ranging from 0.010 to 0.051 mg/kg (10–51 ppb) from 1986 to 1989. Hexachlorobenzene concentrations in Cooper's hawk eggs ranged from 0.005 to 0.012 mg/kg (5–12 ppb) during the same period. Cobb et al. (1994) reported mean residues of 18.0 ng/g (18 ppb) in the chorio-allantoic membranes removed from great blue heron eggs collected from Puget Sound, Washington. Jarman et al. (1996) conducted an experiment with prairie falcon eggs that were collected from eyries in northern and central California between 1989 and 1991. Addled and unhatched eggs were frozen until chemical analysis. The following are the geometric mean concentrations (in mg/kg wet weight) of hexachlorobenzene at their respective

sampling sites: Frog/Hand Nest 800; Pig Cyn 17; Crowley Tower 11; Willow Sp. 8.0; Goat Rock 10; Mt. Dome 20; and Mt. Diablo 81. Mean hexachlorobenzene residues in peregrine falcon eggs from Rankin Inlet (Hudson Bay, Canada) were 0.03 μg/g wet weight (n=2; range 0–0.15 μg/g wet weight) between 1982 and 1986, and 0.030 μg/g wet weight (n=20; range 0–0.165 μg/g wet weight) between 1991 and 1994 (Braune et al. 1999).

Hexachlorobenzene residues were also detected in snapping turtle eggs collected from a wetland area on Lake Ontario. Residues in the snapping turtle clutches ranged from 43.9, 16.6, and 20.9 ng/g (ppb) (wet weight) to 494.7, 282.1, and 262.2 ng/g (ppb) (lipid weight) for the first five eggs that were layed, a composite sample of five eggs, and the last five eggs that were layed, respectively (Bishop et al. 1995). Bishop et al. (1996) conducted another study with snapping turtle eggs that were collected from nests at five locations from the Great Lakes Basin in 1990–1991. The eggs were analyzed for hexachlorobenzene and the results were compared to data collected from the same sites in the years 1981, 1984, 1988, and 1989. Based on the results, the hexachlorobenzene concentration was the highest in eggs from Cootes Paradise (170–350 ng/g) and lowest in eggs from Algonquin Park (3–20 ng/g). On the whole, the hexachlorobenzene mean concentration from the five sites in the Great Lakes Basin showed a decrease from the years 1984 to 1990 (Bishop et al. 1996).

Langlois and Langis (1995) reported that the concentration of hexachlorobenzene in the blubber of beluga whales from the St. Lawrence Estuary to Northern Quebec Province ranged from 0.22 to 0.93 mg/kg (220–930 ppb) wet weight. Consumption of blubber and organ meats from these whales by native peoples may constitute a potential health risk if these tissues are a significant part of the diet (Langlois and Langis 1995). In 1997, Gauthier et al. (1997) analyzed blubber biopsies from Northwestern Atlantic Balaenopterid whales summering in the Gulf of St. Lawrence. Samples were collected in the summer and fall of 1991 and 1992 from 21 minke, 15 fin, 6 blue, and 8 humpback whales. Hexachlorobenzene concentrations in the blubber of these whales were 101, 96, 110, and 177 ng/g lipid, respectively, and in all species, the concentrations were higher in males (140 ng/g lipid) than in females (103 ng/g lipid).

Becker et al. (1997) analyzed the blubber of 7 pilot whales, 5 harbor porpoises, 12 beluga whales, 2 northern fur seals, and 2 ringed seals that were obtained from the U.S. National Biomonitoring Specimen Bank. Hexachlorobenzene concentration ranges were 43–465 ng/g wet weight for pilot whales, 223–1,070 ng/g wet weight for harbor porpoises, 81.9–952 ng/g wet weight for beluga whales, 138–741 ng/g wet weight for northern fur seals, and 125–156 ng/g wet weight for ringed seals (Becker et al. 1997). Elkin and Bethke (1995) reported that hexachlorobenzene was the most predominant organo-

chlorine residue present in tissues of the caribou collected in the Northwest Territory of Canada. Residues ranged from a lipid corrected mean of 32.83 ng/g (ppb) in fat from Bathurst caribou to 129.41 ng/g (ppb) in Lake Harbor animals (Baffin Island). Consumption of meat and organs from these animals by native peoples, including native American populations of Inuit in Alaska, may constitute a potential human health risk if these tissues are a significant part of the diet.

The Florida Department of Environmental Protection and the Florida Marine Research Facility in St. Petersburg, Florida maintain archives of tissues obtained from dead Florida Manatees (*Trichechus manatus latirostris*). In 1996, Ames and Van Vleet (1996) obtained and analyzed 19 manatee samples from the Florida EPA. Of these samples, hexachlorobenzene was found at concentrations of 0.038 and 0.085 μg/g in one blubber and one liver sample, respectively. The authors did not find a correlation between the contamination in manatees by hexachlorobenzene and other pesticides and the location in which the manatees were found dead; thus, the authors concluded that these manatees must have been contaminated elsewhere, especially since manatees are known to travel long distances.

Corsolini et al. (1999) analyzed chlorinated hydrocarbon concentrations in muscle and fat samples of the red fox collected from Sienna, Italy in 1992. Hexachlorobenzene mean concentrations in muscle and fat were 0.47 and 0.23 μg/g lipid basis, respectively, and were the lowest of all of the chlorinated hydrocarbons that were tested (Corsolini et al. 1999).

Sitarska et al. (1995) collected tissue samples from 18 cows just after slaughtering, always from the same parts of the studied organs. Hexachlorobenzene mean concentrations were 1.394 μg/kg wet mass in the ovaries, 1.061 μg/kg wet mass in the mammary glands, and 0.550 μg/kg wet mass in the liver. Hexachlorobenzene was detected in the follicular fluid of cattle, sheep, goats, and pigs raised on local farms in Greece at mean concentrations of 1.77, 1.25, 1.63, and 0.78 ng/mL, respectively (Kamarianos et al. 2003).

Beyer (1996) subjected earthworms (*Lubricous terrestris*) to hexachlorobenzene in artificial soil. Over the course of 28 weeks, hexachlorobenzene concentrations in these earthworms ranged from 1.8 to 3 ppm. Beyer (1996) also conducted three 8-week experiments in which earthworm survival rates were 97, 99, and 100%, respectively.

Hexachlorobenzene was detected in composited milk samples collected through the U.S. Pasteurized Milk Network during 1990–1991 (Trotter and Dickerson 1993). The milk samples were collected at

approximately 63 sites located in the United States, Puerto Rico, and the Panama Canal Zone. Hexachlorobenzene was detected in trace amounts in one sample collected in each of seven metropolitan areas (Cristobal, Panama Canal Zone; Kansas City, Missouri; Los Angeles, California; Memphis, Tennessee; Portland, Oregon; Spokane, Washington; Wichita, Kansas) and was detected at 0.001 ppm (1 ppb) in one sample from Rapid City, South Dakota.

Pesticide residue data from the FDA Adult Total Diet Study conducted from 1980 to 1982 were evaluated by Gartrell et al. (1986). These authors reported that hexachlorobenzene was detected in a wide variety of domestic foods: dairy products, meat, fish and poultry, oils and fats, and sugar and adjuncts. The highest mean concentrations of hexachlorobenzene were detected in oils and fats (0.9 ppb) and in meat, poultry, and fish (0.2 ppb). Concentrations of hexachlorobenzene in ready-to-eat foods were monitored for 10 years from 1982–1991 through the FDA's Revised Market Basket Survey. Hexachlorobenzene was detected in 618 samples of 81 different foods at a mean concentration of 0.0006 μg/g (0.6 ppb) (KAN-DO Office and Pesticide Teams 1995). In food composites from six Canadian cities, the mean concentration of hexachlorobenzene in positive samples (4.8% of 913 total analysis) was 0.5 ng/g (Newsome et al. 2000). The U.S. Food and Drug Administration monitored domestic and imported apples and rice by collecting random samples for a period of 12 months beginning in October, 1993. Hexachlorobenzene was not determined to be in violation according to the concentration limits set for this compound in any of the domestic and imported apple and rice samples; however, it was found in 1 out of 612 imported rice samples (0.02 ppm), but this concentration does not violate any limit set by the EPA (Roy et al. 1997). The concentrations and occurrences of hexachlorobenzene residues in butter from Spain (n=36, 89% positive) and the rest of Europe (n=20, 70% positive) were 5.864±3.171 ng/g wet weight and 3.022±3.964 ng/g wet weight, respectively (Badia-Vila et al. 2000).

The frequency of detection of hexachlorobenzene in the FDA Total Diet Study conducted in 1982–1984 was 9% (Gunderson 1988). Hexachlorobenzene intakes, in μg/kg body weight/day, estimated for these total diet analyses were 0.0020 and 0.0011 for 14–16-year-old males and 60–65-year-old females, respectively. In additional FDA Total Diet Studies, the frequency of detection of hexachlorobenzene residues declined to 7% in 1988 (FDA 1989), 5% in 1989 (FDA 1990), 4% in 1990 (FDA 1991), 2% in 1991 (FDA 1992), <2% on 1991–1993 (FDA 1994), and <2% in 1994 (FDA 1995). Hexachlorobenzene intakes (μg/kg body weight/day) estimated for the Total Diet Analyses also declined from intakes estimated in the 1982–1984 analysis and were 0.0011 and 0.0006 in 1988 (FDA 1989); 0.0009 and 0.0005 in 1989 (FDA 1990); 0.0005 and 0.0002 in 1990 (FDA 1991); and 0.0004 and 0.0002 in 1991 (FDA 1992) for 14–16-year-old males and 60–65-year-old women, respectively.

Hexachlorobenzene levels in food products collected from FDA's Total Diet Study (TDS) market basket surveys are summarized in Tables 6-3 and 6-4. The TDS is FDA's ongoing market basket survey of core foods in the U.S. food supply to determine levels of various pesticide residues, contaminants, and nutrients in foods and to estimate exposures of these substances in representative diets of specific age and gender groups in the United States. For each market basket, food samples are collected from grocery stores and fast food restaurants in different cities, prepared table-ready (i.e., as they would be consumed), and composited for analysis.

Domestic samples of mixed feed rations were collected and analyzed by the FDA for pesticide surveillance during fiscal years 1989–1994. Hexachlorobenzene residue was detected in 1 of 457 samples in trace amounts (Lovell et al. 1996).

Burton and Bennett (1987) estimated a human body burden for hexachlorobenzene of 0.7 mg derived primarily from dietary intake of fatty foods (0.2 μg/day). Inhalation was estimated to contribute 100 times less than dietary intake (0.002 μg/day) and consumption of drinking water was also considered to contribute only negligible amounts of hexachlorobenzene (0.06 μg/year).

Hexachlorobenzene was detected in 98% of cheese samples obtained from of 61 commercially available brands in the Canary Islands (Almeida-Gonzalez et al. 2012). The average concentrations of hexachlorobenzene were 6.95±7.77 and 2.27±1.46 ng/g fat for conventional and organic cheeses, respectively.

An exploratory study of chemical exposure was conducted among Vietnamese, Bangladeshi, and local resident sportfish consumers in the Montreal region of the St. Lawrence River. The concentration ranges for the respective groups are as follows: 0.01–0.04; 0.01–0.02; and 0.01–0.07 μg/L, indicating a positive correlation between local residents consuming sportfish from the St. Lawrence River and hexachlorobenzene concentrations (Kosatsky et al. 1999). Anderson et al. (1998) conducted a study to assess hexachlorobenzene contamination in human serum and urine samples from frequent consumers of sport fish from Lakes Michigan, Erie, and Huron. A telephone survey was conducted requesting fish eating habits with special attention to lake trout, brown trout, rainbow trout, or chinook or coho salmon, carp or catfish, and walleye or perch or smelt. After the survey, each angler was invited to give a serum sample. The minimum and maximum hexachlorobenzene concentrations of all 30 participating subjects were 0.02 and

Table 6-3. Hexachlorobenzene Levels in Food Items from the Food and Drug Administration's Total Diet Study Market Baskets 1991–1993 Through 2003–2004 Collected Between September 1991 and October 2003[a]

Food	Number	Number ≥LQ	Number Trace	Mean (ppm)	Minimum (ppm)	Maximum (ppm)
Milk, lowfat (2%), fluid	44	0	1	0.00000	0.0001	0.0001
Cheese, American, processed	44	0	8	0.00006	0.0001	0.0001
Cheese, cheddar, natural (sharp/mild)	44	0	5	0.00005	0.0003	0.0006
Beef, ground, regular, pan-cooked	44	0	11	0.00005	0.0001	0.0004
Beef roast, chuck, oven-roasted	44	0	2	0.00001	0.0001	0.0003
Pork chop, pan-cooked with oil	44	0	2	0.00001	0.0002	0.0003
Pork sausage (link/patty), oven-cooked	44	1	0	0.00005	0.0020	0.0020
Pork bacon oven cooked	44	0	2	0.00002	0.0003	0.0006
Lamb chop, pan-cooked with oil	44	4	18	0.00030	0.0001	0.0020
Frankfurter (beef/pork), boiled	44	0	8	0.00004	0.0001	0.0004
Tuna canned in oil, drained	40	0	1	0.00001	0.0005	0.0005
Peanut butter, creamy	44	0	1	0.00000	0.0002	0.0002
Peanuts, dry roasted, salted	44	0	1	0.00000	0.0002	0.0002
Bread rye	44	0	1	0.00001	0.0003	0.0003
Watermelon, raw/frozen	44	1	4	0.00006	0.0001	0.0010
Summer squash, fresh/frozen, boiled	44	0	5	0.00003	0.0001	0.0007
Squash, winter (Hubbard/acorn) Fresh/frozen broiled	44	0	3	0.00003	0.0001	0.0009
Potato chips	44	0	1	0.00000	0.0002	0.0002
Chili con carne with beans, canned	4	0	1	0.00003	0.0001	0.0001
Quarter-pound hamburger on bun, fast food	44	0	5	0.00001	0.0001	0.0002
Meatloaf, beef, homemade	44	0	9	0.00003	0.0001	0.0003
Butter salted	44	6	29	0.00087	0.0004	0.0070
Half & half cream	44	0	2	0.00000	0.0001	0.0001
Sandwich cookies with crème filling	44	0	1	0.00000	0.0001	0.0001
Cheese, Swiss natural	44	0	9	0.00009	0.0003	0.0005
Cream cheese	44	0	8	0.00007	0.0003	0.0005
Lunch meat (ham)	44	0	2	0.00001	0.0002	0.0004
Chicken nuggets, fast-food	44	0	1	0.00000	0.0001	0.0001
Chicken, fried (breast, leg, and thigh) fast food	40	1	0	0.00005	0.0020	0.0020
Haddock	20	0	6	0.00009	0.0002	0.0003

Table 6-3. Hexachlorobenzene Levels in Food Items from the Food and Drug Administration's Total Diet Study Market Baskets 1991–1993 Through 2003–2004 Collected Between September 1991 and October 2003[a]

Food	Number	Number ≥LQ	Trace	Mean (ppm)	Minimum (ppm)	Maximum (ppm)
Crackers butter type	44	0	3	0.00005	0.0004	0.0008
Quarter-pound cheeseburger on bun, fast food	44	0	2	0.00001	0.0001	0.0002
Fish sandwich on bun, fast-food	44	2	9	0.00015	0.0001	0.0020
Frankfurter on bun, fast-food	40	1	4	0.00007	0.0001	0.0020
Taco/tostada with beef and cheese, from Mexican carry out	44	0	3	0.00003	0.0003	0.0004
Ice cream, regular, vanilla	44	0	2	0.00000	0.0001	0.0001
Sugar cookies	44	0	1	0.00001	0.0003	0.0003
Sour cream	44	0	8	0.00004	0.0001	0.0004
Olive/safflower oil	44	0	1	0.00002	0.0008	0.0008
Salmon, steaks/fillets, baked	24	11	12	0.00098	0.0003	0.0020
Baby food, squash	44	3	2	0.00008	0.0002	0.0010
Baby food, cereal, mixed, dry, prepared with water	44	0	5	0.00003	0.0002	0.0005

[a]Number ≥LQ: number of result(s) that were greater than the limit of quantification (LQ). Number of traces: number of result(s) that were greater than or equal to the limit of detection but less than the LQ. Statistics were calculated using value of 0 for results below the limit of detection.

Source: FDA 2006a

Table 6-4. Hexachlorobenzene Levels in Food Items from the Food and Drug Administration's Total Diet Study Market Baskets 2004-1 Through 2005-4 (Eight Market Baskets) Collected Between October 2003 and August 2005[a]

Food	Number	Number ≥LQ	Trace	Mean (ppm)	Minimum (ppm)	Maximum (ppm)
Cheese, American	8	0	1	0.00003	0.2002	0.0002
Beef, ground	8	1	1	0.00014	0.0001	0.0010
Lamb, chop	8	0	2	0.00003	0.0001	0.0001
Bologna	8	0	1	0.00004	0.0003	0.0003
Peanut butter, smooth	8	0	1	0.00001	0.0001	0.0001
Fruit-flavored sweetened cereal	8	0	1	0.00001	0.0001	0.0001
Summer squash, fresh/frozen	8	0	3	0.00005	0.0001	0.0001
Meatloaf	8	1	2	0.00004	0.0001	0.0001
Butter	8	0	2	0.00014	0.0002	0.0009
Half & half cream	8	0	1	0.00001	0.0001	0.0001
Cheese, Swiss, natural	8	0	1	0.00004	0.0003	0.0003
Cream cheese	8	0	1	0.00003	0.0002	0.0002
Sugar cookies	8	0	1	0.00001	0.0001	0.0001
Sour cream	8	0	1	0.00001	0.0001	0.0001
Salmon	8	0	6	0.00023	0.0002	0.0004
Baby food, squash	8	0	1	0.00001	0.0001	0.0001
Granola bar with raisins	8	0	1	0.00001	0.0001	0.0001

[a]Number ≥LQ: number of result(s) that were greater than the limit of quantification (LQ). Number of traces: number of result(s) that were greater than or equal to the limit of detection but less than the LQ. Statistics were calculated using value of 0 for results below the limit of detection.

Source: FDA 2006b

0.2 ppb, respectively, with a median concentration of 0.1 ppb. Eight participants from Lake Michigan had minimum and maximum concentrations of 0.09 and 0.2 ppb, respectively, with a median concentration of 0.1 ppb, and 11 participants from Lake Huron and Lake Erie had respective minimum and maximum concentrations of 0.04 and 0.2 ppb and 0.02 and 0.2 ppb with median concentrations of 0.1 and 0.09 ppb. A comparison group from Arkansas (180 serum samples) had hexachlorobenzene concentrations ranging from not detected to 0.3 ppb with a median of 0.1 ppb (Anderson et al. 1998). This study illustrates that since the comparison group had the highest range in concentration, there is a wide spread of hexachlorobenzene contamination and any population may possibly be affected.

Sinkkonen et al. (1995) discovered concentrations of hexachlorobenzene in pine needles in the vicinity of a metal reclamation plant. Five sites sampled and analyzed for hexachlorobenzene in the wax of the needles and the rest of the needles in 1991 (0.257–0.731 ng/g; 0.758–3.170 ng/g), 1992 (0.142–0.692 ng/g; 0.255–1.785 ng/g), and 1993 (0 concentration found in the wax of the needles; 0.217–0.885 ng/g) show decreasing concentrations (Sinkkonen et al. 1995). In 1996, Sinkkonen et al. (1996) analyzed pine needles in and around the metal reclamation plant again. Composite samples from the years 1993, 1994, and 1995 had hexachlorobenzene concentrations of 6.9–8.3 ng/g in the wax of the needles and 0.2–2.2 ng/g in the rest of the needles, contrary to the decreasing trends found from the analysis conducted during 1991–1993.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Hexachlorobenzene is no longer produced (as an end-product) or used as a pesticide in the United States. Consequently, the current potential for exposure of the general population appears to be very limited. Members of the general population may be exposed to very low concentrations of hexachlorobenzene through ingestion of contaminated foodstuffs, particularly those with high lipid content such as meat, poultry, and fish. General population exposure to hexachlorobenzene via inhalation or dermal contact would be much less. Occupational exposure is possible for workers involved in the production of chlorinated hydrocarbons, which releases hexachlorobenzene as a byproduct.

Brock et al. (1998) investigated four families in Iowa and two families in North Carolina for exposure to several organochlorine pesticides by analyzing the pesticide levels in their serum. Among the farmers from Iowa, mean hexachlorobenzene concentrations in serum ranged from 0.12 to 0.26 ng/mL, and their spouses had mean levels ranging from 0.05 to 0.24 ng/mL. The two farmers from North Carolina had mean levels of 0.15 and <0.05 ng/mL, and their respective spouses had levels of 0.16 and 0.17 ng/mL. It

was shown that in one family, the pesticide serum level in the spouse (0.17 ng/mL) was higher than that in the farmer (<0.05).

The concentration of hexachlorobenzene in 12 San Francisco, California firefighters' serum was evaluated by high resolution gas chromatography-high resolution mass spectrometry (Shaw et al. 2013). The samples were obtained within 24 hours of responding to a fire. All samples of serum contained hexachlorobenzene; the concentrations ranged from 8 to 46 ng/g lipid weight basis.

Glynn et al. (2000) studied the serum concentration of hexachlorobenzene in a group of 790 men ages 40–75 who were randomly selected from both rural and urban areas of Uppsala, Sweden. The mean concentration of hexachlorobenzene was 83.1±133.6 ng/g (ppb) lipid for this group. This study group had lower serum concentrations than males who had recent occupational exposure or high environmental exposure. Hagmar et al. (2001) examined blood samples from 110 men who consumed varying amounts of fish from the Baltic Sea. The median plasma level of hexachlorobenzene in the 43 Swedish and 67 Latvian adult males in this study group was 84 ng/g (ppb) lipid. In a study of intra-individual variation in serum levels of 39 Swedish men, mean hexachlorobenzene concentrations measured in 1991 and 2001 were 77 and 33 ng/g (ppb) lipid, respectively, displaying an average decrease of 53% after a decade (Hagmar et al. 2006). The average decrease in hexachlorobenzene concentration was associated with high fish consumption in 1991 as compared to 2001, but this only explained 12% of the variation. The serum concentrations of pregnant women from the Disko Bay area, Greenland were studied by Bjerregaard and Hansen (2000). The women in this study consume a high level of meat and blubber from marine animals. The concentration of hexachlorobenzene in plasma taken from these women between the years 1994 and 1996 was 1.2 ng/mL wet weight (range, 0.1–7.0 ng/mL wet weight). Hexachlorobenzene concentrations in serum in study groups of active smoking mothers, passive smoking mothers, and nonsmoking mothers from Germany were 0.87 ng/mL (range, 0.23–4.38 ng/mL), 0.55 ng/mL (range, <0.10–3.27 ng/mL), and 0.46 ng/mL (range, <0.10–2.73 ng/mL) (Lackman et al. 2000). The mean concentration of hexachlorobenzene in whole blood from infant children ranged from 0.13 to 0.23 ng/mL (Karmaus et al. 2001). The highest concentration was observed in children who were breastfed for over 12 weeks after birth.

Serum samples from French adults aged 18–74 years had a reported geometric mean hexachlorobenzene concentration of 24.3 ng/g lipid in The French National Nutrition and Health Study (Saoudi et al. 2014). The median concentration of hexachlorobenzene in serum samples from 101 subjects in the National Health System Patients Roster for Calatafimi, Italy was 18.63 ng/g lipids and the detection frequency was

84.2% (Amodio, 2012). The average concentration of hexachlorobenzene in human serum samples from Bizerte, Tunisia was 49.1±29.6 (Ben Hassine et al. 2014).

In Western Australia, almost all (99%) of the plasma samples collected from pregnant women 2 weeks before delivery contained hexachlorobenzene at levels above the limit of detection (Reid et al. 2013). The range of hexachlorobenzene concentration in the 167 blood plasma samples was 0.005–2.00 μg/L and the average concentration was 0.08 μg/L.

Nonoccupational exposure to hexachlorobenzene for residents of two U.S. cities (Jacksonville, Florida and Springfield, Massachusetts) were studied over three seasons; summer 1986, spring 1987, and winter 1988 (Whitmore et al. 1994). The study focused primarily on inhalation exposures with primary environmental monitoring consisting of 24-hour indoor, personal, and outdoor air. For the Jacksonville, Florida population, the estimated mean hexachlorobenzene concentration ranged from 0.3 to 1.3 ng/m^3 for indoor air, from not detected to 0.2 ng/m^3 for outdoor air, and from 0.4 to 0.9 ng/m^3 for personal air. For the Springfield, Massachusetts population, mean exposures were much less. The estimated hexachlorobenzene concentrations ranged from not detected to 0.1 ng/m^3 for indoor air, were not detected in outdoor air, and ranged from not detected to <0.05 ng/m^3 for personal air. The mean air exposure estimated for hexachlorobenzene in Jacksonville, Florida, was 10 ng/day, while dietary exposure ranged from 70 to 120 ng/day. The mean air exposure for Springfield, Massachusetts, was not detected in the personal air samplers, while the dietary exposure was 105 ng/day. In both the Jacksonville, Florida population, characterized as a high pesticide use area, and in the Springfield, Massachusetts population, characterized as a low pesticide use area, the dietary exposure to hexachlorobenzene was the predominant exposure pathway.

In the National Human Adipose Tissue Survey, hexachlorobenzene was found in 35 of 46 human adipose tissue samples from all regions of the United States at levels ranging from 12 to 1,300 ppb (EPA 1986c). In other studies of the general population, hexachlorobenzene has been found in human fat samples from residents of the Texas Gulf Coast at concentrations ranging from 18 to 35 ppb (Ansari et al. 1986). Kutz et al. (1991) summarized data on hexachlorobenzene residues in human adipose tissue collected in the United States from 1973 through 1983. The geometric mean concentrations increased slightly from 0.02 ppm (20 ppb) in 1973 to 0.05 ppm (50 ppb) in 1976, and then declined to 0.031 ppm (31 ppb) in 1983.

Human breast adipose tissue samples from 36 females of 50–80 years in age were collected from the Yale-New Haven hospital in Connecticut. A correlation was made between breast adipose tissue and serum residues using Pearson's correlation coefficient. On a lipid adjusted basis, all 36 human adipose tissue samples were found to contain residues of hexachlorobenzene. The range of concentration was 2.5–33.3 ng/g (ppb) with a median of 17.7 ng/g (ppb) (Archibeque-Engle et al. 1997). A study conducted in British Columbia, Canada, Mes (1992) reported median and maximum hexachlorobenzene residues in biopsied fatty tissue of 18.8 and 87 ng/g (ppb), respectively. In a more recent study, small amounts of breast tissue were collected from 60 women undergoing breast surgery at Stanford University, California. The mean hexachlorobenzene concentration was 46 ng/g (ppb) fat with a minimum and maximum of 14 and 170 ng/g (ppb) fat, respectively (Petreas et al. 1998). Weistrand and Noren (1998) collected adipose tissue and liver samples from five Swedish men and two Swedish women. Hexachlorobenzene concentrations ranged from 12 to 129 ng/g (ppb) lipids with a mean of 56 ng/g (ppb) lipids in adipose tissue and 17 to 156 ng/g (ppb) lipids with a mean of 58 ng/g (ppb) lipids in the liver. Hexachlorobenzene levels in human adipose tissue from 64 mothers living in Veracruz, Mexico averaged 0.065 mg/kg (65 ppb) (range, 0.010–0.401 mg/kg) on a lipid adjusted basis (Waliszewski et al. 2000a). The mean concentrations of hexachlorobenzene in autopsy tissue samples from Greenlanders were 594 μg/kg (ppb) lipid (range, 476–742 μg/kg lipid), 588 μg/kg (ppb) lipid (range, 156–1,890 μg/kg lipid), 260 μg/kg (ppb) lipid (range, 175–387 μg/kg lipid), and 754 μg/kg (ppb) lipid (range, 603–943 μg/kg lipid) for subcutaneous fat, omental fat, brain, and liver tissues, respectively (Dewailly et al. 1999). Moreover, in a study evaluating hexachlorobenzene in 70-year-old Uppsala, Sweden citizens, plasma concentrations of hexachlorobenzene, ranging from 27.8 to 53.7 ng/g lipid, positively related to both visceral and subcutaneous adipose tissue content measured using abdominal magnetic resonance imaging (Roos et al. 2013).

In the National Health and Nutrition Examination Survey (NHANES II) conducted by CDC hexachlorobenzene levels in blood from the general population collected from 1976 to 1980 reported a median level for quantifiable positive results of 1.7 ppb, which did not vary among the four age groups studied (Murphy and Harvey 1985). Table 6-5 lists mean serum hexachlorobenzene concentrations (by whole weight and lipid-weight bases) in the U.S. population from NHANES IV (updated in 2015) using serum samples collected during the years 2003–2004 (CDC 2009, 2015). Mean concentrations of hexachlorobenzene were highest for adults ≥20 years of age, slightly higher among females than males, and highest among Mexican Americans and lowest among non-Hispanic whites.

Table 6-5. Geometric Mean Serum Hexachlorobenzene Concentrations (Whole Weight and Lipid Adjusted) for the Years 2003–2004 in the U.S. Population from the National Health and Nutrition Examination Survey

	Geometric mean serum hexachlorobenzene (95% CI)	
Group	Whole weight (ng/g serum or ppb)	Lipid adjusted (ng/g lipid)
Age group		
All ages	0.092 (0.088–0.097)	15.2 (14.5–15.9)
12–19 years of age	0.065 (0.062–0.069)	13.3 (12.5–14.1)
≥20 years	0.097 (0.092–0.102)	15.5 (14.7–16.2)
Gender		
Males	0.090 (0.085–0.095)	14.5 (13.8–15.3)
Females	0.095 (0.089–0.100)	15.8 (15.0–16.6)
Race/ethnicity		
Non-Hispanic whites	0.094 (0.088–0.099)	15.1 (14.4–16.0)
Non-Hispanic blacks	0.081 (0.077–0.085)	14.5 (13.9–15.0)
Mexican Americans	0.098 (0.089–0.109)	16.2 (14.9–17.7)

Source: CDC 2009, 2015

Table 6-6 lists mean serum hexachlorobenzene concentrations (by whole weight and lipid-weight bases) in the U.S. population from NHANES IV (updated in 2015) using serum samples collected during the years 2007–2008 (CDC 2015). Grouped by race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American), gender, and age, mean serum hexachlorobenzene levels were highest in the oldest age group of females (≥60 years of age) for each race/ethnicity, and higher among Mexican-American females than non-Hispanic white or black females.

Hexachlorobenzene levels in normal human blood serum samples were reported to be 2.2 ppb and were higher (4.6 ppb) in uremic serum samples (Rutten et al. 1988). In a study conducted in British Columbia, Canada, Mes (1992), reported median and maximum whole blood levels of 0.11 and 0.34 ng/g (ppb) in individuals from the general population. Hexachlorobenzene was detected in 100% of blood samples taken from young adults between the ages of 17 and 21 years of age from the Akwesasne Mohawk Nation on the St. Lawrence River at mean and maximum concentrations of 0.036 and 0.112 ppb, respectively (Gallo et al. 2011). Data indicated that Akwesasne young adults have sustained a lower overall exposure to hexachlorobenzene than similarly aged people from the general U.S. population, probably due to relatively lower legacy contamination and lower contributions to background body burden.

Butler et al. (2003) reported hexachlorobenzene concentrations in maternal and umbilical cord blood plasma for Inuit, Dene, Metis, Caucasian, and other non-Aboriginal participants living in the Northwest Territories and Nunavut areas of Arctic Canada. Overall, 523 women participated resulting in the collection of 386 maternal blood samples and 407 cord blood samples taken between May 1994 and June 1999. Hexachlorobenzene was detected in 100% of 385 maternal blood plasma samples with concentrations ranging from 0.02 to 4.51 μg/L, and a mean value of 0.35 μg/L. Hexachlorobenzene was detected in 97.5% of 400 cord blood plasma samples with concentrations ranging from not detected (detection limit was 0.02 μg/L) to 1.01 μg/L and a mean value of 0.10 μg/L.

Although all uses of hexachlorobenzene as a pesticide in the United States were voluntarily canceled in 1984, occupational exposures may still occur among workers in the chlorinated solvent manufacturing industry, and workers currently involved in the manufacture and application of pesticides contaminated with hexachlorobenzene. Military or firefighting personnel who use pyrotechnic mixtures that release hexachlorobenzene and workers involved in the disposal of hexachlorobenzene contaminated materials, via combustion processes associated with municipal incinerators or those involved in the handling and treatment of wastes at hazardous waste sites, may be exposed to higher than background concentrations.

Table 6-6. Geometric Mean Serum Hexachlorobenzene Concentrations (Whole Weight and Lipid Adjusted) for the Years 2007–2008 in the U.S. Population from the National Health and Nutrition Examination Survey

Group	Age (years)	Geometric mean serum hexachlorobenzene (± standard error)	
		Whole weight (ng/g serum or ppb)	Lipid adjusted (ng/g lipid)
Non-Hispanic white male	12–19	0.062±0.003	12.4±0.8
	20–39	0.062±0.003	9.45±0.46
	40–59	0.074±0.003	10.1±0.3
	≥60	0.075±0.003	11.8±0.5
Non-Hispanic white female	12–19	0.033±0.001	7.06±0.29
	20–39	0.047±0.002	7.94±0.23
	40–59	0.071±0.005	10.8±0.7
	≥60	0.102±0.004	15.1±0.7
Non-Hispanic black male	12–19	0.046±0.004	9.67±0.79
	20–39	0.051±0.003	9.11±0.58
	40–59	0.061±0.003	9.58±0.43
	≥60	0.062±0.003	10.3±0.6
Non-Hispanic black female	12–19	0.034±0.003	7.20±0.57
	20–39	0.035±0.001	7.04±0.3
	40–59	0.059±0.004	9.90±0.58
	≥60	0.090±0.006	15.1±1.2
Mexican-American male	12–19	0.050±0.003	9.49±0.7
	20–39	0.065±0.006	9.47±0.57
	40–59	Not reported	20.6±8.3
	≥60	0.089±0.011	12.1±0.8
Mexican-American female	12–19	0.033±0.002	6.81±0.44
	20–39	0.060±0.004	10.4±0.7
	40–59	0.138±0.016	20.6±2.6
	≥60	0.141±0.013	20.7±1.9

Source: CDC 2015

NIOSH conducted a study in May, August, and November 2004 to investigate the extent of exposure to 30 U.S. magnesium workers during the processing of magnesium (NIOSH 2005). Hand wipes contained hexachlorobenzene concentrations of 0.14–3.5 μg. The means of lipid-adjusted and whole-blood hexachlorobenzene from the 30 workers were 891 (range 253.0–6790.0) and 0.7 (range 0.2–3.4) ng/g (ppb) lipid, respectively. Full-shift air sampling results for hexachlorobenzene ranged from 0.096 $\mu g/m^3$ for a sample collected on a foundry operator to 5.3 $\mu g/m^3$ for a maintenance helper working on a reactor. Bulk sample results indicated the presence of 250 μg/g (ppb) of hexachlorobenzene in collected dust (NIOSH 2005). In a 10-year study (1976–1985), human adipose tissue samples and human milk from patients exposed to PCBs or pesticides in Ontario, Canada were analyzed for a variety of pesticides and industrial chemicals (Frank et al. 1988). Residues of hexachlorobenzene in adipose tissues averaged at or below 0.2 ppb in the extractable fat. Concentrations of hexachlorobenzene in milk ranged from a mean of 0.52 ppb (in whole milk) in 1983–1984 to a mean of 0.26 ppb (in whole milk) in 1985. The highest mean concentration (1.33 ppb) was observed in central Ontario residents. Urban residents had a higher mean concentration (0.57 ppb) as compared to rural residents with a mean of 0.27 ppb.

Plasma hexachlorobenzene concentrations in a Louisiana population living in a hexachlorobenzene-contaminated area averaged 3.6±4.3 ppb. The highest plasma level (345 ppb) was detected in a waste disposal facility worker, while the highest plasma level in the general population was 23 ppb (Burns and Miller 1975). Workers at a carbon tetrachloride and perchloroethylene production facility had plasma hexachlorobenzene levels of up to 223 ppb. Hexachlorobenzene blood levels were determined over a 4-year period in men employed in the manufacture of chlorinated solvents (Currier et al. 1980). Blood levels ranged from 5 to 1,121 ppb (310.7 ppb mean) in 1974, 30–990 ppb (311.5 mean) in 1975, 3–600 ppb (159.9 mean) in 1976, and 22–467 ppb (170.3 mean) in 1977. The hexachlorobenzene blood levels were strongly associated with the number of years worked in the chlorinated solvents plant, but they were poorly correlated with airborne hexachlorobenzene concentrations ranging from <1 to 13 ppb or wipe samples from work areas ranging from 0.03 to 124 $\mu g/100\ m^2$.

Vegetable sprayers applying hexachlorobenzene-contaminated dimethyltetrachloroterephthalate (DCPA) had plasma levels of hexachlorobenzene ranging from 0 to 310 ppb (mean 40±63 ppb), accompanied by elevated levels of delta-aminolevulinic acid, but no health related adverse effects (Burns et al. 1974). Elevated urinary uroporphyrin and coproporphyrin were found in 1 of 54 men occupationally exposed to hexachlorobenzene (Morley et al. 1973).

Workers at a new hazardous waste incinerator in Constanti, Spain had mean plasma levels of hexachlorobenzene at 152 μg/kg lipid (range, 19.4–854.0 μg/kg lipid) (Domingo et al. 2001). Employees at a hazardous waste incinerator in Constanti, Spain working in the plant, laboratory, and administration had mean plasma levels of hexachlorobenzene of 134 and 84, 182 and 179, and 223 and 179 μg/kg lipid in 1999 and 2000, respectively (Schuhmacher et al. 2002). Residents (n=608) living near an electrochemical factory in Catalonia, Spain had mean serum concentrations of hexachlorobenzene as follows (units ng/mL): general population, male (50.2), female (48.0), all (48.9); nonfactory workers, male (39.8), female (47.9), all (46.3); and living with a worker of the factory, yes (46.8), no (46.8) (Ballester et al. 2000).

Individuals employed in industries that manufacture or process hexachlorobenzene or products containing hexachlorobenzene may be exposed to the highest concentrations. The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, estimated that 1,038 workers employed at 10 facilities were potentially exposed to hexachlorobenzene in the United States (NOES 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.

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 are particularly susceptible to hexachlorobenzene by ingestion of breast milk. Table 6-7 summarizes the concentrations of hexachlorobenzene in human breast milk taken from women living in different regions of the world. Hexachlorobenzene was detected in human milk samples collected from

Table 6-7. Mean Levels of Hexachlorobenzene in Breast Milk

Location of study	Concentration[a]	Reference
Australia (Victoria, 1985/1986)	0.005[b]	Monheit and Luke 1990
Australia (Victoria)	0.41	Quinsey et al. 1995
Bavaria	0.019	Raab et al. 2013
Brazil (Porto Alegre)	0.02	Beretta and Dick 1994
Canada (1967–1992)	0.002–0.00044[b]	Craan and Haines 1998
Quebec	0.002–0.00040[b]	
Ontario	0.002–0.00048[b]	
Canada (Arctic Quebec)		Dewailly et al. 1993
Inuit women	0.136	
Caucasian women	0.028	
Canada (multiple locations, 1992)	0.0044[b]	Newsome et al. 1995
	0.015[c]	
Canada	0.026	Mes et al. 1993
Czech Republic		Schoula et al. 1996
Prague	0.639	
Kladno	0.570	
Uherske Hradiste	0.482	
France (multiple locations)	0.147	Bordet et al. 1993
Ghana (Akomadan)	0.04	Ntow 2001
Italy		Larsen et al. 1994
Rome	0.25	
Pavia	0.12	
Milan	0.20	
Mexico (Veracruz)	0.047	Waliszewski et al. 1996
Mexico (Guerrero)	0.013±0.014	Chavez-Almazan et al. 2014
New Zealand		Bates et al. 1994
Auckland, urban	0.020	
Northland, rural	0.021	
Christchurch, urban	0.030	
Canterbury, rural	0.063	
New Zealand	0.006763	Mannetje et al. 2014
Slovak Republic (Bratislava)	0.339	Prachar et al. 1993
Spain (Madrid)	1.0	Conde et al. 1993
Industrialized area	1.74	
Sweden (1972–1980)	0.110–220	Norén and Meironyté 2000

Table 6-7. Mean Levels of Hexachlorobenzene in Breast Milk

Location of study	Concentration[a]	Reference
Thailand (Northern Thailand)	0.0051[b]	Stuetz et al. 2001
United States (Arkansas)	0.03	Mattison et al. 1992
United States (Hawaii, 1979–1980)	0.046±0.049	Takei et al. 1983

[a]µg/g on lipid basis.
[b]Whole milk sample.
[c]Milk fat sample.

54 residents of Hawaii during 1979–1980 (Takei et al. 1983). The incidence of detection of hexachlorobenzene in the sampled population was 100% and the mean concentration of positive detections was 46±49 ppb (ng/g lipid basis) with residues ranging from 18 to 38 ppb (ng/g lipid basis). The authors state that the levels of hexachlorobenzene in human milk from residents of Hawaii are consistent with levels detected in an earlier human milk study conducted on women on the mainland United States. Schecter et al. (1998) found that hexachlorobenzene residues in the breast milk of a mother with nursing twins decreased from 10.7 ng/g (ppb) lipid to not detectable in 30 months. Thus, nursing infants are particularly susceptible to hexachlorobenzene poisoning due to the mother's decrease in body burden and the infant's intake (Schecter et al. 1998). In human milk samples collected from 2002 to 2007 from women residing in the agricultural region of Salinas, California and the urban San Francisco Bay Area, California, median concentrations of hexachlorobenzene in breast milk were 191 and 223 pg/g (0.191 and 0.223 ppb), respectively (Weldon et al. 2011). A study of organochlorine pesticide concentrations in human milk sampled throughout Canada during 1992 found that hexachlorobenzene was present in all 497 milk samples at mean concentrations of 0.44 ng/g (ppb) in whole milk and 14.5 ng/g (ppb) in milk fat (Newsome et al. 1995). A comparison by Canada Health of human milk contamination in whole milk from 1967 to 1992 was conducted in Canada, Quebec and Ontario. This study showed a decrease in hexachlorobenzene concentration in Canada (mean concentration; 2–0.44 ng/g), Quebec (median concentration; 2–0.40 ng/g), and Ontario (median concentration; 2–0.48 ng/g) (Craan and Haines 1998). Newsome et al. (1995) reported that concentrations of hexachlorobenzene were higher in women from the Great Lakes Basin area as compared to the rest of Canada. Concentrations of hexachlorobenzene were higher in the breast milk of women who consumed more than 100 g of fish weekly. Hexachlorobenzene has also been detected in the breast milk of women from China. For example, samples from women from Beijing had hexachlorobenzene concentrations ranging from 0.40 to 3.79 μg/kg whole milk (Song et al. 2013).

Children may also be exposed to chemicals via ingestion of contaminated foods. Hexachlorobenzene residues have been detected in 76% of samples analyzed as part of the National Human Adipose Tissue Survey (FY82) (EPA 1986c). These hexachlorobenzene residues are most likely the result of consumption of low levels of hexachlorobenzene in food, with calculated yearly intakes of 68, 22, and 5 μg for adults, toddlers, and infants, respectively (EPA 1986b). Yess et al. (1993) evaluated hexachlorobenzene residues from 1985 to 1991 detected in the Total Diet Studies of infant and adult foods that are consumed by infants and young children. These authors reported maximum hexachlorobenzene residues detected in various food groups as follows: combination meat dinners—pork (0.4 ppb), beef (0.3 ppb), chicken/turkey (0.3 ppb), chicken/turkey/vegetable (0.3 ppb), beef and vegetable (0.1 ppb); vegetables

and fruits—pears (1.0 ppb), apples (0.4 ppb), and carrots (0.2 ppb); milk products—canned evaporated milk (0.5 ppb), whole milk (0.2 ppb), and low-fat (2%) milk (0.1 ppb); and peanut butter (5.0 ppb).

Hexachlorobenzene intakes, in μg/kg body weight/day, estimated for these total diet analyses (1982–1984) were 0.0015 for 6–11-month-old infants. A follow-up study found a decrease in intakes that were estimated in 1982–1984. Hexachlorobenzene intakes (μg/kg body weight/day) were estimated to be 0.0016 in 1988 (FDA 1989); 0.0007 in 1989 (FDA 1990); 0.0004 in 1990 (FDA 1991); and 0.0003 in 1991 (FDA 1992) for 6–11-month-old infants.

Hexachlorobenzene plasma concentrations were studied for a population of 4-year-old children born between 1997 and 1999 in an urban area under the influence of hexachlorobenzene emissions from a chloro-alkali plant and in a rural area where hexachlorobenzene was present at relatively low levels (Carrizo et al. 2008). In the urban area of Ribera d'Ebre, Catalonia, Spain, the measured average hexachlorobenzene plasma concentration was 1.4 ng/mL, with a range of 0.17–5.8 ng/mL. The measured average plasma concentration in the rural area of Menorca Island in the Mediterranean Sea was 0.42 ng/mL, with a range of 0.067–2.1 ng/mL. Higher concentrations of hexachlorobenzene were measured in children who were breastfed as opposed to formula fed, showing that a major portion of the compound was incorporated into the serum during the lactation period. The average concentrations were 1.5 and 0.99 ng/mL for Ribera d'Ebre and 0.47 and 0.23 ng/mL for Menorca for breastfed and formula-fed children, respectively (Carrizo et al. 2008). Dallaire et al. (2002) studied umbilical cord plasma of newborns collected between 1993 and 2000 from the Lower North Shore of the St. Lawrence River in Quebec, Canada, and found a 69% decrease in the mean hexachlorobenzene concentration in that time span, falling from 35.5 μg/kg in 1993 to 11.6 μg/kg in 2000. In a study of umbilical cord blood samples taken from Inuit infants born in Nunavik, Quebec between 1994 and 2001, the mean hexachlorobenzene concentration decreased an average of 6.6% per year during that time span (Dallaire et al. 2003). Hexachlorobenzene was detected in 100% of the 251 cord blood samples collected (Dallaire et al. 2003).

Although inhalation exposures of hexachlorobenzene in children have not been studied, it is anticipated that exposure by this route will not be significant in outdoor environments. The Henry's law constant of hexachlorobenzene is 5.8×10^{-4} atm-m^3/mol (Ten Hulscher et al. 1992), indicating that this compound will volatilize, especially in moist soils with low organic content. Hexachlorobenzene's high log K_{oc} of 6.08 (EPA 1981), however, indicates that volatilization from soil surfaces will be attenuated. Considering that hexachlorobenzene concentrations in the environment are extremely low, exposure of children by inhalation is expected to be insignificant. After a hexachlorobenzene spill, inhalation

exposure may be important before environmental equilibrium is attained. Under these conditions, high concentrations of hexachlorobenzene would be found in the atmosphere, due to hexachlorobenzene's calculated vapor density of 10. This situation, however, is not expected to occur since hexachlorobenzene is no longer produced or used commercially and is only found as an impurity in pesticides and as a byproduct of chlorinated hydrocarbons.

The EPA issued a warning regarding pesticides and advised that potential exposure of pesticides to young children via dermal absorption and ingestion was more important than inhalation routes (Jantunen et al. 1997).

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to those individuals occupationally exposed to hexachlorobenzene identified in Section 6.5, several groups within the general population may receive potentially higher exposures to hexachlorobenzene. These groups within the general population include individuals living near facilities where hexachlorobenzene is produced as a byproduct, individuals living near the current or former NPL hazardous waste sites where this compound is present, recreational and subsistence fishermen who consume higher amounts of fish than the general population, and native populations (including Native American populations such as the Inuit of Alaska) who may be exposed to higher levels of hexachlorobenzene associated with dietary intakes of caribou and other game species.

Recreational and subsistence fishermen who consume appreciably higher amounts of locally caught fish from contaminated water bodies may be exposed to higher levels of hexachlorobenzene associated with dietary intake than members of the general population (EPA 1995a). Hexachlorobenzene contamination in fish and shellfish has triggered the issuance of several human health advisories. As of September 1994, hexachlorobenzene was identified as the causative pollutant in fish consumption advisories in Louisiana and Ohio. This information is summarized in Table 6-8. EPA has identified hexachlorobenzene as a target analyte and recommended that this chemical be monitored in fish and shellfish tissue samples collected as part of state toxics monitoring programs. EPA recommends that residue data obtained from these monitoring programs should then be used by the states to conduct risk assessments to determine the need for issuing fish and shellfish consumption advisories for the protection of the general public as well as recreational and subsistence fishermen (EPA 1997a).

Table 6-8. Fish Consumption Advisories[a]

State	Waterbody	Extent	Species
Louisiana	Devil's Swamp Lake and Bayou Baton Rouge	7 miles	All fish
	Calcasieu and Cameron parishes	6 miles	All fish and shellfish
Ohio	Tuscarawas River	Turkey Foot Road. (SR 619), Barberton, to South Broadway Street (SR 416), New Philadelphia (Tuscarawas County)	Carp-common, bass-smallmouth, bullhead-yellow, bass-rock, catfish-channel, bass-largemouth

[a]From EPA 1997a National Listing of Fish Consumption Advisories

Native American populations such as the Inuit of Alaska or other subsistence hunters living in high latitude areas of the United States and Canada may be exposed to hexachlorobenzene residues in caribou, beluga whales, polar bears, seals, and other game species. Significantly higher concentrations of hexachlorobenzene (mean 136 ng/g [ppb]) were reported in breast milk of Inuit mothers from eastern Canada (Quebec Province) as compared with residues of 28 ng/g (ppb) in Caucasian mothers (Dewailly et al. 1993). By analogy, it is possible that Inuit populations in western North America (Alaska) may receive potentially higher hexachlorobenzene exposures from their dietary habits. In a follow-up study by Dewailly et al. (1999) 26 subcutaneous fat samples, 41 omental fat samples, 17 brain samples, and 26 liver samples were collected in November 1992 to Mid-October 1994 from Inuit Greenlanders. Mean hexachlorobenzene concentrations were 594, 588, 260, and 754 μg/kg lipid basis, respectively. A comparison of these data clearly suggest an increase in Inuit population's hexachlorobenzene levels from dietary habits. Maternal body burden and lactational transfer of hexachlorobenzene can increase tissue levels in the neonate (Ando et al. 1985; Frank et al. 1988).

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 hexachlorobenzene 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 hexachlorobenzene.

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 hexachlorobenzene are sufficiently well documented to permit estimation of the compound's environmental fate (EPA 1981; Hansch et al. 1995; Haynes and Lide 2010; ten Hulscher et al. 1992; O'Neil et al. 2006; Verschueren 2001). No further information is needed.

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 2013, became available in October of 2014. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Hexachlorobenzene is not currently manufactured as a commercial end-product in the United States and has not been commercially produced since the late 1970s (EPA 1986b). However, hexachlorobenzene currently is produced as a by-product or impurity in the manufacture of chlorinated solvents, other chlorinated compounds, and several currently registered pesticides (Bailey 2001; EPA 1986b; IARC 1979).

The total amount of hexachlorobenzene released as a byproduct in the production of all chlorinated solvents was estimated as 0.3 kg/year in the mid-1990s (Bailey 2001), while hexachlorobenzene released through use of eight major pesticides containing hexachlorobenzene accounted for 1,270 kg/year (Bailey 2001). Current production estimates for hexachlorobenzene as a by-product or impurity are not available. Current, quantitative estimates of production of hexachlorobenzene from all sources are needed to evaluate potential exposures and risks to human health.

There are no current commercial uses of hexachlorobenzene in the United States, although the compound was used as a fungicide until 1984 when the last registered use as a pesticide was voluntarily cancelled (Beyer 1996). Prior to the registration cancellations, hexachlorobenzene was registered as a seed protecting for use on several grains (principally wheat) and field crops (EPA 1986b). Hexachlorobenzene was also used in pyrotechnic and ordinance materials and in synthetic rubber production (EPA 1986b). Impurities of hexachlorobenzene in currently registered pesticides (picloram, PNCB, chlorothalonil, Dacthal®, atrazine, simazine, and pentachlorophenol) (Bailey 2001; EPA 1986b, 1993) appear to be a continuing source of hexachlorobenzene exposure for the general population. Currently, data regarding the release of hexachlorobenzene into the environment as an impurity via the use of other pesticides is not available.

No current information was located on import/export volumes for hexachlorobenzene, although import/export volumes for hexachlorobenzene/DDT combined are available (NTDB 1995).

Hexachlorobenzene is listed as a hazardous waste. It is regulated under the Clean Water Effluent Guidelines as stated in Title 40, Section 400–475, of the Code of Federal Regulations and the Resource Conservation and Recovery Act (RCRA) (see Chapter 8). Past disposal methods have included incineration, landfills, discharges to municipal sewage treatment plants, and emissions to the atmosphere. The recommended method of disposal for hexachlorobenzene is incineration (Clayton and Clayton 1981; EPA 1988a, 1989a; HSBC 2012; Lamb et al. 1994). No further information on disposal practices is needed; however, estimates on the volume of hexachlorobenzene disposed of annually and the disposal method used are needed to assess exposure pathways.

Environmental Fate. Hexachlorobenzene released to the environment partitions to several environmental compartments (air, water, soil and sediment, and biological organisms). Hexachlorobenzene partitions to the atmosphere from soil surfaces through volatilization (Nash and Gish 1989). The remainder is adsorbed strongly to soil where it persists for extended periods (half-life of months to years) due to its resistance to biodegradation (Beall 1976; Beck and Hansen 1974; Isensee et al. 1976). Leaching of hexachlorobenzene into groundwater is not expected to occur very rapidly under most circumstances due to the compound's high sorption characteristics (Swann et al. 1983). Yuan et al. (1999) have reported that hexachlorobenzene can be dechlorinated to dichlorobenzenes under anaerobic conditions in the laboratory using sewage sludge as inoculum.

Once in the atmosphere, hexachlorobenzene exists in both the vapor and particulate phase; however, the vapor phase predominates (Ballschmiter and Wittlinger 1991; Bidleman et al. 1989; Lane et al. 1992). Degradation of hexachlorobenzene in the atmosphere is quite slow (1.69 years) (Brubaker and Hites 1998). Since hexachlorobenzene is hydrophobic, wet deposition will not be an important loss process. In cold high latitude zones, dry deposition of hexachlorobenzene aerosols is encouraged (Ballschmiter and Wittlinger 1991; Lane et al. 1992; Wania and Mackay 1993). Atmospheric transport of hexachlorobenzene is a major mechanism for global translocation of this compound (Eisenreich et al. 1981; Kelly et al. 1991). Long-range global transport of hexachlorobenzene released anywhere in the world can occur via atmospheric or oceanic systems (Ballschmiter and Wittlinger 1991; Wania and MacKay 1993).

Hexachlorobenzene released to water will volatilize, adsorb to sediments, or bioaccumulate in fish and other aquatic organisms (Bishop et al. 1995; EPA 1992; Kelly et al. 1991; Langlois and Langis 1995; Oliver and Nichol 1982a; Quemerais et al. 1994; Rostad et al. 1993; Schmitt et al. 1990; Zabik et al. 1995). Hydrolysis and biodegradation are not significant processes in water. Information on biodegradation of hexachlorobenzene under anaerobic conditions in a laboratory study exists (Yuan et al. 1999),

but degradation data under field conditions were not found. Further information on these processes, including degradation products, are needed to determine potential mechanisms and sources of hexachlorobenzene releases from soils and the potential for the compound and its degradation products to contaminate groundwater.

Both bioaccumulation and biomagnification of hexachlorobenzene were reported to occur in an aquatic laboratory microcosm system (Burkhard et al. 1997; Isensee et al. 1976); however, data by Russell et al. (1995) suggests that hexachlorobenzene bioaccumulates, but is not biomagnified in certain fish populations in Lake Erie. In terrestrial ecosystems, hexachlorobenzene can also be accumulated in several agricultural plant species in the roots and parts of the plants closest to the soil (Kraaij and Connell 1997; Scheunert et al. 1983; Schroll et al. 1994; Smelt and Leistra 1974). In lichens, a high latitude forage food for caribou, hexachlorobenzene was found to be bioconcentrated 8,800,000–17,000,000 times the concentration in the atmosphere (Muir et al. 1993). Although the issue of biomagnification in some ecosystems needs to be clarified, there are adequate data on the bioconcentration of hexachlorobenzene in both aquatic and terrestrial ecosystems.

Bioavailability from Environmental Media. Hexachlorobenzene can be absorbed following inhalation of contaminated workplace air (Burns et al. 1974; Currier et al. 1980; Richter et al. 1994). Since hexachlorobenzene is moderately volatile, inhalation may not be a major concern except at hazardous waste sites or in industrial settings. Hexachlorobenzene can be absorbed following ingestion of contaminated food or water. Exposure to hexachlorobenzene through ingestion of food contaminated with low levels of the compound is probably the greatest source of exposure for the general population. Exposure to hexachlorobenzene through ingestion of contaminated drinking water is not expected to be an important source of concern since the compound is not very soluble in water. Although there are no quantitative data on the human absorption of orally administered hexachlorobenzene, gastrointestinal absorption has been demonstrated for rats (Albro and Thomas 1974; Ingebrigtsen and Nafstad 1983; Ingebrigtsen et al. 1981). The lymphatic system has also been shown to play an important part in the absorption of hexachlorobenzene in the intestines. Hexachlorobenzene is absorbed by the lymphatic system in the region of the duodenum and jejuno-ileum and is deposited in the adipose tissue, bypassing the portal circulation (Iatropoulos et al. 1975). Since hexachlorobenzene is tightly bound to soil particles, ingestion of hexachlorobenzene-contaminated soil, particularly by children, may also be an important route of exposure near production and processing facilities or near hazardous waste disposal sites. No information was available regarding absorption of hexachlorobenzene following dermal contact. Information regarding the bioavailability of hexachlorobenzene from both ingestion of soil-bound hexa-

chlorobenzene particularly in children and from dermal contact with contaminated soils are needed, particularly in assessing health risks to populations living near hazardous waste sites.

Food Chain Bioaccumulation. Like many of the other organochlorine pesticides, hexachlorobenzene is lipophilic and has a high bioaccumulation potential. Hexachlorobenzene is bioaccumulated in fish and other aquatic organisms (Bishop et al. 1995; EPA 1992; Langlois and Langis 1995; Murray et al. 1980, 1981; Oliver and Nichol 1982a; Schmitt et al. 1990; Zabik et al. 1995) as well as waterfowl (Cobb et al. 1994; Foley 1992; Gebauer and Weseloh 1993; Somers et al. 1993; Swift et al. 1993; Yamashita et al. 1992). Hexachlorobenzene is bioaccumulated in aquatic food chains with virtually no degradation of the compound by the exposed organisms (Isensee et al. 1976). The results of a laboratory aquatic ecosystem study suggest that bioaccumulation as well as biomagnification of hexachlorobenzene occurs (Isensee et al. 1976). In terrestrial ecosystems, several agricultural crops have been found to accumulate hexachlorobenzene in their roots and in portions growing closest to soil level (Ecker and Horak 1994; Scheunert et al. 1983; Schroll et al. 1994; Smelt and Leistra 1974). The edible root portion of carrots accumulated the highest hexachlorobenzene concentration with a BCF of 19 (Smelt and Leistra 1974). Lichens, a primary forage for caribou, were also shown to bioaccumulate hexachlorobenzene (Muir et al. 1993). A field study on a terrestrial ecosystem suggested that hexachlorobenzene was biomagnified through various trophic levels of the food web (Hebert et al. 1994). Further studies are needed to resolve whether hexachlorobenzene is biomagnified in both aquatic and terrestrial ecosystems.

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

Environmental monitoring data are available for hexachlorobenzene in air (Currier et al. 1980; Davis and Morgan 1986; Eisenreich et al. 1981; EPA 1975b, Hoff et al. 1996; Lee et al. 2000b; Mann et al. 1974; Poissant et al. 1997; Tiernan et al. 1985), water (Chan et al. 1994; Davis and Morgan 1986; EPA 1976a; Hoff et al. 1996; Laska et al. 1976; Oliver and Nichol 1982a; Quemerais et al. 1994), soil (Elder et al. 1981; Laseter et al. 1976; Laska et al. 1976), and sediment (Davis and Morgan 1986; Elder et al. 1981; Murray et al. 1981; Oliver and Nichol 1982a; Ray et al. 1983; Rostad et al. 1999). Current information on hexachlorobenzene concentrations in groundwater is needed. Human intake estimates for exposure from environmental media are available (Whitmore et al. 1994), but are limited. In general, while

monitoring data are available for most environmental media, much of the information on environmental levels was collected during the 1970s through the mid 1980s. More recent monitoring data from all environmental media would provide more accurate information for estimating human and animal intakes.

Exposure Levels in Humans. Hexachlorobenzene has been detected in human adipose tissue (Ansari et al. 1986; EPA 1986c; Frank et al. 1988; Kutz et al. 1991; Mes 1992), blood (Burns and Miller 1975; Burns et al. 1974; CDC 2009, 2015; Currier et al. 1980; Murphy and Harvey 1985; Rutten et al. 1988), and milk (Craan and Haines 1998; Frank et al. 1988; Newsome et al. 1995; Schecter et al. 1998; Takei et al. 1983). Studies exist that relate occupational exposure to blood levels of hexachlorobenzene (Burns et al. 1974; Currier et al. 1980). Studies to compare the steady-state intake of hexachlorobenzene as measured by urinary and fecal excretion as it relates to blood levels in occupationally exposed workers would be particularly useful. Since hexachlorobenzene has been detected in both urine and feces, a study of this nature could be conducted. These studies might also address possible individual differences in the metabolism of this compound.

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

Exposures of Children. Exposure pathways for children have been well documented in breast milk (Newsome et al. 1995; Schecter et al. 1998) and diet (FDA 1992; Yess et al. 1993). Data documenting body burdens for children are needed. Data addressing exposure to children who live, play, or attend school near NPL sites, industrial sites, such as chlorinated hydrocarbon production factories, or on farmlands where hexachlorobenzene is being released as an impurity of another pesticide would allow for a better assessment of hexachlorobenzene exposure. As hexachlorobenzene is released due to the use of other pesticides on foods, an evaluation of possible hexachlorobenzene residues in children's food substances would further enhance the ability to construct a complete picture of exposure. Studies revealing contamination of drinking water or groundwater would also prove essential in this assessment. Attention should also focus on the use of tap water as a contaminant source when used to prepare infant formulas from condensed or powdered forms. As children are often bound to pick up soil off the ground and maybe even put this soil in their mouths, studies regarding exposure to children through soil would be helpful. Information concerning childhood-specific means to decrease exposure would be useful.

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

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

Currently, Inuit communities appear to have potentially higher exposures to hexachlorobenzene and should be further monitored (Dewailly et al. 1993, 1999).

6.8.2 Ongoing Studies

No NIH or EPA ongoing studies regarding the potential human exposures to hexachlorobenzene were identified.

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring hexachlorobenzene, its metabolites, and other biomarkers of exposure and effect to hexachlorobenzene. 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

Methods for the determination of organochlorine compounds such as hexachlorobenzene generally consist of the following steps: extraction of the analyte from the sample matrix; clean-up to remove interfering compounds; and analysis (separation and quantitation). The primary method of analysis is gas chromatography (GC) coupled with electron capture detection (ECD) or mass spectrometry (MS). Analytical methods have been developed for the determination of hexachlorobenzene in blood or serum, urine, feces, adipose tissue, and breast milk. A summary of methods is shown in Table 7-1.

Several cautions should be noted. Interferences may result from organics of biological origin that are extracted from the sample, and from contaminated glassware, solvent, etc. Sample interferences are usually removed using fractionation and clean-up procedures. Rigorous sample collection and preparation methods must be followed to prevent contamination of the sample. Good quality control procedures must be used to identify and remove interferences caused by sample contamination.

Blood (or serum) is a body fluid often utilized to assess human exposure to chlorinated organics, including hexachlorobenzene. Blood is usually extracted with solvent (Bristol et al. 1982; Burse et al. 1990; EPA 1980b; Langhorst and Nestrick 1979; Mes et al. 1982), and the extract is cleaned up (and sometimes fractionated) by column chromatography utilizing silica gel (Langhorst and Nestrick 1979), Florisil (Mes et al. 1982), a combination of columns (Burse et al. 1990), or by solid-phase extraction (Dmitrovic et al. 2002). Hexachlorobenzene may also be extracted by automated solid-phase extraction

Table 7-1. Analytical Methods for Determining Hexachlorobenzene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Extraction, GPC cleanup, Florisil fractionation, optional additional cleanup	Capillary GC/MS	12 ng/g	No data	EPA 1986c
Adipose tissue	Maceration with sodium sulfate, extraction and back extraction, Florisil fractionation	GC/ECD	No data	No data	EPA 1980b
Adipose tissue	Soxhlet extraction, cleanup on Florisil	Capillary GC/ECD; confirmation on second column	0.001 µg/g	82	Alawi et al. 1992
Adipose tissue	Solvent extraction, filtration, Florisil fractionation	Capillary GC/ECD; confirmation by GC/MS	0.12 ng/g	86	Mes et al. 1982
Adipose tissue	SFE with alumina (to remove lipids, purification by column chromatography	Capillary GC/ECD	10 µg/kg (fatty tissue)	115	Djordjevic et al. 1994
Breast milk	Separation of fat; column cleanup	cap GC/ECD	0.4 ng/g fat	No data	Abraham et al. 1994
Breast milk	Acid treatment, elute from silica gel, concentrate	GC/ECD	0.009 mg/kg	91	Stachel et al. 1989
Breast milk	Solvent extraction, concentration, SPE	GC/MS/MS EI	0.068 ng/mL	63.23–83.07	Chen et al. 2014
Blood	Solvent (hexane) extraction, concentration	GC/ECD	No data	No data	EPA 1980b
Blood	Solvent extraction, cleanup on silica gel, concentration	GC/PID	16 ng/g	79	Langhorst and Nestrick 1979
Blood	Homogenization with benzene, filtration, Florisil fractionation	Capillary GC/ECD; confirmation by GC/MS	0.2 ng/g	80	Mes et al. 1982
Blood	Hexane extraction, concentration	GC/ECD; confirmation by GC/MS	0.16 ng/g	72	Bristol et al. 1982
Serum	Solvent extraction of denatured serum, fractionation on micro-Florisil column, acid treatment/silica gel cleanup	GC/ECD	1 ppb	58–76	Burse et al. 1990
Serum	Solvent extraction, cleanup with solid-phase solvent extraction cartridges, concentration	GC/NICI MS	0.05 ng/mL	~100	Dmitrovic et al. 2002

Table 7-1. Analytical Methods for Determining Hexachlorobenzene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Serum	Solid-phase extraction, cleanup on acid/silica gel column	GC/MS	0.1–56 pg/g	93	Thomsen et al. 2007
Serum	Rapid headspace solid-phase micro-extraction	GC/MS EI	0.79	103–104	R. Flores-Ramírez et al. 2014
Plasma	SPE, cleanup on small multilayer silica gel columns	HRGC/HRMS	89.1 pg/mL	98.6	Salihovic et al. 2012
Urine	Solvent extraction, cleanup on silica gel, concentration	GC/PID	4.1 ng/g	84	Langhorst and Nestrick 1979
Semen	Solvent extraction, cleanup on Florisil, concentration	Capillary GC/ECD; confirmation by NICI	~0.3 ng/mL	80	Stachel et al. 1989
Feces	Boiling with solvent, cleanup on alumina	Capillary GC/ECD	No data	No data	Abraham et al. 1994

ECD = electron capture detector; EI = electron ionization; GC = gas chromatography; GPC = gel permeation chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; MS = mass spectrometry; NICI = negative ionization chemical ionization; PID = photoionization detector; SFE = supercritical fluid extraction; SPE = solid-phase extraction

using a polystyrene-divinylbenzene sorbent, with additional cleanup by column chromatography utilizing a sulfuric acid-silica column (Thomsen et al. 2007). Analysis is usually by GC/ECD (Bristol et al. 1982; Burse et al. 1990; EPA 1980b; Mes et al. 1982), although GC/MS (Thomsen et al. 2007), and GC coupled with photoionization detection (PID) (Langhorst and Nestrick 1979), high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) (Salihovic et al. 2012) or MS with negative chemical ionization (NICI) (Dmitrovic et al. 2002) may be used as well. Confirmation by GC/MS is recommended (Bristol et al. 1982; Mes et al. 1982). Recovery for all methods is acceptable (≈70–90%) (Bristol et al. 1982; Burse et al. 1990; Dmitrovic et al. 2002, Langhorst and Nestrick 1979; Mes et al. 1982; Thomsen et al. 2007); precision is also acceptable (≤20% relative standard deviation [RSD]) (Bristol et al. 1982; Burse et al. 1990; Langhorst and Nestrick 1979; Mes et al. 1982; Thomsen et al. 2007). Detection limits are in the low-ppb (ng/g) range (Bristol et al. 1982; Burse et al. 1990; Dmitrovic et al. 2002; Langhorst and Nestrick 1979; Mes et al. 1982; Thomsen et al. 2007) and the ppt (pg/g) range (Thomsen et al. 2007).

Adipose tissue is usually solvent extracted (EPA 1980b; Mes et al. 1982), and the hexachlorobenzene is separated from the extracted fat by Florisil column fractionation (Mes et al. 1982). Analysis is by GC/ECD (EPA 1980b; Mes et al. 1982). Confirmation by GC/MS (Mes et al. 1982) or a second GC column is recommended. Recovery is good (82–86%) (Mes et al. 1982); precision is very good (<10% RSD) (Mes et al. 1982). Solvent extraction followed by gel permeation chromatography (GPC) clean-up and Florisil column fractionation was utilized for a large adipose tissue monitoring study (EPA 1986c). Additional clean-up measures may be required if fractions are not clean enough for capillary GC/MS analysis (EPA 1986c). Supercritical fluid extraction (SFE) and treatment with alumina for lipid removal have been combined; additional purification was carried out by column chromatography (Djordjevic et al. 1994). Recovery was 115%, precision 10.5% RSD. Detection limits for all methods are in the low-ppb (ng/g) range (Alawi and Ababneh 1991; Djordjevic et al. 1994; EPA 1986c; Mes et al. 1982).

Few methods are available for monitoring other tissues and fluids. Breast milk has been analyzed with a combination of fat separation, column clean-up, and capillary GC/ECD (Abraham et al. 1994). Detection limits were 0.4 ng/g fat; other performance data were not reported. Methods for urine (Langhorst and Nestrick 1979) and semen (Stachel et al. 1989) have been reported. Both provide good recovery (80–84%). A method for feces has been reported, and involves boiling with solvent and clean-up on alumina followed by capillary GC/ECD analysis (Abraham et al. 1994). Performance data were not reported.

It is well known that ingestion of hexachlorobenzene can produce porphyria (see Section 3.2.2.2). Urinary porphyrins from humans with porphyria cuntanea tarda (PCT) can be analyzed using thin layer chromatography (TLC). Separation and estimation of porhyrins are carried out on a TLC plate by extraction and esterification of porphyrins, 2-dimensional development, and fluorescent scanning (Miura and Torinuki 1977). Other analysis methods for porphyrins include spectrophotometry. Analysis by this method is carried out by extraction of porphyrins using an anion exchange column, esterification of porphyrins, separation by chromatography, and quantification spectrophotometrically (Grinstein 1977).

7.2 ENVIRONMENTAL SAMPLES

Most environmental analyses have been performed using multiresidue methods involving solvent extract of the analytes from the sample matrix, clean-up to remove interfering compounds, determination by GC with ECD, and confirmation using an ancillary method such as MS. New methods and technologies are evolving, and this has resulted in lower detection limits. For example, detection limits are in the low ppb to ppt range for water matrices and the low ppm to ppb range for food. Analytical methods for the determination of hexachlorobenzene in environmental samples are given in Table 7-2.

Atmospheric hexachlorobenzene is usually sampled by pulling a volume of air through an adsorbent trap (EPA 1988b, 1990b; Hippelein et al. 1993; Langhorst and Nestrick 1979). A filter may be included in the sampling system in order to determine the amount of hexachlorobenzene in particulate (Atlas and Giam 1981; Brorström-Lundén et al. 1994; Farrar et al. 2006; Hippelein et al. 1993). Filters and polyurethane foam (PUF) adsorbent are Soxhlet extracted (EPA 1990b, 1991; Hippelein et al. 1993); filters and sorbent-impregnated polyurethane foam (SIP) adsorbent are Soxhlet extracted (Koblizkova et al. 2012); XAD-2 adsorbent is extracted in a Soxhlet apparatus (Hippelein et al. 1993) or by solvent desorption (Langhorst and Nestrick 1979); polymer-coated glass (POG) adsorbent is extracted by solvent extraction (Farrar et al. 2006). Clean-up on adsorbent columns may be utilized (EPA 1988b; Farrar et al. 2006; Hippelein et al. 1993). A variety of analytical methods are used: GC/ECD (Atlas and Giam 1981; EPA 1991), capillary GC/ECD (Brorström-Lundén et al. 1994; EPA 1990b), GC/PID (Langhorst and Nestrick 1979), and capillary GC/MS (Hippelein et al. 1993; Farrar et al. 2006; Koblizkova et al. 2012). Confirmation on a second GC column or by GC/MS is recommended (Atlas and Giam 1981; EPA 1990b). Reported recovery is good (82–103%) (EPA 1990b; Farrar et al. 2006; Langhorst and Nestrick 1979); precision is also good (<10–20% RSD) (Farrar et al. 2006; Hippelein et al. 1993). Detection limits depend upon the amount of air sampled, but may be in the ppb to sub-ppt range (EPA 1990b; Farrar et al. 2006; Hippelein et al. 1993; Langhorst and Nestrick 1979).

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on PUF; Soxhlet extraction; cleanup on alumina	(EPA Method TO-10) GC/ECD	No data	No data	EPA 1988b
Ambient air	≈2,200 m^3 collected on GFF and XAD-2; Soxhlet extraction; cleanup on layered silica gel; alumina partition	Capillary GC/MS	0.18 pg/m^3 (calculated)	No data	Hippelein et al. 1993
Ambient air	Collection on XAD-2; solvent desorption	GC/PID	70 ppb	95	Langhorst and Nestrick 1979
Ambient air	Collection on PUF; Soxhlet extraction; concentration	dual column megabore GC/ECD or GC/ECD and GC/MS	5 ng/m^3	82–103	EPA 1990b
Ambient air	Collection on SIP; Soxhlet extraction; concentration	GC/MS	No data	No data	Koblizkova et al. 2012
Ambient air	Collection on POG; solvent extraction; clean up on silica gel column	Capillary GC/MS	17 pg/m^3	94	Farrar et al. 2006
Rain, snow	Modified collector; solvent extraction; solvent exchange; cleanup on silica gel	Capillary GC/ECD	0.4 ng/L	No data	Chan et al. 1994
Drinking water	Solid-phase extraction (disk or cartridge)	(EPA Method 525.1) capillary GC/MS	0.111 µg/L	80	EPA 1991
Drinking water	Solvent extraction; solvent exchange	(EPA Method 508) capillary GC/ECD; confirmation using second column	0.077 µg/L (estimated)	68–82	EPA 1988c
Drinking water	Solvent extraction	(EPA Method 505) GC/ECD, confirmation using second column	0.003 µg/L	91–100	EPA 1989b
Drinking water	pH adjustment; concentration on XAD-4; cleanup on silica gel	(Master Scheme) capillary GC/MS	0.1 µg/L (target)	73	Garrison and Pellizzari 1987

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	Functionalized polysulfone membrane extraction (membrane); solvent desorption	GC/MS	1.3 ng/L	72.9	Nuhu et al. 2011
Groundwater	Solvent extraction; solvent exchange	(National Pesticide Survey Method 2) capillary GC/ECD, confirmation using second column	0.12 µg/L	96	Munch et al. 1990
River water	Centrifugation; chromic acid digestion; extraction	Capillary GC/ECD	No data	97.5	Driscoll et al. 1991
Drinking water and river water	DLLME; phase separation	Capillary GC/ECD	0.0005–0.05 µg/L	71.1–81.3	Kozani et al. 2007
Tap water	Add CH_3OH 5% (v/v) and NaCl 5% (w/v) to sample; sorptive extraction by rotation of C18 extraction disk and Teflon disk	GC/ECD	0.08 µg/L	84±2% (C18 phase); 85±3% (PDMS phase)	Canas and Richter 2012
Municipal and industrial waste	Solvent extraction; solvent exchange; optional cleanup on Florisil	(EPA Method 612) GC/ECD	0.05 µg/L	95	EPA 2012a
Municipal and industrial waste	pH adjustment; solvent extraction; concentration	(EPA Method 625) GC/MS	1.9 µg/L	79	EPA 1984
Waste water, soil, sediments, solid wastes	Solvent extraction	(EPA Method 8410) capillary GC/FTIR	20 µg/L	Not applicable	EPA 1986a
Groundwater, soils, solid wastes	Various extraction; cleanup methods	(EPA Method 8270B) capillary GC/MS	660 µg/kg (soil, sediment); 10 µg/L (groundwater)	72.6 (automated Soxhlet extraction)	EPA 1994
Soil, sediment, solid waste	Liquid-liquid extraction	HPLC	0.3 µg/L	97	Khan et al. 2011
Soil	Solvent extraction; liquid-liquid partition; cleanup by sulfuric acid treatment	GC/ECD	No data	98	Waliszewski and Szymczyn-ski 1985

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Soxhlet and sonication extraction; acetylation; solvent extraction; fractionation on silica gel	dual column capillary GC/ECD	No data	83–106	Ojala 1993
Soil	Homogenization with solvent; microwave-assisted solvent extraction	GC	No data	27–115	de Andrea et al. 2001
Sediments	Microwave extraction centrifugation; filtration	Capillary GC/ECD	No data	91.7	Onuska and Terry 1993
Fish tissue	Grind with sodium sulfate; extract with hexane/acetone	GC/ECD	No data	No data	Oliver and Nicol 1982b
Fish	Homogenization; Soxhlet extraction; GPC fractionation; silica gel fractionation	Capillary GC/MS	12.5 ng/g	96	Tiernan et al. 1990
Fish	Maceration; Soxhlet extraction; cleanup with sulfuric acid/silica gel	Dual capillary GC/ECD	5 ng/g (lipid basis)	95	Rahman et al. 1993
Fish	Solvent extraction; column clean-up	GC/IDMS	3.7 ng/g	90–110	Majoros et al. 2013
Fish, aquatic biota	Homogenization with solvent; solvent exchange; cleanup on Florisil	Capillary GC/ECD, confirmation on second column	0.01 mg/kg	~94	Miskiewicz and Gibbs 1994
Aquatic organisms	Homogenization; Soxhlet extraction; GPC fractionation; SPE fractionation; solvent exchange	(USGS method) capillary GC/ECD	No data	50–75	Shan et al. 1994
Butterfat, fish	Isolation on Florisil column; solvent partition; partition on Florisil	GC/ECD	No data	95–98 (fish), 99–104 (butterfat)	Bong 1975
Fatty foods	SFE/SFC (on-line cleanup)	Capillary GC/ECD	4 ppb	85	Nam and King 1994
Fatty foods	Extraction and pretreatment; Florisil cleanup	(DFG Method S9) GC/ECD; confirmation by TLC	0.01 mg/kg	90	Thier and Zeumer 1987b
Milk	Extraction; silica, alumina, and carbon column cleanup	GC/HRMS	1.86 pg/g fat	No data	Kim et al. 2013
Milk	Solid-phase extraction	GC/ECD	No data	88–94	Manes et al. 1993

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Solvent extraction; solvent partition; solvent exchange; GPC cleanup; optional alumina cleanup	GC/ECD, confirmation on second column	<0.5 ppb	88–91	Trotter and Dickerson 1993
Vegetable oils, oil seeds	Sandwich-type extraction fractionation	GC/ECD	1–2 ppb	80–100	Seidel and Linder 1993
Fruits, vegetables	Chop and blend; blend with solvent; partition with water; dry	GC/ECD, confirmation by GC/MS	0.002 ppm	93	Pylypiw 1993
Crops and foods	Solvent extraction; GPC cleanup; optional silica gel cleanup	(DFG Method S19) dual GC/ECD	No data	>70	Thier and Zeumer 1987a
Tobacco	Solvent extraction; SPE	GC/MS/MS	5 µg/kg	72–95	Chen et al. 2014
Pine needles	Dry and mince; homogenization; Soxhlet extraction; sulfuric acid cleanup; fractionation on Florisil	Capillary GC/ECD	0.1 ng/g (dry weight)	80–100	Calamari et al. 1994

DLLME = dispersive liquid-liquid microextraction; ECD = electron capture detector; EPA = Environmental Protection Agency; FTIR = Fourier transform infrared spectrometry; GC = gas chromatography; GFF = glass fiber filter; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; HRMS = high resolution mass spectrometry; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; PDMS = polydimethylsiloxane; PID = photoionization detector; POG = polymer-coated glass; PUF = polyurethane foam; SFC = supercritical fluid chromatography; SFE = supercritical fluid extraction; SIP = sorbent-impregnated polyurethane foam; SPE = solid phase extraction; TLC = thin-layer chromatography; USGS = U.S. Geological Survey

Hexachlorobenzene is usually extracted from water with organic solvents for analysis (EPA 1988c, 1989b; Kozani et al. 2007; Munch et al. 1990). Hexachlorobenzene may also be extracted and concentrated by adsorption on adsorbent cartridges, disks, or membranes, with subsequent solvent desorption (EPA 1988a; Nuhu et al. 2011). Clean-up of the extracts is usually not necessary; however, methods are available for samples that contain interfering compounds (Chan et al. 1994; Driscoll et al. 1991; Garrison and Pellizzari 1987). Analysis is usually by capillary GC/ECD (Chan et al. 1994; Driscoll et al. 1991; EPA 1988c; Kozani et al. 2007; Munch et al. 1990). Confirmation using a second method is recommended (EPA 1988c, 1989b; Munch et al. 1990). Capillary GC/MS and GC/MS are also utilized for analysis (EPA 1991; Garrison and Pellizzari 1987; Nuhu et al. 2011). Accuracy ranges from acceptable (≈60–80%) (EPA 1988a; Garrison and Pellizzari 1987; Kozani et al. 2007; Nuhu et al. 2011) to excellent (>90%) (Driscoll et al. 1991; EPA 1989b, 1991; Munch et al. 1990). Precision is rarely reported; 16% RSD was reported for the Master Scheme (Garrison and Pellizzari 1987), 0.52–2.8% RSD was reported for dispersive liquid-liquid microextraction (DLLME) (Kozani et al. 2007), and 9.2% RSD was reported for functionalized polysulfone membrane extraction (Nuhu et al. 2011). Detection limits are in the low- to sub-ppb range (EPA 1991; Garrison and Pellizzari 1987; Kozani et al. 2007; Munch et al. 1990). Detection limits in the ppt range have been achieved by methods utilizing solvent extraction and functionalized polysulfone membrane extraction with capillary GC/ECD and GC/MS analysis, respectively (Chan et al. 1994; EPA 1989b; Nuhu et al. 2011). Waste water is solvent extracted with analysis by GC/ECD (EPA 2012a) or GC/MS (EPA 1989b). Reported recovery is good (79–95%) (EPA 1984, 2012a). Detection limits are in the low-ppb range, with lower detection limits reported for the GC/ECD analysis (EPA 2012a).

Soxhlet or sonication extraction is most commonly used to extract hexachlorobenzene from solid matrices such as soils and sediments, and wastes (EPA 2012a; Ojala 1993). Solvent extraction (Waliszewski and Szymczynski 1985) and microwave extraction techniques (de Andrea et al. 2001; Onuska and Terry 1993) may be used as well. Clean-up is usually required for the extracts (EPA 1994; Ojala 1993; Waliszewski and Szymczynski 1985), with subsequent analysis by GC (de Andrea et al. 2001), GC/ECD (Waliszewski and Szymczynski 1985), capillary GC/ECD (Ojala 1993; Onuska and Terry 1993), or capillary GC/MS (EPA 1994). Reported recovery is good (73–106%) (de Andrea et al. 2001; EPA 1994; Ojala 1993; Onuska and Terry 1993; Waliszewski and Szymczynski 1985). Precision, where reported, is acceptable (≤20% RSD) (de Andrea et al. 2001; EPA 1984, 2012a; Ojala 1993). Little information is available on detection limits. Detection limits of 660 µg/kg (ppb) have been reported for automated Soxhlet extraction with capillary GC/MS analysis (EPA 1994). A high performance liquid chromatography (HPLC) based method for the determination of hexachlorobenzene and its possible

metabolites, including chlorophenolic and chloroquinolic intermediates, by liquid-liquid extraction has been developed (Khan et al. 2011). Sample cleanup or derivatization is not needed. Analysis is done by HPLC with a reported recovery of 97%. Precision is acceptable (2.1% RSD). A detection limit of 0.3 µg/L is reported (Khan et al. 2011).

Fish and aquatic organisms are homogenized, then extracted with solvent (Miskiewicz and Gibbs 1994; Oliver and Nichol 1982b), isolated on Florisil columns (Bong 1975), or Soxhlet extracted (Rahman et al. 1993; Shan et al. 1994; Tiernan et al. 1990). Clean-up is usually necessary to remove lipids and interfering substances (Bong 1975; Miskiewicz and Gibbs 1994; Rahman et al. 1993; Shan et al. 1994; Tiernan et al. 1990). Capillary GC/ECD analysis is used most often (Miskiewicz and Gibbs 1994; Rahman et al. 1993; Shan et al. 1994). Capillary GC/MS (Tiernan et al. 1990) and GC/ECD (Bong 1975; Oliver and Nichol 1982b) are also utilized. Reported recovery ranges from moderate (50–75%) (Shan et al. 1994) to excellent (>90%) (Bong 1975; Miskiewicz and Gibbs 1994; Rahman et al. 1993; Tiernan et al. 1990). Precision is usually not reported; however, 4–6% RSD has been achieved (Shan et al. 1994). Detection limits, where reported, are in the low-ppb range (ng/g) (Miskiewicz and Gibbs 1994; Rahman et al. 1993; Tiernan et al. 1990).

Fatty foods, including milk, have been extracted with solvent to remove the fat, and then cleaned up to separate the hexachlorobenzene from the fat (Bong 1975; Thier and Zeumer 1987b; Trotter and Dickerson 1993). Newer methods for combined separation and clean-up are supercritical fluid techniques (Nam and King 1994), solid-phase extraction (SPE) (Manes et al. 1993), and a sandwich system (Seidel and Linder 1994). Analysis is by GC/ECD (Bong 1975; Manes et al. 1993; Seidel and Linder 1993; Thier and Zeumer 1987b; Trotter and Dickerson 1993). Confirmation on a second GC column (Trotter and Dickerson 1993) or by TLC (Thier and Zeumer 1987b) is recommended. Capillary GC/ECD has also been utilized (Nam and King 1994). Reported recoveries are good (>80%) (Bong 1975; Manes et al. 1993; Nam and King 1994; Seidel and Linder 1993; Trotter and Dickerson 1993). Precision, where reported, is very good (<15% RSD) (Bong 1975; Nam and King 1994; Thier and Zeumer 1987b; Trotter and Dickerson 1993). Limit of detection, where reported, is in the low-ppb (ng/g) range (Nam and King 1994; Seidel and Linder 1993; Thier and Zeumer 1987b; Trotter and Dickerson 1993).

Fruits, vegetables, and crops are blended, solvent extracted, and then cleaned up and fractionated (Pylypiw 1993; Thier and Zeumer 1987a). Capillary GC/ECD is the analytical method. Recovery is acceptable (>70%) (Pylypiw 1993; Thier and Zeumer 1987a). Precision was not reported. The reported detection limit is 2 ppb (Pylypiw 1993).

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 hexachlorobenzene 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 hexachlorobenzene.

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. Methods exist for measuring hexachlorobenzene in blood (Bristol et al. 1982; Burse et al. 1990; Langhorst and Nestrick 1979; Mes et al. 1982) and adipose tissue (Alawi and Ababneh 1991; Djordjevic et al. 1994; EPA 1980b, 1986c; Mes et al. 1982). The methods for blood and adipose are sensitive (low-ppb range), but improved accuracy is needed for blood analysis. The data on determination of hexachlorobenzene in urine, breast milk, and tissues are limited, and the methods may not be sufficiently sensitive. 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. Improved methods to detect phenolic metabolites are not needed since these metabolites are not unique to hexachlorobenzene. Representative methods for determining pentachlorophenol and other phenolic metabolites using GC/ECD and GC/MS are shown in Table 7-3.

Biomarkers for effects of hexachlorobenzene are porphyric symptoms and increased gamma-glutamic transferase activity. Since these effects are also indicative of exposure to other toxicants, additional studies are needed for more specific biomarkers for effects of hexachlorobenzene exposure.

Table 7-3. Analytical Methods for Determining Biomarkers of Hexachlorobenzene

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (pentachloro-phenol)	pH adjustment, solvent extraction, derivatization	GC/ECD	10 ppb	92	EPA 1980b
Urine (chlorinated phenol metabolites)	Hydrolysis; solvent extraction, derivatization	GC/ECD, confirmation by GC/MS	No data	>90 (PCP); most other metabolites >80	EPA 1980b

ECD = electron capture detector; GC = gas chromatography; MS = mass spectrometry; PCP = pentachlorophenol

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining hexachlorobenzene in air (EPA 1988b, 1990b; Hippelein et al. 1993; Langhorst and Nestrick 1979) and water (Chan et al. 1994; EPA 1988c, 1989b, 1991; Garrison and Pellizzari 1987), the media of most concern for human exposure, are reliable, but may not be sensitive enough to measure background levels in the environment. Limited performance data are available for methods for soil and other solid media. In addition, there is insufficient performance information for methods for determining hexachlorobenzene in media such as shellfish, fish, and plants. Some exposure to hexachlorobenzene may occur via ingestion of food and standardized methods for foods are needed. Methods with sufficient sensitivity for measuring background levels in foods would be helpful as well.

7.3.2 Ongoing Studies

No ongoing studies regarding analytical methods sponsored by NIH or EPA were identified for hexachlorobenzene.

8. REGULATIONS, ADVISORIES, AND GUIDELINES

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

ATSDR has derived an acute-duration oral MRL of $8x10^{-3}$ mg/kg/day based on a LOAEL of 2.5 mg/kg/day for hyperactivity in rat offspring whose mothers were exposed to hexachlorobenzene for 4 days prior to mating (Goldey and Taylor 1992).

ATSDR has derived an intermediate-duration oral MRL of $1x10^{-4}$ mg/kg/day based on degenerative changes in the ovaries of female monkeys exposed to hexachlorobenzene doses ≥0.01 mg/kg/day for 90 days (Babineau et al. 1991; Bourque et al. 1995; Jarrell et al. 1993).

ATSDR has derived a chronic-duration oral MRL of $7x10^{-5}$ mg/kg/day based on a LOAEL of 0.022 mg/kg/day for peribiliary lymphocytosis and fibrosis of the liver in adult F_1 generation male rats fed hexachlorobenzene for 130 weeks in a 2-generation study (Arnold et al. 1985).

The EPA (IRIS 2003) has derived an oral reference dose (RfD) of $8x10^{-4}$ mg/kg/day for hexachlorobenzene based on a NOAEL of 0.08 mg/kg/day for liver effects in the Arnold et al. (1985) rat chronic feeding study. An uncertainty factor of 100 was used (10 for intraspecies variability and 10 for interspecies extrapolation). No reference concentration (RfC) for chronic inhalation exposures to hexachlorobenzene was reported.

The International Agency for Research on Cancer (IARC) classifies hexachlorobenzene as Group 2B carcinogen (*possibly carcinogenic to humans*) (IARC 2015). The National Toxicology Program (NTP) concluded that hexachlorobenzene is *reasonably anticipated to be a human carcinogen* (NTP 2014). EPA has classified hexachlorobenzene in weight-of-evidence Group B2 (*probable human carcinogen*) (IRIS 2003). EPA derived an oral slope factor of 1.6 per (mg/kg)/day and an inhalation unit risk of $4.6x10^{-4}$ per ($\mu g/m^3$) based on hepatocellular carcinoma in female Sprague-Dawley rats exposed orally. The American Conference of Governmental Industrial Hygienists (ACGIH) has classified hexachlorobenzene as an A3 carcinogen (*confirmed animal carcinogen with unknown relevance to humans*) (ACGIH 2014).

Although no Occupational Safety and Health Administration (OSHA) standards exist for hexachlorobenzene, the ACGIH (2014) has set a threshold limit value (8-hour time-weighted average) of 0.002 mg/m^3, based on a route-to-route extrapolation from an oral study in Rhesus monkeys (Rozman et al. 1978).

Hexachlorobenzene is on the list of chemicals designated Hazardous Air Pollutants (HAPs) under Section 112 of the Clean Air Act (EPA 2013a). Hexachlorobenzene also appears on the list of chemicals in "The Emergency Planning and Community Right-to-Know Act of 1986" (EPCRA) and has been assigned a reportable quantity (RQ) limit of 10 pounds (EPA 2014d). Section 313 of Title III of EPCRA requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually their release of those chemicals to any environmental media.

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

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

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	2B[a]	IARC 2015
WHO	Air quality guidelines	No data	WHO 2010
	Drinking water quality guidelines	No guideline value[b]	WHO 2011
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)	0.002 mg/m^3 [c]	ACGIH 2014
AIHA	ERPGs	No data	AIHA 2014
DOE	PAC-1[d]	0.006 mg/m^3	DOE 2012a
	PAC-2[d]	8.9 mg/m^3	
	PAC-3[d]	160 mg/m^3	
EPA	AEGLs	No data	EPA 2014a
	Hazardous air pollutant	Yes	EPA 2013a 42 USC 7412
	NAAQS	No data	EPA 2012c
NIOSH	REL (10-hour TWA)	No data	NIOSH 2015
	IDLH	No data	
OSHA	PEL (8-hour TWA) for general industry	No data	OSHA 2013 29 CFR 1910.1000, Table Z-1
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	No data	EPA 2013b 40 CFR 116.4
	Drinking water standards and health advisories		EPA 2012b
	1-day health advisory for a 10-kg child	0.05 mg/L	
	10-day health advisory for a 10-kg child	0.05 mg/L	
	DWEL	0.03 mg/L	
	10^{-4} Cancer risk	0.002 mg/L	

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

Agency	Description	Information	Reference
NATIONAL (*cont.)*			
EPA	National primary drinking water standards	No data	EPA 2009b
	MCL	0.001 mg/L	
	Potential health effects from long-term exposure above the MCL	Liver or kidney problems; reproductive difficulties; increased risk of cancer	
	Common sources of contaminant in drinking water	Discharge from metal refineries and agricultural chemical factories	
	Public Health Goal	Zero	
	National recommended water quality criteria: human health for the consumption of		EPA 2013c
	Water plus organism	0.28 mg/L[e,f]	
	Organism only	0.29 mg/L[e,f]	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act	No data	EPA 2013d 40 CFR 117.3
c. Food			
FDA	EAFUS	No data[g]	FDA 2013
d. Other			
ACGIH	Carcinogenicity classification	A3[h]	ACGIH 2014
EPA	Carcinogenicity classification	B2[i]	IRIS 2003
	RfC	No data	
	RfD	$8x10^{-4}$ mg/kg/day	
	Inhalation unit risk	$4.6x10^{-4}$ per µg/m^3	
	Oral slope factor	1.6 per mg/kg/day	
	Identification and listing of hazardous waste	U127	EPA 2013e 40 CFR 261, Appendix VIII
	Inert pesticide ingredients in pesticide products	No data	EPA 2014c
	Superfund, emergency planning, and community right-to-know		EPA 2014d 40 CFR 302.4
	Designated CERCLA hazardous substance and reportable quantity	10 pounds[j]	
	Effective date of toxic chemical release reporting	01/01/1987	EPA 2014e 40 CFR 372.65
EPA	Extremely hazardous substances and its threshold planning quantity	No data	EPA 2013f 40 CFR 355, Appendix A
	TSCA chemical lists and reporting periods	No data	EPA 2014f 40 CFR 712.30

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

Agency	Description	Information	Reference
NATIONAL (*cont.)*			
EPA	TSCA health and safety data reporting	No data	EPA 2014g 40 CFR 716.120
NTP	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen	NTP 2014

[a]Group 2B: possibly carcinogenic to humans.
[b]The WHO noted that when hexachlorobenzene is detected in drinking water, it occurs at concentrations well below those at which toxic effects might be expected, and considered it unnecessary to establish a guideline value.
PAC-1: mild, transient health effects. PAC-2: irreversible or other serious health effects that could impair the ability to take protective action. PAC-3: life-threatening health effects.
[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]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2012b).
[e]This criterion based on carcinogenicity of 10^{-6} risk and has been revised to reflect the EPA's q1* or RfD, as contained in IRIS as of May 17, 2002. The fish tissue bioconcentration factor (BCF) from the 1980 Ambient Water Quality Criteria document was retained in each case.
[f]EPA has updated its national recommended water quality criteria for human health. The comment period for the draft water quality criteria ended August 13, 2014. A final update has not been issued yet, but the proposed criteria for hexachlorobenzene is 0.0064 mg/L for water + organism and 0.0064 mg/L for organism only (EPA 2014b).
[g]The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.
[h]A3: confirmed animal carcinogen with unknown relevance to humans
[i]B2: probable human carcinogen
[j]Designated CERCLA hazardous substance pursuant to Section 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 exposure guideline levels; AIHA = American Industrial Hygiene Association; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; NAAQS = National Ambient Air Quality Standards; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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10. GLOSSARY

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

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

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

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

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

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

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

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

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

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

Carcinogen—A chemical capable of inducing cancer.

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

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

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

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

Chlorinated hydrocarbon—An organic compound containing chlorine.

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.

Combustion—The process of burning. A chemical change, especially oxidation, accompanied by the production of heat and light.

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.

Metabolite—Any substance produced by metabolism or by a metabolic process.

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.

10. GLOSSARY

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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 Human Health Sciences, 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 Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Hexachlorobenzene
CAS Numbers: 118-74-1
Date: June 2015
Profile Status: Final, Post-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 31
Species: Rat

Minimal Risk Level: 0.008 [X] mg/kg/day [ ] ppm
(LOAEL = 2.5 mg/kg/day; total uncertainty factors = 300)

Reference: Goldey ES, Taylor DH. 1992. Developmental neurotoxicity following premating maternal exposure to hexachlorobenzene in rats. Neurotoxicol Teratol 14:15-21.

Experimental design: (human study details or strain, number of animals per exposure/control group, sex, dose administration details): Groups of 30 virgin female Sprague-Dawley rats were dosed by gastric intubation for 4 days with 0, 2.5, or 25 mg/kg/day hexachlorobenzene to achieve a total dose of 0, 10, or 100 mg/kg for the 4-day period. Dosing was completed 2 weeks before breeding. The developmental neurotoxicity of hexachlorobenzene was assessed using a battery of behavioral tests. Negative geotaxic response was assessed in two male and two female pups from each litter on postnatal day (PND) 6, 8, and 10. Olfactory discrimination/homing was assessed in two male and two female pups from each litter on PND 9, 10, and 11. This test simultaneously measures sensory discrimination, motivation, and locomotor ability. The development of exploration and locomotion was assessed in whole litters between PND 15 and 20. Acoustic startle response (ASR) was assessed on PND 23 and 90. Visual discrimination learning, as measured in the water-filled T-maze, was assessed in offspring on PND 40. Motor activity in mature offspring (PND 60) was measured in an open area. These adult animals were again tested for exploratory activity on PND 100.

Effect noted in study and corresponding doses: Hexachlorobenzene affected multiple pathways throughout the developing nervous system, manifested as slight hyperactivity, at a LOAEL of 2.5 mg/kg/day. The offspring rats showed faster response times in negative geotaxis and olfactory discrimination/homing tests at the 2.5 or 25 mg/kg/day maternal dose level. Offspring exposed to maternal doses of 2.5 or 25 mg/kg/day showed either significantly increased exploratory behavior, slight hyperactivity, or both during the early life (19–20 days of age). Hexachlorobenzene-exposed offspring at the 25 mg/kg/day dose level exhibited significantly decreased ASR (23-day-old pups). When rats were tested later as adults (90 days old), response amplitude was significantly elevated in males in both groups exposed *in utero* to 2.5 and 25 mg/kg/day, compared to controls. Maternal exposure of rats to hexachlorobenzene did not result in any significant changes in learning ability, locomotor activity (60-day-old offspring), or exploratory activity (100-day-old offspring).

Dose and end point used for MRL derivation:

[ ] NOAEL [X] LOAEL

2.5 mg/kg/day; hyperactivity in offspring.

The data for mean exploratory activity (on a per litter basis) on postnatal days 19 and 20 were fit to available continuous models in the EPA Benchmark Dose Software (Version 2.1.2); however, the models

did not provide adequate fit to the data. Therefore, a NOAEL/LOAEL approach was employed to identify a point of departure (POD) for deriving an acute-duration oral MRL for hexachlorobenzene.

Uncertainty Factors used in MRL derivation:

[ ] 1 [X] 3 [ ] 10 for use of a LOAEL
[ ] 1 [ ] 3 [X] 10 for extrapolation from animals to humans
[ ] 1 [ ] 3 [X] 10 for human variability
Total uncertainty factors: 3 x 10 x 10 = 300

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

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? No.

Other additional studies or pertinent information that lend support to this MRL: Review of the located human and animal oral acute toxicity data for hexachlorobenzene indicate that the 4-day developmental study by Goldey and Taylor (1992) provides the most appropriate data for use deriving oral acute MRL for hexachlorobenzene. The LOAEL of 2.5 mg/kg/day is the most refined LOAEL for the acute toxicity of hexachlorobenzene.

Adverse neurological signs and symptoms have also been observed in human offspring of maternally exposed to hexachlorobenzene. Children of mothers who had eaten hexachlorobenzene-contaminated bread (estimated hexachlorobenzene intake of 0.05–0.2 g/day) in Turkey exhibited muscle weakness, pinched facies, cogwheel rigidity, and sensory shading. Hexachlorobenzene was detected in the breast milk of the mothers, indicating lactational transfer (Cam and Nigogosyan 1963; Peters et al. 1982, 1987). Since hexachlorobenzene crosses the placenta and accumulates in fetal tissues in several animal species including the rat (Cripps 1990; Villeneuve and Hierlihy 1975), rabbit (Villeneuve et al. 1974a), and mouse (Courtney and Andrews 1985; Courtney et al. 1979), it is likely that the human offspring were also exposed to hexachlorobenzene during gestation. Development of neurotoxic signs has also been reported in other neonatal animals. These signs included convulsions, tremors, and progressive weakness in rats (Cripps 1990). Oral hexachlorobenzene has also been shown to interfere with the function of the nervous system in adult animals, inducing tremors, ataxia, and paralysis in unspecified strain of rats (Ockner and Schmid 1961); clonic convulsions, tremors, hyper-excitability, reversible muscle fasciculations, and lethargy in adult Wistar rats (Kennedy and Wigfield 1990; Koss et al. 1978; Nikolaev et al. 1986); mild reduction in conduction velocity of sciatic nerve, denervation, fibrillations, and chronic repetitive discharges in adult Sprague-Dawley rats (Sufit et al. 1986); tremor and hyperexcitability in adult Sherman rats (Kimbrough and Linder 1974); dysrhythmic electroencephalogram in adult Beagle dogs (Sundlof et al. 1981); tremor in adult C57B1/6J mice (Hahn et al. 1988); severe tremors and muscular weakness in adult Rhesus monkeys (Knauf and Hobson 1979); and tremors, panting, and unsteady gait in adult SPF pigs (Den Tonkelaar et al. 1978).

Agency Contact (Chemical Manager): Robert Williams

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Hexachlorobenzene
CAS Numbers: 118-74-1
Date: June 2015
Profile Status: Final, Post-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 165
Species: Monkey

Minimal Risk Level: 0.0001 [X] mg/kg/day [ ] ppm
(LOAEL = 0.01 mg/kg/day; total uncertainty factors = 90)

References: Bourque AC, Singh A, Lakhanpal N, et al. 1995. Ultrastructural changes in ovarian follicles of monkeys administered hexachlorobenzene. Am J Vet Res 56: 1673–1677.

Jarrell JF, MacMahon A, Villeneuve D, et al. 1993. Hexachlorobenzene toxicity in the monkey primordial germ cell without induced porphyria. Reproductive Toxicology 7:41–47.

Experimental design: (human study details or strain, number of animals per exposure/control group, sex, dose administration details): In the Jarrell et al. (1993) study, groups of four female Cynomolgus monkeys were administered 0, 0.1, 1, or 10 mg/kg/day hexachlorobenzene in gelatin capsules for 90 days. No systemic toxicity was noted in any of the animals. After treatment, the animals were sacrificed and one ovary was removed from each animal from each dose group (including controls) and examined by transmission electron microscopy for alterations to surface epithelium. A cycle of *in vitro* fertilization with oocytes removed from exposed females during the menstrual cycle was performed to evaluate fertility. Induction of ovarian hyperstimulation (performed with human menopausal gonadotropin) was conducted to evaluate oocyte function. In the follow-up Bourque et al. (1995) study, groups of four female Cynomolgus monkeys were administered 0, 0.01, 0.1, 1, or 10 mg/kg/day hexachlorobenzene in gelatin capsules for 90 days. Monkeys were then given a preparation containing follicle-stimulating and luteinizing hormones on days 2–7 of the next menstrual cycle to stimulate follicle development, and human chorionic gonadotropin was administered on day 8 of the cycle. Oophorectomy was performed on day 10 of the cycle via laparotomy. The ovary was examined by transmission electron microscopy.

Effect noted in study and corresponding doses: In the Jarrell et al. (1993) study, *in vitro* fertilization and ovarian hyperstimulation in terms of percent fertilization, estradiol response to gonadotropin, follicular development, oocyte recovery rates and maturation, and early embryo development were not significantly different from control animals. Hexachlorobenzene treatment caused a decrease in the total number of oocytes and primordial follicles. Ultrastructural changes in ovarian epithelium included a decrease in nuclear membrane distinction, an increase in density and granularity of oocyte nuclei, an increase in vacuoles, aggregated lysosomes in ooplasm of follicular cells, and pyknotic granulosa cells. The ooplasm of some follicles was necrotic, and some follicles had mild to moderate degenerative changes. These changes were observed in all exposed animals; the severity of symptoms increased in a dose-dependent manner. The follow-up study by Bourque et al. (1995) extended the observation of ultrastructural effects in the ovary to 0.01 mg/kg/day. At this dose, mitochondria in developing follicles were condensed and deformed. At higher doses, the mitochondria were progressively more damaged and additional changes, such as indentation of nuclear membranes and abnormal accumulation of lipid in the cytoplasm of follicular cells, were noted.

Dose and end point used for MRL derivation:

[ ] NOAEL [X] LOAEL

0.01 mg/kg/day; degenerative lesions in ovarian follicles.

Benchmark dose analysis was not attempted because the principal study (Bourque et al. 1995) did not include quantitative data (incidence, severity) for the critical effect of hexachlorobenzene-induced ultrastructural changes in developing follicles. The intermediate-duration oral MRL for hexachlorobenzene is based on the identified LOAEL of 0.01 mg/kg/day for mitochondrial changes in developing follicles.

Uncertainty Factors used in MRL derivation:

[ ] 1 [X] 3 [ ] 10 for use of a LOAEL
[ ] 1 [X] 3 [ ] 10 for extrapolation from animals to humans
[ ] 1 [ ] 3 [X] 10 for human variability
Total uncertainty factors: 3 x 3 x 10 = 90

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

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? No.

Other additional studies or pertinent information that lend support to this MRL: Review of the located human and animal oral intermediate toxicity data for hexachlorobenzene indicate that the 90-day studies by Bourque et al. (1995) and Jarrell et al. (1993) provide the most appropriate data for use in deriving an oral intermediate MRL for hexachlorobenzene.

In humans, 15 fetal deaths (comprising 13 miscarriages and 2 stillbirths) and 173 live births occurred in 42 females in a 4-year period (1977–1981) several years after the widespread accidental ingestion of hexachlorobenzene-treated seed grain in Turkey (Peters et al. 1982, 1987). These mothers also had 0.51 ppm hexachlorobenzene in their breast milk as compared to 0.07 ppm in unexposed controls (Gocmen et al. 1989). It has also been demonstrated that hexachlorobenzene in human milk can readily cross to the child during lactation and accumulate in the offspring (Ando et al. 1985; Weisenberg 1986; Weisenberg et al. 1985).

Animal studies provide additional evidence that hexachlorobenzene is toxic to the mammalian ovary and may interfere with mechanisms regulating ovarian steroidogenesis. Female Cynomolgus monkeys exhibited a dose-dependent decrease in serum progesterone levels during the luteal phase of the menstrual cycle when administered hexachlorobenzene doses of ≥0.1 (0.1, 1, 10) mg/kg/day as capsules for 90 days; the decrease in levels of progesterone was not observed during the follicular and periovulatory phases. Lengthening of the menstrual cycle was also observed, as well as dose-dependent ultrastructural changes in surface epithelium of the ovary (indicative of cellular degeneration) and changes in ovary surface epithelial cell shape (length to width ratio), in all treatment groups, the severity of which was increased in a dose-dependent manner (Foster et al. 1992a; Sims et al. 1991). Increased serum progesterone levels and elevated ovarian weights were observed in superovulated female Sprague-Dawley rats orally administered ≥1 mg/kg/day hexachlorobenzene by gavage (in corn oil) for 21 days (Foster et al. 1992b). Serum levels of estradiol and progesterone of female Sprague-Dawley rats receiving daily doses of 50 mg/kg/day

hexachlorobenzene by gavage in corn oil for 5 days were not significantly affected, although super-ovulated rats (dosed with pregnant mare serum gonadotropin and human chorionic gonadotropin) exposed to hexachlorobenzene in this study exhibited significant elevation of serum levels of progesterone (Foster et al. 1993). In a subsequent study with ovariectomized female Sprague-Dawley rats administered daily oral doses of 1, 10, or 100 mg/kg/day hexachlorobenzene in corn oil by gavage for 30 days, circulating levels of corticosterone levels were reduced by 25 and 51% at the 1 and 10 mg/kg/day hexachlorobenzene dose levels, respectively. Circulating cortisol levels were also significantly reduced ($p<0.05$). Hexachlorobenzene treatment had no effect on the levels of circulating aldosterone and progesterone levels, or on absolute and relative weights of the adrenal glands. The investigators concluded that hexachlorobenzene exposure induces alterations in steroidogenesis of cells of the adrenal cortex inner zone (Foster et al. 1995b).

A study conducted with female Rhesus monkeys given gavage doses of 8, 32, 64, or 128 mg/kg/day hexachlorobenzene in methylcellulose for 60 days found degenerative changes of the ovarian follicle, stroma, and germinal epithelium at dose levels of 64 mg/kg/day (Iatropoulos et al. 1976). In another monkey study, adult female Rhesus monkeys given oral doses of 8, 16, 32, 64, or 128 mg/kg/day hexachlorobenzene for 60 days showed significantly depressed (29%, $p<0.01$) whole serum cholesterol levels in weeks 3, 5, and 8. On day 60, depressed serum potassium and elevated SGOT were seen at the 128 mg/kg/day dose level. The authors suggested that the changes in potassium and cholesterol levels may be due to liver histopathology. The authors suggested that the changes in potassium levels may be due to unusual steroidogenic activity associated with changes in ovarian morphology (Knauf and Hobson 1979).

Agency Contact (Chemical Manager): Robert Williams

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Hexachlorobenzene
CAS Numbers: 118-74-1
Date: June 2015
Profile Status: Final, Post-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 196
Species: Rat

Minimal Risk Level: 0.00007 [X] mg/kg/day [ ] ppm
(LOAEL = 0.022 mg/kg/day; total uncertainty factors = 300)

Reference: Arnold DL, Moodie CA, Charbonneau SM, Grice HC, et al. 1985. Long-term toxicity of hexachlorobenzene in the rat and the effect of dietary vitamin A. Food Chem Toxicol 23(9): 779–793.

Experimental design: (human study details or strain, number of animals per exposure/control group, sex, dose administration details): Groups of Sprague-Dawley rats (50 per sex) of the F_1 generation were exposed to dietary hexachlorobenzene at 0, 0.32, 1.6, 8, or 40 ppm (approximate doses of 0, 0.022, 0.11, 0.55, and 2.8 mg/kg/day, respectively, for the F_1 males and 0, 0.026, 0.13, 0.64, and 3.2 mg/kg/day, respectively, for the F_1 females) from weaning for life (130 weeks). The groups of F_1 rats had also been exposed via their mothers during gestation and lactation. The F_1 rats in this study were sacrificed after the animals had been on test for 130 weeks. A total of 35 tissues and organs (including brain, heart, liver, extrahepatic bile duct, lungs, spleen, pancreas, small intestine, adrenals, kidneys, bladder, ovaries, uterus, skin, pituitary, thyroid, parathyroid, thymus, prostate, testes, and bone) were histopathologically examined.

Effect noted in study and corresponding doses: Significant dose-response trends were observed in both sexes for hepatic basophilic chromogenesis at >0.55 mg/kg/day, and in males for peribiliary lymphocytosis and fibrosis at or greater than the lowest dose tested (0.022 mg/kg/day). Chronic nephrosis, severe in males, and reduced pup viability were observed at the high dose (2.8 mg/kg/day for males and 3.2 mg/kg/day for females). Tumors were also increased at the high dose, including neoplastic liver nodules in females, parathyroid adenoma in males, and adrenal pheochromocytoma in both males and females. No treatment related effects in the rat offspring were observed with respect to feed consumption or body weight.

For derivation of the MRL, the increased incidences of peribiliary lymphocytosis and fibrosis in treated males were considered to represent a minimal effect. These are common spontaneous lesions in aging rats and occurred in approximately 30% of controls in this study. For peribiliary fibrosis, incidence was increased in all treated groups (statistically significant in the 0.022 and 2.8 mg/kg/day groups), but there was no clear evidence of a dose-response (13/48, 23/48, 21/48, 21/49, and 23/49 in the control, 0.022, 0.11, 0.55, and 2.8 mg/kg/day groups, respectively). For peribiliary lymphocytosis, the incidence was increased in all treated groups (statistically significant in the 0.022, 0.11, and 2.8 mg/kg/day groups), and while the trend with dose was not very impressive, it was statistically significant (16/48, 27/48, 26/48, 21/49, and 32/49, respectively). Incidences of these lesions in the control and treated females were similar to the control males (ranging from 6/49 to 14/49), suggesting that the incidence levels in control males were not unusually low. Overall, these findings suggest that hexachlorobenzene produced a minimal hepatic effect in male rats at the lowest doses administered by increasing the incidence of age-related hepatic lesions.

Dose and end point used for MRL derivation:

[ ] NOAEL [X] LOAEL

0.022 mg/kg/day; peribiliary lymphocytosis and fibrosis of the liver.

The incidence data for peribiliary lymphocytosis and peribiliary fibrosis in the F_1 male rats were fit to available dichotomous models in the EPA Benchmark Dose Software (Version 2.1.2); however, the models did not provide adequate fit to the data. Therefore, a NOAEL/LOAEL approach was employed to identify a POD (LOAEL of 0.022 mg/kg/day) for deriving a chronic-duration oral MRL for hexachlorobenzene.

Uncertainty Factors used in MRL derivation:

[ ] 1 [X] 3 [ ] 10 for use of a LOAEL
[ ] 1 [ ] 3 [X] 10 for extrapolation from animals to humans
[ ] 1 [ ] 3 [X] 10 for human variability
Total uncertainty factors: 3 x 10 x 10 = 300

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Yes. U.S. EPA (1988) chronic reference body weight (male: 0.523 kg; female: 0.338 kg) and food consumption (male: 0.036 kg/day; female 0.027 kg/day) for Sprague-Dawley rats were used to calculate hexachlorobenzene dose from concentration in food. Sample calculation for males: (0.32 mg hexachlorobenzene/kg food [0.32 ppm] x 0.036 kg food consumed/day) / 0.523 kg body weight = 0.022 mg hexachlorobenzene/kg/day.

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? No.

Other additional studies or pertinent information that lend support to this MRL: The liver appears to be a target organ of hexachlorobenzene; therefore, using hepatic effects (peribiliary lymphocytosis and fibrosis) to calculate the MRL is appropriate. Also, review of the located human and animal oral chronic toxicity data for hexachlorobenzene indicate that the 130-week rat study by Arnold et al. (1985) provide the most appropriate data to derive a chronic MRL because the study provides the most refined LOAEL for the characteristic chronic toxicity (hepatic effects) of hexachlorobenzene.

Other studies in several animal species have demonstrated that the liver is the major target organ of hexachlorobenzene exposure. Typical signs included microscopic lesions, increased porphyrin levels, and interference with hepatic enzymes involved in the heme biosynthesis pathway. Hexachlorobenzene doses as low as 5–51 mg/kg/day have been reported to produce porphyrinogenic effects, such as increased liver weight, inhibition of hepatic uroporphyrinogen decarboxylase, accumulation of porphyrins in liver, excretion of porphyrins in urine, and increased hepatic δ-aminolevulinic acid synthestase activity, in female rats exposed for intermediate durations (Alvarez et al. 2000; Den Besten et al. 1993; Goldstein et al. 1978; Kennedy and Wigfield 1990; Kuiper-Goodman et al. 1977; Michielsen et al. 2001, 2002; Smith et al. 1979, 1985; Sweeney et al. 1986; Wolfe and Pepperl 2005). In studies of chronic exposure duration, hexachlorobenzene doses of 7–18 mg/kg/day from the feed produced complete inhibition of uroporphyrinogen decarboxylase and high levels of porphyrins in the liver and urine in both male and female rats (Smith and Cabral 1980; Smith et al. 1985, 1993).

Uroporphyria and hepatic porphyrin accumulation were reported in a 13-week study in female Wistar rats receiving 9.5 or 19 mg/kg/day hexachlorobenzene from the feed. Rats in the 9.5 and 19 mg/kg/day dose group exhibited elevated d-ALA synthase levels (94 and 483%, respectively). At the low dose (9.5 mg/kg/day), relative liver weight increased by 31%, and at the highest dose tested (19 mg/kg/day), relative liver weight increased by 103%. Hypertrophic hepatocytes with eosinophilic cytoplasm with thready basophilic structures, as well as inflammatory cell infiltrates, were observed in the livers of animals dosed with 19 mg/kg/day hexachlorobenzene. By the end of the study, uroporphyria in the 19 mg/kg/day dose group was 1,400% that of control animals. Liver accumulations of porphyrins were 1,054 and 15,104%, respectively, for the 9.5 and 19 mg/kg/day dose group animals compared to undosed controls. Liver retinoid and plasma retinol were decreased by 70 and 53%, respectively, at the 19 mg/kg/day dose level (den Besten et al. 1993).

Female Sprague-Dawley rats exhibited elevated urinary and hepatic porphyrins following administration of 50 mg/kg/day hexachlorobenzene for 12 consecutive days (cumulative dose of 600 mg/kg), and 10 days (5 days/week for 2 weeks, cumulative dose of 500 mg/kg), or 100 mg/kg/day for 5 consecutive days (cumulative dose of 500 mg/kg). The porphyria seen was reported to be similar in severity to that observed in female rats receiving a cumulative dose of 1,500 mg/kg over a 6-week period. Porphyria induced by a cumulative dose of 500 mg/kg (either protocol) persisted for >500–600 days after exposure (Krishnan et al. 1991). Similarly, female Wistar rats receiving hexachlorobenzene from the diet at an estimated dose of 308 mg/kg/day for 107 days exhibited a 91% decrease in uroporphyrinogen decarboxylase activity and a 2,888-fold increase in hepatic porphyrin concentration (Elder and Urquhart 1986).

Oral exposure to hexachlorobenzene induced liver histopathology and altered liver histochemistry in rats. The relative liver weights of male rats were increased by 46% while those of females were increased by 23% in a study in which both sexes of Sprague-Dawley rats were given oral hexachlorobenzene doses of 27.5 mg/kg/day for 4 weeks (Richter et al. 1981). Cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes were seen in the liver of female Sprague-Dawley rats administered single gavage doses of 400 or 600 mg/kg hexachlorobenzene in corn oil and observed for 14 days. Relative liver weights were increased by 16–18 and 13–18% in the 400 and 600 mg/kg dose group animals, respectively. Serum cholesterol levels were increased by 13–30 and 7–31% in the 400 and 600 mg/kg dose group animals, respectively. No changes in serum sodium, potassium, inorganic phosphorus, total bilirubin, alkaline phosphatase, aspartate aminotransferase, total protein, calcium, glucose, and lactate dehydrogenase content were found. Liver microsomal aminopyrine demethylase, aniline hydroxylase, and EROD activities were unchanged by hexachlorobenzene exposure (Lecavalier et al. 1994). Liver weight was significantly increased by nearly 45% in animals treated with 1,000 mg/kg/day for 7 days. Liver porphyrin carboxylase activity was significantly decreased in animals receiving 1,000 mg/kg/day (Kleiman de Pisarev et al. 1990). In rats receiving hexachlorobenzene from the diet at an estimated dose level of 172 mg/kg/day for 60 days, treatment-related effects included enlarged, degenerative liver lesions and increased hepatic porphyrins (Ockner and Schmid 1961). Male Wistar (WAG/MBL) rats given oral doses of 1,000 mg/kg hexachlorobenzene, 3 times a week for 4 weeks (van Raaij et al. 1993b) exhibited 67% increased liver weight. Similarly, liver weight was increased by 81% in a group of Fischer 344 rats administered hexachlorobenzene by gavage at 10 mg/kg/day for 15 weeks (Andrews et al. 1989).

Oral exposure to hexachlorobenzene resulted in altered liver function and histology in: adult female Rhesus monkeys given oral doses of 8, 16, 32, 64, or 128 mg/kg/day hexachlorobenzene for 60 days (Knauf and Hobson 1979); female Wistar rats exposed to 50 mg/kg/day hexachlorobenzene by gavage for 15 weeks (Koss et al. 1978, 1983); female Agus Wistar rats receiving hexachlorobenzene at 7 mg/kg/day (with 2% arachis oil) from the diet for 90 weeks (Smith and Cabral 1980); female Wistar rats receiving 12.95 or 129 mg/kg hexachlorobenzene from the diet for 56 days (Kennedy and Wigfield 1990); both sexes of Charles River rats receiving hexachlorobenzene from the diet at doses of 0.5, 2, 8, or

32 mg/kg/day (Kuiper-Goodman et al. 1977); monkeys given doses of 8 mg/kg/day for 60 days (Iatropoulos et al. 1976); and adult female Beagle dogs administered ≥25 mg/kg/day hexachlorobenzene in gelatin capsules for 21 days (Sundlof et al. 1981). Induction of liver microsomal enzymes, increased liver weight, and microscopic lesions were demonstrated at a hexachlorobenzene dose of 5 mg/kg/day, while centrilobular hypertrophy, elevated urinary coproporphyrinogen, and depressed glucose-6-phosphatase activity were observed at a lower dose level (0.5 mg/kg/day) in pigs treated for 90 days (Den Tonkelaar et al. 1978).

Several animal studies also found increased induction of P-450 isozymes and other hepatic enzymes, usually accompanied by hepatic or uroporphyria, as an index of adverse effects in the liver (Adjarov et al. 1982; Hahn et al. 1988, 1989; Kitchin and Brown 1989; Kleiman de Pisarev et al. 1995; Li et al. 1989; Linko et al. 1986; Lissner et al. 1975; Mehendale et al. 1975; Rajamanickam and Padmanaban 1974; Smith et al. 1985; Wada et al. 1968).

Agency Contact (Chemical Manager): Robert Williams

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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

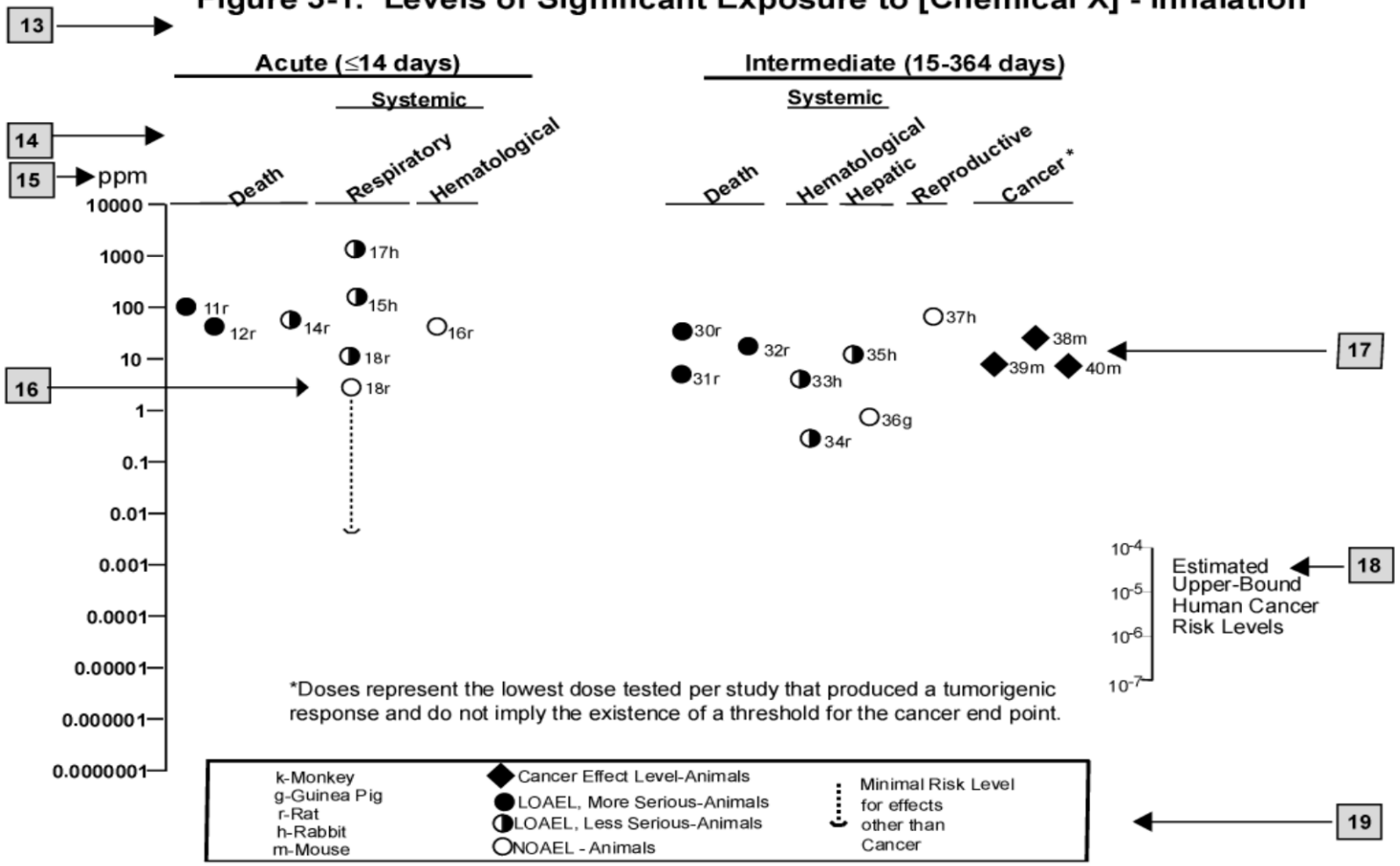
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	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/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
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

APPENDIX C

DOT	Department of Transportation
DOT/UN/ NA/IMDG	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