

**TOXICOLOGICAL PROFILE FOR
METHOXYCHLOR**

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

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UPDATE STATEMENT

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

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FOREWORD

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

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

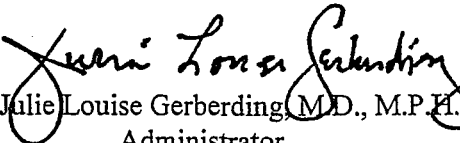
The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels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

Each profile includes the following:

- (A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;
- (B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and
- (C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

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

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


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*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepared toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on November 17, 1997 (62 FR 61332). For prior versions of the list of substances, see *Federal Register* notices dated April 29, 1996 (61 FR 18744); April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); and February 28, 1994 (59 FR 9486). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

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

Primary Chapters/Sections of Interest

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

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

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by *type of health effect* (death, systemic, immunologic, reproductive), by *route of exposure*, and by *length of exposure* (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.

NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

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

- Section 1.6** **How Can Methoxychlor Affect Children?**
- Section 1.7** **How Can Families Reduce the Risk of Exposure to Methoxychlor?**
- 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: 1-888-42-ATSDR or (404) 498-0110 **Fax:** (404) 498-0057
E-mail: atsdric@cdc.gov **Internet:** <http://www.atsdr.cdc.gov>

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

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

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

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

Other Agencies and Organizations

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

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

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

Referrals

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

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. *Contact:* ACOEM, 55 West Seegers Road, Arlington Heights, IL 60005 • 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 Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

PEER REVIEW

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

1. Dr. Martin Alexander, Professor, Department of Crop and Soil Sciences, Cornell University, Ithaca, New York.
2. Dr. Neena Schwartz, Professor, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois.
3. Dr. William Toscano, Professor and Division Head, Environmental and Occupational Health, University of Minnesota, Minneapolis, Minnesota.

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

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

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

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about methoxychlor and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup activities. Methoxychlor has been found in at least 58 of the 1,613 current or former NPL sites. However, the total number of NPL sites evaluated for methoxychlor is not known. As more sites are evaluated, the sites at which methoxychlor is found may increase. This information is important because exposure to methoxychlor may harm you and because these sites may be sources of exposure.

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

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

1.1 WHAT IS METHOXYCHLOR?

Methoxychlor, also known as DMDT, Marlate[®], or Metox[®], is a manufactured chemical now used in the United States for controlling insects. Methoxychlor is effective against flies, mosquitos, cockroaches, and a wide variety of other insects. This insecticide is used on agricultural crops and livestock, and in animal feed, barns, and grain storage bins. Some pesticide products that contain methoxychlor are used for controlling insects in gardens or on pets.

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Pure methoxychlor is a pale-yellow powder that has a slightly fruity or musty odor. It does not readily evaporate into air or dissolve in water. Pesticide workers usually dissolve methoxychlor in a petroleum-based liquid and apply it as a spray, or they mix it with other chemicals and apply it as a dust. Application of methoxychlor as an insect killer accounts for most of the methoxychlor that enters the environment. Since the use of methoxychlor is highly seasonal, the amount that is released to the environment tends to be greater during periods of insect control (spring and summer). Some methoxychlor is released to the environment from chemical manufacturing plants that make methoxychlor or from manufacturing sites that formulate products containing methoxychlor. A small amount may also be released from hazardous waste sites where it has been disposed of.

More complete information on the sources, properties, and uses of methoxychlor can be found in Chapters 4 and 5 of this profile.

1.2 WHAT HAPPENS TO METHOXYCHLOR WHEN IT ENTERS THE ENVIRONMENT?

Methoxychlor does not occur naturally in the environment. Most methoxychlor enters the environment when it is applied to forests, agricultural crops, and farm animals. Methoxychlor can be applied to forests and crops by aerial spraying. This process can contaminate nearby land and water. Methoxychlor that is released into the air will eventually settle to the ground, although some may travel long distances before settling. Rain and snow cause methoxychlor to settle to the ground more quickly.

Once methoxychlor is deposited on the ground, it becomes bound to the soil. Because of this, methoxychlor does not tend to move rapidly from one place to another. However, soil particles that contain methoxychlor can be blown by the wind or be carried by rainwater or melted snow into rivers or lakes. Most methoxychlor stays in the very top layer of soil, but some of the products that it breaks down into may move deeper into the ground. Smaller amounts of methoxychlor in air may settle directly into rivers, lakes, and other surface waters. Once

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methoxychlor is in water, it usually binds to sediments or organic matter and settles to the bottom.

Methoxychlor is broken down in the environment by several processes. However, these processes are slow and may take months. In soil, some methoxychlor is broken down by bacteria and other microorganisms, and some is broken down by a reaction with water or materials in soil. In air and water, some methoxychlor is broken down by sunlight.

Methoxychlor is also broken down by reactive chemicals normally present in the air. Some of the breakdown products are capable of producing harmful effects similar to those caused by exposure to methoxychlor.

Methoxychlor can accumulate in some living organisms, including algae, bacteria, snails, clams, and some fish. However, most fish and animals change methoxychlor into other substances that are rapidly released from their bodies, so methoxychlor does not usually build up in the food chain.

More complete information on the environmental fate of methoxychlor can be found in Chapter 6 of this profile.

1.3 HOW MIGHT I BE EXPOSED TO METHOXYCHLOR?

Most people are not exposed to methoxychlor on a regular basis. Although methoxychlor is not usually detected in air, people can be exposed to low levels of methoxychlor by inhaling dusts and aerosols in air surrounding areas where methoxychlor is used. Since methoxychlor is not usually detected in surface or well water sources, exposure from drinking water is not likely to be significant for the general public. However, surface water that has been treated with methoxychlor for control of insect larvae should be avoided until methoxychlor residue has decreased below the level of concern. Methoxychlor is not usually found in food. However, low levels are sometimes detected in foods obtained from areas where methoxychlor has been used.

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Fish usually do not contain detectable levels of methoxychlor, but people who eat fish caught in water contaminated with methoxychlor may occasionally have above-average intakes of methoxychlor.

People who make or use methoxychlor may be exposed by breathing in the dust or aerosol, or by getting it on their skin. For example:

- C If you work in a factory that makes methoxychlor or products containing methoxychlor, you could be exposed to methoxychlor in air or on your skin during work hours. The government has estimated that approximately 3,400 people may be exposed to methoxychlor in this way.

- C Methoxychlor is present in some pesticides used for home gardening or for spraying pets (such as cats and dogs). If you use these products, you could be exposed to above-average levels of methoxychlor in air and on your skin.

- C If you live or work on or near a farm where methoxychlor is used on crops or livestock, you could be exposed to above-average levels of methoxychlor in air, soil, and possibly in water.

If you live near a hazardous waste site that contains methoxychlor, you could be exposed by breathing in methoxychlor from the air, by swallowing contaminated soil or water, or by getting contaminated soil or water on your skin. The amount of exposure you receive depends on conditions specific to where you live and can only be evaluated on a case-by-case basis.

More information on how you may be exposed to methoxychlor can be found in Chapter 6.

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1.4 HOW CAN METHOXYCHLOR ENTER AND LEAVE MY BODY?

Scientists do not know how much or how quickly methoxychlor is absorbed into your body if you breathe it in or if it contacts your skin. If you get methoxychlor-contaminated soil or water on your skin, some of it may pass through your skin and enter your bloodstream. If you breathe methoxychlor-containing dust into your lungs, some of the dust will deposit in your lungs. Dust that deposits in the upper part of your lungs is likely to be coughed up and swallowed. Dust that deposits deep in your lungs is likely to remain long enough for the methoxychlor to pass through the lining of your lungs and enter your bloodstream. If you swallow food, water, or soil containing methoxychlor, most of it will rapidly pass through the lining of your stomach and intestines and enter your bloodstream.

Once methoxychlor enters your bloodstream, it is distributed to all parts of your body. Animal studies suggest that methoxychlor is changed into other substances called metabolites by your liver. Most of these metabolites leave your body within 24 hours, primarily in your feces, with lesser amounts in your urine. Some methoxychlor can enter the fat in your body, but methoxychlor does not accumulate or build up in fat.

More information on how methoxychlor enters and leaves your body can be found in Chapter 3.

1.5 HOW CAN METHOXYCHLOR AFFECT MY HEALTH?

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

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

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compassion. Laws today protect the welfare of research animals, and scientists must comply with strict animal care guidelines.

Very few reports exist on the health effects of methoxychlor in humans. In animals, exposure to high levels of methoxychlor caused effects on the nervous system. These effects included tremors, convulsions, and seizures. These effects are probably caused by methoxychlor itself and not by its metabolites. Because methoxychlor is quickly transformed into metabolites by the liver, you are not likely to experience nervous system effects unless you are exposed to very high levels.

Some of the breakdown products of methoxychlor cause effects similar to those produced by estrogen. Estrogens are naturally occurring hormones that are important in women for the development and maintenance of their ovaries, uterus, and breasts, and also play a role in the development of the reproductive system in men. Studies in animals show that exposure to methoxychlor adversely affects the ovaries, uterus, and mating cycle in females, and the testes and prostate in males. Fertility is decreased in both female and male animals. These effects can occur both in adult animals and in developing animals exposed prenatally or shortly after birth. Effects of methoxychlor on reproduction have been studied mainly in animals given methoxychlor in food or water, but it is expected that these effects could occur following inhalation and skin exposures as well. Likewise, it is expected that reproductive effects seen in animals could occur in humans exposed to methoxychlor, but this has not been reported.

There is not enough information available to definitely state whether methoxychlor causes cancer. However, most of the information that we have indicates that methoxychlor does not cause cancer. One very small study in humans indicated a possible link with increased incidence of leukemia. However, a definitive connection between a cause of leukemia and exposure to methoxychlor cannot be made with so little information. Most animal studies with methoxychlor have been negative for cancer. Therefore, the International Agency for Research on Cancer (IARC) has determined that methoxychlor is not classifiable as to its carcinogenicity to humans. Similarly, the EPA has determined that methoxychlor is not classifiable as to its human carcinogenicity.

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More information on how methoxychlor can affect your health can be found in Chapters 2 and 3.

1.6 HOW CAN METHOXYCHLOR AFFECT CHILDREN?

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

Children are likely to be exposed to methoxychlor in the same way as adults, primarily from low-level contamination of food. Other possible sources of methoxychlor exposure for children include swallowing soil, crawling on home carpets, breathing in house dust and residues from methoxychlor-containing pesticides used for home gardening or pet care, and touching work clothes or equipment used to apply products that contain methoxychlor.

Little information is available regarding the effects of methoxychlor in children. Exposure to very high doses of methoxychlor may cause nervous system effects such as tremors or convulsions. The reproductive system is likely to be the most sensitive target of methoxychlor in both adults and children. Methoxychlor is metabolized in the liver to substances that act like estrogen in the body. This probably occurs similarly in children and adults. Estrogens are naturally occurring substances that are necessary for the proper development and function of the male and female reproductive system. Elevated levels of estrogen, or substances like methoxychlor that mimic estrogen, have been shown to disrupt reproductive development and function in animals. This has resulted in early puberty in females, delayed puberty in males, disruption of the reproductive cycle in females, decreased fertility in males and females, and altered hormone levels in the blood. These effects can happen when the exposure occurs before birth or between birth and sexual maturity. It is thought that similar effects could occur in humans, but this has not been reported.

There is no evidence in humans that methoxychlor causes birth defects. Methoxychlor does not cause structural birth defects in animals, but exposure to high levels of methoxychlor during pregnancy caused reduced survival of fetuses. It is unclear whether or at what level of exposure this might occur in humans.

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Methoxychlor or its metabolites can probably be transferred from a pregnant mother to a developing fetus in animals, since abnormal reproductive development has been seen in the newborn animals born to mothers exposed during pregnancy. In animals, methoxychlor and metabolites of methoxychlor that are estrogenic can be transferred from a nursing mother to her newborn babies through breast milk. Methoxychlor and its metabolites can probably cross the placenta and have been detected in human breast milk.

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

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

You can purchase products containing methoxychlor as an over-the-counter pesticide product to apply yourself. If you do purchase such a product, be sure that the product is in an unopened pesticide container that is labeled and contains an EPA registration number. If you plan to apply the pesticide indoors, make sure that the pesticide is approved for indoor use. Be sure to carefully follow the instructions on the label and follow any warning statements. Children can be exposed to pesticides by entering a room or playing on a lawn too soon after a pesticide has been applied. Carefully read and follow the directions on the pesticide label about how long to wait before re-entering the treated area. If you or any member of your family feels sick after a pesticide has been applied, consult your doctor or local poison control center. Pesticides and household chemicals should be stored out of reach of young children to prevent unintentional poisonings. Always store pesticides and household chemicals in their original labeled containers. Never store pesticides or household chemicals in containers that children would find attractive to eat or drink from, such as old soda bottles. Your children may be exposed to methoxychlor if an unqualified person applies pesticides containing it around your home. Make sure that any person you hire is licensed. Your state licenses each person who is qualified to apply pesticides according to EPA standards. Ask to see the license. Also ask for the brand name of the pesticide, a Material Safety Data Sheet (MSDS), the name of the product's active

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ingredient, and the EPA registration number. Ask what are the EPA approved uses. This information is important if you or your family react to the product.

Children may be exposed to methoxychlor if they come in contact with family pets or farm animals that have been treated with the pesticide. Exposure may occur through skin contact with the animal or application devices, or by breathing vapor from animal-dipping solutions and baths. Dipping solution that contains methoxychlor should be disposed of according to the directions on the product label. Waste methoxychlor should never be discarded in any area where children might play.

Although methoxychlor has been found in some foods, it occurs at very low levels. To reduce your family's risk of exposure, you should thoroughly wash all fruits and vegetables before preparing them for consumption.

Methoxychlor may be released to soil and water, especially near hazardous waste sites. Hazardous waste sites are often clearly marked, but children have a tendency to ignore signs that are designed to alert us to dangers. Your children should be encouraged not to play at or near hazardous waste sites. Low levels of methoxychlor have also been found in carpet and house dust.

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

Specific and sensitive tests have been developed to detect methoxychlor in blood, fat, semen, and breast milk of exposed individuals. Because methoxychlor is removed from the body relatively quickly, these tests are only useful in detecting recent exposures (within 24 hours) and are not useful for detecting past exposures to methoxychlor. These tests currently cannot be used to estimate how much methoxychlor you have been exposed to or whether adverse health effects will occur. These tests are not usually performed in a doctor's office because special equipment is required and samples must be sent to a laboratory for testing.

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More information on tests that detect methoxychlor and its metabolites can be found in Chapter 7.

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

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

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

The federal government has taken several actions to help protect humans from excess exposure to methoxychlor. EPA limits the amount of methoxychlor that may be present in drinking water to 0.04 parts of methoxychlor per million parts of water (0.04 ppm). EPA has also set limits of 1–100 ppm on the amount of methoxychlor that may be present in various agricultural products (crops, fruits, vegetables, grains, meats, milk, and food for livestock). FDA limits the amount of methoxychlor in bottled water to 0.04 ppm. EPA restricts the amount of methoxychlor that may

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be released to the environment during burning or by disposal in landfills. OSHA has set a Permissible Exposure Limit (PEL) of 15 milligrams per cubic meter of air (mg/m^3) for the average amount of methoxychlor that may be present in air during an 8-hour workday. A court decision struck down a proposed PEL of $10 \text{ mg}/\text{m}^3$. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a Threshold Limit Value (TLV) of $10 \text{ mg}/\text{m}^3$.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, Mailstop E-29
Atlanta, GA 30333
Web site: <http://www.atsdr.cdc.gov>

* Information line and technical assistance

Phone: 1-888-42-ATSDR (1-888-422-8737)
Fax: 1-404-498-0057

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

* To order toxicological profiles, contact

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000

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2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO METHOXYCHLOR IN THE UNITED STATES

Methoxychlor is produced commercially in the United States, typically as a technical-grade product containing 88–90% of the pure chemical and 10–12% of impurities consisting of isomers of methoxychlor and other reaction products. Methoxychlor has been used as an insecticide against a wide range of pests, including houseflies, mosquitos, cockroaches, chiggers, and various arthropods commonly found on field crops, vegetables, fruits, stored grain, livestock, and domestic pets. There are no known natural sources of methoxychlor. It is moderately soluble in water and is soluble in a variety of organic solvents. Annual usage of methoxychlor in the United States was estimated to range from 500,000 to 900,000 pounds in 1986. The available data show that use patterns have remained fairly constant since 1974.

Methoxychlor is released to the environment mainly as a result of its application to crops and livestock as a pesticide. Smaller amounts may be released during its production, formulation, storage, shipment, and disposal. Most methoxychlor is probably removed from the air by wet and dry deposition processes in less than a month. Methoxychlor binds tightly to soils, but most does not persist due to degradation by microorganisms in the soil. Degradation products of methoxychlor are generally detected in lower levels of soil, suggesting that they are more mobile than methoxychlor. Methoxychlor is generally not detected in surface water or groundwater in the United States, probably due to its degradation and its affinity for sediments and organic matter. However, methoxychlor may be detected in waters near release sources. Although methoxychlor (a derivative of DDT) is not as persistent as DDT, it does have the potential for bioconcentration and has been shown to bioaccumulate to varying degrees in fish, insects, and mammals.

For the general population, the most likely source of methoxychlor exposure is from low-level contamination of food. The FDA's Total Diet Study program monitors chemical contaminants in the U.S. food supply and has calculated average daily intakes of methoxychlor in adults (age 25–65) ranging from 0.1 to 0.3 ng/kg/day for the period 1986–1991. Exposure to methoxychlor from food may be elevated in persons who consume large amounts of fish and seafood from methoxychlor-contaminated waters. Because methoxychlor is usually not detected in ground or surface water sources, exposure to methoxychlor from drinking water is not expected to be significant for the general population. Based on the results of the Non-Occupational Pesticide Exposure Study (conducted between 1986 and 1988),

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inhalation exposure to methoxychlor ranged from 0.002 to 0.012 $\mu\text{g}/\text{day}$ in one U.S. city. In a monitoring study of nonoccupational exposure to pesticides used in and around the home, methoxychlor was detected (air concentrations were not provided) in both indoor and outdoor samples.

Exposures may be greater in individuals who use methoxychlor-containing products for home gardening or animal-care purposes. Populations that live or work on or near a farm where methoxychlor has been used recently on crops or livestock or that live near a hazardous waste site that contains methoxychlor could be exposed to above-average levels of methoxychlor in soil and possibly in water.

The available data suggest that exposure of children to methoxychlor differs from that of adults. Small children may also play close to the ground and are therefore more likely than adults to come in contact with dirt and dust found in home carpets, dirt found outside the home, and lawns. Children also may intentionally or unintentionally ingest soil that contains low levels of methoxychlor.

Methoxychlor has been found in at least 58 of the 1,613 current or former NPL sites. However, the total number of NPL sites evaluated for methoxychlor is not known.

2.2 SUMMARY OF HEALTH EFFECTS

Available data on the toxicity of methoxychlor in humans are limited to a study that found no clinical or histopathological changes in four men and four women who ingested 2 mg/kg/day of methoxychlor for 6 weeks. These data are too limited to allow assessment of the health risks of methoxychlor to humans.

Oral exposure of animals to methoxychlor has shown that high doses of methoxychlor are capable of causing neurological injury (tremors, convulsions), but most studies indicate that the reproductive system is the most sensitive target for methoxychlor. The resultant types of reproductive effects are indicative of interference with the normal actions of estrogen or androgen. Mechanistic studies have confirmed that metabolites of methoxychlor can compete with estrogen for binding to estrogen receptors and can mimic some and antagonize other effects of estrogen. Additional studies have shown that methoxychlor or its metabolites can interact with the androgen receptor and antagonize androgenic effects. In females, these interactions can result in disruption of estrus cyclicity, reduced fertility, and increased pre- and post-implantation losses. Effects in males can include delayed sexual maturation, atrophy of reproductive organs and accessory glands, and altered sexual or socio-sexual behavior. Many of these effects may be mediated through altered hormone levels. Because methoxychlor and its metabolites are cleared fairly

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rapidly from the body (approximately 92% in 24 hours in mice), there does not appear to be much potential for cumulative toxicity.

Observable changes in the liver (altered liver weight, altered enzyme and protein levels, pale and mottled appearance) and kidneys (cystic tubular nephropathy, elevated blood urea nitrogen [BUN]) of animals, as well as weight loss, are caused only by relatively large doses of methoxychlor; these effects are probably not mediated by an estrogenic mechanism.

Most carcinogenicity studies have not shown an increase in the cancer incidence following exposure to methoxychlor. Based on a review of all the available data, IARC has classified methoxychlor as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans). Similarly, EPA has classified methoxychlor as a Group D carcinogen (not classifiable as to human carcinogenicity).

Reproductive Effects. Although human data on the reproductive effects of methoxychlor are limited, the animal and *in vitro* data strongly suggest that sufficient exposure to methoxychlor may adversely affect the development, histopathology, and function of the human reproductive system.

Data from oral studies in animals indicate that the reproductive system is the primary and most sensitive target of methoxychlor-induced toxicity in both adult and developing animals. Some metabolites and contaminants of methoxychlor are estrogenic or anti-androgenic and are capable of producing adverse effects on the male and female reproductive system. These effects are thought to be mediated by interaction of methoxychlor or its metabolites with the estrogen receptor α , estrogen receptor β , or an as yet unknown estrogen receptor, or with the androgen receptor. These interactions can cause disruption of reproductive development or can alter reproductive function in adults. Altered serum and pituitary hormone levels have frequently been seen in animal studies, which may contribute to the changes in reproductive development and function. Developmental reproductive changes include precocious puberty and abnormal estrus cyclicity in females, delayed puberty in males, altered weights of reproductive organs and accessory glands, and impaired reproductive function in adulthood, including decreased pups/litter and increased resorptions. Similar effects have also been seen following exposure of adult animals. Additionally, gross and microscopic cellular changes have been observed in the reproductive organs of exposed adult females and males. While methoxychlor does have estrogenic properties, it is important to note that it is at least several thousand fold less potent than endogenous estrogen.

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There are no human data that report adverse effects on the reproductive system following exposure to methoxychlor, but *in vitro* studies reveal that human liver microsomes are capable of metabolizing methoxychlor to estrogenic compounds. Therefore, it is likely that methoxychlor could produce reproductive estrogen-like effects in humans.

Developmental Effects. In animals, signs of fetotoxicity (decreased fetal body weight, increased incidence of wavy ribs, resorptions, and death) were noted following exposure to methoxychlor *in utero*. These effects may be due to the maternal toxicity of methoxychlor and may not be true signs of teratogenicity.

Methoxychlor exposure during development can adversely affect the reproductive system of both developing and adult animals. These effects were discussed with the reproductive effects above. These effects are the result of the disruption by estrogenic methoxychlor metabolites of the normal delicate balance of time-sensitive hormone levels during fetal and post-natal development. Taken together, the animal data suggest that human exposure to methoxychlor during critical stages of development may adversely affect reproductive development, causing effects that may not be detected until after puberty.

Neurological Effects. Animal studies suggest that exposure to large amounts of methoxychlor can produce neurological effects, such as apprehension, nervousness, increased salivation, decreased locomotor activity, tremors, convulsions, and death. Methoxychlor has been demonstrated to be a neurotoxicant even in the absence of metabolism. This suggests that it is the parent compound that is neurotoxic, and that neurotoxicity is of concern only when the metabolic capacity for *O*-demethylation is exceeded. This is supported by the observation that the neurological effects of methoxychlor are similar to those associated with exposure of humans and animals to DDT, a structurally similar chemical that is very slowly metabolized. The mechanism by which DDT, and therefore possibly methoxychlor, produces neurological effects has been proposed to involve the membrane-association of a lipophilic species, which alters ion transport across neural membranes.

2.3 MINIMAL RISK LEVELS

Inhalation MRLs

No inhalation MRLs for methoxychlor have been derived because adequate data were not available concerning the effects of methoxychlor via this route of exposure.

Oral MRLs

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No acute oral MRL was derived for methoxychlor. A variety of candidate acute-duration MRL studies were considered. The acute oral MRL derived in the previous 1994 toxicological profile for methoxychlor was based on precocious vaginal opening (early puberty) observed in the study by Gray et al. at 25 mg/kg/day. However, since this study did not test doses as low as Chapin et al. (used as the basis of the intermediate-duration MRL), which demonstrated the same effect at 5 mg/kg/day administered from gestation day 14 to postnatal day 42, it is not known whether the premature puberty would have occurred at a lower acute dose than the 25 mg/kg/day observed in the Gray et al. study. There were several hypothesis-generating studies at extremely low doses that were not definitive enough to use for MRL derivation. The male reproductive parameters observed in these studies included increased prostate weight, increased territorial urine marking, increased killing of young mice by adults, and changes in aggression. These studies were not used for MRL derivation because the biological significance and relevance to human health of extremely low-dose effects has not been definitively established. Additionally, prostate weight is normally highly variable, even within a litter, and therefore may not be a good indicator of low-dose effects. These studies were all performed by the same laboratory and need to be replicated by other laboratories. See Appendix A for further discussion.

- C An MRL of 0.005 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to methoxychlor.

This MRL is based on a LOAEL of 5 mg/kg/day from gestation day 14 to postpartum day 42 for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning. Precocious vaginal opening was evident (statistically significant) in all methoxychlor-treated groups (vaginal opening occurred on postnatal days 37.4, 35.2, 30.8, and 33.4, respectively, for groups 0, 5, 50, and 150 mg/kg/day). The LOAEL was divided by an uncertainty factor of 1,000 (10 for variation in sensitivity among humans, 10 for extrapolation of animal data to humans, and 10 for extrapolation from a LOAEL to a NOAEL). The MRL is supported by other observations of reproductive effects associated with intermediate-duration exposure including elevated levels of prolactin in the pituitary of male rats exposed to 50 mg/kg/day, decreased seminal vesicle weight, caudal epididymal weight, and caudal epididymal sperm count, and increased gonadotropin releasing hormone in the mediobasal hypothalamus in male rats exposed to 50 mg/kg/day. At higher exposure levels, intermediate-duration studies show decreased fertility in male rats at doses of 60–400 mg/kg/day and in female rats at doses of 50–150 mg/kg/day. A study in humans that identified a NOAEL of 2 mg/kg/day for effects to the testes and menstrual cycle was not chosen as the basis for an MRL because reproductive function was not evaluated and the number of subjects was small (4/sex/exposure group), and such an MRL may not be protective of reproductive and developmental effects in the fetus or child.

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No chronic oral MRL was derived for methoxychlor. Studies in rats and mice reported no adverse histopathological effects on a number of organ systems, including the reproductive system, following chronic exposure to methoxychlor at dose levels of 77–599 mg/kg/day. However, these chronic studies did not evaluate sensitive indices of reproductive toxicity. A 3-generation study in rats reported a LOAEL of 79 mg/kg/day methoxychlor for decreased fertility, and a NOAEL of 18 mg/kg/day for this effect. An MRL was not based on this study because sensitive reproductive end points such as precocious vaginal opening were not monitored, and the resultant MRL might not be protective for these types of effects.

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 methoxychlor. 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.

The health effects of methoxychlor have been investigated mainly in studies using technical grade methoxychlor, although a few studies have used laboratory grade (98% pure) or recrystallized (>99% pure) preparations. Technical grade methoxychlor is the grade used in industrial preparations and typically contains about 80–90% methoxychlor, with the remainder being composed of >50 chemically-related compounds (IARC 1979; Lamoureux and Feil 1980). Please refer to Chapter 4 for more detailed information on the composition of methoxychlor. Some *in vivo* studies suggest that technical grade methoxychlor is approximately 2–4 times more potent with respect to reproductive and developmental effects than is pure methoxychlor (Bitman and Cecil 1970; Tullner 1961). This is because several of the contaminants in technical grade methoxychlor are directly estrogenic (Kupfer and Bulger 1987b), whereas pure methoxychlor is proestrogenic and requires metabolic activation before exhibiting estrogenic activity (Bulger et al. 1978d; Kupfer and Bulger 1979). Some of the estrogenic contaminants of technical grade methoxychlor are the same as those formed by metabolism *in vivo* (Bulger et al. 1985). It is important to recognize that because of the biological activity of these contaminants, dose-response relationships obtained using technical grade methoxychlor may not be directly applicable to pure methoxychlor. For this reason, the chemical purity of the methoxychlor used in key quantitative studies is provided. *Since most studies employ technical grade methoxychlor, the text refers to purity only when other grades are used.*

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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.

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

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

A User's Guide has been provided at the end of this profile (see Appendix 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

In air, methoxychlor is generally associated with particulate matter, and inhalation exposure to methoxychlor can occur by inspiration of methoxychlor-laden dust. Although the health effects resulting from inhalation exposure to methoxychlor have not been extensively investigated, there are a few reports that describe health effects following this type of exposure.

3.2.1.1 Death

A single case study reported the death of a 49-year-old male after an acute inhalation exposure to a pesticide mixture containing methoxychlor and captan (Ziem 1982). The exposure level was not reported. Death occurred 6 months after exposure and was attributed to aplastic anemia. Because only a single case was described and because the exposure was to a mixture of pesticides, it is not possible to deduce the role of methoxychlor in this outcome.

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No studies were located regarding death in animals after inhalation exposure to methoxychlor.

3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, dermal, or ocular effects in humans or animals after inhalation exposure to methoxychlor.

Hematological Effects. A single case study reported the development of aplastic anemia in a 49-year-old male after an acute inhalation exposure to a pesticide mixture containing methoxychlor and captan (Ziem 1982). Symptoms of fatigue and bruising developed several weeks after exposure. The authors stated that although there are published case studies of aplastic anemia in humans following exposure to structurally-related pesticides such as dichlorodiphenyltrichloroethane (DDT) and lindane, no other reports of methoxychlor-induced aplastic anemia were located. Due to the limited database and the exposure to other chemicals (captan) in this study, a causal relationship between methoxychlor and aplastic anemia cannot be established. It is possible that this illness had an etiology unrelated to the pesticide exposure.

Body Weight Effects. There is no information regarding body weight effects of methoxychlor in humans. One animal study showed a 24% reduction in weight gain in rats intermittently exposed to 360 or 430 mg/m³ methoxychlor for 4–5 weeks (Haag et al. 1950). However, the vehicle used to deliver the methoxychlor (Pyrax plus 3% Santo-Cel) was toxic and resulted in several deaths in the controls; therefore, the results of this study are difficult to interpret.

3.2.1.3 Immunological and Lymphoreticular Effects

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

3.2.1.4 Neurological Effects

A single case study described neurological effects in a 21-year-old male acutely exposed (15–20 minutes) to a pesticide mixture that contained 15% methoxychlor and 7.5% malathion (Harell et al. 1978). The subject noted blurred vision and nausea 8–9 hours after exposure. He was admitted to the hospital

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36 hours after exposure in a state of dehydration with severe abdominal cramps and diarrhea. Approximately 4 days later, he experienced dizziness and complete deafness followed by difficulty moving the extremities, hypoesthesias, paresthesias in the limbs, bilateral foot drop, and leg pain. There was no improvement in any of these neurological effects 6 years after exposure. The authors noted that neither methoxychlor nor malathion typically produced such profound effects, and attributed the special susceptibility of this individual to a deficiency in the enzyme responsible for the detoxification of malathion. Whether methoxychlor contributed to the effects is not known.

Only one study was located regarding the neurological effects of inhalation exposure to methoxychlor-laden dust in animals. Intermittent exposure (2 hours/day, 5 days/week for 4 weeks) of rabbits to 430 mg/m³ methoxychlor produced hind leg paralysis and disseminated nodules in the cerebral cortex in one out of two animals (Haag et al. 1950). Neurological effects were not observed in the two control animals. The results from this study are limited by the small number of animals tested, and by the possibility that the observed paralysis in the affected animal was the result of a disease that is endemic to rabbits (Haag et al. 1950). Thus, no firm conclusions can be made on the neurotoxicity of methoxychlor in animals after inhalation exposure.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after inhalation exposure to methoxychlor.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to methoxychlor.

3.2.1.7 Cancer

A single epidemiological study was located on the potential association between methoxychlor exposure and occurrence of leukemia in farmers (Brown et al. 1990). In this study, 11/578 cases of leukemia and 16/1,245 controls were found to have been occupationally exposed to methoxychlor. After adjustment for vital status, age, state, tobacco use, family history of lymphopietic cancer, high risk occupations and high risk exposures, an odds ratio of 2.2 was calculated. This increase was statistically significant;

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however, it is difficult to make firm conclusions on the carcinogenicity of methoxychlor in humans based on a single study.

No studies were located regarding cancer in animals after inhalation exposure to methoxychlor.

3.2.2 Oral Exposure

3.2.2.1 Death

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

Reported acute LD₅₀ values for recrystallized and technical grade methoxychlor range from 3,460 to 7,000 mg/kg in male and female rats (Cannon Laboratories 1976; Hodge et al. 1950; Smith et al. 1946) and 2,900 mg/kg in mice (Coulston and Serrone 1969). A more recent study reported an acute LD₅₀ in male and female Wistar rats of 2,828 mg/kg for methoxychlor that contained 88% *p,p'* isomer and 12% *o,p'* isomer, and an acute LD₅₀ of 1,782 mg/kg for a methoxychlor formulation that contained only 25% methoxychlor (*p,p'* isomer) (Dikshith et al. 1990). Mortality following a single exposure to 1,000, 2,000, 4,000, or 8,000 mg/kg of the mixed isomer methoxychlor was 0, 25, 100, and 100%, respectively, in male rats, and 25, 50, 50, and 75%, respectively, in female rats (Dikshith et al. 1990). Two out of 17 pregnant rabbits died following exposure to 251 mg/kg/day methoxychlor on days 7–19 of gestation (Kincaid Enterprises 1986). Exposure to 790–4,200 mg/kg/day recrystallized and technical grade methoxychlor in feed for 4–16 weeks produced significant increases in mortality in rats (Davison and Cox 1976; Haag et al. 1950; Hodge et al. 1950). Mortality rates were higher in female Wistar rats (0–25%) than in males (0–10%) exposed to 100–1,000 mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). Mortality was low in male and female Wistar rats that received 200–800 mg/kg/day of a formulation containing 25% methoxychlor for 90 days (Dikshith et al. 1990). Increases in mortality were also reported in dogs exposed to 2,000 mg/kg/day for 8–24 weeks (Tegeris et al. 1966). In dogs and rats, death was preceded by neurological effects (tremors, convulsions), as discussed below. LOAEL values for the lethal effects of methoxychlor are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, musculoskeletal, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans after oral exposure to methoxychlor. Data on the remaining

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systemic effects after methoxychlor ingestion are available from a single study in humans. The highest NOAEL values and all LOAEL values from each reliable study for the systemic effects of methoxychlor in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1. These studies are discussed below.

Respiratory Effects. A single study reported pulmonary edema in rabbits exposed to 200 mg/kg/day methoxychlor by gavage for 1–3 weeks, but the authors attributed this effect to possible gavage error resulting in aspiration of the chemical; no control group was included in the study (Smith et al. 1946). Chronic exposure of rats to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967) or 107 mg/kg/day technical grade methoxychlor (NCI 1978) or of mice to 599 mg/kg/day technical grade methoxychlor (NCI 1978) did not produce any significant histopathological effects in the respiratory tract. These data are too limited to draw firm conclusions, but suggest that the lung is not especially sensitive to ingested methoxychlor.

Cardiovascular Effects. A single case report documented the ingestion of approximately 125 mL of a commercial product that contained methoxychlor (about 15 mg of methoxychlor) by a 62-year-old man in an attempted suicide (Thompson and Vorster 2000). Testing of a serum sample collected at the time of admission to the hospital showed a methoxychlor level of 0.67 µg/mL serum. His blood pressure was very low (58/40) and his pulse rate was high (88 beats per minute). After initiation of treatment, his blood pressure recovered to 110/70.

In animals, one study reported the development of fatty hearts in two of four rabbits administered lethal doses of methoxychlor (200 mg/kg/day) for 1–3 weeks (Smith et al. 1946). However, no gross or histopathological changes in the heart were noted in rats or mice following chronic oral exposure to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978). Although no firm conclusion can be

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat	Once (GO)				3460 (LD50)	Cannon Laboratories 1976 TG
2	Rat (Wistar)	1x (GO)				2828 (LD50)	Dikshith et al. 1990 mixed isomers
3	Rat	Once (GO)				5000 (LD50)	Hodge et al. 1950 RC TG
4	Mouse (NS)	Once (G)				2900 (LD50)	Coulston and Serrone 1969 TG
5	Rabbit (New Zealand)	Gd 7-19 1x/d (GW)				251 (2/17 deaths)	Kincaid Enterprises 1986 TG
Systemic							
6	Rat	1x/d Gd 6- 15 (GO)	Bd Wt		50 (reduced maternal body weight gain)		Khera et al. 1978 TG
7	Rat	Once (GO)	Hepatic	640			Morgan and Hickenbottom 1979 TG
8	Rabbit	Gd 7-19 1x/d (GW)	Hepatic	5	35.5 (pale/mottled appearance of the liver)		Kincaid Enterprises 1986 TG
			Bd Wt	5	35.5 (anorexia and 14.4% decreased body weight gain)		
Neurological							
9	Rat (Wistar)	Once (GO)			2500 (decreased locomotor activity)	3000 (tremors)	Cannon Laboratories 1976 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Reproductive							
10	Rat	Gd 6-15 (F)				97.3	(dose-related increases in post-implantation loss) Culik and Kaplan 1976 TG
11	Rat (Holtzman)	8d 1x/d (GO)		50 F	75 F (induction of uterine decidualization)		Cummings 1993 98% pure
12	Rat	1x/d 8 d (GO)		100	200 (decreased uterine receptivity to implantation)		Cummings and Gray 1987 LG
13	Rat	1x/d 3-8 d (GO)			100 (decreased serum progesterone)	200	(decreased number of implantation sites and uterine weight; increased number of resorptions) Cummings and Gray 1989 LG
14	Rat	Gd 1-8 1x/d (GO)		25	50 (decreased serum progesterone)	250	(decreased number of implantation sites) Cummings and Laskey 1993 LG
15	Rat	Gd 1-3 1x/d (GO)			100 (accelerated embryo transport into uterus)	200	(decreased number of implantations) Cummings and Perreault 1990 LG
16	Rat	1x/d 5-76 d, starting at weaning at 21 ppd (GO)			25 (precocious vaginal opening and estrus as early as 3 d after exposure started)		Gray et al. 1989 TG
17	Mouse	1x/d 5 d/wk 2 wk (GO)			25 (persistent vaginal cornification)		Martinez and Swartz 1991 TG 50%
18	Rabbit	Gd 7-19 1x/d (GW)		5		35.5	(increased frequency of abortion and late resorptions) Kincaid Enterprises 1986 TG

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Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Developmental							
19	Rat	Gd 6-15 (F)			40.8	(increased incidence of wavy ribs)	Culik and Kaplan 1976 TG
20	Rat	1x/d Gd 14-20 (GO)		30			Gellert and Wilson 1979 NS
21	Rat	1x/d Gd 6-15 (GO)				200 (increased percent dead, resorbed, or anomalous fetuses)	Khera et al. 1978 TG
22	Rabbit	Gd 7-19 1x/d (GW)		5	35.5	(10-11% decreased fetal body weight and percentage of male fetuses)	Kincaid Enterprises 1986 TG
INTERMEDIATE EXPOSURE							
Death							
23	Rat (Sherman)	16 wk (F)				1200 (4/12 died)	Davison and Cox 1976 TG
24	Rat	4 wk (F)				917 (6/7 females died)	Haag et al. 1950 RC
25	Rat	45 d (F)				4200 (16/20 died)	Hodge et al. 1950 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
Systemic							
26	Rat (Sprague- Dawley)	Gd 14 - ppd 42 1x/d (GO)	Hemato	50 M	150 M (24% increase in relative spleen weight)		Chapin et al. 1997 95% pure
			Hepatic	50 M	150 M (13% increase in relative liver weight)		
			Renal	50 M	150 M (16% increase in relative kidney weight)		
			Endocr	50 M	150 M (36% increase in relative and absolute adrenal gland weight)		
			Bd Wt	5	50 (10% decrease in body weight gain)		
27	Rat (Sprague- Dawley)	16 wk (F)	Hepatic	90	1200 (66% increased relative liver weight; decreased total and relative vitamin A content)		Davison and Cox 1976 TG
			Bd Wt	90	1200 (22% decreased body weight gain)		
28	Rat (Long- Evans)	309d 1x/d (GO)	Hepatic		200 M (37% decrease in absolute liver weight)		Gray et al. 1999 90% purity (TG)
			Renal		200 M (37% decrease in absolute kidney weight)		
			Endocr		200 M (17% decrease in absolute pituitary weight; 60% increase in relative adrenal gland weight)		
			Bd Wt			200 M (37% decrease in body weight gain)	

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
36	Rat	1x/d 55-66 d (GO)				400 (persistent vaginal cornification; atrophy of ovary and uterus)	Gray et al. 1988 TG
37	Rat	1x/d 56-66 d (GO)				100 (80% decreased in fertility when treated males and females mated; decreased number of liver pups per litter; decreased caudal epididymal sperm count)	Gray et al. 1989 TG
38	Rat	1x/d 59-76 d (GO)		25 M (elevated levels of prolactin in the pituitary) 25 F (precocious vaginal opening)			Gray et al. 1989 TG
39	Rat	1x/d 76-99 d (GO)		100 M (elevated prolactin, FSH and TSH in the pituitary)		100 F (40% decreased fertility when treated females were mated with untreated males; decreased number of live pups per litter; ovarian atrophy and histopathology)	Gray et al. 1989 TG
40	Rat (Long- Evans)	309d 1x/d (GO)				200 M (delayed puberty; decreased testes weight, fertility, and sperm counts; altered mating behavior)	Gray et al. 1999 90% purity (TG)
41	Rat (Sprague-Dawley)	6-9 wk (F)		60 (decreased ovary weight)		150 (decreased ovary weight; increased uterus weight; decreased mating frequency and fertility in females)	Harris et al. 1974 TG

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form	
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)		
INTERMEDIATE EXPOSURE								
42	Rat (Long- Evans) (F)	12 wk				300	(persistent vaginal cornification; decreased mating frequency)	Harris et al. 1974 TG
43	Rat (Long- Evans) (F)	6 wk				60	(precocious vaginal opening in females; decreased mating frequency and fertility in males and females)	Harris et al. 1974 TG
44	Rat (F)	30-45 d				140		Hodge et al. 1950 TG
45	Rat (Long- Evans) GO	15d 1x/d					50 F (precocious vaginal opening)	Laws et al. 2000 TG 95%
46	Rat (CD)	28d; 1x/d (GO)				20		Okazaki et al. 2001 LG
							100 M (atrophy of seminiferous tubules and Leydig cells in testes; decreased sperm; cell debris in lumen of epididymis; atrophy of mammary acinus)	
							100 F (abnormal estrous cyclicity)	
47	Mouse (GO)	1x/day 5 d/wk 4 wk				50	(persistent vaginal cornification; decreased ovary weight)	Martinez and Swartz 1991 TG
48	Mouse (GO)	1x/d 5 d/wk 4 wk				100	(increased lipid in interstitial and thecal cells of the ovary)	Martinez and Swartz 1992 TG 50%

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
Developmental							
49	Rat (Sprague- Dawley)	Gd 14 - ppd 21 1x/d (GO)			5 (decreased click response in M and F; decreased approach stimulus in M; no dose-response relationship)	50 F (decreased fertility; 68% reduction in number of implants)	Chápin et al. 1997 95% pure (LG)
50	Rat	1x/d 59-76 d (GO)				50 (precocious vaginal opening; 45.3% decreased fertility in offspring; acyclic estrus; pituitary abnormalities)	Gray et al. 1989 TG
51	Rat	6-9 wk (F)			60 (precocious vaginal opening in female offspring)		Harris et al. 1974 TG
52	Rat (Sprague- Dawley)	9 wk (F)				60 (reduced fertility in males and female offspring)	Harris et al. 1974 TG
CHRONIC EXPOSURE							
Systemic							
53	Rat (Osborne- Mendel)	24-27 mo (F)	Resp	77			Deichman et al. 1967 RC
			Gastro	77			
			Hepatic	77			
			Renal	77			

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
54	Rat (albino)	1 yr (F)	Resp	917			Haag et al. 1950 RC
			Cardio	917			
			Gastro	917			
			Musc/skel	917			
			Hepatic	917			
			Renal	917			
			Dermal	917			
			Ocular	917			
			Bd Wt		197 M (>10% decrease in body weight)		

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
55	Rat (Osborne- Mendel)	78 wk (F)	Resp	107			NCI 1978 TG
			Cardio	107			
			Gastro	107			
			Hemato	107			
			Musc/skel	107			
			Hepatic	107			
			Renal	107			
			Dermal	107			
			Ocular	107			
			Bd Wt		107 F (decreased body weight gain)		

Table 3-1 Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
56	Mouse (B6C3F1)	78 wk (F)	Resp	599			NCI 1978 TG
			Cardio	599			
			Gastro	599			
			Hemato	599			
			Musc/skel	599			
			Hepatic	599			
			Renal	599			
			Dermal	599			
			Ocular	599			
			Bd Wt		172 F (12% decrease in body weight)		
			Immuno/ Lymphoret				
57	Rat (Osborne- Mendel)	78 wk (F)		107			NCI 1978 TG
58	Mouse (B6C3F1)	78 wk (F)		599 M			NCI 1978 TG
			Neurological				
59	Rat (Osborne- Mendel)	78 wk (F)		107 F			NCI 1978 TG
60	Mouse	78 wk (F)		599 M			NCI 1978 TG

Levels of Significant Exposure to Methoxychlor - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
Reproductive							
61	Rat (CD)	3 generations (F)		18		92 F (decreased fertility)	Haskell Laboratories 1966 TG

a The number corresponds to entries in Figure 3-1

b Used to derive a minimal risk level (MRL) of 0.005 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Figure 3-1. Levels of Significant Exposure to Methoxychlor - Oral
Acute (≤ 14 days)

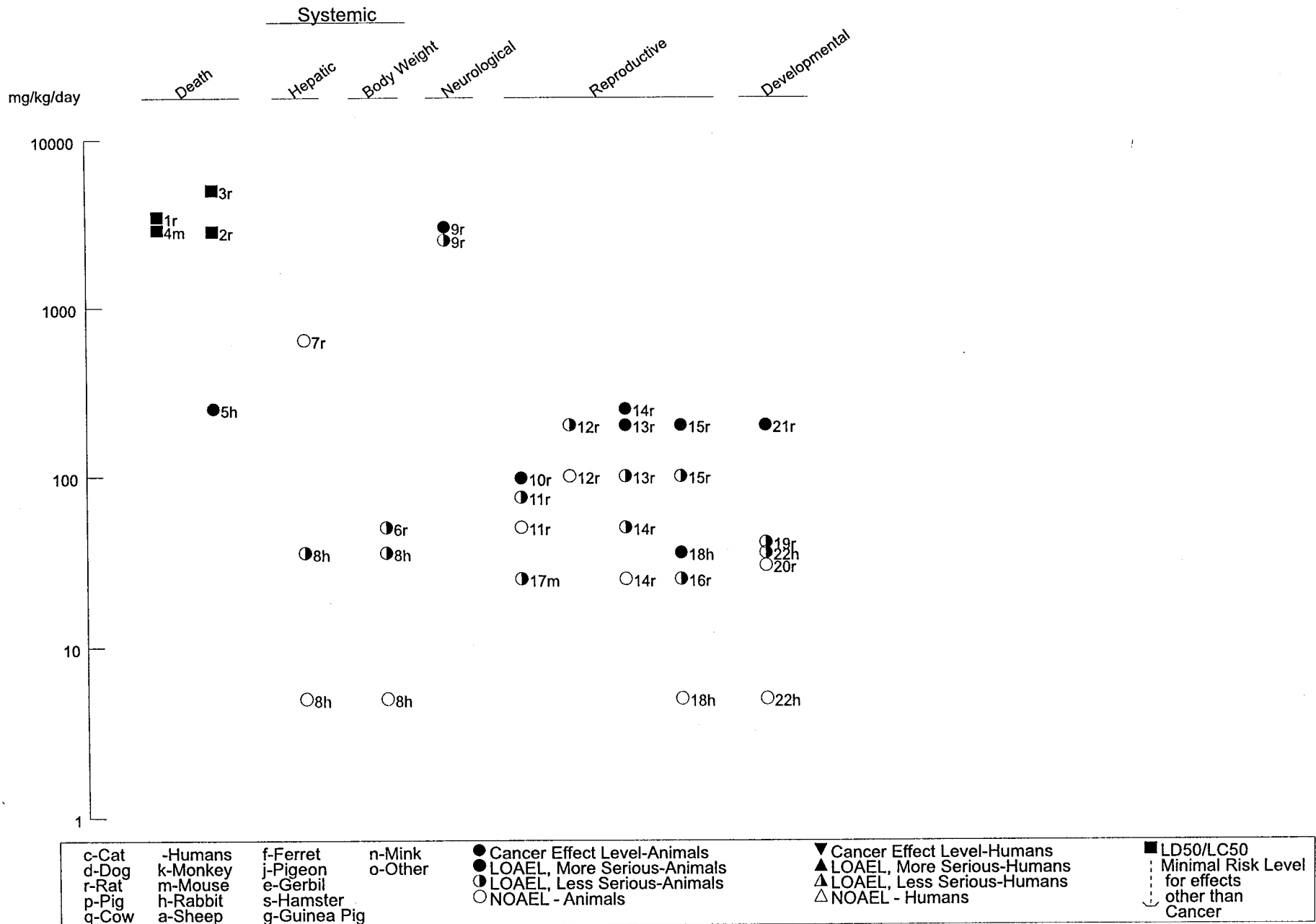
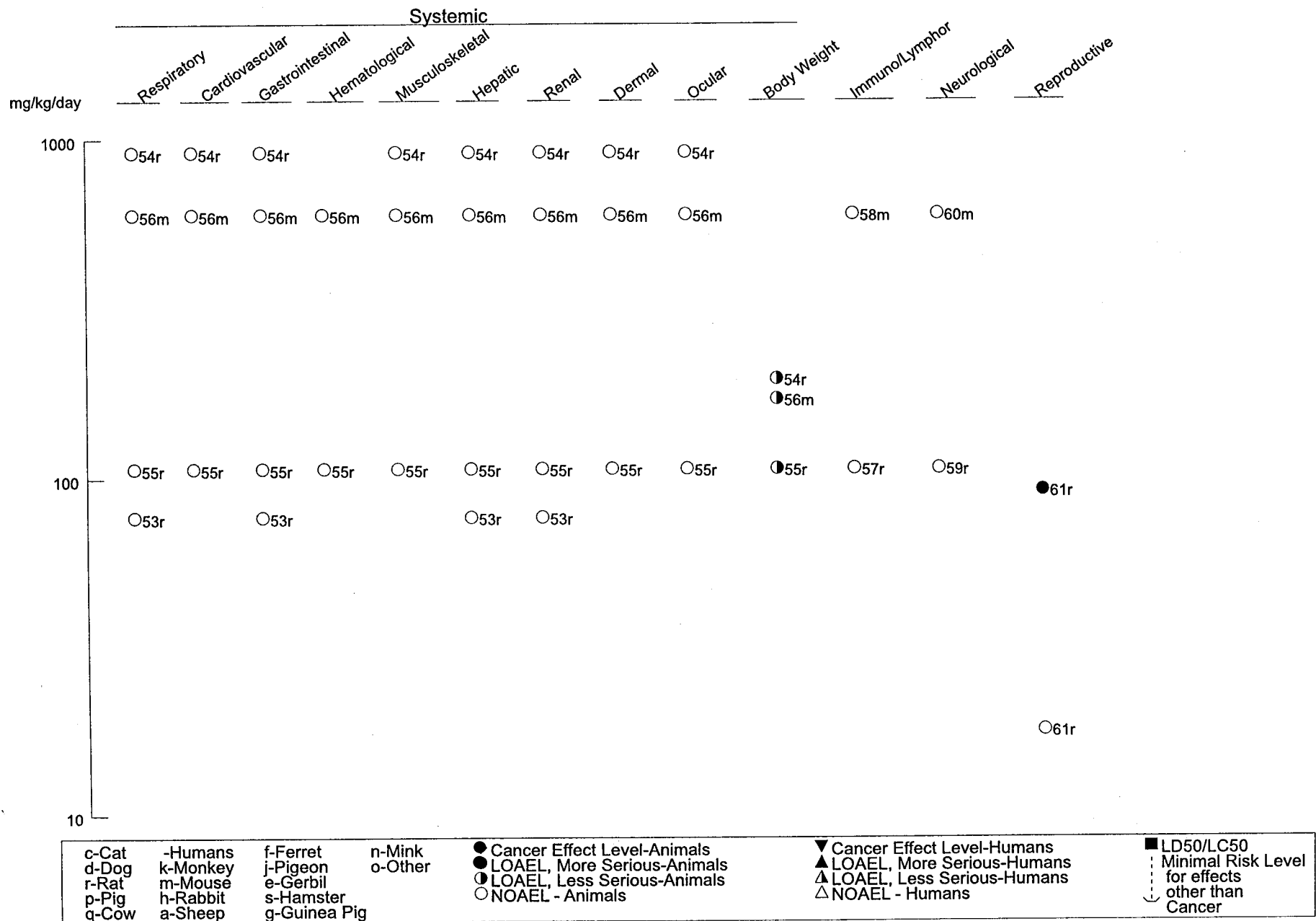


Figure 3-1. Levels of Significant Exposure to Methoxychlor - Oral (continued)
Chronic (≥365 days)



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drawn regarding cardiovascular effects of methoxychlor, the data suggest that the heart is not a primary target organ for methoxychlor.

Gastrointestinal Effects. No histological evidence of injury to the small intestine was detected in biopsy samples from four men and four women who ingested 2 mg/kg/day of methoxychlor for 6 weeks (Wills 1969).

Diarrhea was noted in rats given acutely lethal doses (7,000 mg/kg) of methoxychlor (Smith et al. 1946). However, no histopathological changes of the gastrointestinal tract were noted following chronic oral exposure of rats to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967) or 107 mg/kg/day technical grade methoxychlor (NCI 1978), or mice to 599 mg/kg/day technical grade methoxychlor (NCI 1978). These data suggest that the gastrointestinal tract is not a sensitive target organ for methoxychlor.

Hematological Effects. Only one study was located regarding the hematological effects of methoxychlor in humans after oral exposure. Electron microscopy of bone marrow biopsy samples and chemical analysis of the blood from four male and four female volunteers exposed to 2 mg/kg/day for 6 weeks revealed no significant changes (Wills 1969).

Rabbits administered lethal doses of methoxychlor (200 mg/kg/day) for 1–3 weeks developed hemosiderosis of the spleen, but no controls were included in the study (Smith et al. 1946). Rats exposed to 150, but not 50, mg/kg/day methoxychlor for 35 days had increased relative spleen weights (Chapin et al. 1997). However, no gross or histopathological changes were noted on the hematopoietic system (bone marrow, spleen) in rats or mice exposed to 107 or 599 mg/kg/day methoxychlor in the feed for 78 weeks (NCI 1978). No changes in red blood cell, white blood cell, or hemoglobin levels were observed in rats exposed to up to 1,000 mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). These data are insufficient to evaluate the hematological effects of methoxychlor.

Musculoskeletal Effects. In animals, no histopathological effects were detected in the skeletal muscles or bones of rats or mice following chronic oral exposure to doses of 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978). This suggests the musculoskeletal system is not an important target for methoxychlor.

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Hepatic Effects. Only one study was located regarding the effects of methoxychlor on the liver in humans after oral exposure. Electron microscopy of liver biopsy samples from 4 men and 4 women exposed for 6 weeks to 2 mg/kg/day revealed no histopathological changes (Wills 1969). In addition, there were no changes in serum enzyme levels such as glutamate-oxaloacetate aminotransferase (SGOT; also called aspartate aminotransferase or AST), glutamate-pyruvate aminotransferase (SGPT; also called alanine aminotransferase or ALT), and alkaline phosphatase (AP) that are indicative of liver injury. Based on these limited data, the liver of humans does not appear to be affected by low doses of methoxychlor for intermediate durations. The hepatic effects of chronic oral exposure to low doses of methoxychlor have not been studied in humans.

Several studies in animals have described effects on the liver following oral exposure to methoxychlor, but the effects were usually mild or moderate, and effects were generally observed only at high doses (lethal or near lethal). In male rats given a single oral dose of 640 mg/kg methoxychlor, increased hepatic levels of glucose-6-phosphatase and decreased levels of glycogen phosphorylase and lactate were detected, but there were no observable histopathological changes (Morgan and Hickenbottom 1979). These enzymatic changes suggest that methoxychlor may promote the utilization of liver glycogen (Morgan and Hickenbottom 1979). The livers of pregnant rabbits administered 35.5 mg/kg/day methoxychlor on days 7–19 of gestation were pale and mottled in appearance (Kincaid Enterprises 1986). This effect was not noted in rabbits administered 5.01 mg/kg/day methoxychlor. "Fatty degeneration" of the liver (not otherwise described) was noted in two out of four rabbits given lethal doses of methoxychlor (200 mg/kg/day) for 1–3 weeks; no controls were included in this study (Smith et al. 1946). Levels of serum enzymes (SGOT or AST, SGPT or ALT, AP) were elevated in dogs receiving 2,000, but not 1,000, mg/kg/day methoxychlor in feed for 8–24 weeks (Tegeris et al. 1966). Rats that received 100–1,000 mg/kg/day methoxychlor for 90 days had small, but statistically significant, nondose related changes in serum and/or liver enzyme activities (GOT, GPT, and AP) and serum protein levels (Dikshith et al. 1990). Significant increases in liver weight were reported in rats administered lethal doses of methoxychlor (500–1,200 mg/kg/day for 13–16 weeks) (Davison and Cox 1976; Dikshith et al. 1990). Rats fed nonlethal doses of methoxychlor (60 mg/kg/day) for 9 weeks, spanning mating through weaning, had enlarged livers and elevated liver vitamin A concentrations prior to mating, but no significant differences from controls following weaning (Harris et al. 1974). Although reductions in liver weight have also been reported in animals receiving nonlethal doses of methoxychlor for intermediate or chronic durations (Cecil et al. 1974; Deichmann et al. 1967; Gray et al. 1989, 1999), these reductions were generally associated with decreases in body weight gain and are not considered adverse. No histopathological changes were found in the liver of rats or mice chronically exposed to 107 or

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599 mg/kg/day methoxychlor, respectively (NCI 1978), or rats exposed to 77 mg/kg/day (Deichmann et al. 1967). Taken together, these data suggest that high doses of methoxychlor may cause hepatic effects in some animals, but that the liver is not a key target organ for methoxychlor.

Because DDT (a structural analogue of methoxychlor) is known to cause vitamin A depletion in the livers of exposed animals, a number of studies have investigated the effects of methoxychlor on the vitamin A content of the liver. A significant reduction in total liver vitamin A content was observed in rats receiving doses of 9 mg/kg/day or more for 16 weeks (Davison and Cox 1976), and vitamin A concentration ($\mu\text{g}/100\text{ mg liver}$), but not total vitamin A, was significantly reduced in rats exposed to 60 mg/kg/day for 9 weeks (Harris et al. 1974). No significant change in vitamin A content was reported in rats administered 5 mg/kg/day methoxychlor for 8–16 weeks (Cecil et al. 1974; Phillips and Hatina 1972). In an *in vitro* assay, methoxychlor did not react with human transthyretin, a carrier protein for vitamin A and thyroid hormones (Van den Berg et al. 1991). However, no microsomal activation system was employed to determine if methoxychlor metabolites could bind transthyretin. Decreases in the vitamin A content of liver are not necessarily adverse by themselves, so the significance of this effect is uncertain.

Renal Effects. Adverse effects on the kidneys were reported in animals administered lethal or near-lethal doses of methoxychlor. Renal nephrosis was observed in four out of four rabbits administered 200 mg/kg/day methoxychlor for 1–3 weeks, but the significance is unclear since no controls were included (Smith et al. 1946). Basophilic tubules, dilatation of renal tubules, and casts were seen in rats exposed to 500, but not 100, mg/kg/day for 28 days; no further description of the lesions was provided (Okazaki et al. 2001). Cystic tubular nephropathy was reported in rats exposed to 861 mg/kg/day methoxychlor in the feed for 33–55 days (Tullner and Edgcomb 1962). Cystic tubular nephropathy was accompanied by elevated blood urea nitrogen (BUN) in pigs exposed to 1,000 mg/kg/day methoxychlor in the feed for 24 weeks (Tegeris et al. 1966). No histopathological changes were detected in rats and mice exposed to 107 or 599 mg/kg/day, respectively, in the feed for 78 weeks (NCI 1978) or in rats exposed to up to 1,000 mg/kg/day for 90 days (Dikshith et al. 1990). One study showed a 37% reduction in kidney weight in rats treated with 200 mg/kg/day for 4 weeks, but this was associated with a 37% reduction in body weight and therefore, may not be adverse (Gray et al. 1999). Although limited, these data suggest that high doses may injure the kidneys, but that renal effects are not of major concern at lower doses.

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Endocrine Effects. Only limited data were located regarding the effects of methoxychlor on endocrine glands in animals following oral exposure. A decrease in absolute pituitary weight was seen in rats exposed to 200–400 mg/kg/day for 44 weeks or 100–200 mg/kg/day for 11–16 weeks (Gray et al. 1989, 1999). This was, however, associated with a reduction in body weight and, in most cases, a slight increase in relative pituitary weight. In these same studies, Gray et al. (1989) observed an increase in absolute and relative adrenal gland weight in male and female rats exposed to 100–200 mg/kg/day for 11–16 weeks. No treatment-related histopathological effects were seen. No histopathological effects or changes in adrenal gland weight were seen in male or female rats that received up to 1,000 mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). Increased relative and absolute adrenal gland weights were seen in male rats exposed to 150 mg/kg/day methoxychlor from gestation day 14 through postnatal day 42 (Chapin et al. 1997). This effect was not seen in female rats or in male rats at 5 or 50 mg/kg/day. No histopathological data were reported. Adrenal gland weight returned to normal (compared to controls) with cessation of methoxychlor administration (Chapin et al. 1997).

In female laboratory animals, exposure to methoxychlor results in a number of effects involving the reproductive tract and the major hormone-producing glands that regulate it. Atrophic and degenerative changes in the ovaries were seen in female mice and rats exposed to 25–400 mg/kg/day methoxychlor for intermediate durations (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991). Decreased serum progesterone levels were observed in female rats acutely exposed to 50–100 mg/kg/day laboratory grade methoxychlor, but not to 25 mg/kg/day (Cummings and Gray 1989; Cummings and Laskey 1993). Ultrastructural changes in ovarian cells of mice exposed to 100 mg/kg/day for 4 weeks included an accumulation of lipid in the interstitial and thecal cells (Martinez and Swartz 1992). It has been postulated that methoxychlor disrupts the feedback regulation of pituitary hormones that normally exert an effect on the uterus and other estrogen-sensitive tissues (Martinez and Swartz 1992).

Oral exposure to methoxychlor also produces a number of effects in male laboratory animals. A 21% decrease in ventral prostate weight was observed in male rats exposed to 154 mg/kg/day for 90 days (Shain et al. 1977). Decreased weight of the testes was observed in male rats and mice exposed to methoxychlor at 50–1,400 mg/kg/day for intermediate durations (Bal 1984; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962), and regression of the seminiferous epithelium was seen in mice exposed to 60 mg/kg/day for 50 days or more (Wenda-Rozewicka 1983). A dose-related inhibition of testes development, fewer mature germ cells, and fewer germ cells of each type were observed in the testes of male rats exposed to 50 or 150 mg/kg/day methoxychlor from gestation

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day 14 through postnatal day 42 (Chapin et al. 1997). Decreased caudal epididymal sperm counts were observed in rats exposed to 50–400 mg/kg/day for intermediate-duration exposures (Gray et al. 1989, 1999). Exposure to 25–50 mg/kg/day methoxychlor produced increased levels of prolactin in the pituitary of male rats (Goldman et al. 1986; Gray et al. 1989). The hypothalamus of rats exposed to 50 mg/kg/day developed elevated levels of gonadotropin releasing hormone (GnRH) (Goldman et al. 1986). Serum levels of thyroid stimulating hormone (TSH) were decreased in rats following exposure to 100–200 mg/kg/day methoxychlor (Gray et al. 1989). Serum T₃ (triiodothyronine) levels were increased in female rats at 100 and 500 mg/kg/day, and T₃ and TSH levels were increased in male rats exposed to 500, but not 100, mg/kg/day for 28 days (Okazaki et al. 2001). Serum luteinizing hormone (LH) levels were decreased in female rats exposed to 100, but not 20, mg/kg/day methoxychlor, and serum FSH and prolactin levels were increased and testosterone levels were decreased in male rats exposed to 500, but not 100, mg/kg/day (Okazaki et al. 2001). Serum LH, prolactin, testosterone, and interstitial fluid testosterone were unaffected in male rats exposed to 200–400 mg/kg/day for 44 weeks (Gray et al. 1999).

Effects of methoxychlor on the endocrine system are likely related to methoxychlor-associated reproductive disturbances. For example, decreased serum progesterone levels (Chapin et al. 1997; Cummings and Gray 1989; Cummings and Laskey 1993) may result from the estrogenic effects of methoxychlor on the ovaries that cause decreased follicular and corpus luteum development (Chapin et al. 1997). The effects on the ovaries may be caused directly by methoxychlor metabolites or may result from effects on the pituitary and hypothalamus, which alter the release of regulatory hormones that affect the reproductive and accessory sex glands (Chapin et al. 1997; Martinez and Swartz 1992). There is a high level of interdependence between the hypothalamus, pituitary, and peripheral endocrine glands; therefore, feedback mechanisms between the peripheral endocrine glands and the pituitary and hypothalamus and between the pituitary and the hypothalamus are probably also involved in the alteration of hormone levels by methoxychlor. Effects of methoxychlor on the reproductive system are discussed in more detail in Section 3.2.2.5 Reproductive Effects, and mechanisms for effects on the endocrine and reproductive systems are discussed in Section 3.5.2 Mechanisms of Toxicity and Section 3.6 Toxicities Mediated Through the Neuroendocrine Axis.

Dermal Effects. Gross and microscopic examination of the skin did not reveal any treatment related effects in rats or mice chronically orally exposed to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978). These data are too sparse to permit a firm conclusion to be made on the dermal effects of methoxychlor, but suggest that the skin is not a target system.

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Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to methoxychlor. No ocular effects were seen in rats or mice chronically exposed to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978).

Body Weight Effects. Several animal studies suggest that oral exposure to methoxychlor can lead to decreased body weight. Acute exposure of rats to 40.8–400 mg/kg/day or intermediate exposure to 25–400 mg/kg/day resulted in decreased growth (Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989, 1999; Khera et al. 1978). A 14.4% decrease in body weight gain was noted in pregnant rabbits administered 35.5 mg/kg/day methoxychlor, but not 5.01 mg/kg/day methoxychlor (Kincaid Enterprises 1986). Rats exposed to 100–1,000 mg/kg/day methoxychlor for 90 days had a 5–43% decrease in body weight gain (Dikshith et al. 1990). In dogs exposed to 1,000 mg/kg/day for 8–24 weeks, decreased body weight was accompanied by an absence of body fat in normal depot areas (Tegeris et al. 1966). Chronic exposure of rats to 107 mg/kg/day (NCI 1978) or 229 mg/kg/day (Haag et al. 1950) or mice to 599 mg/kg/day (NCI 1978) produced a decrease in body weight gain. Since many of these studies also reported depressed food intake in methoxychlor-treated animals, it is possible that this is responsible, in part, for the observed decreases in body weight gain. However, in studies in which pair-fed controls were used, a 22% decreased body weight was observed in rats fed diets containing 861 mg/kg/day for 33–55 days (Tullner and Edgcomb 1962) or 1,200 mg/kg/day for 16 weeks (Davison and Cox 1976). These data indicate that body weight gain may be adversely affected by relatively high doses of methoxychlor.

Metabolic Effects. No studies were located regarding metabolic effects in animals after oral exposure to methoxychlor.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to methoxychlor. In animals, no adverse histological effects were detected in the thymus, spleen, or lymph nodes of rats chronically exposed to 107 mg/kg/day or mice chronically exposed to 599 mg/kg/day (NCI 1978). However, histological examination may not be sensitive enough to detect changes in immune system function. In male (but not female) rats exposed to 5 or 50 mg/kg/day methoxychlor from gestation day 14 through postnatal day 42, a respective 35 or 42% decrease in plaque-forming cells/spleen was observed, indicating a possible attenuation of the primary immune response (Chapin et al. 1997). The plaque-forming cell assay quantitates the production of a specific antibody following administration of an

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antigen. The physiological functioning of a number of immune system components, including B cells, T helper cells, and macrophages, are reflected in this assay. Thymus weights were decreased by about 15 and 30% in rats exposed to 50 and 150 mg/kg/day, respectively, from gestation day 14 through postnatal day 42 (Chapin et al. 1997). No histological data were provided. Spleen weight, splenic natural killer cell activity, splenic lymphoproliferative response, and splenic cell-surface phenotypes did not differ from controls. The study authors suggested that the observed effects on plaque-forming cell numbers in males may have been anomalous because no such effects were observed in females and no changes were observed in males in related end points such as splenic lymphoproliferative response (Chapin et al. 1997). However, atrophy of the thymus was also observed in male, but not female, rats exposed to 500, but not 100, mg/kg/day methoxychlor for 28 days (Okazaki et al. 2001). Relative thymus weights were lower in female rats exposed to 500, but not 100, mg/kg/day, but the difference did not reach statistical significance (Okazaki et al. 2001). No histological abnormalities were noted. These data suggest that high exposure levels of methoxychlor may affect certain immunological parameters in rodents and that male rats may be more sensitive to the immunological effects of methoxychlor than females. Considering the available data, the immune system does not appear to be a primary target for methoxychlor.

3.2.2.4 Neurological Effects

A single case report documented the ingestion of approximately 125 mL of a commercial product that contained methoxychlor (about 15 mg of methoxychlor) by a 62-year-old man in an attempted suicide (Thompson and Vorster 2000). The man showed no response to pain or verbal stimuli and had pale skin with profuse sweating. Testing of a serum sample collected at the time of admission to the hospital showed a methoxychlor level of 0.67 µg/mL serum.

Neurological effects have been observed in some animals exposed to methoxychlor. Large acute doses of 1,000 mg/kg methoxychlor or more administered to rats produced neurological effects such as decreased locomotor activity, tremors, lacrimation, salivation, nasal bleeding, dyspnea, diarrhea, convulsions, and paralysis (Cannon Laboratories 1976; Dikshith et al. 1990). In dogs, exposure to 1,000–4,000 mg/kg/day methoxychlor for 8–24 weeks produced a dose-dependent increase in neurological effects including apprehension, nervousness, increased salivation, tremors, convulsions, and death (Tegeris et al. 1966). When rats with liver damage (induced by carbon tetrachloride) were exposed to methoxychlor, they exhibited tremors similar to those described above for dogs (Lehman 1952). Assuming that one of the effects of the liver injury is decreased metabolism of methoxychlor, this observation suggests that the

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parent compound (rather than a metabolite) may be mainly responsible for these types of neurological effects. This idea is consistent with the observation that DDT (a poorly metabolized analogue of methoxychlor) also produces similar neurological signs (Agency for Toxic Substances and Disease Registry 1994). An increased incidence (significance not reported) of hunched posture and rough fur was reported in rats exposed to 22–107 mg/kg/day methoxychlor in the feed for 78 weeks (NCI 1978). No changes in brain weight or histopathology were noted in rats or mice chronically exposed to 107 or 599 mg/kg/day methoxychlor, respectively (NCI 1978) or in rats exposed to up to 1,000 mg/kg/day for 90 days (Dikshith et al. 1990).

Exposure to methoxychlor has also produced behavioral changes in animals. Doses of 200 or 400 mg/kg/day administered for 55–66 days produced an increased running wheel activity in ovariectomized and intact female rats, respectively (Gray et al. 1988). In rats, running wheel activity is regulated by estrogen and is synchronous with the 5-day estrus cycle, being greater during proestrus than during estrus and diestrus. Ovariectomy reduces running wheel activity and abolishes cyclicity. Administration of progesterone to methoxychlor-treated ovariectomized rats significantly lowered running wheel activity (Gray et al. 1988). Because progesterone blocks the synthesis of estrogen receptors in the central nervous system and reproductive tract but does not lower running wheel activity induced by nonestrogenic mechanisms, the authors concluded that the observed inhibition of methoxychlor-induced running wheel activity by progesterone was evidence of an estrogenic effect of methoxychlor on the central nervous system.

The administration of 200 mg/kg/day methoxychlor for 2 weeks also increased receptivity to mating in ovariectomized hamsters (Gray et al. 1988). Behavioral estrus remained in more than half of the hamsters at 1 week post-exposure. Although the magnitude of the effects of methoxychlor on mating receptivity in ovariectomized hamsters was not as great as that observed with estradiol (1.0 mg/kg), the effects persisted longer following cessation of exposure (Gray et al. 1988). Nine of 14 hamsters exposed to methoxychlor for 13 days displayed lordosis behavior, and 15/15 hamsters exposed to weekly administration of estrogen displayed lordosis behavior. Neurobehavioral effects were also observed following exposure of rats to 5, 50, or 150 mg/kg/day methoxychlor from gestation day 14 through postnatal 21 (Chapin et al. 1997). Male rats treated with 150 mg/kg/day, but not 5 or 50 mg/kg/day, showed an increased handling reactivity. Non-dose-related but statistically significant changes were seen for sensorimotor parameters: locomotor activity (increased in females in the 50 mg/kg/day group, but not at 5 or 150 mg/kg/day), click response (decreased in males at 5 and 150 mg/kg/day, but not 50 mg/kg/day, and in females at 5 mg/kg/day, but not 50 or 150 mg/kg/day), and approach stimulus (lower in males at 5 mg/kg/day, but

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not 50 or 150 mg/kg/day). Temporary but statistically significant changes that occurred at various times during the study included increased urination in the open field in females at 50 and 150 mg/kg/day, but not 5 mg/kg/day on postpartum day 66, increased defecation in females at 150 mg/kg/day, but not 5 or 50 mg/kg/day on postpartum day 31, and decreased hindlimb grip strength in females at 50 mg/kg/day, but not 5 or 150 mg/kg/day on postpartum days 47 and 66. There were no differences between the controls and treatment groups in the passive avoidance/cognitive function tests.

An increase in urine-marking behavior was observed in adult male offspring of mouse dams exposed to 0.02 mg/kg/day on gestation days 11–17 (vom Saal et al. 1995). This experiment is also described in Parmigiani et al. (1998). Parmigiani et al. (1998) also observed a statistically significantly increased incidence of aggressive behavior (infanticide) toward unrelated pups in adult male offspring of mice treated with 1.8 mg/kg/day during gestation days 11–17, but not at lower (0.02 or 0.18 mg/kg/day) or higher (18.2 or 90.0 mg/kg/day) exposure levels. Exposure to 0.02–182 µg/kg/day of the potent estrogen, DES, had no effect on this behavior. However, limitations in the study designs of vom Saal et al. (1995) and Parmigiani et al. (1998) make these studies difficult to interpret, (please refer to Appendix A for a more detailed discussion of the study limitations). Aggressive grooming behavior was altered in a non-monotonic fashion in male mice orally exposed *in utero* to 0.02, 0.2, or 2.0 mg/kg/day methoxychlor (Palanza et al. 1999). This behavior was statistically significantly decreased in the 0.02 mg/kg/day group, and was increased and decreased in the 0.2 and 2.0 mg/kg/day groups, respectively, but without statistical significance. This change in behavior was only seen on day 39, but not on day 54, postpartum. Unlike the neurological effects (tremors, convulsions) discussed above, these behavioral effects of methoxychlor (increased running wheel activity and receptivity to mating, urine-marking, infanticide), which have been shown previously to be influenced by estrogen or testosterone, are most likely attributable to one or more of the metabolites or contaminants of methoxychlor that exhibit estrogenic or anti-androgenic activity.

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

3.2.2.5 Reproductive Effects

A single study was located regarding effects of oral exposure to methoxychlor on reproductive tissues in humans. Electron microscopic examination of biopsy samples from the testes of men exposed to 2 mg/kg/day (three men) for 6 weeks revealed no histopathological changes from controls (three men)

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(Wills 1969). In addition, no effects on menstrual cycles were noted in similarly dosed women at 0.5, 1.0, or 2.0 mg/kg/day (four per exposure group).

The reproductive effects of methoxychlor have been well studied in animals. These studies indicate that the reproductive system is a sensitive target of methoxychlor toxicity in both males and females. Effects associated with methoxychlor exposure include histopathological changes in the reproductive organs and accessory glands, impaired pubertal development and reproductive function, and altered hormone levels. These effects are due to the estrogenic activity of both the *O*-demethylated metabolites of methoxychlor and some of the *O*-demethylated contaminants of technical grade methoxychlor (Bulger et al. 1985). Information regarding the reproductive effects of methoxychlor in male and female animals is presented separately below.

Reproductive Effects in Female Animals. Methoxychlor adversely affects the development of the female reproductive system. The day of vaginal opening and first estrus was significantly earlier in female rats exposed to 25–100 mg/kg/day methoxychlor beginning on postpartum day 21 and extending through mating, gestation, and lactation (Gray et al. 1989). In these experiments, vaginal opening occurred at an average age of 32 days in controls, but in exposed groups, occurred as early as 3 days after beginning exposure. Average age at vaginal opening ranged from 24 to 26 days in the exposed groups. The onset of estrus cyclicity was accelerated in animals exposed to 25 mg/kg/day and normal in animals exposed to 50–100 mg/kg/day. The onset of estrus cyclicity was delayed in animals dosed with 200 mg/kg/day. Similarly, precocious vaginal opening and estrus were noted in female rats exposed *in utero*, during lactation and postweaning to 5–150 mg/kg/day (Chapin et al. 1997; Harris et al. 1974). In female rats exposed to 5, 50, or 150 mg/kg/day during gestation days 14–21, via lactation during postpartum days 1–7, and then directly via oral gavage through postpartum day 42 (dams were no longer treated after postpartum day 7 and pups stayed with the dams until postpartum day 21), vaginal opening was accelerated by 2–7 days (35.2, 30.8, and 33.4 days postpartum in the 5, 50, and 150 mg/kg/day groups, respectively, compared to 37.4 days for the controls) (Chapin et al. 1997). Female rats exposed *in utero* throughout gestation to 0, 60, or 150 mg/kg/day methoxychlor and maintained on the same oral dose postnatally had earlier vaginal opening than controls (23 and 19 days postpartum in the 60 and 150 mg/kg/day groups, respectively, compared to 39 days for the controls) (Harris et al. 1974). Fertility was significantly impaired in females exposed to 50 or 150 mg/kg/day, but not to 5 mg/kg/day, between gestation day 14 and postnatal day 42 (Chapin et al. 1997). Incidences for females that became pregnant after cohabitating with untreated males were 13/15 (control), 11/15 (5 mg/kg/day), 3/15 (50 mg/kg/day),

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and 0/15 (150 mg/kg/day) (Chapin et al. 1997). Descriptions of developmental effects can be found in greater detail in Section 3.2.2.6 Developmental Effects.

Methoxychlor produces gross and histopathological changes in the mature female reproductive system after oral exposure. An accumulation of lipid was observed in ovarian interstitial and thecal cells of mice exposed to 100 mg/kg/day for 4 weeks (Martinez and Swartz 1992). Cellular hypertrophy of the uterine epithelial cells was observed in adult female mice exposed to 50 or 100 (but not 2, 10, or 20) mg/kg/day methoxychlor (as Marlate, a 50% mixture; contaminants unknown) 5 days/week for 4 weeks; this effect was similar to the response to estradiol (Swartz et al. 1994). Electron microscopic examination revealed dose-related ultrastructural changes, including one or more of the following at all dose levels: elongated microvilli, decreased numbers of microvilli, increased vacuoles, dilated endoplasmic reticulum and Golgi complexes, enlarged and disrupted mitochondria, and irregular apical cell surface. A marked uterotrophic effect (as indicated by a 2–3-fold increase in uterine weight) was observed in ovariectomized mice exposed to 16.7 mg/kg/day and in ovariectomized plus adrenalectomized rats and hypophysectomized rats exposed to 95.2 mg/kg/day for 3 days (Tullner 1961). From the time of vaginal opening until mating, increased vaginal cornification and a decreased percentage of vaginal smears with leukocytes was observed in female rats acutely exposed to 50–200 mg/kg/day pre- and postnatally (Chapin et al. 1997) or beginning on postpartum day 21 (Gray et al. 1989) or exposed to 400 mg/kg/day for intermediate durations (Gray et al. 1988). An enlarged uterus was observed in rats exposed to 150 mg/kg/day for 6 weeks (Harris et al. 1974) or 100 or 500 mg/kg/day for 28–31 days (Okazaki et al. 2001) and in pigs exposed to 1,000 mg/kg/day for 24 weeks (Tegeris et al. 1966). However, absolute uterine weight was reduced by 35% (and relative uterine weight by 27%) in intact virgin female rats exposed to 150 mg/kg/day from gestation day 14 through postnatal 42 (Chapin et al. 1997). This effect was even more pronounced on the pregnant uterus (21–50% reduction in weight of empty uterus at exposures of 5–50 mg/kg/day) (Chapin et al. 1997). Mice treated initially with ENU (a uterine cancer initiator) and then administered 390 mg/kg/day methoxychlor for 26 weeks had no increased incidence of tumors, but had increased absolute and relative uterine weights (Mitsumori et al. 2000). Mammary gland hyperplasia was also observed in rats exposed to 500, but not 100, mg/kg/day for 28–31 days (Okazaki et al. 2001) or pigs exposed to 1,000 mg/kg/day for 24 weeks (Tegeris et al. 1966), and decreased ovarian weight was reported in adult rats exposed to 500, but not 100, mg/kg/day methoxychlor for 90 days (Dikshith et al. 1990). Intermediate-duration exposures to 25–500 mg/kg/day methoxychlor produced atrophic changes in the ovaries of exposed female mice and rats (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991; Okazaki et al. 2001). Some of these effects mimic those of estrogen, but some differ from estrogenic effects. Methoxychlor (60–300 mg/kg/day) and estradiol

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benzoate (0.015–0.075 mg/kg/day) resulted in decreased mating frequency and ovarian weight, increased uterine weight, and precocious vaginal opening, whereas only methoxychlor caused increased estrus (Harris et al. 1974). In adult ovariectomized female rats in which endometriosis had been surgically induced, administration of 250 mg/kg/day methoxychlor for 3 weeks resulted in maintenance of endometriotic sites, similar to that seen following administration of estrone (1 µg/rat), and also similar to intact rats (endogenous estrogen present) in which endometriosis had been induced (Cummings and Metcalf 1995b). Ovariectomized vehicle control rats (no endogenous or exogenous estrogen present) showed a substantial regression in size of endometriotic sites. These data indicate that estrogens maintain, or prevent regression of, endometriotic sites, and that sufficient levels of methoxychlor produce results similar to those of estrogen.

Oral exposure to methoxychlor adversely affects reproductive function in mature females by interfering with estrus cyclicity and decreasing fertility. Female rats exposed to 500, but not 100, mg/kg/day methoxychlor for 28–31 days had significantly fewer estrus cycles and remained in estrus longer than controls (Okazaki et al. 2001). Rats that received 100 mg/kg/day methoxychlor showed a nonsignificant decrease in the number of estrus cycles (Okazaki et al. 2001). Persistent vaginal cornification (sometimes referred to as persistent estrus) was observed in female mice acutely exposed to 25 mg/kg/day (Martinez and Swartz 1991) and in female rats exposed to 300–400 mg/kg/day for intermediate durations (Gray et al. 1988; Harris et al. 1974). Female pigs exposed to 1,000 mg/kg/day for 24 weeks failed to come into estrus during the exposure period (Tegeris et al. 1966). Female mice exposed to a wide range of doses of methoxychlor (0.02, 0.2, 2, 20, or 100 mg/kg/day) on gestational days 11–17 showed no decrease in number of pups/litter, and in fact showed an increase at 0.2 mg/kg/day (Palanza et al. 2001). The study authors interpreted the non-monotonic response as an overall lack of effect on number of pups/litter. Female rats exposed to 100 mg/kg/day methoxychlor beginning on postpartum day 21 showed a 40% decrease in fertility and live pups/litter when mated with untreated males and an 80% decrease when mated with similarly treated males (Gray et al. 1989). Higher doses (200 mg/kg/day) produced infertility in 100% of the animals. Females that did not become pregnant had no implantation sites indicating that the effect occurs prior to implantation. Significant decreases in fertility or complete infertility occurred in female rats following intermediate-duration exposure to 100 mg/kg/day (Bal 1984) or to 50 or 150 mg/kg/day (Chapin et al. 1997). With chronic-duration exposure, decreases in fertility were noted in the second and third generation male and female rats exposed to 79 and 92 mg/kg/day methoxychlor, respectively, for three generations (Haskell Laboratories 1966); no effects were seen in rats exposed to 18 mg/kg/day for 3 generations. These decreases were predominantly attributed to effects on female animals rather than male animals. No effects on fertility were noted in rats similarly treated with

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16 (males) or 18 (females) mg/kg/day methoxychlor. In female rats, a decreased mating frequency and a decreased fertility in those that mated were noted following intermediate-duration exposure to 60–150 mg/kg/day (Harris et al. 1974). An increased number of resorptions have been consistently reported in female rats following acute- and intermediate-duration exposure to 35.5–200 mg/kg/day laboratory grade and technical grade methoxychlor (Culik and Kaplan 1976; Cummings and Gray 1989; Cummings and Perreault 1990; Gray et al. 1989; Harris et al. 1974; Kincaid Enterprises 1986).

The effects of methoxychlor on uterine decidualization (the buildup of the uterine lining necessary for implantation and pregnancy) vary with exposure level. Acute exposures to 100–250 mg/kg/day laboratory grade methoxychlor in female rats reportedly inhibit the decidual growth of the uterus (Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Cummings and Perreault 1990). Further investigation by Cummings (1993) showed that methoxychlor acts as an estrogen to induce uterine decidualization only over a narrow exposure range. The artificially induced decidual cell response in the rodent mimics the implantation of the blastocyst and induces the development of the uterine decidual tissue. This response assay was used by Cummings (1993) to investigate the estrogenicity of methoxychlor and its mechanism of reducing fertility in female rats. Estrogen and progesterone are required to induce the decidualization of the uterus. In ovariectomized female rats (used because no endogenous estrogen or progesterone is present and pseudopregnancy can be initiated with exogenous estrone and progesterone administration), exposure to low doses (5 or 50 mg/kg/day) of laboratory grade methoxychlor plus 2 mg progesterone for 8 days produced the same degree of decidualization as in controls (progesterone alone). Higher doses (75 or 100 mg/kg/day) of methoxychlor plus progesterone produced maximal decidualization similar to estrone (0.004 mg/kg/day) plus progesterone. Decidualization response decreased with further increased methoxychlor dose (500 mg/kg/day) plus progesterone (Cummings 1993). Such a decrease in decidualization might contribute to preimplantation loss. Methoxychlor also has been shown to accelerate embryo transport into the uterus, which may further contribute to increases in preimplantation loss (Cummings and Laws 2000; Cummings and Perreault 1990). These findings provide insight into the possible mechanisms by which methoxychlor reduces fertility in female rats.

Exposure to methoxychlor produces changes in a number of hormone levels in female animals after oral exposure. Decreased serum progesterone levels were observed in female rats acutely exposed to 50–500 mg/kg/day laboratory grade methoxychlor, but not to 25 mg/kg/day (Cummings and Gray 1989; Cummings and Laskey 1993). Serum estradiol levels were not altered (Cummings and Laskey 1993). When ovaries from methoxychlor-treated rats were removed and incubated with human chorionic

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gonadotropin (to stimulate steroid production and release), ovarian progesterone concentration was unaffected, but ovarian estradiol and testosterone concentrations were significantly reduced (Cummings and Laskey 1993). Pituitary levels of prolactin were decreased in intact female rats but increased in ovariectomized rats exposed to 400 mg/kg/day for intermediate-durations (Gray et al. 1988). In female rats dosed with 100 mg/kg/day methoxychlor in oil by gavage for 4 weeks, a number of ultrastructural changes were noted in the ovarian cells, including the accumulation of lipid (Martinez and Swartz 1992). Similar effects were seen in rats exposed to 1 mg/kg/day estradiol-17 β . The authors suggested that these cells retained their ability to synthesize lipids but have lost the ability to convert lipids to steroid hormones. The changes in hormone levels discussed above may also be an important mechanism by which methoxychlor can affect female reproduction. Martinez and Swartz (1992) speculated that methoxychlor causes a feedback inhibition of pituitary hormone secretions resulting in a lack of stimulation of ovarian cells to produce hormones that would have otherwise exerted an effect on the uterus and other estrogen-sensitive tissues.

An intermediate-duration oral MRL of 0.005 mg/kg/day was derived based on the LOAEL of 5 mg/kg/day for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning (Chapin et al. 1997), as described in the footnote in Table 3-1.

Reproductive Effects in Male Animals. Methoxychlor adversely affects the development of the male reproductive system. Preputial separation was significantly delayed in a dose-dependent manner (days 40–42 in controls, day 43.8 in the 100 mg/kg/day methoxychlor group, days 50–53 in the 200 mg/kg/day, day 68 in the 300 mg/kg/day group, and day 74 in the 400 mg/kg/day group) in male rats exposed to 100–400 mg/kg/day beginning on postpartum day 21 for 56 days to 10 months (Gray et al. 1989, 1999), suggesting that sexual maturity was delayed. Male fertility in rats exposed to methoxychlor from gestation day 14 to postpartum day 42 was significantly impaired at 150 mg/kg/day, but not at 5 or 50 mg/kg/day; only 2/15 150 mg/kg/day rats successfully impregnated untreated females, compared with 13/15 in the control group (Chapin et al. 1997). Gray et al. (1989) also observed a slight decrease in testes weight and caudal epididymal sperm count in male offspring of rats exposed to 50–200 mg/kg/day methoxychlor for 59 days. Welshons et al. (1999) observed a substantial increase in prostate weight in adult male mice exposed *in utero* (gestation days 11–17) to 0.02 and 2.0 mg/kg/day, and seminal vesicle weight was increased in a dose-dependent manner. Preputial gland, testes, and adrenal gland weights were not significantly affected. Lateral, but not ventral, prostate weight was increased by 44%, and inflammation was evident in male rats exposed to 50 mg/kg/day methoxychlor from gestation

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day 18 through postpartum day 5 (Stoker et al. 1999). In a simultaneous experiment, lateral prostate inflammation was also seen in male rats exposed to estradiol-17 β from gestation day 18 through postpartum day 5 (dams received 134 μ g on gestation day 18–22, then pups received 6.7 μ g on postpartum days 1–5), but lateral prostate weight was decreased by 59% (Stoker et al. 1999). Testis weight was not affected. Mating frequency and fertility in rats were decreased in male offspring of rats exposed to 60 mg/kg/day methoxychlor (Harris et al. 1974). In addition to structural and functional effects, urine-marking behavior and infanticide behavior toward unrelated pups were altered in male offspring of mice exposed to 0.02–91 mg/kg/day methoxychlor *in utero* (Parmigiani et al. 1998; vom Saal et al. 1995). These reproductive/developmental effects are discussed in more detail in Section 3.2.2.6 Developmental Effects.

Oral exposure to methoxychlor can produce gross and histopathological changes in the mature male reproductive system. Decreased weight of the testes was observed in male rats and mice exposed to 50–1,400 mg/kg/day for intermediate-durations (Bal 1984; Dikshith et al. 1990; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962; Wenda-Rozewicka 1983). In addition, a decreased ventral prostate weight was observed in male rats exposed to 154 mg/kg/day for 90 days (Shain et al. 1977) or 100 mg/kg/day for 28 days (Okazaki et al. 2001). Atrophy of the dorso-lateral prostate was also seen in rats following exposure to 500 mg/kg/day for 28 days (Okazaki et al. 2001). Three studies reported a decreased caudal epididymal sperm count in rats exposed to 50–500 mg/kg/day for intermediate-duration exposures (Gray et al. 1989, 1999; Okazaki et al. 2001). No gross or histopathological changes were noted in the testes of male mice acutely exposed to 60 mg/kg/day (Wenda-Rozewicka 1983) or 200–400 mg/kg/day (Gray et al. 1999). A dose-related inhibition of testes development, fewer mature germ cells, and fewer germ cells of each type were observed in the testes of male rats exposed to 5, 50, or 150 mg/kg/day from gestation day 14 through postnatal day 42 (Chapin et al. 1997). Additionally, testis, epididymis, seminal vesicle, and prostate weights were decreased by 23–85% in males exposed to 50 or 150 mg/kg/day (Chapin et al. 1997) or to 100 or 500 mg/kg/day for 28 days (Okazaki et al. 2001). Decreases in prostate weight have also been seen following exogenous estradiol administration (Rajfer and Coffey 1978; Stoker et al. 1999; vom Saal et al. 1997).

Exposure to methoxychlor by the oral route adversely affects reproductive function in mature male animals. Fertility was decreased by 80% when males exposed to 100 mg/kg/day from postpartum day 21 until necropsy (on postpartum days 77–87) were mated with similarly treated females, compared to only a 50% decrease when untreated males were mated with treated females (Gray et al. 1989). Since pseudopregnancies were not observed in females that were mated with methoxychlor-treated males, the

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authors concluded that treated males failed to provide sufficient copulatory stimulation to induce changes in the females necessary for pregnancy (Gray et al. 1989). Fertility was decreased by 50–75% when males exposed to 200–400 mg/kg/day methoxychlor from postpartum day 21 until necropsy at 11 months of age were mated with untreated females (Gray et al. 1999). Decreased fertility was also reported in male mice exposed to 60 mg/kg/day for intermediate durations (Wenda-Rozewicka 1983) and in male rats exposed to 150 mg/kg/day from gestation day 14 through postnatal day 42 (Chapin et al. 1997). In addition, mating frequency and fertility in those that mated were significantly reduced in the male rats exposed *in utero*, during lactation, and postweaning to 60 mg/kg/day (Harris et al. 1974) or 200–400 mg/kg/day (Gray et al. 1999). These data collectively indicate that methoxychlor can decrease fertility in male animals and that the reduced fertility in male animals may be due to impaired mating behavior, inadequate stimulation of the female animal, or deficits in other reproductive parameters (decreased sperm count, testicular atrophy).

Methoxychlor also produces changes in hormone levels in male animals after oral exposure. Changes in hormone levels often accompany or precede the reproductive effects discussed above. Exposure to 25–50 mg/kg/day methoxychlor produced increased levels of prolactin, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) in the pituitary of male rats (Goldman et al. 1986; Gray et al. 1989; Stoker et al. 1999); serum prolactin levels were non-statistically significantly increased at 200 mg/kg/day and serum FSH levels were not affected (Gray et al. 1989). Prolactin levels in adult male rats were not affected by estradiol administration during gestation (gestation days 18–22) and postpartum days 1–5 (Stoker et al. 1999). The hypothalamus of rats exposed to 50 mg/kg/day developed elevated levels of gonadotropin releasing hormone (GnRH) (Goldman et al. 1986). Serum levels of several hormones (TSH, testosterone, progesterone) were decreased in rats as a result of exposure to doses of 100 mg/kg/day laboratory grade or technical grade methoxychlor (Cummings and Gray 1989; Gray et al. 1989), although serum LH, prolactin, testosterone, and interstitial fluid testosterone were unaffected in male rats exposed to 200–400 mg/kg/day for 44 weeks (Gray et al. 1999). Similarly, reduced levels of testosterone were reported in the interstitial fluid and epididymis of male rats exposed to 100 mg/kg/day from postpartum day 21 to postpartum day 77 (Gray et al. 1989). Effects of methoxychlor on the endocrine system are likely related to some of the histopathological, functional, and behavioral changes described above (see Section 3.5.2, Mechanisms of Toxicity for further discussion).

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

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3.2.2.6 Developmental Effects

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

In animals, exposure to methoxychlor during gestation can produce signs of maternal and fetal toxicity. The incidence of offspring with wavy ribs was significantly increased in rats exposed to 40.8 mg/kg/day or more methoxychlor on days 6–15 of gestation. This effect was also noted in rats administered 17.8 mg/kg/day, however, the increase (2–3-fold) was not statistically significant (Culik and Kaplan 1976). Acute doses of 50 mg/kg/day or more caused a decreased body weight in pregnant rats, whereas doses of 200 mg/kg/day produced an increased percentage of dead, resorbed, or anomalous fetuses and decreased fetal weight (Khera et al. 1978). The anomalies also consisted primarily of wavy or extra ribs. The skeletal effects were judged by the authors to be due to delayed development of alkaline phosphatase activity and to arrested calcium deposition in the ribs due to the toxicity of methoxychlor, and were not considered to be true signs of teratogenicity. Fetal body weights were decreased by 10% when pregnant rabbits were administered 35.5 mg/kg/day methoxychlor on days 7–19 of gestation (Kincaid Enterprises 1986). In addition the percentage of offspring which were male was significantly decreased. These effects were not observed in rabbits administered 5.01 mg/kg/day methoxychlor (Kincaid Enterprises 1986).

Methoxychlor can adversely affect the reproductive development of rats and mice exposed *in utero*, during lactation, or after weaning. Effects of postweaning exposure on reproductive development are discussed in Section 3.2.2.5 Reproductive Effects. No effects were detected on reproductive development in male and female rats acutely exposed *in utero* (gestation days 14–20) to 30 mg/kg/day methoxychlor (Gellert and Wilson 1979). Female offspring of rats exposed for intermediate durations to 25 mg/kg/day methoxychlor from weaning through gestation and lactation exhibited precocious vaginal opening, and at 50 mg/kg/day also exhibited abnormal estrus cycling and pituitary abnormalities (Gray et al. 1989). Precocious vaginal opening and delayed prepuce separation were also seen in female and male offspring, respectively, of rats exposed to 50 or 150 mg/kg/day methoxychlor from gestation day 14 through postnatal day 7, followed by direct exposure of the pups through postnatal day 42 (Chapin et al. 1997). Male offspring had slightly decreased testes weight and caudal epididymal sperm count compared to controls, but these changes were not statistically significant (Gray et al. 1989).

A substantial and statistically significant increase in prostate weight (61% at 0.02 mg/kg/day and 51% at 2.0 mg/kg/day) was seen in adult (9.5 months of age) male offspring of CF-1 mice exposed to 0, 0.02, and

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2.0 mg/kg/day methoxychlor on gestation days 11–17 (Welshons et al. 1999), and seminal vesicle weight was increased by 20% in the 2.0 mg/kg/day group. Although no positive control was included in this study, the methoxychlor-induced prostate enlargement was greater than the enlargement observed in previous studies by the same investigators examining effects from gestational exposure to other estrogen receptor ligands (estradiol or DES) (vom Saal et al. 1997). There were no statistically significant exposure-related changes in body weight or weights of preputial glands, testes, or adrenals. These findings are in agreement with studies showing increased prostate weight in adult mice exposed to slightly elevated levels of estrogen perinatally (Nonneman et al. 1992; Timms et al. 1999; vom Saal 1989; vom Saal et al. 1997) and are consistent with the estrogenic activity of some methoxychlor metabolites. Only a small number of mice were assessed (one pup/litter from five to six litters) in this study. A more detailed analysis of Welshons et al. (1999) is presented in the Acute MRL Worksheet in Appendix A of this profile. Additionally, prostate weight in adult mice has been shown to be affected by intrauterine position of the fetus. Male mice that are between two female mice during *in utero* development have about a 35% higher serum level of estradiol due to diffusion from the adjacent females and have larger, heavier adult prostates than male mice that develop between two male mice (Nonneman et al. 1992; Timms et al. 1999; vom Saal 1989). Low levels of exogenous estrogen and estrogenic substances can also result in increased adult prostate weights (Rajfer and Coffey 1978; vom Saal et al. 1997). Higher levels of these same substances result in decreased adult prostate weights (Rajfer and Coffey 1978; vom Saal et al. 1997; Welshons et al. 1999). The exposure-response curve for this effect of estrogens takes on an inverted U-shape. This type of dose-response curve has also been reported for prostate weight following exposure to DES (vom Saal et al. 1997). U-shaped curves are recognized curvilinear dose-response relationships in toxicological and epidemiological studies, especially at low doses in the threshold region of response (Davis and Svendsgaard 1990). Inflammation, and a corresponding weight increase, of the lateral prostate was observed in male offspring of rats exposed to 50 mg/kg/day methoxychlor from day 18 of gestation to postpartum day 5 (Stoker et al. 1999). No difference was noted in body weight, ventral prostate weight, testes weight, testosterone level, mean serum prolactin, or prostate deoxyribonucleic acid (DNA) levels. Reductions in testes, epididymis, seminal vesicle, and ventral prostate weights were seen in male offspring of rats exposed to 50 or 150 mg/kg/day from gestation day 14 through postnatal day 7, followed by direct exposure of the pups through postpartum day 42 (Chapin et al. 1997). Some *in vitro* data also suggest that methoxychlor and its metabolite, bis-hydroxy methoxychlor (HPTE), can alter rat embryonic testis development (Cupp and Skinner 2001).

Breeding of rats (exposed *in utero* and through lactation to 50 mg/kg/day methoxychlor, but not exposed thereafter) resulted in a 30% decrease in fertility and a significant reduction in the number of pups/female

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and litters/female (Gray et al. 1989). Treated F1 females mated with treated F1 males had 0, 0, 80, and 100% decreases in fertility in the 25, 50, 100, and 200 mg/kg/day groups, respectively. Treated F1 females mated with untreated F1 males had 50 and 0% fertility, respectively, compared to 83% for controls. The fertility of treated males was not reduced when they were mated with untreated females. These data suggest that the decrements in fertility in the offspring were most likely attributed to effects in the female. Similar results were reported by Harris et al. (1974) in which pregnant female rats were exposed to 60–300 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation. After weaning, pups were maintained on the same treatment as the dams. The onset of puberty was accelerated in female offspring exposed to 60 mg/kg/day. Both males and females exhibited a decreased mating frequency and a decreased fertility in those animals that mated.

A number of reproductive abnormalities were observed in male and female rats that were exposed to 50 or 150 (but not 5) mg/kg/day from gestation day 14 through postpartum day 42 (Chapin et al. 1997). Female rats in both exposure groups had highly irregular or absent estrus cycles, and there was a severe reduction in the number of females that conceived when mated to nontreated males. In females that did conceive, a severe reduction in the number of implants was seen. This was accompanied by an increased incidence of endometrial squamous metaplasia, endometrial hyperplasia, vaginal cornification (an estrogenic response), vaginal epithelial hyperplasia, underdeveloped mammary tissue, and atrophied ovaries with little or no detectable follicular development and few or no corpora lutea. The serum estrogen:progesterone ratio was elevated, which the authors attributed to a lack of progesterone due to absence of ovulation and corpora lutea formation, and the FSH level was decreased. Males exposed to 50 or 150 mg/kg/day from gestation day 14 through postnatal day 42 also displayed reproductive deficits including significantly decreased ability to impregnate untreated females. Fewer nontreated females had detectable sperm in their vagina when mated with male offspring exposed to 150 mg/kg/day, and sperm motility, epididymal sperm count, and testicular spermatid numbers were reduced in these male rats (Chapin et al. 1997).

The offspring of mice (F1a) exposed to 50 mg/kg/day methoxychlor on days 6–15 of gestation and through lactation had an increased incidence of atretic follicles (Swartz and Corkern 1992). In addition, a second (unexposed) litter (F1b) produced following weaning of the F1a offspring exhibited precocious vaginal opening. It is unclear why the F1a exposed litter did not experience precocious vaginal opening and by what mechanism this effect was produced in the unexposed F1b offspring.

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Effects on neurobehavioral development were also observed following exposure of rats to 5, 50, or 150 mg/kg/day from gestation day 14 through postnatal 21 (Chapin et al. 1997). Male rats treated with 150 mg/kg/day showed an increased handling reactivity. Non-dose-related but statistically significant changes were seen for sensorimotor parameters: locomotor activity (increased in females in the 50 mg/kg/day group), click response (decreased in males and females at 5 mg/kg/day and males at 150 mg/kg/day), and approach stimulus (lower in males at 5 mg/kg/day). Temporary but statistically significant changes that occurred at various times during the study included increased urination in the open field in females at 50 and 150 mg/kg/day, increased defecation in females at 150 mg/kg/day, and decreased hindlimb grip strength in females at 50 mg/kg/day. There were no differences between the controls and treatment groups in the passive avoidance/cognitive function tests. Male and female rats exposed *in utero*, via lactation, and then directly in the feed to 98, but not 9.8, mg/kg/day had increased intake of a sodium solution (an estrogen-responsive behavior) on postpartum days 69–75 (Ferguson et al. 2000).

A series of recent studies examined behavioral effects in adult mice after exposure to low levels of methoxychlor or other estrogenic agents during critical *in utero* developmental periods. A dose-dependent increase in urine-marking behavior was observed in adult male offspring of mice exposed to 0.02–91 mg/kg/day methoxychlor on gestation days 11–17 (vom Saal et al. 1995; also reported in Parmigiani et al. 1998). However, only two male pups/litter were tested, and each pup was tested only one time. It was also difficult to assess the accuracy of the measurement of the number of urine marks and to know how reproducible the results are, and there was no indication of what, if any, statistical methods were used to evaluate the results. The low level of exposure in this study was considered by the authors to be more reflective of the physiological levels at which hormonal effects from steroid receptor binding alone occur, without more wide ranging toxic effects caused by high levels of the (estrogenic) chemical. Urine-marking behavior is known to be influenced by testosterone levels and the male mouse's social status, and urine marking also influences the social and reproductive behaviors of other mice, both male and female, that come in contact with deposited urine (Parmigiani et al. 1998). Therefore, changes in urine-marking behavior may disrupt reproductive behaviors. This group of investigators showed that *in utero* exposure to low levels of other estrogenic chemicals, such as diethylstilbestrol (DES) and *o,p*-DDT, also increased the rate of depositing urine marks by mature male mice when they were placed in novel environments (Palanza et al. 1999). An increase in aggressive behavior (infanticide) toward unrelated pups was also observed in adult male offspring of mice treated with 1.8 (but not 0.02, 0.18, 18.2, or 91) mg/kg/day methoxychlor during gestation days 11–17 (Parmigiani et al. 1998). This behavior was not affected by 0.02–182 µg/kg/day DES. The significance of these results were difficult to

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determine due to the lack of biological information on the strain of mouse used in the study (“house mouse”), the small number of pups tested per litter (two males and two females), and the lack of dose-response (the effect was only seen at one middle dose). These socio-sexual behaviors (urine-marking and infanticide) in mice have been proposed to be influenced by similar neuroendocrine and genetic mechanisms that may not always exhibit monotonic dose-response relationships (Palanza et al. 1999; Parmigiani et al. 1998).

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

3.2.2.7 Cancer

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

Evidence concerning the carcinogenicity of methoxychlor from animal studies is mainly negative, although this is somewhat controversial. The National Cancer Institute (NCI) conducted a study in which mice and rats were fed up to 599 and 107 mg/kg/day methoxychlor, respectively, for 78 weeks. The types and incidences of tumors in methoxychlor-fed animals did not differ significantly from control animals (NCI 1978). NCI concluded that methoxychlor was not carcinogenic to mice or rats under the conditions of this assay. Recrystallized methoxychlor was not carcinogenic to rats when administered at doses of 77 mg/kg/day for 24–27 months, either alone or in combination with other chemicals (aldrin, aramite, DDT, and thiourea) (Deichmann et al. 1967). Similar results were reported by Radomski et al. (1965) with doses of 4 mg/kg/day. Hodge et al. (1952) reported that the tumors observed in methoxychlor-fed rats exposed to up to 80 mg/kg/day for 2 years occurred at a similar frequency as controls. Pituitary tumors were observed in the female rats exposed *in utero* to 50 mg/kg/day (Gray et al. 1989), but data regarding tumor incidence were not provided in this study. Adult female mice heterozygous for the *p53* allele that received a single intraperitoneal injection of N-ethyl-N-nitrosourea (ENU) to induce uterine tumor formation showed no enhancement of endometrial stromal sarcoma or atypical hyperplasia induction, whereas ethinylestradiol enhanced the development of ENU-induced uterine tumors (Mitsumori et al. 2000).

The data from the cancer studies discussed above (Deichmann et al. 1967; Hodge et al. 1952; NCI 1978; Radomski et al. 1965), in addition to data from a number of unpublished studies by the FDA, were

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examined and interpreted in a series of reports by Reuber (1979a, 1979b, 1980). Reuber (1979a) re-examined the histological slides from an unpublished FDA study, and reported an increased incidence of liver carcinomas (3/9 females and 3/8 males, compared to none observed in controls) in Osborne-Mendel rats fed 100 mg/kg/day for 2 years. In addition, Reuber reported an incidence of 1/10 and 5/10 for ovarian tumors in female rats fed 5 and 25 mg/kg/day, respectively, for 2 years, although data concerning ovarian tumor incidence in control animals or in animals at higher doses was not provided. The incidence of other tumors was similar in both treated and control animals (Reuber 1979a). In a re-analysis of data from another unpublished FDA study, Reuber (1979b) reported an increased incidence of testicular tumors (27/51) in male Balb/c mice exposed to 97.5 mg/kg/day methoxychlor in the feed for 2 years compared to an incidence of 8/71 in controls. In addition, the tumors observed in treated animals were reported to be larger, less differentiated and more invasive than those observed in control animals (Reuber 1979b). Based on the available data, Reuber concluded that methoxychlor produces liver tumors in mice and rats, and possibly in dogs (Reuber 1980). In addition, he concluded that methoxychlor is carcinogenic to the testes of male mice, bone of female mice, and the ovaries of female rats. However, there is considerable disagreement between Reuber and the original authors in the interpretation of the histopathological data. EPA (1987b) concluded that the differences observed between Reuber's interpretation and those of the original authors may be due in part to the use of inappropriate control data in Reuber's analysis and the difficulty in distinguishing histopathological lesions as regenerative hyperplasia, hyperplastic nodules, benign tumors, and malignant tumors.

Based on a review of all the available data, EPA has classified methoxychlor as a Group D carcinogen, not classifiable as to human carcinogenicity (IRIS 2000). Similarly, IARC (1987) has classified methoxychlor as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) and NCI (1978) concluded there was insufficient evidence to classify methoxychlor as a carcinogen.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to methoxychlor. Only two studies were located regarding the lethality of methoxychlor in animals after dermal exposure. One out of three rabbits died after intermediate-duration exposure to 3 mL/kg/day of a 30% solution (900 mg/kg/day) of recrystallized methoxychlor in dimethyl phthalate (Haag et al. 1950). No deaths were observed in animals exposed to lower doses. No decrease in survival occurred in mice dermally exposed to

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recrystallized methoxychlor at doses of 10 mg/day, 1 day/week for 2 years (Hodge et al. 1966). LOAEL values for the lethal effects of methoxychlor are recorded in Table 3-2.

Table 3-2 Levels of Significant Exposure to Methoxychlor - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
INTERMEDIATE EXPOSURE						
Death						
Rabbit	1x/d 5 d/wk 13 wk				3 mL/kg/day 30%	(1/3 died after 1.4 weeks) Haag et al. 1950 RC
Systemic						
Rabbit	1x/d 5 d/wk 13 wk	Hepatic	1 mL/kg/day 30%	2 mL/kg/day 30%	(fatty degeneration)	Haag et al. 1950 RC
		Bd Wt	1 mL/kg/day 30%	2 mL/kg/day 30%	(decreased body weight gain)	
CHRONIC EXPOSURE						
Mouse C3H/AnF	1x/d 1 d/wk 104 wk	Dermal	10 mg/day			Hodge et al. 1966 RC
		Bd Wt	10 mg/day			

Bd Wt = body weight; d = day; wk = week(s); x = times(s)

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3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, renal, endocrine, ocular, or body weight effects in humans or animals after dermal exposure to methoxychlor. Limited data are available on hepatic, dermal/ocular, and other systemic effects in animals. The highest NOAEL values and all LOAEL values from each reliable study for the systemic effects of methoxychlor in each species and duration category are recorded in Table 3-2. These studies are discussed below.

Hepatic Effects. Fatty degenerative changes in liver were observed in one of three rabbits exposed to 1 mL/kg/day of a 30% solution (300 mg/kg/day) of recrystallized methoxychlor in dimethyl phthalate, and in 4 of 6 rabbits administered higher doses (2–3 mL/kg/day; equivalent to 600–900 mg/kg/day), 5 days/week for 13 weeks (Haag et al. 1950). The authors state that no apparent effects from dimethyl phthalate were observed.

Dermal Effects. No gross or histopathological changes were observed in the skin of mice exposed to dermal doses of up to 10 mg/day recrystallized methoxychlor in acetone, 1 day/week for 2 years (Hodge et al. 1966).

3.2.3.3 Immunological and Lymphoreticular Effects

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

3.2.3.4 Neurological Effects

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

Neurological effects of methoxychlor in animals after dermal exposure were observed in a single study. Disseminated nodules and petechial hemorrhages were noted in the brains of rabbits exposed dermally to 1 mL/kg/day or more of a 30% solution (300 mg/kg/day) of recrystallized methoxychlor in dimethyl phthalate, 5 days/week for 13 weeks (Haag et al. 1950). Similar effects were not observed in control animals. These lesions were accompanied by foreleg paralysis in two out of six animals exposed to the higher doses (2–3 mL/kg/day). The authors suggested the effects may be manifestations of an underlying

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disease that is endemic to rabbits and was somehow potentiated by exposure to methoxychlor. The foreleg paralysis seen in two of the rabbits may have been related to the lesions of the central nervous system.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to methoxychlor. Although no quantitative data were provided, Tullner (1961) reported marked uterine stimulation in immature female mice dusted with an insecticide containing methoxychlor. Inhalation and oral exposures to the dust were likely to have occurred as well.

3.2.3.6 Developmental Effects

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

3.2.3.7 Cancer

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

Two studies were located that investigated the carcinogenic potential of methoxychlor in animals after dermal exposure. Dermal exposure of mice to 10 mg/day recrystallized methoxychlor, 1 day/week for 2 years did not produce any treatment-related tumors (Hodge et al. 1966). However, in this study, only the skin underwent histopathological examination. Other tissues were only examined grossly. Another study examined the effects of topically applied methoxychlor on skin tumor-promotion in mice (Dwivedi and Tabbert 1994). Application of 300 nmol methoxychlor in 100 μ L acetone to the shaved back skin (previously initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA)) of mice twice a week for 20 weeks did not result in papilloma development, while 100% of the mice treated with DMBA followed by 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA, a known tumor promoter) developed tumors (Dwivedi and Tabbert 1994). Methoxychlor also did not induce ornithine decarboxylase (ODC) activity in mouse skin (Dwivedi and Tabbert 1994).

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3.3 GENOTOXICITY

Numerous *in vivo* and *in vitro* studies have assessed the genotoxic potential of methoxychlor, and the results of these studies are presented in Tables 3-3 and 3-4, respectively.

The genotoxicity of methoxychlor has been well studied *in vitro* and to a lesser extent *in vivo*. Methoxychlor does not appear to be genotoxic in prokaryotic test systems. Negative results were obtained in mutagenicity tests using many different strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, and D3052), with or without metabolic activation by rat liver microsomal fractions (Grant et al. 1976; Mortelmans et al. 1986; Probst et al. 1981; Waters et al. 1980). Negative results were also reported for tests of differential toxicity in DNA repair-proficient and repair-deficient strains of *Bacillus subtilis* or *Escherichia coli* and for mitotic recombination in *Saccharomyces cerevisiae* (Probst et al. 1981; Waters et al. 1980). When selected contaminants of methoxychlor were tested for their mutagenic potential, only 3,6,11,14-tetramethoxydibenzo(g,p)chrysene produced an increased mutation frequency (Grant et al. 1976), however, most of the known contaminants of methoxychlor were not tested in this study. This compound is a relatively minor contaminant of technical methoxychlor comprising only 0.0005% (5 ppm) by weight (Mitchell et al. 1978).

Mixed results have been obtained in genotoxicity tests using mammalian cells *in vitro*. Methoxychlor did not induce unscheduled DNA synthesis in human lung fibroblasts (Waters et al. 1980) or rat hepatocytes (Probst et al. 1981). In cultured bovine uterine epithelial and stromal cells, DNA synthesis (thymidine incorporation) was inhibited at high methoxychlor concentrations and stimulated at low methoxychlor concentrations (Tiemann et al. 1996). Single-stranded DNA breaks were not induced in human or rat testicular cells by methoxychlor (Bjørge et al. 1996b). Methoxychlor did not produce mutations at the thymidine kinase (TK) locus in human lymphoma cells, with or without metabolic activation (Caspary et al. 1988). However, in mouse lymphoma cells, metabolically activated methoxychlor induced increases in mutation frequencies at the TK locus (Caspary et al. 1988; Mitchell et al. 1988; Myhr and Caspary 1988). Mixed results were reported on ability of methoxychlor to induce neoplastic transformations. A dose-dependent increase in neoplastic transformations were reported in cultured mouse fibroblasts (Dunkel et al. 1981), but not in Syrian hamster embryo cells or virally infected rat embryo cells (Dunkel et al. 1981; Pienta 1980). The reason for differing results in mouse lymphoma cells compared to human lymphoma cells, and in mouse fibroblasts compared to Syrian hamster embryo cells and rat embryo cells is uncertain, but may represent important species differences. In the absence of

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

Species (test system)	End point	Results	Reference
Mammalian cells:			
Mouse hepatic cells	Single-stranded DNA breaks	–	Umegaki et al. 1993
Mouse bone marrow cells	Chromosome aberration	–	Degraeve and Chollet 1984
Mouse sperm cells	Chromosome aberration	–	Degraeve and Chollet 1984
Nonmammalian cells:			
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutation	–	Waters et al. 1980
<i>D. melanogaster</i>	Sex-linked recessive lethal mutation	–	Benes and Sram 1969
<i>D. melanogaster</i>	Sex-linked recessive lethal mutation	–	Valencia 1981

– = negative result; + = positive result

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

Species (test system)	End point	With activation	Without activation	Reference
Prokaryotic organisms:				
<i>Salmonella typhimurium</i>	Reverse mutation	–	–	Mortelmans et al. 1986
<i>S. typhimurium</i>	Reverse mutation	–	–	Grant et al. 1976
<i>S. typhimurium</i>	Reverse mutation	–	–	Waters et al. 1980
<i>S. typhimurium</i>	Reverse mutation	–	–	Probst et al. 1981
<i>Escherichia coli</i>	Reverse mutation	–	–	Waters et al. 1980
<i>E. coli</i>	Reverse mutation	–	–	Probst et al. 1981
<i>E. coli</i>	Differential toxicity ^a	–	No data	Waters et al. 1980
<i>Bacillus subtilis</i>	Differential toxicity ^a	–	No data	Waters et al. 1980
Eukaryotic organisms:				
<i>Saccharomyces cerevisiae</i>	Mitotic recombination	–	–	Waters et al. 1980
Mammalian cells:				
Human testicular cells	Single-stranded DNA breaks	No data	–	Bjørge et al. 1996b
Human lung fibroblasts	Unscheduled DNA synthesis	–	–	Waters et al. 1980
Human lymphoma cells	Mutation of TK locus	–	–	Caspary et al. 1988
Mouse lymphoma cells	Mutation at TK locus	+	–	Caspary et al. 1988
Mouse lymphoma cells	Mutation at TK locus	+	–	Myhr and Caspary 1988
Mouse lymphoma cells	Mutation at TK locus	+	–	Mitchell et al. 1988
Chinese hamster cells	Inhibition of metabolic cooperation	No data	+	Kurata et al. 1982
Syrian hamster embryo cells	Neoplastic transformation	–	–	Pienta 1980
Syrian hamster embryo cells	Neoplastic transformation	No data	–	Dunkel et al. 1981

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Table 3-4. Genotoxicity of Methoxychlor *In Vitro* (continued)

Species (test system)	End point	With activation	Without activation	Reference
Mouse fibroblasts	Neoplastic transformation	No data	+	Dunkel et al. 1981
Rat embryo cells (virus-infected)	Neoplastic transformation	No data	-	Dunkel et al. 1981
Rat hepatocytes	Unscheduled DNA synthesis	No data	-	Probst et al. 1981

^a Comparison between DNA repair proficient and repair deficient strains
- = negative result; + = positive result

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a metabolic activation system, methoxychlor inhibited metabolic cooperation in Chinese hamster cells, an activity possibly associated with tumor promoters (Kurata et al. 1982). Exposure to methoxychlor did not induce morphological transformation in Syrian hamster embryo cells (Pienta 1980).

In vivo genotoxicity studies generally yielded negative results (Table 3-3). In mice intraperitoneally injected with 10 mg/kg methoxychlor, the frequency of chromosomal aberrations was not increased in bone marrow cells or sperm cells (Degraeve and Chollet 1984). Single-stranded DNA breaks were not increased in hepatic cells of mice injected intraperitoneally with 170 mg/kg/day for 5 days (Umegaki et al. 1993). No increased frequency of sex-linked recessive lethal mutations were noted in *Drosophila melanogaster* exposed to methoxychlor (Benes and Sram 1969; Valencia 1981; Waters et al. 1980).

No studies were located regarding genotoxic effects in humans or animals after inhalation, oral, or dermal exposure to methoxychlor.

3.4 TOXICOKINETICS

No data were located concerning the toxicokinetics of methoxychlor in humans following any route of exposure, or in animals following inhalation exposure. Studies in animals indicate that methoxychlor is well absorbed by the gastrointestinal tract and to a lesser extent by the skin. However, some of the data from animal studies come from ruminant animals, which may have limited relevance to humans and other nonruminant species. Once in the bloodstream, methoxychlor appears to distribute to most tissues of the body, with highest levels usually found in fat. Methoxychlor is metabolized rapidly by the liver and neither the parent compound nor the metabolites tend to accumulate in fat or other tissue. The metabolism of methoxychlor has been fairly well studied *in vitro* and *in vivo* in animals and with human liver microsomes. Both sets of data indicate that methoxychlor undergoes demethylation to form phenolic derivatives, with dechlorination and dehydrochlorination reactions occurring to a lesser extent. Most of the ingested dose of methoxychlor is eliminated in the feces via biliary excretion of metabolites. Urinary excretion contributes to a lesser extent (approximately 10% of the total administered dose as indicated in mouse studies). The toxicokinetics of methoxychlor in humans is expected to be similar to the toxicokinetics of methoxychlor observed in animals.

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3.4.1 Absorption

No quantitative studies were located regarding absorption of methoxychlor in humans after exposure by any route. There is no information regarding age-related differences in rate or extent of absorption of methoxychlor in animals or humans after exposure by any route. Thus, it is unknown whether absorption of methoxychlor by children differs from that by adults.

3.4.1.1 Inhalation Exposure

No studies were located regarding absorption of methoxychlor in animals after inhalation exposure.

3.4.1.2 Oral Exposure

Observations of adverse health effects in animals following oral exposure provide indirect evidence that ingested methoxychlor is absorbed by the gastrointestinal tract (see Section 3.2.2 Health Effects: Oral Exposure). Data from studies examining fecal and urinary excretion of radioactivity after oral administration of radiolabeled methoxychlor to mice and goats indicate that methoxychlor is rapidly and efficiently absorbed by the gastrointestinal tract.

In mice administered single doses of 50 mg/kg recrystallized, radiolabeled methoxychlor in oil by gavage, 90% of the dose was excreted as metabolites in the feces (85% polar metabolites) and 10% was excreted in urine (63.8% polar metabolites) within 48 hours (Kapoor et al. 1970). Assuming that the fecal metabolites (primarily demethylated, dechlorinated, and dehydrochlorinated compounds) resulted from biliary excretion of absorbed material and not from degradation of unabsorbed parent by enteric bacteria, gastrointestinal absorption of methoxychlor appears to exceed 90% in mice.

In two lactating female goats administered single oral doses of 3.6 or 11.6 mg/kg laboratory grade, radiolabeled methoxychlor encapsulated in gelatin, 40.5 and 67.5% of the doses were excreted in the feces within 3 days, respectively, and 58.4 and 27.1% were excreted in the urine, respectively (Davison et al. 1982). Metabolites (demethylated, dechlorinated, and dehydrochlorinated products of methoxychlor) were estimated to represent 70 and 81% of radioactivity in the feces, respectively. Assuming that metabolites in feces resulted from biliary excretion of absorbed material (and that the remaining radioactivity in feces was not absorbed), the data indicate that at least 87 and 82% of the respective administered doses were absorbed. In a bile-cannulated, castrated male goat given an oral dose

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of 25.6 mg/kg radiolabeled methoxychlor, 7.8, 35.2, and 44.4% of the radioactivity was excreted within 3 days in the bile, feces, and urine, respectively (Davison et al. 1983). The profile of metabolites in the collected bile was reported to be similar to the metabolite profile in the feces collected from the female goats, but Davison et al. (1983) did not report any chemical analysis of the feces collected from the bile-cannulated goat. The data provide support that methoxychlor is rapidly and efficiently absorbed by the mammalian gastrointestinal tract. However, interpretation of the goat studies is limited, because only one animal was tested per dose, bile cannulation may have influenced absorption, and ruminant toxicokinetic properties are not always relevant to nonruminant mammals.

3.4.1.3 Dermal Exposure

Two studies in animals suggest dermal absorption of methoxychlor may range from 5 to 20%. In one study, a single dermal dose of 200 mg laboratory grade methoxychlor in dichloromethane was applied to the shaved backs of two goats. Three days later, 5–8% of the dose was recovered in the carcass, urine, and feces (Davison et al. 1983). In the second study, four cows were dermally exposed to a single dose of 5 g methoxychlor in an emulsion (Skaare et al. 1982). The levels of methoxychlor detected in milk were comparable to the levels in milk from cows given an intravenous dose of 1 g methoxychlor. The authors concluded that approximately 20% of the dermal dose was absorbed. Because of differences in skin, dermal absorption by goats and cows may not be a good model for dermal absorption by humans.

3.4.2 Distribution

There are no studies regarding distribution of methoxychlor in humans after exposure by any route, nor any studies examining possible age-related differences in distribution of methoxychlor in animals or humans after exposure by any route. Thus, it is unknown whether distribution of methoxychlor and metabolites in children differs from that in adults.

There are no studies directly examining whether methoxychlor and metabolites cross the placenta in humans or animals. Subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); however, it should be noted that exposure was not exclusively during gestation in any of these studies. It is unclear if these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes.

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3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in animals after inhalation exposure to methoxychlor.

3.4.2.2 Oral Exposure

In animals, methoxychlor preferentially distributes to the fat but does not appear to persist. Furthermore, metabolic adaptation may enhance elimination. In rats exposed to 1.25, 5, or 25 mg/kg/day methoxychlor for 4–18 weeks, fat levels of methoxychlor were reported to be nondetectable, 1–7 mg/kg, and 14–36 mg/kg, respectively (Kunze et al. 1950). Levels of methoxychlor in fat peaked during the first 9 weeks of exposure, after which time a gradual decline was noted during the last 9 weeks of exposure (Kunze et al. 1950). Methoxychlor was not detected in the fat following the 2-week recovery period. In female rats exposed to 50, 125, or 250 mg/kg/day in the feed for 6 weeks, the levels of methoxychlor detected in abdominal fat were 21, 68, and 61 mg/kg, respectively (Harris et al. 1974). The levels of methoxychlor in fat were 34 and 140 mg/kg in female rats when exposure to 50 and 125 mg/kg/day was continued through pregnancy and weaning (exposure duration=12 weeks) (Harris et al. 1974). The authors speculated that the higher levels of methoxychlor in fat of female rats after pregnancy and weaning was due to a lower fat content in these animals. However, exposure duration (12 vs. 6 weeks) may have been a factor as well. Levels of methoxychlor in the fat of sheep exposed to 6 and 49 mg/kg/day for 18 weeks peaked at 7.8 mg/kg by week 10, and at 24 mg/kg by week 6, respectively (Reynolds et al. 1976). A steady decline of methoxychlor in fat was noted after week 10 in the low dose group, suggesting that metabolic adaptation enhanced elimination (Reynolds et al. 1976). Methoxychlor was not detected in fat from either group following a 12–14-week recovery period. In dogs administered 20 or 100 mg/kg/day methoxychlor for 1 year, the levels of methoxychlor in the fat were 8.9 and 85 mg/kg, respectively (Hodge et al. 1952). The levels of methoxychlor in the fat of rats exposed to 1.25, 10, or 80 mg/kg/day for 2 years were 3.7, 2.3–6.8, and 11–22.7 mg/kg, respectively (Hodge et al. 1952). In rats receiving 80 mg/kg/day, the highest levels of methoxychlor detected in the kidneys, liver, and brain were less than 4.2, 0.5, and 0.2 mg/kg, respectively (Hodge et al. 1952).

Three days after oral administration of 3.6 or 11.6 mg/kg radiolabeled methoxychlor to lactating goats, radioactivity was detected in adrenals, brain, gall bladder, heart, kidneys, and liver (Davison et al. 1982), thus indicating that absorbed methoxychlor and metabolites are widely distributed by the blood. However, the radioactivity in these tissues represented <1% of the administered dose. These observations

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are consistent with other information indicating that methoxychlor is rapidly metabolized and excreted from the body.

Methoxychlor and/or methoxychlor metabolites have been detected in milk following oral exposure of animals to methoxychlor during lactation. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated. In lactating female goats given oral doses of 3.6 or 11.6 mg/kg radiolabeled methoxychlor, radioactivity in milk collected for 3 days was below limits of detection for one goat and represented only 0.065% of the dose given to the other goat (Davison et al. 1982). In female rats fed 5, 50, or 150 mg/kg/day methoxychlor during late gestation and early lactation, milk levels of methoxychlor at postnatal day 7 were 25, 128, and 221% of plasma methoxychlor levels, respectively (Chapin et al. 1997). Mono-hydroxy methoxychlor and bis-hydroxy methoxychlor, two major metabolites of methoxychlor, showed similar patterns of concentration in the milk with increasing methoxychlor exposure level. The data suggest that methoxychlor and metabolites concentrate in milk, relative to maternal plasma levels, after intermediate-duration dose levels ≤ 50 mg/kg/day. Mean plasma levels of methoxychlor in suckling pups were <5 (below detection limit), 12.4, 37.8, and 59.9 ng/mL in the 0-, 5-, 50-, and 150-mg/kg/day groups, respectively (Chapin et al. 1997). Mono-hydroxy methoxychlor was detected only in 150-mg/kg/day pup plasma (6.2 ng/mL), and bis-hydroxy methoxychlor was detected in the 50- and 150-mg/kg/day pup plasma (6.4 and 11.5 mg/kg/day). Pup plasma was not drawn for analysis until 27–30 hours after dams received the last dose; therefore, measured methoxychlor and metabolites may not have been indicative of peak body burden.

No studies were located that examined whether preconceptional or pregestational exposure of females to methoxychlor would result in exposure to the developing embryo/fetus or neonate. The evidence that methoxychlor is rapidly metabolized and eliminated from the body (e.g., all radioactivity from radiolabeled oral doses of 50 mg/kg methoxychlor was excreted by mice in feces and urine within 48 hours [Kapoor et al. 1970]) suggests, however, that it is unlikely that methoxychlor at low background exposure levels would be stored in maternal tissues and subsequently mobilized during pregnancy or lactation. It is unknown what mechanism produced the results reported by Swartz and Corkern (1992) (discussed in Section 3.2.2.6 Developmental Effects); in this experiment, females from a litter produced after maternal exposure had ceased exhibited precocious puberty, but earlier offspring of these same dams exposed during gestation did not.

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3.4.2.3 Dermal Exposure

A single study was located concerning the distribution of methoxychlor in animals after dermal exposure. Three days after two goats were administered a single dermal dose of 200 mg methoxychlor, low levels of methoxychlor (<0.3 mg/kg tissue) were detected in the skin, muscle, liver, fat, and kidneys (Davison et al. 1983). Less than 0.1% of an applied dermal dose of methoxychlor was excreted in the milk of cows after 30 days (Ivey et al. 1983; Skaare et al. 1982). Interpretation of data from these studies is limited since only two goats were tested, and goats and cows may not be good models for dermal exposures in humans.

3.4.2.4 Other Routes of Exposure

Further evidence, albeit indirect, that methoxychlor and/or metabolites can be excreted in milk is provided by observations of morphological changes in the reproductive tract of 15-day-old female offspring of lactating mice given 14 daily intraperitoneal injections of 1, 2, or 5 mg technical-grade methoxychlor in sesame oil (30, 60, or 150 mg/kg/day) on postnatal days 1–14 (Appel and Eroschenko 1992). Exposure-related, statistically significant changes included increased reproductive tract weight, increased thickness of epithelia of vagina and uterine horns, and increased incidence of mucified and/or cornified vagina. These stimulatory effects on development of the female reproductive tract are consistent with the estrogenic activity of methoxychlor metabolites.

The estrogenic activity of methoxychlor administered intraperitoneally has also been demonstrated in adult ovariectomized ND4 Swiss Webster mice (Eroschenko et al. 2000). Three daily intraperitoneal doses of 0.125 mg (4 mg/kg/day) methoxychlor resulted in an increase in uterine epithelial height of approximately the same magnitude as three intraperitoneal doses of 25 ng (0.9 µg/kg/day) of estradiol 17-β. However, the potency of methoxychlor was much less than estradiol (approximately 4,400 times less) and the range of effects elicited by methoxychlor was different from those elicited by estradiol. While estradiol increased reproductive tract weight and uterine luminal fluid albumin content, methoxychlor did not, and in fact appeared to reduce the stimulatory response of sensitive uterine cells to estradiol. The study authors speculated that these differences may be due to the different affinities of methoxychlor for the various forms of ER (ERα and ERβ) compared to estradiol and its possible interaction with other receptors that have not yet been elucidated.

Estrogenic effects have also been observed in female mice following subcutaneous administration. Methoxychlor (10 mg/kg/day) administered for 5 days resulted in a significant increase in uterine weight,

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similar to the effects of estradiol-17 β (Al-Jamal and Dubin 2000). However, this effect was eliminated by co-administration with raloxifene (a selective estrogen receptor modulator), whereas the uterine effects of estradiol-17 β were not. This supports other studies (Eroschenko et al. 2000; Gaido et al. 1999; Ghosh et al. 1999) that have suggested that the estrogenic effects of methoxychlor may be mediated via different interactions with the estrogen receptors or different mechanisms than estradiol-17 β . Increased uterine weight was also seen in immature mice administered methoxychlor (50 mg/kg) or its estrogenic metabolite HPTE (100 mg/kg) subcutaneously for 1 or 3 days (Newbold et al. 2001). Further examination of the uterine tissue showed increases in uterine epithelial cell height, uterine gland number, cell proliferation, and estrogen-inducible protein production (Newbold et al. 2001).

Methoxychlor administered subcutaneously to adult male rats resulted in a seemingly complicated alteration of serum and hypothalamic hormone levels (Lafuente et al. 2000). Prolactin release from the hypothalamus is affected by circadian rhythm. Methoxychlor exposure resulted in increased median serum prolactin levels and absolute pulse amplitude and in decreased relative pulse amplitude, but did not affect the frequency or duration of prolactin peaks or the half-life of prolactin in serum (Lafuente et al. 2000). Decreases in serum testosterone and luteinizing hormone (LH) were also noted in this study. Testosterone can stimulate prolactin release; therefore, the increase in prolactin does not appear to be mediated by testosterone. The study authors speculated that the alteration in prolactin release may be a consequence of direct effects of methoxychlor on the hypothalamus (Lafuente et al. 2000). Dopamine, which is an inhibitory neuromodulator of prolactin release, was increased in the anterior hypothalamus and decreased in the median eminence, which suggests a decrease in dopamine release; this could explain the increased prolactin release (Lafuente et al. 2000).

3.4.3 Metabolism

Figure 3-2 presents a summary of methoxychlor metabolic pathways. Following Figure 3-2 is a key to alternative chemical names for methoxychlor metabolites. There are no data to suggest that metabolism of methoxychlor is dependent on the way it enters the body, so the metabolism of methoxychlor is discussed below without reference to route of exposure.

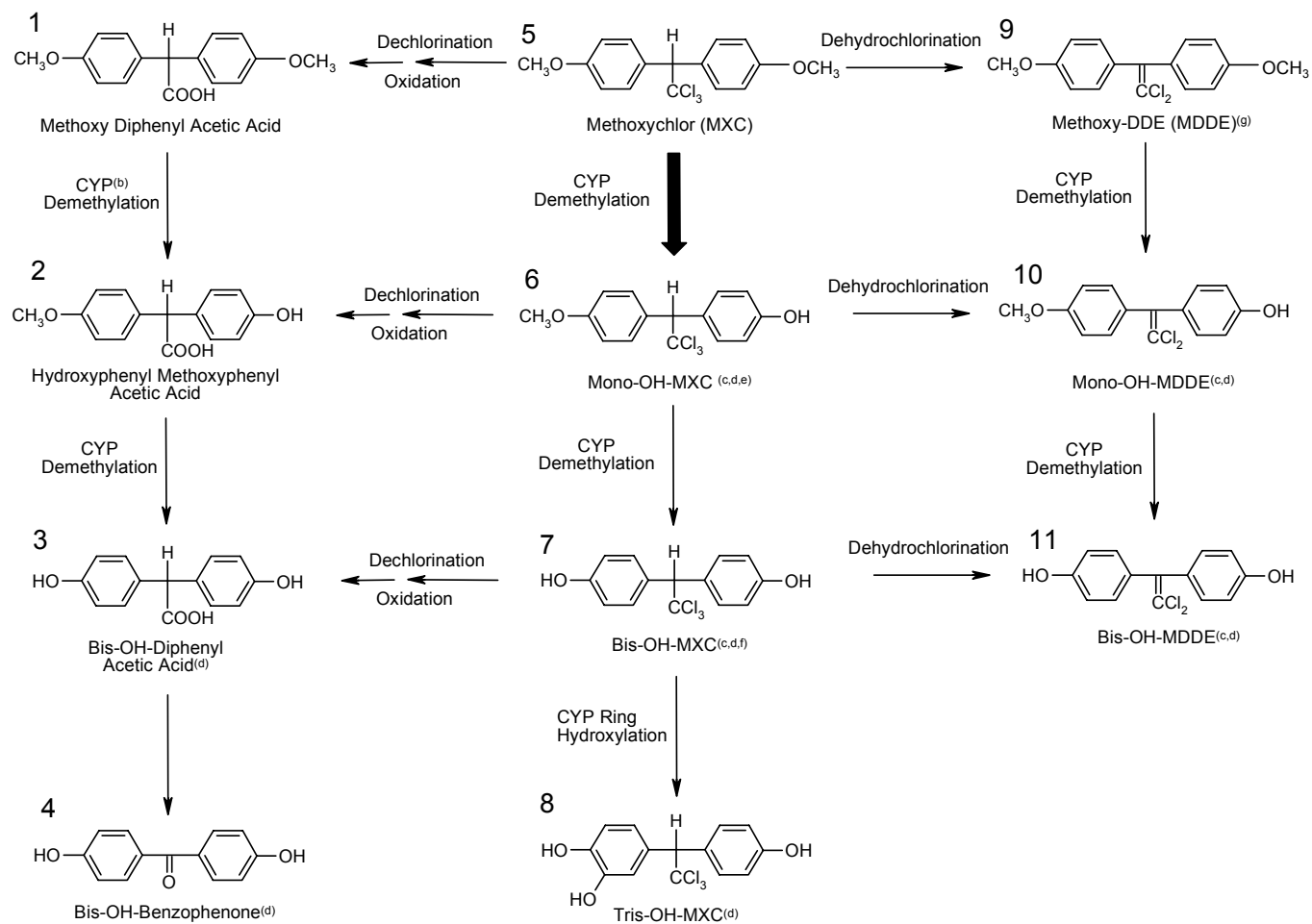
Methoxychlor is metabolized mainly in the liver. In *in vivo* studies in which rats were exposed to carbon tetrachloride to induce liver damage, the metabolism and excretion of methoxychlor were reduced, whereas tissue retention and toxicity were increased (Lehman 1952; Weikel and Laug 1958).

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The primary pathway by which methoxychlor is metabolized in the liver is sequential demethylation reactions to yield mono- and bis-hydroxy methoxychlor. Alternative names for these metabolites are 2-(*p*-methoxyphenyl)-2-(*p*-hydroxyphenyl)-1,1,1-trichloroethane and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (sometimes abbreviated as HPTE). Dechlorinated metabolites such as bis-hydroxydiphenyl acetic acid and bis-hydroxybenzophenone have also been identified (see Figure 3-2).

Methoxychlor and 5 metabolites were identified by thin layer chromatography in urine and feces collected from mice for up to 11 days after administration of single oral doses of 50 mg/kg/day radiolabeled, recrystallized methoxychlor in oil (Kapoor et al. 1970). Mono-hydroxy methoxychlor and bis-hydroxy-methoxychlor, the major metabolites, accounted for approximately 30 and 23% of the administered dose, respectively. Bis-hydroxy-diphenylacetic acid and bis-hydroxy-benzophenone, presumably formed via dechlorination and subsequent oxidation as shown in Figure 3-2, accounted for about 11% of the administered radioactivity. Nonmetabolized methoxychlor and methoxy diphenyl dichloroethylene (bis-OH-MDDE) accounted for about 8 and 1% of the administered radioactivity, respectively (Kapoor et al. 1970). The latter metabolite has been proposed to be formed by dechlorination of bis-hydroxy methoxychlor (i.e., HPTE), or by alternative pathways in which dechlorination precedes demethylation (see Figure 3-2). About 27% of administered radioactivity was not recovered in the thin layer chromatography procedure used in this analysis.

In goats administered single doses of 3.6–25.6 mg/kg, most of the dose was metabolized to demethylated, dechlorinated, and dehydrochlorinated derivatives of methoxychlor, although a ring hydroxylated species

Figure 3-2. Proposed Metabolic Pathways of Methoxychlor^a

(a) Adapted from Kapoor et al. 1970; Kupfer and Bulger 1987b; Kupfer et al. 1990

(b) CYP = cytochrome P-450

(c) Estrogenic compound

(d) Metabolite identified in excreta

(e) Mono-OH-MXC = 2-(p-Methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane

(f) Bis-OH-MXC = 2,2-Bis(p-hydroxyphenyl)-1,1,1-trichloroethanol (HPTE)

(g) MDDE = Methoxydiphenyldichloroethylene

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**Figure 3-2 Proposed Metabolic Pathways of Methoxychlor
Key to Metabolite Chemical Names**

1. Bis(4-methoxyphenyl)acetic acid
Methoxy Diphenyl Acetic Acid
2. α -(4-hydroxyphenyl)- α -(4-methoxyphenyl)acetic acid
Hydroxyphenyl Methoxyphenyl acetic acid
3. Bis(4-hydroxyphenyl)acetic acid
Bis-OH-Diphenyl acetic acid
CASRN: 40232-93-7
4. 4,4-Dihydroxybenzophenone
Bis-OH-Benzophenone
CASRN: 611-99-4
5. 1,1,1-Trichloro-2,2-bis(4-methoxyphenyl)ethane
Methoxychlor (MXC)
CASRN: 72-43-5
6. 1,1,1-Trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane
Mono-OH-MXC
2-(p-Methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane
7. 1,1,1-Trichloro-2,2-bis(4-hydroxyphenyl)ethane
Bis-OH-MXC
2,2-Bis(p-hydroxyphenyl)-1,1,1-trichloroethanol (HPTE)
CASRN: 2971-36-0
8. 1,1,1-Trichloro-2-(3,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethane
Tris-OH-MXC
9. 1,1-Dichloro-2,2-bis(4-methoxyphenyl)ethene
Methoxy-DDE (MDDE)
Methoxydiphenyldichloroethylene
CASRN: 2132-70-9
10. 1,1-Dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene
Mono-OH-MDDE
11. 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethene
Bis-OH-MDDE
CASRN: 14868-03-2

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was also identified in the urine as a minor metabolite (Davison et al. 1982, 1983). Most of the urinary metabolites were conjugated with glucuronic acid.

In vitro studies using human and rat liver microsomal preparations confirm the generation of phenolic compounds via demethylation by hepatic cytochrome P450 (CYP) enzymes (Bulger et al. 1978d, 1985; Dehal and Kupfer 1994; Elsby et al. 2001; Kishimoto and Kurihara 1996; Kupfer et al. 1990; Kurihara and Oku 1991; Stresser and Kupfer 1997, 1998; Stresser et al. 1996). Early studies demonstrated that methoxychlor produced type I spectral changes when added to CYP enzymes from rats, mice, rabbits, sheep, and houseflies (Donovan et al. 1978; Kulkarni et al. 1975; Tsujita and Ichikawa 1993), indicative of substrate binding to CYP enzymes.

Several partially purified CYP isozymes from rat liver microsomes can demethylate methoxychlor: CYP2C6 and 2A1 showed lower K_m and V_{max} values than values for CYP2B1 and CYP2B2, indicating that the former have a higher affinity, but lower capacity, for methoxychlor than the latter (Kishimoto et al. 1995); thus, which pair of enzymes is likely to be performing most of methoxychlor metabolism will depend on the amount of methoxychlor available to the liver. Antibodies to CYP2B1 and CYP2C6 were used with rat liver microsomes and purified preparations of CYP2C6 to provide evidence that CYP2C6 and another unidentified CYP isozyme may represent the most important isozymes in the initial demethylation of methoxychlor in rats (Kishimoto and Kurihara 1996).

Repeated exposure to methoxychlor appears to induce hepatic CYP enzymes involved in its metabolism. Intraperitoneal injection of mature female rats with 200 mg/kg/day methoxychlor twice daily for 4 days increased western blot-detected hepatic levels of CYP2B1, 2B2, and 3A proteins by 3-, 2.8-, and 1.6-fold compared with controls, but did not change levels of CYP2E1 (Li et al. 1995). Injection of immature female rats with 300 mg/kg/day for 4 days increased levels of CYP2B1, 2B2, and 3A proteins by 9.0-, 7.8-, and 5.1-fold without inducing levels of CYP2E1 (Li et al. 1995). In these experiments, methoxychlor caused a markedly greater increase in the amount of CYP2B and 3A proteins than in actual enzyme activity. A recent *in vitro* study indicated that induction of the hepatic CYP2B enzyme by methoxychlor and its metabolites may be mediated via the constitutive androstane receptor (CAR), which initiates transcription of CYP2B RNA and results in increased CYP2B enzyme (Blizard et al. 2001).

In vitro studies with human liver microsomes and human recombinant CYP isozymes indicate that multiple CYP isozymes are involved in the demethylation of methoxychlor in humans (Stresser and Kupfer 1998). Incubation of pooled liver microsomes from three human subjects with saturating levels of

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methoxychlor (25 μM) and NADPH for up to 125 minutes produced mono-hydroxy methoxychlor, bis-hydroxy methoxychlor, and tris-hydroxymethoxychlor consistent with the central demethylation and ring hydroxylation reactions noted in Figure 3-2 (Stresser and Kupfer 1998). Rates of formation were highest for mono-hydroxy methoxychlor followed by bis-hydroxy methoxychlor and tris-hydroxy methoxychlor. Rates of mono-demethylation at non-saturating concentrations of 1 μM using liver preparations from 14 individuals varied 80-fold, whereas rates from preparations from 26 individuals assayed at saturating concentrations varied 23-fold. Rates of formation of mono- and bis-hydroxy methoxychlor with human liver microsomes were generally lower than rates with rat liver microsomes, but some human liver samples displayed higher rates. At nonsaturating methoxychlor concentrations, demethylation by human microsomes was strongly inhibited by CYP2C19 inhibitors, tranlycypromine (also inhibits CYP2A6), and S-mephenytoin (substrate for CYP2C19). Moderate inhibition was produced by tolbutamide (substrate for CYP2C9), furafylline (inhibitor of CYP1A2), sulfaphenazole (inhibitor of CYP2C9), and coumarin (substrate of CYP2A6), whereas weak inhibition was produced by quinidine (substrate of CYP3A4) and ketoconazole (inhibitor of CYP3A4). The involvement of multiple CYP isozymes was supported by the observation of biphasic enzyme kinetics in Eadie-Hofstee plots of methoxychlor demethylation rates with human liver microsomes. Recombinant CYP2C19 expressed in lymphoblast cells was more active in demethylating methoxychlor than similarly expressed 1A2, and human cDNA-expressed CYP2C19, purified from bacterial lysates, catalyzed methoxychlor demethylation at rates 4-fold higher than rates for CYP2C9 and CYP2C18. Stresser and Kupfer (1998) proposed that CYP2C19 and CYP1A2 may be the major CYP demethylases for methoxychlor, but noted that other forms, including CYP2A6, CYP2C9, and CYP2B6, are likely to be major contributors, especially in individuals with low levels of CYP2C19 or CYP1A2.

Ring hydroxylation of methoxychlor or of hydroxy-methoxychlor derivatives (see Figure 3-2 for formation of tris-hydroxy methoxychlor from bis-hydroxy methoxychlor) may involve a different set of CYP isozymes than CYP-catalyzed demethylation. Studies using nine human cDNA-expressed CYP isozymes in microsomes from lymphoblastoid cells (CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, and 3A4) indicated that significantly increased ring hydroxylation of methoxychlor was catalyzed only by CYP1A2 or CYP2B6 (Stresser and Kupfer 1997). Ring hydroxylation of mono-hydroxy methoxychlor in this system was catalyzed by five isozymes (CYP1A2, 2B6, 2D6, 2E1, and 3A4), with CYP3A4 displaying the highest rates of ring hydroxylation (Stresser and Kupfer 1997). Ring hydroxylation of methoxychlor or bis-hydroxy methoxychlor (ortho to the methoxy or hydroxy moieties) was shown to be enhanced in liver microsomes from phenobarbital-induced rats compared with microsomes from non-induced rats and to be markedly inhibited by anti-CYP2B monoclonal antibodies (Stresser et al. 1996).

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These data suggest that CYP2B isozymes may be most important in catalyzing ring hydroxylation of methoxychlor and hydroxy-methoxychlor derivatives in rats.

Activation of Methoxychlor to Estrogenic Metabolites. The rapid demethylation of methoxychlor decreases its neurotoxicity and leads to a rapid elimination from the body (Lehman 1952), making it significantly less toxic than its structural analogue, DDT. However, this detoxification pathway also is thought to act as an activation pathway for reproductive and developmental effects. Data from *in vitro* and *in vivo* rat studies indicate that the phenolic metabolites of methoxychlor resulting from demethylation (and contaminants in technical grade and laboratory grade methoxychlor) are responsible for most of the estrogenic activity rather than methoxychlor itself (Bulger et al. 1978b, 1978d; Charles et al. 2000; Sumida et al. 2001). Highly purified methoxychlor did not interfere with *in vitro* binding of estradiol-17 β to 8S estrogen receptors in rat uterine cytosol, but the demethylated metabolite, 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane (i.e., HPTE), was a potent inhibitor of this binding (Bulger et al. 1978b). In this assay system, technical grade methoxychlor, and even laboratory grade (99% pure), inhibited estradiol binding to cytosolic estrogen receptors, indicating that some contaminants in methoxychlor preparations have estrogenic activity (Bulger et al. 1978b). In ovariectomized rats, intraperitoneal injection of 3 or 10 mg laboratory grade methoxychlor in corn oil significantly increased activities of uterine ornithine decarboxylase activity (ODC, a sensitive marker of estrogenic activity) within 7 hours by 15- or 136-fold, respectively, and significantly increased relative uterine weight by 1.5-fold (i.e., 53% increase), at the higher dose only (Bulger et al. 1978b). Injection of lower doses of HPTE, 0.1 or 0.5 mg per rat, significantly increased ODC activities by 17- or 607-fold, respectively; the 0.5 mg dose level also significantly increased relative uterine weight by 1.3-fold (Bulger et al. 1978b).

Two estrogen receptors, α and β , have recently been identified with overlapping and differential roles in mediating estrogenic responses in mammals. The complexity of the mechanism(s) by which methoxychlor metabolites induce estrogenic responses has also been recently demonstrated by results showing that HPTE acts as an agonist for estrogen receptor α expressed in human hepatoma cells (i.e., similarly to estradiol-17 β , it induced gene expression mediated by estrogen receptor α) and acts as an antagonist for estrogen receptor β (i.e., it abolished estradiol-17 β -induced gene expression mediated by estrogen receptor β) (Gaido et al. 1999). This was further supported by studies showing differential expression of certain ER-related mRNAs in tissues of mice treated with HPTE or estradiol-17 β , depending on ER α and ER β content of the tissues (Waters et al. 2001).

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The dehydrochlorination of HPTE to methoxydiphenyldichloroethylene (bis-OH-MDDE) (see Figure 3-2) also acts as an activation pathway, since bis-OH-MDDE was even more active than HPTE in *in vitro* assays of binding to rat uterine cytosol receptors and in *in vivo* rat assays for induction of uterine ODC activity and increase in relative uterine weight following intraperitoneal exposure (Bulger et al. 1985). Although the enzyme responsible for catalyzing the dehydrochlorination of methoxychlor and its demethylated metabolites has not been studied, it is probably the same glutathione-requiring dehydrochlorinase which catalyzes the conversion of DDT to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (DDE) (Hodgson and Levi 1987). Data were not located regarding the estrogenic activity of methoxydiphenylacetic acid and methoxy benzophenone, so it is not known whether dechlorination and oxidation of methoxychlor to these products act as activating or detoxifying pathways.

Age and Metabolism. Although there is no direct information regarding age-related differences in metabolism of methoxychlor in children, multiple CYP enzymes are involved in methoxychlor metabolism. It is likely that CYPs 2C19, 1A2, 2B6, 2C9, 2A6, and perhaps 2D6, 2E1, and 3A4 play a role in phase I human metabolism (Hong et al. 1987; Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Ratenasavanh et al. 1991; Rich et al. 1990; Sonnier and Cresteil 1998; Treluyer et al. 1997; Vieira et al. 1996; Yang et al. 1994). Many of these phase I enzymes are likely to have overlapping roles. Although nothing is known about phase II metabolism in humans, Davison et al. (1982, 1983) found glucuronic acid conjugates of methoxychlor metabolites in goats; these conjugates are a product of glucuronosyltransferase and it is possible that this enzyme may be involved in human methoxychlor metabolism as well. Although it is unknown whether an overall age-related difference in methoxychlor metabolism would be observed *in vivo* in humans, there is some information that the following enzymes may be developmentally regulated: CYP2C19 (Leeder and Kearns 1997), CYP1A2 (Leeder and Kearns 1997; Rich et al. 1990), CYP2C9 (Ratenasavanh et al. 1991; Treuler et al. 1997), CYP2D6 (Leeder and Kearns 1997; Sonnier and Cresteil 1998), CYP2E1 (Hong et al. 1987; Vieira et al. 1996), CYP3A4 (Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Yang et al. 1994), and glucuronosyltransferase (Leeder and Kearns 1997). In contrast, other researchers have found that CYPs 2A6, 2C9, 2D6, 2E1, and 3A are expressed at the same levels in perinatal (37 weeks of gestation) to geriatric human livers (72 years) (Ratenasavanh et al. 1991; Tateishi et al. 1997).

Metabolites and Covalent Adducts. *In vitro* studies indicate that methoxychlor can be metabolized to reactive intermediates capable of forming covalent adducts with cellular proteins. Studies with rat liver microsomes demonstrated that methoxychlor undergoes CYP mediated activation and the resultant reactive metabolites covalently bind to hepatic microsomal proteins (Bulger et al. 1983). Adduct

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formation requires the presence of NADPH and oxygen, and is inhibited by metyrapone, SKF-525A, hexobarbital and ethylmorphine, indicating that the activation of methoxychlor is catalyzed by the cytochrome P450 enzyme system. Liver microsomes from rats pretreated with phenobarbital, an inducer of CYP2B1 and 2B2, exhibited enhanced covalent binding to microsomal proteins, and antibodies against phenobarbital-induced CYP isozymes inhibited covalent binding, indicating that the major portion of this activity was facilitated by catalysis by CYP2B1 and/or CYP2B2 (Bulger and Kupfer 1989, 1990). Comparative studies with liver microsomal preparations from humans and rats indicate that the mechanism of methoxychlor covalent modification of microsomal proteins is similar in the two species (Bulger and Kupfer 1989). Free radical scavengers act to inhibit adduct formation without affecting the production of polar (demethylated) metabolites (Bulger et al. 1983). Although the formation of methoxychlor adducts has not been well characterized, these adducts contain intact methoxy groups, suggesting that this reaction occurs independently from demethylation reactions (Bulger and Kupfer 1990). The phenolic metabolites of methoxychlor, as well as the dehydrochlorinated product MDDE and its phenolic derivatives, are also capable of undergoing CYP mediated covalent binding. It was postulated that the formation of a reactive intermediate involves modification of the side chain, possibly through homolytic cleavage of the C-H or C-Cl bond (Bulger and Kupfer 1990). The relevance of protein adduct formation to mechanisms by which methoxychlor produces health effects is uncertain, but may be related to possible inactivation of CYP3A by tris-hydroxy methoxychlor (Li et al. 1993).

Comparative Toxicokinetics. The available data comparing rat and human liver microsomal metabolism of methoxychlor indicate qualitative similarities as well as some indications of quantitative differences. Rat liver microsomes were observed to have a higher capacity than human liver microsomes to metabolize methoxychlor to covalent binding intermediates (Bulger and Kupfer 1990). *In vitro* data also indicate that covalent binding of methoxychlor to human liver microsomes is similar across age and sex, whereas in rats, covalent binding in mature males is much higher than in mature females and immature males and females (Bulger and Kupfer 1989). Another comparison found that *in vitro* rates of demethylation of methoxychlor and mono-hydroxy methoxychlor with human liver microsomes were generally higher in rat liver microsomes than in human liver microsomes (Stresser and Kupfer 1998).

3.4.4 Elimination and Excretion

Direct information regarding possible age-related differences in the rate, extent, or route of elimination of methoxychlor and metabolites was not located.

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3.4.4.1 Inhalation Exposure

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

3.4.4.2 Oral Exposure

In comparison to DDT (a pesticide which is relatively persistent and slowly eliminated), animal studies indicate that ingested methoxychlor is excreted rapidly, predominantly in the feces and to a lesser extent in the urine. Approximately 90% of an oral dose of 50 mg/kg recrystallized methoxychlor was recovered in the feces of mice within 48 hours, and 10% was excreted in the urine (Kapoor et al. 1970). Only 7–8% of the material in the feces was excreted as the parent compound. In contrast, only 1.02 and 4.3% of the administered radioactivity was recovered in feces and urine collected from mice within 24 hours and 11 days, respectively, of administering single oral doses of 12.5 mg/kg radiolabeled DDT.

Assuming that the fecal metabolites (primarily demethylated, dechlorinated, and dehydrochlorinated compounds) did not result from degradation of unabsorbed parent compound by enteric bacteria, these data suggest that biliary excretion of metabolites contributes significantly to methoxychlor clearance. Supporting these observations are those that methoxychlor metabolites identified in bile collected from a bile-cannulated male goat were similar to those in feces collected from lactating female goats given 3.6 or 11.6 mg/kg methoxychlor (Davison et al. 1982, 1983). In the female goats, 40.5 and 67.5% of administered doses were excreted in the feces within 3 days, respectively, and metabolites accounted for 70 and 81% of radioactivity in the feces, respectively (Davison et al. 1982).

3.4.4.3 Dermal Exposure

No studies were located regarding excretion in humans after dermal exposure to methoxychlor. Three days after applying a single dose of 200 mg laboratory grade methoxychlor to the shaved backs of goats, 0.37–0.91% of the dose was excreted in the feces and 0.53–0.72% of the dose was excreted in the urine (Davison et al. 1983).

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3.4.4.4 Other Routes of Exposure

Parenteral exposure studies in animals indicate that biliary excretion of methoxychlor accounts for a significant fraction of fecal excretion. In bile-duct cannulated rats given a single intravenous dose of radiolabeled methoxychlor, the radiolabel was first detected in the bile within 1 minute (Weikel 1957). Fifty percent of the dose was excreted in the bile after 4 hours. The urinary excretion of label in bile-cannulated rats was only 0.1–0.2% compared to 5–10% in noncannulated rats, suggesting that the appearance of urinary metabolites was largely due to material that was reabsorbed in the gut (i.e., enterohepatic circulation).

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 et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (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

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1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

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

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

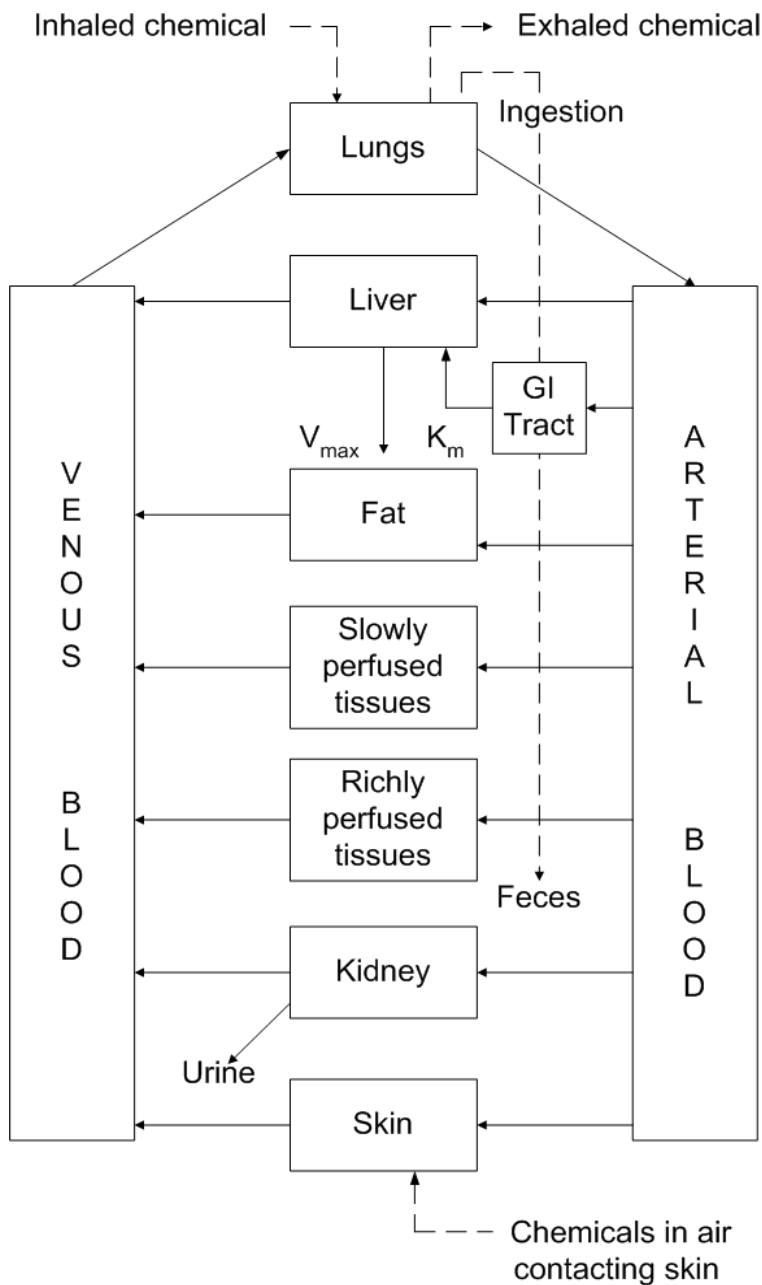
No studies were located regarding PBPK models for methoxychlor.

3.5 MECHANISMS OF ACTION

As discussed in Section 3.2, the chief effects of methoxychlor in animals are on the reproductive system. Although reproductive effects have mainly been observed in animals following oral exposures, it is likely that these types of effects could occur following inhalation and dermal exposures as well, if absorption of

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



Source: adapted from Krishnan et al. 1994

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.

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equivalent amounts occurred. For this reason, the mechanism of action for methoxychlor is discussed below without reference to route of exposure.

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies were located regarding the mechanism of absorption of methoxychlor in humans or animals by any route.

Distribution. No studies were located regarding the mechanism of distribution of methoxychlor in humans or animals after exposure by any route.

Metabolism. Methoxychlor is metabolized to estrogenic compounds in animals and humans by hepatic CYP enzymes (Dehal and Kupfer 1994; Kishimoto and Kurihara 1996; Stresser and Kupfer 1997, 1998; Stresser et al. 1996). In rats, *in vivo* and *in vitro* studies have shown that metabolism of methoxychlor to its mono-, bis-, tris-, and ring-hydroxy metabolites is mediated by CYP2C6, 2A1, 2B1, 2B2, and 3A (Kishimoto and Kurihara 1996; Li et al. 1995). The K_m values for partially purified preparations of CYP2C6, 2A1, 2B1, and 2B2 were 0.36, 0.38, 1.07, and 2.34 μM , respectively, and the V_{max} values were 0.40, 0.36, 1.20, and 1.22 mol/mol P450/minute, respectively (Kishimoto et al. 1995). Studies examining inhibition by antibodies to CYP2B1 and CYP2C6 of methoxychlor demethylation in rat liver microsomes and purified preparations of CYP2C6 provided evidence that CYP2C6 and another unidentified CYP isozyme make important contributions in the initial demethylation of methoxychlor in rats (Kishimoto and Kurihara 1996). Ring hydroxylation of methoxychlor and hydroxy methoxychlor derivatives in rats involves CYP2B isozymes, based on observations of inhibition of *in vitro* activity by anti-CYP2B monoclonal antibodies and enhanced ring hydroxylation activity in liver microsomes from phenobarbital-pretreated rats (Stresser et al. 1996).

Results from *in vitro* studies with human liver microsomes (discussed in Section 3.4.3 Metabolism) have led to the proposal that CYP2C19 and CYP1A2 may be the major CYP isozymes catalyzing the demethylation of methoxychlor and its mono-hydroxy derivative, and that other CYP forms, including CYP2A6, 2C9, and 2B6, may make major contributions, especially in individuals with low levels of CYP2C19 or CYP1A2 (Stresser and Kupfer 1998). Nonlinear regression analysis of data from insect cells overexpressing human CYP2C19 or 1A2 showed standard Michaelis-Menten kinetics for a one-enzyme model. The K_m (μM) values for cytochrome CYP2C19 and 1A2 were similar (0.43 and 0.51), but the V_{max} (nmol/minute/nmol P450) for 2C19 (26.8) was twice that for 1A2 (12.4), indicating greater

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intrinsic clearance of methoxychlor by CYP2C19 (Stresser and Kupfer 1998). Similar analysis of data from human liver microsomes (containing multiple CYPs) showed a fit for a two-enzyme system, even though there were wide variations for V_{\max} (20–576 pmol/minute/mg) and K_m (0.35–12 μM) between samples (Elsby et al. 2001; Stresser and Kupfer 1998). The discrepancy in kinetic values is probably an indication of individual genetic variability in expression of CYP enzymes (Shimada et al. 1994). Studies with human cDNA-expressed CYP isozymes suggest that ring hydroxylation of methoxychlor or its hydroxy derivatives may involve a different set of CYP isozymes than CYP-catalyzed demethylation, including CYP1A2, 2B6, 2D6, 2E1, and 3A4 (Stresser and Kupfer 1997).

Excretion. No studies were located regarding the mechanism of excretion of methoxychlor in humans or animals after exposure by any route.

3.5.2 Mechanisms of Toxicity

The primary effects of methoxychlor in animal models involve the reproductive system, and result from the interaction of methoxychlor and its metabolites with estrogen receptors, and possibly androgen receptors, although the data indicating androgen receptor binding are limited. Many of the reproductive effects of methoxychlor are similar to those caused by estrogen, and are believed to be due to the ability of the mono- and bis-hydroxy derivatives of methoxychlor and MDDE to act as estrogen analogues (Bulger et al. 1978a, 1978b, 1978c, 1978d, 1985; Ousterhout et al. 1981). Androgens and estrogens are very lipid soluble and thus, they diffuse easily through the cell membrane into the cytosol and nucleus (DeFranco 1999). Once a steroid binds to its receptor and the hormone-receptor complex reaches the nucleus, it binds to hormone response elements in the enhancers, silencers, or promoters upstream of the genes controlled by the steroid in question. The hormone-receptor complex acts as a transcription factor to either stimulate or repress transcription of RNA from the steroid responsive gene. The resulting RNA transcript is spliced to form messenger RNA, which in turn directs the synthesis of specific proteins that direct the characteristic steroid hormone responses (Fregly and Luttge 1982). Some ancillary models for immediate action of steroid hormones have recently been proposed and involve either cross-talk interactions with, or possibly direct binding to, other hormone and growth factor cell surface receptors, and cell surface receptor mediated uptake of serum carrier proteins bound to steroid hormones (Chen and Farese 1999).

In vitro studies have shown that methoxychlor itself does not bind to the estrogen receptor (Bulger et al. 1978a, 1978b; Matthews et al. 2000; Ousterhout et al. 1981) but that the mono-hydroxy and bis-hydroxy

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derivatives of methoxychlor and MDDE do bind to the receptor and cause nuclear translocation with the following order of potency: bis-OH-MDDE >> bis-OH-methoxychlor > mono-OH-MDDE > mono-OH-methoxychlor >> MDDE = methoxychlor (inactive) (Bulger et al. 1985). A similar hierarchy (bis-OH-MDDE > bis-OH-methoxychlor >> MDDE > methoxychlor) has been observed *in vivo* for the induction of uterine ornithine decarboxylase and increased uterine weight (Bulger et al. 1985). It was long thought that estrogen mediated its effects by binding to a single receptor, estrogen receptor α (ER α). Recently, however, a second estrogen receptor, ER β , has been discovered (Kuiper et al. 1996; Mosselman et al. 1996). Although bis-hydroxy methoxychlor has been shown to bind to ER α and ER β (Gaido et al. 1999, 2000), none of the earlier (before 1996) studies examining the difference in estrogen receptor binding affinity between methoxychlor and its metabolites differentiated between ER α and ER β .

It has not been fully established what effects are mediated by each estrogen receptor. However, there appears to be tissue-specific distribution of the two receptors (Kuiper et al. 1997), which may allow for tissue-specific effects by estrogens. In tissues where both receptors are expressed, ligand binding to the receptors results in heterodimer formation (an ER α receptor pairs up with an ER β receptor) (Cowley et al. 1997; Pace et al. 1997; Pettersson et al. 1997), which may result in different patterns of gene regulation than seen with homodimeric pairing (an ER α with an ER α or an ER β with an ER β). Additionally, each different estrogenic compound might act as an estrogen agonist at one receptor type and an estrogen antagonist at the other receptor type. This is apparently the case with the bis-hydroxy metabolite of methoxychlor, which has been shown to be an ER α agonist and an ER β antagonist in some *in vitro* assay systems (Gaido et al. 1999, 2000).

Adding further to the mechanistic complexity, Ghosh et al. (1999) have suggested that methoxychlor acts through a third, as yet unelucidated, mechanism, not necessarily via an ER. Ghosh et al. (1999) showed that methoxychlor induced an increase in mRNA of two estrogen-responsive genes (lactoferrin, LF, and glucose-6-phosphate dehydrogenase, G6PD) in uteri of ovariectomized wild-type and ovariectomized ER α -knockout mice. Induction of LF and G6PD mRNA by methoxychlor was slightly greater in wild-type mice than in ER α -knockout mice at 15–30 mg/kg/day. Since no functional ER α receptors were present in the ER α knockout mice, the involvement of ER β was indicated. However, when an estrogen inhibitor (ICI 182,780) was added, which should have eliminated methoxychlor interaction with ER β , methoxychlor still induced an increase in LF and G6PD mRNA at a slightly lower level than without inhibitor in wild-type mice and at the same level in ER α knockout mice. In the same experiment, estradiol-17 β resulted in large increases in LF and G6PD mRNA in wild-type mice, but in low levels of LF and G6PD mRNA (similar to control mice) in ER α -knockout mice (the authors point out that the

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concentration of ER β in uterus of the wild-type and ER α -knockout mice was very low). When an inhibitor was added, no increase in LF and G6PD mRNA was induced by estradiol-17 β in wild-type mice. Thus, the “estrogenic” activity of methoxychlor was not attenuated by the absence of functional ER α receptors or the presence of an estrogen inhibitor effective at ER β , suggesting that an additional mechanism of toxicity may exist besides interaction with ER α or ER β .

A study that examined the effects of methoxychlor, DDE, and estradiol-17 β on steroidogenesis and FSH responsiveness in ovarian cells provides additional support for a non-estrogenic mechanism of endocrine disruption by methoxychlor (Chedrese and Feyles 2001). In CHO-FSH-R cells (a Chinese hamster ovary cell line genetically modified to express the FSH receptor) exposed to DDE in the presence of FSH, DDE inhibited FSH-stimulated cAMP synthesis, which likely resulted in the observed decrease in progesterone synthesis and in decreased activity or synthesis of steroidogenic enzymes. Methoxychlor did not affect FSH-stimulated cAMP synthesis, but did inhibit estradiol-17 β -stimulated progesterone synthesis in primary culture pig granulosa cells (Chedrese and Feyles 2001). Since progesterone is required for normal ovulation and implantation, the study authors speculated that this mechanism may partially explain the detrimental effects of methoxychlor on reproduction (Chedrese and Feyles 2001). The involvement of an ER is unlikely because no metabolic activation system was included, and methoxychlor, unlike its metabolite HPTE, does not interact with the ERs to any great extent (Bulger et al. 1978a, 1978b, 1985; Matthews et al. 2000; Ousterhout et al. 1981).

In contrast, the results of another *in vitro* study indicated that purified methoxychlor may have weak intrinsic estrogenicity (Elsby et al. 2001). A yeast estrogenicity assay was used that did not incorporate a microsomal metabolic activation system. The yeast had the DNA sequence of the human ER α integrated into the genome and contained transfected expression plasmids with the yeast 3-phosphoglycerate kinase promoter, estrogen responsive sequences, and a β -galactosidase reporter gene. Binding of an active ligand to the ER initiated transcription of the reporter gene, secretion of β -galactosidase into the medium, and ultimately, a color change in the medium from yellow to red. Methoxychlor was at least 100,000 times less potent than estradiol-17 β and about 100 times less potent than the methoxychlor metabolite HPTE (Elsby et al. 2001).

An *in vitro* assay in human breast cancer MCF-7 cells examined the estrogenicity of methoxychlor and other xenoestrogens, as well as estradiol-17 β and DES, by quantitatively assaying the induction or repression of four endogenous estrogen-regulated marker genes (pS2, transforming growth factor β 3, monoamine oxidase A, and α 1-antichymotrypsin) (Jørgensen et al. 2000). Methoxychlor induced pS and

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α 1-antichymotrypsin and repressed monoamine oxidase A and transforming growth factor β 3, as did estradiol-17 β , but methoxychlor was at least 100,000 times less potent than estradiol-17 β (10^{-13} – 10^{-11} M for estradiol-17 β and 10^{-6} – 10^{-5} M for methoxychlor). These estrogenic activities of methoxychlor were apparently mediated through the ER α since no ER β was detected in MCF-7 cells.

Methoxychlor, like estradiol-17 β , stimulated an increase in uterine peroxidase activity (a marker of estrogen action) (Cummings and Metcalf 1994). Also similar to estradiol-17 β , methoxychlor stimulation of peroxidase was inhibited by actinomycin D (an RNA synthesis inhibitor) and cycloheximide (a protein synthesis inhibitor), indicating a similar mechanism of action. Estrogen-induced protein synthesis, an early effect of estradiol in the immature rat uterus and indicator of receptor occupancy and mRNA synthesis, was stimulated by 99% pure methoxychlor or estrone in an identical manner (Cummings and Metcalf 1995a). Estrogen-induced protein synthesis by methoxychlor and estrone was inhibited similarly by actinomycin D and cycloheximide. Methoxychlor induced the secretion of proteins in mature ovariectomized mice (Rourke et al. 1991) and neonatal mice (Eroschenko and Rourke 1992). Although the proteins secreted by methoxychlor were similar to those observed following estradiol treatment, some differences were noted, particularly so for neonatal mice. It was postulated by the study authors that posttranslational alterations to the estrogen receptors might be responsible for these differences (Rourke et al. 1991). These observations strongly support the view that the estrogenic effects of methoxychlor are mediated via binding of *O*-demethylated metabolites to estrogen receptors resulting in protein synthesis. Secretion of altered or different proteins into the uterine fluid during implantation and pregnancy could alter the essential uterine environment which may ultimately be responsible for decrements in fertility.

There are a number of physiological mechanisms by which the estrogenic effects of *O*-demethylated metabolites or contaminants of methoxychlor can interfere with reproduction. In female animals, possible mechanisms for decreased fertility include decreased mating frequency (Harris et al. 1974) decreased decidualization and decreased uterine receptivity to implantation (Cummings and Gray 1987, 1989), accelerated tubal transport of fertilized ova (Cummings and Perrault 1990), complete inhibition of implantation and altered preimplantation embryonic development and transport in mice (Hall et al. 1997; seen after intraperitoneal methoxychlor administration), and atresia of preovulatory follicles with reduced corpus luteum formation (Gray et al. 1989). In male animals, possible mechanisms for decreased fertility include decreased mating frequency (Gray et al. 1999; Harris et al. 1974), inadequate cervical stimulation of female animals to induce events necessary for implantation (Gray et al. 1989), and decreased Leydig cell function (Akingbemi et al. 2000; Gray et al. 1989).

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Many of the effects described above are probably mediated by a direct effect of methoxychlor metabolites on estrogen-sensitive tissues. Alternatively, some of these changes may result from the effects of methoxychlor metabolites on the endocrine system. For example, methoxychlor has been shown to cause increased levels of gonadotropin releasing hormone in the hypothalamus (Goldman et al. 1986), elevated levels of prolactin, and thyroid stimulating hormone in the pituitary (Goldman et al. 1986; Gray et al. 1989), and reduced levels of thyroid-stimulating hormone, testosterone, and progesterone in the serum (Cummings and Gray 1989; Gray et al. 1989). These methoxychlor-induced effects on the endocrine system may be partially responsible for methoxychlor-induced effects such as uterine and mammary gland hyperplasia (Tegeris et al. 1966) and gonadal atrophy (Bal 1984; Gray et al. 1988; Hodge et al. 1950; Tullner and Edgcomb 1962), and could also be important in impaired reproduction associated with methoxychlor exposure (Bal 1984; Gray et al. 1989; Harris et al. 1974).

Some of the reproductive effects seen in male rodents following methoxychlor exposure are similar to those produced by either estrogens or antiandrogens (Gray et al. 1999). Delayed puberty and reduced accessory sex gland size can be caused by an estrogen or an antiandrogen. Estrogenic compounds cause a decrease in LH secretion from the pituitary and in serum testosterone and an increase in pituitary tumors and hyperprolactinemia, whereas antiandrogens enhance LH secretion from the pituitary, cause an increase in serum testosterone, and have no effect on prolactin or on pituitary tumorigenesis (Gray et al. 1999). In male rats dosed with 0, 200, 300, or 400 mg/kg/day methoxychlor from weaning (postpartum day 21) through 11 months of age, preputial separation was delayed in a dose-dependent manner, pituitary size was decreased at all exposure levels, and there were no statistical differences from controls in serum LH, prolactin, and testosterone (Gray et al. 1999). These results suggest that methoxychlor might interact with the androgen receptor (AR) as an antagonist; however, it should be noted that in some *in vitro* assays, estradiol-17 β can itself act as a weak androgen antagonist (Kelce et al. 1995). Only one study has investigated the potential androgen antagonism of methoxychlor and its metabolites; this experiment was not a direct receptor binding assay. The methoxychlor metabolite HPTE was a weak AR antagonist of dihydrotestosterone in HepG2 human hepatoma cells transiently transfected with the human androgen receptor and a reporter gene linked to an androgen responsive promoter; methoxychlor itself showed even less androgen antagonism in this experiment (Maness et al. 1998). *p,p'*-DDE, an isomer of the methoxychlor structural analog DDT, is also an AR antagonist of dihydrotestosterone (Danzo 1997; Kelce et al. 1995, 1997; Maness et al. 1998). It is thought that androgen antagonism explains some of the reproductive and developmental effects seen in male rats exposed to *p,p'*-DDE, including reduced anogenital distance, retention of thoracic nipples, delayed puberty, and reduced accessory sex organ weights (Kelce et al. 1997; Loeffler and Peterson 1999; You et al. 1998). Some of these effects (delayed

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puberty and reduced accessory sex organ weights) are also seen following exposure to methoxychlor (Bal 1984; Gray et al. 1989, 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962). More exhaustive studies regarding methoxychlor metabolite binding to the AR are necessary to be able to determine the contribution of androgen antagonism to the reproductive and developmental effects resulting from methoxychlor exposure.

While the reproductive effects of methoxychlor appear to be due to the estrogenic nature of the *O*-demethylated metabolites, parent methoxychlor may be responsible for the neurotoxic effects observed at high exposure levels in animals (Cannon Laboratories 1976; Tegeris et al. 1966). The chief reason for suspecting this is that methoxychlor is a close structural analogue of DDT (differing in that the methoxy groups of methoxychlor are replaced by chlorine groups in DDT), and high levels of DDT are known to be neurotoxic in animals (Agency for Toxic Substances and Disease Registry 1994). DDT prevents the deactivation of the sodium gate after neuron activation and membrane depolarization (Brown et al. 1981; Coats 1990; Wu et al. 1975), resulting in hyperexcitability of the nerve. Other molecular mechanisms may contribute to DDT induced hyperexcitability of the nerve; these include prolonging the action potential by preventing the full opening of the potassium gates (Narahashi and Haas 1967) and interference with two specific neuronal adenosine triphosphatases (ATPases) thought to be involved in controlling sodium, potassium, and calcium fluxes through the nerve membrane (Matsumura and Patil 1969; Matsumura 1985). *In situ* studies have shown that methoxychlor (and some other DDT structural analogs) increase and prolong the depolarizing afterpotential of nerves, similar to DDT (Wu et al. 1975). It is important to note that if the neurotoxic effects (tremors, convulsions) of methoxychlor are indeed attributable to the parent compound, then *O*-demethylation to the phenolic metabolites represents a detoxification pathway for neurotoxicity. This is consistent with the fact that this type of neurotoxicity has only been noted at doses high enough to surpass metabolic capacity (Cannon Laboratories 1976; Tegeris et al. 1966) or in animals in which hepatic metabolism has been impaired (Lehman 1952).

3.5.3 Animal-to-Human Extrapolations

Some species differences in sensitivity to methoxychlor have been observed. Rabbits appear to be more sensitive than rats to short-term exposure to methoxychlor (Kincaid Enterprises 1986; Smith et al. 1946). All rats administered a single gavage dose of 5,000 mg/kg methoxychlor survived, whereas 7 of 13 rats given 7,000 mg/kg died (Smith et al. 1946). Four of 4 rabbits died following the administration of 4–15 daily doses of 200 mg/kg/day methoxychlor (Smith et al. 1946). Fatty degeneration of the liver and nephrosis were observed in some or all of the rabbits that died following exposure to 200 mg/kg/day.

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Two of 17 pregnant rabbits died within 10 days of receiving 13 daily oral exposures to 251 mg/kg/day (Kincaid Enterprises 1986).

Recent studies in mice show effects that may be mediated through the estrogen receptor (increased prostate weight and altered urine-marking behavior) at low exposure levels (0.02 mg/kg/day) (vom Saal et al. 1995; Welshons et al. 1999). No other studies were located that examined similar end points in other species exposed to similarly low levels of methoxychlor; therefore, it is unclear whether there are significant differences between species in susceptibility to these effects of methoxychlor.

Several *in vitro* studies have indicated the presence in human hepatic microsomes of enzymatic activities similar to those in rats that are responsible for the metabolism of methoxychlor to its estrogenic metabolites (Dehal and Kupfer 1994; Stresser and Kupfer 1998). Therefore, there is some indication that rats may be an adequate animal model for the estrogenic effects of methoxychlor in humans. Rates of formation of mono- and bis-hydroxy methoxychlor from methoxychlor with human liver microsomes were generally lower than rates with rat liver microsomes, but some human liver samples displayed higher rates than the rates for rat microsomes (Stresser and Kupfer 1998).

Another *in vitro* study examined the relative binding affinities (RBA) of methoxychlor, its metabolite HPTE, and other estrogenic substances (compared to estradiol-17 β) to bacterially expressed estrogen receptor fusion proteins from humans (ER α), mouse (ER α), chicken, green anole (a lizard), and rainbow trout (Matthews et al. 2000). The ER fusion proteins contained three of the six domains of the receptor, including the ligand binding domain, fused to glutathione-S-transferase. Methoxychlor did not bind to the human or mouse receptor proteins (displaced <10% of radiolabeled estradiol-17 β), bound only weakly to the chicken and green anole receptor proteins (displaced 10–50% of radiolabeled estradiol-17 β), and had an RBA of 0.95 with the rainbow trout receptor protein. HPTE had RBAs of 1.2 with the human and mouse receptor proteins, 4.8 with the chicken and green anole receptor proteins, and 14 with the rainbow trout receptor protein. These data indicate some species differences in receptor binding, but support the mouse as an adequate model for the study of estrogenic effects of methoxychlor mediated through ER binding.

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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 Colborn and Thomas (1992) and again by Colborn (1993), was also used in 1996 when Congress mandated the Environmental Protection Agency (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), which in 1998 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 isoflavonoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is ample evidence, *in vitro* and *in vivo*, that methoxychlor has estrogenic properties. Estrogens, both endogenous and exogenous, or environmental, mediate many of their effects by binding to estrogen receptors (ER) in the cytoplasm of target tissue cells. Estrogens are very lipid soluble and diffuse easily through the cell membrane into the cytosol, where they bind to empty estrogen receptors. The occupied

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receptor is translocated to the nucleus, where the receptor-estrogen complex binds to hormone response elements upstream of the gene or genes controlled by the estrogen and induces or inhibits the production of specific proteins that then produce a characteristic effect(s). The effect may be seen in the tissue in which the protein was synthesized or in a distant tissue following secretion and transport of the newly synthesized protein through the bloodstream. For many years, it was thought that estrogens mediated their effects by binding to a single receptor, estrogen receptor α (ER α). However, a second estrogen receptor, ER β , has recently been discovered (Kuiper et al. 1996; Mosselman et al. 1996). It has not been fully established what effects are mediated by each receptor. However, there appears to be tissue-specific distribution of the two receptors (Kuiper et al. 1997), which may allow for tissue-specific effects by endogenous and exogenous estrogens. This adds complexity to the effects of estrogenic compounds because each compound may, theoretically, interact with each receptor as an estrogen agonist or antagonist. The estrogenicity of methoxychlor derives primarily from its metabolites, mono- and bis-hydroxy methoxychlor. Bis-hydroxy methoxychlor is known to bind to ER α and ER β and is thought to be an ER α agonist and an ER β antagonist (Gaido et al. 1999, 2000). Recent data by Ghosh et al. (1999) indicate that methoxychlor may also act via another, as yet unknown, mechanism not mediated by either ER α or ER β . Estrogenic mechanisms are discussed in more detail in Section 3.5.2 Mechanisms of Toxicity.

There are no data showing estrogenic effects of methoxychlor in humans. However, there are numerous *in vivo* animal studies showing the estrogenic nature of methoxychlor and its metabolites. Below is a brief overview of the effects of exposure to methoxychlor on the endocrine and reproductive systems. A more detailed discussion of these studies can be found in Section 3.2.

Exposure to methoxychlor has been shown to affect the reproductive systems of male and female mammals in ways that mimic or antagonize the effects of estrogen. Effects have been observed following acute, intermediate, and chronic duration exposures and over a wide range of exposure levels. Effects have been seen following exposures *in utero*, during lactation, or post-weaning. There is also a wide range of effects, encompassing the structural, functional, and behavioral realms, in a number of different species. In female mammalian species, sexual maturity was affected, as demonstrated by precocious vaginal opening and delayed onset of estrus cyclicity in rats and mice exposed to 5–200 mg/kg/day (Appel and Eroschenko 1992; Chapin et al. 1997; Cooke and Eroschenko 1990; Eroschenko 1991; Eroschenko and Cooke 1990; Gray et al. 1989; Harris et al. 1974; Swartz and Corkern 1992). A number of structural and functional effects were also seen, including mammary gland hyperplasia in pigs exposed to 1,000 mg/kg/day (Tegeris et al. 1966) and ovarian atrophy in rats and mice exposed to 25–400 mg/kg/day

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(Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991), gross and cellular ultrastructural alterations of the lining of the uterus in mice exposed to 50–100 mg/kg/day (Swartz et al. 1994), persistent estrus in rats and mice exposed to 25–400 mg/kg/day (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1991), decreased mating frequency rats exposed to 60–160 mg/kg/day (Harris et al. 1974), decreased fertility in rats and mice exposed to 35.5–200 mg/kg/day (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966; Kincaid Enterprises 1986; Wenda-Rozewicka 1983), decreased live offspring in rats exposed to 100–150 mg/kg/day (Chapin et al. 1997; Gray et al. 1989), and increased number of resorptions of fetuses in rats and rabbits exposed to 17.8–400 mg/kg/day (Culik and Kaplan 1976; Khera et al. 1978; Kincaid Enterprises 1986). Serum progesterone and pituitary prolactin hormone levels were also affected in rats exposed to 400 mg/kg/day (Gray et al. 1988), and cellular biochemistry of ovarian cells was altered (an accumulation of lipid in the interstitial and thecal cells) in mice exposed to 100 mg/kg/day (Martinez and Swartz 1992).

Similar types of effects are seen in male animals. Delayed preputial separation indicated delayed sexual maturity. Structural and functional effects seen included decreased testes, ventral prostate, epididymis, and seminal vesicle weights in rats exposed to 50–1,400 mg/kg/day (Bal 1984; Chapin et al. 1997; Goldman et al. 1986; Gray et al. 1989, 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962), increased adrenal gland, seminal vesicle, and prostate weights in rats exposed to 0.02–400 mg/kg/day (Chapin et al. 1997; Gray et al. 1989, 1999; Stoker et al. 1999; Welshons et al. 1999), inhibition of testes development in rats exposed to 5–150 mg/kg/day (Chapin et al. 1997), decreased caudal epididymal sperm count in rats exposed to 50–400 mg/kg/day (Gray et al. 1989, 1999), decreased mating frequency in rats exposed to 60–400 mg/kg/day (Gray et al. 1999; Harris et al. 1974), decreased copulatory stimulation of females necessary for pregnancy in male rats exposed to 100 mg/kg/day (Gray et al. 1989), and decreased fertility in rats exposed to 35.5–300 mg/kg/day (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966; Kincaid Enterprises 1986; Wenda-Rozewicka 1983). Hormone levels were also affected in males, with increased levels of pituitary prolactin, FSH, TSH, and hypothalamic GnRH in rats exposed to 25–50 mg/kg/day (Goldman et al. 1986; Gray et al. 1989), decreased levels of serum TSH in rats exposed to 100–200 mg/kg/day (Cummings and Gray 1989; Gray et al. 1989), decreased serum testosterone and progesterone in mice and rats exposed to 33–100 mg/kg/day (Amstislavsky et al. 1999; Cummings and Gray 1989; Gray et al. 1989), and decreased interstitial fluid and epididymide testosterone in rats exposed to 100 mg/kg/day (Gray et al. 1989). Some hormone alterations occurred at methoxychlor

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exposure levels below those causing reproductive effects and may have contributed to or caused those effects.

It is thought that most, if not all, of the above endocrine disruptive effects are mediated by the interaction of the bis-hydroxy metabolite of methoxychlor (HPTE) with one or both of the estrogen receptors and/or the androgen receptor. It is also possible that tissue toxicity or some other indirect route of influence by methoxychlor or its metabolites resulted in some of these effects. Numerous *in vitro* studies have verified that methoxychlor binds to ERs, although with much lower affinity than estradiol. Methoxychlor has been shown to bind ER (probably both ER α and ER β) from MCF-7 cells (a human breast cancer cell line) (Dodge et al. 1996), rabbit uterine cells (Danzo 1997), bovine uterine endometrial explants (Tiemann et al. 1996), GT1 cells (a murine immortal hypothalamic neuron cell line) (Roy et al. 1999), and MtT/E-2 cells (a estradiol-17 β -responsive rat pituitary cell line) (Maruyama et al. 1999). Methoxychlor has also been shown to bind to human ER α and ER β expressed in Sf9 (insect) cells (Kuiper et al. 1998). The methoxychlor metabolite bis-OH-methoxychlor (or HPTE) has been shown to bind to human and rat ER α and ER β expressed in human hepatoma (HepG2) cells (Gaido et al. 1999, 2000), and to be an ER α agonist and an ER β antagonist. Methoxychlor and HPTE can also weakly antagonize dihydrotestosterone at the human androgen receptor (AR) (expressed in HepG2 cells) (Maness et al. 1998). In male rats exposed to 200 mg/kg/day methoxychlor from weaning (postpartum day 21) through 11 months of age, preputial separation was delayed, pituitary size was decreased, and there were no statistical differences from controls in serum LH, prolactin, and testosterone (Gray et al. 1999); these effects are typical of antiandrogenic, but not estrogenic, compounds. The binding affinity and potency of methoxychlor was generally observed to be 1,000–100,000 times less than estradiol (Dodge et al. 1996; Kuiper et al. 1998). However, it is thought that estrogen is bound to serum proteins (>95%) *in vivo* that render it less available for migration into cells and receptor binding (Nagel et al. 1999; vom Saal et al. 1995). The fact that methoxychlor is not bound by these proteins must be taken into account when using *in vitro* assays in serum-free media to simulate *in vivo* exposures (Nagel et al. 1999; vom Saal et al. 1995). Receptor binding of methoxychlor is discussed in more detail in Section 3.5.2, Mechanisms of Toxicity.

Effects on Wildlife. Whether methoxychlor causes estrogenic effects in wildlife has been investigated in a number of experiments. Although methoxychlor has certainly produced effects in fish, amphibians, and sea urchins, only a subset of these effects seem to be due to estrogenic activity. The mechanism for these other effects is not known.

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The estrogenic activity of methoxychlor has been demonstrated in catfish. In catfish, estrogen receptors have been found in liver, brain, and testes. The liver is the primary target tissue of estrogen in fish and is the site of the greatest ER concentration. Interaction with fish liver ER stimulates the synthesis of vitellogenin (the precursor to egg yolk) and also stimulates the synthesis of more ER. Nimrod and Benson (1997) tested methoxychlor, the *O*-demethylated metabolite of methoxychlor and several other environmentally relevant compounds for their ability to compete with estradiol for binding to catfish ER *in vitro*. Methoxychlor was only about 1/6,700 as potent and *O*-demethylated methoxychlor was 1/1,000 as potent as estradiol. Even though *O*-demethylated methoxychlor was not a very strong catfish ER ligand, it was the most potent environmentally relevant compound tested in this study, including *o,p'*-DDT and *o,p'*-DDE, which had extremely low binding ability. When injected into catfish intraperitoneally, methoxychlor did not induce a detectable estrogenic response, as measured by vitellogenin synthesis (Nimrod and Benson 1997). Curiously, after depletion of the liver enzyme thought to be responsible for methoxychlor metabolic transformation, serum vitellogenin and estradiol increased (Schlenk et al. 1997).

Methoxychlor causes subtly different effects from estradiol in trout. Trout exposed every third day to 0, 0.5, 1, 2, or 4 mg/L methoxychlor (laboratory grade) by immersion for 2 hours followed by rinsing from 6 days prior to hatching through 24 days post-hatching showed increased mortality. Mortality following estradiol exposure was 3 times that of methoxychlor-induced mortality (Krisfalusi et al. 1998a). Further experiments showed that methoxychlor caused increased mortality at day 16 post-hatch regardless of number of treatments, whereas estradiol caused increased mortality after 10 treatments regardless of developmental time. Therefore, methoxychlor does not appear to be acting by the same mechanism as estradiol. Also noted in this study was that the skin of methoxychlor-treated fish was lighter than controls by 6 days post-hatching, and the skin of estradiol-treated fish was darker than controls between days 9 and 12 post-hatching, again indicating different mechanisms. The authors speculated that the lighter skin may be due to decreased synthesis and/or release of melanophore stimulating hormone from the pituitary and that the darker skin may be due to toxicity. Fish treated with methoxychlor also showed a dose-dependent decrease in weight. Estradiol caused weight reduction as well, but in a non-dose-dependent manner. A dose-dependent increase in mortality and decrease in growth in trout was also observed (Krisfalusi et al. 1998b). Methoxychlor treatment did not disrupt male sex differentiation or early testicular development in these trout.

Methoxychlor did not produce estrogenic effects in Japanese medaka (a teleost, or bony fish) at doses comparable to an effective dose of estradiol (Nimrod and Benson 1998). In Japanese medaka, no

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developmental or reproductive toxicity was seen following exposure to 0, 0.2, 0.6, or 2.3 µg/L laboratory grade methoxychlor in the water for the first month after hatching. Parameters monitored included ovarian or testicular size, sex ratio, fertility, viability of eggs, and hatchability of eggs. These results contrast with the definitive effects that estradiol causes in these fish. Medaka are susceptible to phenotypic sex reversal when exposed to estrogens and androgens through the diet during early life stages. Exposure to 0.01, 0.12, or 1.66 µg/L estradiol as above resulted in 100% female populations. Fish (female) in the 1.66 µg/L estradiol group also had lower fecundity than controls.

In vitro experiments using cultured carp hepatocytes showed that methoxychlor was 1,000-fold less potent than estradiol. The order of estrogenic potency, as measured by vitellogenin induction, of compounds tested was methoxychlor > *o,p*-DDT > chlordecone . bisphenol-A . 4-t-pentylphenol (Smeets et al. 1999). When cells were exposed simultaneously to estradiol and methoxychlor, estradiol antagonized the effects of methoxychlor. Methoxychlor did not appear to be metabolized to more estrogenic metabolites in carp by CYP1A2 (one of the CYP liver enzymes in mammals responsible for metabolizing methoxychlor to more estrogenic compounds), as induction of this enzyme did not enhance the estrogenicity of methoxychlor.

In salamanders, methoxychlor sometimes causes subtly different effects from estradiol and other times completely different effects. Salamander eggs exposed to up to 10 mg/L laboratory-grade methoxychlor experienced no increase in mortality through post-hatching day 10 (Ingermann et al. 1997). Exposures as low as 0.1 mg/L resulted in precocious hatching and reduced startle response. The effects of methoxychlor were compared to estradiol in salamanders (Ingermann et al. 1999). Salamander eggs were exposed to 0, 0.2, 1.0, or 5.0 µM laboratory grade methoxychlor, recrystallized methoxychlor, HPTE (the estrogenic metabolite of methoxychlor in mammals), estradiol, or deoxycorticosterone (DOC, an adrenal steroid) until 10 days post-hatching. Laboratory grade and recrystallized methoxychlor were equally potent at causing precocious hatching and a blunted startle response, suggesting that contaminants are not responsible for these effects. Only the highest exposure level of estradiol resulted in precocious hatching and an altered startle response. The startle response in estradiol-treated hatchlings frequently involved swimming in circles, whereas the startle response in methoxychlor-treated hatchlings involved a shorter straight distance traveled (never circles). Exposure of eggs and hatchlings to HPTE resulted in no effect on day of hatch or startle response. DOC had no effect on hatch time and resulted in reduced startle response only at the highest exposure level. The effects of laboratory grade and recrystallized methoxychlor and HPTE on maturation of frog oocytes *in vitro* were compared to the effects of estradiol (Pickford and Morris 1999). Both grades of methoxychlor caused a highly significant inhibition of

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progesterone-induced germinal vesicle breakdown (GVBD, necessary for oocyte maturation), while estradiol and HPTE had no effect. This indicates that methoxychlor is the compound responsible for this effect and that this effect is not estrogenic in nature. Failure of ICI 182,780 (an ER inhibitor) to alter the effect of methoxychlor on oocyte maturation indicates that it is not mediated through the ER. The mechanism of this effect by methoxychlor also does not appear to involve the progesterone receptor, as neither methoxychlor nor HPTE exhibited any competitive binding affinity for this receptor.

In sea urchins, exposure of sperm to 3 ppm laboratory grade methoxychlor for 10 or 30 minutes caused a 30 or 100% reduction in fertilization, respectively (Mwatibo and Green 1997). Similar exposure of eggs did not result in a decrease in fertilization, but 5.5% of the embryos resulting from these eggs showed abnormal, stunted or absent gut, malformed spicules (part of the skeleton), and abnormal shape. Embryos from sperm exposed to methoxychlor for 10 minutes showed a slight, but statistically insignificant, increase in the same anomalies. The mechanism of the effect was not examined.

Taken together, these data indicate that methoxychlor has endocrine disruptive effects in fish, amphibian, and sea urchin fertility, growth, and development. Sometimes the effects of methoxychlor parallel those of estradiol and sometimes there are either subtle or drastic differences from the effects of estradiol. Thus, it is likely that not all of the effects of methoxychlor in aquatic wildlife are mediated through estrogen receptors. There may be many different mechanisms involved in the varied effects and the different species.

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.

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

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

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There is no direct information on the toxicity of methoxychlor in children or on effects in adults that were exposed as children. Animal data indicate that the primary target of methoxychlor is the reproductive system and that methoxychlor can both affect adult animals and influence the development of the reproductive system in males and females. Thus, there is concern about whether sufficient exposure to methoxychlor might potentially affect the developing reproductive system of fetuses, children, and adolescents. Although there is no direct evidence that children are more susceptible to health effects from methoxychlor exposure than adults, lower doses of methoxychlor were generally required to produce reproductive effects in developing animals than in adults.

Numerous studies have shown that adult animals, exposed during development or as adults, exhibited effects attributed to the “estrogenic” activity of methoxychlor, indicating that metabolism of methoxychlor to its estrogenic metabolites occurs in developing and adult animals. These estrogenic effects included altered hormone levels, abnormal histology, and decreased fertility (Bal 1984; Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Martinez and Swartz 1991). The exact mechanisms of disruption of normal reproductive function may differ between developmental and adult exposure. The reproductive organs and tissues of adult animals are already developed and capable of responding appropriately to normal hormone levels. Methoxychlor disrupts the normal hormone balance by interacting with estrogen, and possibly androgen receptors, which causes abnormal reproductive responses. Methoxychlor probably also directly affects tissues containing these receptors. Exposure to methoxychlor during critical periods of reproductive development may cause abnormal or under- or over-development of certain reproductive organs or tissues, as well as an altered tissue distribution of these same receptors, so that the organs/tissues have no capability to respond to hormones in a normal fashion, even if hormone levels return to normal following cessation of exposure.

There is ample information from animal studies to indicate that the developing organism is susceptible to the effects of methoxychlor and its metabolites. Wavy ribs have been observed in the offspring of female rats exposed to 7.8 mg/kg/day methoxychlor on gestation days 6–15 (Culik and Kaplan 1976). Khera et al. (1978) also noted wavy or extra ribs and decreased fetal weight, as well as an increased percentage of dead and resorbed fetuses, in offspring of female rats exposed to 200 mg/kg/day during gestation. Body weights of fetuses from female rabbits receiving 35.5 (but not 5.01) mg/kg/day methoxychlor on days 7–19 of gestation were decreased by 10% and the percentage of male offspring was decreased (Kincaid Enterprises 1986).

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Methoxychlor also causes numerous functional changes in the adult and developing reproductive system of animals. Unlike its structural cousin, DDT, methoxychlor is metabolized rapidly to its mono- and bis-hydroxy derivatives. Therefore, the neurotoxicity associated with DDT is not seen following methoxychlor exposure (except at extremely high exposure levels). However, methoxychlor and its metabolites, especially bis-hydroxy methoxychlor, or HPTE, are estrogenic and can bind to estrogen receptors, enhancing or attenuating the effects of endogenous estrogens (Bulger et al. 1978d; Gaido et al. 1999, 2000; Kuiper et al. 1998; Kupfer and Bulger 1979, 1987b; Mosselman et al. 1996). Estrogen receptors are found in many tissues in males and females, including mammary gland, uterus, vagina, ovary, testes, epididymis, prostate, thymus, bone, central nervous system, pituitary, hypothalamus, and cardiovascular system (Kuiper et al. 1998). When the delicate balance of endogenous estrogen levels is disrupted, transient or permanent functional and/or structural abnormalities may occur in a variety of organs or tissues, especially in the reproductive system. Exposure to methoxychlor during critical stages of development or in the adult animal has been shown to result in a number of reproductive effects. Effects associated with methoxychlor exposure include histopathological changes in the reproductive organs and accessory glands (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Martinez and Swartz 1991, 1992; Stoker et al. 1999; Swartz et al. 1994; Tegeris et al. 1966), impaired pubertal development (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974) and reproductive function (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Haskell Laboratories 1966; (Wenda-Rozewicka 1983), and altered hormone levels (Cummings and Gray 1989; Cummings and Laskey 1993; Goldman et al. 1986; Gray et al. 1988, 1989; Martinez and Swartz 1992; Stoker et al. 1999). There is a wide range of doses reported in the literature that cause effects in developing animals. The great majority of studies report effects from *in utero*, neonatal, and adult exposure beginning at around 5–150 mg/kg/day (Bal 1984; Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Martinez and Swartz 1991). Reproductive effects in female mice and rats from *in utero* and postnatal exposures include precocious vaginal opening (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974), delayed onset of estrus (Gray et al. 1989), altered vaginal and uterine histology (Chapin et al. 1997; Gray et al. 1988, 1989), altered ovarian histology (Bal 1984; Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Martinez and Swartz 1991), and decreased fertility (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974). In male animals, delayed preputial separation, decreased testes, epididymis, seminal vesicle, prostate weight, and decreased fertility have been observed following developmental exposure (Chapin et al. 1997; Gray et al. 1999; Harris et al. 1974). A few studies have shown that *in utero* exposures as low as 0.02 mg/kg/day caused changes in sex accessory organs and changes in neurobehavioral and sociosexual behavioral parameters (Palanza et al. 1999; Parmigiani et al. 1999; vom Saal et al. 1999; Welshons et al. 1999). For

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a more detailed discussion of the reproductive and developmental effects of methoxychlor, see Sections 3.2.2.5 Reproductive Effects and 3.2.2.6 Developmental Effects.

There are no studies directly examining whether methoxychlor and metabolites cross the placenta in humans or animals. Subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); however, it should be noted that exposure was not exclusively during gestation in any of these studies. It is unclear if these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes. Levels of methoxychlor and its metabolites were determined in breast milk and plasma of female rats orally administered 5, 50, or 150 mg/kg/day methoxychlor during gestation and early lactation (Chapin et al. 1997). Breast milk levels of methoxychlor and mono- and di-hydroxy methoxychlor were very high compared to plasma levels (25–215% higher). Levels in pup serum were much lower (1.3–5.9% of dam plasma levels), but were still detectable at most exposure levels. Pup serum levels may actually reach much higher levels, since more than 24 hours elapsed between administration of the last dose of methoxychlor to the dam and collection of pup blood for analysis. Therefore, children may be exposed to significant levels of methoxychlor and metabolites via breast milk if their mother is orally exposed to sufficient amounts of methoxychlor. One study analyzed human breast milk for the presence of methoxychlor and found none (Hooper et al. 1997); however, two other studies did detect methoxychlor in human breast milk (Campoy et al. 2001a, 2001b). Unlike its structural cousin, DDT, methoxychlor is metabolized and eliminated from the body rapidly. Some methoxychlor is distributed to tissues throughout the body, but does not appear to persist, even in fat. Therefore, it is unlikely that methoxychlor from pre-conception exposure of the mother would be available for mobilization during pregnancy and lactation. However, puzzling, unexplained data suggest that it is possible that previous exposure of the mother to methoxychlor results in damage to her reproductive system in some way that causes future offspring to experience reproductive anomalies, although the evidence of such occurrences in animals is very limited. Swartz and Corkern (1992) noted precocious vaginal opening in female offspring of a second unexposed pregnancy (F1b) in mice. It is unclear why the F1a exposed litter did not experience precocious vaginal opening and by what mechanism this effect was produced in the unexposed F1b offspring.

The primary pathway by which methoxychlor is metabolized in the liver (the main site of methoxychlor metabolism) is sequential demethylation reactions to yield the estrogenic mono- and bis-hydroxy methoxychlor. *In vitro* studies with human liver microsomes and human recombinant cytochrome P450

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(CYP) isozymes indicate that multiple CYP isozymes are involved in the demethylation of methoxychlor in humans (Stresser and Kupfer 1998). This study indicates that CYP2C19 and CYP1A2 may be the major CYP demethylases for methoxychlor, but that other forms, including CYP2A6, CYP2C9, and CYP2B6, are likely to be major contributors, especially in individuals with low levels of CYP2C19 or CYP1A2. Other CYP enzymes that may play a role include CYP2D6, CYP2E1, and CYP3A4. Many of these phase I enzymes are likely to have overlapping roles. Although nothing is known about phase II metabolism in humans, Davison et al. (1982, 1983) found glucuronic acid conjugates of methoxychlor metabolites in goats; these conjugates are a product of glucuronosyltransferase, and it is possible that this enzyme may be involved in human methoxychlor metabolism as well. Although it is unknown whether an overall age-related difference in methoxychlor metabolism would be observed *in vivo* in humans, there is some information that the following enzymes may be developmentally regulated: CYP2C19 (Leeder and Kearns 1997), CYP1A2 (Leeder and Kearns 1997; Rich et al. 1990), CYP2C9 (Ratenasavanh et al. 1991; Treluyer et al. 1997), CYP2D6 (Leeder and Kearns 1997; Sonnier and Cresteil 1998), CYP2E1 (Hong et al. 1987; Vieira et al. 1996), CYP3A4 (Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Yang et al. 1994), and glucuronosyltransferase (Leeder and Kearns 1997). While CYP2C19 and CYP1A2 are not present in appreciable levels in human fetal liver, their activities increase to adult levels by 4 months to >1 year of age (Leeder and Kearns 1997; Ratenasavanh et al. 1991; Sonnier and Cresteil 1998). In contrast, other researchers have found that CYPs 2A6, 2C9, 2D6, 2E1, and 3A are expressed at the same levels in perinatal (37 weeks of gestation) to geriatric human livers (72 years) (Ratenasavanh et al. 1991; Tateishi et al. 1997).

There are no PBPK models for children, fetuses/pregnant women, infants/lactating women, or humans at any other stage of development.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in

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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 methoxychlor 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 methoxychlor 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 Methoxychlor

Methods exist for determining the levels of methoxychlor and its metabolites in biological media (tissues, fluids, and excreta) (LeBel and Williams 1986; Mes 1981; Steinberg et al. 1989), although data from human samples are sparse and detectable levels of methoxychlor have generally not been reported. A more detailed discussion of the methods used to detect methoxychlor in biological and environmental samples can be found in Chapter 7 Analytical Methods. In a human population exposed to heavy agricultural spraying of pesticides (area sprayed 16–30 times per year), serum levels of methoxychlor were generally below the level of detection (detection limit of 0.24–4.07 mg/L) (Steinberg et al. 1989).

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Similar results were also obtained with adipose tissue obtained from human autopsies (LeBel and Williams 1986).

Data from animal studies indicate that blood and tissue (fat) levels of methoxychlor do not remain elevated for very long after exposure, owing to its rapid metabolism to more polar, readily excretable metabolites (Davison et al. 1982; Hodge et al. 1952; Kapoor et al. 1970; Kunze et al. 1950; Reynolds et al. 1976). Because methoxychlor is rapidly metabolized, detection of these metabolites (e.g., the mono- and bis-hydroxy derivatives) may serve as a better biomarker of exposure than the parent compound, especially in excreta such as feces (Kapoor et al. 1970). Methoxychlor and its metabolites have been measured in the milk of lactating rats (exposed to 5, 50, or 150 mg/kg/day from gestation day 14 through lactation day 7) and were found to concentrate in the milk with increasing methoxychlor exposure level (Chapin et al. 1997). The data also suggest that methoxychlor and metabolites concentrate in milk, relative to maternal plasma levels, after intermediate-duration dose levels ≥ 50 mg/kg/day. Plasma levels of methoxychlor and its metabolites (mono-hydroxy methoxychlor and di-hydroxy methoxychlor) in suckling rat pups also increased with increasing dose of methoxychlor (Chapin et al. 1997). Metabolites in dam milk and pup plasma were below the level of detection (50 and 5 ng/mL, respectively) at the 5 mg/kg/day dose level. Pup plasma was not drawn for analysis until 27–30 hours after dams received the last dose; therefore, measured methoxychlor and metabolite levels may not have been indicative of peak body burden. Because of the relatively rapid clearance of these metabolites, measurements would probably only be useful in detecting recent exposures (within the past 24 hours). In fact, in one study that tested for methoxychlor in human breast milk, none was detected among regional populations in Kazakhstan (Hooper et al. 1997). However, two other studies have detected methoxychlor in human milk samples (Campoy et al. 2001a, 2001b).

Only one study was found in which concentrations of methoxychlor and methoxychlor metabolites were measured in biological fluids at doses at which health effects were being observed in animals (Chapin et al. 1997). Precocious vaginal opening, decreased absolute ovarian weight, decreased absolute and relative uterine weight, and decreased serum FSH levels during estrus were observed in female offspring of rat dams exposed to ≥ 5 mg/kg/day from gestation day 14 to postpartum day 7 (Chapin et al. 1997). Exposure of dams was discontinued at postpartum day 7 and pups were dosed directly through postpartum day 42. The 5 mg/kg/day dose used in the Chapin et al. (1997) study is one of the lowest doses at which health effects have been observed; only a few studies (Palanza et al. 1999; Parmigiani et al. 1999; vom Saal et al. 1995; Welshons et al. 1999) have observed health effects at lower doses.

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3.8.2 Biomarkers Used to Characterize Effects Caused by Methoxychlor

In animals, the primary target of methoxychlor-induced toxicity is the reproductive system and endocrine-related end points. Reproductive effects include accelerated development of the female reproductive system and delayed development of the male reproductive system in young animals and gonadal atrophy or hypertrophy in adult animals (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989, 1999; Hodge et al. 1950; Martinez and Swartz 1991, 1992; Tullner 1961; Tullner and Edgcomb 1962; Welshons et al. 1999). These effects may cause a decrease in fertility in either sex. In some cases, the onset of these effects may be significantly delayed from the time of exposure.

Although none of the above reproductive effects have been observed in human studies or case reports, it is possible that clinical examination of the reproductive organs of exposed humans might reveal some of the changes observed in animals. In exposed human females, a Pap smear may reveal increased cornification of the vaginal epithelium, which is seen in response to methoxychlor exposure in female rats (Gray et al. 1989). Changes in the menstrual cycle might also occur (Gray et al. 1988, 1989; Martinez and Swartz 1991; Tegeris et al. 1966), although this was not observed in a small (n=four females) experimental study in humans (Wills 1969). In exposed human males, measurement of sperm count might reveal decreases which have been reported in exposed male rats (Bal 1984; Gray et al. 1989), although tissue biopsies revealed no adverse effects on the testes of male subjects (n=4) administered doses of up to 2 mg/kg/day for 6 weeks (Wills 1969). The serum levels of certain hormones, including progesterone, prolactin, and testosterone were decreased in methoxychlor-fed animals, therefore, monitoring hormone level changes in the serum of potentially exposed humans might provide a convenient biomarker for exposure to methoxychlor (Bal 1984; Goldman et al. 1986; Gray et al. 1989). However, none of these potential biomarkers have been validated or are specific for exposures to methoxychlor since other estrogenic compounds (other DDT analogs, chlordecone, polychlorinated biphenyls (PCBs), 3,9-hydroxybenz[a]anthracene, and cyclosiloxanes) may also produce similar effects (Bulger and Kupfer 1985).

Additional information concerning biomarkers for effects on the reproductive system can be found in the National Research Council Report on Biologic Markers in Reproductive Toxicology (NAS/NRC 1989), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

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The neurological system may also be affected by methoxychlor. Neurological effects such as decreased locomotor activity, tremors, apprehension, nervousness, and convulsions may be observed following large acute doses of methoxychlor. DDT, a poorly metabolized analogue of methoxychlor, has been shown to cause similar effects (Agency for Toxic Substances and Disease Registry 1994). In people with compromised liver function, neurological signs may occur at lower methoxychlor exposure levels.

3.9 INTERACTIONS WITH OTHER CHEMICALS

The joint toxic actions of binary mixtures of methoxychlor and other pesticides (including organophosphates and other chlorinated hydrocarbons) on acute lethality were examined in mice (Keplinger and Deichmann 1967). After determining oral LD₅₀ values for the individual compounds, binary mixtures (with components at equitoxic doses based on LD₅₀ values) were administered to the mice at the same dose ranges as the individual compounds. Based on the assumption of joint additive action, an expected LD₅₀ value was calculated for each mixture and compared with the observed LD₅₀. The ratio of expected:observed LD₅₀ values for the methoxychlor/DDT mixture (0.66) indicated a less than additive action (i.e., mutual protection). Ratios for the methoxychlor/aldrin (0.81), methoxychlor/diazinon (0.82), methoxychlor/malathion (0.84), methoxychlor/toxaphene (0.92), and methoxychlor/aramite (1.25) mixtures were close to one, indicating joint additive action. Ratios for the methoxychlor/parathion (1.51), methoxychlor/delnav (1.96), methoxychlor/dieldrin (2.06), and methoxychlor/chlordane (2.26) mixtures were suggestive of greater than additive joint action (i.e., potentiation or synergism). In rats fed diets containing a mixture of methoxychlor, Aramite, DDT, and thiourea for 2 years, no treatment-related synergisms or antagonisms were observed on mortality, food consumption, weight gain, or tumor incidence (Radomski et al. 1965). Similar results were reported by Deichmann et al. (1967) in rats fed diets containing the same chemical mixture at higher concentrations.

When methoxychlor was administered orally to rats previously treated with carbon tetrachloride, DDT-like neurological symptoms were observed (Lehman 1952). In addition, methoxychlor was found to accumulate in the fat and liver in amounts approximately 15–19 times the levels observed in control animals. Carbon tetrachloride is known to inactivate certain hepatic enzymes (CYPs or cytochrome P450s) which metabolize xenobiotics, thereby increasing their retention. These data suggest that carbon tetrachloride and other chemicals which inhibit the metabolism of methoxychlor may increase the risk of neurotoxicity.

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In mice receiving 25 mg/kg/day methoxychlor along with 12 mg/kg/day bromfenvinphos for 6 weeks, inflammatory infiltrations of the liver were larger and denser than observed in animals receiving bromfenvinphos alone (Zaleska-Freljan et al. 1983). Small changes were observed in the kidneys at similar frequencies and severities in both treatment groups. Although it was not investigated in this study, methoxychlor usually does not produce effects of the liver by itself at such low doses. Thus, the results of this study suggest that there may be an interaction between methoxychlor and bromfenvinphos in producing hepatic effects.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

As noted earlier, neurotoxic effects were reported following exposure of rats with liver damage to methoxychlor (Lehman 1952). This suggests that individuals who have hepatic damage or who otherwise have their *O*-demethylation metabolic pathway compromised may be more susceptible to the DDT-like neurotoxic effects of methoxychlor, but this has not been studied in humans.

There is no information on the effects of methoxychlor in human fetuses, children, or adolescents. By extrapolation from animal studies, developing fetuses and young children may be the most susceptible human population to the reproductive effects of methoxychlor because the estrogenic and possibly antiandrogenic activity of methoxychlor metabolites may interfere with normal development of the reproductive tract. The offspring of nursing mothers exposed to methoxychlor may be susceptible since methoxychlor has been detected in human milk samples (Campoy et al. 2001a, 2001b) and animal studies indicate that methoxychlor and/or its biologically active metabolites can be released in milk (Chapin et al. 1997; Davison et al. 1982). Acute exposures to methoxychlor during critical periods of development may adversely affect the reproductive system. Such effects may not appear until later in life (onset of puberty or adulthood/childbearing).

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A more detailed discussion of children's susceptibility can be found in Section 3.7 Children's Susceptibility.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

Ellenhorn MJ, Barceloux DG. 1988. *Medical toxicology: Diagnosis and treatment of human poisoning*. New York, NY: Elsevier, 1078–1080.

Haddad LM, Winchester JF. 1990. *Clinical management of poisoning and drug overdose*. Second edition. Philadelphia, PA: W.B. Sanders Company, 1084–1085.

3.11.1 Reducing Peak Absorption Following Exposure

Data regarding the reduction of methoxychlor absorption in humans after inhalation exposure were not located. Oral absorption of methoxychlor can be reduced with gastric lavage, activated charcoal, sodium sulfate, and cathartics (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Since many commercial formulations of organochlorine insecticides contain organic solvents, emesis is not usually recommended due to the hazard of solvent aspiration (Ellenhorn and Barceloux 1988). In addition, oils should usually not be used as cathartics since they may enhance the absorption of methoxychlor (Haddad and Winchester 1990).

Dermal absorption of methoxychlor can be reduced by removing contaminated clothing and thoroughly washing the exposed skin with a mild soap (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Oils should not be used as a cleansing agent since they may facilitate dermal absorption (Haddad and Winchester 1990).

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3.11.2 Reducing Body Burden

Since animal studies indicate that methoxychlor is rapidly metabolized and cleared from the body, methods for reducing body burden are not expected to be especially effective in reducing human exposures. Activated charcoal is sometimes administered in serial doses to minimize enterohepatic recirculation of persistent chemicals (Ellenhorn and Barceloux 1988). Although a study in rats did establish that methoxychlor undergoes enterohepatic recirculation, the extent to which this occurred was minimal (5–10% of the dose) (Weikel 1957). Thus, it is not likely that the administration of activated charcoal will facilitate the excretion of methoxychlor to any significant extent, but this has not been studied.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Methods for managing the seizures and convulsions which may occur following large exposures to methoxychlor or other organochlorine pesticides include the intravenous administration of Diazepam, Valium, or phenobarbital. Patients should be monitored for the possibility of cardiopulmonary arrest (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990).

Methods for interfering with the reproductive effects of methoxychlor were not located. These effects are presumed to be due to the estrogenic action of the phenolic metabolites of methoxychlor. Therefore, it would seem that by blocking the metabolism of methoxychlor to these derivatives, the reproductive effects would also be blocked. However, such an approach might actually increase the risk of other effects that are due to the parent compound. For example, limited data suggest that the neurotoxicity of methoxychlor is most likely due to the parent molecule (Lehman 1952) which may prevent the deactivation of the sodium gate after neuron activation and membrane depolarization (Brown et al. 1981; Coats 1990; Wu et al. 1975). More information is needed on the mechanism of action of methoxychlor in producing effects on the reproductive system (direct or indirect action) before methods for interfering with the mechanism can be determined.

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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 methoxychlor 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 methoxychlor.

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 Methoxychlor

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

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Figure 3-4. Existing Information on Health Effects of Methoxychlor

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation	•	•								•
Oral		•	•		•	•				
Dermal										

Human

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation			•		•					
Oral	•	•	•	•	•	•	•		•	
Dermal	•		•	•	•	•				•

Animal

• Existing Studies

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interpreted as a “data need”. A data need, as defined in ATSDR’s *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989b), 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 seen in Figure 3-4, information in humans is limited to a single clinical study that investigated systemic and reproductive effects following intermediate-duration oral exposure to methoxychlor. A single epidemiological study was located regarding cancer and occupational exposure to methoxychlor. Oral studies in animals provide information on death, systemic effects for acute, intermediate, and chronic exposures, neurological effects, developmental effects, reproductive effects, and cancer. Limited data are available from animal studies regarding systemic effects for intermediate and chronic exposures, neurological effects, and cancer for dermal exposure to methoxychlor. Limited data are also available for intermediate-duration exposures and neurological effects for inhalation exposure to methoxychlor.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No data were located regarding the acute toxicity of methoxychlor in humans after inhalation, oral, or dermal exposure. Likewise, no data were located regarding acute toxicity in animals following inhalation or dermal exposure.

Acute oral studies in mice and rats identified LD₅₀ values of 2,900–7,000 mg/kg/day (Coulston and Serrone 1969; Hodge et al. 1950; Smith et al. 1946). The cause of death was not determined in these studies. Acute oral exposure of animals to sublethal doses of methoxychlor indicate that reproductive development is the most sensitive target of methoxychlor (Cummings and Gray 1987, 1989; Cummings and Perreault 1990; Gray et al. 1989; Khera et al. 1978; Kincaid Enterprises 1986; Martinez and Swartz 1991; Parmigiani et al. 1998; Tullner 1961; vom Saal et al. 1995; Welshons et al. 1999). Precocious vaginal opening was noted in female rats administered oral doses of 25 mg/kg/day (Gray et al. 1989). The data from animal studies are insufficient to derive an acute oral MRL. A number of candidate MRL studies were considered. Although Gray et al. (1989) observed precocious vaginal opening (early puberty) in rats at 25 mg/kg/day, this study did not test doses as low as the current intermediate-duration MRL study (Chapin et al. 1997). The same effect, precocious vaginal opening, was demonstrated at 5 mg/kg/day administered from gestation day 14 to postnatal day 42 (Chapin et al. 1997), so there is some question as to whether the premature puberty would have occurred at a lower acute dose than the

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25 mg/kg/day dose observed in the Gray et al. (1989) study. There were several hypothesis-generating studies at extremely low doses that were not definitive enough to use for MRL derivation; they are discussed further in both the Developmental Toxicity data needs subsection and Appendix A. These acute oral studies identified LOAEL values of 0.02–1.8 mg/kg/day for reproductive/developmental effects in mice (Parmigiani et al. 1998; vom Saal et al. 1995; Welshons et al. 1999). Further oral studies that better define the threshold dose for reproductive effects in rats, mice, and in other species would be useful.

Studies which identify the inhalation or dermal exposure levels which produce reproductive and developmental effects in animals may be valuable for predicting effects in persons acutely exposed to methoxychlor. Studies examining the effects of dermal and inhalation exposure to methoxychlor in other organ systems would also prove useful. Better pharmacokinetic information on the extent of inhalation and dermal absorption would help determine the importance of more acute duration toxicological data from these routes. Data from farmers and pesticide workers who have been exposed acutely to methoxychlor via these routes would also be useful to determine whether the acute effects observed in animals also occur in humans.

Studies are also needed that examine the effects of exposure to pesticide mixtures that contain methoxychlor.

Intermediate-Duration Exposure. Data regarding the toxicity of methoxychlor in humans exposed via the inhalation and dermal routes for intermediate durations are lacking. A very small study in humans (four males and four females) reported that oral exposure to 2 mg/kg/day for 6 weeks caused no adverse effects on the liver, small intestines, bone marrow, or testes (Wills 1969). A number of oral studies in animals indicate that the reproductive system is a target of methoxychlor toxicity. In females, reported effects of intermediate-duration oral exposure to methoxychlor include: precocious vaginal opening in rats exposed to 25–100 mg/kg/day (Gray et al. 1989; Harris et al. 1974); increased vaginal cornification and decreased number of vaginal smears with leukocytes in rats exposed to 50–400 mg/kg/day (Chapin et al. 1997; Gray et al. 1988, 1989); effects on uterine weight in rats and pigs exposed to 150–1,000 mg/kg/day (Chapin et al. 1997; Harris et al. 1974; Tegeris et al. 1966); atrophy and degeneration of the ovaries in mice and rats exposed to 25–400 mg/kg/day (Bal 1984; Chapin et al. 1997; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1991); persistent vaginal cornification in rats and mice, and a lack of estrus in pigs exposed to 25–1,000 mg/kg/day (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1991; Tegeris et al. 1966); decreased mating frequency in rats exposed to

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60–150 mg/kg/day (Harris et al. 1974); decreased fertility in rats exposed to 35.5–200 mg/kg/day (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Kincaid Enterprises 1986); and altered hormone levels in the serum or pituitary of rats exposed to 100–400 mg/kg/day (Cummings and Gray 1989; Gray et al. 1988). A single multigeneration study reported decreased fertility in female rats of the second and third generations exposed to 92 but not to 18 mg/kg/day methoxychlor (Haskell Laboratories 1966). In males, the effects of intermediate-duration oral exposure to methoxychlor include: delayed preputial separation (an indicator of puberty) in rats exposed to 100–400 mg/kg/day (Gray et al. 1989, 1999); decreased testes weight, ventral prostate weight, and caudal epididymal sperm count in rats exposed to 50–1,400 mg/kg/day (Bal 1984; Gray et al. 1989, 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962); decreased mating frequency in rats exposed to 60 mg/kg/day (Harris et al. 1974) or 200–400 mg/kg/day (Gray et al. 1999); decreased fertility in rats and mice exposed to 60–100 mg/kg/day (Gray et al. 1989; Harris et al. 1974; Wenda-Rozewicka 1983); and altered pituitary and serum hormone levels in rats exposed to 25–100 mg/kg/day (Goldman et al. 1986; Gray et al. 1989). These types of effects are typical of exposures to estrogen, and are consistent with the observation that phenolic metabolites and contaminants of methoxychlor exhibit estrogenic activity *in vitro* and *in vivo* (Bulger et al. 1985).

These data are sufficient to derive an MRL for intermediate-duration oral exposures. A LOAEL of 5 mg/kg/day was identified for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning (Chapin et al. 1997). However, a NOAEL was not identified in this study. Further oral studies would be helpful to: define the threshold dose for reproductive effects and narrow down the critical developmental window during which this effect can be produced.

Data regarding inhalation and dermal exposures to methoxychlor are limited. They include permanent neurological effects (Harell et al. 1978) and death (Ziem 1982) following exposure to pesticide mixtures containing methoxychlor and other pesticides; partial paralysis and disseminated nodules in the brains of rabbits exposed to methoxychlor dermally or via inhalation (Haag et al. 1950; the authors attributed these effects to a disease endemic to rabbits that was potentiated by exposure to methoxychlor); fatty degenerative changes of the liver in rabbits (Haag et al. 1950); death in rabbits (Haag et al. 1950); and uterine stimulation in mice following exposure to a pesticide mixture containing methoxychlor (Tullner 1961). No deaths or histopathological changes in the skin were noted in mice receiving intermittent dermal exposure to methoxychlor for 2 years (Hodge et al. 1966). Effects on the reproductive system have not been adequately investigated following inhalation or dermal exposures to methoxychlor. Studies

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that identify reliable NOAELs and LOAELs for reproductive effects in animals exposed to methoxychlor via inhalation for intermediate-duration exposures would be valuable for predicting effect levels in humans occupationally exposed to methoxychlor. Information from farmers, pesticide workers, or persons who live near pesticide facilities or hazardous waste sites who are exposed to methoxychlor for intermediate-durations may serve to indicate whether the effects observed in animals also occur in humans at similar response levels. Better pharmacokinetic information on the extent of inhalation and dermal absorption would help determine the importance of more intermediate duration toxicological data from these routes.

The results of several studies indicated that methoxychlor exposure might result in alterations of the vitamin A content of the liver (Davison and Cox 1976; Harris et al. 1974), while others found no effect (Cecil et al. 1974; Phillips and Hatina 1972). An *in vitro* assay showed that methoxychlor itself does not interact with human transthyretin (a carrier protein for vitamin A and thyroid hormones), but did not include experiments with a microsomal activation system to determine the interaction of methoxychlor metabolites with transthyretin (Van den Berg et al. 1991).

Chronic-Duration Exposure and Cancer. There are no data regarding the chronic toxicity of methoxychlor in humans for inhalation, oral, or dermal exposures. Chronic oral exposure studies have been performed in animals (Deichmann et al. 1967; NCI 1978). No histopathological changes were detected in the lungs, gastrointestinal tract, liver, kidneys, brain, or pituitary of Osborne-Mendel rats chronically exposed to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967). Similarly, histopathological changes were not observed in the lungs, heart, gastrointestinal tract, bone, liver, kidneys, skin, lymph nodes, thymus, brain, testes, ovaries, and uterus of B6C3F1 mice chronically exposed to 599 mg/kg/day methoxychlor or Osborne-Mendel rats chronically exposed to 107 mg/kg/day (NCI 1978). No histopathological changes were noted in the skin of C3H/AnF mice dermally exposed to 10 mg/day recrystallized methoxychlor, 1 time/week, for 2 years (Hodge et al. 1966). A single multigeneration study reported decreased fertility in female rats exposed to 92 mg/kg/day, but not to 18 mg/kg/day, and not in males exposed to up to 79 mg/kg/day (Haskell Laboratories 1966). These studies do not support the derivation of a chronic oral MRL, since most of the studies did not evaluate reproductive function, which is likely to be the most sensitive target. Derivation of a chronic oral MRL based on the NOAEL of 18 mg/kg/day (Haskell Laboratories 1966), would result in a value greater than MRLs derived for acute and intermediate durations, and therefore is not possible. Additional multigeneration studies in animals which investigate the chronic toxicity of methoxychlor with particular reference to reproductive and developmental effects in both sexes from inhalation, oral, or dermal

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exposures to methoxychlor would provide valuable information. This information would be useful in establishing reliable NOAELs and LOAELs for the purposes of estimating risk to humans who live near hazardous waste sites or are occupationally exposed to methoxychlor. Epidemiological studies on agricultural or pesticide workers exposed to methoxychlor would also serve to indicate whether the effects observed in animals also occur in humans. Since animal studies indicate that the offspring of females that are exposed to methoxychlor either prior to or during pregnancy may also be affected, follow-up studies on the offspring of these exposed human populations may also prove to be valuable.

One epidemiological study reported that the risk of leukemia was slightly increased in farmers exposed to methoxychlor (Brown et al. 1990). Further epidemiological studies on agricultural and pesticide workers would be valuable to determine if a relationship between cancer and methoxychlor exposure exist. No increased tumor incidence was detected in the lungs, gastrointestinal tract, liver, kidneys, brain, or pituitary of Osborne-Mendel rats chronically exposed to 77 mg/kg/day recrystallized methoxychlor (Deichmann et al. 1967). Similarly, increased tumor incidences were not observed in the lungs, heart, gastrointestinal tract, bone, liver, kidneys, skin, lymph nodes, thymus, brain, testes, ovaries, and uterus of B6C3F1 mice chronically exposed to 599 mg/kg/day methoxychlor or Osborne-Mendel rats chronically exposed to 107 mg/kg/day (NCI 1978). No increased tumor incidence was noted in the skin of C3H/AnF mice dermally exposed to 10 mg/day recrystallized methoxychlor, 1 time/week, for 2 years (Hodge et al. 1966). Since prolonged exposure to elevated levels of estrogenic compounds have been linked to cancers in the reproductive tract of women and their female offspring, multigenerational oral animal studies focused on evaluation of tumor incidences in estrogen-sensitive tissues for parental and subsequent generations may provide information needed to assess the possible carcinogenicity of methoxychlor in humans.

Data regarding chronic-duration inhalation and dermal exposures are very limited. They include a possible link to leukemia seen in a single very small epidemiological study of farmers exposed via inhalation (Brown et al. 1990); and a lack of gross or histopathological changes in the skin of mice and lack of tumor formation in mice (gross examination) exposed dermally for 2 years (Hodge et al. 1966). Effects on the reproductive system have not been adequately investigated following chronic inhalation or dermal exposures to methoxychlor. Studies that identify reliable NOAELs and LOAELs for reproductive effects in animals exposed to methoxychlor via inhalation for chronic-duration exposures would be valuable for predicting effect levels in humans occupationally exposed to methoxychlor. Additional information from farmers, pesticide workers, or persons who live near pesticide facilities or hazardous waste sites who are exposed to methoxychlor for chronic durations may serve to indicate whether the

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effects observed in animals also occur in humans at similar response levels. Better pharmacokinetic information on the extent of inhalation and dermal exposure would help determine the importance of more chronic-duration toxicological data from these routes.

Genotoxicity. The mutagenicity of methoxychlor has been well studied *in vitro* and there are sufficient data to indicate that methoxychlor is not mutagenic in prokaryotic systems (Grant et al. 1976; Mortelmans et al. 1986; Probst et al. 1981; Waters et al. 1980). However, *in vitro* studies in mammalian cells have yielded conflicting results. DNA synthesis (thymidine incorporation) was inhibited at high methoxychlor concentrations and stimulated at low methoxychlor concentrations in cultured bovine uterine epithelial and stromal cells (Tiemann et al. 1996). Several studies in mouse lymphoma cells with metabolic activation indicate that methoxychlor can induce mutations at the TK locus (Caspary et al. 1988; Mitchell et al. 1988; Myhr and Caspary 1988). However, mutations of this nature were not noted in human lymphoma cells (Caspary et al. 1988). Further *in vitro* studies using human lymphoma cells would confirm whether an important species difference exists between human and mouse lymphoma cells for this end point. Single-stranded DNA breaks were not induced in human or rat testicular cells by methoxychlor (Björge et al. 1996b). Several *in vivo* studies have shown that intraperitoneal exposure of mice does not increase the frequency of chromosomal aberrations (Degraeve and Chollet 1984) or single-stranded DNA breaks (Umegaki et al. 1993), and that methoxychlor does not increase sex-linked recessive lethal mutations in *Drosophila melanogaster* (Benes and Sram 1969; Valencia 1981; Waters et al. 1980). Additional *in vivo* studies that utilize human lymphocytes from workers or farmers exposed to methoxychlor would be useful in evaluating whether chromosomal aberrations develop in humans exposed to methoxychlor.

Reproductive Toxicity. There is no information regarding the reproductive effects of methoxychlor in humans after inhalation or dermal exposures, or in animals after inhalation or dermal exposures. In a single very small human study (16 males and 16 females), tissue biopsies revealed no adverse effects on the testes of male subjects (3 males at 2 mg/kg/day) or on the menstrual cycles of female subjects (4 per exposure level) administered doses of up to 2 mg/kg/day for 6 weeks (Wills 1969).

However, oral studies in animals indicate that the reproductive system, particularly the developing reproductive system, is the primary target of methoxychlor toxicity. In females, oral exposure to methoxychlor caused changes in pituitary and serum hormone levels in rats (Cummings and Gray 1989); early onset of puberty in rats (Anderson et al. 1994; Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); atrophy of the ovaries in rats and mice (Anderson et al. 1994; Bal 1984; Gray et al. 1988, 1989;

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Harris et al. 1974; Martinez and Swartz 1991), abnormal estrus in rats, mice, and pigs (Gray et al. 1988; Martinez and Swartz 1991; Tegeris et al. 1966); an enlarged uterus in rats and pigs (Cummings 1993; Harris et al. 1974; Tegeris et al. 1966); decreased fertility in rats (Bal 1984; Chapin et al. 1997; Culik and Kaplan 1976; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966; Kincaid Enterprises 1986); and decreased number of corpora lutea, decreased number of live pups, and increased resorptions in mice (Swartz and Eroschenko 1998). In addition, dermal exposure to methoxychlor had a uterotrophic effect in immature female mice (Tullner 1961) and injection with methoxychlor accelerated the onset of puberty in newborn female mice (Martinez and Swartz 1991).

In males, methoxychlor exposure produced changes in pituitary and serum hormone levels in rats (Fail et al. 1994; Goldman et al. 1986; Gray et al. 1989); delayed puberty in rats (Anderson et al. 1994; Chapin et al. 1997; Gray et al. 1999); testicular atrophy in rats (Bal 1984; Gray et al. 1999; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962) and mice (Wenda-Rozewicka 1983); decreased prostate weight in rats (Chapin et al. 1997; Shain et al. 1977; Tullner and Edgcomb 1962); decreased fertility in rats and mice (Gray et al. 1989, 1999; Harris et al. 1974; Wenda-Rozewicka 1983); and altered sexual behavior in mice and rats (Gray et al. 1999; Parmigiani et al. 1999; vom Saal et al. 1995). In addition, testicular atrophy was noted in newborn male mice intraperitoneally injected with methoxychlor (Martinez and Swartz 1991). A study reporting early onset of puberty (precocious vaginal opening) in rats (Chapin et al. 1997) has been utilized to derive an intermediate duration oral MRL value. However, no NOAEL was identified in this study. Further oral studies that better define the threshold dose for reproductive effects in rats, mice, and in other species, and examine the similarities and differences in response between species would be useful. Further data needs relating to the developing reproductive system are discussed in the Developmental Toxicity subsection.

In vitro competitive binding assays have shown that methoxychlor itself does not bind to the estrogen receptor (Bulger et al. 1978a, 1978b; Ousterhout et al. 1981), but that the mono-hydroxy and bis-hydroxy derivatives of methoxychlor do bind to the receptor and cause nuclear translocation. An additional study has shown that bis-hydroxy methoxychlor binds to the ER α and ER β subtypes of the estrogen receptor (Gaido et al. 1999). However, not all effects resulting from methoxychlor exposure mimic those of estrogen. Some effects (delayed preputial separation, decreased pituitary size, and a lack of effect on serum LH, prolactin, and testosterone) could be explained by androgen antagonism (Gray et al. 1999). Only one study has investigated the potential androgen antagonism of methoxychlor and its metabolites; this experiment was not a direct receptor binding assay. The methoxychlor metabolite bis-hydroxy methoxychlor (HPTE) was a weak AR antagonist of dihydrotestosterone in HepG2 human hepatoma cells

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transiently transfected with the human androgen receptor and a reporter gene linked to an androgen responsive promoter; methoxychlor showed even less antagonism in this experiment (Maness et al. 1998). While it seems clear that methoxychlor metabolites mediate at least some of their effects via estrogen and androgen receptors, it is not entirely clear whether the effects are mediated directly in the tissues in which the receptor binding takes place, or if some effects are mediated indirectly through feedback mechanisms with the hypothalamus and pituitary and resulting altered hormone levels. Changes in pituitary prolactin, TSH, and FSH levels have been noted in rats exposed to methoxychlor (Goldman et al. 1986; Gray et al. 1988, 1989). Studies are needed that examine the effects of methoxychlor on the hypothalamus-pituitary-gonadal axis and all related hormones and the distribution of the ER and AR within the axis to better understand the varied effects of methoxychlor on development and reproduction.

Induction of two estrogen-responsive genes (lactoferrin [LF] and glucose-6-phosphate dehydrogenase, [G6PD]) in uteri of mice was not attenuated by an absence of ER α (in ER α knockout mice) or by an ER inhibitor (which would eliminate ER β availability) (Ghosh et al. 1999). This suggests that not all of the effects from methoxychlor are mediated through a known ER. Additional studies are needed investigating this effect and incorporating different estrogen-responsive endpoints in mice and rats to clarify this alternative mechanism resulting in estrogen-like effects. There is also evidence in a non-mammalian species (frog) that methoxychlor acts via a non-ER mechanism (Pickford and Morris 1999). Methoxychlor caused a highly significant inhibition of progesterone-induced germinal vesicle breakdown (GVBD, necessary for oocyte maturation), while estradiol and HPTE had no effect. Studies investigating the mechanism of this effect of methoxychlor in frogs might also provide important information.

Animal studies which describe the long-term consequences of methoxychlor exposure during gestation, early postnatal life, or adulthood on reproductive parameters and function in both sexes would also be useful. Animal studies are also needed to identify reliable NOAELs and LOAELs for the inhalation and dermal routes of exposure. Studies in farmers, pesticide workers, or persons living at or near hazardous waste sites who are exposed to methoxychlor may serve to indicate whether the effects observed in animals also occur in humans. More information is needed regarding which metabolites, contaminants, and degradation products of methoxychlor are estrogenic, and therefore, of health concern to the public.

One study reported a decrease in the percentage of male offspring of rabbit dams exposed orally to methoxychlor (Kincaid Enterprises 1986). Other studies have not reported this effect. It is unclear what the mechanism for this alteration in sex ratio is. The same study also reported increased incidences of

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dead and resorbed fetuses (Kincaid Enterprises 1986). It would be interesting to know if there is a sex-specific fetotoxic effect of methoxychlor or if sex ratio is really affected by methoxychlor.

Developmental Toxicity. No data were located regarding the developmental effects of methoxychlor in humans for inhalation, oral, or dermal routes of exposure or in animals for inhalation and dermal routes of exposure. Although oral is by far the predominant route of exposure, studies focusing on developmental effects resulting from inhalation and dermal exposure may be necessary to assess the possible impact of such exposures.

Oral studies in rats and mice indicate that exposure to methoxychlor during gestation can produce decreased number of implants, decreased fetal body weight, increased frequency of dead or resorbed fetuses, or fetuses with wavy ribs (Chapin et al. 1997; Culik and Kaplan 1976; Khera et al. 1978; Kincaid Enterprises 1986; Swartz and Eroschenko 1998).

Methoxychlor can also cause numerous functional changes in the reproductive system of both adult and developing animals. Methoxychlor accelerated the onset of puberty in female rats exposed for intermediate durations *in utero*, during lactation, and/or postweaning (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974; Swartz and Corkern 1992). Additional studies examining this effect following shorter exposures, during gestation, and beginning at weaning (separate experiments), at doses at or below the lowest LOAEL (5 mg/kg/day in Chapin et al. 1997) are necessary to more closely define the critical interval and lowest level of exposure at which this occurs. Chapin et al. (1997) observed a reduction in testes, epididymis, seminal vesicle, and ventral prostate weights in male offspring of rats exposed to 50 or 150, but not 5 mg/kg/day from gestation day 14 through postnatal day 7, followed by direct exposure of the pups through postpartum day 42. Studies examining these effects following exposures for different durations and during specific life stages, including gestation and pre- and postweaning, are also needed to more closely define the critical interval and lowest level of exposure at which this occurs. Decreased performance in neurobehavioral tests in female rats (increased locomotor activity, decreased click response, and decreased hindlimb grip strength) and male rats (increased handling reactivity, decreased click response, and approach stimulus) was seen following exposure to 5–150 mg/kg/day for intermediate durations *in utero*, during lactation, and/or postweaning (Chapin et al. 1997). However, there was no dose-response relationship for these effects. Methoxychlor caused a slight (but not significant) decrease in testes weight and sperm counts of male rats exposed during gestation and lactation (Gray et al. 1989).

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There were several hypothesis-generating studies using extremely low doses that were not definitive enough to use for MRL derivation. Adult male mice exposed *in utero* to 0.02 mg/kg/day methoxychlor had increased prostate weights compared to vehicle controls (Welshons et al. 1999). Male mice exposed *in utero* to 0.02 mg/kg/day methoxychlor exhibited increased urine-marking behavior when placed in a new territory (vom Saal et al. 1995). A LOAEL of 1.8 mg/kg/day was observed for aggressive behavior (infanticide) in male mice exposed *in utero* toward an unrelated pup (Parmigiani et al. 1998), and a LOAEL of 0.02 mg/kg/day was observed for decreased aggression of young male mice exposed *in utero* toward male siblings (Palanza et al. 1999). Further oral studies that better define the threshold dose for developmental effects in rats, mice, and in other species, and examine the similarities and differences in response between species would be useful. Specifically, additional studies in mice using exposure levels encompassing the LOAEL of 0.02 mg/kg/day identified in the Welshons et al. (1999) study, and studies in species other than mice, may prove very useful. The Welshons et al. (1999) results need to be replicated using a larger number of exposed dams appropriate for a developmental toxicity study (Tyl 2000), testing all males in all litters for prostate size (Elswick et al. 2000a, 2000b), including estradiol and/or DES as simultaneous positive controls, and perhaps including more doses of methoxychlor above and below 0.02 mg/kg/day. A comparative study also needs to be done quantifying how much prostate weight in adult mice varies as a function of intrauterine position relative to female littermates and the estrogen that diffuses from them (Nonneman et al. 1992; Timms et al. 1999; vom Saal 1989); the results of such a study would aid in interpreting the biological significance of the Welshons et al. (1999) findings. Intrauterine position can be identified by caesarian delivery just prior to natural parturition. Additional studies are needed examining the effects of very low doses of methoxychlor on neurobehavioral parameters in mice and other species. The behavioral effects seen in male mice following *in utero* exposure need to be verified and investigated further to determine their mechanism and their significance to human health.

New studies would be helpful to address the factors which make vom Saal et al. (1995), Parmigiani et al. (1998), and Palanza et al. (1999) difficult to interpret. Male mice exposed *in utero* to 0.02 mg/kg/day methoxychlor exhibited increased urine-marking behavior when placed in a new territory (vom Saal et al. 1995). This study was not used for MRL derivation because only two male pups/litter were tested and apparently each was only tested one time; the accuracy of the assessment of the test and how reproducible the results are were not determinable; and there was no indication of what, if any, statistical methods were used to evaluate the results. A LOAEL of 1.8 mg/kg/day for aggressive behavior (infanticide) in male mice exposed *in utero* toward an unrelated pup (Parmigiani et al. 1998) was not used for MRL derivation because only two male pups/litter were tested; there was no dose-response relationship, and no effect was

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seen with DES, a positive estrogenic control. Similarly, a LOAEL of 0.02 mg/kg/day for decreased aggression of young male mice exposed *in utero* toward male siblings at postpartum day 39 but not at postpartum day 54 (Palanza et al. 1999) was not a suitable basis for an MRL because there was no dose-response relationship and the effect was transient.

There is puzzling, limited evidence that exposure of adult female animals may cause effects in subsequent, unexposed offspring. Female offspring (F1a) of mice dams exposed during gestation (gestation days 6–15) to 0, 50, or 100 mg/kg/day methoxychlor showed no change in day of vaginal opening and no change in ovarian weight (Swartz and Corkern 1992), although methoxychlor-exposed female pups had an increase in atretic follicles. Female mice exposed only via lactation and females exposed during gestation and lactation showed similar results. However, female offspring (F1b) from a second unexposed pregnancy showed a slight, but statistically significant, advance in the day of vaginal opening (25.0, 23.9, and 23.2 days for the control, 50–100-mg/kg/day groups, respectively). Since methoxychlor is eliminated rapidly from the body (Kapoor et al. 1970), the above data imply that methoxychlor might cause a long-term alteration in reproductive physiology that can affect subsequent offspring. More studies looking at effects on offspring of previous exposures to dams are needed in order to verify this effect. Upon confirmation, subsequent studies would be required to elucidate the mechanism(s) by which this effect on the offspring occurred.

Additional animal studies that identify reliable NOAELs and LOAELs for developmental effects for inhalation and dermal exposures would be helpful for extrapolating data to human exposures. Information on the offspring of female agricultural workers, pesticide workers, or residents who live at or near a hazardous waste site who may be exposed to methoxychlor may indicate whether the developmental effects observed in animals also occur in humans.

Immunotoxicity. No data were located regarding the immunological effects of methoxychlor in humans following inhalation, oral, or dermal exposure to methoxychlor, or in animals following inhalation or dermal exposure to methoxychlor. The immunotoxicity of methoxychlor has not been well investigated in animals following oral exposure to methoxychlor. A single study reported no histopathological changes in the thymus, spleen, and lymph nodes of mice and rats following chronic oral exposure to methoxychlor (NCI 1978). Male rats, but female rats, exposed to 5 or 50 mg/kg/day during gestation and maturation showed a decrease in plaque-forming cells/spleen, indicating a possible attenuation of primary immune responses (Chapin et al. 1997). Thymus weights were decreased in rats exposed to 50–150 mg/kg/day during prenatal and postnatal development (Chapin et al. 1997) and in

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adult male rats exposed to 500 mg/kg/day (Okazaki et al. 2001). No histological data were provided. Spleen weight, splenic natural killer cell activity, splenic lymphoproliferative response, and splenic cell-surface phenotypes did not differ from controls (Chapin et al. 1997). These results provide only limited data that methoxychlor may influence immune responses. The lack of any immune effects in females or on other immune end points in males that are related to plaque-forming cell numbers led Chapin et al. (1997) to suggest that the observed effect may have been anomalous. Additional studies examining more sensitive functional immune end points following oral exposure would help to clarify the impact of methoxychlor on the immune system.

Neurotoxicity. Data regarding the neurological effects of methoxychlor in humans after inhalation, oral, or dermal exposure are limited to a single case report which described the development of deafness and peripheral neuropathies following inhalation exposure to a mixture of pesticides; no causality regarding methoxychlor could be determined (Harell et al. 1978). Based on its structural similarity to DDT, methoxychlor may be expected to have the potential to produce DDT-like neurological effects (tremors, convulsions) in animals and humans at very high acute doses. Effects of this nature have been reported following oral exposure to high doses in rats and dogs (Cannon Laboratories 1976; Tegeris et al. 1966) and in rats whose hepatic metabolic capacity was diminished by coadministration of carbon tetrachloride (Lehman 1952), but are not usually associated with lower exposures to methoxychlor. Neurological symptoms were also observed in rabbits following inhalation and dermal exposure, but since these effects were similar to those produced by a disease which is endemic to rabbits, these effects may not have been treatment related (Haag et al. 1950). Since these neurological effects of methoxychlor are probably due to the parent compound, additional studies which investigate species differences in the capacity to metabolize methoxychlor and differences in sensitivity to the neurotoxic effects of methoxychlor may help in evaluating neurotoxic risk to humans. Behavioral effects were noted in male and female rats and mice administered methoxychlor (Chapin et al. 1997; Gray et al. 1988; Parmigiani et al. 1998; vom Saal et al. 1995), which unlike the neurological effects discussed above, are most likely due to one or more metabolites of methoxychlor which exhibit estrogenic activity (Bulger et al. 1985). Additional studies that further characterize which neurotoxic effects are caused by methoxychlor and which are caused by its metabolites would be useful. Information obtained from humans accidentally exposed to large doses of methoxychlor may also serve to reveal whether the neurotoxic effects observed in animals also occur in humans.

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Epidemiological and Human Dosimetry Studies. A single epidemiological study was located which identified a possible association between exposure to methoxychlor and leukemia in farmers (Brown et al. 1990). However, the exposed and control groups were small, and the increase only marginally significant. Moreover, exposure levels were not quantified and exposure to other chemicals probably occurred, so confidence in the study is low. Nevertheless, the possible association detected in this study suggests that further investigations should be conducted on agricultural workers, pesticide formulators, and pesticide applicators who are frequently exposed to methoxychlor. Other populations which could also be studied include residents at or near areas that undergo frequent aerial spraying of methoxychlor, areas at or near methoxychlor production facilities, and areas at or near hazardous waste sites that contain methoxychlor. Animal studies indicate that reproductive and developmental effects of methoxychlor are end points that are of primary concern. Therefore, epidemiological studies which focus on reproductive and developmental end points would also be valuable.

Biomarkers of Exposure and Effect

Exposure. The presence of methoxychlor and its phenolic metabolites in biological media may be used as specific biomarkers for exposure to methoxychlor. Methoxychlor has been detected in human breast milk samples from women living in rural and urban areas (Campoy et al. 2001a, 2001b). Methoxychlor and its metabolites have also been measured in the milk of lactating rats and were found to concentrate in the milk with increasing methoxychlor exposure level (Chapin et al. 1997). Plasma levels of methoxychlor and its metabolites (mono-hydroxy methoxychlor and di-hydroxy methoxychlor) in suckling rat pups have also been observed to increase with increasing dose of methoxychlor (Chapin et al. 1997). However, because of the relatively rapid clearance of these metabolites, measurements would probably only be useful in detecting recent exposures (within the past 24 hours). Measurements of methoxychlor and metabolite levels in relevant biological fluids in animal studies would be helpful in establishing a quantitative correlation between exposure level, body burden, and effect level. These correlations might prove useful in assessing risk in human exposures. The Chapin et al. (1997) study is the only study in which concentrations of methoxychlor and methoxychlor metabolites were measured in biological fluids at doses at which health effects were being observed in animals.

Effect. As discussed earlier, the primary effects of methoxychlor are on reproduction. In exposed female rodents, methoxychlor exposure may affect the estrus cycle (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1992; Okazaki et al. 2001; Tegeris et al. 1966), serum hormone levels (Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Gray et al. 1988), the uterus and ovaries (Bal 1984; Chapin et

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al. 1997; Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Cummings and Perreault 1990; Dikshith et al. 1990; Gray et al. 1988, 1989; Harris et al. 1974; Martinez and Swartz 1992; Mitsumori et al. 2000; Okazaki et al. 2001; Swartz et al. 1994; Tegeris et al. 1966; Tullner 1961), and may reduce fertility (Bal 1984; Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966). In the female offspring of exposed female rodents, reproductive development, as indicated by age at vaginal opening and first estrus, may be accelerated (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974). In male rodents, methoxychlor exposure may affect sexual maturity (age of preputial separation) (Gray et al. 1989, 1999), serum hormone levels (Cummings and Gray 1989; Goldman et al. 1986; Gray et al. 1989; Stoker et al. 1999), testes (Bal 1984; Chapin et al. 1997; Dikshith et al. 1990; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962; Wenda-Rozewicka 1983), prostate (Okazaki et al. 2001; Shain et al. 1977; Stoker et al. 1999; Welshons et al. 1999), fertility (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Wenda-Rozewicka 1983), and behavior (Parmigiani et al. 1998; vom Saal et al. 1995). However, these effects are not specific to exposure to methoxychlor, but could occur following exposure to other chemicals with estrogenic activity as well. It is unknown whether any of these effects will occur in humans at environmental exposure levels. Some potential end points that might be examined in human studies include: increased cornification of vaginal epithelium as measured in a pap smear, changes in menstrual cycles or sperm counts, and changes in serum progesterone, prolactin, and testosterone levels. Additional studies which investigate the mechanism of action of methoxychlor at the molecular level, may be useful in developing a specific biomarker of effect for methoxychlor. Development of a specific and sensitive biomarker of effect would be useful to facilitate future medical surveillance that can lead to detection and possible treatment of exposed populations.

Absorption, Distribution, Metabolism, and Excretion. *In vivo* toxicokinetics data for methoxychlor in humans are absent. There are no data on inhalation exposure and only minimal information on dermal exposure. As discussed in Section 3.4, studies in which animals were given oral doses of radiolabeled methoxychlor indicate that methoxychlor is:

- (1) rapidly and efficiently absorbed by the gastrointestinal tract in mice and goats (Davison et al. 1982, 1983; Kapoor et al. 1970);
- (2) widely distributed to tissues and organs with some preferential distribution to fatty tissues in rats (Harris et al. 1974; Hodge et al. 1952; Kunze et al. 1950), sheep (Reynolds et al. 1976), dogs (Hodge et al. 1952), and goats (Davison et al. 1982); and
- (3) very rapidly cleared, predominately as metabolites in feces (via biliary excretion) and urine, in mice (Kapoor et al. 1970), goats (Davison et al. 1982), and sheep (Reynolds et al. 1976).

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Dermal absorption has been estimated to be 5–8% in goats (Davison et al. 1983) and 20% in cows (Skaare et al. 1982); however, because of differences in skin, dermal absorption by goats may not be a good model for dermal absorption by humans. Methoxychlor and methoxychlor metabolites have been detected in milk of lactating goats (Davison et al. 1982) and rats (Chapin et al. 1997) given oral doses of methoxychlor. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated (Chapin et al. 1997).

Studies examining placental transfer of methoxychlor or methoxychlor metabolites were not located, but subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974); however, it should be noted that exposure was not exclusively during gestation in any of these studies. Research examining the possibility of placental transport of methoxychlor and metabolites and quantifying possible distribution to developing fetuses may help to discern if these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes.

As discussed in Section 3.4, the metabolism of methoxychlor has been studied *in vivo* in orally exposed rats (Lehman 1952; Weikel and Laug 1958), mice (Kapoor et al. 1970), and goats (Davison et al. 1982, 1983). Demethylated, dechlorinated, and dehydrochlorinated metabolites of methoxychlor were identified in feces and urine, and minor ring hydroxylated metabolites were also found in urine. Most urinary metabolites were conjugated with glucuronic acid (Davison et al. 1982, 1983). *In vitro* studies with human and rat liver microsomes, partially purified rat CYP isozymes, and/or human c-DNA-expressed CYP isozymes indicate that the predominant metabolic pathway involves initial demethylation of methoxychlor catalyzed by a number of CYP isozymes in rats (Kishimoto and Kurihara 1996; Kishimoto et al. 1995) and in humans (Stresser and Kupfer 1998). In humans, CYP2C19 and CYP1A2 may be the major demethylases for methoxychlor, but other forms may play significant roles in individuals deficient in these isozymes (Stresser and Kupfer (1998). In rats, CYP2C6 and another unidentified CYP isozyme may be the major catalysts for demethylation (Kishimoto and Kurihara 1996). Ring hydroxylation of methoxychlor or hydroxy-methoxychlor derivatives appears to be catalyzed by a different set of CYP isozymes than those catalyzing demethylation (Stresser and Kupfer 1997; Stresser et al. 1996). Studies with immature or mature rats given repeated intraperitoneal injections of methoxychlor indicate that methoxychlor can induce hepatic CYP enzymes involved in its metabolism (Li et al. 1995).

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Animal data indicate that methoxychlor and its metabolites are excreted primarily in the bile with a lesser amount excreted in the urine (Davison et al. 1982, 1983; Kapoor et al. 1970). This is true regardless of the route of exposure. In bile-duct cannulated rats given a single intravenous dose of radiolabeled methoxychlor, 50% of the dose was excreted in the bile after 4 hours (Weikel 1957). The urinary excretion of label in bile-cannulated rats was only 0.1–0.2% compared to 5–10% in noncannulated rats, suggesting that the appearance of urinary metabolites was largely due to material that was reabsorbed in the gut (i.e., enterohepatic circulation). Because methoxychlor does not accumulate significantly in biological tissues and is rapidly eliminated, additional studies examining the excretion of methoxychlor and its metabolites are not warranted.

Comparative Toxicokinetics. The available data comparing rat and human liver microsomal metabolism of methoxychlor indicate qualitative similarities and some indications of quantitative differences. Rat liver microsomes were observed to have a higher capacity than human liver microsomes to metabolize methoxychlor to covalent binding intermediates (Bulger and Kupfer 1990). *In vitro* data also indicate that covalent binding of methoxychlor to human liver microsomes is similar across age and sex, whereas in rats, covalent binding in mature males is much higher than in mature females and immature males and females (Bulger and Kupfer 1989). Another comparison found that *in vitro* rates of demethylation of methoxychlor and mono-hydroxy methoxychlor with human liver microsomes were generally higher in rat liver microsomes than in human liver microsomes (Stresser and Kupfer 1998).

Further research comparing quantitative and qualitative aspects of metabolism with mouse, rat, and human systems may help in developing PBPK models for methoxychlor in these species. New studies comparing species-specific tissue/blood partition coefficients, cross-placental distribution in pregnant animals (mice and rats), distribution to milk in lactating animals (mice and rats), elimination kinetics for methoxychlor from blood (mice and rats), and/or kinetics of metabolite appearance in urine or feces (rats; data are available for mice, Kapoor et al. 1970) would facilitate development of relevant PBPK models predicting distribution of methoxychlor and methoxychlor metabolites to the developing fetus and suckling infants. Such models may be useful in extrapolating observations of reproductive effects in rodents following *in utero* and/or lactational methoxychlor exposure to relevant human exposure scenarios.

3. HEALTH EFFECTS

Methods for Reducing Toxic Effects. No data were located regarding the mechanism of absorption, distribution, or excretion of methoxychlor in humans or animals by any route. Based on the chemical and physical properties of methoxychlor, its absorption most likely occurs by passive diffusion. However, this has not been investigated. Studies which investigate the mechanism by which methoxychlor enters the body may be useful in developing methods for reducing its absorption. Once it enters the body, methoxychlor preferentially distributes to tissues with higher fat content; however, methoxychlor does not persist in tissues (Harris et al. 1974; Hodge et al. 1952; Kunze et al. 1950; Reynolds et al. 1976). Its rapid metabolism to more polar compounds facilitates its rapid clearance from these tissues (Davison et al. 1982, 1983; Lehman 1952; Weikel and Laug 1958). Since the majority of methoxychlor is cleared from the body within 24 hours (Kapoor et al. 1970), it seems unlikely that methods for reducing body burden of methoxychlor would be of much benefit. Therefore, studies examining the mechanism of methoxychlor distribution and excretion are not warranted.

Children's Susceptibility. There is no information on health effects observed in children after methoxychlor exposure via inhalation, oral, or dermal routes, and there is a data need for such information, particularly from the predominant oral route of exposure. Animal data indicate that the primary target of methoxychlor is the reproductive system, and methoxychlor can affect both adult animals and the developing reproductive system in males and females. Thus, there is concern about whether exposure to methoxychlor during any stage of development might potentially affect the developing reproductive system of fetuses, children, and adolescents. 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.

There are no studies of methoxychlor metabolism in children; such information would be useful. Numerous studies have shown that both adult and developing animals exhibit effects attributed to the estrogenic activity of methoxychlor or its metabolites. This suggests that either methoxychlor is metabolized by the mother first and the metabolites then cross the placenta, or that methoxychlor crosses the placenta and is metabolized by the fetus. However, there are no studies that specifically investigated metabolic enzyme activity in fetuses. Although there is no direct information regarding age-related differences in metabolism of methoxychlor in children, multiple CYP enzymes are involved in methoxychlor metabolism. It is likely that CYPs 2C19, 1A2, 2B6, 2C9, 2A6, and perhaps 2D6, 2E1, and 3A4 play a role in phase I human metabolism (Hong et al. 1987; Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Ratenasavanh et al. 1991; Rich et al. 1990; Sonnier and Cresteil 1998; Treuler et al. 1997; Vieira et al. 1996; Yang et al. 1994). Many of these phase I enzymes are likely to have

3. HEALTH EFFECTS

overlapping roles. Although nothing is known about phase II metabolism in humans, Davison et al. (1982, 1983) found glucuronic acid conjugates of methoxychlor metabolites in goats; these conjugates are a product of glucuronosyltransferase and it is possible that this enzyme may be involved in human methoxychlor metabolism as well. Although it is unknown whether an overall age-related difference in methoxychlor metabolism would be observed *in vivo* in humans, there is some information that the following enzymes may be developmentally regulated: CYP2C19 (Leeder and Kearns 1997), CYP1A2 (Leeder and Kearns 1997; Rich et al. 1990), CYP2C9 (Ratenasavanh et al. 1991; Treuler et al. 1997), CYP2D6 (Leeder and Kearns 1997; Sonnier and Cresteil 1998), CYP2E1 (Hong et al. 1987; Vieira et al. 1996), CYP3A4 (Lacroix et al. 1997; Leeder and Kearns 1997; Mote et al. 1990; Yang et al. 1994), and glucuronosyltransferase (Leeder and Kearns 1997). In contrast, other researchers have found that CYPs 2A6, 2C9, 2D6, 2E1, and 3A have been found at the same levels in perinatal (37 weeks of gestation) to geriatric human livers (72 years) (Ratenasavanh et al. 1991; Tateishi et al. 1997).

There are no studies examining placental transfer of methoxychlor or methoxychlor metabolites, but subtle effects on the development of reproductive organs have been observed in male and female offspring of rodents exposed to oral doses of methoxychlor during gestation (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Swartz and Corkern 1992; Welshons et al. 1999). It is unclear whether these effects result directly from methoxychlor and metabolites passing through the placenta or indirectly from a disturbance of maternal physiological processes. Research examining the possibility of placental transport of methoxychlor and metabolites and quantifying possible distribution to developing fetuses may help in elucidating the mechanism by which offspring of an exposed dam are affected.

Methoxychlor and methoxychlor metabolites have been detected in milk of lactating goats (Davison et al. 1982) and rats (Chapin et al. 1997) given oral doses of methoxychlor. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated (Chapin et al. 1997). The Chapin et al. (1997) study measured plasma methoxychlor (and metabolite) levels 27–30 hours after the dam's last exposure to methoxychlor; it would be interesting to know what pup plasma levels would be shortly after the last exposure of the dams. Additional studies to quantitate the plasma levels of methoxychlor in pups exposed only via lactation and to investigate the resultant effects through puberty and reproduction would be helpful in understanding the risk to offspring of maternal exposure to methoxychlor, even after birth.

3. HEALTH EFFECTS

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

3.12.3 Ongoing Studies

Ongoing studies pertaining to methoxychlor have been identified and are shown in Table 3-5.

3. HEALTH EFFECTS

Table 3-5. Ongoing Studies on the Health Effects of Methoxychlor^a

Investigator	Affiliation	Research description	Sponsor
Ahmed, SA et al.	Virginia Polytechnic Institute, College of Veterinary Medicine	In vivo effects of a pesticide on the immune system: Age and gender factors	CSREES VA
Chapin, RE	NIEHS, NIH	Prenatal/Juvenile exposure to pesticides on adult neural, immune function	NIEHS
Lubahn, DB	University of Missouri–Columbia	Environmental estrogen response proteins in ER-minus mice	NIEHS
Chambers, JE	Mississippi State University, College of Veterinary Medicine	Developmental and comparative toxicology of neurotoxic insecticides	U.S. Department of Agriculture, Cooperative Research Service
Gore, A	Mount Sinai School of Medicine	Neuroendocrine mechanisms of environmental toxicity during development	NIEHS
James, MO	University of Florida	Bioavailability of chlorinated compounds	NIEHS
Kupfer, D	Worcester Foundation of Experimental Biology	Effects of chlorinated hydrocarbons on mammalian systems	NIEHS
Poznanski, AA	Midwestern University	Methoxychlor effects on zebrafish forebrain development	NIEHS
Schiffenbauer, J	University of Florida	Autoimmune toxicity of chlorinated compounds	NIEHS
Wilson, EM	University of North Carolina–Chapel Hill	Mechanisms of action of environmental antiandrogens	NIEHS
Zacharewski, TR	Michigan State University	Comprehensive assessment of endocrine active mixtures	NIEHS

^aSource: FEDRIP (2001)

NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health

4. CHEMICAL AND PHYSICAL INFORMATION

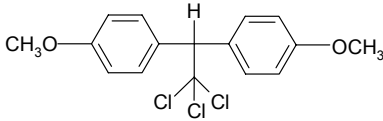
4.1 CHEMICAL IDENTITY

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

Methoxychlor is produced commercially in the United States as a technical grade containing 88–90% of the pure chemical and 10–12% of impurities consisting of isomers and other reaction products (IARC 1979; Lamoureux and Feil 1980). Twenty-five of these impurities were characterized in studies conducted on technical methoxychlor; evidence for >50 impurities was obtained through gas chromatography/mass spectrometry (GC/MS) (Lamoureux and Feil 1980). Purification of technical grade (nominally 90%) 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane (*p,p'*-methoxychlor; *p,p'*-DMDT) by recrystallization gave 76% *p,p'*-methoxychlor (99.8% pure by normal phase high performance liquid chromatography [HPLC]) and 24% impurities (West et al. 1982). The impurities were found to contain approximately 40 components. The major components were identified (HPLC and GC/MS) using reference standards. Component identities and percent (w/w) in technical grade methoxychlor were found to be as follows: 1,1,1,2-tetrachloro-2-(4-methoxyphenyl)ethane (1.73%), 1,1,1-trichloro-2-(2-methoxyphenyl)-2-(4-methoxyphenyl)ethane (*o,p'*-methoxychlor; *o,p'*-DMDT; 4.03%), 1,1-dichloro-2,2-di(4-methoxyphenyl)ethene (DMDE; 0.39%), a condensation product of *p,p'*-methoxychlor (*p,p'*-DMDT; 0.48%), a condensation product of *o,p'*-methoxychlor (*o,p'*-DMDT; 0.4%), and 1,2,2,2-tetrakis(4-methoxybenzyl)ethene (0.5%). The percentages were calculated relative to the quantity of methoxychlor contained in technical grade material and were found to vary depending upon manufacturing conditions. Another impurity is 1-chloro-1,2,2-tris(4-methoxyphenyl)ethene (chlorotrianisene; TACE), a triphenylethylene derivative, that exhibits estrogenic/anti-estrogenic characteristics. Other impurities include polycyclic hydrocarbons (e.g., 3,6-dimethoxy-9,10-bis(p-methoxyphenyl)phenanthrene, tetrakis(p-methoxyphenyl)ethylene, and 3,6,11,14-tetramethoxydibenzo(g,p)chrysene), which have been studied for mutagenicity and putative carcinogenicity (Grant et al. 1976). The substance, 3,6,11,14-tetramethoxydibenzo(g,p)chrysene, was found to be mutagenic.

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-1. Chemical Identity of Methoxychlor

Characteristic	Information	Reference
Chemical name	Methoxychlor	Howard 1991
Synonym(s)	2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane; 1,1,1-trichloro-2,2-bis(4-methoxyphenyl) ethane; methoxy-DDT; 1,1-(2,2,2-trichloroethylidene)-bis(4-methoxybenzene)	HSDB 2000
Registered trade name(s)	Marlate® Metox® Prentox®; Methoxcide®	EPA 1988b; HSDB 2000; Sittig 1980
Chemical formula	C ₁₆ H ₁₅ Cl ₃ O ₂	Howard 1991
Chemical structure	 <p>1,1,1-Trichloro-2,2-bis(4-methoxyphenyl)ethane (<i>p,p'</i>-methoxy chlor; DMDT)</p>	EPA 1988c
Identification numbers:		
CAS registry	72-43-5	Howard 1991
NIOSH RTECS	KJ 3675000	Sax and Lewis 1989
EPA hazardous waste	U247; D014	HSDB 2000
OHM/TADS	OHM 7216536	HSDB 2000
DOT/UN/NA/IMCO shipping	DOT 2761 UN 2761 NA 2761 IMCO 6.1	Sax and Lewis 1989 HSDB 2000
HSDB	1173	HSDB 2000
NCI	C00497	HSDB 2000

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

4. CHEMICAL AND PHYSICAL INFORMATION

4.2 PHYSICAL AND CHEMICAL PROPERTIES

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

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-2. Physical and Chemical Properties of Methoxychlor

Property	Information	Reference
Molecular weight	345.65	Howard 1991
Color	Pale yellow	EPA 1988c
Physical state	Crystalline solid	EPA 1988c
Melting point	89 EC 77 EC (technical grade) No data (decomposes)	HSDB 2000 Montgomery and Welkom 1990
Density: at 25 EC	1.41 g/cm ³	Montgomery and Welkom 1990
Odor	Slightly fruity; musty; chlorine-like	HSDB 2000; Sigworth 1965
Odor threshold: Water 60 EC Air	4.7 ppm No data	Sigworth 1965
Solubility: Water at 25 EC at 15 EC at 24 EC at 35 EC at 45 EC	0.045 mg/L 0.02 mg/L 0.04 mg/L 0.095 mg/L 0.185 mg/L	
Organic solvent(s)	Soluble in chlorinated aromatic solvents, ketonic solvents, ethanol, methylene chloride, methylated naphthalene, carbon tetrachloride, chloroform, xylene, methanol, petroleum ether, benzene	Budavari et al. 1989; EPA 1988a; HSDB 2000; Montgomery and Welkom 1990
Partition coefficients: Log K _{ow} Log K _{oc}	4.68–5.08 4.9	Howard 1991 Montgomery and Welkom 1990
Vapor pressure at 25 EC	1.4x10 ⁻⁶ mmHg (estimated)	Howard 1991
Henry's law constant: at 25 EC	1.6x10 ⁻⁵ atm-m ³ /mol (estimated)	Howard 1991
Autoignition temperature	No data	
Flashpoint	No data	
Flammability limits	No data	
Conversion factors ^a	No data	
Explosive limits	No data	

^aExists partially in particulate form in air. Conversion factors are only applicable for compounds that are entirely in the vapor phase.

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

5.1 PRODUCTION

Methoxychlor was first synthesized in 1893 by the reaction of chloral hydrate with anisole in the presence of acetic acid and sulfuric acid. It is produced commercially by the condensation of anisole with chloral in the presence of an acidic condensing agent (IARC 1979; Sittig 1980). Commercial production of methoxychlor in the United States was first reported in 1946. In 1975, three U.S. companies produced approximately 5.5 million pounds of methoxychlor (IARC 1979). U.S. production in 1982 was 3 million pounds (EPA 1988c). No data were found for current production volume levels for methoxychlor.

Methoxychlor is currently registered under the trade names of Prentox[®], Methoxide[®], Marlate[®], and Metox[®] (EPA 1988b; HSDB 1993). Currently, Kincaid Enterprises Inc. in Nitro, West Virginia is the sole producer and distributor of methoxychlor in the United States. Production volumes of the technical product in pounds were 631,550 in 1986, 672,000 in 1987, 584,000 in 1988, 476,000 in 1989, 301,235 in 1990, and 423,832 in 1991 (Kincaid Enterprises 1992). On January 14, 2000, EPA issued a suspension order to Kincaid enterprises, Inc. to prevent further manufacture and sale of their methoxychlor products (http://www.epa.gov/oppfead1/cb/csb_page/updates/methox.html). The order affects the technical product and three products manufactured by Kincaid, but does not directly affect other companies that manufacture methoxychlor products. The order was issued because the registrant failed to submit overdue (per an agreement signed in September of 1998) environmental fate studies. At the time of the writing of this profile (September 2002), EPA is in the process of issuing a notice of intent to suspend to all companies that use methoxychlor in their products.

A number of U.S. facilities use methoxychlor as a component in formulated pesticide products. Methoxychlor is produced commercially in the United States as a technical grade containing 88–90% of the pure chemical and 10–12% of impurities consisting of isomers and other reaction products (IARC 1979; Lamoureux and Feil 1980). Twenty-five of these impurities were characterized in studies conducted on technical methoxychlor; evidence for more than 50 impurities was obtained through gas chromatography/mass spectrometry (GC/MS) (Lamoureux and Feil 1980). Purification of technical grade (nominally 90%) 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane (*p,p'*-methoxychlor; *p,p'*-DMDT) by recrystallization gave 76% of *p,p'*-methoxychlor (99.8% pure by normal phase high performance liquid chromatography [HPLC]) and 24% impurities (West et al. 1982). The impurities were found to contain approximately 40 components. The major components were identified (HPLC and gas

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

chromatography/mass spectrometry [GC/MS]) using reference standards. Component identities and percent (w/w) in technical grade methoxychlor were found to be as follows: 1,1,1,2-tetrachloro-2-(4-methoxyphenyl)ethane (1.73%), 1,1,1-trichloro-2-(2-methoxyphenyl)-2-(4-methoxyphenyl)ethane (*o,p'*-methoxychlor; *o,p'*-DMDT; 4.03%), 1,1-dichloro-2,2-di(4-methoxyphenyl)ethene (DMDE; 0.39%), a condensation product of *p,p'*-methoxychlor (*p,p'*-DMDT; 0.48%), a condensation product of *o,p'*-methoxychlor (*o,p'*-DMDT; 0.4%), and 1,2,2,2-tetrakis(4-methoxybenzyl)ethene (0.5%). These percentages were calculated relative to the quantity of methoxychlor contained in technical grade material and were found to vary depending upon manufacturing conditions. Formulations of methoxychlor for various uses in the United States include wettable powders, dust, granules, emulsifiable concentrates, flowable concentrates, liquid soluble concentrates, ready-to-use products (liquids), and pressurized liquids (EPA 1988a, 1988b).

Table 5-1 lists information on U.S. companies that reported the manufacture and use of methoxychlor in 1999 (TRI99 2001). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

5.2 IMPORT/EXPORT

Data on import and export volumes of methoxychlor are limited. In 1978, 17,700 pounds of methoxychlor were imported (HSDB 1993). There is no information on current import volumes (HSDB 2000). Kincaid Enterprises, Inc. reported information on export volumes of technical grade methoxychlor in pounds as 25,750 in 1986, 86,000 in 1987, 22,600 in 1988, 47,150 in 1989, 10,350 in 1990, and 49,750 in 1991 (Kincaid Enterprises 1992). However, there is no information on current export volumes of formulated products containing methoxychlor.

5.3 USE

Because of its low toxicity in animals and humans, methoxychlor has been viewed as an attractive replacement for DDT (EPA 1988b; IARC 1979). Methoxychlor is registered as an insecticide against a wide range of pests, including houseflies and mosquitos, cockroaches, chiggers, and various arthropods commonly found on field crops, vegetables, fruits, stored grain, livestock, and domestic pets (EPA

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

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

State ^a	Number of facilities	Minimum amount on site in pounds ^b	Maximum amount on site in pounds ^b	Activities and uses ^c
AR	1	10,000	99,999	13
OH	1	1,000	9,999	13
TX	1	10,000	99,999	13

Source: TRI99 2001

^aPost office state abbreviations used

^bAmounts on site reported by facilities in each state

^cActivities/Uses:

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

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

1988b; Verschueren 1983). The EPA has approved the use of methoxychlor as a pesticide and fumigant on more than 85 crops, including cranberries (EPA 1988a, 1988b). This substance can be applied to large areas such as beaches, estuaries, lakes, and marshes for control of fly and mosquito larvae by aerial application (EPA 1988b). Other uses include the spray treatment of barns, grain bins, mushroom houses, and other agricultural premises and the spraying or fogging of garbage containers, sewer manholes, and sewage disposal areas (EPA 1988b).

Annual usage of methoxychlor in the United States was estimated to range from 500,000 to 900,000 pounds in 1986 (EPA 1988b). Use patterns for methoxychlor, which is a General Use Pesticide (GUP) that is available over the counter, have remained fairly constant since 1974. At that time, home and garden applications constituted 30% of usage; livestock and poultry, 15%; alfalfa, 10%; soybeans, 10%; forests, 10%; ornamental shrubs, 10%; deciduous fruits and nuts, 5%; and vegetables, 5% (IARC 1979). Likewise, in 1988, EPA reported that predominant use of methoxychlor in the United States was as an insecticide for alfalfa, livestock, home orchards, and ornamentals. These applications accounted for a total of 37.3% of the total U.S. annual usage (EPA 1988b). More recent information indicates that approximately 28% is used for home and garden purposes, 15% for industrial and commercial purposes, and 57% for agricultural purposes (Kincaid Enterprises 1992).

5.4 DISPOSAL

Methoxychlor and waste containing methoxychlor are classified as hazardous wastes by EPA. Generators of waste containing this contaminant must conform to EPA regulations for treatment, storage, and disposal (see Chapter 8). The recommended method for disposal of methoxychlor wastes is incineration with scrubbing (HSDB 1993; Sittig 1980). Empty containers may be returned to manufacturers for reuse or buried in designated landfills away from water supplies. Small quantities of waste methoxychlor may be landfilled (HSDB 1993).

According to the Toxics Release Inventory (TRI99 2001), about 14 pounds of methoxychlor were transferred to landfills and/or treatment/disposal facilities in 1999 (see Table 5-1). No information was located on disposal trends or past disposal methods.

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

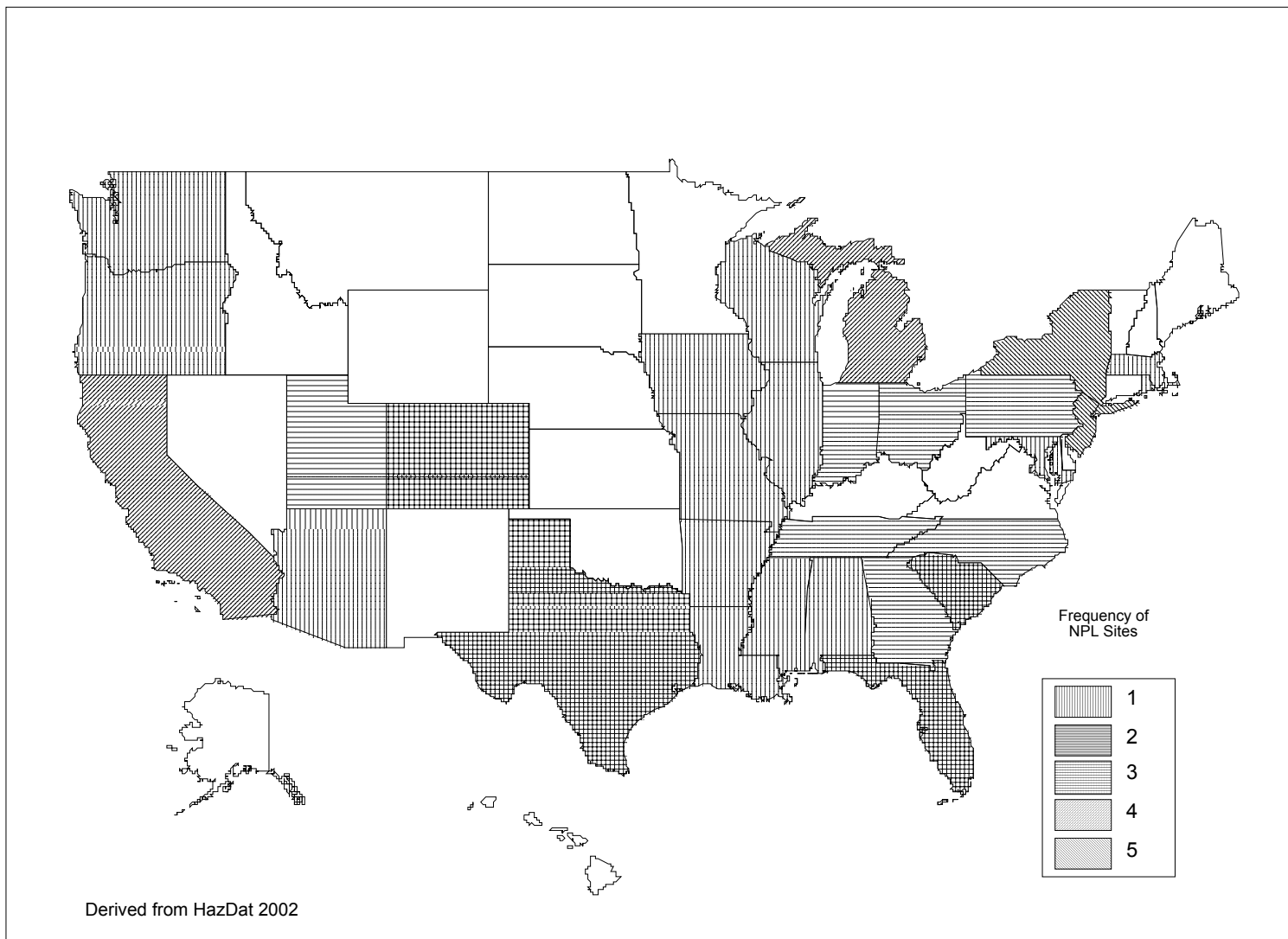
Methoxychlor has been identified in at least 58 of the 1,613 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2002). However, the number of sites evaluated for methoxychlor is not known. The frequency of these sites can be seen in Figure 6-1.

Methoxychlor is released to the environment mainly as a result of its application to crops and livestock as a pesticide. Smaller amounts may be released during its production, formulation, storage, shipment, and disposal. There are no known natural sources of methoxychlor.

In air, methoxychlor exists in both the particulate and, to a small degree, vapor phase. Air levels exhibit seasonal variations, paralleling its use for pest control. Levels ranging from 0.002 to 0.6 ng/m³ have been reported in ambient air in Canada and the United States. Methoxychlor may react with photochemically-produced hydroxyl radicals in air, but most is probably removed from the atmosphere by wet and dry deposition processes. The residence time for methoxychlor in the atmosphere is <1 month. In water, methoxychlor preferentially binds to sediments and organic matter, although some methoxychlor may remain dissolved in water. Methoxychlor bioconcentrates in a number of aquatic organisms including microorganisms, snails, clams, and some fish (see Section 6.3.1), but probably does not accumulate in mammalian species due to rapid metabolism and elimination. Methoxychlor in water and sediment is degraded to dechlorinated, dehydrochlorinated, and demethylated products by chemical, photochemical, and biological processes. Depending on the availability of sunlight, air, plant materials, and microorganisms, the half-life of methoxychlor in water may range from 2 to 5 hours to approximately 1 year. Both its degradation and its affinity for sediments and organic matter may explain, in part, why methoxychlor is generally not detected in surface water or groundwater in the United States. However, methoxychlor may be detected in waters near release sources.

Methoxychlor binds tightly to soils, but is not usually detectable in soil except in areas where it has been applied as a pesticide. Wind and rain can erode contaminated soils, resulting in the migration of methoxychlor-containing particulates. Some methoxychlor can persist in soils for more than a year after its application. However, most is degraded to dechlorinated, dehydrochlorinated, and demethylated products. The degradation of methoxychlor is mediated by microorganisms and is affected by the level

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



6. POTENTIAL FOR HUMAN EXPOSURE

of aeration of the soil. Half-lives of <30 days in anaerobic soils (Fogel et al. 1982; Muir and Yarechewski 1984) and >100 days in aerobic soils were observed (Muir and Yarechewski 1984) for methoxychlor. In a study of the effect of methoxychlor on soil microflora, methoxychlor was found to be moderately persistent (30 days < $t_{1/2}$ < 1 year) (Howard et al. 1991) in soil under anaerobic conditions. Six months after the application of methoxychlor to the soil, 42% remained (Polyakova et al. 1984).

Methoxychlor is generally not detected in foods, although low levels (ranging from 0.001 to 0.004 mg/kg) have occasionally been detected in dairy products, grains, fruits, and vegetables. Higher levels (ranging from 10 to 120 $\mu\text{g}/\text{kg}$) have been infrequently reported in fish (Camanzo et al. 1987; Devault 1985). Most members of the general population have little or no exposure to methoxychlor. People who use products containing methoxychlor in farming, home gardening, professional landscaping, or animal care are more likely to be exposed to methoxychlor.

6.2 RELEASES TO THE ENVIRONMENT

Methoxychlor has been identified in a variety of environmental media (air, surface water, leachate, groundwater, soil, and sediment) collected at 58 of 1,613 current or former NPL hazardous waste sites (HazDat 2002).

According to the Toxics Release Inventory (TRI), methoxychlor processing facilities listed for 1998 (TRI99 2001) report that the major portion of methoxychlor released to the environment is released to the land. Table 6-1 lists releases to the environment in 1999 from facilities that manufacture or process methoxychlor. One facility that processes methoxychlor reports that 29 pounds were released to the air and none was released to land or water. Only certain types of facilities are legally required to report; therefore, this is not an exhaustive list.

The TRI data should be used with caution because only certain types of facilities are required to report. This is not an exhaustive list.

6.2.1 Air

Release of methoxychlor to the atmosphere occurs mainly as a result of its use as a pesticide. However, no data were located on the amount of methoxychlor released to air by this route. Releases to the

6. POTENTIAL FOR HUMAN EXPOSURE

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Methoxychlor

State ^b	Number of facilities	Reported amounts released in pounds per year ^a					Total on-site release ^d	Total off-site release ^e	Total on and off-site release
		Air ^c	Water	Underground injection	Land				
AR	1	29	No data	No data	No data	29	14	43	
OH	1	0	0	No data	No data	0	0	0	
TX	1	0	No data	No data	No data	0	No data	0	
Total	3	29	0	0	0	29	14	43	

Source: TRI99 2001

^aData in TRI are maximum amounts released by each facility.

^bPost office state abbreviations are used.

^cThe sum of fugitive and stack releases are included in releases to air by a given facility.

^dThe sum of all releases of the chemical to air, land, water, and underground injection wells

^eTotal amount of chemical transferred off-site, including to publicly owned treatment works (POTW)

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atmosphere during production, formulation, and disposal of methoxychlor have been estimated to be 0.1 kg/metric ton produced (HSDB 2000). Based on the 423,832 pounds of methoxychlor produced in the United States in 1991 (Kincaid Enterprises 1992), atmospheric release during production may be estimated to be 42 pounds (19 kg). According to TRI (TRI99 2001), three processing facilities in the United States reported the release of 29 pounds of methoxychlor to the air in 1999. The TRI data (Table 6-1) should be used with caution since only certain types of facilities are required to report; therefore, this is not an exhaustive list. Methoxychlor has been identified in 1 air sample collected from 1,613 current or former NPL hazardous waste sites where it was detected in some environmental media (HazDat 2002).

Methoxychlor has been detected in air samples taken during monitoring studies conducted during the years 1986–1988 in the Jacksonville, Florida (EPA 1990e) and seasonal variation was reported. The mean outdoor concentration of methoxychlor was highest in winter months (0.1 ng/m³; detection limits not reported); methoxychlor was not present at detectable levels during the spring and summer months.

6.2.2 Water

Methoxychlor can be released directly to surface waters on farms when used to control larvae of insects (Stoltz and Pollock 1982). Methoxychlor is approved for use on cranberries (EPA 1988b), which are grown in bogs, and therefore methoxychlor could be released directly to surface waters where cranberries are grown. Methoxychlor may be released to water from runoff from soil containing methoxychlor, industrial effluents or from leaks at storage and waste sites. According to TRI (TRI99 2001), three processing facilities in the United States reported that no methoxychlor was released to water in 1999. The TRI data (Table 6-1) should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Methoxychlor has been identified in 19 groundwater and 7 surface water samples collected from 58 NPL hazardous waste sites, where it was detected in some environmental media (HazDat 2002).

6.2.3 Soil

Methoxychlor is released to soils primarily through its use as an insecticide for agricultural crops, home orchards, and ornamentals. Some methoxychlor may be released to soils through leaks at storage waste sites. According to TRI (TRI99 2001), three processing facilities in the United States reported that no

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methoxychlor was released to soil in 1999. The TRI data should be used with caution since only certain types of facilities are required to report; therefore, this is not an exhaustive list.

Methoxychlor has been identified in 46 soil and 11 sediment samples collected from 58 NPL hazardous waste sites, where it was detected in some environmental media (HazDat 2002).

6.3 ENVIRONMENTAL FATE

This discussion applies primarily to *p,p'*-methoxychlor, the major component in technical grade methoxychlor. Please see Section 4.1 Chemical Identity for a detailed discussion of impurities and contaminants in technical grade methoxychlor, which is the primary form used for pesticide formulation. Little information is available on the environmental fate of impurities and contaminants in technical grade methoxychlor. Methoxychlor can contain as many as 50 contaminants (impurities) that are introduced during commercial production. The contaminants include 1-chloro-1,2,2-tris[4-methoxyphenyl]ethene (chlorotrianisene; TACE), a triphenylethylene derivative that exhibits estrogenic/anti-estrogenic characteristics. Other contaminants include polycyclic hydrocarbons (e.g., 3,6-dimethoxy-9,10-bis(p-methoxyphenyl)phenanthrene, tetrakis(p-methoxyphenyl)ethylene, and 3,6,11,14-tetra-methoxydibenzo(g,p)chrysene), which have been studied for mutagenicity and putative carcinogenicity (Grant et al. 1976).

6.3.1 Transport and Partitioning

Methoxychlor is expected to exist in both the particulate (bound to particulate matter) and, to a small degree, vapor phase in the atmosphere (Kelly et al. 1994). The residence time and dispersion of methoxychlor in air is, therefore, a function of particle size, windspeed, and precipitation. Based on monitoring data, the majority of methoxychlor is removed by wet or dry deposition processes with a residence time of <1 month (Hoff et al. 1992). However, evidence of wide dispersion of methoxychlor in the atmosphere by its detection in Canadian arctic snow suggests that some methoxychlor may remain in air for extended periods of time (Welch et al. 1991). Methoxychlor has been frequently detected in rain (Strachan 1985, 1988). In a 6-year study (1986–1991) conducted in the Great Lakes Region, the mean annual concentration of methoxychlor in rain was 2.4 ng/L (Chan et al. 1994). These data suggest that wet deposition processes significantly contribute to the removal of methoxychlor from the atmosphere. However, wet deposition of methoxychlor will depend upon the amount of precipitation and will vary from year to year. Dry deposition due to gravity will also act to remove methoxychlor from air. In the

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Great Lakes region, dry deposition of chlorinated pesticides was estimated to be 1.5–5.0 times as great as wet deposition (Eisenreich et al. 1981).

Methoxychlor is a relatively hydrophobic compound with estimated log octanol/water partition coefficient ($\log K_{ow}$) values ranging from 4.68 to 5.08 (Howard 1991). Because of this, methoxychlor in water is expected to partition mainly to sediment and organic matter, although a significant fraction may remain in solution when the ratio of sediment mass to water volume is low (Wolfe et al. 1977). In sediments, the partitioning of methoxychlor was higher for silts and clays than it was for sand (Karickhoff et al. 1979). Sorption of methoxychlor to bacteria, algae, and fungi has been reported (Paris and Lewis 1976). Methoxychlor has an estimated vapor pressure of 1.4×10^{-6} mmHg (Howard 1991) and an estimated Henry's law constant of 1.6×10^{-5} atm-m³/mole (Howard 1991). These values indicate that volatilization of methoxychlor from water may occur. A half-time of 4.5 days has been estimated for the volatilization of methoxychlor from a shallow river (Howard 1991). In addition, methoxychlor has been observed to slowly volatilize from foliage (Howard 1991). This process may contribute to the environmental cycling of methoxychlor.

Experimental adsorption coefficients (K_{oc}) that have been reported for methoxychlor are as follows: 9,700–41,000 in sand, 80,000–100,000 in fine silt, and 73,000–92,000 in clay (Kenaga 1980; Prasad 1992). Because methoxychlor has a relatively high K_{oc} value, for example, an experimental K_{oc} of 79,433 was reported by Montgomery and Welkom (1990), methoxychlor has the potential to undergo significant adsorption to soils, especially those with high organic carbon content (Karickhoff et al. 1979; Richardson and Epstein 1971). Methoxychlor retention is also greater in finer-textured soils (particle size $<0.08 \mu\text{m}$) than in coarse-textured soils (particle size $\geq 0.08 \mu\text{m}$) (Richardson and Epstein 1971). The mobility of methoxychlor may be higher in sandy soils, since adsorption was significantly less in soil with lower organic carbon content and larger particle size (Karickhoff et al. 1979; Richardson and Epstein 1971). In addition to soil adsorption, methoxychlor may become structurally bound to soil humic materials (Mathur and Morley 1978).

Methoxychlor is generally detected only in the uppermost layer (top 5 cm) of soil on which it was applied (Golovleva et al. 1984). Dechlorinated, dehydrochlorinated, and demethylated degradation products of methoxychlor were generally detected in lower levels of soil, suggesting that they are more mobile than methoxychlor (Golovleva et al. 1984).

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The bioconcentration of methoxychlor has been investigated in microorganisms, lower invertebrates, and in fish. Because methoxychlor has a relatively high octanol/water partition coefficient, it is expected to have potential for bioconcentration. Reported bioconcentration factors (BCFs) for methoxychlor were 411–2,758 in *Aerobacter aerogenes* bacteria, 2,114–8,138 in *Bacillus subtilis* bacteria (Johnson and Kennedy 1973), 348–1,130 in stoneflies, 5,000–8,570 in snails (Anderson and DeFoe 1980), and 1,500 in clams (Hawker and Connell 1986). Methoxychlor has been reported to bioaccumulate in phytoplankton, and levels up to 80 µg/kg have been reported in samples from Lake Erie, Ontario. In a model ecosystem, BCFs of 1,500 for fish and 120,000 for mosquitos were reported (Kapoor et al. 1970). BCFs of 185 for fish (flowing water) and 1,550 for trout (static water) have also been reported (Prasad 1992). In sheepshead minnows, BCFs were found to be concentration dependent, ranging from 113 at 3 µg/L to 264 at 23 µg/L (Parrish et al. 1977). In fathead minnows, a BCF of 8,300 was reported (Veith et al. 1979). These data suggest that considerable species variation exists in the bioconcentration of methoxychlor in fish, perhaps as the result of species differences in the capacity to metabolize and eliminate methoxychlor. Methoxychlor has been reported to bioaccumulate in the blubber (0.68 µg/kg wet weight) and liver (0.1 µg/kg wet weight) of harp seals (Zitko et al. 1998). The primary source of methoxychlor contamination in seals is thought to be their diet, which consists largely of fish.

The persistence and disappearance (washoff or dryfall) of methoxychlor from mature soybean foliage was investigated in a small field plot study under natural rainfall conditions in 1977 and 1978 (Smith et al. 1981). Methoxychlor washoff rate was 8±4% of the amount on plants (prior to rain) per cm of rainfall, regardless of time after application. Total seasonal washoff for 1978 accounted for 33.5% of the applied pesticide; however, 30.5% of the total loss was removed by washoff on the second day after application. Dryfall or dislodgeable residue accounted for <1% of the amount applied. The amount of dryfall was greater in plots entered by workers than in those where entry was avoided.

6.3.2 Transformation and Degradation

The structures of many of the methoxychlor degradation products mentioned here are shown in Figure 3-2, Proposed Metabolic Pathways of Methoxychlor.

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6.3.2.1 Air

Methoxychlor is expected to exist in both the particulate and, to a small degree, vapor phase in the atmosphere (Kelly et al. 1994). The rate constant for the vapor-phase reaction of methoxychlor with photochemically-produced hydroxyl radicals has been estimated to be 5.2×10^{-11} cm³/molecule-second at 25 EC using a structure estimation method (Meylan and Howard 1993). This corresponds to an atmospheric half-life of about 7 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals/cm³ (Meylan and Howard 1993). Particulate-phase methoxychlor is not expected to react with photochemically produced hydroxyl radicals and, therefore, will not be readily degraded in the atmosphere.

6.3.2.2 Water

Methoxychlor can be degraded in water by chemical, photochemical, and biological processes. Methoxychlor undergoes a spontaneous elimination reaction in aqueous solution to yield dehydrochlorinated products, including 1,1-bis(4-methoxyphenyl)-2,2-dichloroethylene (a proestrogenic derivative of methoxychlor discussed in Section 3.4.3; also known as methoxy-DDE). The half-life for the degradation of methoxychlor by this process was estimated to be approximately 1 year (Wolfe et al. 1977). Methoxychlor may also be oxidized by hydroxyl radicals or ozone in ozonated waters (Haag and Yao 1992; Yao and Haag 1991). The half-life of methoxychlor when reacted with ozone was estimated to be 2.1 minutes.

Methoxychlor is photochemically degraded by sunlight through loss of one chlorine atom to form a radical intermediate, which rearranges to the more stable 1,1-bis(4-methoxyphenyl)-2,2-dichloroethylene (also known as MDDE) (Zepp et al. 1976). A dramatic difference in half-life was observed for the photochemical degradation of methoxychlor in distilled water (4.5 months) and natural water (2–5 hours). Methoxychlor was found to form adducts extensively with plant materials via a photochemically-induced radical mechanism (Schwack 1988). This observation may explain the dramatic differences in the half-life of methoxychlor in distilled and natural waters.

DDE and 1,1-bis(4-methoxyphenyl)-2,2-dichloroethylene, respective degradation products of DDT and methoxychlor, rapidly undergo an unusual photoisomerization in solution when exposed to sunlight (Zepp et al. 1977). The isomerization involves the exchange of a vinyl chlorine and an ortho aromatic

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hydrogen. Other photodegradation products identified were corresponding benzophenones and 1,1-bis(4-methoxyphenyl)-2-chloroethylene.

Methoxychlor is also degraded by bacteria under aerobic conditions to form dechlorinated products (Baarschers et al. 1982). Algae are also capable of degrading methoxychlor; however, the degradation products were not determined (EPA 1976b). The half-life of methoxychlor by this degradation process was reported to be <2 weeks with some organisms. Data regarding the biodegradation of methoxychlor in water under anaerobic conditions were not located. However, based on biodegradation of methoxychlor in soil under anaerobic conditions (see below), transformation via this process is likely to occur.

6.3.2.3 Sediment and Soil

First-order kinetics have been used to model the dissipation of methoxychlor in the environment (under anaerobic and aerobic conditions) because a half-life for the chemical can be defined. The half-life represents the calculated time for loss of the first 50% of the substance, but the time required for the loss of half of that which remains may be substantially longer, and the rate of disappearance may decline further as time progresses. The term half-life in this document is used to indicate the estimated time for the initial disappearance of 50% of the compound and does not necessarily imply that first-order kinetics were observed throughout the experiment unless otherwise noted.

Half-lives of <30 days in anaerobic soils (Fogel et al. 1982; Muir and Yarechewski 1984) and >100 days aerobic soils were observed (Muir and Yarechewski 1984) for methoxychlor. In a study of the effect of methoxychlor on soil microflora, methoxychlor was found to be moderately persistent (30 days < $t_{1/2}$ < 1 year) (Howard et al. 1991) under anaerobic conditions. Six months after the application of methoxychlor to the soil, 42% remained (Polyakova et al. 1984).

In soils and sediments, methoxychlor can be biodegraded under either aerobic or anaerobic conditions. Biodegradation of methoxychlor has been reported to be greater under anaerobic conditions than aerobic conditions; biodegradation of the compound has been observed to be more rapid as conditions become more strongly reducing (i.e., more anaerobic). For anaerobic biodegradation in flooded sediment and nitrogen atmosphere (reducing environment), methoxychlor degraded with a half-life of <28 days compared with half-lives of 49–50 days in flooded sediment maintained in static aerobic conditions and 115–206 days in aerobic (artificially aerated, oxidizing environment) flooded sediment (Fogel et al. 1982; Muir and Yarechewski 1984). The major degradation products consisted of 1,1-dichloro-

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2,2-di(4-methoxyphenyl)ethene (DMDE), 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane (DMDD), 1-chloro-2,2-bis(4-methoxyphenyl)ethene (DMDU), 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane, 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene, and 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethene (Muir and Yarechewski 1984). In another study, methoxychlor was found to degrade by >95% over 160 days in flooded sediment (anaerobic conditions; reducing environment) and by 70% over 185 days in aerated soil (aerobic conditions; oxidizing environment) Golovleva et al. (1984). Out of 709 strains of microorganisms found in soils, 17 were able to convert methoxychlor to 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethene (DMDE), and 5 were able to convert it to nonchlorinated products (Golovleva et al. 1984). Some of the degradation products resulting from partial dechlorination of methoxychlor may accumulate in soils (degradation products were identified by chromatography/MS analysis; Golovleva et al. 1984). Data regarding the photodegradation of methoxychlor in surface soils were not located, but based on the photodegradability of methoxychlor in water (see Section 6.3.2.2) and the photodegradation of a structural analogue (ethoxychlor) in soil (Coats et al. 1979), this process is likely to occur, but only at the very surface of soil. Because methoxychlor is mostly found in the upper layer of soil, the photochemical and aerobic degradative processes would probably be more important for methoxychlor applied to crops.

6.3.2.4 Other Media

The degradation of methoxychlor has been studied under anaerobic conditions (incubation time = 48 hours) in microbial suspensions (Van Duck and Van de Voorde 1976). The bacterial strains studied, *Hydrogenomonas sp.*, *Mycoplana bullata*, *Mycoplana dimorpha*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, and *Escherichia coli* were found to be capable of degrading 85% of the methoxychlor at concentration levels of 20,000–160,000 µg/L. The microbial degradation of methoxychlor has been studied under anaerobic and aerobic conditions using four cultures: *Bacillus sphaericus*, *Rhodococcus erythropolis*, and *Acinetobacter calcoaceticus* 5 and 21 (Golovleva et al. 1984). All four cultures were found to degrade methoxychlor at a concentration of 100,000–200,000 µg/L. The most extensive degradation was observed to occur under anaerobic conditions (incubation time=7 days; degradation=75%) compared to aerobic conditions (incubation time=7 days; degradation=18%). In another study of the degradation of methoxychlor by the bacteria, *B. sphaericus*, methoxychlor was shown to be degraded under anaerobic and aerobic conditions (Polyakova et al. 1984). The bacteria degraded methoxychlor (at a concentration of 50,000 µg/L) more completely

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under anaerobic conditions (incubation time = 10 days; degradation=83%) compared to aerobic conditions (incubation time=10 days; degradation=30%).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

6.4.1 Air

In a survey (conducted during the years 1987, 1988, and 1989) of pesticide levels in air in two U.S. cities, the mean levels of methoxychlor in indoor, outdoor, and personal air samples from Jacksonville, Florida were 200–300, 0–100, and 100–600 pg/m³, respectively (EPA 1990e). Levels of methoxychlor were below the level of detection (approximately 36 pg/m³) in these air samples in Springfield, Massachusetts.

In a survey of ambient air measurements, atmospheric levels of methoxychlor (from data taken at two locations in the United States from 301 samples) ranged from not detected to 7,000 pg/m³ (Kelly et al. 1994). In Canada, the yearly mean level of methoxychlor in air was 1.7 pg/m³ from 1988 to 1989 (Hoff et al. 1992). Air levels tended to be higher during insect control periods (up to 27 pg/m³), whereas levels were generally below the detection limit (0.04–0.1 pg/m³) during non-use periods. In the arctic, mean concentrations of methoxychlor in air samples were found to be 0.07–0.72 pg/m³ for the year 1993 and 0.18–1.43 pg/m³ for the year 1994 (Halsall et al. 1998). No data were found for methoxychlor degradation products and their levels in air.

6.4.2 Water

Methoxychlor is not commonly detected in surface, ground, or drinking waters. In a survey of major rivers in the United States, methoxychlor was not detected (detection limit =100 ng/L) at approximately 180 sites (Gilliom et al. 1985). Methoxychlor was occasionally detected in surface waters from the Great Lakes regions at concentrations ranging from 0.032 to 15 ng/L (Biberhofer and Stevens 1987; Konasewich et al. 1978; Kuntz and Warry 1983; Maguire et al. 1983). In a survey of 783 rural domestic wells and 566 community water systems across the United States, methoxychlor was generally not found above the reporting limit (300 ng/L) (EPA 1990f). Methoxychlor was not found above the detection limit (5,000 ng/L) in 54 wells in California (Maddy et al. 1982), was not detected above the detection limit (5–10 ng/L) in groundwater below irrigated farmland in Nebraska (Spalding et al. 1980), and was not detected above the detection limit (150 ng/L) in groundwater tested from 53 residential drinking wells in residential areas (non-agricultural past use) of Connecticut where home and garden pesticide use is

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reported by 66% of homeowners (Eitzer and Chevalier 1999). In a survey of the drinking water in Jacksonville, Florida and Springfield, Massachusetts, methoxychlor was not detected in any samples (EPA 1990e). In the Great Lakes region, methoxychlor has been detected in rain and snow at concentrations ranging from 0.43 to 13.1 and 0.1 to 5.8 ng/L, respectively (Strachan 1985, 1988; Strachan and Huneault 1979). Methoxychlor has also been detected in snow in the Canadian arctic at levels of 0.2 ng/L (Welch et al. 1991). In an irrigation canal in which methoxychlor was intentionally added to control fly larvae, peak levels of 1,000 ng/L were detected at a site nearly 75 miles downstream, 45–46 hours after application (Stoltz and Pollock 1982). In another study, conducted during 1981–1986, methoxychlor residues were monitored in the Athabasca River in Canada after treatment for control of black fly larvae (Kumar and Byrtus 1993). Peak levels of 5,000 ng/L were detected (detection limit=0.03 µg/L) 47 miles downstream of the treatment location. Methoxychlor has been detected at high concentrations in waters near points of methoxychlor use or application. Shortly after the aerial application of methoxychlor to elm trees, levels of 40–160 mg/L were detected downstream in the top foot of a nearby river (Wallner et al. 1969). Methoxychlor was not detected (detection limit not reported) 100 feet downstream after 24 hours. Methoxychlor has also been detected in groundwater at waste disposal sites. A review of groundwater monitoring data from 479 site investigations indicates that methoxychlor was detected in groundwater at 14 (. 3%) of the sites (Plumb 1991). No data were found for methoxychlor degradation products and their levels in water.

6.4.3 Sediment and Soil

In general, methoxychlor is infrequently detected in soils and sediments in the United States, but higher levels may be detected more frequently near release sources. In a survey of soils in the United States, methoxychlor was detected in 1 of the 1,729 cropland soil samples at a level of 0.28 µg/kg (IARC 1979). Methoxychlor was detected in 1 of the 45 random soil samples in Alabama at a level <100 µg/kg (Albright et al. 1974). The detection limit was not reported. At a hazardous waste site in Fresno, California, methoxychlor was detected at levels ranging from <150 to 17,000 µg/kg in subsurface soil (Agency for Toxic Substances and Disease Registry 1989a). Soil beneath elm trees sprayed with 12–16% methoxychlor was reported to contain 2,900–14,600 µg/kg 3 days after application, and 1,000–8,400 µg/kg 130 days later (Wallner et al. 1969). These data suggest that methoxychlor is moderately persistent ($30 \text{ days} < t_{1/2} < 1 \text{ year}$) (Howard et al. 1991) in soil. In a survey of major rivers in the United States, methoxychlor was detected in sediment samples from only 1 out of 160 sites (detection limit =1 µg/kg) (Gilliom et al. 1985). In contrast, 51% of 70 sediments samples from the Niagara River contained detectable levels of methoxychlor, with a mean level of 7 µg/kg (standard deviation=14 µg/kg)

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dry weight (Kuntz and Warry 1983). No data were found for methoxychlor degradation products and their levels in soil or sediment.

6.4.4 Other Environmental Media

Methoxychlor is generally not detectable or detectable only at very low levels in food. Methoxychlor was reported in only 5% of nearly 14,000 composite food samples obtained from 10 states in 1988, and in 11 of 13,000 samples in 1989 (Minyard and Roberts 1991). However, the levels of methoxychlor detected were not provided. In a market basket survey performed in 1980–1982, dairy products and cereals/grain products contained levels ranging from a trace to 4 µg/kg (Gartrell et al. 1986a).

Methoxychlor was infrequently (0.3%) encountered in vegetables in the United States in 1969–1976, with a mean level of 1 µg/kg (Duggan et al. 1983). In a study conducted by the Food and Drug Administration (FDA) for incidence/level monitoring of domestic and imported fruits and vegetables, methoxychlor was not detected in tomatoes or pears (Roy et al. 1995). In Canada from 1980 to 1985, methoxychlor was generally not detected in vegetables, fruits, meats, or dairy products (Davies 1988; Frank et al. 1987b). However, low levels (4 µg/kg) were detected in strawberries (Frank et al. 1987a). Fish from the Great Lakes generally did not contain detectable levels of methoxychlor, but occasionally, some samples contained levels ranging from 10 to 120 µg/kg wet weight (Camanzo et al. 1987; Devault 1985). Giesy et al. (1994) studied several species of migratory fish in rivers above and below the Great Lakes.

Methoxychlor was detected at levels ranging from not detectable to 1.4 µg/kg. Fish (bluegill and carp), taken from rivers that are downstream from irrigated farmland in California, did not contain detectable levels (limit of detection=0.04 mg/kg) of methoxychlor (Saiki and Schmitt 1986).

Methoxychlor has been detected in the blubber and liver of harp seals (Zitko et al. 1998). Levels detected were 0.68 µg/kg (wet weight) in the blubber and 0.1 µg/kg (wet weight) in the liver of male harp seals. Seal muscle tissue is used for human consumption, and there is a growing market for seal meat. The primary source of methoxychlor contamination in seals is thought to be their diet, which consists largely of fish.

Methoxychlor was detected on grain storage building surfaces in which methoxychlor was used for insect control, and this resulted in detectable levels in grain (NIOSH 1986; Watters and Grussendorf 1969).

In a survey of methoxychlor residues in house dust in 28 homes in an agricultural area of Colorado where pesticide use was common, levels (limit of detection for methoxychlor was not reported) ranged from 1.6

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to 103 mg/kg (mean=14.9 mg/kg) in 8% of the homes of farmers, from 1.9 to 144 mg/kg (mean=18.2 mg/kg) in 9% of the homes of pesticide formulators, and from 1.5 to 29 mg/kg in 2% of the homes of the control group (Starr et al. 1974). It is not clear if the presence of methoxychlor in the homes of the control group was due to migration of methoxychlor from nearby buildings or fields where it was applied by farmers, or to in-house use of methoxychlor-containing products by the residents.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

For the general population, the most likely source of methoxychlor exposure is from low-level contamination of food. In a study of exposure of the general population to chemical contaminants in food, the average daily exposure to methoxychlor was estimated by calculating exposures from individual foods and summing across food types (Dougherty et al. 2000). The sources of data for contaminant concentrations included the USDA Pesticide Data Program (for years 1992–1993 covering fruits and vegetables), FDA Total Diet Study (for years 1988–1993 covering 261 foods), USEPA Dioxin Report and USEPA/USDA Dioxin in Beef Study (for years 1989–1991, covering meat, dairy products, pork, chicken, milk, and eggs), and National Sediment Inventory (for years 1988–1993, covering fish and shellfish). Methoxychlor was detected in 93 of 29,180 samples and exposure to methoxychlor was estimated to be 5 ng/kg/day (nondetects = 0) and 70 ng/kg/day (nondetects = one half the limit of detection). The 5 ng/kg/day value is larger than the value calculated in the FDA's Total Diet Study Program, which may be attributed to higher concentrations of methoxychlor in raw fish than in other foods. The FDA's Total Diet Study program monitors chemical contaminants in the U.S. food supply and has calculated average daily intakes of methoxychlor (the limit of detection was not reported) in adults (age 25–65) ranging from 0.1 to 0.3 ng/kg/day for the period 1986–1991 (Gunderson 1995b), 0.6–0.9 ng/kg/day for the period 1984–1986 (Gunderson 1995a), and 4 ng/kg/day for the period 1980–1982 (Gartrell et al. 1986b). A decrease in the average daily intakes of methoxychlor is noted for period 1980–1991. Average daily intakes of methoxychlor for infants and children were also monitored (see Section 6.6). Exposure to methoxychlor from food may be elevated in persons who consume large amounts of fish and seafood from methoxychlor-contaminated waters. Because methoxychlor is usually not detected in ground or surface water sources, exposure to methoxychlor from drinking water is not expected to be significant for the general population. Based on the results of the Non-occupational Pesticide Exposure Study, inhalation exposure to methoxychlor ranged from 0.002 to 0.012 µg/day in one U.S. city (EPA 1990e). In a monitoring study of residential and commercial air and dust samples for the occurrence of air born endocrine disruptors, methoxychlor was detected in four out of six dust samples at 0.6–3.5 µg/g of dust. A methoxychlor metabolite, 2,2-bis (*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) was also detected at

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0.14 µg/g in three of the four dust samples that contained methoxychlor. Methoxychlor was not reported to be detected in collected air samples. Exposures may be greater in individuals who use methoxychlor-containing products for home gardening or animal-care purposes. In a monitoring study of nonoccupational exposure to pesticides used in and around the home, methoxychlor was detected (air concentrations were not provided) in both indoor and outdoor samples (Lewis et al. 1988). Inhalation and dermal exposures may be greater for workers and farmers exposed to methoxychlor. The National Occupational Exposure Survey of 1981–1983 estimated that approximately 3,418 workers (agricultural services and personal services) were exposed to methoxychlor in the United States in 1980 (NIOSH 1992b). However, this number does not include farmers who are exposed by using methoxychlor on their crops and livestock. No quantitative data were located regarding blood, tissue, or urine levels of methoxychlor following occupational exposure to methoxychlor.

The occurrence of methoxychlor in blood serum was studied in 39 men of varying occupations and similar age and weight, living Southern Honduras (Steinberg et al. 1989). Of the population studied, 20 men, representing the test group, lived and worked in and near farming cooperatives where pesticide use is extensive (16–30 times/year), and 19 of the men, representing a comparison group, lived in an area where pesticides are applied only once per season. The blood serum for all participants was analyzed using HPLC and GC/MS (EC detection; detection limits not specified). Methoxychlor was detected in the serum of one of the comparison group at 5.16 mg/L, but none was detected at or above the detection limit in the remaining participants. The absence of detectable levels of methoxychlor in serum of the majority of the participants may suggest a low exposure to the pesticide; the detection limit was 0.24–4.07 mg/L. However, since the majority of the participants were of lean weight (145 pounds; low adipose tissue), it may also suggest that in the absence of adipose, methoxychlor is not stored in the body.

In a study of human adipose tissue (taken from cadavers at autopsy) conducted in Kingston and Ottawa, Ontario, Canada, methoxychlor was not detected (using gel permeation chromatography and GC/MS) at the minimum level of detection of 13.5 ng/g of adipose (LeBel and Williams 1986). However, it could not be determined from the study whether any of the deceased individuals had ever lived in regions of high pesticide use or worked with products that contain methoxychlor.

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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).

There are little data about children's exposure to methoxychlor or its degradation products. Like adults, children may be exposed to methoxychlor upon inhalation of contaminated air, ingestion of contaminated groundwater that is used as drinking water, ingestion of contaminated food, and dermal contact with contaminated soil or products treated with the substance. In addition, since small children may play close to the ground they are more likely than adults to come in contact with dirt and dust found in home carpets, dirt found outside the home, and lawns. Children and infants exhibit frequent hand-to-mouth activities, tend to put foreign objects in their mouths, and sometimes intentionally eat soil. This behavior may result in the ingestion of methoxychlor contaminated soil and dust. Playing in dirt may also cause dermal exposure and inhalation of airborne soil particles. Children may come in contact with and ingest pesticide products that are stored in the home or garage area. If a methoxychlor containing product is used on a pet, a child may be exposed through dermal contact or through contact with an applicator or animal dipping solution.

Children are more likely than adults to ignore no trespassing signs and wander into areas that may contain hazardous waste. A child playing in such an area may be at risk of exposure to elevated levels of methoxychlor from contact with or ingestion of soil since methoxychlor applied at the surface is known to adsorb to soil and remain near the surface. No data concerning the risk of exposure to methoxychlor or its degradates from ingestion of soil were found. There is no information on the bioavailability of methoxychlor from oral ingestion, dermal exposure, or inhalation of soil or dust.

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A pilot study was conducted of nine residential homes to monitor potential exposures of children (age 6 months to 5 years) to pesticides (Lewis et al. 1994). Methoxychlor was detected in two of the older nine households (constructed in 1930 and 1962), but was not detected in newer homes. The mean concentration of methoxychlor found in sample test areas that included house dust, child hand rinse, air, entryway soil, walkway soil, and play area soil was not reported. In a later study conducted in residential areas of North Carolina (the Research Triangle area), house dust was collected, separated into seven size fractions (<4–500 μm in diameter), and monitored for pesticide residues (Lewis et al. 1999). The concentration of methoxychlor reported by the authors for various dust particle sizes is as follows: 250–500 μm , 120 ng/g; 150–250 μm , 210 ng/g; 106–150 μm , 310 ng/g; 53–106 μm , 570 ng/g; 25–53 μm , 740 ng/g; 4–25 μm , 680 ng/g; and <4 μm , 1,000 ng/g. The authors state that ingestion, dermal exposure, and inhalation of house dust may represent a substantial portion of a child's exposure to pesticides.

The FDA tested the occurrence of pesticide residues in domestic and imported adult foods eaten by infants/children. The foods were prepared for consumption prior to analysis using the FDA's regulatory monitoring methodology (Yess et al. 1993). Methoxychlor was found in apples (0.16% of samples, 0.38 ppm) and in plain milk (0.22% of samples, 0.38 ppm).

In another study, foods that are representative of the diets of eight population groups (ranging from infants to elderly adults) were prepared for consumption and analyzed for pesticide residues using the methods in the FDA's revised (April 1982) Total Diet Study (Gunderson 1995a). The mean daily intakes for children and adults for the test period 1986–1991 were calculated to be 0.4 ng/kg/day for 6–11-month-old infants, 0.9 ng/kg/day for 2-year-old children; 0.3 ng/kg/day for 14–16-year-old females; 0.4 ng/kg/day for 14–16-year-old males; and 0.1–0.3 ng/kg/day for 25–65-year-old adults (Gunderson 1995b). Intakes of methoxychlor for the test period 1984–1986 were calculated to be 1.0 ng/kg/day for 6–11-month-old infants; 2.4 ng/kg/day for 2-year-old children; 0.8 ng/kg/day for 14–16-year-old females; 0.6 ng/kg/day for 14–16-year-old males; and 0.6–0.9 ng/kg/day for 25–65-year-old adults (Gunderson 1995a). Intakes of methoxychlor for the test period 1980–1982 were calculated to be 19 ng/kg/day for 6–11-month-old infants, 4 ng/kg/day for 2-year-old toddlers, and 4 ng/kg/day for 25–65-year-old adults (Gartrell et al. 1986b). A reduction in the estimated daily intake of methoxychlor is noted for the period 1980–1991 for all population groups. More recent residue analysis from the total diet study 1991–1999 is available for various foods, but intakes for various age groups have not been calculated (FDA Residue Monitoring 1999).

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The FDA has also collected and analyzed a number of baby foods (12 fruit juices or fruits, 4 fruit desserts, 4 grain products, and 1 vegetable) in addition to those covered under the Total Diet Study Program (FDA Residue Monitoring 1999). In 1998, methoxychlor was found in 1% of samples at a level of 0.001 ppm. In 1999, 20 different food items (7 fruit juices, 5 fruits, 4 fruit deserts, and 4 grain products) were analyzed and methoxychlor was not reported to be found at a detectable level (FDA Residue Monitoring 1999).

A possible source of exposure in infants to methoxychlor is breast or formula milk. No data were found on the presence of methoxychlor in breast milk in the United States. However, in a recent study conducted among regional populations in Kazakstan (represented by large urban centers, an agricultural region, a petrochemical region, and rural regions), human milk obtained from 92 donors was analyzed according to the World Health Organization Protocol for organochlorine pesticides (Hooper et al. 1997). Methoxychlor was not detected (limit of detection=5–100 pg/g of fat) in any of the samples. Methoxychlor was also not detected in infant formulas, canned milk, with and without iron (Gunderson 1995b; Yess et al. 1993). Methoxychlor was not detected in whole milk, infant formula (with or without iron), or soy-based infant formula according to the most recent FDA Total Diet Study (FDA Residue Monitoring 1999).

Other possible sources of concern for exposure of methoxychlor to children are parents' work clothes and equipment used to apply products that contain methoxychlor. This mode of exposure would be of special concern in agricultural areas and around homes where pesticides are applied for lawn and garden or indoor use. However, no data were found regarding increased levels of methoxychlor in children living in agricultural areas and no information is available on methoxychlor take-home exposure. See *Report to Congress on Workers' Home Contamination Study Conducted Under The Workers' Family Protection Act* (NIOSH 1995) for a good review of the literature and examples of other chemicals likely to be taken home inadvertently.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Farmers and pesticide applicators who use methoxychlor are the populations most likely to receive above average exposures. In addition, people who live on or near farms, methoxychlor-production plants, formulation plants, or hazardous-waste sites may be exposed to above-average levels of methoxychlor. Individuals who consume large amounts of seafood obtained from methoxychlor-contaminated waters may also be exposed to above-average levels of methoxychlor.

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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 methoxychlor 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 methoxychlor.

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 relevant physical and chemical properties of methoxychlor (see Table 4-2) including water solubility (Budavari et al. 1989; EPA 1988a; HSDB 2000; Montgomery and Welkom 1990), K_{ow} (Howard 1991), K_{oc} (Montgomery and Welkom 1990), vapor pressure (Howard 1991), and Henry's law constant (Howard 1991) have either been measured experimentally or have been estimated accurately enough to permit the evaluation of the environmental fate and transport of methoxychlor. Technical grade methoxychlor contains between 10 and 12% impurities (IARC 1979; Lamoureux and Feil 1980), and GC/MS studies have identified >50 individual impurities at varying percentages (Lamoureux and Feil 1980). Additional data regarding the physical and chemical properties of the most abundant impurities in the technical grade product would be useful in assessing the environmental fate of methoxychlor.

Production, Import/Export, Use, Release, and Disposal. According to the Hazardous Substances Data Bank (HSDB), 17,700 pounds of methoxychlor were imported in 1978 (HSDB 1993). No information on current import volumes are available (HSDB 2000). A manufacturer of methoxychlor, Kincaid Enterprises, Inc., reported information on export volumes of technical grade methoxychlor in pounds as 25,750 in 1986; 86,000 in 1987; 22,600 in 1988; 47,150 in 1989; 10,350 in 1990; and 49,750 in 1991 (Kincaid Enterprises 1992). However, there is no information on current export volumes

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of formulated products containing methoxychlor. No recent data were found for current production volume and import/export levels of methoxychlor. Information regarding current production volumes and import/export amounts are necessary in order to understand the current trends of usage and potential for human exposure in the United States.

Methoxychlor is a General Use Pesticide (GUP) and is available over the counter. It is predominately used for agriculture (57%) with lesser usage for industrial and commercial purposes (15%) and home and garden use (28%) (Kincaid Enterprises 1992). However, data regarding the widespread use of methoxychlor in the environment, in the workplace, or at home were not found. It would be useful to know the extent to which methoxychlor is used for estimating the percent of the population exposed to methoxychlor.

Approximately 25,486 pounds of methoxychlor were released to the environment in 1998, which included 12 pounds to air and 25,474 pounds to land (TRI99 2001). However, only certain types of facilities are legally required to report; therefore, this is not an exhaustive list. Releases of methoxychlor from industrial sources and operations are most likely minimal compared to the release to the environment from its use as a pesticide. Quantitative information on the frequency, extent, and actual application rate of methoxychlor when used as a pesticide would be valuable in estimating potential exposures to people who live or work at or near agricultural areas that use methoxychlor. Currently, the EPA does restrict the amount of methoxychlor that can be disposed of in landfills (EPA 1990d). Data for the quantity of methoxychlor disposed of in landfills would be useful for estimating exposure potentials for persons living near landfills.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The TRI, which contains this information for 1998, became available in May of 2000. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. In the atmosphere, methoxychlor exists in both the particulate and, to a small degree, vapor phase (Kelly et al. 1994). Vapor-phase methoxychlor is rapidly degraded by photochemically produced hydroxyl radicals with an estimated half-life of 7 hours, calculated from its estimated rate constant (Meylan and Howard 1993). Particulate-phase methoxychlor is removed from the atmosphere by wet and dry deposition (Hoff et al. 1992). Since methoxychlor is a hydrophobic substance, it is expected to partition to sediment and organic matter (Karickhoff et al. 1979; Richardson

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and Epstein 1971). Methoxychlor is degraded in water and soil by chemical, photochemical, and biological processes, making it less persistent in the environment than its structural analogue, DDT (Baarschers et al. 1982; Golovleva et al. 1984; Wolfe et al. 1977; Zepp et al. 1976). Methoxychlor is biodegraded more rapidly under anaerobic conditions than under aerobic conditions. Half-lives of <28 days were reported in anaerobic flooded soil, compared with a half-lives of 49–50 days in flooded sediment (static aerobic conditions) and 115–206 days in aerobic artificially flooded sediment (Fogel et al. 1982; Muir and Yarechewski 1984). These half-lives only represent the time that it takes for 50% of the initial concentration to degrade, and may not be representative of true first-order kinetics.

Degradation products of methoxychlor studied under laboratory conditions using lake and pond sediment have been identified as: 1,1-dichloro-2,2-di(4-methoxyphenyl)ethene (DMDE), 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane (DMDD), 1-chloro-2,2,-bis(4-methoxyphenyl)ethene (DMDU), 1,1,1-trichloro-2(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane, 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 1,1-dichloro-2-(4-hydroxyphenyl)2-(4-methoxyphenyl)ethene, and 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethene (Muir and Yarechewski 1984). Additional data that not only identifies, but also quantifies degradation products of methoxychlor under field conditions would be useful in order to gain a complete understanding of the biodegradation process of methoxychlor in the environment. Since many of these derivatives are more polar than methoxychlor, they may be more mobile in soils and more water soluble than the parent compound. Further studies that investigate the persistence, mobility, and degradation of the estrogenic degradation products of methoxychlor (particularly estrogenic ones) in water and soil would be valuable in predicting their fate and transport at hazardous waste sites and in assessing exposure and hazard potential. Biodegradation data for the degradation products of methoxychlor were not located and information regarding the rate of degradation and possible degradation byproducts of these compounds would be useful in the determination of the persistence of toxic methoxychlor degradates.

Bioavailability from Environmental Media. No information was located regarding the bioavailability of methoxychlor from environmental media. Toxicokinetic and toxicity studies indicate that methoxychlor is absorbed following oral (Bal 1984; Chapin et al. 1997; Davison et al. 1982, 1983; Gray et al. 1988, 1989; Harris et al. 1974; Kapoor et al. 1970; Martinez and Swartz 1991; Morgan and Hickenbottom 1979) and dermal exposures (Davison et al. 1983; Haag et al. 1950; Skaare et al. 1982). No data are available regarding absorption following inhalation exposure. Studies regarding the absorption of methoxychlor following inhalation exposure would be useful since inhalation of ambient air and house dust may represent a potential route of human exposure (Lewis et al. 1999). The extent to which absorption occurs could be affected by the media in which methoxychlor is contained, but there are

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no data on these subjects. Methoxychlor is strongly adsorbed to soil surfaces and this may ultimately limit methoxychlor's bioavailability from soils. Furthermore, it has been shown that the bioavailability of certain pesticides, such as DDT, in soil declines with time as the compound becomes sequestered into the soil (Alexander 1995, 1997; Robertson and Alexander 1998). Studies regarding the bioavailability of methoxychlor from aged soils would be useful in assessing its potential toxicity from soil surfaces. No data were located regarding the bioavailability of methoxychlor's degradation products. Since these compounds can accumulate in soil surfaces, studies regarding the bioavailability of these degradation products from soils would be useful.

Quantitative studies that determine whether methoxychlor contained in soil or food is as well absorbed as methoxychlor dissolved in water or oil (form used in most toxicity and pharmacokinetic studies) would be useful in estimating the absorbed dose of methoxychlor received by individuals living at or near hazardous waste sites. Since young children might be exposed to methoxychlor (or its degradation products) orally, dermally, or by inhalation of soil, information on bioavailability from air, water, soil, or plant material would be desirable in evaluating risk to the subpopulation.

Food Chain Bioaccumulation. There are no data regarding food chain biomagnification of methoxychlor. BCFs have been measured for microorganisms, lower invertebrates, and fish (Anderson and DeFoe 1980; Hawker and Connell 1986; Johnson and Kennedy 1973; Prasad 1992; Veith et al. 1979). Although the lower species tend to bioconcentrate methoxychlor, fish generally have low BCFs. There are sparse data regarding the concentrations of methoxychlor in aquatic and terrestrial animals. However, harp seals had detectable concentrations of methoxychlor in various tissues which was attributed to the consumption of fish in the seal's diet (Zitko 1998). Since some populations use seals as a dietary meat source, it is possible that this may be a potential route of exposure in the human food chain. Studies in laboratory animals indicate that methoxychlor is rapidly metabolized and eliminated (Kapoor et al. 1970), and thus, biomagnification of methoxychlor further up the food chain does not appear to be of concern. More monitoring data of animals are required in order to ascertain that methoxychlor is not bioaccumulated up the aquatic and terrestrial food chains. There is no information regarding the plant uptake of methoxychlor following its application as a pesticide. Such information would be useful in assessing human exposure via ingestion of agricultural products, including home grown vegetables treated with methoxychlor.

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Exposure Levels in Environmental Media. Information on the levels of methoxychlor in air (EPA 1990e; Kelly et al. 1994), surface water (Biberhofer and Stevens 1987; Konasewich et al. 1978; Kuntz and Warry 1983; Maguire et al. 1983), groundwater and drinking water (EPA 1990f; Maddy et al. 1982; Plumb 1991), and soil (Albright et al. 1974; IARC 1979) are available. In general, methoxychlor is detected in the pg/m³ to the ng/m³ range in the ambient atmosphere of the United States. Methoxychlor is not frequently detected in groundwater, drinking water, or surface water, but has been detected at high concentrations in waters near points of methoxychlor use or application (Wallner et al. 1969). Methoxychlor is infrequently detected in soils and sediments in the United States, but is detected more frequently near release sources that use methoxychlor as a pesticide. Common ranges in soils have been reported as <150–17,000 µg/kg (Agency for Toxic Substances and Disease Registry 1989a; Wallner et al. 1969). Methoxychlor is generally found in low levels in foods. Methoxychlor was identified, but not quantified, in 5% of nearly 14,000 composite food samples obtained from 10 states in 1998, and in 11 of 13,000 samples in 1989 (Minyard and Roberts 1991).

No data exist for methoxychlor degradation products and their levels in environmental media. Since these degradation products may accumulate in soils (Golovleva et al. 1984), information regarding the frequency and levels detected in soils would be useful.

For the general population, exposure to methoxychlor is expected to occur primarily from low-level contamination of foods. Levels of methoxychlor contamination in a variety of foods and food groups as well as estimates of human intake from those foods have been studied (Dougherty et al. 2000; EPA 1992, 1994; FDA 1994; USDA 1995; Winters et al. 1994). Estimates of the average daily intake of methoxychlor in adults (ages 25–65) range from 0.1 to 0.3 ng/kg/day for the period 1986–1991 (Gunderson 1995b), 0.6–0.9 ng/kg/day for the period 1984–1986 (Gunderson 1995a), and 4 ng/kg/day for the period 1980–1982 (Gartrell et al. 1986b). The intake for children and young adults has been estimated as 0.4 ng/kg/day for 6–11-month-old infants, 0.9 ng/kg/day for 2-year-old children; 0.3 ng/kg/day for 14–16-year-old females; 0.4 ng/kg/day for 14–16-year-old males; and 0.1–0.3 ng/kg/day for 25–65-year-old adults (Gunderson 1995b).

Exposure Levels in Humans. Methoxychlor has been detected in blood of 1 of 39 males living at or near farming cooperatives in southern Honduras where there is extensive use (16–30 times/year) of pesticides, but levels are generally below the limit of detection in blood (Steinberg et al. 1989), adipose tissue (LeBel and Williams 1986), and milk (Mes 1981). No biomonitoring data for methoxychlor were

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located for populations living near hazardous waste sites or working at methoxychlor production, formulation, or disposal facilities.

Collecting data on the level of methoxychlor in human blood or tissue along with estimates of environmental exposure levels would be useful in estimating recent exposures in these populations. This information would aid in assessing the need to conduct health studies on these populations.

Exposures of Children. Data on exposure levels and body burden measurements of methoxychlor in children are needed to determine what extent children are exposed to this compound. Children may be exposed to methoxychlor through the ingestion of contaminated media (food or drinking water) and inhalation of ambient air or household dust particles since methoxychlor has been detected in household dusts in homes where pesticides have been used (Starr et al. 1974). Additionally, children may be exposed dermally through play activities from contaminated soils, gardens, or lawns that have recently had methoxychlor applied. Since methoxychlor is also commonly used for controlling insects on animals, children may also be exposed through contact with family pets or livestock. Exposure may also arise when children ingest soil either intentionally through pica or unintentionally through hand-to-mouth activity. Methoxychlor has been measured in residential house dust (Lewis et al. 1999); children might be exposed to such dust by crawling on the floor. Bioavailability data needs related to dust and soil exposure have been discussed above under the subsection for Bioavailability from Environmental Media. In the FDA's Total Diet Study Program for 1986–1991 exposures, daily average intakes for various age ranges were calculated; ranges were from 0.9 ng/kg/day for 2-year-old children to 0.1–0.3 ng/kg/day for 25–65-year-old adults (Gunderson 1995b). More recent data about residue amounts on various food products are available for the period of 1991–1999, but the weight adjusted intakes have not been published yet (FDA Residue Monitoring 1999). A possible source of exposure in infants to methoxychlor is breast or formula milk. No data were found on the presence of methoxychlor in breast milk in the United States. However, in a recent study conducted among regional populations in Kazakstan (represented by large urban centers, an agricultural region, a petrochemical region, and rural region), human milk obtained from 92 donors was analyzed according to the World Health Organization Protocol for organochlorine pesticides (Hooper et al. 1997). Methoxychlor was not detected (limit of detection=5–100 pg/g of fat) in any of the samples. Methoxychlor was also not detected in infant formulas, canned milk, with and without iron (Gunderson 1995b; Yess et al. 1993). Methoxychlor was not detected in whole milk, infant formula (with or without iron), or soy-based infant formula according to the most recent FDA Total Diet Study (FDA Residue Monitoring, 1999). Monitoring data on levels of

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methoxychlor in breast milk in the United States are needed because this is an important potential route of exposure for infants.

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 methoxychlor were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

No ongoing studies were located regarding the environmental fate or potential for human exposure to methoxychlor.

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/ monitoring methoxychlor, its metabolites, and other biomarkers of exposure and effect to methoxychlor. 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

Gas chromatography (GC) with an electron-capture detector (ECD) has been used to measure methoxychlor concentrations in human milk, serum, semen, and adipose tissue (LeBel and Williams 1986; Mes 1981; Steinberg et al. 1989; Szymczynski and Waliszewski 1982). In GC, samples dissolved in a volatile solvent are injected into a heated column with a stationary phase consisting of silica coated with a liquid phase. An inert gas carries the sample through the column, and the partitioning of methoxychlor between the mobile and stationary phases gives it a characteristic retention time that is used to identify it. ECDs use a radioactive source such as ^{63}Ni to generate electrons that are captured by the chlorine atoms of methoxychlor. Reduction in electron flow by this capture produces a signal for methoxychlor.

Most methoxychlor in biological samples is dissolved in fat, and thus, samples are prepared for GC/ECD by extraction of the fat with organic solvents. The fat extract is generally "cleaned up" by gel permeation chromatography (GPC), which separates methoxychlor from higher molecular weight lipids, and/or by passage through a Florisil[®] column which retains lipids and other contaminants (LeBel and Williams 1986; Mes 1981; Szymczynski and Waliszewski 1982). These methods provide 71–104% recovery of methoxychlor (LeBel and Williams 1986; Szymczynski and Waliszewski 1982). Methoxychlor has been determined in human serum in women living in agricultural areas of Spain (Frenich et al. 2000). Samples of serum were purified using a hexane extraction procedure followed by cleanup using high performance liquid chromatography (HPLC) and detection using tandem mass spectrometry

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(GC/MS/MS). Using this method, the analytical limit of detection (LOD) was found to be 2.0 µg/L and the percent recovery was 113%. The advantage of using GC/MS/MS is a much higher sensitivity than GC/ECD. However, the GC/ECD gives greater precision, accuracy, and limit of detection values. In serum, the detection limit for methoxychlor was reported to be 0.24–4.07 mg/L (Steinberg et al. 1989). No data were located on the specificity or precision of these methods for biological samples.

As discussed previously, because methoxychlor is metabolized fairly quickly, measurement of metabolites may prove to be more useful for assessing exposure than measurement of methoxychlor itself. Pure samples of methoxychlor impurities (such as, 1,1,2,2-tetrachloro-2-(4-methoxyphenyl)ethane, 1,1-dichloro-2,2-(4-methoxyphenyl)ethene, and 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane), that may also be metabolic products of methoxychlor, may be analyzed by GC with a flame ionization detector (FID), HPLC with an ultraviolet (UV) detector, or by GC with detection by mass spectrometry (MS) (Lamoureux and Feil 1980; West et al. 1982); however, no information was located concerning the application of these methods to detecting metabolites in biological samples.

Table 7-1 summarizes the methods used for sample preparation and analysis of methoxychlor in biological samples.

7.2 ENVIRONMENTAL SAMPLES

Methoxychlor in environmental samples is also usually measured using GC/ECD (APHA 1992; Ault and Spurgeon 1984; EPA 1989b, 1990a, 1990c; Gillespie and Walters 1986; Hopper and King 1991; Hsu et al. 1991; Ivey et al. 1983). A halogen electrolytic conductivity detector (HECD) may be used instead of, or in conjunction with, an ECD, and GC/MS may be used to confirm the identity of methoxychlor (EPA 1990b; Hopper and King 1991; NIOSH 1978). Use of MS as the primary detection system can be achieved by establishing a library of reference pesticide spectra and inclusion of appropriate standards (Liao et al. 1991). Recent work has concentrated on improving methods for extraction of methoxychlor from environmental samples. Since methoxychlor in air is usually associated with particulate matter, standard methods involve collection of air samples on glass fiber or polyurethane foam and extraction with organic solvents prior to GC analysis (EPA 1990a; NIOSH 1978). Methoxychlor has been detected in dust samples at levels of 0.6–3.5 µg/g using GC/MS selective ion monitoring (Rudel et al. 2001). Reports in the development and application of semipermeable membrane devices (SPMDs) describe new

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Table 7-1. Analytical Methods for Determining Methoxychlor in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum	Extract with hexane/diethyl ether, concentrate, acidify (H ₂ SO ₄), centrifuge, extract aqueous layer with hexane, cleanup via HPLC	GC/MS/MS	500 ng/L	113	Frenich et al. 2000
Human serum	No data	GC/ECD and GC/MS	0.24–4.07 mg/L	No data	Steinberg et al. 1989
Adipose tissue	Extract with acetone/hexane, cleanup with GPC and FAC	GC/ECD and GC/MS	No data	71–98	LeBel and Williams 1986
Human milk	Extract with ethanol/hexane/ethyl ether, cleanup via GPC	GC/ECD	10 mg/kg	No data	Hooper et al. 1997
Human milk	Extract with hexane or benzene, cleanup with FAC	GC/ECD	No data	No data	Mes 1981
Human semen	Extract with petroleum ether, cleanup with FAC	GC/ECD	No data	104	Szymczynski and Waliszewski 1982

ECD = electron capture detector; FAC = Florisil® adsorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry.

7. ANALYTICAL METHODS

approaches to determining bioavailable contaminants in a variety of aquatic systems (Petty et al. 1998). The SPMD membrane mimics the diffusion transfer of organic contaminants through the respiratory membranes of aquatic organisms. Methoxychlor in water, soil, solid waste, and food is also generally extracted with organic solvents, and the extracts are cleaned up by chromatographic methods using GPC, Florisil[®] adsorption, Attagel[®], magnesia-silica gel, and/or semi-preparative HPLC (APHA 1992; Ault and Spurgeon 1984; EPA 1990c; Gillespie and Walters 1986; Hopper and King 1991; Hsu et al. 1991; Ivey et al. 1983). Modifications to extraction and cleanup methods have been proposed, which reduce the amount of solvent required or increase the speed of analyses (Kraut-Vass and Thoma 1991; Patterson 1991). An analytical procedure for the identification of methoxychlor in a drinking water sample matrix has been developed that involves the use of a two-step pre-concentration followed by a separation step (Fung and Mak 2001). The pre-concentration steps employ a solid-phase extraction (C18 SPE) and cleanup followed by stacking with reverse polarity prior to separation using micellar electrokinetic capillary chromatography (MEKC). The recovery of methoxychlor using the SPE method is 89%. The MEKC procedure was found to give analytical results that are comparable with GC-MS and LC-MS and is more sensitive than LC. The detection limit of methoxychlor in drinking water was determined to be 0.041 µg/L. Supercritical fluid extraction, a process using carbon dioxide liquified above 31 EC at high pressure, provides efficient extraction of large samples (Hopper and King 1991). These extraction methods typically provide recoveries of 84–100%. Gas chromatographic measurements can detect about 0.2 µg methoxychlor per L of hexane extract, with actual detection limits for soil and waste dependent upon matrix interferences, extraction efficiency, and cleanup procedures (EPA 1990b, 1990c). Although detection limits were not reported for analysis of methoxychlor by GC/ECD (Hopper and King 1991; Hsu et al. 1991) a detection limit of 0.05 µg/g food was reported for analysis by GC/MS (Liao et al. 1991). Pure samples of metabolic products of methoxychlor may be analyzed by GC with a FID, HPLC with an UV detector, or by GC with detection by MS (Lamoureux and Feil 1980; West et al. 1982).

Table 7-2 summarizes representative methods used for sample preparation and analysis of methoxychlor in environmental samples.

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 methoxychlor is available. Where adequate information is

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Table 7-2. Analytical Methods for Determining Methoxychlor in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
House dust	Extract with ether/hexane, chromatograph on florisil with acetone/hexane	GC/MS-SIM	No data	No data	Rudel et al. 2001
House dust	Extract with diethyl ether/hexane, cleanup with FAC	GC/MS	0.02–0.1 µg/g	No data	Lewis et al. 1999
Air	Collect on glass fiber filter, extract with isooctane	GC/HECD	7.7 mg/m ³	103	NIOSH 1978
Indoor air	Collect on polyurethane foam, extract with diethyl ether/hexane	GC/ECD	0.01 µg/m ³	65	EPA 1990a
Drinking Water	Adjust pH to 3.0 (HCl), soild phase extraction (eluted with methanol)	MEKC equipped with a variable UV detector	0.041 µg/m ³	89	Fung and Mak 2001
Water	SPMD, extract with hexane, and fractionate	Capillary column GC/ECD	0.01 µg/-SPMD	No data	Petty et al. 1998
Water	Collect analyte on SPE cartridge, elute with hexane/propanol	Capillary column GC/ECD	0.01–0.018 µg/L	No data	Kolpin et al. 1998
Water	Extract with diethyl ether/hexane or methylene chloride/hexane, cleanup with magnesia-silica gel	GC/ECD	No data	No data	APHA 1992
Water	Extract with hexane	GC/ECD	0.96 µg/L	98–100	EPA 1989b
Soil/solid waste	Sonicate, extract with hexane, cleanup with GPC	GC/ECD or GC/HECD	0.12–18 mg/kg wet weight	No data	EPA 1990b

7. ANALYTICAL METHODS

Table 7-2. Analytical Methods for Determining Methoxychlor in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil/solid waste	Extract with hexane/acetone or methylene chloride/acetone, cleanup with silica gel, GPC, and/or FAC	Capillary column GC/ECD	5.7–860 µg/kg wet weight	53–80	EPA 1990c
Food	Extract with SC-CO ₂	GC/ECD	No data	90–105	Lehotay et al. 1995
Food	Extract with SC-CO ₂ and cleanup with GPC and FAC	GC/ECD and GC/HECD	No data	87–112	Hopper and King 1991
Food	Extract with acetonitrile	GC/MS	0.05 µg/g	94	Liao et al. 1991
Food	Extract with acetonitrile, cleanup with Attagel [®] (in benzene), Florisil [®] (in hexane), or C ₁₈ (in acetonitrile)	GC/ECD	No data	89–125	Hsu et al. 1991
Edible fat	Dissolve in hexane, filter, HPLC on semi-preparative silica column	GC/ECD	No data	89–95	Gillespie and Walters 1986
Edible fat	Dissolve in methylene chloride/cyclohexane, cleanup with GPC	GC/ECD	No data	84–86	Ault and Spurgeon 1984
Human milk	Extract with ethanol/hexane/ethyl ether, cleanup via GPC	GC/ECD	10 mg/kg	No data	Hooper et al. 1997
Milk	Cleanup with Florisil [®] , elute with hexane	GC/ECD	0.005 ppm	84–96	Ivey et al. 1983

C₁₈ = Carbon 18; ECD = electron capture detector; FAC = Florisil[®] absorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; HECD = halogen electrolytic conductivity detector; HPLC = high performance liquid chromatography; MEKC = Micellar electrokinetic capillary chromatography; MS = mass spectrometry; SC-CO₂ = superficial fluid-carbon dioxide; SIM = selective ion monitoring; SPE = solid phase extraction; SPMD = semipermeable membrane device

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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 methoxychlor.

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. Methoxychlor can be measured in a variety of human tissues and fluids, including serum (LeBel and Williams 1986), milk (Mes 1981), semen (Steinberg et al. 1989), and adipose tissue (Szymczynski and Waliszewski 1982). Detection limits are not well characterized, but are probably about 0.2 µg/L in extracts of biological samples (EPA 1990b; Steinberg et al. 1989) and 0.05 µg/g in food (Liao et al. 1991). Based on the low frequency of methoxychlor detection in biological samples, it would be helpful if suitable sample extraction and analysis procedures could be developed to measure background levels of methoxychlor in the general population as well as levels at which biological effects occur. These data would be valuable in monitoring environmentally exposed populations. Adaptation of methods for measuring pure samples of methoxychlor metabolic products (Lamoureux and Feil 1980; West et al. 1982) to biological samples could provide additional data on exposure to methoxychlor. Since methoxychlor is rapidly metabolized to phenolic derivatives, some of which are estrogenic, additional studies that investigate the applicability or adaptability of methods for methoxychlor to metabolites of methoxychlor in biological media would be useful in developing other biomarkers of exposure.

No data were located concerning methods to measure biological markers of methoxychlor effects. Methoxychlor produces effects on the reproductive system of exposed animals primarily by the estrogenic action of its metabolites (see Chapter 3). In exposed female rodents, methoxychlor exposure may affect the estrus cycle (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1992; Okazaki et al. 2001; Tegeris et al. 1966), serum hormone levels (Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Gray et al. 1988), and the uterus and ovaries (Bal 1984; Chapin et al. 1997; Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Cummings and Perreault 1990; Dikshith et al. 1990; Gray et al.

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1988, 1989; Harris et al. 1974; Martinez and Swartz 1992; Mitsumori et al. 2000; Okazaki et al. 2001; Swartz 1994; Tegeris et al. 1966; Tullner 1961), and may reduce fertility (Bal 1984; Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966). In the female offspring of exposed female rodents, reproductive development, as indicated by age at vaginal opening and first estrus, may be accelerated (Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974). In male rodents, methoxychlor exposure may affect sexual maturity (age of preputial separation) (Gray et al. 1989, 1999), serum hormone levels (Cummings and Gray 1989; Goldman et al. 1986; Gray et al. 1989; Stoker et al. 1999), testes (Bal 1984; Chapin et al. 1997; Dikshith et al. 1990; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962; Wenda-Rozewicka 1983), prostate (Okazaki et al. 2001; Shain et al. 1977; Stoker et al. 1999; Welshons et al. 1999), fertility (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Wenda-Rozewicka 1983), and behavior (Parmigiani et al. 1998; vom Saal et al. 1995). However, these effects are not specific to exposure to methoxychlor, but could occur following exposure to other chemicals with estrogenic activity as well. Since several *in vitro* studies have indicated the presence in human hepatic microsomes of enzymatic activities similar to those in rats that are responsible for the metabolism of methoxychlor to its estrogenic metabolites (Dehal and Kupfer 1994; Stresser and Kupfer 1998), it is likely that similar effects may occur in exposed humans. Research into biomarkers for the estrogenic effects of methoxychlor would be most useful if performed in conjunction with development of sensitive, specific, and reliable methods for measuring these biomarkers.

Methods for Determining Parent Compounds and Degradation Products in Environmental

Media. Methods for detection of methoxychlor in air (EPA 1989b), water (EPA 1990a), soil (EPA 1990b), solid waste (EPA 1990c), and food (Liao et al. 1991) are all based on GC. The media most likely to be of concern for human exposure to methoxychlor are soil and food. Developments in GC analysis for methoxychlor include development of capillary columns, which provide better resolution than packed columns (EPA 1990c; Lopez-Avila et al. 1990; Tessari and Winn 1991), and the use of MS as the primary means of detection, which is much more specific than retention time monitored by ECD or halogen electrolytic conductivity detector, HECD (Liao et al. 1991; Stan 1989). Immunoassays, which have been developed for other pesticides (Stanker et al. 1989), may provide a rapid, inexpensive, and sensitive, method for detecting methoxychlor in environmental samples. Another area of interest is the complete and selective extraction of methoxychlor from complex environmental samples, such as soil and food; supercritical-fluid extraction shows promise in this area (Hopper and King 1991; Lopez-Avila et al. 1990; Walters 1990). Supercritical-fluid extraction and GC/MS, if validated in additional studies, appears to have the sensitivity, specificity, and reliability to measure background levels of methoxychlor in the environment and levels at which health effects occur.

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Methods have been developed to separate methoxychlor-degradation products (West et al. 1982). These methods are based on complimentary chromatographic techniques using normal phase HPLC and GC. High molecular weight impurities found in technical grade and formulated methoxychlor that are difficult to separate by GC are cleanly separated by HPLC. HPLC separation products are then readily analyzed by GC/MS. However, it is not known if this technique can be used reliably for the analysis of environmental samples. Further studies using HPLC GC/MS are needed to determine if the technique could be used in the analysis of air, water, or soil samples contaminated with methoxychlor degradation products.

7.3.2 Ongoing Studies

The application of microwave assisted solvent extraction, coupled with solid phase extraction cleanup to the recovery of methoxychlor from crops (beets, cucumbers, lettuce, peppers, and tomatoes have been studied thus far) shows promise in screening foods for pesticide residues, and pesticide recoveries compare favorably with standard extraction methods (Pylypiw et al. 1997). Ongoing studies to improve methods for analysis of methoxychlor include further research on applications of supercritical-fluid extraction procedures to recover methoxychlor and other compounds from solid matrices (Ashraf-Khorassani et al. 1992; Lopez-Avila et al. 1992). In addition, research to optimize sensitivity, specificity, reproducibility, and analysis time using fused silica capillary columns in a dual-column, dual-detector arrangement, is providing promising results for analysis of food and solid matrices (Hopper 1991; Lopez-Avila et al. 1992).

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

8. REGULATIONS AND ADVISORIES

Because of methoxychlor's potential to cause adverse health effects in exposed people, a number of regulations and guidelines have been established by international, federal, and state agencies. The international, national, and state regulations and guidelines regarding methoxychlor in air, water, and other media are summarized in Table 8-1.

In addition to these values, ATSDR and EPA have established additional guidelines to protect people from the adverse health effects from ingesting methoxychlor. ATSDR has withdrawn the previous MRL of 0.02 mg/kg/day for acute-duration exposure derived in the 1994 profile (see Appendix A for further discussion). An intermediate-duration oral MRL of 0.005 mg/kg/day was derived based on the LOAEL of 5 mg/kg/day for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning (Chapin et al. 1997). This MRL supercedes the previous MRL of 0.02 mg/kg/day for intermediate-duration exposure derived in the 1994 profile. A chronic-duration oral MRL was not derived. A reference dose (RfD) of 0.005 mg/kg/day was derived (in 1991) by EPA based on the NOEL for maternal toxicity in rabbits dosed during gestational days 7-19 (IRIS 2001; Kincaid Enterprises 1986).

IARC (2001) has classified methoxychlor as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) and NCI (1978) concluded that there was insufficient evidence to classify methoxychlor as a carcinogen. Similarly, EPA has classified methoxychlor as a Group D carcinogen, not classifiable as to human carcinogenicity (IRIS 2002).

On January 14, 2000, EPA issued a suspension order to Kincaid Enterprises, Inc. to prevent further manufacture and sale of their methoxychlor products. The order affects the technical product and three products manufactured by Kincaid, but does not directly affect other companies that manufacture methoxychlor products. The order was issued because the registrant failed to submit overdue (per an agreement signed in September of 1998) environmental fate studies. At the time of the writing of this profile, EPA is in the process of issuing a notice of intent to suspend to all companies that use methoxychlor in their products (EPA 2002) (http://www.epa.gov/oppfead1/cb/csb_page/updates/methox.htm).

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Methoxychlor

Agency	Description	Information	Reference
<u>INTERNATIONAL</u>			
Guidelines:			
IARC	Carcinogenicity classification	Group 3 ^a	IARC 2001
<u>NATIONAL</u>			
Regulations and Guidelines:			
a. Air:			
ACGIH	TLV (8-hour TWA)	10 mg/m ³	ACGIH 2001
EPA	RAC	50 µg/m ³	EPA 2001p 40CFR266, Appendix IV
NIOSH	REL	Potential occupational carcinogen	NIOSH 2001
	IDLH	5,000 mg/m ³	
OSHA	PEL (8-hour TWA)—total dust	15 mg/m ³	OSHA 2001b 29CFR1910.1000
	PEL (8-hour TWA) for construction workers—total dust	15 mg/m ³	OSHA 2001c 29CFR1926.55
	PEL (8-hour TWA) for shipyard workers—total dust	15 mg/m ³	OSHA 2001a 29CFR1915.1000
USC	Listed as hazardous air pollutant		USC 2001 42 USC 7412
b. Water			
EPA	Designated as hazardous substance in accordance with Section 311(b)(2)(A) of the Clean Water Act		EPA 2001s 40CFR116.4
	Drinking water standard	0.04 ppm	EPA 2001c 40CFR141.32 (e)(43)
	Groundwater monitoring Suggested method	<u>PQL</u>	EPA 2001d 40CFR264, Appendix IX
	8080	2 µg/L	
	8270	10 µg/L	

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Methoxychlor (continued)

Agency	Description	Information	Reference
<u>NATIONAL</u> (cont.)			
EPA	Health advisories		EPA 2000
	10-kg child		
	1-day	0.05 mg/L	
	10-day	0.05 mg/L	
	DWEL ^b	0.2 mg/L	
	Lifetime	0.04 mg/L	
	Interim primary drinking water standard for owners and operators of hazardous waste TSD facilities—maximum level	0.1 mg/L	EPA 2001g 40CFR265, Appendix III
	Land disposal restrictions; universal treatment standards		EPA 2001h 40CFR268.48
	Wastewater concentration	0.25 mg/L ²	
	Non-wastewater concentration	0.18 mg/kg ²	
	Maximum concentration of constituents for groundwater protection	0.1 mg/L	EPA 2001i 40CFR264.94
	MCL—apply to community water systems and non-transient, non-community water systems	0.04 mg/L	EPA 2001j 40CFR141.61(c)
	MCL—promulgated under the Safe Drinking Water Act	0.1 mg/L	EPA 2001k 40CFR 257, Appendix I
MCLG	0.04 mg/L	EPA 2001m 40CFR141.50(b)	
National recommended water quality criteria		EPA 1999j	
Freshwater	0.03 µg/L		
Saltwater	0.03 µg/L		
Human health for consumption of water and organism	100 µg/L		
Radiation protection—maximum concentration for groundwater protection	0.1 mg/L	EPA 2001o 40CFR192, Table 1 to Subpart A	
Reportable quantity of hazardous substance designated pursuant to Section 311 of the Clean Water Act	1 pound	EPA 2001b 40CFR117.3	

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Table 8-1. Regulations and Guidelines Applicable to Methoxychlor (continued)

Agency	Description	Information	Reference
<u>NATIONAL</u> (cont.)			
c. Food			
EPA	Tolerances for residues	100 ppm	EPA 2001n 40CFR180.120
	Alfalfa, clover, cowpeas, grass for forage, peanut forage, and soybean forage		
	Apples, apricots, asparagus, beans, beets, blackberries, blueberries, boysenberries, broccoli, brussel sprouts, cabbage, carrots, cauliflower, cherries, collards, corn, cranberries, cucumbers, currants, dewberries, eggplants, gooseberries, grapes, kale, kohlrabi, lettuce, loganberries, melons, mushrooms, nectarines, peaches, peanuts, pears, peas, peppers, pineapples, plums, pumpkins, quinces, radishes, raspberries, rutabagas, spinach, squash, strawberries, summer squash, tomatoes, turnips, youngberries	14 ppm	
	Sweet potatoes and yams from preharvest and postharvest application	7 ppm	
	Fat of meat from cattle, goats, hogs, horses, or sheep	3 ppm	
	Barley, corn, oats, rice, rye, sorghum grain, and wheat from storage-bin treatment	2 ppm	
	Milk fat reflecting negligible residues in milk	1.25 ppm	
	Potatoes and horseradish	1 ppm	
FDA	Beverages—bottled water concentration	0.04 mg/L	FDA 2001 21CFR165.110
USDA	Federal seed act regulations—not harmful when present at a rate less than the number of ppm indicated	2 ppm	USDA 2001 7CFR201.31a

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Table 8-1. Regulations and Guidelines Applicable to Methoxychlor (continued)

Agency	Description	Information	Reference
<u>NATIONAL</u> (cont.)			
d. Other			
ACGIH	Carcinogenicity classification	A4 ^c	ACGIH 2001
EPA	Carcinogenicity classification RfD RfC	Group D ^d 5x10 ⁻³ mg/kg/day Not verifiable	IRIS 2001
	Health based limits for exclusion of waste-derived residues—concentration limits for residues	1x10 ⁻¹ mg/kg	EPA 2001e 40CFR266, Appendix VII
	Identification and listing of hazardous waste—hazardous waste number	U247	EPA 2001f 40CFR261.33(e)
	Maximum concentration of contaminants for toxicity characteristic—regulatory level	10 mg/L	EPA 2001j 40CFR261.24
	Organic pesticide active ingredient—pesticide code	34001	EPA 1999i 40CFR455, Subpart E
	Pesticide class	Chlorinated organic pesticide	EPA 2001q 40CFR180.3(e)(4)
	Reportable quantity of hazardous substance designated pursuant to Section 311(b)(4) of the Clean Water Act and Section 112 of the Clean Air Act	1 pound	EPA 2001a 40CFR302.4
	Toxic chemical release reporting; community right-to-know—effective date for reporting	01/01/87	EPA 2001r 40CFR372.65
<u>STATE</u> Regulations and Guidelines:			
a. Air			
Arkansas	RAC	50 µg/m ³	BNA 2001
Idaho	AAC EL OEL	0.5 mg/m ³ 0.667 pounds/hour 10 mg/m ³	ID Dept. of Health and Welfare 1999
Montana	Occupational air contaminant—TLV	15 mg/m ³	BNA 2001

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Table 8-1. Regulations and Guidelines Applicable to Methoxychlor (continued)

Agency	Description	Information	Reference
<i>STATE (cont.)</i>			
New Hampshire	Regulated toxic air pollutant—OEL	10 mg/m ³	BNA 2001
New York	Dangerous air contaminant—TLV	15 mg/m ³	BNA 2001
South Carolina	RAC	50 µg/m ³	BNA 2001
	Toxic air emissions Category	3 ^e	BNA 2001
	Maximum allowable category concentration	50 µg/m ³	
Tennessee	RAC	50 µg/m ³	BNA 2001
Texas	Occupational health—TLV	15 mg/m ³	BNA 2001
Vermont	Hazardous air contaminant		BNA 2001
Washington	Acceptable source impact level, 24-hour average	33 µg/m ³	WA Dept. of Ecology 1998
	Threshold for hazardous air pollutants—threshold level	0.5 tons/year	BNA 2001
Wyoming	RAC	50 µg/m ³	BNA 2001
b. Water			
Alaska	MCL	0.04 mg/L	AK Dept. of Environ. Conserv. 1999
Arizona	Drinking water standard and guideline	340 ug/L	FSTRAC 1999
California	MCL	0.04 mg/L	CA Dept. of Health Services 2000
Colorado	Groundwater organic chemical standard	40 µg/L	CO Dept. of Public Health and Environ. 1999
Georgia	Groundwater criteria concentration	0.04 mg/L	BNA 2001
Hawaii	MCL applying to community and non-community, non-transient water systems	0.04 mg/L	HI Dept. of Health 1999a

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Methoxychlor (continued)

Agency	Description	Information	Reference
<i>STATE (cont.)</i>			
Hawaii	Toxic pollutant standard		HI Dept. of Health 1999b
	Freshwater		
	Acute	No standard	
	Chronic	0.03 µg/L	
	Saltwater		
	Acute	No standard	
Illinois	Chronic	0.03 µg/L	IL Environ. Protection Agency 1999
	Fish Consumption	No standard	
	Water supply standard	0.1 mg/L	
Kansas	Water quality standard		KS Dept. of Health and Environ. 2001
	Aquatic life		
	Acute	Not available	
	Chronic	0.3 µg/L	
	Public health		
Maine	Food procurement	Not available	FSTRAC 1999
	Domestic water supply	40 µg/L	
Missouri	Drinking water standard and guideline	100 µg/L	BNA 2001
	Water quality standards		
	Aquatic life	003 µg/L	
	Drinking water supply	40 µg/L	
New Jersey	Groundwater	40 µg/L	NJ Dept. of Environ. Protection 1993
	Groundwater quality criteria	40 µg/L	
	South Dakota	MCL	
Vermont			SD Dept. of Environ. & Natural Resources 1998
	Groundwater quality standards		
	Enforcement standard	40 µg/L	
Wisconsin	Preventive action limit	4 µg/L	BNA 2001
	Groundwater quality standards		
c. Food	Enforcement standard	40 µg/L	BNA 2001
	Preventive action limit	4 µg/L	
	No data		

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Methoxychlor (continued)

Agency	Description	Information	Reference
<i>STATE (cont.)</i>			
d. Other			
Arizona	Soil remediation level		BNA 2001
	Residential	330 mg/kg	
	Non-residential	3,400 mg/kg	
California	Characteristics of toxicity		BNA 2001
	Regulatory level	10 mg/L	
	STLC	10 mg/L	
	TTLC	100 wet weight mg/kg	

^aGroup 3: unclassifiable as to its carcinogenicity to humans

^bDWEL: A lifetime exposure concentration protective of adverse, non-cancer health effects, that assumes all of the exposure to a contaminant is from drinking water.

^cA4: not classifiable as a human carcinogen

^dGroup D: not classifiable as to human carcinogenicity

^eCategory 3: High toxicity—those pollutants that may cause chronic effects that result in death or permanent injury after very short exposure to small quantities.

AAC = acceptable ambient concentration; ACGIH = American Conference of Governmental Industrial Hygienists; BNA = Bureau of National Affairs; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EL = emissions screening level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FSTRAC = Federal-State Toxicology and Regulatory Alliance Committee; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NIOSH = National Institute for Occupational Safety and Health; OEL = occupational exposure level; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; PQL = practical quantitation limit; RAC = reference air concentration; ppm = parts per million; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STLC = soluble threshold limit concentration; TLV = threshold limit value; TSD = treatment, storage, and disposal; TTLC = total threshold limit concentration; TWA = time-weighted average; USC = United States Code; USDA = U.S. Department of Agriculture

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

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 which 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.

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

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

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

Cross-sectional Study—A type of epidemiological study of a group or groups which 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.

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.

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

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

Immunological Effects—Functional changes in the immune response.

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

In Vivo—Occurring within the living organism.

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

Lethal Concentration₍₅₀₎ (LC₅₀)—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₎ (LD₅₀)—The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time₍₅₀₎ (LT₅₀)—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

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

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

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

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

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

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

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

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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) which 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 odds ratio of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed.

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 science of quantitatively predicting 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 whereby 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 which 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.

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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 $\mu\text{g/L}$ for water, mg/kg/day for food, and $\mu\text{g/m}^3$ for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentrations 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

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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.

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 min continually. No more than four excursions are allowed per day, and there must be at least 60 min 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₎ (TD₅₀)—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 study of the absorption, distribution and elimination of toxic compounds in the living organism.

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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 one can be used; however a reduced UF of three may be used on a case-by-case basis, three being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

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ATSDR MINIMAL RISK LEVEL 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.

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MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

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Rationale Statement (Acute-duration oral MRL)

An acute-duration oral MRL for methoxychlor has not been derived. ATSDR has withdrawn the previous MRL of 0.02 mg/kg/day for acute-duration exposure derived in the 1994 Toxicological Profile for Methoxychlor. This MRL was based on precocious vaginal opening (early puberty) observed in rats exposed to 25 mg/kg/day for 59–104 days (Gray et al., 1989). This study did not test doses as low as the current intermediate-duration MRL study (Chapin et al. 1997), which demonstrated the same effect from 5 mg/kg/day administered from gestation day 14 to postnatal day 42. Thus, there is some question as to whether the premature puberty would have occurred at a lower acute dose than the 25 mg/kg/day observed in the Gray et al. (1989) study.

A variety of candidate MRL studies were considered for derivation of an acute-duration oral MRL for methoxychlor. There were several hypothesis-generating studies at extremely low doses that were not definitive enough to use for MRL derivation. A synopsis of each candidate MRL study and the reasons for not using it follow; these two candidate studies probably bracket the upper and lower bounds of where the true MRL should lie.

Upper Bound

Reference: Gray LE, Otsby J, Ferrell J, et al. 1989. A dose-response analysis of methoxychlor-induced alterations of the reproductive development and function in the rat. *Fund Appl Toxicol* 12:92-109.

Experimental design: In block 2 of this study, groups of eight immature Long-Evans hooded rats of each sex were exposed to either 0, 25, or 50 mg/kg/day technical grade methoxychlor for 59–104 days beginning at 21 days of age by gavage in corn oil. Females were monitored for onset of vaginal opening, onset of estrus, estrus cyclicity, fertility, litter size, number of implantation sites, organ weights, and ovarian and uterine histology. Males were monitored for preputial separation, testis weight, and sperm count.

Effects noted in study and corresponding doses: Female rats exposed to 25 mg/kg/day or more exhibited younger age at vaginal estrus and vaginal opening after 1 week of exposure. Vaginal opening occurred at an average age of 26 days in rats exposed to 25 mg/kg/day, compared with an average vaginal-opening age of 32–33 days in control rats. Atypical vaginal smears (decreased leukocytes, increased cornification) were noted in females exposed to 50 mg/kg/day. This study identifies an acute oral LOAEL of 25 mg/kg/day for reproductive/developmental effects in female rats.

Lower Bound

Reference: Welshons WV, Nagel SC, Thayer KA, et al. 1999. Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. *Toxicol Ind Health* 15:12-25.

Experimental design: Adult female pregnant CF-1 mice were administered 0 (n=9), 0.02 (n=6), or 2.0 (n=5) mg methoxychlor/kg/day in corn oil on gestation days 11–17. Pups were weaned at postpartum day 23 and males were housed together until 8.5 months of age. One male from each litter was housed individually for 4 weeks, then killed, and the prostate, seminal vesicles, preputial glands, liver, and adrenals were removed and weighed (seminal vesicles and preputial glands were blotted to remove fluid before weighing).

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Effects noted in study and corresponding doses: Prostate weight was statistically significantly increased by 61 and 51% in the 0.02 and 2.0 mg/kg/day groups, respectively; seminal vesicle weight was increased by 20% in the 2.0 mg/kg/day group; and liver weight was decreased by 5% in both treatment groups. Although no positive control was done in this study, the methoxychlor-induced prostate enlargement was greater than the enlargement observed in previous studies by the same investigators examining effects from gestational exposure to other estrogen-receptor ligands (estradiol or DES). There were no statistically significant exposure-related changes in body weight or weights of preputial glands, testes, or adrenals. No histological examinations were performed.

Problems with using this study for risk assessment calculations: This is a hypothesis generating study, not a definitive one. There are several areas in which its design is less than ideal. EPA 1998 Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study (EPA 1998) recommends that for a definitive study, each treatment group has 20 pregnant dams yielding offspring; Welshons et al. (1999) only used 5–6 pregnant dams in the treatment groups. These guidelines do not specify how many offspring from each litter should be measured. In good developmental studies, the litter is considered the unit of measurement for statistical calculations (Tyl 2000), but the measurement of the litter response still needs to be accurate. In many thorough developmental studies, such as the Chapin et al. (1997) study used for intermediate MRL derivation, every animal in the litter is assessed and for sex specific end points, each animal of a given sex is measured. The use of only one male from each litter raises questions about the representativeness of the measurement of the litter response; some type of selection bias might have occurred. In an analysis of a similar study in which only one or two animals out of a litter were measured for effects on prostate size, it was found that only measuring one animal per litter resulted in incorrect conclusions 50% of the time, when compared to a study in which all male offspring in each litter were measured (Elwicks et al. 2000a, 2000b; Janszen et al. 2000).

Measuring only one male out of each litter for a characteristic known to vary between litter members may not be a good experimental design strategy. Prostate size in untreated rodents normally varies between males in a litter depending on how they are positioned relative to the females in the litter; males positioned between two females are exposed to more estrogen and consequently have larger prostates than males positioned between two other males (Timms et al. 1999). It is unknown whether treatment with an exogenous estrogen-like compound would decrease the variability of prostate weight within a litter.

As mentioned above, *in utero* exposure to estrogens is one factor that influences prostate weight and some exposure to estrogen may result from the intrauterine position of male fetuses in relation to females. This is a natural consequence of the physiology of multiparous animals. The magnitude of the prostate weight differences resulting from intrauterine position has not been precisely measured; data on this topic would facilitate comparisons with the magnitude of effects produced by methoxychlor. It would be interesting to have data on prostate weights in adult males whose intrauterine position was known via observation after caesarian section delivery. Timms et al. (1999) did measure cross-sectional areas of prostate histology sections, and lengths of prostate buds and estimated prostate volume in rats with a computer model. Data on differences between cross sectional *area* in prostates from males positioned between two other males versus males positioned between two females is presented in Figure 2 of the publication, but no direct comparisons are made of *volume* or weight. It appears from the figure that budding areas of certain parts of the prostate can vary by a factor of about 2-fold as a function of intrauterine position.

Another problem with the Welshons et al. (1999) study is its lack of appropriate positive controls; ideally, one of these would have included various doses of estradiol. Vom Saal et al. (1997) includes data on the prostate weight effects of *in utero* exposure to estradiol continuously delivered from implanted silastic capsules, but this method differs from the once a day dosing of methoxychlor in the Welshons et al. (1999) paper. The prostate effects of the extremely potent synthetic estrogen DES administered by the

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same methods as Welshons et al. (1999) have been reported from experiments done at a different time (vom Saal et al. 1997). Although lower doses of DES produced the same increased prostate weight effect in these experiments as methoxychlor did in the Welshons et al. (1999) study, big differences in the magnitude of the response and less than expected differences in the effectiveness of DES and methoxychlor in producing this response raise some questions about how exactly reproducible the results of this experimental protocol are. As can be seen in the table below, the maximum percent increase in prostate weight produced by methoxychlor is 61.5% while that produced by DES is only 29%. Also, there is only a 100-fold difference between the doses of methoxychlor and DES producing the maximal prostate weight increase; a greater fold difference would have been expected based on the fact that DES is an extremely potent estrogen receptor agonist while methoxychlor is a weak one (Dodge et al. 1996; Kuiper et al. 1998; Ousterhout et al. 1981).

Welshons et al. 1999		vom Saal et al. 1997	
Methoxychlor µg/kg/day	mg prostate weight adjusted by ANCOVA for body weight (percent increase from controls)	DES µg/kg/day	mg prostate weight thought to be adjusted by ANCOVA for body weight (percent increase from controls)
0	40.0 (-)	0	41.5 (-)
20	64.5 (61%)	0.002	40.0 (-4%)
2000	60.3 (51%)	0.02	48.0 (20%)
		0.2	55.0 (38%)
		2.0	49.0 (21%)
		20	47.0 (19%)
		200	32.0 (-20%)

It has been shown that low levels of estradiol (0.32 pg/mL serum, a 50% increase in free-serum estradiol over the endogenous level, released continuously from a silastic implant) and DES (0.02, 0.2, and 2.0 µg/kg/day) administered to pregnant mice during gestation produces increased prostate weight in adult male offspring, while higher and lower exposure levels of estradiol (0.21 and 0.56 pg/mL and above) and DES (0.002 and 200 µg/kg/day) resulted in a decrease in prostate weight (vom Saal et al. 1997). This results in an inverted U-shaped dose-response curve. Gestational exposure to low levels of methoxychlor (0.02–2.0 mg/kg/day) have also been shown to result in increased prostate weight (Welshons et al. 1999), while exposure of weanling to adult rats to high levels (100–1,400 mg/kg/day) of methoxychlor have been shown to result in decreased prostate weight (Shain et al. 1977; Tullner and Edgcomb 1962), as well as testicular atrophy (Bal 1984; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962). Adult mice exposed to 60 mg/kg/day methoxychlor developed testicular degeneration (Wenda-Rozewicka 1983).

Although the Welshons et al. (1999) study is not definitive enough to be the basis of an MRL, the suggestion that gestational exposure to methoxychlor, and other estrogenic compounds, can increase prostate weight at some dose is worthy of further investigation. This issue has been featured prominently in Section 3.12.2, Identification of Data Needs, of this Toxicological Profile.

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A peer review panel was organized by the National Institute of Environmental Health Sciences (NIEHS), National Institute of Health (NIH), National Toxicology Program (NTP) to examine low-dose effects of endocrine disruptors (NTP 2001). The panel examined a number of studies involving estrogen and several estrogenic chemicals (including methoxychlor), androgens, and antiandrogens, as well as biological factors and study design, statistics, and dose-response modeling. The panel's overall conclusions included:

- (1) While low-dose effects have been observed in some laboratory animals with certain endocrine disruptors, they are compound- and end point-specific, and in some cases they have not been replicated in studies by other investigators. Additionally, the toxicological significance of some of the end points is not known.
- (2) The shape of the dose-response curve may be low-dose linear, threshold appearing, or non-monotonic. The curve shape varies with the end point and dosing regimen.
- (3) Previously reported key low-dose findings need to be replicated, and studies are needed to characterize target tissue dosimetry, identify sensitive molecular markers, and determine the long-term health consequences of low-dose effects.
- (4) The current testing paradigm for reproductive and developmental toxicity should be revisited to determine if changes need to be made regarding dose selection, animal model selection, age of animals when evaluated, and end points measured.

Conclusions regarding methoxychlor studies included:

- (1) Methoxychlor is a weakly estrogenic chemical that can induce uterotrophism in immature rodents.
- (2) There is a wide range of changes in estrogen sensitive organs at doses of 5 mg/kg/day and higher.
- (3) Some immune effects, which need to be further evaluated to determine their toxicological significance, were seen following exposure to 1 mg/kg/day.
- (4) More data are needed on the differences in toxicology of technical grade and pure methoxychlor.

Therefore, ATSDR has concluded that without additional data to confirm the causal relationship between exposure to extremely low doses of methoxychlor and increased prostate weight in adult male offspring (and other estrogen-related effects), derivation of an MRL in the nanogram range is not justified. However, the current data do suggest that low-dose effects of methoxychlor may be real, and more definitive studies are necessary to examine them further. An intermediate oral MRL of 0.005 mg/kg/day has been derived based on data that are well supported by the database and based on an end point that is well-established for methoxychlor (accelerated onset of puberty).

A potential acute-duration oral MRL of 0.00002 mg/kg/day can be derived from the LOAEL of 0.02 mg/kg/day in the Welshons et al. (1999) study dividing by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

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Other Studies Considered for Use as the Basis for an Acute Oral MRL:

- (1) A LOAEL of 0.02 mg/kg/day for increased urine-marking behavior in mice when placed in a new territory (vom Saal et al. 1995). Only two male pups per litter were tested and apparently, each was only tested one time; the accuracy of the assessment of the test was somewhat questionable; it is unknown how reproducible the results are, and there was no indication of what, if any, statistical methods were used to evaluate the results.
- (2) A LOAEL of 1.8 mg/kg/day for aggressive behavior (infanticide) in mice toward an unrelated pup (Parmigiani et al. 1998). The strain of mouse used was the “house mouse,” and no information was provided on the specifics of the strain or whether they were inbred; only two male pups per litter were tested; the effect was only seen at one middle dose (no dose-response); and no effect was seen with DES, a positive estrogenic control.
- (3) A LOAEL of 0.02 mg/kg/day for decreased aggression of young male mice toward male siblings at postpartum day 39, but not at postpartum 54 (Palanza et al. 1999). The effect was transient and there was no dose-response (no effect was seen at 200 or 2,000 mg/kg/day).
- (4) A LOAEL of 16.7 mg/kg/day methoxychlor for a 2-fold increase in uterine weight in ovariectomized mice (Tullner 1961). There was little information about the condition of the animals used in the experiments; the estrogenic activity of methoxychlor was discovered serendipitously following the dusting of the mice (being used for an experiment not related to methoxychlor) for parasite control.

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MINIMAL RISK LEVEL WORKSHEET

Chemical Name: Methoxychlor
CAS Number: 77-43-5
Date: September 2002
Profile Status: Final Post Public
Route: Inhalation Oral
Duration: Acute Intermediate Chronic
Graph Key: 33
Species: Mice

Minimal Risk Level: 0.005 mg/kg/day ppm

Reference: Chapin RE, Harris MW, Davis BJ, et al. 1997. The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *Fundam Appl Toxicol* 40:138-157.

Experimental design: Pregnant female rats were administered 0, 5, 50, or 150 mg 95% pure methoxychlor/kg/day from gestation day 14 to postpartum day 7 by gavage in corn oil, then pups were dosed directly in the same manner until postpartum day 42. At birth, pups were weighed, measured for anogenital distance, and checked for external malformations. The first day of vaginal opening or preputial separation was recorded. At about 12 weeks of age, one male and one female from each litter were mated: each treated male to two untreated females and every two treated females to one fertile untreated male. Pups were counted, sexed, and weighed at birth and removed from the dam at postpartum day 11. The dams were mated again and killed at gestation day 19. Uterine contents were analyzed for fetal number, sex, and number of dead implants and resorptions. Blood was drawn from treated males for hematology and clinical chemistry. The gonads and associated reproductive organs were examined for structural malformations, weighed, and fixed for histology. Testicular spermatid head count and epididymide sperm motility and sperm count were performed. Blood was collected from 10 unmated treated females for determination of serum estradiol, progesterone, and follicle stimulating hormone (FSH).

Effects noted in study and corresponding doses: Dams exposed to 150 mg/kg/day methoxychlor had fewer live pups/litter than controls. Precocious vaginal opening was evident (statistically significant) in all methoxychlor-treated groups (postnatal days 37.4, 35.2, 30.8, and 33.4, respectively, for groups 0, 5, 50, and 150 mg/kg/day). Estrus cycles were highly irregular or absent in the 50 and 150 mg/kg/day females. There was a severe reduction in the number of females that conceived in the 50 and 150 mg/kg/day groups (3/15 and 0/15, respectively, compared to 13/15 in the controls). There were no statistically significant effects on litter size or pup mortality or weight gain to postpartum day 10. In the second mating, the 50 mg/kg/day litters had only 32% of the implants of the controls. Empty uterine weight of pregnant females was reduced by 20 and 51% in the 5 and 50 mg/kg/day groups, respectively. Non-pregnant females also showed reduced uterine weight at the high dose. Histologically, uteri from the rats in the 50 mg/kg/day group that did not get pregnant showed mild to severe endometrial squamous metaplasia and endometrial hyperplasia; vaginas of six of the rats were cornified, and one rat had vaginal epithelial hyperplasia. Ovaries in this group were generally polycystic, two females had cystic oviducts, there were few corpora lutea, and mammary tissue was underdeveloped. The effects in the 150 mg/kg/day group were similar.

In the male mating trials, the number of females with vaginal sperm was statistically significantly reduced in the 150 mg/kg/day group. Litter size, postpartum pup death, and pup weight were not affected, and

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there was no increase in resorptions or preimplantation losses. Preputial separation was delayed by 8 and 34 days in the 50 and 150 mg/kg/day groups, respectively. Weights of the testes and right epididymis were significantly reduced in the 50 and 150 mg/kg/day group, and weights of the left cauda epididymis, seminal vesicles, and ventral prostate were significantly reduced at 150 mg/kg/day. Sperm motility and epididymal sperm density were also reduced at 150 mg/kg/day. A dose-related inhibition of testes development was observed. Mild epithelial disorganization was seen in the testes of one rat in each of the 50 and 150 mg/kg/day groups and the testes of one rat in the 150 mg/kg/day group were completely atrophic. No treatment-related changes were noted in clinical chemistries and hematology. The serum estrogen:progesterone ratio was significantly elevated in the 50 and 150 mg/kg/day groups, and the FSH levels were significantly suppressed in all methoxychlor-treated rats verified to be in estrous.

Dose and end point used for MRL derivation: 5 mg/kg/day from gestation day 14 to postpartum day 42, accelerated onset of puberty

[] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

- [X] 10 for use of a LOAEL
- [X] 10 for extrapolation from animals to humans
- [X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose?

If so, explain: None needed.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:

N/A

Other additional studies or pertinent information which lend support to this MRL: The MRL is supported by other observations of reproductive effects associated with intermediate-duration exposure including elevated levels of prolactin in the pituitary of male rats exposed to 50 mg/kg/day (Goldman et al. 1986; Gray et al. 1989), decreased seminal vesicle weight, caudal epididymal weight, and caudal epididymal sperm count (Gray et al. 1989), and increased gonadotropin releasing hormone in the mediobasal hypothalamus in male rats exposed to 50 mg/kg/day (Goldman et al. 1986). At higher exposures levels, intermediate-duration studies show decreased fertility in male rats at doses of 60–400 mg/kg/day (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974), and in female rats at doses of 50–150 mg/kg/day (Bal 1984; Chapin et al. 1997). A study in humans described by Stein (1968), Coulston and Serrone (1969), and Wills (1969) that identified a NOAEL of 2 mg/kg/day for effects to the testes and menstrual cycle was not chosen as the basis for an MRL because reproductive function was not evaluated, and such an MRL may not be protective of reproductive and developmental effects in the fetus or child.

Comparison with acute study on which EPA RfD was based.

An RfD of 0.005 mg/kg/day has been derived by EPA (IRIS 2002; RfD derived 1991) based on a NOEL of 5.01 mg/kg/day administered on gestation days 7–19 in New Zealand White rabbits for maternal toxicity observed as excessive loss of litters (Kincaid Enterprises 1986) and an uncertainty factor of 1,000 (10 for interspecies differences; 10 for intraspecies differences; and 10 for the poor quality of the critical study and for the incompleteness of the database on chronic toxicity).

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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 they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, 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.

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Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "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 the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive 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 NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

Chapter 3**Health Effects****Tables and Figures for Levels of Significant Exposure (LSE)**

Tables (3-1, 3-2, and 3-3) and figures (3-1 and 3-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an 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 No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

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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 LSE Table 3-1**

- (1) **Route of Exposure** One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 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 2 "18r" data points in 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 regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.
- (7) **System** This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

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- (8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").
- (9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific 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 Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.
- (12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND**See Figure 3-1**

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

- (13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.
- (14) Health Effect These are the categories of health effects for which reliable quantitative data 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/m³ or ppm and oral exposure is reported in mg/kg/day.
- (16) NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

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- (17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.
- (18) 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

Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation

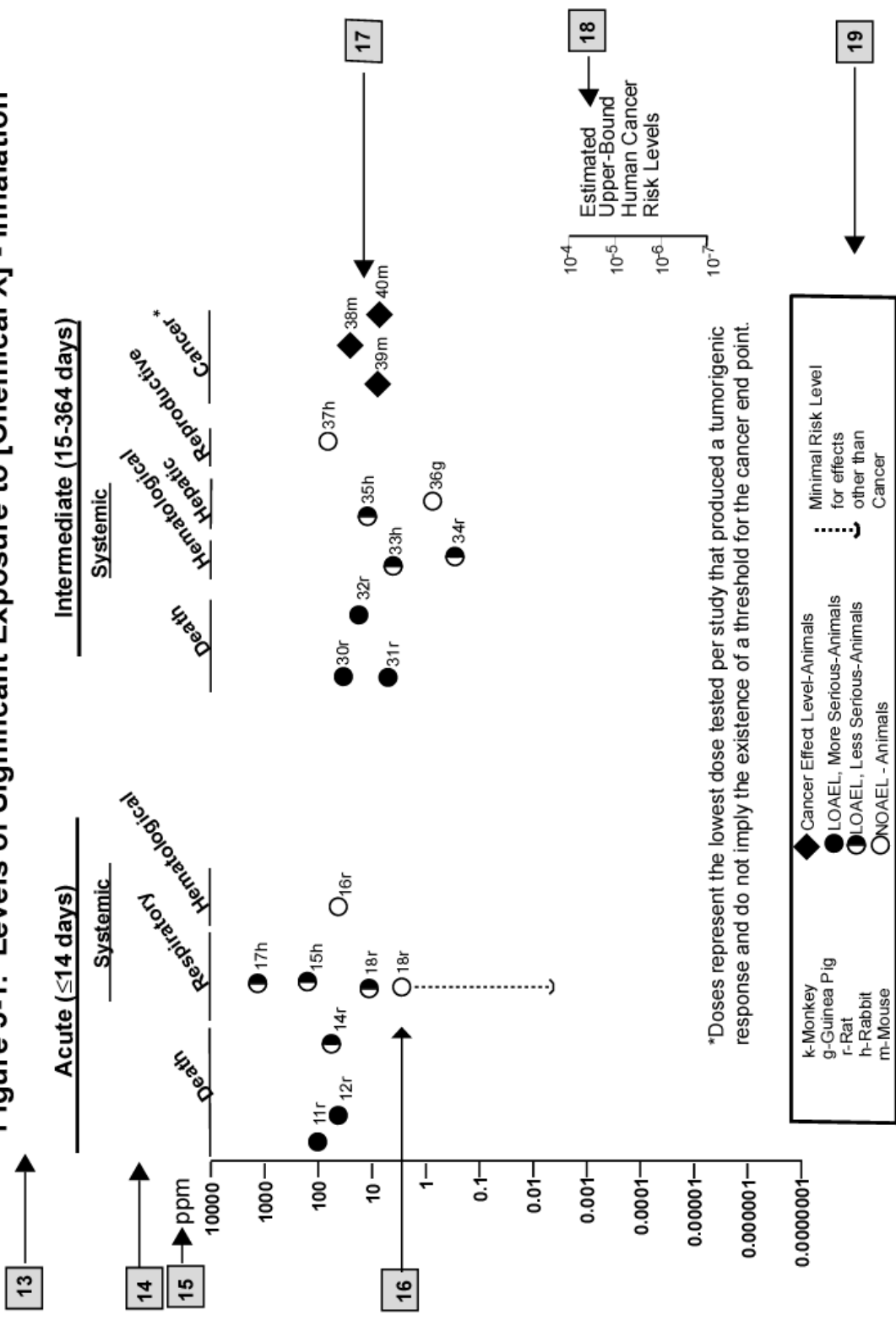
Key to figure ^a	Species	Exposure frequency/duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
INTERMEDIATE EXPOSURE							
1	5	6	7	8	9	10	10
2	9	9	9	9	9	9	9
3	9	9	9	9	9	9	9
4	Rat	13 wk 5 d/wk 6 hr/d	Resp	3 ^b	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
6	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

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

^b Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

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DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/International 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	<i>Federal Register</i>
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
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
K _d	adsorption ratio
kg	kilogram
K _{oc}	organic carbon partition coefficient
K _{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC _{Lo}	lethal concentration, low
LC ₅₀	lethal concentration, 50% kill
LD _{Lo}	lethal dose, low
LD ₅₀	lethal dose, 50% kill
LDH	lactic dehydrogenase
LH	luteinizing hormone
LT ₅₀	lethal time, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	<i>trans,trans</i> -muconic acid
MAL	maximum allowable level
mCi	millicurie

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MCL	maximum contaminant level
MCLG	maximum contaminant level goal
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
OW	Office of Water

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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

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\$	greater than or equal to
=	equal to
<	less than
#	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
-	negative
+	positive
(+)	weakly positive result
(-)	weakly negative result

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