TOXICOLOGICAL PROFILE FOR SULFUR MUSTARD (UPDATE)

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

September 2003

DISCLAIMER

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

UPDATE STATEMENT

A Toxicological Profile for Sulfur Mustard (previously Mustard Gas), Draft for Public Comment was released in September 2001. This edition supersedes any previously released draft or final profile.

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

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE, Mailstop E-29
Atlanta, GA 30333

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:

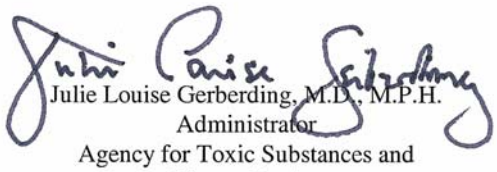
(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(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.



Julie Louise Gerberding, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on October 25, 2001 (66 FR 54014). For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17,1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); and October 21, 1999 (64 FR 56792). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

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

Primary Chapters/Sections of Interest

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

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

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

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

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

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

Other Sections of Interest:

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

ATSDR Information Center

Phone: 1-888-42-ATSDR or (404) 498-0110 ***Fax:*** (404) 498-0093
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.

CONTRIBUTORS

CHEMICAL MANAGER(S)/AUTHOR(S):

Zemoria A. Rosemond, B.A.
ATSDR, Division of Toxicology, Atlanta, GA

Dolores A. Beblo, Ph.D.
Syracuse Research Corporation, North Syracuse, NY

Richard Amata, Ph.D.
Syracuse Research Corporation, North Syracuse, NY

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 sulfur mustard. The panel consisted of the following members:

1. Dr. Vincent Garry, M.D. D.A.B.T., Director, Laboratory of Environmental Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota;

2. Dr. Shane Que Hee, Ph.D., Professor, Center for Occupational and Environmental Health, UCLA School of Public Health, Los Angeles, California; and

3. Dr. James Withey, Ph.D., Retired Senior Research Scientist, Environmental Health Science Center, Ontario, Canada, Ottawa, Ontario.

These experts collectively have knowledge of sulfur mustard'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 sulfur mustard 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. Sulfur mustard has been found in at least 3 of the 1,636 current or former NPL sites. However, the total number of NPL sites evaluated for this substance is not known. As more sites are evaluated, the sites at which sulfur mustard is found may increase. This information is important because exposure to this substance may harm you and because these sites may be sources of exposure.

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

If you are exposed to sulfur mustard, 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 SULFUR MUSTARD?

Sulfur mustard is a thick liquid, which was made for use as a chemical weapon. Presently, the chemical is found at a few Army facilities in large quantities and at several locations in smaller quantities. It is often called by its common name, ‘mustard gas.’ However, the term ‘mustard gas’ can be confusing, since it is stored as a liquid and is not likely to change into a vapor immediately if it is released at ordinary temperatures. As a liquid, it is colorless when pure and it is brown when mixed with other chemicals. It is odorless when pure, but can have a slight garlic

smell when mixed with other chemicals. It dissolves easily in fats, oils, alcohol, and gasoline. Sulfur mustard dissolves slowly in unstirred water, but within minutes in stirred water. When it does dissolve, it reacts with water and turns into different chemicals. It was used in chemical warfare as early as World War I and as late as the Iran-Iraq War in 1980–1988. It is not used in the United States, except for laboratory testing of health effects and antidotes. More information on the physical and chemical properties of sulfur mustard can be found in Chapters 4 and 5. Information about mustard agents other than sulfur mustard, such as nitrogen mustard, thickened mustard, and lewisite, is not included in this document.

1.2 WHAT HAPPENS TO SULFUR MUSTARD WHEN IT ENTERS THE ENVIRONMENT?

Sulfur mustard is not found naturally in the environment in any amount. If sulfur mustard is accidentally spilled at an Army base where it is stored, it could be released into the environment. Currently, all of the sulfur mustard at these Army bases is being destroyed by burning or neutralization. U.S. law requires that the Department of Defense destroy all sulfur mustard by 2004. However, complete destruction of sulfur mustard may continue beyond this date. Once all of the sulfur mustard is destroyed, it will no longer be dangerous. If sulfur mustard is put on soil, it will remain there for at least a day, but may remain for several days or longer. The time it takes for sulfur mustard to disappear from soil depends on how hot it is outside and how strongly the wind is blowing. If it is hot and the wind is strong, then sulfur mustard will disappear faster. When sulfur mustard disappears from soil, it becomes a vapor or changes into other compounds if the soil is wet. If sulfur mustard is buried underground, it may not disappear for several years. Sulfur mustard will not move through soil to underground water. If sulfur mustard is put in water, it dissolves within minutes if the water is stirred, and slowly if is not. When it does dissolve, it reacts with water and changes to other compounds. The time necessary for a quantity of sulfur mustard that is dissolved in water to decrease by half is about 2 minutes at 40 °C (104 °F). If large amounts of sulfur mustard are spilled into water, most of the sulfur mustard will change to other compounds very slowly or not at all. If sulfur mustard is released into air, it will react with components in the air to form other compounds. The time necessary for a quantity of sulfur mustard in air to decrease by half is about 2 days at 25 °C (77 °F). Because

sulfur mustard changes to other chemicals in the environment, it will not concentrate in plants or animals. For more information on what happens when sulfur mustard enters the environment, see Chapter 6.

1.3 HOW MIGHT I BE EXPOSED TO SULFUR MUSTARD?

Sulfur mustard is not currently being produced in the United States. The general public might be exposed through accidental release from the Army bases where it is stored. These storage areas are heavily guarded, and storage buildings are sealed. People who work at Army bases that store sulfur mustard are more likely to be exposed than the general public. However, the Army has taken many precautions to protect the public from exposure to sulfur mustard. The general public may be exposed to sulfur mustard at hazardous waste sites that contain sulfur mustard. In addition, the use of sulfur mustard by terrorists is of concern. Persons involved in the transport or disposal of sulfur mustard may be exposed to mustard agents generated unintentionally through mishap. Spouses, children, and others may be exposed if workers unknowingly bring the mustard agents out of the factory on their skin or clothing. Sulfur mustard readily passes through ordinary clothing. Mixed in water, sulfur mustard changes its form within minutes, so it is very unlikely that you would drink it. The likelihood of the general population being exposed by way of water (drinking, cooking, bathing, and swimming) is therefore very small. Sulfur mustard does not occur naturally; therefore, there are no background levels in the soil, air, water, or food. If it is accidentally released, it will stay in the air or on the ground for 1–3 days. Under certain conditions, it may remain on the ground or in water for long periods. For more information on possible exposures, see Chapter 6.

1.4 HOW CAN SULFUR MUSTARD ENTER AND LEAVE MY BODY?

Sulfur mustard can enter your body easily and quickly if it gets in your eyes or on your skin, or if you breathe sulfur mustard vapors. It can easily pass through your clothing to get onto your skin. It is possible that you could come into contact with sulfur mustard at hazardous waste sites that contain this material. Sulfur mustard changes into other chemicals in your body, and these

chemicals mostly leave your body in the urine within a few weeks. For more information, see Chapter 3.

1.5 HOW CAN SULFUR MUSTARD AFFECT MY HEALTH?

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

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

Sulfur mustard can harm you depending on how much of the chemical you were exposed to and for how long. Sulfur mustard may make your eyes burn, your eyelids swell, or cause you to blink a lot. Sulfur mustard may burn your skin and cause skin blisters within a few days. Your eyes and the parts of your body that are sweaty are the most likely to be harmed. If you breathe it, sulfur mustard can cause coughing, bronchitis, and long-term respiratory disease. Sulfur mustard may affect reproduction. Some men exposed to sulfur mustard during war have reported decreased sexual drive and have had problems with sexual function due to scarring of genital tissues and lower sperm counts. The International Agency for Research on Cancer has determined that sulfur mustard is carcinogenic to humans. The Department of Health and Human Services has also determined that sulfur mustard is a known carcinogen. It can cause cancer in your airways, lungs, skin, and maybe other areas of your body later in life. If you are exposed to a very large amount of sulfur mustard, you can eventually die from it. Some of the chemicals that are formed when sulfur mustard is burned or spilled into water can also be irritating to the skin.

1.6 HOW CAN SULFUR MUSTARD AFFECT CHILDREN?

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

Sulfur mustard causes the eyes and skin of children to burn similarly to adults; however, the burns may be more severe in children. Blisters may appear sooner in children than adults, as early as 4 hours after sulfur mustard contacts the skin. Coughing and vomiting have been reported as early symptoms of exposure to sulfur mustard in children. Sulfur mustard vapors are heavier than air and since young children are closer to the ground or floor because of their height, they may be exposed to more sulfur mustard vapors than adults during accidental exposures. Sulfur mustard may cause birth defects or affect the development of children. An increased incidence of birth defects has been reported among newborn babies of sulfur mustard victims exposed during war. Studies in animals also indicate that sulfur mustard may affect development. It is not known if sulfur mustard can cross the placenta or be passed to infants in breast milk.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO SULFUR MUSTARD?

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

The risk of exposure to sulfur mustard to the general public may be slightly greater for those who live or work near Army bases and other facilities that store it. However, the Army has instituted precautions to protect the public from exposure to sulfur mustard. Sulfur mustard is currently being destroyed at these facilities, and thus the risk of exposure due to accidents is decreasing.

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

Sulfur mustard or some of the chemicals that it makes in your body can be found by testing your urine or blood. However, a test for sulfur mustard exposure is not readily available at local physicians' offices or hospitals. A urine or blood sample may be sent to a special laboratory for testing. For further assistance, see Section 1.10.

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 sulfur mustard include the following:

The federal government considers sulfur mustard an extremely hazardous substance. In 1985, Congress directed the U.S. Army to begin destroying the stockpile of U.S. chemical agents

including sulfur mustard. As a result, the U.S. Army's Chemical Stockpile Disposal Program (CSDP) was started. As part of this program, the U.S. Army has continued to study how workers and the general public might best be protected from harm by sulfur mustard. The U.S. Army is the primary source of safety recommendations for sulfur mustard. The federal government has recommended maximum concentrations in air to which the general public should be exposed for different lengths of time. The maximum concentration for long-term exposure is 0.0001 milligrams per cubic meter of air. Higher concentrations may be tolerated for shorter periods. Stored quantities of 500 pounds or more must be reported to the State Emergency Response Commission, the fire department, and the Local Emergency Planning Committee. Spills of over 1 pound must be reported to the National Response Center. For more information, see Chapter 8.

The National Advisory Committee has developed acute exposure guideline levels (AEGLs) to protect people from the harmful effects of a short-term (8 hours or less) exposure to sulfur mustard. Three types of AEGLs have been developed: AEGL-1, AEGL-2, and AEGL-3. For sulfur mustard, the AEGL-1 values range from 0.40 mg/m^3 for a 10-minute exposure to 0.008 mg/m^3 for an 8-hour exposure; exposure to higher concentrations may result in eye irritation. The AEGL-2 values range from 0.60 mg/m^3 for 10 minutes to 0.013 mg/m^3 for 8 hours; exposure to higher concentrations may result in swelling of the eyes, sensitivity to light, and eye irritation. The AEGL-3 values range from 3.9 mg/m^3 for 10 minutes to 0.27 mg/m^3 for 8 hours; exposure to higher concentrations may result in death. For more information on the AEGLs, see Appendix D.

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: 1-404-498-0093

As part of the Homeland Defense Program, the U.S. Army Soldier and Biological Chemical Command (SBCCOM) has a mission to enhance the response capabilities of military, federal, state, and local emergency responders to incidents involving biological and chemical agents, including sulfur mustard. For more information, you may go to the SBCCOM Web site: http://hld.sbccom.army.mil/ip/bca_qr.htm.

The following agencies may also be contacted for assistance regarding sulfur mustard exposure:

National Response Center: 800-424-8802
U.S. Public Health Service: 800-USA-NDMS
Chemical Transportation Emergency Center: 800-424-9300

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: (800) 553-6847 or (703) 605-6000
Web Site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO SULFUR MUSTARD IN THE UNITED STATES

Sulfur mustard (bis[2-chloroethyl]sulfide; $C_4H_8Cl_2S$; CASRN: 505-60-2) or as it is commonly called, 'mustard gas', is one of a class of vesicant chemical warfare agents with the ability to form vesicles or blisters on exposed skin. Sulfur mustard is a viscous liquid at ambient temperature, but becomes a solid at 58 °F (14 °C). It is heavier than water as a liquid and heavier than air as a vapor. Sulfur mustard is a component of the H-series blister agents including undistilled sulfur mustard (H; sulfur mustard with 20–30% impurities, also known as Levinstein mustard), distilled sulfur mustard (HD or HS; approximately 96% pure), a mustard-lewisite mixture (HL), an HD/agent T mixture (HT; a mixture of HD and nonvolatile agent T), and an HD/agent Q mixture (HQ; a mixture of HD and nonvolatile agent Q; agent Q is also known as sesqui-mustard).

Sulfur mustard was first manufactured in 1822. It was utilized as early as the late 1880s, when it was used as a pesticide and to treat minor tumors. It was first used as a war gas in 1917, during World War I by the Germans on the British at Ypres. For this reason, sulfur mustard is also called yperite. Sulfur mustard was used in the Iran-Iraq War of 1980–1988, and there are reports of sulfur mustard being utilized in other conflicts. The production of sulfur mustard in the United States was discontinued in 1968. Sulfur mustard does not naturally occur, and, therefore, there are no background levels in the soil, air, water, or food. The major possibilities of exposure of the general public are through accidental release from the Army bases where it is stored. Occupational exposures in the United States are expected when handling, disposing and treating hazardous wastes containing sulfur mustard. It is also possible that workers involved in plastics manufacturing may be exposed to mustard agents inadvertently, resulting from process contamination with sulfur or nitrogen impurities, as occurred in a vinyl chloride monomer manufacturing facility in Plaquemine, Louisiana in 1996. If sulfur mustard was released into the air, the primary routes of exposure would be contact with eyes and skin or inhalation. Children are expected to be exposed to sulfur mustard by the same routes as adults.

The U.S. stockpile of sulfur mustard is currently stored at seven sites in the continental United States, and formerly at one site located on Johnston Island in the Pacific Ocean. All of these locations are heavily

guarded, and storage buildings are sealed. Sulfur mustard may also be found in non-stockpile locations at current and former military base sites and testing sites across the United States. Non-stockpile sites include locations that may contain buried chemical munitions or contaminated sites formerly used in production and storage of sulfur mustard. The Army has taken many precautions to protect the public from exposure to sulfur mustard. People who work at Army bases that store sulfur mustard are more likely to be exposed than the general public. Currently, all of the sulfur mustard at these Army bases will be destroyed by either incineration or neutralization. U.S. law requires that the Department of Defense destroy all sulfur mustard by 2004. However, complete destruction of the entire stockpile of sulfur mustard may continue beyond this date. The United States also ratified the international Chemical Weapons Convention treaty, according to which, the United States must destroy its stockpile of mustard agents by April 2007. Sulfur mustard has been found in at least 3 of the 1,636 current or former NPL sites, the EPA's listing of the most serious hazardous waste sites in the nation. At hazardous waste sites, exposure to sulfur mustard is also possible by dermal contact with contaminated soil or containers.

The National Advisory Committee for the Acute Exposure Guideline Levels for Hazardous Substances has developed acute exposure guideline levels (AEGLs) for sulfur mustard. The AEGLs are threshold exposure limit values for the general public applicable to emergency exposure periods ranging from 10 minutes to 8 hours. For each chemical, three levels of AEGLs, distinguished by varying degrees of severity of toxic effects, are developed: at exposure levels above the AEGL-1, the general population could experience notable discomfort, irritation, or asymptomatic, nonsensory effects; above AEGL-2, the general population could experience irreversible or other serious, long-lasting health effects or impaired ability to escape; and above AEGL-3, the general population could experience life-threatening health effects or death. At each AEGL level, values are developed for five exposure periods: 10 minutes, 30 minutes, 1 hour, 4 hours, and 8 hours. The AEGLs for sulfur mustard are summarized below. For sulfur mustard, the AEGL-1 values are based on human data for conjunctivitis; the AEGL-2 values are based on human data for conjunctivitis, edema, photophobia, and eye irritation, and the AEGL-3 values are for lethality in mice. For a more detailed description of the derivation of the sulfur mustard AEGLs, see Appendix D.

Acute Exposure Guideline Level (AEGL) Values for Sulfur Mustard (mg/m^3)

AEGL Level	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-1	0.40 mg/m^3	0.13 mg/m^3	0.067 mg/m^3	0.017 mg/m^3	0.008 mg/m^3
AEGL-2	0.60 mg/m^3	0.20 mg/m^3	0.10 mg/m^3	0.025 mg/m^3	0.013 mg/m^3
AEGL-3	3.9 mg/m^3	2.7 mg/m^3	2.1 mg/m^3	0.53 mg/m^3	0.27 mg/m^3

Information about mustard agents other than sulfur mustard, such as nitrogen mustard and thickened mustard is not included in this document (see Chapter 4).

2.2 SUMMARY OF HEALTH EFFECTS

Sulfur mustard or other chemical warfare agents containing sulfur mustard are no longer produced or used commercially in the United States and general population exposures are expected to be low. People whose work is connected with chemical weapons or who work at military sites where these compounds are stored have the potential of being exposed. The primary adverse health effects of sulfur mustard exposure are ocular, respiratory and dermal direct contact effects, reproductive effects and cancer following inhalation, oral, and dermal exposure. Numerous reports of battlefield exposures provide strong evidence of the toxic potential of sulfur mustard; however, combat sulfur mustard exposure levels have not been quantified, and blast effects may be present concurrently. Additional information on the acute health effects of sulfur mustard is available from studies of sulfur mustard testing of volunteer subjects. Clinical studies of mustard agent filling plant workers provide evidence of health effects following chronic exposure to sulfur mustard; however, these studies are complicated by possible concurrent exposure to other toxic agents because factories generally produced multiple chemical warfare agents. Some evidence has also surfaced regarding delayed toxic effects several years after acute sulfur mustard exposure during battlefield operations or occupational exposure. The following symptoms have appeared from 2 months to several years after exposure to sulfur mustard: cough, chest pain, shortness of breath, fatigue, wheezing, insomnia, fever, relapsing keratitis (inflammation of the cornea), marked sensitivity to pulmonary irritants, and increased susceptibility to respiratory infections. Animal studies have shown that sulfur mustard induces similar toxic effects in animals and humans, with the exception of blistering of animals that have fur.

Ocular Effects. The eye is one of the organs that is most sensitive to the acute effects of sulfur mustard vapor. Studies with volunteer soldiers wearing respirators indicated conjunctivitis (inflammation of the conjunctiva), manifested as early as 30 minutes after exposure, as the first sign of exposure to sulfur mustard. The ocular effects are due to direct contact of sulfur mustard with the corneal/ conjunctival epithelium. This is supported by experiments in animals that have shown little involvement of the eye when sulfur mustard was administered parenterally at dose levels known to be systemically toxic and lethal. The severity of ocular injury is a function of dose/concentration, duration, and

temperature. Respiratory tract and skin irritations have occurred at about the same threshold vapor concentration, but the latency periods were generally longer (≥12 hours). Other signs and symptoms of acute exposure include ocular irritation, redness, lacrimation, burning pain, swelling of the eyelids, photophobia (sensitivity of the eyes to light), blepharospasm (spasm of eyelid muscle), and corneal damage. When the severity of the injury was such that corneal damage occurred, necrotic ulcers, with or without bacterial infection, have developed. It has been reported that normal corneal epithelial regeneration occurred rapidly if the underlying stroma was intact, but if damaged, regeneration could be incomplete with continued erosion and neovascularization. A range of ocular effects, including conjunctivitis, chronic keratitis, and corneal ulcerations, have been reported in dogs and rabbits following acute exposure to sulfur mustard depending on the concentration and duration of exposures.

Follow-up studies of battlefield exposures and long-term animal studies indicate that delayed or recurrent keratitis and/or ulceration of the cornea may result from severe burns. A sudden increase in the number of veterans with these signs of disease has been observed 8–25 years after the initial sulfur mustard injury. Long-term studies examining delayed ocular effects in rabbits acutely exposed to sulfur mustard showed that, similar to the human condition, migration of fatty and/or cholesterin deposits to the surface of the eye occurred 7–8 months after the initial injury, causing secondary ulceration. Exposure of the eye to liquid droplets of sulfur mustard can result in severe corneal damage, with possible perforation of the cornea and loss of the eye.

There are no rigorous human studies evaluating the occurrence of ocular sensitivity to sulfur mustard. From early chamber tests that indicated conjunctivitis as the initial sign of toxicity, conducted with three groups of men, those having no previous exposure, those who were exposed to “very low”, but unspecified, concentrations of sulfur mustard through their work, but who experienced no symptoms or burns, and those with unspecified occupational exposure who experienced one or more burns at various times, one investigator concluded that the toxicity of sulfur mustard did not appear to increase with previous exposure. However, details upon which this conclusion was based were lacking. Animal data suggest that ocular sensitization occurs following exposures to levels in the air that produce severe effects. While quantitative exposure data are not available, conjunctivitis, altered corneal pigmentation, photophobia, lacrimation, impaired vision, and blepharospasm have been reported in studies of workers at sulfur mustard research laboratories and manufacturing plants with longer-than-acute (>14 days) exposure durations. However, these studies are limited by possible exposures to multiple toxic chemicals, confounding factors of age and smoking history, and comparisons to controls. Chronic keratitis has been observed in dogs and rats exposed to sulfur mustard vapor for ≥7.5 months.

The Agency for Toxic Substances and Disease Registry (ATSDR) has derived acute and intermediate-duration inhalation Minimum Risk Levels (MRLs) based on ocular effects (see Appendix A). The National Advisory Committee for Acute Exposure Guideline Levels (AEGLs) for Hazardous Substances has established AEGLs and the Army has established an air exposure limit for the general population for chronic exposures (GPL) based on ocular effects (see Chapter 8).

Respiratory Effects. Early respiratory effects of sulfur mustard exposure include hoarseness, sore throat, a burning sensation of the vocal cords, shortness of breath, and hemorrhagic inflammation of the tracheobronchial mucosa accompanied by severe erosions or membranous lesions. In children, cough was the first respiratory symptom. Erosions of the airway mucosa have also been reported in animals. Respiratory infections are often a secondary complication following sulfur-mustard-induced injury, and pulmonary edema and bronchopneumonia may develop. Acute exposures to sulfur mustard have resulted in long-term damage manifested as asthma-like conditions, emphysematous bronchitis, and increases in incidence of secondary respiratory infections (bronchopneumonia and tuberculosis). Epidemiological studies of World War I victims exposed to sulfur mustard revealed an association between acute respiratory exposure and the risk of developing respiratory tract cancer. Prolonged inhalation exposure, as experienced by workers exposed to factory ambient levels of sulfur mustard for a number of years, can also result in these same conditions and/or cancer. Several studies of workers occupationally exposed to sulfur mustard have revealed elevated risks of respiratory tract tumors after long-term exposure.

Dermal and Other Direct Contact Effects. Data from soldiers and civilians exposed during combat, mustard agent factory workers, sulfur mustard testing volunteers, and people who were accidentally exposed to sulfur mustard provide ample evidence of the toxic potential of sulfur mustard to tissues coming into direct contact with sulfur mustard. Sulfur mustard exposure results in burning of the skin, which begins several hours after exposure. The severity of cutaneous injury is a function of dose, duration, temperature, humidity, and/or perspiration and is directly related to the sulfur mustard alkylation levels in skin. It is likely that direct contact with other tissues would have these same dependencies. Stomach irritation and inflammation and bleeding of the gastric mucosa were reported in victims of combat exposure where at least small amounts were likely ingested. Similar effects have been observed in animal studies. Occupational dermal exposure to sulfur mustard has produced abnormal skin pigmentation and Bowen's disease (precancerous dermatitis) in humans. There is also some evidence that former sulfur mustard factory workers may have an increased risk of developing digestive tract and skin tumors.

ATSDR has derived an intermediate-duration oral MRL based on gastrointestinal effects (see Appendix A). The U.S. Army has derived an oral reference dose (RfD) based on gastrointestinal effects (see Chapter 8).

Reproductive Effects. Reduced sperm counts were reported in a follow-up study of men who were exposed to sulfur mustard during the Iran-Iraq War. An increased rate of fetal deaths and an altered sex ratio were reported in progenies of Iranian survivors of chemical attacks that included sulfur mustard. It is also important to note that reproductive success can be adversely affected by impaired sexual function caused by scarring of genital tissues resulting subsequent to blistering from direct contact with sulfur mustard. While the routes of exposure differ, animal reproductive toxicity data support the long-term effects reported in humans. Increases of early fetal resorptions and preimplantation losses and decreases in live embryo implants were observed in male dominant studies in which male rats, orally administered sulfur mustard, were mated with untreated females. An increase in the percentage of abnormal sperm was also detected in these treated rats. The reproductive effects appear to be male dominant as no female dominant lethal effects have been observed. The timing of the dominant lethal effects is consistent with an effect during the post-meiotic stages of spermatogenesis, possibly involving the generally sensitive spermatids. An altered sex ratio and a decrease in growth rate during nursing were observed in the offspring of parental rats that had been orally exposed to sulfur mustard during fetal and neonatal development, as well as premating, mating, and gestation. Sulfur mustard has also induced dominant and sex-linked recessive lethal mutations in *Drosophila*.

Cancer. There is sufficient evidence that sulfur mustard is carcinogenic to humans. Epidemiological studies of World War I victims exposed to sulfur mustard revealed an association between respiratory exposure and the risk of developing respiratory tract cancer. Factory workers exposed to sulfur mustard for a number of years have been shown to develop respiratory cancer. Although most human studies have found an association between sulfur mustard exposure and respiratory cancer, some studies have not found a significant relationship, possibly due to lower exposure levels. It is also documented that occupational dermal exposure to sulfur mustard produces Bowen's disease (precancerous dermatitis) in humans. There is some evidence that former sulfur mustard factory workers may have an increased risk of developing digestive tract and skin tumors. Two animal studies, of low predictive quality due to species strain tendency to develop lung tumors, insufficient animals, and inadequate doses, have also shown increases in tumors from exposure to sulfur mustard in the air. Subcutaneous, intramuscular, and intravenous injections of sulfur mustard into mice have also produced increased tumors at the site of the

injection, in the mammary glands, or in the lungs. Sulfur mustard has been shown to induce a wide variety of genetic mutations in many types of mammalian cells *in vitro* in a dose-related fashion. Sulfur mustard has also induced genetic damage *in vivo* in peripheral blood lymphocytes from exposed individuals at low doses. This is not unexpected considering sulfur mustard is a bi-functional alkylating agent that can cross-link DNA strands.

IARC has classified sulfur mustard as "carcinogenic to humans" (Group 1) based on sufficient evidence in humans, limited evidence in experimental animals, supporting evidence that sulfur mustard is a bi-functional alkylating agent, and positive results in a number of assays for genotoxic effects.

The Army's current health-based environmental screening levels (HBESLs) for sulfur mustard include an oral cancer potency value (slope factor), a cancer inhalation unit risk value, and an inhalation cancer potency value. However, ongoing evaluations of alternative approaches for quantitatively estimating cancer risk may result in changes to these values (see Chapter 8).

2.3 MINIMAL RISK LEVELS

Inhalation MRLs

- An MRL of 0.0007 mg/m^3 has been derived for acute-duration inhalation exposure (14 days or less) to sulfur mustard.

The acute-duration inhalation MRL was based on a concentration of sulfur mustard vapors of 0.06 mg/m^3 at which minimal ocular effects occurred in men who underwent a 3-day chamber test with sulfur mustard (Guild et al. 1941). The corresponding time-weighted average (TWA) concentration of 0.02 mg/m^3 was considered a minimal lowest-observed-adverse-effect level (LOAEL) for the MRL derivation. An uncertainty factor of 30 (3 for use of a minimal LOAEL and 10 for human variability) was applied to derive the MRL. Male soldiers wearing respirators (2–6 men/group) were exposed to sulfur mustard vapor concentrations ranging from 0.06 to 320 mg/m^3. Continuous exposure durations ranged from 15 seconds to 10 hours, yielding concentration time (Ct) products in the range of 42–144 mg-minute/m^3. Two repeated-exposure tests were also conducted; a group of four men was exposed to 0.22 mg/m^3, 2.5 hours/day for 2 days, and another group of four men was exposed to 0.06 mg/m^3, 8 hours/day, for 3 days (intermittent Cts of 66 and 86 mg-minute/m^3, respectively). At the lowest continuous Ct of 42 mg-minute/m^3 (1.4 mg/m^3 for 30 minutes), four of four soldiers showed a slight generalized conjunctival

reaction. Slight to moderate degree of conjunctival congestion was reported for the Ct range of 80–90 mg-minute/m^3. Photophobia occurred at Cts ≥99 mg-minute/m^3. A scarcely discernable generalized conjunctival reaction (incidence unspecified) was reported in subjects undergoing the 3-day repeated exposure. The severity of conjunctivitis for the 3-day intermittent exposure was described as far slighter than the moderate degree of conjunctivitis observed from continuous exposures with Cts ≥80 mg-minute/m^3.

Support for ocular effects as a critical detection of effect end point comes from other chamber tests with human subjects (Anderson 1942; Reed 1918) and numerous reports of eye injuries in humans following combat exposure to sulfur mustard (Hughes 1942; Pechura and Rall, 1993; Philips 1940). A range of ocular effects, including conjunctivitis, chronic keratitis, and corneal ulcerations, has been reported in dogs and rabbits following acute exposure to sulfur mustard depending on the concentration and duration of exposures (Balali-Mood 1986; Gates and Moore 1946; Laughlin 1944a; Maumenee and Scholts 1948; Reed 1918; Warthin and Weller 1919).

- An MRL of 0.00002 mg/m^3 has been derived for intermediate-duration inhalation exposure (15–364 days) to sulfur mustard.

The intermediate-duration inhalation MRL was based on a no-observed-adverse-effect level (NOAEL) of 0.001 mg/m^3 for ocular effects (conjunctivitis and chronic keratitis) in dogs (McNamara et al. 1975). Male and female beagle dogs were exposed to sulfur mustard vapor at a concentration of 0.001 mg/m^3 for 24 hours/day, or to 0.1 mg/m^3 for 6.5 hours followed by exposure to 0.0025 mg/m^3 for the remaining 17.5 hours of the day, 5 days/week (TWAs of 0.0007 and 0.0206 mg/m^3, respectively), for durations up to 1 year. No dogs died during the study. No clinical signs of toxicity were observed in the dogs at the low concentration. Ocular effects at the high concentration first appeared after 16 weeks of exposure and included corneal opacity, pannus, chronic keratitis, vascularization, pigmentation, and granulation. The TWA concentration of 0.0007 mg/m^3 was used in the MRL derivation.

In addition to the supporting information for ocular lesions as provided for the acute-duration MRL, there are numerous reports of eye burns in workers accidentally exposed to large quantities of sulfur mustard vapor, as well as to slow leaks that were not detected by smell (Pechura and Rall 1993; Uhde and Dunphy 1946).

A chronic-duration (365 days or more) inhalation MRL for sulfur mustard was not derived because quantitative data were not available to determine NOAELs or LOAELs. However, using a different

derivation procedure than that used for chronic-duration inhalation MRLs, the Army has established an air exposure limit for the general population for chronic exposures (GPL) of 0.00002 mg/m^3 as a 24-hour time-weighted average, 7 days/week (USACHPPM 2000a). The key critical effect chosen for the GPL was ocular effects, as the data indicate the eyes to yield the most sensitive response to vapor exposures of sulfur mustard. A previously established GPL of 0.0001 mg/m^3 for sulfur mustard was promulgated by the Centers for Disease Control and Prevention (CDC) in 1988 (DHHS 1988). The Army has also derived an inhalation reference dose (RfD; an estimate of a daily ingestion exposure level for the human population, including sensitive subpopulations, that is likely to be without appreciable risk of deleterious noncancer health effects during a lifetime) of 0.00003 mg/kg/day from this GPL by assuming a human inhalation rate of 20 m^3/day and a body weight of 70 kg (USACHPPM 1999).

Oral MRLs

- An MRL of 0.0005 mg/kg/day (0.5 µg/kg/day) has been derived for acute-duration oral exposure (14 days or less) to sulfur mustard.

The acute-duration oral MRL was based on a LOAEL of 0.5 mg/kg/day for inflamed mesenteric lymph nodes in rat dams administered sulfur mustard in sesame oil by gavage (DOA 1987). The dose is also a LOAEL for reduced ossification in the fetuses. An uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) was applied to the LOAEL to derive the MRL. In the teratology study that formed the basis for the acute-duration oral MRL, there were no treatment-related deaths in groups of 25–27 mated Sprague-Dawley female rats (10–11 weeks old) that were dosed acutely on gestation days 6–15 (10 days) with 0, 0.5, 1.0, or 2.0 mg/kg/day sulfur mustard (95.9–96.1% purity) dissolved in sesame oil. Significant incidences of inflamed mesenteric lymph nodes in dams and reduced ossification in fetuses occurred with sulfur mustard doses ≥0.5 mg/kg/day.

There is some evidence for sulfur mustard-induced lymph system effects in humans. Lymph node discoloration and spleen pathology were found in autopsies of sulfur mustard victims (Alexander 1947). Additional animal studies also indicate sulfur mustard-induced damage to the lymph system (Cameron et al. 1946; Coutelier et al. 1991; Venkateswaran et al. 1994a). In other oral studies in animals in which sulfur mustard dissolved in sesame oil was administered by gavage, increased incidence of inflamed mesenteric lymph nodes occurred in rats at ≥0.4 mg/kg/day in dose-range experiments (DOA 1987) and another lymphoretic effect, enlarged Peyer's patches, was observed in rabbits at 0.5 mg/kg/day in a range-

finding study and at 0.4 mg/kg/day in the subsequent full-scale teratology study (incidence data not reported) (DOA 1987).

- An MRL of 0.00007 mg/kg/day (0.07 µg/kg/day) has been derived for intermediate-duration oral exposure (15–364 days) to sulfur mustard.

The intermediate-duration oral MRL was based on a LOAEL of 0.03 mg/kg/day for forestomach epithelium lesions in rats administered sulfur mustard in sesame oil by gavage in a 2-generation reproduction study (Sasser et al. 1996a). In that study, groups of 8-week-old Sprague-Dawley rats (27 females and 20 males/group/generation) were dosed with 0, 0.03, 0.1, or 0.4 mg/kg/day sulfur mustard (97.3% purity) dissolved in sesame oil. Male and female rats were dosed 5 days/week for 13 weeks before mating and during a 2-week mating period. Females were dosed daily (7 days/week) throughout the 21-day gestation period and 4–5 days/week during the 21-day lactation period. Males were dosed 5 days/week during the 21-day gestation period and sacrificed at the birth of their pups. Dams were sacrificed when their pups were weaned. Male and female F1 pups were treated with sulfur mustard until they were mated and the females became pregnant and gave birth. The dosing of F1 dams continued until pup weaning, at which time, the study was terminated. Significant dose-related severity and incidences of forestomach squamous epithelium lesions occurred in both sexes with sulfur mustard doses ≥0.03 mg/kg/day. This LOAEL corresponds to a TWA LOAEL of 0.02 mg/kg/day, which was used for MRL derivation. An uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) was applied to the TWA LOAEL to derive the MRL.

In support of the forestomach epithelial lesions as the key critical effect for the intermediate-duration MRL, gastrointestinal effects (stomach irritation and inflammation, hyperemia, epithelial loss, necrosis, ulceration, vomiting, nausea, bleeding, anorexia, and abdominal pain) have been reported in humans following combat exposure to sulfur mustard, in sulfur mustard testing volunteers, and in sulfur mustard factory workers (Alexander 1947; Momeni and Aminjavaheri 1994; Pierard et al. 1990; Sinclair 1948; Yamakido et al. 1985). Gastrointestinal effects (edema, hemorrhage or sloughing of the mucosa, and ulceration) were also observed in rabbits following 14-day exposures at ≥0.4 mg/kg/day (DOA 1987), in rats following 10-day exposures at ≥2.0 mg/kg/day (DOA 1987), and in rats following 13-week exposures at ≥0.1 mg/kg/day (Sasser et al. 1996b).

A chronic-duration (365 days or more) oral MRL for sulfur mustard was not derived because a chronic bioassay was not located. However, using a derivation procedure different than that used for chronic-duration oral MRLs, the Army has derived an oral RfD of 0.007 µg/kg/day (Oak Ridge National

Laboratory 1996; Opresko et al. 1998, 2001; USACHPPM 1999, 2000b) that has also been approved by the National Research Council (NRC 1999b).

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 sulfur mustard. 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.

Sulfur mustard [bis(2-chloroethyl)sulfide; $C_4H_8Cl_2S$; CASRN: 505-60-2] or as it is commonly called, 'mustard gas', is one of a class of vesicant chemical warfare agents with the ability to form vesicles or blisters on exposed skin. Sulfur mustard is a component of the H-series blister agents including undistilled sulfur mustard (H; sulfur mustard with 20–30% impurities, also known as Levinstein mustard), distilled sulfur mustard (HD or HS; approximately 96% pure), a mustard-lewisite mixture (HL), an HD/agent T mixture (HT; a mixture of HD and nonvolatile agent T), and an HD/agent Q mixture (HQ; a mixture of HD and nonvolatile agent Q; agent Q is also known as sesqui-mustard) (Gates and Moore 1946). Mustard agents, including sulfur mustard, are regulated under the Chemical Weapons Convention (CWC) (USCWCR1999). Three classes of chemicals are monitored under the CWC (1993), with sulfur mustard grouped in the highest risk class, "Schedule 1." Information about mustard agents other than sulfur mustard, such as nitrogen mustards, thickened mustard, or the mixtures listed above are not discussed in this document.

Sulfur mustard is actually a clear, colorless, oily liquid. As a warfare or terrorist agent, sulfur mustard has been dispersed by spraying or by explosive blasts producing a vapor, aerosol, and/or liquid droplets, earning the chemical the name 'mustard gas.' Persons involved in the transport or disposal of sulfur mustard may be exposed occupationally. It is also possible that workers involved in plastics manufacturing may be exposed to mustard agents inadvertently, resulting from process contamination with sulfur or nitrogen impurities, as occurred in a vinyl chloride monomer manufacturing facility in Plaquemine, Louisiana in 1996 (Johnson 1998). Spouses, children, and others may be exposed if workers

unknowingly bring the mustard agents out of the factory on their skin or clothing. Both liquid and vapor forms readily penetrate ordinary clothing.

Sulfur mustard is slightly soluble in water, but both the liquid and vapor forms are readily soluble in alcohol, gasoline, kerosene, oils, fats, and organic solvents. Sulfur mustard is environmentally persistent. Evaporation in air increases with increasing temperatures, but at temperatures below 58 °F (14 °C), it freezes while retaining its vesicant properties. Both liquid and vapor forms readily penetrate ordinary clothing. The effects of sulfur mustard poisoning may be local, systemic, or both, depending on environmental conditions, exposed organs, and extent and duration of exposure. Because of the high lipid solubility, sulfur mustard quickly penetrates the lipid cell membrane. Although sulfur mustard may be lethal, it is more likely to cause extensive incapacitating injuries to the eyes, skin, and respiratory tract of exposed persons. Alkylation reactions (replacement of a hydrogen atom in an organic compound by an alkyl group [C_nH_{2n+1}]) of sulfur mustard with tissue are rapid and irreversible; however, there is a latency period before effects become apparent. Eye and cutaneous lesions do not become apparent for 30 minutes to several hours after exposure. Burns caused by sulfur mustard may require long healing periods. Local effects are manifested at concentrations/doses far lower than those that produce systemic effects (NRC 1997).

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.

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

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs.

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.

There is a considerable amount of information regarding the effects of exposure to sulfur mustard in humans and in animals dating from over a century ago. A substantial amount of information is derived from the use of sulfur mustard as a chemical weapon or from research related to this use and the original documents are not readily available. However, there are numerous reviews of the literature that include very early data as well as more recent information. The information in this Toxicological Profile is based both on reviews from the literature and original studies.

3.2.1 Inhalation Exposure

While sulfur mustard (mustard gas) is described as smelling like mustard, horseradish, garlic, or onions, it can be difficult to smell and may not be recognized by the general population. Olfactory fatigue, resulting in discontinued ability to detect the sulfur mustard odor, occurred within 3–8 minutes of initial exposure in subjects participating in sulfur mustard chamber tests (Reed 1918). Due to the delayed symptoms and difficulties associated with detection by smell, individuals may not know that they are being exposed, and consequently, appropriate actions may not be taken. Inhalation exposure to sulfur mustard can result in local, followed by systemic, effects and death depending on concentration, duration, temperature, humidity, and/or perspiration. Because of sulfur mustard's ability to penetrate cell membranes rapidly, injury resulting from inhalation exposure is characterized initially by local effects on the epithelial tissues through which it is absorbed (Papirmeister et al. 1991). In environmental exposures to sulfur mustard, the most sensitive target tissues are primarily the eyes, skin, and respiratory tract (Papirmeister et al. 1991; Reed 1918). Although the local effects of sulfur mustard on these tissues are often of most immediate concern, only a small portion of the dose that penetrates the tissue may induce these. The remainder of the absorbed dose passes into the circulation, is distributed throughout the body, and may result in systemic effects (Papirmeister et al. 1991). Local effects are manifested at concentrations/doses far lower than those that produce systemic effects.

3.2.1.1 Death

Human deaths associated with sulfur mustard exposure occurred during World War I (Prentiss 1937; Pechura and Rall 1993) and during the Iran-Iraq War in 1980–1988 (D'Halluin and Roels 1984; Eisenmenger et al. 1991; Mandl and Frielinger 1984; Momeni et al. 1992); however, no exposure doses for any of these wartime cases are available. During chemical warfare, exposure to sulfur mustard generally occurred by multiple routes. In the case of exposure by multiple routes, it is often difficult to

determine the relative importance of local and systemic effects in causing death. Heavy and painful coughing, vomiting, burning eyes, and shock often closely preceded death. Deaths have occurred immediately following exposure in the battlefield, most likely due to acute chemical-induced pulmonary edema (Freitag et al. 1991). Deaths, which occurred in 1–3% of the soldiers exposed during World War I, were largely due to secondary respiratory infections (Uhrig 1962). Battlefield air concentrations of sulfur mustard vapor during attacks in World War I were estimated in the range 19–33 mg/m^3 (Solberg et al. 1997). While sulfur mustard was not used during World War II, German planes bombed cargo vessels in the Italian port of Bari carrying sulfur mustard and explosive munitions. In the resulting explosion, sulfur mustard was released into the air and water, exposing survivors to sulfur mustard vapor and to a mixture of sulfur mustard in oil. Sulfur mustard caused death within a few hours of exposure by inducing shock in victims of the Bari Harbor incident and in civilians who accidentally recovered unspent World War I sulfur mustard shells (Alexander 1947; Papirmeister et al. 1991). Deaths beyond the second day after the Bari Harbor incident were attributed to decreased leukocyte counts, which reached levels below 100 cell/cm^3 (Dacre and Goldman 1996). Accidental death of a family of two adults and two children occurred in 1919 in Salaise, France after exposure to sulfur mustard, which evaporated from a leaking can of sulfur mustard-contaminated alcohol that was being stored in the house (Dacre and Goldman 1996).

One death among 14 children (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) admitted to a hospital in Iran 18–24 hours following exposure to sulfur mustard from air bombs during the Iran-Iraq War was reported (Momeni and Aminjavaheri 1994). The 13-month-old girl developed pancytopenia and respiratory failure, and died 8 days after exposure. Deaths have also occurred from delayed responses (DOA 1988; Somani and Babu 1989). Further information on delayed death due to inhalation of sulfur mustard by humans is discussed below in the sections on respiratory effects in Section 3.2.1.2 and on cancer in Section 3.2.1.7.

As summarized by NRC (1997), the Army's Chemical Defense Equipment Process Action Team (CDEPAT) estimated a lethal concentration-time product (LCt_{50}) for humans of 900 mg-minute/m^3 for 2–10-minute exposures. In the absence of better data, the CDEPAT derived this value by averaging toxicity data from several animal species.

Rabbits and monkeys that had undergone tracheal cannulation were exposed to nominal chamber concentrations of sulfur mustard ranging from 30 to 350 mg/m^3 (5–54 ppm) for 10 minutes (Cameron et al. 1946). While incidence data were not provided, Cameron et al. (1946) reported that sulfur mustard vapor produced lethal effects in rabbits and monkeys in the absence of lung damage, indicating that lethal

doses may be absorbed through the mucous membrane of the nose. No deaths attributable to sulfur mustard were noted in mice, rats, guinea pigs, rabbits, or dogs exposed to 0.1 mg/m^3 (0.015 ppm) of sulfur mustard vapor, 6.5 hours/day, 5 days/week, for up to 1 year (McNamara et al. 1975).

Gates and Moore (1946) reported undistilled sulfur mustard (agent H) median LCt_{50} for several different animal species exposed whole-body to sulfur mustard for a 10-minute exposure: dog (600 mg-minute/m^3); cat (700 mg-minute/m^3); monkey (800 mg-minute/m^3); rat (800 mg-minute/m^3); rabbit (900 mg-minute/m^3); mouse (1,200 mg-minute/m^3); guinea pig (1,700 mg-minute/m^3); and goat (1,900 mg-minute/m^3).

3.2.1.2 Systemic Effects

The highest NOAEL and all LOAEL values for each study for systemic effects in each species are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. There is extensive evidence in humans that the respiratory tract is one of the primary targets of sulfur mustard toxicity following inhalation exposure. Respiratory effects have occurred in humans following acute and/or chronic exposures to sulfur mustard. In general, warm environmental conditions increased the severity of the respiratory effects of sulfur mustard. Reviews of the literature (Papirmeister et al 1991; Pechura and Rall 1993; Watson and Griffin 1992) indicate that symptoms of exposure are not immediate, but develop over a period of hours to days. Hoarseness and irritation of the nasal mucosa may develop 12 hours to 2 days after exposure to 12–70 mg-minute/m^3; recovery may occur after approximately 2 weeks. Pulmonary effects are evident after exposure to 100–500 mg-minute/m^3. Exposure to 200 mg-minute/m^3 causes sneezing and lacrimation, rhinorrhea, sore throat, and nosebleed; recovery may occur after approximately 2 weeks following exposure. Exposure to ≥1,000 mg-minute/m^3 may result in injuries progressing to edema in the pharynx and tracheobronchial tree, followed by death due to severe edema, secondary infection, or necrotic bronchopneumonia. There is evidence that pulmonary injury is the leading cause of mortality in the first few days to weeks after sufficiently high concentrations of sulfur mustard (Case and Lea 1955; Hosseini et al. 1989; Papirmeister et al. 1991; Pechura and Rall 1993; Willems 1989).

In a clinical study of soldiers exposed to sulfur mustard during the Iran-Iraq War, Momeni et al. (1992) reported respiratory effects in 15% of 535 patients (95% male; 3% children) examined. Respiratory

Table 3-1 Levels of Significant Exposure to Sulfur Mustard - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Human	33 min	Ocular		1.7 M (Injection band over sclera)		Anderson 1942
2	Human	8 h/d, 3 d	Ocular		0.06 M[b] (Slight generalized conjunctival reaction)		Guild et al. 1941
3	Human	15 min	Ocular		0.1 M (Conjunctival injection)		Reed 1918
4	Human	10 min	Ocular	0.1 M			Reed 1918
5	Mouse (albino)	1 h	Renal		21.3 F (Increased blood and urine uric acid levels)		Kumar and Vijayaraghavan 1998
6	Mouse (albino)	1 h	Resp		84.6 F (Decreased lung/Bd Wt ratio)		Pant and Vijayaraghavan 1999
			Bd Wt		84.6 F (14% reduction)		
7	Mouse (albino)	1 h	Resp	16.9 F	21.3 F (Decreased respiratory frequency)		Vijayaraghavan 1997
8	Gn Pig Not reported	10 min	Bd Wt		125 (14% reduction)		Allon et al. 1993
	Immuno/ Lymphoret						
9	Mouse (albino)	1 h			84.6 F (Decreased spleen/Bd Wt ratio)		Pant and Vijayaraghavan 1999

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

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Systemic						
10	Dog (Beagle)	6.5 h/d, 5 d/wk	Ocular		0.1 (Conjunctivitis and chronic keratitis)		McNamara et al. 1975
11	Dog (Beagle)	24 h/d, 5 d/wk	Ocular	0.001[c]			McNamara et al. 1975

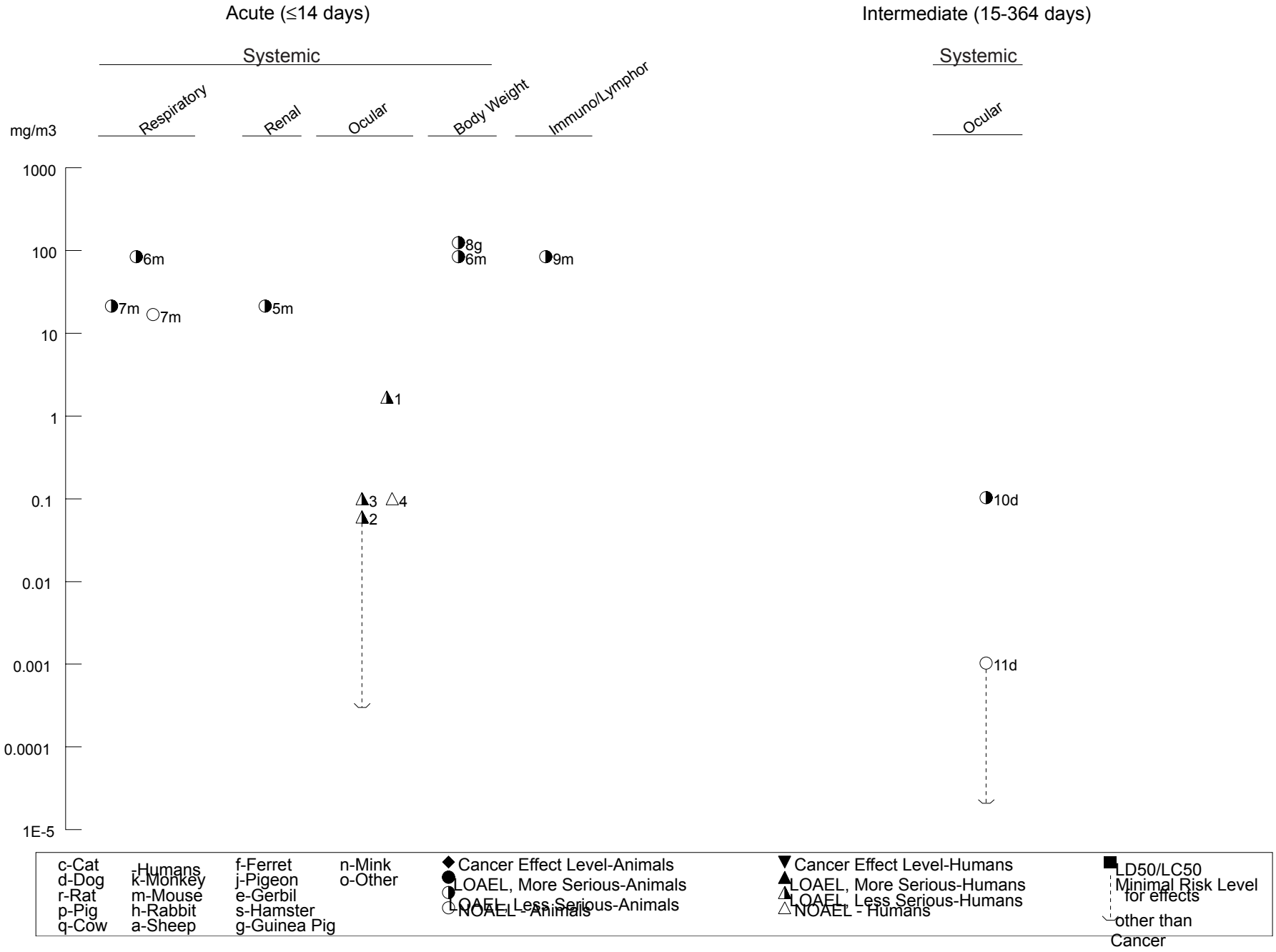
a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation MRL of 0.0007 mg/m3; concentration adjusted to a TWA of 0.02 mg/m3 for intermittent exposure (see Appendix A) divided by an uncertainty factory of 30 (3 for use of a minimal LOAEL and 10 for human variability).

c Used to derive an intermediate-duration inhalation MRL of 0.00002 mg/m3; concentration adjusted to a TWA of 0.0007 mg/m3 for intermittent exposure (see Appendix A) divided by an uncertainty factory of 30 (3 for extrapolation from animals to humans and 10 for human variability).

Bd Wt = body weight; d = day(s); F = female; H = hour(s); M = male; Min = minute(s); Resp = respiratory; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Sulfur Mustard - Inhalation



symptoms included those pertaining to the upper respiratory tract such as burning sensation in the mouth, pharyngodysphonia (difficulty in speaking due to disorder of the pharynx), and cough. Some exposed soldiers became temporarily aphonic due to an acid-like burning sensation of the vocal cords. Lower respiratory tract symptoms, such as shortness of breath and tachypnea, were reported less frequently. In children exposed to sulfur mustard during the Iran-Iraq War, cough was the first respiratory symptom; in a cohort of 14 children and teenagers examined 18–24 hours following exposure, cough developed in 11 children (79%)(Momeni and Aminjavaheri 1994). Other respiratory effects included crepitation (57%), dyspnea (57%), wheezing (36%), and sore throat (14%). Secondary complications consisted of extensive stenosis of sections or the entire tracheobronchial tree, suppurative bronchitis, and chronic respiratory infections with *Staphylococcus aureus*, *Hemophilus influenzae*, and *Pseudomonas aeruginosa* resistant to appropriate antibiotic therapy. Scars, ulcers, strictures, and nonspecific fibrous granulation developed in central airways after a delay up to 15 months. Progressive deterioration of lung compliance and gas exchange with resulting hypoxemia and hypercapnia, were common with injury. Momeni and Aminjavaheri (1994) reported that children had higher occurrences and earlier onset of pulmonary symptoms than adults.

The incidence of respiratory sequelae has been studied in subjects exposed to sulfur mustard in the battlefield, workers, and volunteers exposed under controlled conditions. In a study of 197 veterans admitted to the hospital in 1986 due to acute respiratory symptoms, exposed to sulfur mustard 10 years earlier asthma was newly diagnosed in 21 (10.7%), chronic bronchitis in 116 (58.9%), bronchiectasis in 17 (8.6%), airway narrowing due to scarring or granulation tissue in 19 (9.6%), and pulmonary fibrosis in 24 (12.2%)(Emad and Rezaian 1997). None of these were found in a control group of 84 subjects. A significant positive correlation was reported between the age of the subject and the severity of asthma, but not with the severity of pulmonary fibrosis. There was a significant correlation between age and incidence, but not the severity, of chronic bronchitis. There was a significant correlation between the severity of pulmonary fibrosis with the spirometry measurement of carbon monoxide diffusion capacity, but not the other physiological parameters of forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1). Also, British soldiers exposed to sulfur mustard during combat in World War I had a significantly higher incidence of death due to bronchitis than the general population (Case and Lea 1955).

Workers who were apparently exposed to sulfur mustard for a few years (exact quantity and duration not reported) also developed acute and chronic respiratory effects. Workers in a Japanese poison gas factory were more likely to have chronic bronchitis, chronic cough, and decreased respiratory volume than non-exposed persons (Nishimoto et al. 1970). Manning et al. (1981) reported a significantly increased

incidence of mortality from pneumonia among 428 former workers of a sulfur mustard manufacturing facility. Factory workers in Britain who were exposed to sulfur mustard also showed increased deaths due to acute and chronic nonmalignant respiratory disease, including influenza and pneumonia (Easton et al. 1988).

A retrospective mortality study of 1,545 white male Navy recruits who were exposed to >120–960 mg-minute/L of sulfur mustard under controlled conditions at a single site between 1944 and 1945 found no excess of any cause specific mortality associated with exposure to sulfur mustard relative to a control group of 2,663 white male Navy veterans who served at the same location and time as the exposed group, but did not participate in sulfur mustard chamber tests (Bullman and Kang 2000). Causes of death investigated included laryngeal, lung, and skin cancers, chronic obstructive pulmonary and parenchymal respiratory diseases, external causes, and suicide. The veterans who participated in the sulfur mustard chamber tests, while exposed to lower levels than estimated for combat-exposed World War I veterans, did have sufficient exposure to produce skin reactions of erythema and edema.

Respiratory effects similar to those described in humans have been reported in experimental animals. Information summarized by Pechura and Rall (1993) indicate that inhalation exposure of rabbits to sulfur mustard produced concentration-related damage particularly prominent in the upper respiratory tract, including nasal passages, pharynx, larynx, trachea, and large bronchi. Low levels of exposure caused congestion of these areas without hemorrhage. An Army report noted that dogs exposed to unspecified levels of sulfur mustard developed irregular respiration 8 hours after exposure (Winternitz and Finney 1920). Animals that died 1–3 days after exposure displayed destruction of the epithelial lining, the presence of pseudomembrane, and leukocytic infiltration in the trachea and bronchi. Evidence of necrotizing bronchopneunomia was present in dogs that died 2–10 days after exposure. In dogs that recovered and were killed 1–5 weeks later, there were ulcerations or constrictions of the trachea, but chronic changes in the lung were infrequent.

More recent information is available from studies in mice. A study by Vijayaraghavan (1997) showed that a single 1-hour exposure, head-only, to 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 sulfur mustard produced sensory irritation and, 15–20 minutes after the start, decreased respiratory frequency. characterized by a pause between inspiration and expiration. The respiratory frequency decreased approximately 20% at 8.5 mg/m^3 and a maximum of 64% at concentrations ≥42.3 mg/m^3. The concentration that depressed 50% of the respiratory frequency (RD_{50}) was calculated as 27.4 mg/m^3. Normal respiration pattern was recovered after inhalation exposure was terminated. While sensory

irritation was reversible, delayed effects of sulfur mustard were indicated by a significant reduction in respiratory frequency beginning 48 hours after exposure at concentrations of ≥21.3 mg/m^3. The depression in respiratory frequency following exposure was related to both concentration and postexposure time. Airflow limitation was evidenced by a lengthening of expiration time and a decreased respiratory rate and is thought to occur due to the effect of sulfur mustard on the tracheal secretory cells. Reversible respiratory effects were also observed in similar experiments in mice by Rao et al. (1999) (10.6–42.3 mg/m^3) and by Pant and Vijayaraghavan (1999) (84.6 mg/m^3). Pant and Vijayaraghavan (1999) measured a significant 13% reduction in lung-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour.

Guinea pigs were exposed by inhalation to 1,200–1,900 μg-minute/L of sulfur mustard for 10 minutes (120–190 mg/m^3) (Allon et al. 1993). A decrease in respiratory rate and minute volume, and an increase in tidal volume occurred immediately after the onset of exposure and lasted for up to 7 days after exposure. The changes in respiratory parameters were accompanied by a significant reduction in oxygen diffusion capacity in the lung.

These reports indicate similar respiratory effects of sulfur mustard in several animal species (rabbits, dogs, mice, and guinea pigs) and humans, which suggests that knowledge obtained regarding respiratory effects in animal models can be usefully applied to humans.

Cardiovascular Effects. In 12 of 53 (23%) autopsies of Bari Harbor victims, small sub-epithelial hemorrhages were noted in the hearts, but in all instances, the parietal pericardium showed no pathology (Alexander 1947). There was a slight increase in the pericardial fluid having normal color in four cases (8%). In 18 cases, the myocardium was described as pale and lacking normal firmness.

Studies of 65 sulfur mustard casualties of the Iran-Iraq War treated in European hospitals did not indicate any heart abnormalities (Willems 1989). However, mild tachycardia without fever was reported in a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) who were examined in a hospital in Iran 18–24 hours following exposure to sulfur mustard from air bombs during the Iran-Iraq War (incidence not reported) (Momeni and Aminjavaheri 1994). However, the tachycardia may have been due to stress caused by the bombing episode. In a 1996 follow-up study of Iran-Iraq War veterans, 10 years after hospital admission in 1986 due to acute respiratory symptoms with confirmed sulfur mustard exposure, only 3/212 (1.4%) had cardiovascular disease, which was not

confirmed attributable to exposure (Emad and Rezaian 1997) (see study description under Respiratory Effects).

Gastrointestinal Effects. Victims of the World War II Bari Harbor incident suffered local lesions of the oropharynx and upper portion of the esophagus (Alexander 1947). In a few cases, there was intense congestion of the first inch of the esophagus, which may or may not have been due to the blast. In 19 of 53 (36%) cases autopsied, stomach irritation and inflammation were documented. The lesions varied from simple hyperemia to focal loss of epithelium, necrosis, and ulceration. Some lesions were located near the cardiac end, but most were in the region of the pylorus. In some cases, the hyperemia extended into the duodenum, and in one case, congestion of the jejunum was noted (Alexander 1947). In a review of the clinical manifestations of sulfur mustard exposure in Iran-Iraq War victims, Pierard et al. (1990) reported that endoscopy frequently revealed acute gastritis. Gastrointestinal effects of nausea and vomiting were reported in 10% of 535 patients (95% male; 3% children) exposed to sulfur mustard during the Iran-Iraq War (Momeni et al. 1992). Gastrointestinal symptoms were more frequent in children and teenagers, compared to adults; incidences of gastrointestinal effects of nausea (9 patients, 64%), vomiting (6 patients, 43%), and bleeding (2 patients, 14%) were reported in a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) who were admitted to a hospital in Iran 18–24 hours following exposure to sulfur mustard from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Gastrointestinal neoplasms were reported in Japanese sulfur mustard factory workers who were involved with the production of chemical agents during World War II (Yamakido et al. 1985).

Gastrointestinal effects were not reported in rats, mice, rabbits, guinea pigs, and dogs exposed continuously to sulfur mustard concentrations up to 0.001 mg/m^3, 5 days/week, for ≥7.5 months (McNamara et al. 1975). Angelov et al. (1996a) observed changes in the intestinal muscosa consisting of villi necrosis, dilatation of blood vessels, and increased cellular presence in broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of sulfur mustard for 30 minutes.

Hematological Effects. There are reports of changes in white blood cell (WBC) counts in victims of sulfur mustard exposure during World War I and the Iran-Iraq War. In a group of children and teenagers who were admitted to a hospital in Iran 18–24 hours following exposure to sulfur mustard from air bombs during the Iran-Iraq War, admission WBC counts ranged from 9,500 to 11,200 cells/µL (normally 4,500–10,000 cells/µL), indicating mild leukocytosis (Momeni and Aminjavaheri 1994). During days 1–3 following exposure in World War I, increases of 3–5 times normal levels in WBC counts in peripheral

blood were measured (Marrs et al. 1996). The increase was due mainly to an increase in polymorpho-nuclear cells, while lymphocytes were reduced in numbers during this period. In severe cases, a subsequent leukopenia occurred with WBC counts falling to <200 cells/μL. Leukopenia and pancytopenia were also observed in casualties of sulfur mustard exposure of the World War II Bari Harbor incident and during the Iran-Iraq War (Dacre and Goldman 1996; Marrs et al. 1996; Momeni and Aminjavaheri 1994; Zakerinia et al. 1998). A 13-month-old Iranian girl developed pancytopenia and respiratory failure, and died 8 days after exposure (Momeni and Aminjavaheri 1994).

In a review of the clinical manifestations of sulfur mustard exposure in the Iran-Iraq War victims, Pierard et al. (1990) reported that in addition to the leukocytosis followed by leukopenia and pancytopenia described above, the ratio of T and B lymphocytes decreases while the phagocytic function of neutrophils remains intact. A primary decrease in albumin and increase in α-globulin content, especially α1 antitrypsin, occurs. Both serum protein complement component C3 and C4 titers first increase, followed by a gradual decrease. Aplastic anemia or pancytopenia is not uncommon. Increases in serum tumor markers, α-fetoprotein, β-HCG, and CA-125, have been observed, but the relevance of these increases to the oncogenic potential of sulfur mustard is not yet known. Aplastic anemia was reported in a prospective study of Iran-Iraq War victims (Zakerinia et al. 1998).

Dogs and rabbits exposed to 0.1 mg/m^3 of sulfur mustard in the air for 1 year showed no hematological changes in a study that did not report further experimental details (McNamara et al. 1975). Specific parameters monitored included red blood cell counts, total and differential white cell counts, hematocrit, and hemoglobin concentration.

Changes in the coloring and formation of erythrocyte nuclei and fatty dystrophy of bone marrow cells were observed in broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of sulfur mustard for 30 minutes (Angelov et al. 1996a).

Musculoskeletal Effects. No evidence of sulfur mustard-related changes to the musculoskeletal system was reported in any of 53 autopsies of victims of the World War II Bari Harbor incident (Alexander 1947).

Hepatic Effects. In 39 of 53 (74%) autopsies of Bari Harbor victims, yellow streaks and patches of fatty change appearing as fatty necrosis were observed throughout the liver (Alexander 1947). Several pale liver sections and atypical liver texture were mentioned. In 3 of 53 (6%) autopsies, small

subcapsular hemorrhages, and in one instance, a small rupture near the diaphragmatic attachment, were noted. The gall bladder contained bile with a thick appearance. Microscopic examinations were performed on 31 of the 39 livers with gross changes. Five showed fatty change and two showed focal necroses.

Renal Effects. Renal complications, consisting of acute hemorrhagic nephritis, oliguria, albuminuria, and casts, have been reported in near-death stages of sulfur mustard warfare victims (Papirmeister et al. 1991).

Microscopic examinations of kidney sections from Bari Harbor sulfur mustard casualties revealed tubules containing casts in 25 of 32 (78%) cases. In three cases, casts appeared to be calcified. Casts appeared to contain hemoglobin, as judged by their color in hematoxylin-stained sections, in eight cases. Both cast types were present in the remaining 14 cases (Alexander 1947).

Blood uric acid increased significantly in a dose- and time-related manner in mice exposed nose-only to 21.2, 42.3, or 84.6 mg/m^3 of sulfur mustard in the air for 1 hour, suggesting development of kidney damage (Kumar and Vijayaraghavan 1998). Blood uric acid levels peaked at 2 days after exposure, but were still significantly elevated above controls at 7 days postexposure.

Endocrine Effects. No significant findings were noted grossly in the thyroid or adrenal glands in any of 53 autopsies of victims of the World War II Bari Harbor incident (Alexander 1947).

The time course of changes in serum concentrations of total and free testosterone, luteinizing hormone (LH), dehydroepiandrosterone (DS), follicle-stimulating hormone (FSH), 17 α-OH progesterone, and prolactin were studied in 16 men during the first 3 months after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing sulfur mustard (Azizi et al. 1995). A group of 34 healthy unexposed men of similar age served as controls. At 1 week after exposure, total testosterone, free testosterone, and DS were significantly lower, 57, 72, and 53%, respectively, in exposed men than in controls, while levels of the remaining hormones were comparable between groups. Total testosterone, free testosterone, and DS levels continued to decrease during the first 5 weeks after exposure. Dehydroepiandrosterone mean values reached as low as 18% of the mean of control subjects. After the 5^{th} week, these three hormone levels returned to normal levels at 12 weeks after injury. Small but significant increases in mean serum concentration of LH at the 3^{rd} week and that of FSH and prolactin at the 5^{th} week, were measured. Normal levels of LH, FSH, and prolactin were measured at 12 weeks.

FSH and LH response levels to 100 μg of gonadotropin releasing hormone (GnRH) administered intravenously during the first week after exposure, were subnormal in four of five patients.

In another study, the time course of changes in thyroid indices, serum T3, T4, TSH, reverse T3, thyroglobulin and cortisol, plasma adrenocorticotropic hormone (ACTH), and free T3 and T4 (FT3, FT4) were studied in 13 male soldiers, ages 21–32 years, during the first 5 weeks after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing sulfur mustard (Azizi et al. 1993). A group of 34 healthy unexposed men of similar age served as controls. T4 and FT4 were not consistently affected following injury; compared to controls, significantly decreased values were measured at 1 and 5 weeks after exposure, but values slightly above normal were measured at 3 weeks. T3 and FT3 were significantly lower (11–23%) than control at 1, 3, and 5 weeks after injury. Reverse T3 concentration in injured men was significantly higher (29%) than mean control value at 1 week, but was normal at weeks 3 and 5. TSH and thyroglobulin levels in the injured soldiers were comparable to controls during the 5 postexposure weeks. Cortisol was significantly higher (40%) than normal 1 week after exposure, within the normal range at week 3, and significantly decreased (50%) below normal at week 5. ACTH was significantly increased (57–80%) above the normal control value at 1, 3, and 5 weeks after exposure.

In a follow-up study of 42 men, ages 18–37, injured by sulfur mustard during the Iran-Iraq War, serum testosterone, LH, and prolactin concentrations were normal in all men 1–3 years following exposure (Azizi et al. 1995). A comparison of the mean serum FSH concentration in 13 subjects with sperm count below 20 million and in 20 subjects with sperm counts above 60 million, revealed a nearly 2-fold increase in FSH concentration in the those with the lower sperm count; the increased FSH level was 38% above the mean FSH concentration in a group of 34 health unexposed males.

Dermal Effects. Since the dermal effects of sulfur mustard are due to direct contact of the airborne chemical with the skin, which is supported by experiments in animals that have shown little involvement of the skin when sulfur mustard was administered parentally at dose levels known to be systemically toxic and lethal (Papirmeister et al. 1991), dermal effects in humans and animals are described under Dermal Exposure, Section 3.2.3.2.

Ocular Effects. There is extensive evidence in humans and animals that the eyes are one of the most sensitive targets of sulfur mustard toxicity following vapor exposure. This is attributed to the constant presence of a tear film over the eye's surface and mucous membranes (Pechura and Rall 1993). Ocular

effects are due to direct contact and absorption of sulfur mustard by ocular tissues. In studies with soldiers, Guild et al. (1941) and Anderson (1942) reported conjunctivitis (inflammation of the conjunctiva) as the first sign of exposure to sulfur mustard without symptoms. An acute inhalation MRL of 0.0007 mg/m^3 (see Appendix A) was derived based on a concentration of 0.06 mg/m^3 at which minimal ocular effects (slight generalized conjunctival reaction) occurred in men who underwent a 3-day chamber test with sulfur mustard (Guild et al. 1941). The National Advisory Committee for Acute Exposure Guideline Levels (AEGLs) for Hazardous Substances has established AEGLs for sulfur mustard (see Chapter 8) based on ocular effects (NAC/AEGL 2001). Other acute signs and symptoms described in literature reviews include ocular irritation, redness, lacrimation, burning pain, swelling of the eyelids, photophobia, blepharospasm (spasm of eyelid muscle), and corneal damage (Papirmeister et al. 1991; Pechura and Rall 1993; Somani 1992; USACHPPM 2000a). As with skin and the respiratory tract, an asymptomatic latent period precedes the first signs of ocular injury. Both the severity of ocular effects and the latency period are dependent on the exposure concentration and duration (Ct, concentration-time product) (Eisenmenger et al. 1991; Papirmeister et al. 1991; Pechura and Rall 1993). The latency period is generally shorter in eye injuries than in skin (Papirmeister et al. 1991). As the concentration of sulfur mustard increases, the injury to the eye appears to parallel that of the respiratory tract. Varying degrees of humidity do not influence the degree of injury to the eye; this is attributed to the constant presence of fluid on the surface of the eye (Papirmeister et al. 1991). An increase in temperature appears to increase the severity of dermal effects to a greater extent than ocular effects. Anderson (1942) concluded, based on a comparison on his observations at tropical temperatures (>80 °F) with those of Guild et al. (1941) at cooler temperatures (≤80 °F), that an eye lesion, of any particular degree of severity, would result under tropical conditions from exposure to a Ct slightly lower than that required to produce the same result under cool conditions. Guild et al. (1941) concluded, based on experiments at gas chamber temperatures between 55 and 80 °F, that the degree of sulfur mustard-induced ocular lesions is less related to temperature than that of skin lesions. Reviews from the literature (Papirmeister et al. 1991; Pechura and Rall 1993; Watson and Griffin 1992) indicate that exposure to concentration-time products of ≤12 mg-minute/m^3 produced conjunctivitis and reddening with a latency of hours to days, whereas exposure to 50–100 mg-minute/m^3 produced corneal edema and clouding, eyelid edema, photophobia, severe blepharospasm, and temporary blindness in 3–12 hours with recovery occurring in several weeks. Exposure to 400–800 mg-minute/m^3 may produce corneal damage in 1–4 hours accompanied by possible ulceration and secondary infection. Recovery in this case may take months with the possibility of permanent eye damage. Exposure to higher concentrations of sulfur mustard increases the severity of these signs and symptoms, and may produce systemic effects and incapacitation.

In a clinical study of Iran-Iraq War victims exposed to sulfur mustard, Momeni et al. (1990) reported ocular effects including conjunctivitis, blepharokeratoconjunctivitis, a burning sensation, and lacrimation in 85%, photophobia in 62%, edema of the eyelids in 12%, and corneal edema and abrasion in 8% of 535 patients (95% male; 3% children) examined. Most patients had blurred vision and a few were temporarily blinded. Of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), who were examined 18–24 hours following exposure to sulfur mustard during the Iran-Iraq War (Momeni and Aminjavaheri 1994), ocular effects of conjunctivitis and photophobia were most prevalent, each occurring in 93% of the children, with lower incidences of edema of the eyelids (57%), closure of the eyes (43%), keratitis (43%), blepharospasm (43%), subconjunctival hemorrhage (14%), and corneal ulcer in one child (7%). Burning sensation (71%) and pain (36%) were also noted. The burning sensation developed 3–4 hours after exposure and was followed by photophobia and conjunctivitis. While incidences of mild ocular effects were only slightly higher, Momeni and Aminjavaheri (1994) reported that the severity of ophthalmic manifestations was greater in children and teenagers than adults, so that children may be a sensitive sub-group.

Humans who have experienced eye injury due to acute sulfur mustard exposure may continue to have recurrent corneal erosions and inflammatory keratitis for an indefinite number of years after the initial injury (Amalric et al. 1965; Dahl et al. 1985; Mann 1944; Scholz and Woods 1945). In the acute stage, the limbal region has been reported to present a marbled appearance in which areas of ischemia are surrounded by blood vessels of irregular diameter. Later, the vascularized scars of the cornea often contain deposits of cholesterin, calcium, and fat (Pechura and Rall 1993; Scholz and Woods 1945). While inflammatory keratitis has developed intermittently in veterans injured by sulfur mustard, a sudden increase in the number of cases with these symptoms has been observed some 8–25 years after the initial injury (Pechura and Rall 1993).

There are no rigorous experimental human studies evaluating the occurrence of ocular sensitivity to sulfur mustard. From early chamber tests that indicated conjunctivitis as the initial sign of toxicity, conducted with three groups of men, those having no previous exposure, those who were exposed to very low concentrations of sulfur mustard through their work, but who experienced no symptoms or burns, and those with unspecified occupational exposure who experienced one or more burns at various times, one investigator concluded that the toxicity of sulfur mustard did not appear to increase with previous exposure (Reed 1918). However, details upon which this conclusion was based were lacking. As reported by USACHPPM (2000a), animal data suggest that ocular sensitization occurs following inhalation exposures that produce severe effects. McNamara et al. (1975) cite an earlier study in rabbits

by Laughlin (1944a) in which the severity of ocular effects increased after a second exposure, subsequent to recovery from an initial exposure, to a sulfur mustard Ct of 400 mg-minute/m^3.

While quantitative exposure data are not available, conjunctivitis, altered corneal pigmentation, photophobia, lacrimation, impaired vision, and blepharospasm have been reported in studies of workers at sulfur mustard research laboratories and manufacturing plants with longer-than-acute (>14 days) exposure durations (Laughlin 1944b; Morgenstern et al. 1947). However, these studies are limited by possible exposures to multiple toxic chemicals, confounding factors of age and smoking history, and lack of comparisons to controls.

Scholz (1945) summarized the ocular histological changes that developed in rabbits after sulfur mustard exposure. Changes included corneal basal epithelial cell edema and nuclei relocation, loss of mucus and sloughing off of goblet cells, edema and loss of the conjunctival and corneal epithelial cells, and edema of the stroma as a consequence of corneal endothelial cell damage and loss. When the endothelium of the blood vessels was lost, an infiltrate, composed primarily of neutrophils, accumulated. The conjunctival epithelium began to regenerate about 2 days after injury. If the corneal and limbal epithelium had been lost, conjunctival epithelium was observed to cross the limbus to resurface the cornea. Conjunctival epithelium thickened in 1–2 weeks after injury, but the corneal epithelial layer remained thinner than normal, often with "skip" areas referred to as defects. When these defects were long-lasting, necrotic ulcers, with or without bacterial infection, often developed. Depending on the severity of the original injury, a scarring or "hazing" of the corneal stroma was noted. Normal corneal epithelial regeneration could occur rapidly if the underlying stroma was intact, but if damaged, regeneration could be incomplete with recurrent erosion and vascularization (Somani 1992).

Long-term studies examining delayed ocular effects in rabbits acutely exposed to sulfur mustard showed that, similar to the human condition based on the lifetime of a rabbit as one-tenth that of a human, migration of fatty and/or cholesterin deposits to the surface of the eye occurred 7–8 months after initial injury, causing secondary ulceration (Mann and Pullinger 1944).

Chronic keratitis has been observed in dogs and rats exposed to sulfur mustard vapor for ≥7.5 months; however, this lesion occurred only at the lower of two test concentrations in rats (McNamara et al. 1975). McNamara et al. (1975) reported chronic keratitis (inflammation of the cornea) in 5 of 79 of rats exposed to 0.001 mg/m^3 of sulfur mustard for 12 months, compared to a single occurrence in 29 control animals. However, in this same study (McNamara et al. 1975), no keratitis was observed in a group of 79 rats

exposed to a higher concentration (0.1 mg/m^3) of sulfur mustard. In dogs exposed to these same concentrations, 3 of 10 dogs exposed to 0.1 mg/m^3 for ≥7.5 months developed ocular effects, first appearing after 16 weeks of exposure and including corneal opacity, pannus, chronic keratitis, vascularization, pigmentation, and granulation, compared to no incidences of these lesions in control or low-dose animals (McNamara et al. 1975). An intermediate-duration inhalation MRL of 0.00002 mg/m^3 (see Appendix A) was based on the NOAEL of 0.001 mg/m^3 for ocular effects in dogs identified in this study (McNamara et al. 1975). McNamara et al. (1975) reported no signs of increased ocular sensitivity in dogs or guinea pigs exposed for 1 year to 0.021 mg/m^3 (TWA).

Using a different derivation procedure than that used for chronic-duration inhalation MRLs, the Army has established an air exposure limit for the general population for chronic exposures (GPL) of 0.00002 mg/m^3 as a 24-hour time-weighted average, 7 days/week (USACHPPM 2000a). The key critical effect chosen for the GPL was ocular effects in humans. A previously established GPL of 0.0001 mg/m^3 for sulfur mustard was promulgated by the Centers for Disease Control and Prevention (CDC) in 1988 (DHHS 1988).

Body Weight Effects. No information was located regarding effects on body weight in humans following inhalation exposure to sulfur mustard.

In female Swiss albino mice exposed head only to 0, 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 sulfur mustard in the air for 1 hour, decreases in body weight began 24 hours after exposure, were concentration-related, and achieved statistical significance ($p<0.05$) at concentrations of ≥16.9 mg/m^3 (Vijayaraghavan 1997). Seven days postexposure, body weights were decreased by 2, 13, 28, 25, 32, and 34% in the control, 8.5, 16.9, 21.3, 26.8, and 42.3 mg/m^3 exposure groups. In another study in female albino mice, in which the animals were exposed to 84.6 mg/m^3 sulfur mustard for 1 hour, a progressive fall in body weight was observed starting on the third post-exposure day; on post-exposure day 7, body weight was reduced by 14%, compared to control animals (Pant and Vijayaraghavan 1999). Food and water intakes were also significantly decreased. Since histopathological examination of the esophagus was apparently not conducted, it is not known whether the reduced food and water intake may have been due to discomfort produced by esophageal lesions.

Guinea pigs administered nominal concentrations of 1,250, 1,650, or 1,750 μg-minute/L (125, 165, or 175 mg/m^3) of sulfur mustard (head only) for 10 minutes exhibited a dose-related significant decrease in body weight, with no recovery evident at 6–7 days post-exposure (Allon et al. 1993). At 6–7 days post-

exposure, body weight was reduced compared to controls by ~14, ~24, and ~27% at the low-, mid-, and high-concentrations, respectively (data presented graphically).

3.2.1.3 Immunological and Lymphoreticular Effects

The spleen demonstrated evidences of gross pathology in 33 of 53 (62%) autopsies of Bari Harbor victims (Alexander 1947). In the majority of cases, the spleen was described as shrunken in size with pale color. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted. No significant findings were noted grossly in the thymus in any of the autopsies. Microscopically only 2 of 32 spleens examined showed degeneration or necrosis; pyknosis and karyorrhexis of lymphocytes in some corpuscles was observed in one and slight necrosis of the malpighian follicle in the other. Consistent with observations of the human spleen, Pant and Vijayaraghavan (1999) measured a significant 38% reduction in spleen-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour.

Cameron et al. (1946) provided a general description of pathological changes in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of sulfur mustard ranging from 30 to 350 mg/m^3 (5–54 ppm). After 12 hours, damage was found in the cervical lymph nodes, which drain the nose and lymphoid tissue throughout the body. In experiments where the time sequence was studied, damage to the cervical lymph nodes could not be attributed solely to lymphatic absorption from nasal mucosa, since identical changes resulted from topical skin application or subcutaneous injection of sulfur mustard. Angelov et al. (1996a) detected atrophy of the lymphoid tissue in the bursa Fabricii of broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of sulfur mustard for 30 minutes.

No generalized hypersensitization reaction, as indicated by the lack of release of bradykinin or histamine in the plasma, was seen in dogs exposed to 0.029 mg/m^3 (TWA) of sulfur mustard for 6 months (McNamara et al. 1975).

3.2.1.4 Neurological Effects

No significant findings were noted grossly in the central nervous system in any of 53 autopsies of World War II Bari Harbor victims (Alexander 1947).

Dogs exposed to unspecified levels of sulfur mustard showed no tremors or convulsions during exposure, but no examination of the nervous system was conducted (Winternitz and Finney 1920).

3.2.1.5 Reproductive Effects

In a follow-up study of 42 men, ages 18–37, conducted 1–3 years after injury by sulfur mustard during the Iran-Iraq War, the mean sperm count was 84 million cells per mL, ranging from 0 to 328 million cells per mL (Azizi et al. 1995). Thirteen (29%) had decreased sperm count below 20 million. Serum testosterone, LH, and prolactin concentrations in the 13 subjects with sperm count below 20 million were comparable to the levels in 20 subjects with sperm count above 60 million. FSH measured in these same groups was higher in the group with lower sperm counts. The increased FSH level was 38% above the mean FSH concentration in a group of 34 healthy unexposed males. Complete or relative arrest of spermatogenesis was evident in each testicular biopsy (100% incidence) performed on six men with sperm count below 20 million cells per mL.

Pour-Jafari (1992, 1994a) reported an increased rate of fetal deaths and an increased secondary sex ratio (57.2 vs. 51.0% in controls, percent of males) in progenies of Iranian survivors of chemical attacks that included sulfur mustard.

In a survey of 800 Iranian men who were exposed to sulfur mustard during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

In a study available only in abstract form, exposure of male rats to 0.1 mg/m^3 sulfur mustard 6 hours/day, 5 days/week for up to 52 weeks significantly increased the rate of dominant lethal mutations (Rozmiarek et al. 1973). A maximum rate of 9.4% was observed at 12–52 weeks, compared to 3.9% in controls. In an additional study in which unexposed female rats were mated to males exposed to 0, 0.001, or 0.1 mg/m^3 sulfur mustard for up to 52 weeks, the percentage of fetal deaths in the high-exposure group appeared higher than in the low-exposure group, but no statistical analysis of the results was presented (McNamara et al. 1975). The percentages of fetal deaths at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001, and 0.1 mg/m^3 exposure groups, respectively. In that same study, the investigators stated that the percentage of fetal deaths in rats exposed to 0.001 or 0.1 mg/m^3 sulfur mustard at various times

during pregnancy was within normal limits, but no statistical analyses of the results was presented. No firm conclusions can be drawn from these limited reports.

3.2.1.6 Developmental Effects

Pour-Jafari (1994b) reported an increased incidence of congenital malformations among offspring of Iranian chemical victims. While sulfur mustard was a common chemical agent, the victims may have been exposed to other agents instead of or in addition to sulfur mustard.

Rozmiarek et al. (1973) reported that exposure of pregnant rats to 0.1 mg/m^3 vaporized sulfur mustard did not produce fetal toxicity or gross teratogenic effects, but little additional detail was provided in this abstract. No excess fetal abnormalities were noted when female rats were mated with males exposed to up to 0.1 mg/m^3 for up to 52 weeks (McNamara et al. 1975), but no further details were provided. No conclusions regarding developmental effects of sulfur mustard can be made based on the information available.

3.2.1.7 Cancer

Human Cancer Studies. Data on cancer in humans after inhalation exposure to sulfur mustard are from two primary sources: inhalation for several years by sulfur mustard factory workers and inhalation as the result of a few or of single exposures during combat in World War I and in the Iran-Iraq War. While several epidemiologic studies provide sufficient evidence that sulfur mustard is carcinogenic in humans, particularly in the upper respiratory tract, in no case was the exposure level or duration quantified, and therefore, these data are inadequate for deriving dose-response relationships. Typically, factories produced several different poisonous gases and workers involved with sulfur mustard production were exposed to other toxic chemicals, confounding any study findings.

Other studies provide epidemiological evidence that World War I veterans who were exposed to sulfur mustard in combat had slight, but statistically significant, increased incidences of lung cancer deaths. British retired veterans who were studied 15 years after their exposure to sulfur mustard in World War I showed twice the expected number of deaths due to lung cancer (standardized mortality ratio [SMR]=2; $p<0.01$) compared to controls and also had excessive deaths from bronchitis (SMR=10, $p<0.001$), as compared to nonexposed soldiers (Case and Lea 1955). Veterans who were not exposed to sulfur

mustard, but who did have bronchitis also had excess mortality due to lung cancer (SMR=2; $p<0.01$), as compared with controls. The authors suggest that the finding of a high incidence of lung cancer in both sulfur-mustard-exposed veterans and in non-sulfur-mustard-exposed veterans who had bronchitis does not support the action of sulfur mustard as a direct carcinogen. Deaths from neoplasms other than cancer of the lung were not significantly increased.

A cohort of 7,151 white male American World War I soldiers was studied 1–37 years (Beebe 1960) and 47 years (Norman 1975) postexposure. Deaths from respiratory cancer occurred in 2.5% of those exposed to sulfur mustard, 1.8% of those who had had pneumonia, and in 1.9% of a control group (Norman 1975). The risk of death from lung cancer among men gassed relative to that for controls was estimated as 1.3 (95% CI=0.9–1.9), which in contrast to the findings of Case and Lea (1955), did not suggest a strong carcinogenic effect under the exposure conditions. In a 1996 follow-up clinical study of 197 Iran-Iraq War veterans, 10 years after hospital admission in 1986 due to acute respiratory symptoms with confirmed sulfur mustard exposure, no bronchial carcinoma or other lung malignancies were found (Emad and Rezaian 1997) (see study description under Respiratory Effects in Section 3.2.1.2).

Occupational studies from three countries have shown elevated incidence of cancers of the respiratory tract among factory workers who manufactured sulfur mustard and other chemical agents. In Japanese factory workers, histological examination revealed foci of moderate or severe atypical cell lesions or carcinoma in the bronchial epithelium (Tokuoka et al. 1986). Another study of workers from this same factory showed an increased number of deaths (SMR=37; 33 deaths observed vs. 0.9 deaths expected) from cancer of the respiratory passages (Wada et al. 1968). In another study of Japanese factory workers, with estimated exposure to sulfur mustard concentrations of 0.05–0.07 mg/L, of 172 worker deaths, 48 (28%) were due to malignant tumors compared with 7.7 and 8.5% in two groups of unexposed residents of the same area (Nakamura 1956;Yamada 1963). Respiratory tract tumors accounted for 58% of all malignant tumors (16% of all deaths). In the two reference groups, the incidence of respiratory tumors was much lower, 0.5 and 0.3%, respectively. In the occupational cases described above, central lung cancers were more commonly observed than peripheral lung cancers, and the most common histologic types were squamous cell carcinoma and small cell carcinoma (Yamada 1963). The duration of sulfur mustard exposure in cases of lung cancer was 7–9 years, and the latent period for tumor induction was 17–20 years.

Additional studies have been conducted to determine the comparative risk for development of cancer in Japanese males who worked in a poison gas factory between 1927 and 1945 that produced sulfur mustard,

lewisite, diphenylcyanarsine, hydrocyanic acid, chloracetophenone, and phosgene (Nishimoto et al. 1983; Yamakido et al. 1996). In an attempt to establish a dose-relationship, the workers were divided into three groups according to type of work and association with sulfur mustard. Among 2,068 cases investigated, the number of deaths from cancer of the lungs in the two groups with the highest sulfur mustard exposure potential was more than 3 times the number in the local male population (SMR≥3, $p<0.01$) (Nishimoto et al. 1983). Deaths due to cancers of the gastrointestinal tract and liver or other type were not significantly elevated. Yamakido et al. (1996) studied 1,632 male workers from this same factory and found that the SMRs for lung cancer were significant ($p<0.001$) in the group working directly in the production of sulfur mustard and lewisite for >6 months (SMR=3.24 [0.5–5 years], SMR=7.35 [>5 years]). In a different group of workers who had less contact with sulfur mustard, the SMR for lung cancer was significant only in the subgroup with >5 years of employment (SMR=4.92), further supporting a dose-relationship for lung cancer. However, there were no data presented to weight relatively the exposure to sulfur mustard and lewisite.

British sulfur mustard workers also showed increased deaths from cancers of the respiratory passages and from lung cancer (Manning et al. 1981). In a cohort study of 502 workers involved in sulfur mustard manufacturing between 1940 and 1945, a significant excess mortality was observed for carcinoma of the larynx and trachea (SMR=7.5, $p<0.02$). While not listed as cause of death, seven subjects developed cancer of the larynx, compared with 0.75 expected, yielding a rate ratio of 9.3 ($p<0.001$). Increased mortality due to cancers of other organs was not statistically significant. In another study of 3,354 British sulfur mustard workers, significant excesses were observed compared with national death rates for deaths from cancer of the larynx (SMR=2.7, 11 deaths observed, 4.04 deaths expected, $p=0.003$), pharynx (SMR=5.5, 15 observed, 2.73 expected, $p<0.001$), lung (SMR=1.4, 200 observed, 138.39 expected, $p<0.001$), upper respiratory sites combined (lip, tongue, salivary gland, mouth, and nose) (SMR=2.8, 12 observed, 4.29 expected, $p=0.002$), esophagus (SMR=1.9, 20 observed, 10.72 expected, $p<0.01$), and stomach (SMR=1.4, 70 observed, 49.57 expected, $p<0.001$) (Easton et al. 1988). The risks of cancers of the pharynx and lung, but not of the esophagus and stomach, were significantly related to duration of employment.

In a study of 245 German factory workers with previous occupational exposure to sulfur mustard and followed for over 20 years there was a statistically significant increase in malignant tumors, especially bronchial carcinoma, bladder carcinoma, and leukemia (Weiss and Weiss 1975).

A retrospective mortality study was conducted in World War II veterans who participated in U.S. military experiments testing the effectiveness of various protective clothing and equipment in preventing injury due to sulfur mustard (Bullman and Kang 2000). The study identified 1,545 white male Navy recruits who were exposed to nonlethal levels (>120–960 mg-minute/L) of sulfur mustard at a single site between 1944 and 1945. A control group consisted of 2,663 white male Navy veterans who served at the same location and time as the exposed, but did not participate in sulfur mustard chamber tests. Sulfur mustard chamber test documentation included concentration of sulfur mustard in the chamber, length of exposure, and subject physiological reactions, so a dose-response analysis could be conducted. The veterans who participated in the sulfur mustard chamber tests, while exposed to lower levels than estimated for combat exposed World War I veterans, did have sufficient exposure to produce skin reactions of erythema and edema. Causes of death investigated included laryngeal, lung, and skin cancers, chronic obstructive pulmonary and parenchymal respiratory diseases, external causes, and suicide. The mortality rate ratios for all cancer types among the total exposure group and all subgroups were less than unity. The greatest mortality rate ratio, 1.57 (95% CI=0.70–3.54), resulted for chronic obstructive pulmonary disease among veterans with exposure levels in the range of 121–960 mg-minute/L. The authors indicated that this value was not statistically significant and that there was no excess of any cause-specific mortality associated with sulfur mustard exposure or associated with the level of sulfur mustard exposure among veterans. The authors noted that reliance on death certificates for cause of death and lack of data on potential confounders (smoking, drinking habits, and occupational history/exposure to carcinogens) were potential study weaknesses.

Animal Cancer Studies. Two animal studies showed tumors following inhalation exposure to sulfur mustard. Male and female Strain A mice exposed once for 15 minutes to an unquantified level of sulfur mustard had a significantly higher incidence of pulmonary tumors than did their littermate controls (Heston 1953b). The significance of this finding for humans is difficult to determine since these Strain A mice are used due to their specific genetic tendency to develop lung tumors. Guinea pigs, mice, rabbits, and dogs that were exposed to sulfur mustard in the air for 3–12 months did not develop tumors, although rats did develop squamous cell carcinoma of the skin (McNamara et al. 1975).

IARC has classified sulfur mustard as “carcinogenic to humans” (Group 1) based on sufficient evidence in humans, limited evidence in experimental animals, supporting evidence that sulfur mustard is a bifunctional alkylating agent, and positive results in a number of assays for genotoxic effects (IARC 1975, 1987).

The Army's current health-based environmental screening levels (HBESLs) for sulfur mustard include a cancer inhalation unit risk value, and an inhalation cancer potency value (USACHPPM 1999) (see Chapter 8). However, ongoing evaluations of alternative approaches for quantitatively estimating cancer risk may result in changes to these values.

3.2.2 Oral Exposure

Victims of battlefield exposures may have ingested small amounts of airborne sulfur mustard. Sulfur mustard aerosol, with aerodynamic diameters greater than 10 μm, entering the nose or mouth will be ingested, if not expectorated. However, no studies were located regarding the health effects in humans after specific oral exposure to sulfur mustard. While exposure to sulfur mustard by the oral route can occur, the dermal and inhalation routes of exposure are the primary routes of exposure.

3.2.2.1 Death

Limited information is available regarding the acute oral toxicity of sulfur mustard. Without providing any information on how the value was derived, an Army report indicates that an oral LD_{50} of 0.7 mg/kg has been estimated for humans (SBCCOM 1999). In a review of the literature on sulfur mustard, Opresko et al. (1998) stated that the oral LD_{50} for rats is 17 mg/kg.

Significant maternal mortality occurred in a teratology study in which sulfur mustard in sesame oil was administered acutely by oral gavage to pregnant rats and rabbits on gestation days 6–15 and 6–19, respectively (DOA 1987). Rabbits were dosed with 0, 0.4, 0.6, or 0.8 mg/kg/day. In rabbits, maternal mortality was dose-related with deaths occurring at doses ≥0.8 mg/kg/day: 3/18 (17%) at 0.8 mg/kg/day, 3/7 (43%) at 1.0 mg/kg/day, 5/8 (63%) at 2.0 mg/kg/day, and 4/6 (75%) at 2.5 mg/kg/day. Female rats were dosed with 0, 0.4, 0.8 1.0, 1.6, 2.0, or 2.5 mg/kg/day. One of three rats died on gestation day 12 at the highest dose of 2.5 mg/kg/day. No maternal deaths in rats were attributed to sulfur mustard at doses <2.5 mg/kg/day.

The lethal dose levels for the rats and rabbits are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal effects in animals after oral exposure to sulfur mustard The respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, dermal, ocular, and body weight effects observed in animals after oral exposure to sulfur mustard are discussed below. Sparse animal data indicate no respiratory, cardiovascular, hepatic, renal, endocrine, dermal, or ocular effects following oral exposure to sulfur mustard. The highest NOAEL and all LOAEL values for each study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Gross examinations of the lungs of rats orally gavaged with 0.3 mg/kg/day of sulfur mustard in sesame oil, 5 days/week, for 13 weeks, did not reveal any significant treatment-related lesions (Sasser et al. 1996b).

Cardiovascular Effects. Microscopic examinations of the heart of rats orally gavaged with 0.3 mg/kg/day of sulfur mustard in sesame oil, 5 days/week, for 13 weeks, did not reveal any significant treatment-related lesions (Sasser et al. 1996b).

Gastrointestinal Effects. Dose-related gastrointestinal effects (mucosal irritation and/or inflammation) have occurred in experimental animals following acute and subchronic oral administration of sulfur mustard in sesame oil (DOA 1987; Sasser et al. 1996a,1996b).

In pregnant rats, orally gavaged acutely with 0.2–2.5 mg/kg/day of sulfur mustard on gestation days 6–15, gastric mucosal inflammation was observed at doses ≥2.0 mg/kg/day (DOA 1987). Inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of sulfur mustard on gestation days 6–19 incurred dose-related damage to the gastric mucosa at doses ≥0.4 mg/kg/day (DOA 1987).

A significant increase in the incidence of epithelial hyperplasia of the forestomach was observed in rats treated with 0.3 mg/kg/day sulfur mustard by gavage for 13 weeks (Sasser et al. 1996b). No significant increase was seen at ≤0.1 mg/kg/day. The hyperplastic change was characterized by cellular disorganization of the basilar layer, apparent increase in mitotic activity of the basilar epithelial cells, and thickening of the epithelial layer.

Table 3-2 Levels of Significant Exposure to Sulfur Mustard - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE							
	Death							
1	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)					2.5 F	DOA 1987
2	Rabbit (NS)	14 d Gd 6-19 (GO)					0.8 F	DOA 1987
	Systemic							
3	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)	Dermal	2.5				DOA 1987
			Bd Wt	1	1.6	(9.1-16.6% decrease after 7 days of exposure)		
4	Rabbit (NS)	14 d Gd 6-19 (GO)	Hemato	0.6	0.8	(9.1% decreased hematocrit)		DOA 1987
			Dermal	2.5				
			Bd Wt	0.6	0.8			
	Immuno/ Lymphoret							
5	Rat (Sprague-Dawley)	10 d, Gd 6-15 (GO)			0.5[b] F	(Inflamed mesenteric lymph nodes)		DOA 1987
	Developmental							
6	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)			0.5[b]	(Reduced ossification)		DOA 1987

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

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
7	Rabbit (NS)	14 d Gd 6-19 (GO)		0.8			DOA 1987
	INTERMEDIATE EXPOSURE						
	Systemic						
8	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)	Dermal	0.5			Sasser et al. 1993
			Bd Wt	0.5 M			
9	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)	Gastro		0.03[c] (29/47 M, 42/47 F; epithelial acanthosis of the forestomach)		Sasser et al. 1996a
			Dermal	0.4			

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

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
10	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)	Resp	0.3			Sasser et al. 1996b
			Cardio	0.3			
			Gastro	0.1	0.3 (epithelial hyperplasia of forestomach)		
			Hemato	0.3			
			Hepatic	0.3			
			Renal	0.3			
			Endocr	0.3			
			Dermal	0.3			
			Ocular	0.3			
			Bd Wt	0.1	0.3 (>10% decrease in females, >8% decrease in males)		
	Immuno/ Lymphoret						
11	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)		0.3			Sasser et al. 1996b

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

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Neurological						
12	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)		0.5			Sasser et al. 1993
	Reproductive						
13	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)				0.08 (4-fold increase in resorptions; increased preimplantation losses; 7% decrease in live fetuses) 0.5 M (2-fold increase in abnormal sperm head morphology)	Sasser et al. 1993
14	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)		0.1	0.4 (Increased fraction of males, 58%)		Sasser et al. 1996a
15	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)		0.3			Sasser et al. 1996b
	Developmental						
16	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)		0.4			Sasser et al. 1996a

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

b Used to derive an acute oral MRL of 0.0005 mg/kg/day; dose divided by an uncertainty factory of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

c Used to derive an intermediate-duration oral MRL of 0.00007 mg/kg/day; dose adjusted to a TWA of 0.02 mg/kg/day for intermittent exposure (see Appendix A) divided by an uncertainty factory of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endo = endocrine; F = female; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; hemato = hematological; M = male; resp = respiratory; wk = week(s); x = time(s)

Figure 3-2. Levels of Significant Exposure to Sulfur Mustard - Oral

Acute (≤14 days)

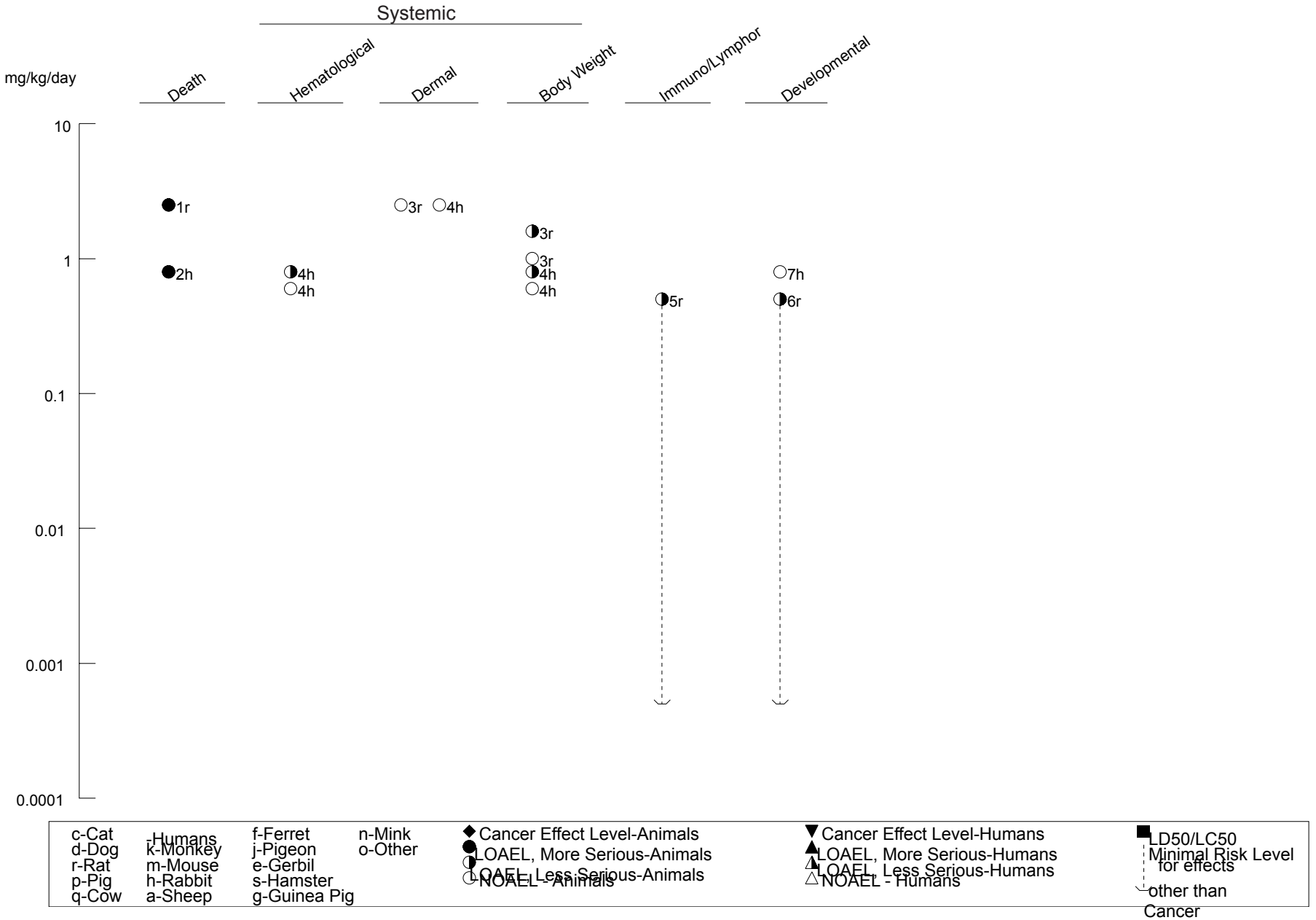
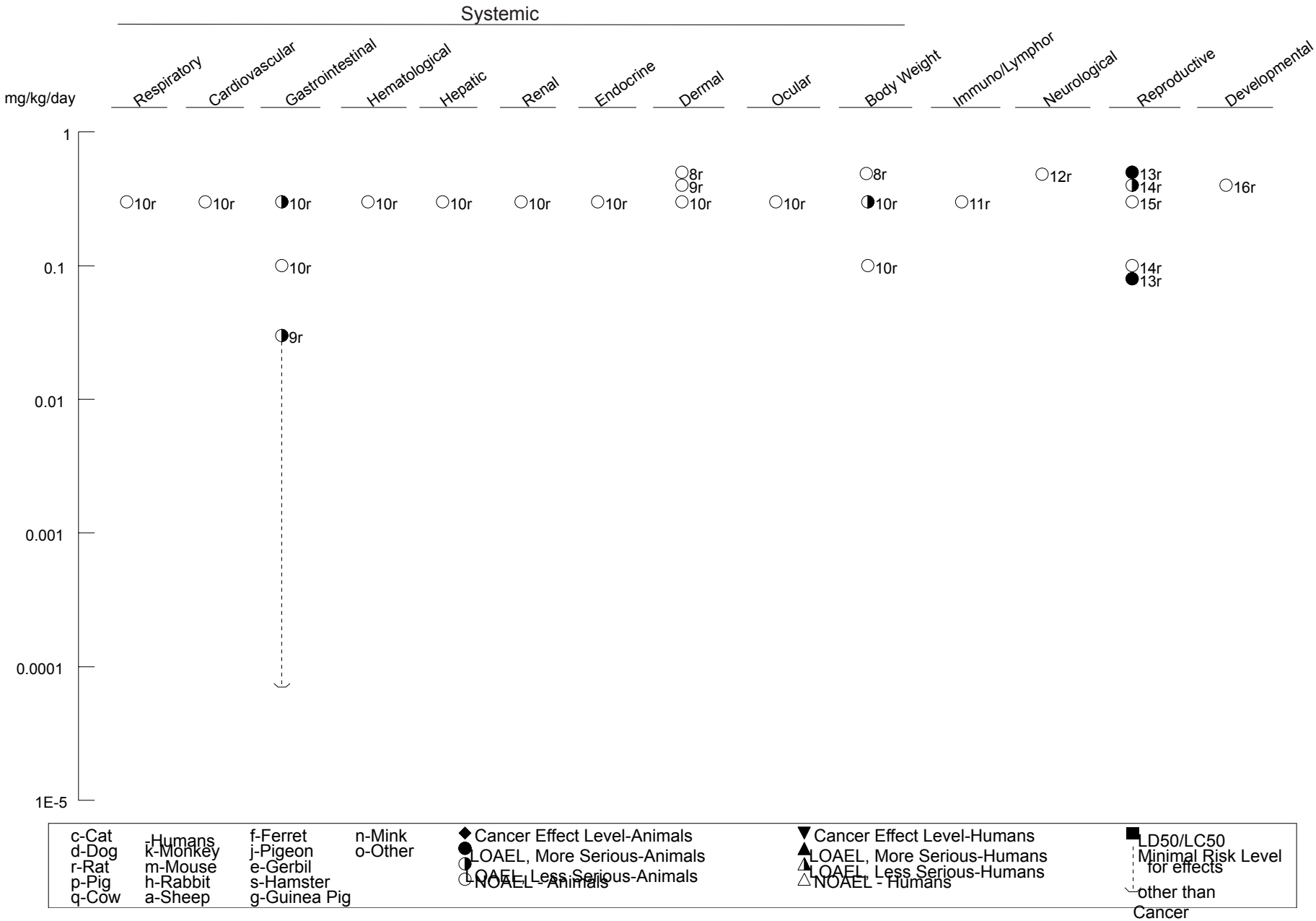


Figure 3-2. Levels of Significant Exposure to Sulfur Mustard - Oral (*Continued*)

Intermediate (15-364 days)



In a 2-generation reproduction study, dose-related incidence and severity of lesions of the squamous epithelium of the forestomach occurred in both sexes of rats orally gavaged with 0, 0.03, 0.1, or 0.4 mg/kg/day of sulfur mustard dissolved in sesame oil for 18–21 weeks (Sasser et al. 1996a). The incidences of hyperplasia [combined F0 and F1 males and females: 0/94 controls, 71/94 (76%; 29 male/42 female) in the low-dose groups, 89/94 (95%; 37 male/ 52 female) in the mid-dose groups, and 94/94 in the high-dose groups] were significantly increased in all treated groups, compared to controls. An intermediate-duration oral MRL of 0.07 µg/kg/day was derived based on the LOAEL of 0.03 mg/kg/day, the lowest dose tested, for gastric lesions from this study (See Appendix A for details).

Hematological Effects. In pregnant rats gavaged with 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, or 2.0 mg/kg/day of sulfur mustard on gestation days 6–15, maternal hematocrit values were significantly reduced by 10.8% at 0.8 mg/kg/day and 5.4% at 1.0 and 2.0 mg/kg/day (DOA 1987). While hematocrit at 1.6 mg/kg/day was reduced, the decrease was not significant. A dose-related decrease in maternal hematocrit was reported in pregnant rabbits following acute oral administration of 0.4, 0.6, or 0.8 mg/kg/day sulfur mustard on gestation days 6–19, with statistical significance achieved only at the highest dose (DOA 1987). No other hematological parameter was evaluated. The biological significance of these changes is unknown and, according to the investigators, may have been due to changes in plasma volume during pregnancy or to anorexia in some of the animals.

Hepatic Effects. No significant hepatic effects were observed in rats treated by gavage with up to 0.3 mg/kg/day sulfur mustard in sesame oil, 5 days/week, for 13 weeks, as judged by no significant changes in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and no microscopical alterations in the liver (Sasser et al. 1996b).

Renal Effects. Neither blood urea nitrogen (BUN) nor serum creatinine levels were significantly altered in rats treated with up to 0.3 mg/kg/day sulfur mustard 5 days/week for 13 weeks (Sasser et al. 1996b). In addition, microscopic examination of the kidneys did not reveal any treatment-related effects.

Endocrine Effects. Only limited animal data exist on endocrine effects following oral exposure to sulfur mustard. Microscopic examination of adrenals from rats orally gavaged with 0.3 mg/kg/day of sulfur mustard in sesame oil, 5 days/week, for 13 weeks revealed no lesions (Sasser et al. 1996b).

Dermal Effects. In animal studies, no systemic dermal effects were induced following acute or sub-chronic oral exposure to sulfur mustard in sesame oil. No dermal effects were observed in rats or rabbits

acutely dosed with up to 2.5 mg/kg/day of sulfur mustard (DOA 1987) or following longer exposures in rats orally gavaged with 0.08–0.5 mg/kg/day of sulfur mustard, 5 days/week, for 10 weeks (Sasser et al. 1993), with 0.003–0.3 mg/kg/day of sulfur mustard, 5 days/week, for 13 weeks (Sasser et al. 1996b), or with 0.03–0.4 mg/kg/day of sulfur mustard for 18–21 weeks (Sasser et al. 1996a).

Ocular Effects. Animal data indicate that no systemic ocular effects result from oral exposure to sulfur mustard in sesame oil. Ophthalmology evaluations of rats orally gavaged with 0.003–0.3 mg/kg/day of sulfur mustard, 5 days/week, for 13 weeks, revealed no abnormalities (Sasser et al. 1996b).

Body Weight Effects. In pregnant rats gavaged with 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, or 2.0 mg/kg/day of sulfur mustard in sesame oil on gestation days 6–15, a significant dose-related decrease in maternal body weight was observed by gestation day 9 at 1.0 mg/kg/day (4.7–9.1%) and 2.0 mg/kg/day (6.5–16.0%) and by gestation day 12 at 0.5 mg/kg/day (4.1–6.6%) and 1.6 mg/kg/day (9.1–16.6%) (DOA 1987). Reductions in extragestation weight gain was also dose-related with decreases of 10, 27, 25, 29, 38, 53, and 57% measured in 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, and 2.0 mg/kg/day groups, respectively, compared to concurrent controls, with statistical significance achieved at ≥0.4 mg/kg/day.

Inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of sulfur mustard in sesame oil on gestation days 6–19, showed a significantly decreased maternal body weight at 0.8 mg/kg/day (7.9–10.5% decrease after gestation day 10, 5 days of exposure) and 2.0 mg/kg/day (12.0–18.3% decrease after gestation day 14, 9 days of exposure), but not at 1.0 mg/kg/day (DOA 1987).

Females in the highest-dose group of rats orally gavaged with 0.003–0.3 mg/kg/day sulfur mustard in sesame oil, 5 days/week, for 13 weeks, weighed significantly less than controls at week 4 and during the last 5 weeks of exposure (reduced >10%) (Sasser et al. 1996b). Males in the highest-dose group weighed significantly less than controls during 6 of the weeks in the weeks 3–12 of the study period (reduced by >8%). There was no indication of a dose response in body weight in lower dose groups.

In a two-generation reproductive study of sulfur mustard in sesame oil administered intragastrically at doses of 0.03–0.4 mg/kg/day, the body weights of the F0 exposed rats were not significantly different from controls; however, the growth rate of the high-dose males tended to decline after about 7 weeks of exposure (Sasser et al. 1996a). Body weight gain beginning 1 or 2 weeks after treatment was started (approximately 20% for males and 15–24% for females) was significantly lower ($p < 0.05$) than control

values in F1 rats of both sexes born to high-dose parents. No significant dose-response in body weight occurred at the lower doses.

Body weights of female rats treated by gavage with 0.5 mg/kg/day sulfur mustard for 10 weeks were slightly lower than controls during most of the study, but at 10 weeks, it appeared no different than controls in the figure from the study (Sasser et al. 1993). In males, body weight was lower than in controls beginning at week 2, and final body was reduced approximately 9% relative to controls.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans following oral exposure to sulfur mustard.

In a range-finding teratology study in pregnant rats in which sulfur mustard was administered by gavage in oil (0.2, 0.4, 0.8, 1.6, 2.0, and 2.5 mg/kg/day), a significant increased incidence of inflamed mesenteric lymph nodes was found in all treated groups except the lowest dose group (DOA 1987). In the final study, inflamed mesenteric lymph nodes were found in 11/25 (44%), 16/25 (64%), and 15/27 (56%) animals at 0.5, 1.0, and 2.0 mg/kg/day, respectively, compared to no occurrences in a group of 25 control animals (DOA 1987). Also, enlarged Peyer's patches (flat patches of lymphatic tissue located in the small intestine) were found in inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of sulfur mustard in sesame oil on gestation days 6–19; however, incidences were not reported (DOA 1987). The teratology study (DOA 1987) was selected as the key study for acute oral MRL derivation. An acute oral MRL of 0.5 µg/kg/day was derived based on the LOAEL of 0.5 mg/kg/day, the lowest dose tested, for inflamed mesenteric lymph nodes in the rat dams. An uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) was applied to the LOAEL to derive the MRL.

3.2.2.3 Neurological Effects

No studies were located regarding neurological effects in humans following oral exposure to sulfur mustard.

In rats, orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day sulfur mustard in sesame oil, 5 days/week, for 10 weeks, excessive salivation (drooling) following dosing was observed in the highest dose group (Sasser et al. 1993). No further relevant information was located.

3.2.2.4 Reproductive Effects

No information was located regarding reproductive effects in humans following oral exposure to sulfur mustard.

In a teratology study (DOA 1987), pregnant rats were treated by gavage with 0, 0.5, 1.0, or 2.0 mg/kg/day of sulfur mustard in sesame oil on gestation days 6–15. A significant decrease in gravid uteri weight (16%) was measured in dams at the highest dose of 2.0 mg/kg/day. The number of corpora lutea and implantation sites, and the incidence of pre-implantation failure and intrauterine mortality were unaffected by sulfur mustard treatment.

In a study in rats, oral exposure to sulfur mustard resulted in significant dominant lethal effects in male rats mated to untreated females, whereas female dominant lethal effects were not observed (Sasser et al. 1993). In that study, rats were treated by gavage with 0.08, 0.2, or 0.5 mg/kg/day sulfur mustard in sesame oil, 5 days/week, for 10 weeks (Sasser et al. 1993). In female dominant lethality experiments (treated or untreated males were mated with treated females), the overall mean pregnancy rate in treated groups was 86% with a range from 70 to 100%, and with no significant differences between treatment groups. Reproductive performance indicators (number of live or dead implants, resorptions, and preimplantation losses) in treated female rats mated to treated or nontreated males were not significantly different from controls. In male dominant lethality experiments (treated males were mated with untreated females), the overall mean pregnancy rate in treatment groups was 91%; treatment means ranged from 65 to 100%, with no significant differences between treatment groups. There was no indication of a dose relationship with the number of live implants. In the highest exposure group, the mean number of total and early resorptions per litter was significantly greater than control during the 2nd and 3rd postexposure weeks. The number of total and late resorptions in the mid-dose group was also significantly greater than controls during the 3rd postexposure week. Preimplantation losses in the mid- and high-dose groups were significantly elevated during the 2nd postexposure week. High-dose male sperm morphology data at all postexposure sampling times (0, 5, and 12 weeks) showed a statistically significant decrease in the percentage of normal sperm. Blunthook and banana-shaped sperm heads were observed at 0, 5, and 12 weeks, whereas amorphous and short head abnormalities were observed only at 5 and 12 weeks.

Overall, there was a total 2-fold increase in abnormal sperm heads in high-dose sulfur mustard-treated males. Sperm morphology and motility were not examined in the low- and mid-dose groups. In summary, female fertility was not affected by these sulfur mustard exposures; however, a male dominant lethal effect was demonstrated at the mid and high doses of sulfur mustard.

In a two-generation study in rats, reproductive performance was not adversely affected following exposure to sulfur mustard administered by gavage at levels of 0.03–0.4 mg/kg/day, 5 days/week, for 13 weeks prior to mating and through gestation, parturition, and lactation (Sasser et al. 1996a). Reproductive performance was measured by assessing the number of matings, pregnant females and females delivering live pups, fertility index, and mating index. The only significant birth measurement was an altered sex ratio (58% males) in the high-dose F0 offspring (Sasser et al. 1996a). Futhermore, microscopic examination of the reproductive organs revealed no evidence of treatment-related effects. Microscopic examinations of the testes from rats orally gavaged with up to 0.3 mg/kg/day of sulfur mustard in sesame oil, 5 days/week, for 13 weeks also revealed no lesions (Sasser et al. 1996b).

3.2.2.5 Developmental Effects

No information was located regarding developmental effects in humans following oral exposure to sulfur mustard.

In a study in which rats were treated by gavage with 0, 0.5, 1.0, or 2.0 mg/kg/day sulfur mustard, the numbers of live fetuses per litter were comparable between dose groups. Fetal body weights were significantly decreased in litters exposed to doses ≥1.0 mg/kg/day; however, the depressed fetal weights were not accompanied by a corresponding decrease in crown-rump length. Sex ratio was significantly different from control only at the high-dose. Placental weights were significantly lower in the high-dose group, compared to control. The number of minor skeletal anomalies, mostly commonly misaligned sternebrae, was significantly increased in the high-dose group. The incidences of reduced ossification of the vertebrae and/or sternebrae in all treated groups were significantly increased, compared to controls (DOA 1987). In the companion study in rabbits with doses of 0, 0.4, 0.6, or 0.8 mg/kg/day, no adverse effects to fetal body weight or skeletal morphology were observed. However, in the preliminary dose-range study in rabbits, fetal body weight was significantly reduced at 2.0 mg/kg/day (DOA 1987).

See Table 3-4 for ongoing studies of developmental effects of sulfur mustard.

3.2.2.7 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to sulfur mustard.

The Army's current health-based environmental screening levels (HBESLs) for sulfur mustard include an oral cancer potency value (slope factor) (USACHPPM 1999). In the absence of a chronic bioassay for sulfur mustard, the oral cancer potency value was estimated as the geometric mean of slope factors developed using various data sets (potency relative to benzo(a)pyrene, forestomach hyperplasia incidence, and maximum tolerated dose). However, ongoing evaluations of alternative approaches for quantitatively estimating cancer risk may result in changes to this value (see Chapter 8).

3.2.3 Dermal Exposure

3.2.3.1 Death

There are reports of human deaths from skin contact with liquid sulfur mustard from old deteriorating artillery shells (Aasted et al. 1985; Heully et al. 1956; Jorgensen et al. 1985). In France, two children died after a 40-year-old sulfur mustard shell accidentally exploded spraying the liquid onto their skin and clothing (Heully et al. 1956). Two fishermen died from handling sulfur mustard bombs disposed of in the Baltic Sea, which became caught in their nets (Aasted et al. 1985; Jorgensen et al. 1985). Dermal dose estimates are not available for these accidental exposures. Other surviving fishermen suffered skin lesions, erythema, blistering, and eye lesions. Without providing any further details, SBCCOM (1999) indicates that in humans the LD_{50} for skin exposure is 100 mg/kg. LD_{50} values in animals for sulfur mustard administered topically range from 9 to 100 mg/kg (Dacre et al. 1995). Of the species studied (rat, mouse, dog, rabbit, guinea pig, and goat), the rat was the most sensitive to acute lethal effects, with a dermal LD_{50} of 9 mg/kg.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, renal, or body weight effects in humans or animals after dermal exposure to sulfur mustard.

Gastrointestinal Effects. Volunteers who were wearing respirators and who were exposed to unspecified levels of sulfur mustard vapors and liquids not only had skin burns, but also complained of nausea, vomiting, anorexia, abdominal pain, diarrhea, headache, and lassitude (Sinclair 1948). These signs could have been primary effects of the sulfur mustard on the rapidly dividing cells of the gastrointestinal epithelium, secondary effects from the skin burns, or psychological effects not related to the sulfur mustard exposure at all.

In a study designed to determine lethal dermal doses, rats stopped eating and drinking, had diarrhea, and lost weight prior to death (Young 1947).

Hematological Effects. Intense skin exposure to sulfur mustard sufficient to cause severe vesiculation and skin necrosis may result in systemic effects to bone marrow. Absorbed sulfur mustard may destroy or diminish bone marrow activity denoted by reduced numbers of or destruction of replicating marrow stem cells (Pechura and Rall 1993). Sulfur mustard-induced reduction of granulocyte and other marrow-derived cells in peripheral blood cause a diminished protective effect from polymorphonuclear leukocytes, macrophages, monocytes, and other cell types that are active in the destruction and scavenging of organisms that invade and impede wound healing.

A reduction in lymphocytes was noted in mice whose shaved backs were topically treated a single time with 3.88–15.5 mg/kg of sulfur mustard diluted in olive oil (Venkateswaran et al. 1994a); hematology revealed a significant dose-related increase in packed cell volume (10–16%). The increase in hemoglobin concentration was also dose-related and significantly increased (13–19%) at 7.75–15.5 mg/kg of sulfur mustard.

Hepatic Effects. Topical application of a single dose of 3.88, 7.75, or 15.5 mg/kg sulfur mustard to the shaved backs of Balb/c mice induce a dose-related decrease in liver weight was observed, with a significant reduction of 14% measured at the high dose (Venkateswaran et al. 1994a).

Twenty-four hours after application of a single dose of 51.3 mg/kg (1 LD_{50}) of neat sulfur mustard to the hair-clipped backs of male guinea pigs the liver showed fatty degeneration accompanied by infiltration with red blood cells, lipidolysis, and distortion of cell structure (Chauhan and Murty 1997). Three days after dosing, infiltration with macrophages was observed in addition to the above alterations. Liver injury was also indicated by increases in serum AST and ALT activities. Both enzymes increased after

exposure, reaching maximum levels of nearly twice control values at 3 days, and returned toward normal levels at 6 days postexposure. The AST recovery was slower than ALT as the 6-day level, while submaximal, was still significantly elevated (33%) above control.

Endocrine Effects. Adrenal weight was significantly increased in a dose-related manner in mice whose shaved backs were treated topically with sulfur mustard doses of 7.8–15.5 mg/kg once (Venkateswaran et al. 1994a).

Dermal Effects. When sulfur mustard gets on human skin, it causes itching, erythema, and/or blister formation (Pechura and Rall 1993). Australian soldiers, who were wearing respirators, volunteered to be exposed to skin contact with sulfur mustard during World War I. They had erythema on the exposed areas, and skin burns on the genitalia (Sinclair 1948, 1950). Men who were exposed to sulfur mustard from leaking artillery shells picked up by fishing vessels off the coast of Denmark showed inflamed skin, blisters, eye irritation, and transient blindness (Wulf et al. 1985). These reactions are usually delayed by at least several hours, up to 48 hours after initial exposure (Jakubowski et al. 2000; Renshaw 1946; Smith et al. 1919).

A review of the literature prior to 1950 indicates that drops containing 0.1% or more sulfur mustard can cause skin blisters on humans (Sulzberger et al. 1947). Tissue injury does not develop when low, therapeutically effective doses of sulfur mustard are used to control the hyperproliferation of psoratic keratinocytes. The severity of cutaneous injury is dependent on dose, exposure duration, temperature, humidity, and/or perspiration and is directly related to the sulfur mustard alkylation levels in skin (Papirmeister 1993). Sulfur mustard is more harmful to the skin on hot, humid days or in tropical climates (Papirmeister et al. 1991; Sulzberger et al. 1947).

Humans show varying degrees of sensitivity to sulfur mustard (Renshaw 1946; Sulzberger et al. 1947). In particular, people with fair skin are more sensitive than those with dark skin. Affected skin usually changes color in response to stimulation of melanogenesis (Pechura and Rall 1993). Increased darkening of the skin at the periphery of sulfur mustard-induced blisters is characteristically observed. There may be variations in the skin's response to the same sulfur mustard dose and exposure duration depending on the contacted dermal site (Pechura and Rall 1993). For a given dose and duration of exposure, loose tissue, as around the eyes and on the genitalia, may respond with edema without blistering, while tissue sites having a very dense dermis, such as on the back, may respond with erythema and blister formation without edema (Pechura and Rall 1993). Scar formation following sulfur mustard injury may be

disabling. Individuals with previous exposure are more sensitive to the dermal effects of sulfur mustard (Renshaw 1946; Sulzberger et al. 1947). SBCCOM (1999) reports a maximum safe Ct of 5 mg-minute/m^3 for human skin exposure to sulfur mustard vapor.

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined in a hospital in Iran 18–24 hours following exposure to sulfur mustard from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Cutaneous effects included erythema (in 94% of patients), itching (71%), bulla (71%), ulceration (64%), hyperpigmentation (50%), and hypopigmentation (21%). Burning sensation (in 71% of patients) and pain (36%) were also noted. Skin lesions first appeared 4–18 hours after exposure, and were accompanied by an itching and burning sensation, especially over the face and neck. Thereafter, the patients developed erythema and gradually, after 20–30 hours, blisters. Most of the lesions in children developed of the face (79%), followed by genital (43%), thoracic (21%), trunkal (14%), and axillar lesions (14%). No direct relation was found between sex of the individual and the site of the lesions. The time of onset of sulfur mustard manifestations in children was shorter (4–18 hours) and the severity of the lesions higher than in adults (8–24 hours), possibly due to more delicate skin and epithelial tissues. Genital lesions were less frequent in children and teenagers (42%) than adults (70%); however, even within the group of children, the incidence and severity of genital lesions increased with age. Other skin lesions had no apparent age-relation.

Sulfur mustard applied to the skin of rats produced local edema, which subsided after 3 days (Young 1947). In mice, rabbits, and guinea pigs, sulfur mustard produced vascular leakage, leukocytic infiltration, and slow death of basal epidermal cells; this damage reached its peak 1–3 days after application (Chauhan et al. 1993a, 1993b, 1995, 1996; Vogt et al. 1984). Healing occurred within 10 days. Suckling rats (which had not yet grown hair) developed inflammatory changes and epidermal thickening after dermal exposure to sulfur mustard for 1–15 minutes (McAdams 1956). This damage was evident 1–7 days postexposure. Blisters did not develop, but the basal cells were destroyed.

Application of single doses of 3.88, 7.75, or 15.5 mg/kg sulfur mustard to the shaved backs of Balb/c mice resulted in mild skin lesions, first appearing on postexposure day 4 (Venkateswaran et al. 1994a). The lesions progressed to severe, with fluid loss, on postexposure day 7.

Ocular Effects. Ocular effects that occur during or following exposure to sulfur mustard in the air are due to direct contact of sulfur mustard with the eye. This is supported by experiments in animals that

have shown little involvement of the eyes when sulfur mustard was administered parentally at dose levels known to be systemically toxic and lethal (Papirmeister et al. 1991). Ocular effects due to exposure to sulfur mustard in the air are summarized in Section 3.2.1.2.

Body Weight Effects. Application of a single dose of 3.88, 7.75, or 15.5 mg/kg sulfur mustard to the shaved backs of Balb/c mice resulted in a progressive dose-dependent fall in body weight beginning 3–5 days after exposure (Venkateswaran et al. 1994a). The decrease was significant at the mid and high doses, 11 and 27%, respectively. Reduced food consumption was noted only in the high-dose group.

Guinea pigs treated with a single dose of 51.3 mg/kg (1 LD_{50}) of neat sulfur mustard applied to their hair-clipped backs showed a gradual loss of weight up to 35% on postexposure day 6 (Chauhan and Murty 1997).

3.2.3.3 Immunological and Lymphoreticular Effects

Sulfur mustard was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (16/group/dose) (Venkateswaran et al. 1994a). Sulfur mustard produced a significant dose-related decrease in the weight of the spleen (12–59%), and peripheral (12–44%) and mesenteric lymph nodes (significant only at high dose, 18%). Incidence and severity of histological changes in the thymus and spleen were also dose-related. Spleen histopathology included hypocellularity, atrophy of the lymphoid follicles, degeneration of germinal centers, and red pulp infiltrated with macrophages. The cortex and medulla regions of the thymus showed atrophy and hypocellularity. Red blood cells replaced cortical thymocytes with severe atrophy. A significant dose-related decrease in the cellularity of the spleen (24–45%) was measured. A dose-related decrease in the cellularity of the thymus was also found, significant at the mid and high doses (36–42%).

Cameron et al. (1946), after observing damage to the cervical lymph nodes and lymphoid tissue throughout the body in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of sulfur mustard ranging from 30 to 350 mg/m^3 (5–54 ppm), administered sulfur mustard to animal skin and found identical changes to the lymph tissue, suggesting that lymphoid tissue damage may be due to systemic absorption. Only a general discussion, lacking experimental details, was reported.

3.2.3.4 Neurological Effects

Chronic and/or late neurological symptoms of abnormal skin sensation after exposure to sulfur mustard were studied in five patients exposed to sulfur mustard during battlefield operations in the Middle East and five fishermen accidentally exposed to sulfur mustard by pulling shells leaking the chemical agent aboard their fishing vessels. All 10 patients (100%) suffered from neuropathic pain or other deafferentation symptoms, suggesting persistent damage to the afferent nerve system as a frequent complication in persons exposed to sulfur mustard (Thomsen et al. 1998).

Guinea pigs treated with a single dose of 51.3 mg/kg (1 LD_{50}) of neat sulfur mustard applied to their hair-clipped backs became sedated 1 day after exposure (Chauhan and Murty 1997).

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to sulfur mustard. However, it is likely that dermal exposure contributed to the effects observed in subjects exposed to sulfur mustard in the air described in Section 3.2.1.5.

3.2.3.6 Developmental Effects

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

3.2.3.7 Cancer

Five cases of Bowen's disease (a type of skin cancer) were studied among 488 former workers of a Japanese poison gas factory (Inada et al. 1978). Workers were involved in manufacturing sulfur mustard for 3–15 years and the diagnosis was made 31–41 years after exposure. These workers also suffered from acute dermatitis, conjunctivitis, bronchitis, and hyperkeratotic skin eruptions. The occurrence of Bowen's disease, Bowen's carcinoma, basal cell carcinomas, and spinocellular carcinoma has also been reported in survivors of the dismantling of the "Heeres-Munitionsanstalt St. Georgen" who were exposed to poisonous gases including sulfur mustard by skin contact and inhalation (Klehr 1984).

No studies were located regarding cancer in animals after dermal exposure to sulfur mustard, although animals exposed to sulfur mustard in the air could have also had skin exposure. Cancer in these animals is discussed in Section 3.2.1.7.

3.2.4 Other Routes of Exposure

Several animal studies indicate effects of sulfur mustard on the hemopoietic system following intravenous or subcutaneous administration of sulfur mustard. Single intravenous injection of 0.5 mg/kg of sulfur mustard dissolved in thiodiglycol in young male rats caused degenerative damage to the spleen, thymus, and bone marrow (Kindred 1947). This was also observed in rats, mice, rabbits, and dogs following a subcutaneous injection of 3 mg/kg of sulfur mustard (Graef et al. 1948). Within 12 hours of injection, granulocytosis was observed, followed by leukopenia. In addition to hemopoietic tissue damage, injury to the testes with inhibition of spermatogenesis were also observed. An intraperitoneal injection of 15 mg/kg of sulfur mustard in olive oil depressed the activity of bone marrow cells of the femur in mice (Friedberg et al. 1983). Parenteral administration of sulfur mustard to laboratory animals resulted in death due to systemic intoxication, with little or no involvement of the eyes or skin. Damage to the lungs was seen with intravenous administration of neat sulfur mustard or a solution of sulfur mustard in either propylene glycol or thiodiglycol, but not other parenteral routes (Anslow and Houck 1946).

A significant dose-related reduction in spleen cell number was measured in mice 7 days after intraperitoneal injection of sulfur mustard (23% at 5 mg/kg and 49% at 10 mg/kg) (Coutelier et al. 1991). Sv female mice (5–9/group) were injected intraperitoneally with a single dose of 2, 5, or 10 mg/kg sulfur mustard (>90% purity) in a 1% isopropanol solution in saline. A 26% increase in spleen T-lymphocytes and a 44% decrease in B-lymphocytes was measured 7 days following injection with 10 mg/kg of sulfur mustard. B- and T-lymphocyte function, as assayed by *in vitro* thymidine incorporation and/or immunoglobulin secretion, was not significantly affected by sulfur mustard.

The retinas of rats sacrificed 24 hours after subcutaneous injection in the dorsal area with 10 µL of undiluted radiolabeled sulfur mustard showed edematous swelling of the inner layers. Cell degenerative changes included dense cytoplasm, enlarged mitochondria, and Golgi apparatus. Rats sacrificed at 48 hours after injection had highly disorganized and vacuolated outer segment membranes and the choroid vessels contained large clusters of activated platelets (Klain et al. 1991).

Sulfur mustard, administered to guinea pigs by intratracheal injection, induced a 3-fold increase in respiratory system resistance, accompanied by a significant decrease in compliance (Calvet et al. 1993). Capsaicin-sensitive nerves do not have primary involvement in the acute respiratory effects of sulfur mustard as pretreatment with capsaicin did not prevent acute effects. Fourteen days after exposure, substance P induced concentration-dependent bronchoconstriction in guinea pigs, and tracheal epithelium neutral endopeptidase (NEP), the main enzyme that degrades tachykinins, was reduced significantly (40%) from the control level. While hyper-responsiveness to substance P has been attributed to a decrease in the tracheal activity of NEP and corresponding increase in tachykinins, this hypothesis was not upheld, as pretreatment with phosphoramidon, a NEP inhibitor, only increased sulfur mustard-induced hypersensitivity to substance P. Phosphoramidon administered prior to vehicle control ethanol also increased sensitivity to substance P.

3.3 GENOTOXICITY

Low doses of sulfur mustard can inhibit cell division due to its ability to cross-link complementary strands of DNA or produce mutagenesis, which may be caused by replication or repair errors (Papirmeister 1993). DNA is the most functionally sensitive target of sulfur mustard in cells. Men who were exposed to sulfur mustard from leaking shells picked up by fishing vessels showed increased sister chromatid exchanges in their lymphocytes (Wulf et al. 1985). However, the offspring of workers exposed to sulfur mustard in a Japanese factory showed no increases in diseases that would be indicative of genetic damage (Yamakido et al. 1985).

Sulfur mustard induced dose-related interstrand cross-links in the DNA of rat epidermal keratinocytes in primary monolayer cultures (Lin et al. 1996a), thus affecting the cell cycle and DNA synthesis (Lin et al. 1996b). Similar effects on DNA from rat cutaneous keratinocytes were reported by Ribeiro et al. (1991). Sulfur mustard has also been shown to affect the repair of mismatched bases in the DNA in African green monkey kidneys cells (Fan and Bernstein 1991).

DNA extracted from white blood cells of human blood and exposed to [14C]-labeled sulfur mustard *in vitro* was shown to contain the DNA adduct 7-(2-hydroxyethylthioethyl)guanine (Ludlum et al. 1994). Sulfur mustard alkylation has also been shown to affect transcriptional processes by leading to the production of truncated transcripts (Masta et al. 1996). This occurs when the RNA polymerase remains

associated with an alkylated promoter. Analysis of truncated transcripts revealed that sulfur mustard alkylates DNA preferentially at 5'-AA, 5'-GG, and 5'-GNC sequences on the DNA template strand.

Sulfur mustard at concentrations of 0.5 and 0.1 mM produced single strand breaks in bacteriophage lambda DNA (Venkateswaran et al. 1994b), which were prevented by the presence of magnesium ions in the reaction mixture. The authors proposed that the degradation of lambda DNA by its interaction with sulfur mustard may be caused by the breakage of phosphodiester backbone of DNA via the formation of an intermediate phosphotriester bond.

A variety of *in vitro* assays, summarized in Table 3-3, provide positive genotoxicity results. These data support the few human data on *in vivo* exposures to this compound. The *in vitro* data from both prokaryotic organisms (*Salmonella typhimurium* and *Escherichia coli*) and eukaryotic organisms (HeLa cells, mouse lymphoma, mouse L cells, rat lymphosarcoma) all support a mechanism of DNA alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutation, and chromosome and chromatid aberration formation.

There are also data from *Drosophila* experiments in which sulfur mustard was injected into male flies, leading to sex-linked lethal mutations and point mutations at one of the loci affecting bristle formation (Auerbach 1947; Fahmy and Fahmy 1971, 1972). Sulfur mustard has also been shown to be a micronucleus-inducing agent to the mouse bone marrow (Ashby et al. 1991). All of these data are consistent with this agent being a powerful genotoxicant, which supports the recognized carcinogenicity of sulfur mustard.

Transcription, translation, and enzyme catalysis, cellular activities that are dependent on biological entities of much lower molecular size than chromosomal DNA, are much less sensitive to sulfur mustard (Papirmeister 1993). Thus, cells that are prevented from synthesizing DNA continue to generate energy and synthesize RNA and protein. As a result of this unbalanced metabolism, cells may enlarge, differentiate, or be induced to synthesize high levels of certain proteins. While some of these proteins may protect cells, others may hasten cell death.

Vesicant and acute tissue injury only occur at sulfur mustard alkylation levels much higher than those needed to produce genotoxic effects. Tissue injury does not develop when low, therapeutically effective doses of sulfur mustard are used to control the hyperproliferation of psoratic keratinocytes. Therefore, it

Table 3-3. Genotoxicity of Sulfur Mustard *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Escherichia coli	DNA interstrand crosslinks	+	No data	Venitt 1968
E. coli	DNA recombination repair inhibition	+	+	Ichinotsubo et al. 1977
Salmonella typhimurium	Gene mutation	+	+	Ichinotsubo et al. 1977
S. typhimurium	Gene mutation	+	+	Ashby et al. 1991
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	DNA alkylation	+	No data	Kircher and Brendel 1983
Human HeLa cells in culture	DNA crosslinking	+	No data	Ball and Roberts 1971/72
Mouse lymphoma cells	Gene mutation	+	No data	Capizzi et al. 1974
Mouse lymphoma cells	Chromosomal and chromatid aberrations	+	No data	Scott et al. 1974
Rat lymphosarcoma cells	Chromosomal and chromatid aberrations	+	No data	Scott et al. 1974
Rat lymphosarcoma cells	DNA replication repair inhibition	+	No data	Scott et al. 1974
Mouse fibroblasts, L-strain	Inhibition of DNA synthesis	+	No data	Walker and Thatcher 1968

+ = positive result; DNA = deoxyribonucleic acid

is likely that additional mechanisms other those related to genotoxicity are responsible for acute toxicity of sulfur mustard.

3.4 TOXICOKINETICS

There is a substantial toxicokinetic database for intravenous and intraperitoneal routes of sulfur mustard exposure in animals. While these data are useful, there is evidence to suggest that this information does not mimic the scenario resulting from field or accidental conditions that expose humans to sulfur mustard by absorption from the skin, or by the lung or eyes. Sulfur mustard tissue distribution data from an Iranian soldier who died 7 days after inhalation and/or dermal exposure to sulfur mustard indicated distribution: brain > kidney > liver > spleen > lung (Drasch et al. 1987), whereas radiolabel concentration data in rats 4 days after an intravenous injection of radiolabeled sulfur mustard indicate a different distribution pattern to these organs: kidney > lung > liver > spleen > brain (Maisonneuve et al. 1994). While the difference could be due to measurement methods, species variations, or postexposure time, the route of exposure appears to be a significant toxicokinetic factor.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Since sulfur mustard can be found in human tissues following exposure through the air, it can apparently be absorbed through the lungs or skin (Drasch et al. 1987). Analyses of the blood of hairless guinea pigs after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm) of sulfur mustard indicated that the concentration of sulfur mustard in blood peaked within 5 minutes after inhalation exposure (Langenberg et al. 1998).

In rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of 40, 100, and 500 mg/m^3 of sulfur mustard, about 15% of the dose was recovered, indicating that 85% was absorbed through the mucous membrane of the nose (Cameron et al. 1946).

The absorption of sulfur mustard through the cornea was demonstrated in guinea pigs (Klain et al. 1991). Following 30 minutes after a single topical application of 5 µL of radiolabeled sulfur mustard to the

cornea of guinea pigs, radioactivity was detected in kidney, liver, lung, adipose tissue, adrenals, plasma, and muscle.

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans or animals after oral exposure to sulfur mustard. Absorption via the oral route has been demonstrated in animal studies in which sulfur mustard dissolved in sesame oil was administered by gavage to rats (DOA 1987; Sasser et al. 1993, 1996a, 1996b) and rabbits (DOA 1987).

3.4.1.3 Dermal Exposure

Sulfur mustard is readily absorbed through the skin. When applied to human skin, most of the sulfur mustard evaporates (Smith et al. 1919). Some of the absorbed sulfur mustard remains in the skin, whereas the majority passes into the blood stream (Cullumbine 1946, 1947; Nagy et al. 1946; Renshaw 1946). Renshaw (1946) reported that 80% of unoccluded, topically-applied sulfur mustard evaporates from the skin and the remaining fraction penetrates the skin. This finding has been confirmed in studies of human foreskin grafted onto athymic mice (Papirmeister et al. 1984a, 1984b).

The absorption of sulfur mustard through the cornea was demonstrated in guinea pigs (Klain et al. 1991). Following 30 minutes after a single topical application of 5 µL of radiolabeled sulfur mustard to the cornea of guinea pigs, radioactivity was detected in kidney, liver, lung, adipose tissue, adrenals, plasma, and muscle.

Hambrook et al. (1993) reported that after a 6-hour cutaneous exposure with occlusion, >90% of the applied dose was absorbed in rat skin. The initial rate of uptake, within 60 minutes of loading, increased linearly with applied dosage in the range of 3–605 µg/cm^2 (0.2–3.8 µmol/cm^2), and reached a maximum of approximately 7 µg/cm^2/minute (0.044 µmol/cm^2/minute) at a dosage of 955 µg/cm^2 (6 µmol/cm^2) (Hambrook et al. 1993). A range of skin-retention fractions from 10 to 50% has been reported (Cullumbine 1947; Hambrook et al. 1992; Renshaw 1946), while the remaining sulfur mustard is absorbed systemically. The rate of penetration of sulfur mustard into human skin was estimated in the range of 1–4 µg/cm^2/minute (0.006–0.025 µmol/cm^2/minute) (Renshaw 1946). Skin penetration of sulfur mustard is proportional to its temperature (Nagy et al. 1946).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Analyses of body fluids and tissues of an Iranian soldier who died 7 days after exposure to sulfur mustard (by inhalation and/or dermal routes) indicated that sulfur mustard was distributed to cerebrospinal fluid and, in order of decreasing concentrations, fat (from thigh), brain, abdominal skin, kidney, muscle, liver, spleen, and lung (Drasch et al. 1987). Analyses of the blood of hairless guinea pigs after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm; 2,400 mg-minute/m^3) of sulfur mustard indicated that the concentration of sulfur mustard in blood peaked within 5 minutes after exposure, dropped to about 50% of peak at 30 minutes, and gradually increased again to about 60% of peak concentration at 4 hours after exposure (Langenberg et al. 1998). Evidence of tissue sulfur mustard DNA adducts in hairless guinea pigs at 4 hours after 5-minute nose-only exposure to 160 mg/m^3 (25 ppm; 800 mg-minute/m^3) of sulfur mustard indicates absorption and/or distribution to nasal epithelium, nasopharynx, larynx, carina, and lung (Langenberg et al. 1998). Sulfur mustard DNA adducts found in the lung, spleen, and bone marrow in the same species after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm; 2,400 mg-minute/m^3) of sulfur mustard indicates distribution to these tissues (Langenberg et al. 1998).

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans or animals after oral exposure to sulfur mustard.

3.4.2.3 Dermal Exposure

As reported in Section 3.4.2.1, analyses of body fluids and tissues of an Iranian soldier exposed to airborne sulfur mustard indicated sulfur mustard distribution to most major organs (Drasch et al. 1987), consistent with older reports (Cullumbine 1947). Hambrook et al. (1993) reported that after a 6-hour cutaneous exposure to radiolabeled sulfur mustard with occlusion, 10–23% of absorbed radiolabel dose was retained in rat skin, with 3–7% detected in blood. At the end of the 6-hour application, when the level of radiolabel in the blood reached a maximum, greater than 90% of the red cell radiolabel activity was found within the cell, and the rest in the red cell membrane.

In guinea pigs, following a single topical application of 5 µL of radiolabeled sulfur mustard to the cornea, radioactivity at 30 minutes after application, as expressed per unit weight, was greatest in the kidney followed by liver, lung, adipose tissue, adrenals, plasma, and muscle (Klain et al. 1991). At 2 and 5 hours postadministration, the greatest radioactivity per unit weight was again measured in the kidney, whereas the level in the plasma increased and that in the liver and lung decreased with postadministration time. Expressed per organ, the liver contained the highest level of radioactivity, followed by the kidney and lung. At 30 minutes postapplication, radioactivity was widely distributed in the guinea pig eye; the choroid/sclera portion contained the highest level followed by cornea, retina, and lens. Low levels were also detected in the aqueous and vitreous humors. At 5 hours, the only eye compartment in which the radioactivity level had decreased significantly from the 30-minute value was the choroid/sclera portion.

3.4.2.4 Other Routes of Exposure

Boursnell et al. (1946) observed significant radioactivity levels in the kidney, lung, and liver of rabbits after intravenous injection of 5 mg/kg of radiolabeled sulfur mustard. Lower levels of radioactivity were also detected in bone marrow, spleen, stomach wall, duodenal wall, brain, heart, muscle, skin, and thyroid. Six hours after intravenous injection of 8.2 mg/kg of radiolabeled sulfur mustard into male hairless guinea pigs, radiolabel was distributed in decreasing concentrations to the bone marrow, liver, spleen, blood, and lung (Langenberg et al. 1998). In the rat, sulfur mustard is quickly and widely distributed (Maisonneuve et al. 1993, 1994; Zhang and Wu 1987). Maisonneuve et al. (1993) reported a distribution volume of 74.4 L/kg and a half-life of 5.6 minutes following intravenous bolus administration of 10 mg/kg (3 LD_{50}) of sulfur mustard in the rat. The concentration of unchanged sulfur mustard in the blood decreased quickly in the first half hour, but low levels were detectable up to 8 hours after administration. The large volume of distribution, greater than the volume of body water, suggests a wide distribution of sulfur mustard throughout the animal. A quantitative distribution analysis was performed by Maisonneuve et al. (1994) in rats intravenously injected with radiolabeled sulfur mustard. Radioactivity was detected in blood, plasma, kidney, liver, intestine and stomach, heart, lung, brain, spleen, eyes, testicle, and adrenal gland. From 10 minutes to 6 hours after administration, the liver and kidney had higher radiolabel concentrations than the blood. The organs with the lowest levels of radioactivity were the brain, spleen, eye, and testicle. Maximum radioactivity levels in the organs were reached between 2 and 3 hours after injection. Total radioactivity in any organ did not exceed 4% of the administered dose. Most of the administered radioactivity was recovered in the muscle; 51% measured in

muscle at 5 minutes, 36% in muscle at 3 hours, 3% in fat at 35 minutes, and 10% in skin at 35 minutes (radioactivity peaked in fat and skin at 35 minutes).

In vitro studies of plasma and red blood cells treated with radiolabeled sulfur mustard indicate a high affinity of sulfur mustard toward red blood cells (Maisonneuve et al. 1993). The mean equilibrium red blood cell/plasma radiolabel concentration ratios for treatments with 4 and 400 µg/mL radiolabeled sulfur mustard were 2.12 and 4.15, respectively.

Radiolabeled sulfur mustard administered in rats to the femoral or jugular veins resulted in different organ distribution patterns. Subsequent to femoral vein injection, the injected leg was a site of significant sulfur mustard distribution, whereas jugular vein injection did not result in significant accumulation in the lung (Maisonneuve et al. 1994). The heart, lung, brain, and spleen received greater proportionate shares of radioactivity 35 minutes after jugular vein injection compared to femoral vein administration.

In rats subcutaneously injected with 10 µL of undiluted radiolabeled sulfur mustard, examination of the eyes 4 hours after treatment revealed the highest level of radioactivity in the pooled aqueous and vitreous humors (70%), followed by retina (12%), choroid/sclera (8%), lens (6%), and cornea (3%) (Klain et al. 1991).

3.4.3 Metabolism

The metabolism of sulfur mustard has not been studied extensively. Sulfur mustard tends to undergo intramolecular cyclization to create a hyperactive compound (see Section 6.3.2). Conversion to this derivative is facilitated in aqueous solution (Somani and Babu 1989), which accounts for the sensitivity of mucosal tissues, such as the eye, to its action (Solberg et al. 1997). Sulfur mustard cyclic intermediates react with and alkylate electron-rich molecular structures, such as the sulfhydryl (-SH) and amino ($-NH_2$) groups of proteins and nucleic acids (Solberg et al. 1997). Metabolic pathways, including direct alkylation reactions, glutathione reactions, hydrolysis, and oxidation, are presumed based on the finding of sulfur mustard DNA adducts in tissues (Fidder et al. 1994, 1996a; Niu et al. 1996; Somani and Babu 1989; Van der Schans et al. 1994) and the identification of urinary products (Jakubowski et al. 2000; Wils et al. 1985, 1988).

3.4.3.1 Inhalation Exposure

Jakubowski et al. (2000) measured elevated levels of thiodiglycol, the major sulfur mustard hydrolysis product, in human urine following an accidental exposure to sulfur mustard vapor and aerosol. Thiodiglycol was also found in the urine of people exposed to airborne sulfur mustard during the Iran-Iraq War (Wils et al. 1985, 1988).

A sulfur mustard adduct in DNA, the 2′-deoxyguanosine derivative of N7-HETE-guanine, N7-(2-hydroxyethylthioethyl)-2′-deoxyguanosine, as well as albumin- and hemoglobin-sulfur mustard adducts, have been detected in the blood of two sulfur mustard poisoning victims of the Iran-Iraq War (Benschop et al. 1997; Noort et al. 1999). Sulfur mustard DNA adducts were found in the nasal epithelium, nasopharynx, larynx, carina, lung, spleen, and bone marrow of hairless guinea pigs after nose-only exposure to sulfur mustard (Langenberg et al. 1998).

3.4.3.2 Oral Exposure

No studies were located regarding metabolism in humans or animals after specific oral exposure to sulfur mustard.

3.4.3.3 Dermal Exposure

Studies of casualties of the Iran-Iraq War with obvious signs of sulfur mustard-induced cutaneous injuries have identified significant amounts of the sulfur mustard metabolite, thiodiglycol, in human urine for up to 2 weeks after sulfur mustard exposure (Wils et al. 1985, 1988). As reported in Section 3.4.3.1, DNA-, hemoglobin- and albumin-sulfur mustard adducts have been detected in the blood of sulfur mustard poisoning victims (Benschop et al. 1997; Noort et al. 1999). In two subjects following an accidental predominantly cutaneous exposure to sulfur mustard, thiodiglycol, thiodiglycol sulphoxide, and closely related metabolites, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methyl-thio)ethylsulphonyl]ethane, derived from the action of β-lyase on cysteine conjugates, were detected in the urine (Black and Read 1995a). Thiodiglycol sulphoxide concentrations were 20–35 times thiodiglycol concentrations. The β-lyase metabolites were detected at concentrations comparable with those of thiodiglycol sulphoxide (Black and Read 1995a). The presence of urinary biotransformation product thiodiglycol sulphoxide is consistent with findings in animal studies discussed below in which sulfur

mustard was administered by alternate routes (Black et al. 1992a). Sandelowsky et al. (1992) reported the detection of sulfur mustard metabolite, 4-met-1-imid-thiodiglycol, in plasma and urine following dermal exposure of sulfur mustard in pigs.

3.4.3.4 Other Routes of Exposure

In a metabolic study, radiolabeled sulfur mustard dissolved in ethanol was administered intravenously to rats, mice, and two terminal cancer patients (Davison et al. 1961). Several minutes after administration, 80–90% of the radioactivity was cleared from human blood. The residual level remained constant in both plasma and cells for at least 2 days, suggesting binding to some blood constituent. Chromatographic analyses of urine yielded similar eluant patterns for rats and mice, whereas those of the human subjects showed somewhat lower peaks at pH 2.8 and 2.3. The metabolism of sulfur mustard is apparently largely due to glutathione reactions, hydrolysis, and oxidation, since the major urinary metabolites identified in the rat were glutathione-bis-2-chloroethyl sulfide conjugates (45% of total), thiodiglycol and conjugates (14%), and sulfone products (20%). Unchanged sulfur mustard in excess of the 5 µg assay detection limit was not detected in rat urine.

Slightly different results were reported by Roberts and Warwick (1963), who found that at least 50% of the urinary metabolites in rats were conjugated forms of bis-cysteinyl-ethylsulphone. Thiodiglycol accounted for 15–20% of the urinary radioactivity, and 10–15% was a sulfide. Black et al. (1992b) similarly investigated the metabolism of sulfur mustard in the rat and identified urinary metabolites thiodglycol sulphoxide, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane], 1-[S-(N-acetylcysteinyl)]-2-(ethenylsulphonyl)ethane, 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane, two diastereoisomers of 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphinyl)ethane, 1,1'-sulphinylbis-[2-chloroethane], 1,1'-sulphonylbis[2-S(N-acetylcysteinyl)ethane], and 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphonyl)ethane. Black et al. (1992b), while confirming the major metabolic transformations of Davison et al. (1961), identified thiodiglycol sulphoxide as the major urinary excretion product and not the initial hydrolysis product thiodiglycol. The finding of metabolites 1,1'-sulphonylbis-[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane revealed a pathway for the degradation of glutathione conjugates formed via the action of enzyme β-lyase on cysteine conjugates. Renal β-lyase metabolism has also been implicated in the formation of nephrotoxic intermediates from halogenated alkenes.

A comparison of unchanged radiolabeled sulfur mustard and total radiolabel concentrations in the blood following intravenous bolus administration of radiolabeled sulfur mustard in rats indicated that much of the sulfur mustard is metabolized within a half-hour after administration (Maisonneuve et al. 1993).

Metabolites N7-HETE-guanine (Fidder et al. 1996b), derived from sulfur mustard DNA alkylation, and N7-HETE-valine (Fidder et al. 1996a), derived from sulfur mustard hemoglobin alkylation, have been detected in the urine of guinea pigs intravenously injected with sulfur mustard.

3.4.4 Elimination and Excretion

Urinary excretion is the primary route of elimination for sulfur mustard and/or its metabolites (Boursnell et al. 1946; Davison et al. 1961; Hambrook et al. 1992; Maisonneuve et al. 1993).

3.4.4.1 Inhalation Exposure

People who were exposed to sulfur mustard during the Iran-Iraq War could have absorbed the material through the lungs or through the skin. One of the breakdown products of sulfur mustard, thiodiglycol, was detected in the urine of these people (Wils et al. 1985). These authors also report that thiodiglycol was found in unexposed persons and could not be used to determine the exact level of sulfur mustard exposure, although it could possibly be used to show exposures to high levels. Unmetabolized sulfur mustard was also found in urine and feces samples from two Iran-Iraq War victims (Heyndrickx and Heyndrickx 1984; Mandl and Freilinger 1984; Pauser et al. 1984; Vycudilik 1985). No studies regarding animal excretion data from inhalation exposure are available.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans or animals after specific oral exposure to sulfur mustard.

3.4.4.3 Dermal Exposure

Jakubowski et al. (2000) measured the excretion of thiodiglycol in human urine following an accidental exposure to sulfur mustard vapor and aerosol. Detectable levels of thiodiglycol in urine were measured for 13 days after exposure to an undetermined level. The patient's urine was randomly sampled for 6 months after exposure and no further thiodiglycol elimination was detected. Maximum thiodiglycol excretion was seen on postexposure day 4. First-order elimination kinetics was observed, and the half-life of thiodiglycol elimination was estimated to be 1.2 days.

Hambrook et al. (1992) reported that in the rat, following a 6-hour cutaneous exposure to radiolabeled sulfur mustard with occlusion, the urinary excretion of radiolabel had a half-life of 1.4 days; the half-life of excretion in feces, which varied slightly with dose, was approximately 1.6 days. Most of the radioactivity was found in the urine. Most of the dose was eliminated by 3 days; however, measurable urinary excretion of radiolabel continued for >3 months.

3.4.4.4 Other Routes of Exposure

Two terminal cancer patients were injected intravenously with radiolabeled sulfur mustard dissolved in ethanol (Davison et al. 1961). Several minutes after administration, 80–90% of the radioactivity was cleared from the blood. The residual level remained constant in both plasma and cells for at least 2 days, suggesting binding to some blood constituent. Excretion of 21% of the radioactivity in the urine occurred within 3 days.

The major route of elimination of radioactivity in the rat, after intravenous injection of radiolabeled sulfur mustard is by the kidney (Boursnell et al. 1946; Davison et al. 1961; Hambrook et al. 1992; Maisonneuve et al. 1993). Maisonneuve et al. (1993) reported a blood clearance of 21 L/hours-kg, indicating rapid excretion from the body, and elimination half-life of 3.59 hours from blood concentration data following intravenous bolus administration of 10 mg/kg (3 LD_{50}) of radiolabeled sulfur mustard in the rat. Maximum blood radioactivity was observed 1 hour after sulfur mustard administration and, similarly to that found in humans (Davidson et al. 1961), a residual constant level of radioactivity (approaching 70% of maximum) was found in blood 2 days after exposure; a second peak approaching the maximum level was observed between 2 and 4 days. The largest overall recovery of radioactivity was in urine, with about 65% of the administered dose excreted during 24 hours and 80% during 96 hours, a much higher

percentage than that reported for humans (Davison et al. 1961). Fecal excretion accounted for <3% of the administered dose during 96 hours (Maisonneuve et al. 1993).

Rats and mice that were injected intraperitoneally with radiolabeled sulfur mustard excreted 50–78% of the radioactivity within 1 day and 90% within 3–5 days in the urine (Black et al. 1992a; Davison et al. 1961; Roberts and Warwick 1963; Smith et al. 1958). Twelve hours after intraperitoneal injection, 6% was excreted in the feces and 0.05% in the expired air (Davison et al. 1961).

Hambrook et al. (1992) measured the excretion of radiolabel in urine and feces in the rat following intravenous or intraperitoneal injection of radiolabeled sulfur mustard. The half-life varied little with dose, route, or excretion type and an average value of 1.4 days was reported. The pattern of excretion was similar after intraperitoneal and intravenous injections. Most of the dose was eliminated by 3 days; however, urinary excretion of radiolabel continued for greater than 3 months. About 65% of absorbed radiolabel was found in the urine and 11% in feces within 24 hours after administration.

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.

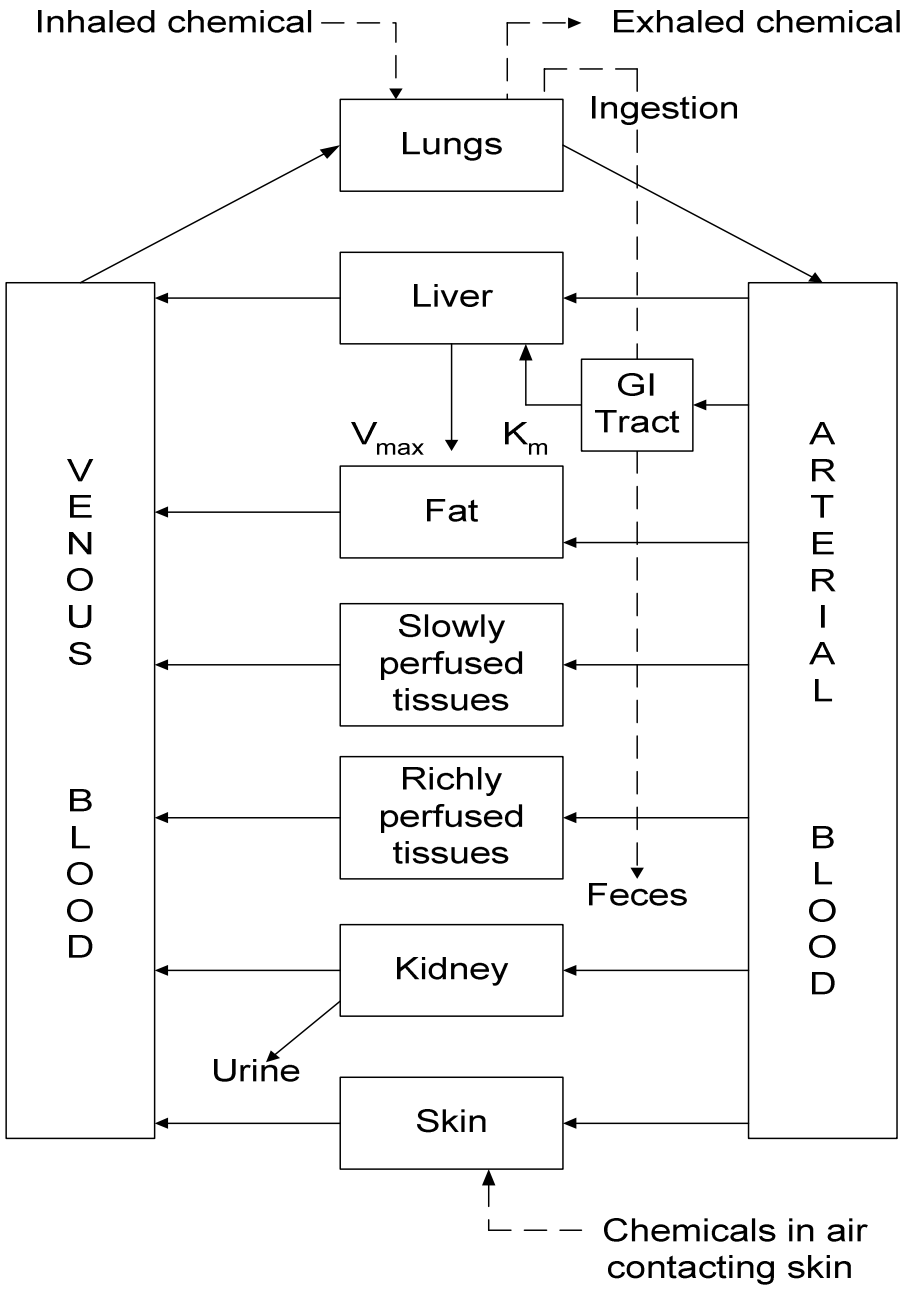
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 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 PBPK models exist for sulfur mustard. Toxicokinetic information is insufficient for modeling.

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 physicologically 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.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. Sulfur mustard is slightly soluble in water, but both the liquid and vapor forms are readily soluble in oils, fats, alcohol, and organic solvents. Because of its high lipid solubility, sulfur mustard quickly penetrates the lipid cell membrane.

Distribution. It has been estimated that about 12% of dermally absorbed sulfur mustard reacts with components in skin and the remainder is distributed in greatest proportion to the kidney and fairly evenly throughout the rest of the body as unreacted sulfur mustard or hydrolyzed sulfur mustard. In studies with radiolabeled sulfur mustard, tissue radioactivity levels increased as early as 5 minutes after intravenous injection and 15 minutes after percutaneous administration.

Metabolism. Sulfur mustard is presumed to be biotransformed by direct alkylation reactions, glutathione reactions, hydrolysis, and oxidation based on the finding of sulfur mustard DNA adducts in tissues and the identification of urinary products.

Excretion. Urinary excretion is the primary route of elimination for sulfur mustard and/or its metabolites. In humans, elimination follows first-order kinetics and the half-life of thiodiglycol elimination is estimated to be 1.2 days (Jakubowski et al. 2000).

3.5.2 Mechanisms of Toxicity

Several studies have shown that sulfur mustard applied topically can diffuse and produce biochemical alterations consistent with free-radical-mediated oxidative stress, including increased lipid peroxidation and antioxidant enzyme activities, depletion of glutathione content, and increased glutathione content in eye, kidney, brain, lungs, and liver of rats and mice (Arroyo et al. 2000). Compounds containing reactive chlorine, preferably a chloroamide group, have been demonstrated as useful neutralizers of sulfur mustard (Arroyo et al. 2000). Sulfur mustard undergoes nucleophilic substitution reactions to form a sulfonium ring (Yang et al, 1992) that, in the presence of oxygen, generates a non-toxic sulfoxide reactive intermediate (Arroyo et al. 2000). More extensive oxidation results in a toxic sulfone species (Arroyo et al. 2000).

At the cellular level, sulfur mustard interacts with nucleophiles on the cell membrane, at intracellular sites, and with nucleic acids (Papirmeister et al. 1991). While sulfur mustard is able to alkylate DNA, RNA, and proteins affecting a variety of cell functions, including altering proteins that have been coded by alkylated RNA and structurally altering cell membranes, DNA is the most functionally sensitive target of sulfur mustard in cells. Low doses of sulfur mustard can inhibit cell division due to its ability to cross-link complementary strands of DNA (Papirmeister 1993). Transcription, translation, and enzyme catalysis, cellular activities that are dependent on biological entities of much lower molecular size than chromosomal DNA, are much less sensitive to sulfur mustard. Thus, cells that are prevented from synthesizing DNA continue to generate energy and synthesize RNA and proteins. As a result of this unbalanced metabolism, cells may enlarge, differentiate, or be induced to synthesize high levels of certain proteins. While some of these proteins may protect cells, others may hasten cell death.

Mechanisms of the toxicity of sulfur mustard have been postulated, but none have been demonstrated with certainty (Papirmeister 1993; Somani and Babu 1989; Whitfield 1987). As discussed in Section 3.3, it appears that different mechanisms are responsible for the acute and delayed effects of sulfur mustard and that additional mechanisms besides genotoxicity mechanisms are responsible for sulfur mustard vesication since acute skin injury develops at a time much earlier than expected from genotoxic effects. Also, tissue injury does not develop when low, therapeutically effective doses of sulfur mustard are used to control the hyperproliferation of psoratic keratinocytes.

While the mechanisms of sulfur mustard toxicity are not currently fully understood, one hypothesis for sulfur mustard cytotoxicity involves poly(ADP-ribose) polymerase (PARP). The following mechanism for skin damage has been proposed: sulfur mustard alkylates DNA, which causes DNA breaks; numerous sulfur mustard-induced DNA strand breaks cause activation of nuclear repair enzyme PARP. This causes cellular depletion of nicotinamide adenine dinucleotide (NAD^+), which decreases glycolysis, which leads to protease release and cellular injury. Dermal-epidermal separation and blister formation may involve the fragmentation of anchoring filaments by protease released from moribund or dead cells (Papirmeister 1993). Clark and Smith (1993) showed that sulfur mustard treatment of HeLa cells produces a rapid stimulation of PARP activity, followed 2 hours later by a decline in NAD^+ levels. Several other studies provide partial support for the hypothesis, but hint that additional pathways may be involved. The hypothesis is almost fully validated in a study by Meier and Kelly (1993), in which PADPRP inhibitors prevent the sulfur mustard-induced losses of ATP, NAD^+, and viability in human peripheral blood

lymphocytes. However, their observation that ATP levels decline before NAD^+ deviates from the expected response.

Niacinamide, an inhibitor of PARP and a substrate for NAD synthesis reduced sulfur mustard-induced loss in NAD (Meier et al. 1987; Mol et al. 1989, 1991; Papirmeister et al. 1985; Smith et al. 1990a) and ATP (Meier et al. 1996). 3-Aminobenzamide, an inhibitor of PADPRP but not a substrate for NAD synthesis, also reduced sulfur mustard-induced loss in ATP (Meier et al. 1996). Niacin, a substrate for NAD synthesis, which does not effect PADPRP, failed to prevent sulfur mustard-induced loss of ATP (Meier et al. 1996). These findings support the hypothesis that PARP plays a substantial role in sulfur mustard-initiated biochemical changes. Cowan et al. (1993) observed that although niacinamide-attenuated sulfur mustard-induced increases in protease activity *in vitro* and *in vivo*, it did not eliminate them, suggesting that pathways other than the one involving PADPRP may contribute to the increase in protease activity. Yourick et al. (1991, 1993) noted that while pretreatment with niacinamide reduced the incidence of sulfur mustard-induced microvesiculation in hairless guinea pig skin, the prediction of the PARP hypothesis, that the loss of NAD^+ precedes tissue injury, was not upheld. Martens and Smith (1993) demonstrated that whereas sulfur mustard treatment of human epidermal keratinocytes (HEK) produces a dose-related depletion of NAD^+ and inhibition of glucose metabolism, preceding cell death, niacinamide did not prevent the inhibition of glycolysis, suggesting that in HEK, other energy-depleting mechanisms may be involved in sulfur mustard cytotoxicity. In contradiction to the hypothesis, results in rat keratinocytes exposed to sulfur mustard indicate that depletion of NAD is not a prerequisite for cell death (Lin et al. 1994). At doses lower than 50 μM, DNA content, viable cell number, and the proliferative capacity of the culture, as assessed by thymidine incorporation, were all reduced, whereas the total NAD level (NAD^+ plus NADH) was not changed. Also supplementing the culture with nicotinamide after exposure to sulfur mustard did not reverse the decrease in DNA content.

As another hypothesis for sulfur mustard-induced cytotoxicity, Whitfield (1987) suggested that sulfur mustard alkylation of glutathione (GSH) removes one of the major cellular defense mechanisms against electrophilic compounds and oxidants. Once GSH is depleted, electrophiles such as sulfur mustard or endogenously-generated reactive oxygen species eventually inactivate critical sulfhydryl proteins involved in calcium homeostasis and/or modify cytoskeletal elements. The subsequent inability of cells to maintain the low intracellular calcium concentration causes activation of catabolic processes leading to cell damage and death. In partial support of this hypothesis, Ray et al. (1993) demonstrated that treatment of neuroblastoma cells and HEKs with sulfur mustard causes depletion of GSH, raises the level of intracellular calcium, and stimulates phospholipase A2, processes that precede and ultimately lead to

membrane damage and cell death. Also, increasing cellular GSH levels decreased the toxic effects of sulfur mustard in human peripheral blood lymphocytes (Gross and Smith 1993).

Apoptosis may be a mechanism by which sulfur mustard exerts its cytotoxic effects. In keratinocytes incubated with sulfur mustard, p53 (a promoter of apoptosis) levels increases, while levels of bcl-2 (a suppressor of apoptosis) decreased (Rosenthal et al. 1998). The immunostaining pattern of these two markers in sulfur mustard treated skin excised from weanling pigs also suggests the involvement of apoptosis in cell death secondary to sulfur mustard exposure (Smith et al. 1997a). Thymocytes, isolated from rats, and incubated with sulfur mustard showed an increase in the production of DNA fragments characteristic of apoptosis (Michaelson 2000). It is possible that sulfur mustard toxicity involves several independent or interacting pathways, some aspects of the various hypotheses.

Lundy et al. (1998) proposed that sulfur mustard-induced cytotoxicity results from activation of purinergic P2 receptors. P2 antagonists were shown to reduce sulfur mustard-induced cytotoxicity, providing support for this hypothesis (Doebler 2002).

Cell cycle kinetics are involved in the cytotoxic processes following sulfur mustard exposure. Sulfur mustard-induced damage at subvesicating concentrations (<50 μM) to genomic DNA in cultured HEK resulted in a dose-related reversible block at the G2/M phase of the cell cycle (Smith et al. 1993a). Okadaic acid and calyculin A, inhibitors of protein phosphatase 2A (PP2A), completely reversed the sulfur mustard-induced G2/M block, whereas tautomycin, an inhibitor of protein phosphatase 1, was ineffective at reversing the block (Hart and Schlager 1997). As total cellular PP2A was not affected by sulfur mustard treatment; these results suggest that PP2A is involved in the G2/M block produced by exposure of HEK to low concentrations of sulfur mustard. Exposure of human peripheral blood lymphocytes (PBL) to vesicating equivalent concentrations of sulfur mustard (≥50 μM) resulted in irreversible blockage at the G1/S interface (Smith et al. 1998). DNA became terminally fragmented.

Theories have been proposed that blistering induced by sulfur mustard may involve cytokine production and a secondary inflammatory response (Dannenberg and Tsuruta 1993; Graham et al. 1994; Papirmeister et al. 1991). In the trachea as in the skin, sulfur mustard appears to preferentially damage the cells that are the most active in regeneration after aggression, basal cells located above the dermal papillae in skin (Papirmeister et al. 1991), and epithelial secretory cells in the trachea (Calvet et al. 1996). In the cell, DNA and proteins are the main targets for sulfur mustard alkylation; therefore, it is not unexpected that the most severe lesions affect cells with the greatest progenitorial and metabolic capacity. Eosinophils,

known to produce growth factor and cytokines, were reduced in guinea pigs at 2 weeks postexposure, which may influence epithelial regeneration and result in the characteristic slow lesion repair or recovery (Calvet et al. 1996). The literature contains conflicting reports of sulfur mustard effects on cytokines. In cultured HEK treated with 1–100 µM sulfur mustard, Pu et al. (1995) observed a dose-related increase in IL-1α at 72 hours after exposure. Zhang et al. (1995) also measured an increase in IL-1α in isolated perfused porcine skin treated with sulfur mustard at 5 hours after exposure. In contrast, Kurt et al. (1998) who tested the effects of sulfur mustard on both adult and neonatal HEK, reported a dose-related decrease in IL-1α in cultured adult HEK treated with 0.5 and 1.0 mM sulfur mustard; however, only a minimal change in IL-1α was seen in neonatal HEK. Sulfur mustard applied to the mouse ear resulted in an increase in IL-6 levels at 6 and 18 hours postexposure, whereas IL-1β and TNF-α levels were unchanged (Casillas et al. 1996). Kurt et al. (1998) reported that in both neonatal and adult HEK, TNF-α was increased at 0.5 mM and decreased at 1.0 mM sulfur mustard, whereas IL-1β, IL-6, and IL-8 were increased at both concentrations. While IL-1α and IL-1β share the same biological activity and recognize the same receptors on target cells, Kurt et al. (1998) suggest that the differences in the amount of each cytokine released relative to the distribution in HEK support different mechanisms of action for sulfur mustard with IL-1α and IL-1β. Since the decrease in IL-1β was the only cytokine of those studied with significant decreases in both neonatal and adult cell types and at both concentrations, Kurt et al. (1998) hypothesized a direct effect of sulfur mustard on IL-1β and indirect actions on the other cytokines.

In order to investigate possible mechanisms of blistering, urokinase, one of two mammalian activators for converting plasminogen into active plasmin, was investigated *in vitro* in cultured 3T3 fibroblasts exposed to 100 µM sulfur mustard (Detheux et al. 1997). Plasmin is a wide-spectrum serine protease, which is capable of degrading most extracellular and basement membrane proteins. Twenty-four hours after exposure, urokinase activity was increased 20-fold compared to control cells. The significance of this proteolytic response in the pathogenesis of blistering is not yet understood.

There have been several studies of protein alkylation by sulfur mustard with possible relevance to blister formation. A potential target for sulfur mustard alykation is uncein, an anchoring filament-associated antigen thought to play a role in maintaining the integrity of the dermal-epidermal basement membrane zone. Fractionation by SDS-PAGE and immonofluorescent staining of uncein treated with sulfur mustard indicated that sulfur mustard chemically modified uncein (Zhang et al. 1998). Male Yorkshire cross weanling pigs were exposed dermally to two vesicating doses, estimated at 21,000 and 42,000 mg-minute/m^3, of sulfur mustard (Smith et al. 1997a). Immunostaining of excised treated skin revealed a progressive decrease with eventual loss of expression of GB3, an antibody to basement membrane

protein, laminin 5, during the time of vesiculation at both doses. Desmosomal proteins, cellular fibronectin, laminin 1, collagen IV, and collagen VII showed no change or inconsistent changes during the same period. The laminins are cystein-rich proteins with multiple thiol groups available for alkylation by sulfur mustard. The pattern of immunostaining for laminin 5 was consistent with electron microscopy findings showing fragmentation of anchoring filaments at the time of vesication and suggests that disruption of laminin 5 may be a factor in sulfur mustard-induced blistering. Laminin 5 regeneration occurs early after injury, whereas cutaneous lesions are slow-healing with no evidence of re-epithelialization at 7 days after exposure in a hairless guinea pig model. The authors suggested that residual alkylated laminin 5 and laminin 1 fragments could inhibit the functioning of the newly formed laminin 5.

DNA arrays were used to study the differential gene expression changes that occur within human epidermal keratinocytes after exposure to sulfur mustard (Platteborze 2000). Several genes were identified that exhibited significant transcriptional upregulation that could have roles in early sulfur mustard injury. Transmembrane serine protease hepsin, which is thought to be involved in cell growth, differentiation, and maintenance of morphology, was upregulated about 8-fold at 10–30 minutes after exposure. Heparin sulfate proteoglycan 2 (HSPG2) was upregulated about 13-fold at 10 minutes and about 8-fold at 30 minutes after exposure. HSPG2 is an integral component of basement membranes and is proposed to be involved in cell binding, basement membrane assembly, calcium binding, LDL metabolism, activation of serine protease inhibitors, and the anchorage of acetylcholinesterase (AChE) to the extracellular matrix of the neuromuscular junction. In addition, heparin sulfate chains carry a fixed negative charge, which is thought to participate in the selective permeability of basement membranes. Human periodic tryptophan protein 2 (yeast) homolog (PWP2H) was also significantly overexpressed, about 7-fold at 10 minutes and about 14-fold at 30 minutes. At present, little is known about the function of PWP2H. A notable absence of upregulation of nucleotide repair genes, ERCC1 (Excision Repair Cross-Complementing repair deficiency group 1) and ERCC2, and enzyme PARP at 10 and 30 minutes postexposure suggests that the recognition or response of human epidermal keratinocytes to sulfur mustard genotoxicity is delayed, since PARP) activation was observed at 4 hours after exposure.

A dose-dependent inhibitory effect of sulfur mustard on the heat shock response (temperature-related synthesis of heat-shock proteins enabling an adaptive response) was found in mononuclear human cells (Sterri 1993). The effect was fully developed at subvesicating doses and was strongly dependent on the order of the exposures to sulfur mustard and stress effector. Heat shock protein expression was inhibited in cells exposed to sulfur mustard and subsequently heat shocked, whereas cells that were heat shocked

first and then exposed to sulfur mustard continued with the normal heat shock response. These results point to both transcriptional and translational sites of effect. The mechanistic coupling between the stress response and sulfur mustard remains to be understood.

Sawyer et al. (1996) examined the possibility that the toxicity of sulfur mustard is due to the induction or activation of nitric oxide synthase (NOS). L-nitroarginine methyl ester (L-NAME), an arginine analog inhibitor of NOS, was found to confer protection to mature primary cultures of chick embryo forebrain neurons against the toxicity of sulfur mustard when administered as a pretreatment or up to 3 hours postexposure. No protection was evident in immature (1-day-old) cultures. While NOS requires L-arginine as a substrate, sulfur mustard toxicity and L-NAME protection were independent of L-arginine concentration. In contrast to L-NAME, L-thiocitrulline (L-TC), another arginine analog NOS inhibitor, was found to protect immature cultures of neurons against sulfur mustard, as well as mature cultures (Sawyer et al. 1998). L-TC increased the LC_{50} of sulfur mustard by approximately 800 and 1,500% with 1- and 24-hour pretreatments, respectively. The protection conferred by L-TC was persistent, unlike L-NAME whose protection was dependent on its continued presence, suggesting that these closely related arginine analogs act at different sites to exert their effects (Sawyer et al. 1996, 1998). A synergistic protective effect was found in mature neuron cultures pretreated with both L-NAME and L-TC (Sawyer 1998). Whereas 1-hour pretreatment with L-NAME and L-TC increased the LC_{50} of sulfur mustard by approximately 200 and 800%, respectively, together up to 1,500% protection was conferred in mature cultures. Based on these findings, Sawyer (1998) proposed that sulfur mustard initiates its toxicity rapidly through a cell-surface mediated event, that can be blocked by L-TC, followed by signal transduction into the cell with an additional event manifested several hours later. The role of NOS in sulfur mustard toxicity remains unclear; however, these arginine analog NOS inhibitors provide protective effects, apparently not mediated through inhibition of NOS.

A study by Zhang et al. (1995) of the protective effects of four pharmacological agents in sulfur mustard-treated isolated perfuse porcine skin flap (IPPSF) suggests that different mechanisms are involved in the production of sulfur mustard-induced dark basal cells, microvesicles, and vascular response. Reduction of sulfur mustard-induced dark basal cells was observed with sulfur mustard scavengers, sodium thiosulfate and cysteine, with niacinamide, an inhibitor of poly(adenosine diphosphoribose) polymerase (PADPRP) and a substrate for NAD synthesis, and with cyclooxygenase inhibitor indomethacin. Treatments with niacinamide and indomethacin, but not sodium thiosulfate or cysteine, resulted in an inhibition of the vascular response in IPPSF exposed to sulfur mustard. Of the four agents, microvesicles were only partially prevented in the indomethacin-perfused IPPSF.

The toxic effects of sulfur mustard have been attributed to DNA modification with the formation of 7-hydroxyethylthioethyl guanine, 3-hydroxyethylthioethyl adenine, and the cross-link, di-(2-guanin-7-yl-ethyl)sulfide. Bacterial 3-methyladenine DNA glycosylase II (Gly II) was found to release both 3-hydroxyethylthioethyl adenine and 7-hydroxyethylthioethyl guanine from calf thymus DNA was modified with [14C]sulfur mustard, suggesting that glycosylase action may play a role in protecting cells from the toxic effects of sulfur mustard (Matijasevic et al. 1996).

Sulfur mustard was found to inhibit blood cell and tissue antioxidant enzyme activities in rats following topical application, which could impair cytoprotective defense mechanisms (Husain et al. 1996). Enzyme activities were measured at 24 hours after dermal treatment with 98 mg/mg (0.5 LD_{50}) of sulfur mustard. Superoxide dismutase (SOD) activity decreased significantly, 70% in white blood cells, 65% in platelets, 72% in the spleen, and 29% in brain. SOD activity in red blood cells, liver, and kidney did not change significantly following treatment. Catalase activity decreased significantly (54% in white blood cells, 23% in red blood cells, and 51% in spleen); activity levels in platelets, liver, kidney, and brain were not significantly altered. Glutathione peroxidase (GSH-Px) activity, as a consequence of glutathione and NADPH depletion, decreased significantly in white blood cells (42%), spleen (43%), and liver (22%). Glutathione activities in red blood cells, platelets, kidney, and brain were within 10% of control values.

A significant depletion of GSH of blood and liver was also observed in mice following dermal application of 38.7 or 77.4 mg/kg of sulfur mustard (Vijayaraghavan et al. 1991).

3.5.3 Animal-to-Human Extrapolations

Various models consisting of human peripheral blood lymphocytes, human skin grafts, porcine skin flaps in explant culture, human epidermal keratinocytes in culture, human eyes, hairless guinea pigs, nude mice, and stratified rat epidermal cultures have been developed to study the biochemical events in sulfur mustard toxicity. However, an appropriate animal model is lacking, as there have been no animals in which it has been possible to reproduce, in its entirety, the effects of sulfur mustard on human skin (Pechura and Rall 1993). Laboratory animals with fur, lacking sweat glands on most of their body, do not provide optimal models for dermal exposure. For a given dose, higher dermal concentrations are achieved in nonhuman mammalian skin, compared to human skin, and more severe tissue damage is noted in the dermis than the epidermis (Pechura and Rall 1993). Injuries to animal skin develop and heal more quickly than same-degree-of-severity injuries to human skin (Pechura and Rall 1993). Blister

character differs between humans and animals. Microblisters, rather than macroblisters, develop in the skin of laboratory species at effective dose levels.

An intermediate-duration inhalation MRL was derived based on ocular effects of conjunctivitis and chronic keratitis in dogs (McNamara et al. 1975). Gates and Moore (1946) reported that the human eye is about 4 times more sensitive to sulfur mustard than the rabbit eye based on the observation of corneal ulceration produced in rabbits at a Ct of 4 times the value at which this effect occurred in humans. Gates and Moore (1946) also reported the observation of sulfur mustard-induced corneal ulceration in dogs at a Ct of twice the value at which this effect occurred in humans, which is consistent with the observation by McNamara et al. (1975) of ocular effects in dogs. Thus, an uncertainty factor of 3 for extrapolation of ocular effects data from dogs to humans, which is closer to the observed Ct difference factor of 2 than a default value of 10, is considered appropriate for derivation of the intermediate-duration inhalation MRL.

An intermediate-duration oral MRL was derived based on mild epithelial acanthosis of the forestomach in rats (Sasser et al. 1996a). Although humans do not have forestomachs, the primary mechanism of toxicity of sulfur mustard is epithelial tissue damage from direct contact and, therefore, epithelial acanthosis is considered a suitable critical noncancer end point for deriving an oral MRL. Tissue damage would be expected to occur at the point of contact, even if it were another part of the gastrointestinal tract. Because sulfur mustard is a highly corrosive agent, epithelial lesions at the point of entry into the stomach are likely to occur across species. For this reason, the typical default value of 10 for the uncertainty factor for extrapolation of data from animals to humans is considered to be too high and a lower value of 3 was applied.

An uncertainty factor default value of 10 for extrapolation from animals to humans was applied in deriving an acute oral MRL based on inflamed mesenteric lymph nodes in the rat dams and reduced ossification in the fetuses (DOA 1987).

As discussed in Section 3.2.1.2, short-term respiratory effects similar to those described in humans have been reported in experimental animals, which suggests that knowledge obtained regarding respiratory effects in animal models can be usefully applied to humans.

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

It is possible that sulfur mustard modifies the feedback of endogenous hormones and, through the complex interactions of central nervous system and endocrine function regulation, behavior (i.e., libido). In a survey of 800 Iranian men who were exposed to sulfur mustard during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido,

and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

There is limited evidence to suggest that sulfur mustard affects follicle stimulating hormone (FSH) levels and thus plays a role in reproductive function. The time course of changes in serum concentrations of total and free testosterone, luteinizing hormone (LH), dehydroepiandrosterone (DS), FSH, 17 α-OH progesterone, and prolactin were studied in 16 men during the first 3 months after chemically confirmed exposure to chemical weapons containing sulfur mustard in 1987 during the Iran-Iraq War (Azizi et al. 1995). A group of 34 healthy unexposed men of similar age served as controls. Released from the pituitary, LH stimulates the Leydig cells to produce testosterone, while FSH stimulates the Sertoli cells to produce sperm. At 1 week after exposure, total testosterone, free testosterone, and DS were significantly lower, 57, 72, and 53%, respectively, in exposed men than in controls, while levels of the remaining hormones were comparable between groups. Total testosterone, free testosterone, and DS levels continued to decrease during the first 5 weeks after exposure. At 1 week, 4 of 16 exposed men (25%) had serum testosterone levels that were reduced by >60% below the control average; by the 5th week, the number increased to 11 (69%). DS mean values reached as low as 18% of the mean of control subjects. After the 5th week, these three hormone levels increased returning to normal levels at 12 weeks after injury. Small but significant increases in mean serum concentration of LH at the 3rd week and that of FSH and prolactin at the 5th week were measured. Normal levels of LH, FSH, and prolactin were measured at 12 weeks. FSH and LH response levels to 100 μg of gonadotropin releasing hormone (GnRH) administered intravenously during the first week after exposure, were subnormal in four of five patients. Testosterone levels in these men returned to normal 12 weeks after exposure.

In a follow-up study of 42 men, ages 18–37, injured by sulfur mustard during the Iran-Iraq War, serum testosterone, LH, and prolactin concentrations were normal in all men 1–3 years following exposure (Azizi et al. 1995). A comparison of the mean serum FSH concentration in 13 subjects with sperm count below 20 million and in 20 subjects with sperm counts above 60 million, revealed a nearly 2-fold increase in FSH concentration in those with the lower sperm count; the increased FSH level was 38% above the mean FSH concentration in a group of 34 healthy unexposed males. Inhibition of spermatogenesis was also observed in male mice following intravenous injection of sulfur mustard (Graef et al. 1948). Elevated FSH has been correlated clinically with testicular failure, germinal aplasia, or hypergonadotropic hypogonadism. It appears unlikely that alteration of FSH levels is related to the effect of sulfur mustard on the pituitary since LH levels were unaffected in males. A possible target is inhibin secretion by testes Sertoli cells, which suppresses pituitary FSH secretion.

Administration of sulfur mustard did not affect the reproductive potential of female mice because the fertility of the mice was not altered and no injurious effects were observed in the ovaries (Graef et al. 1948). Chronic (52 weeks) inhalation exposure of male rats to sulfur mustard (0.1 mg/m^3) was reported to produce significant dominant lethal mutation rates (a maximum of 9.4% at 12–52 weeks), but exposure of pregnant females to the same concentration for a shorter time interval did not (Rozmiarek et al. 1973). McNamara et al. (1975) subsequently concluded from these same data that there were no differences between the control and experimental groups and no evidence of mutagenesis. The conflict between these two reports is not readily resolvable, but the fetal mortality values presented by McNamara et al. (1975) suggest at least a trend for a dominant lethal effect. Complete control data and statistical analyses of the results are not presented, but percentages of fetal death at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001, and 0.1 mg/m^3 exposure groups, respectively.

In a dominant lethal study of sulfur mustard, rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day sulfur mustard 5 days/week for 10 weeks (Sasser et al. 1993). In female dominant lethality experiments, reproductive performance indicators (number of live or dead implants, resorptions, and preimplantation losses) in treated female rats mated to treated or nontreated males were not significantly different from controls. In male dominant lethality experiments (treated males were mated with untreated females), resorptions and preimplantation losses in the mid- and high-dose groups were significantly elevated. High-dose male sperm morphology data at all postexposure sampling times, 0, 5, and 12 weeks, showed a statistically significant decrease in the percentage of normal sperm. Blunthook and banana-shaped sperm heads were observed at 0, 5, and 12 weeks, whereas amorphous and short head abnormalities were observed only at 5 and 12 weeks. Overall, there was a total 2-fold increase in abnormal sperm heads in high-dose sulfur mustard-treated males. In summary, female fertility was not affected by these sulfur mustard exposures; however, a male dominant lethal effect was demonstrated at the mid and high doses of sulfur mustard. This lack of reproductive effects in female animals further supports the testes, rather than the pituitary, as the target organ in connection with possible sulfur mustard-induced alteration in FSH levels.

The time course of changes in thyroid indices, serum T3, T4, TSH, reverse T3, thyroglobulin and cortisol, plasma ACTH, and free T3 and T4 indexes (FT3I, FT4I) were studied in 13 male soldiers, ages 21–32 years, during the first 5 weeks after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing sulfur mustard (Azizi et al. 1993). A group of 34 healthy unexposed men of similar age served as controls. T4 and FT4I were not consistently affected following injury; compared

to controls, significantly decreased values were measured at 1 and 5 weeks after exposure, and but values slightly above normal were measured at 3 weeks. T3 and FT3I were significantly lower (11–23%) than control at 1, 3, and 5 weeks after injury. Reverse T3 concentration in injured men was significantly higher (29%) than mean control value at 1 week, but was normal at weeks 3 and 5. TSH and thyroglobulin levels in the injured soldiers were comparable to controls during the 5 postexposure weeks. Cortisol was significantly higher (40%) than normal 1 week after exposure, within the normal range at week 3, and significantly decreased (50%) below normal at week 5. ACTH was significantly increased (57–80%) above the normal control value at 1, 3, and 5 weeks after exposure. No follow-up studies of thyroid indices were located to determine whether normal levels returned or if any chronic effects exist.

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example,

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

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

Information on children's health effects is provided from reports of children exposed to sulfur mustard from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). In children, as in adults, the most severe effects were contact effects to the eyes, skin, and respiratory tract as might be expected for a vesicant; however, some differences in clinical manifestations were reported. The onset of symptoms in children was sooner than in adults (Momeni and Aminjavaheri 1994). Generally, clinical manifestations of sulfur mustard exposure in the adults examined were delayed by about 8–24 hours, whereas manifestations in children first occurred 4–18 hours after exposure. Cough and vomiting were the first signs of poisoning in the children, but not in adults. Blisters appeared sooner in the children and teenager group than in adults. Cough and vomiting were the first symptoms in the children, but not in adults. The severity of ocular effects was greater in the children and teenager subgroup than in adults. Pulmonary and gastrointestinal symptoms were more frequent in children and teenagers (78% and 69%, respectively), compared with adults (11%). Genital lesions were less frequent in children and teenagers (42%) than

adults (70%); however, even within the group of children, the incidence and severity of genital lesions increased with age. Other skin lesions had no apparent age-relation. The only information located regarding possible adverse developmental effects in humans suggested an association between parental exposure to chemical agents, including, but not limited to, sulfur mustard, and elevated rates for congenital malformations (Pour-Jafari 1994b). Studies of animals administered sulfur mustard by gavage in oil during pregnancy have indicated reduced fetal weight and reduced ossification of the vertebrae and/or sternebrae, but only at levels that were also toxic to the mother (DOA 1987; Sasser et al. 1996a).

No information was located regarding pharmacokinetics of sulfur mustard in children nor it is known whether sulfur mustard can be stored and excreted in breast milk. There have been no direct measurements to determine whether sulfur mustard can cross the placenta. There is no information on whether sulfur mustard can be stored in maternal tissues and be mobilized during pregnancy or lactation.

There are no biomarkers of exposure or effect for sulfur mustard that have been validated in children or in adults exposed as children. No studies were located regarding interactions of sulfur mustard with other chemicals in children or adults.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to sulfur mustard, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects of sulfur mustard in adults might be contraindicated in children.

Kurt et al. (1998) report differential sensitivity related to cytokine release of cultured adult and neonatal human epidermal keratinocytes treated with sulfur mustard, but the significance of these findings are not known.

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 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 sulfur mustard 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 sulfur mustard 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 Sulfur mustard

Several analytical methods are available that can be used to quantitatively determine thiodiglycol, a major sulfur mustard hydrolysis product, and metabolites that yield thiodiglycol under sample preparation

conditions in the urine of persons exposed to sulfur mustard (Jakubowski et al. 2000). However, thiodiglycol, not associated with sulfur mustard exposure, has been detected at low levels in normal human urine. A significantly higher urinary level of thiodiglycol, compared with the range found in normal urine from unexposed individuals, is generally consistent with exposure to sulfur mustard, but does not definitively prove mustard poisoning (Wils et al. 1985, 1988). Elevated levels of thiodiglycol have been detected in the urine of persons exposed to sulfur mustard up to about two weeks after exposure (Jakubowski et al. 2000). Unmetabolized sulfur mustard may be detected in the urine if a person is exposed to very high levels (Heyndrickx and Heyndrickx 1984; Mandl and Freilinger 1984; Pauser et al. 1984; Vycudilik 1985). Thiodiglycol was not unambiguously detected in sulfur mustard-induced blister fluid, but chromatographic components have provided strong evidence for thiodiglycol-related fragments (Jakubowski et al. 2000). However, the need for low-level and retrospective detection of exposure has been illustrated in the attempts to clarify the causes of the significant number of postwar symptoms experienced by soldiers involved in the Persian Gulf War.

Black et al. (1992a) identified, in addition to several other metabolites, thiodiglycol sulphoxide as the major urinary excretion product, and not the initial hydrolysis product thiodiglycol. In two subjects accidentally exposed to sulfur mustard, urine thiodiglycol sulphoxide concentrations were 20–35 times thiodiglycol concentrations (Black and Read 1995a). However, the use of thiodiglycol sulphoxide as a biological marker for sulfur mustard poisoning, as is the case for thiodiglycol, is limited by its presence at low concentrations in normal human urine. Of the remaining metabolites, several are conjugates of sulfur mustard with N-acetylcysteine, most of which have poor mass spectrometric and/or gas chromatography properties mainly due to thermal instability (Black et al. 1991). Two closely related metabolites of sulfur mustard, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methylthio)ethyl-sulphonyl]ethane, derived from the action of β-lyase on cysteine conjugates, have been detected in urine collected from Iran-Iraq War casualties of sulfur mustard poisoning (Black and Read 1995b); there were no background levels of these metabolites detected in human or rat urine (Black et al. 1991).

Since sulfur mustard is known to alkylate DNA, RNA, and proteins, attempts have been made to detect these adducts in blood and, subsequent to release from dying cells, in urine (Somani and Babu 1989). In DNA, N-alkylated purines, such as N7-hydroxyethylguanine, have been identified from enzymatic digests as active sites for sulfur mustard (Fidder et al. 1994, 1996b; Niu et al. 1996; Somani and Babu 1989; Van der Schans et al. 1994). Van der Schans et al. (1994) synthesized N7-(2-hydroxyethylthioethyl)-GMP (N7-HETE-GMP) for use as a hapten to generate monoclonal antibodies against the major adduct, N7-(2-hydroxyethylthioethyl)guanine (N7-HETE-guanine), formed after alkylation of DNA with sulfur

mustard. Another sulfur mustard adduct in DNA, the 2′-deoxyguanosine derivative of N7-HETE-guanine, N7-(2-hydroxyethylthioethyl)-2′-deoxyguanosine, has been detected by immunochemical analysis in the blood of two victims of the Iran-Iraq War (Benschop et al. 1997). Presently, these adducts in white blood cells can be detected after exposure of human blood to sulfur mustard concentrations ≥2 μM (van der Schans et al. 1994). The metabolite, N7-HETE-guanine, derived from sulfur mustard DNA alkylation, was detected by immunochemical analysis in the urine of guinea pigs administered sulfur mustard intravenously (Fidder et al. 1996b). These antibodies also have potential in the development of a single-cell assay with immunofluorescence microscopy to quantify adduct formation in skin exposed to sulfur mustard.

To enable detection of low-level exposure to sulfur mustard, sulfur mustard adducts with proteins have also been explored. Sulfur mustard alkylates hemoglobin (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1996, 1997) and albumin (Noort et al. 1999). In hemoglobin, histidine residues and the N-terminal valine on both the α and β chains were identified as key sites of interaction (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1997). A procedure employing gas chromatography-mass spectrometry with modified Edman degradation has been developed for the determination of the adduct of sulfur mustard with the N-terminal valine residue of hemoglobin (Fidder et al. 1996a). Metabolite N7-HETE-valine was detected in the urine of guinea pigs administered sulfur mustard intravenously (Fidder et al. 1996a). A cysteine residue of albumin was identified as a site of sulfur mustard alkylation (Noort et al. 1999). A mass spectrometric analysis of the adduct of sulfur mustard with the cysteine residue of albumin, S-HETE-Cys-Pro-Phe, provided a detection limit for sulfur mustard an order of magnitude lower than the modified Edman assay for hemoglobin (Noort et al. 1999). Compared to the hemoglobin, the drawback for albumin-sulfur mustard adduct detection is the faster elimination rate. The half-life of albumin is 20–25 days versus the 120-day life span of hemoglobin. Both albumin- and hemoglobin-sulfur mustard adducts have been detected in the blood of two victims of the Iran-Iraq War using the respective assay (Benschop et al. 1997; Noort et al. 1999).

3.8.2 Biomarkers Used to Characterize Effects Caused by Sulfur mustard

There are no specific biomarkers of effects for sulfur mustard. Sulfur mustard is one of many vesicant agents that affect mucosal and non-mucosal surfaces with which it comes in contact. Thus, the primary targets for exposure to sulfur mustard in the air are the skin, eyes, and respiratory tract and, if ingested, the gastrointestinal tract.

3.9 INTERACTIONS WITH OTHER CHEMICALS

No data were located on the interactions of sulfur mustard with other toxicants likely to be found at hazardous waste sites.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

Humans show varying degrees of dermal sensitivity to sulfur mustard (Renshaw 1946; Sulzberger et al. 1947); fair-skinned people are more sensitive than dark-skinned people. These reports also indicate that individuals with previous exposure are more sensitive to the dermal effects of sulfur mustard. It is possible that individuals with respiratory problems (asthma, emphysema, etc.) might be more sensitive to the effects of sulfur mustard and might suffer acceleration of their disease following exposure. Since sulfur mustard has been associated with lung cancer, people who smoke may be at greater risk. Children may be more susceptible to the effects of sulfur mustard than adults (see Section 3.7).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

Augerson WS, Sivak A, Marley WS. 1986. Chemical casualty treatment protocol development - treatment approaches. Vol II-IV. Cambridge, MA: Arthur D. Little, Inc.

Marrs TC, Maynard RL, Sidell FR. 1996. Chemical warfare agents. John Wiley & Sons, New York.

NIOSH. 2003. Mustard emergency response card. National Institute for Occupational Safety and Health. http://www.bt.cdc.gov/agent/mustardgas/erc505-60-2pr.asp. March 20, 2003.

OPCW. 2001. Organization for the prohibition of chemical weapons, decontamination of chemical warfare agents. http://www.opcw.nl/chemhaz/decon.htm. March 13, 2001.

SBCCOM. 2001. Material safety data sheet, sulfur mustard. Aberdeen Proving Ground, MD: U.S. Army Soldier and Biological Chemical Command. http://in1.apgea.army.mil/RDA/msds/hd.htm. March 13, 2001.

U.S. Army. 1995. Treatment of chemical agent casualties and conventional military chemical injuries. Washington, DC: U.S. Department of the Army, FM 8-285. http://www.adtdl.army.mil/cgi-bin/atdl.dll/query/info/FM+8-285. March 22, 2001.

Willems JL. 1989. Clinical management of sulfur mustard casualties. Annales Medicinae Militaris Belgicae. Vol 3: (Suppl.) Heymans Institute of Pharmacology. Ghent, Belgium: University of Ghent Medical School and Royal School of the Medical Services.

3.11.1 Reducing Peak Absorption Following Exposure

Decontamination procedures should be initiated immediately after exposure. Hypochlorite bleaches were the earliest decontaminants used to detoxify mustard. During World War II, both common bleach [$NaOCl^-$] and superchlorinated bleaches [$Ca(OCl^-)_2$] were used. More stable *N*-chloro compounds, such as chloramine, have been used in more modern personal decontamination systems. In the 1950s, a non-aqueous equipment decontamination solution "DS2" (2% NaOH, 70% diethylenetriamine, 28% ethylene glycol monomethyl ether) was developed in which the conjugate base of the glycol ether reacts rapidly with mustard via double elimination. The currently fielded U.S. ARMY M291 skin decontamination kit contains the decontaminant powder XE-555 resin (Amberguard 555) (SBCCOM 2001).

The eyes should be washed immediately with as much water as tolerable, for at least 15 minutes, even if no symptoms are present, since it is known that ocular and dermal symptoms are delayed (Dreisbach and Robertson 1987; Goldfrank et al. 1990; Solberg et al. 1997). Of the many fluids studied for eye irrigation, none has proven more effective than tap water (Solberg et al. 1997). Contaminated clothing should be removed and the skin, particularly the groin, axillae, and perineal areas, should be decontaminated. Rapid removal from skin is critical, as sulfur mustard penetrates skin within minutes of exposure. Skin decontamination may be accomplished with copious amounts of water, a 0.5% hypochlorite solution, or a skin decontamination kit.

Topical decontamination with hypochlorite solutions was examined in euthymic hairless guinea pigs (Gold et al. 1994) and rabbits (Hobson et al. 1993). No significant wound differences were found between decontamination with water only and various concentrations of hypochlorite solutions; however, decontamination with a 0.5% solution is standard in many vesicant exposure management protocols (SBCCOM 2002). It has been suggested that removal of sulfur mustard with water alone may be contra-indicated as sulfur mustard spreads over more skin surface and increases the area of blistering (Kumar et al. 1991). Absorbent decontaminants including fuller's earth, calcium chloride powder, or XE-555 resin may be sprinkled onto the exposed skin, allowed to absorb the sulfur mustard, and then washed off with water (Chilcott et al. 2000, 2001; Kumar et al. 1991; Solberg et al. 1997). Of decontaminants including fuller's earth, Ambergard, and BDH spillage granules, fuller's earth was most effective in reducing skin absorption in *in vitro* studies using human epidermal membranes (Chilcott et al. 2001). When fuller's earth, N,N'-dichloro-bis (2,4,6-trichlorophenyl) urea (CC-2), and their various combinations (w/w ratios) were evaluated for their decontamination efficacy against sulfur mustard applied on mouse skin, maximum protection was obtained with fuller's earth and CC-2 in a combination of 80:20 (w/w) (Kumar et al. 1991); however, disparities have been evident in measured decontaminant efficiencies between animal models and man (Chilcott et al. 2001).

3.11.2 Reducing Body Burden

There is no specific antidote for sulfur mustard, and therapy is supportive. Victims should be removed from contaminated areas. Patient care should include supportive treatment protocols for skin injury, respiratory distress, and cardiac dysrhythmias (Dreisbach and Robertson 1987; Haddad and Winchester 1990). There is usually a delay of onset of toxicity in exposed individuals. Severe respiratory distress may be delayed for up to 72 hours depending on the concentration and duration of exposure (Ellenhorn and Barceloux 1988). In cases of damage to the upper respiratory tract, antibiotic cover is recommended to prevent infection (Murray and Volans 1991). In severely injured victims, administration of systemic analgesics should be considered after examination. Mortality can be reduced by intravenous administration of electrolyte solutions commencing early and continuing throughout the intoxication period (Cullumbine 1947). Electrolyte replacement is needed due to losses from skin locally and in the intestine, and via saliva, vomitus, and diarrheic stools. A single dose of saline or glucose-saline (5 mg glucose/kg) administered intraperitoneally to mice offered protection after topical sulfur mustard exposure; survival was 83% with saline treatment compared to 33% without treatment (Sugendran et al. 1994).

In cases of ocular injury, local anesthetic drops should be avoided other than for ophthalmologic examination, as they are toxic to both healthy and damaged corneas. Patients whose ocular injuries are limited to the conjunctiva require no additional treatment subsequent to irrigation. Corneal lesions may be detected by staining with fluorescein and examining with blue light. Treatment for injury to the cornea should include daily irrigation, mydriatics to ease the eye pain produced by spasm of the ciliary muscle and to prevent posterior iridolenticular adhesions, antibiotic drops to prevent secondary bacterial infections, local medications to control intraocular pressure, and systemic analgesics (Solberg et al. 1997). In cases of ocular injury, local anesthetic drops should be avoided other than for ophthalmologic examination, as they are toxic to both healthy and damaged corneas. Although recommended, the use of sterile petroleum jelly to prevent the eyelid margins from sticking together should be delayed until after sufficient irrigation, since sulfur mustard will dissolve and concentrate in the jelly (Solberg et al. 1997). Ocular bandages should not be applied as they might raise the corneal temperature and increase the toxic effects (Solberg et al. 1997). Delayed keratitis should be treated with ocular lubricants, therapeutic lenses, and in severe cases, tarsorrhaphy (suturing of the eyelids together) (Solberg et al. 1997). Keratoplasty should be considered if there is significant opacification of the cornea.

Faster healing and less scarring have been reported when skin blisters were drained. While aseptic procedures are prudent for handling all bodily fluids, there are conflicting reports as to the danger of the blister fluid itself. There are no reports of sulfur mustard detected in blister fluid (Jakubowski et al. 2000); however, secondary blistering running proximal to an original blister, thought to be due to leaking fluid, was reported in a case of accidental exposure during destruction of sulfur mustard stockpiles (Bide et al. 1993). Canadian Reactive Skin Decontamination Lotion (RSDL), which is a 1.25 molal solution of potassium 2,3-butanedionemonoximate (KBDO) in polyethyleneglycol monoethylether (500 nominal weight) and water, was shown to reduce the severity and scarring of sulfur mustard-induced lesions on the shaved back of guinea pigs (Bide et al. 1993). A case was also reported of an employee who suffered minor sulfur mustard burns to the wrist and forearm during destruction of sulfur mustard stockpiles at the Canadian Defense Research Establishment Suffield (DRES). Treatment was carried out partly at DRES and partly at a local hospital. One set of burns received treatment with RSDL at DRES, where it was available, and another set did not, as RSDL was not available at the local hospital. The blister without RSDL treatment initially burst, and a series of secondary burns running proximal to the original blister formed. The RSDL-treated burn was much less severe and no secondary burns formed (Bide et al. 1993).

Pulsed carbon dioxide (CO_2) laser debridement has been shown to be effective in clearing the epidermis of sulfur mustard damaged cells (Smith et al. 1997b). In weanling pigs, whose skin was exposed to sulfur mustard, CO_2 laser debridement of the exposed skin resulted in clearing of the cytologic atypia, reduced inflammatory infiltrate, and increased numbers of stromal cells within the papillary dermis. At 14 days postexposure, there was no significant difference between skin laser-debrided at 6, 24, or 48 hours after exposure.

Animal experiments have shown that sodium thiosulfate, N-acetyl-L-cysteine, nicotinamide, nicotinic acid, promethazine, dexamethasone, prednisone, and vitamin E have decreased tissue damage, but their efficacy in humans is not known (Dabney 1991; Papirmeister et al. 1991; Vojvodic et al. 1985). Thiosulfate likely acts as a mustard scavenger, vitamin E as an antioxidant, and the corticosteroids by inhibiting lipooxygenase activity leading to synthesis of prostaglandins and leukotrienes (Borak and Sidell 1992). In guinea pigs injected intratracheally with sulfur mustard, subsequent treatment with betamethasone, a glucocorticoid, significantly increased tracheal epithelium height by about 20% and cell density, compared to untreated animals (Calvet et al. 1996). Application of provodine iodine (PI) ointment to the shaved back of guinea pigs up to 10 minutes following sulfur mustard exposure has been shown to provide significant protection from ulceration (Wormser et al. 1997). Histopathological evaluation of PI-treated skin showed only moderate thickening of the epidermis with slight hyperkeratosis, whereas deep epidermal ulceration involving the superficial dermis was evident without PI treatment. In a comparative study of chemical burn therapies in guinea pigs, debridement with trypsin-linked gauze (Debridase) was more effective in reducing the lesion area than surgical excision or laser ablation (Eldad et al. 1998b). A recent study with amifostine, an organophosphorothioate, and its analogues showed that pretreatment of mice with the chemical either intraperitoneally or orally protected against the acute toxicity of dermally applied sulfur mustard (Vijayaraghavan et al. 2001). Amifostine, originally developed as a radioprotector, can neutralize and reduce the concentration of sulfur mustard inside the cell after it is dephosphorylated to its free thiol molecule by membrane-bound alkaline phosphatase.

Topically applied pretreatments have been shown to be effective in reducing the severity of sulfur mustard-induced skin lesions (Kwong and Segers 1996). Superoxide dismutase was effective in reducing the lesion area when administered before, but not after, topical application of sulfur mustard to guinea pigs (Eldad et al. 1998a). In a study of sulfur mustard vesication following pretreatment with topically applied agents, the most promising barrier cream was comprised of petrolatum, sorbitan stearate, and water with either of the N-halo oxidants 1,3,4,6-tetrachloro-7,8-diphenyl-2,5-diiminoglycoluril (S-330) or

1,3-dichloro-5-5-dimethylhydantoin, and optionally, with a barrier-providing polymer such as perfluoroalkylpolyether (FOMBLIN HC/04, HC/25, or HC/R) or a polysiloxane (Kwong and Segers 1996). A topical skin protectant cream containing perfluoroalkylpolyether and polytetrafluoroethylene, ICD 2289, being developed to protect service members from exposure to chemical warfare agents, was shown to reduce the sulfur mustard-induced lesion area to 18% of the untreated lesion area when applied as a pretreatment in rabbits (Liu et al. 1999). A new destructive absorption technology (DAT) employs highly reactive nanoparticles (RNP; small [≥ 4nm] crystals of metal oxides) to neutralize toxic substances including sulfur mustard. Preliminary studies indicate that RNP remain active against chemical agents when incorporated into a base cream and are compatible with skin contact (Koper et al. 1999). Extensive antivesicant research is currently in progress with significant developments likely to be reported in the near future.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Research has elucidated areas of biochemical/pathological alterations induced in cells or tissues by sulfur mustard that provide targets for pharmacological interventions including macromolecular alkylation, DNA damage, activation of poly(ADP-ribose) polymerase (PARP), tissue proteolysis, and inflammation (Papirmeister et al. 1991; Smith et al. 2000).

Sulfur mustard is thought to induce structural changes in cellular DNA, as indicated by altered dye response in flow cytometric studies (Smith et al. 1993a). The unsaturated keto groups of DNA appear to be functional groups that are attacked by mustard alkylating agents (Baskin et al. 2000). Toxic effects of sulfur mustard have been attributed to DNA modification, uncoiling in part (Baskin et al. 2000), with the formation of N7-(2-hydroxyethylthioethyl)guanine (Fidder et al. 1994, 1996a; Matijasevic et al. 1996; Niu et al. 1996; Somani and Babu 1989; Van der Schans et al. 1994), 3-hydroxyethylthioethyl adenine and the cross-link, di-(2-guanin-7-yl-ethyl)sulfide (Matijasevic et al. 1996). Reducing or preventing the ability of sulfur mustard to alkylate DNA and critical target molecules would reduce toxicity. Reversal of secondary consequences of alkylation requires a better understanding of the biochemical pathways of toxicity and may require interventions for more than one mechanism of action. As pointed out by Papirmeister et al. (1991), this strategy would provide temporary measures, slowing down the injury process and buying time for intracellular repair processes, thereby avoiding the simultaneous necrosis of massive numbers of cells as occurs in sulfur mustard-induced epithelial lesions. Tissue function may remain close to normal if cell death can be spread out over a sufficiently long period of time, and dead cells are replaced through endogenous tissue repair and regeneration mechanisms.

Reduction of target structural changes may by possible by the use of compounds that react with or scavenge sulfur mustard and lower target alkylation levels. The speed at which sulfur mustard reacts presents a difficulty to this strategy of treatment. However, several anionic sulfur compounds, such as thiosulfate, have been shown to reduce the toxic effects of mustard agents when administered as a pretreatment (Baskin et al. 2000; Papirmeister et al. 1991). Thiosulfate's protective effect is due, at least in part, to its extracellular detoxification of mustard agents by direct chemical reaction. However, a small percentage (3–5%) of thiosulfate enters cells, but it is not yet known if any intracellular interactions contribute to its efficacy (Baskin et al. 2000).

DNA repair enzymes may offer some protection against the toxic action of sulfur mustard. Li et al. (1997) investigated the action of formamidopyrimidine-DNA glycosylase (Fpg), purified from *E. coli*, on the ring-opened (ro) form of sulfur mustard-DNA adduct N7-(2-hydroxyethylthioethyl)guanine (N7-HETE-guanine). Fpg protein is thought to protect cells from toxicity by removing ring-opened N7-guanine adducts from DNA. Fpg protein released ro-HETE-guanine from DNA modified by [^{14}C]sulfur mustard in an enzyme- and time-dependent manner. Bacterial 3-methyladenine DNA glycosylase II (Gly II) was found to release both HETE-adenine and HETE-guanine from calf thymus DNA modified with [^{14}C]sulfur mustard, also suggesting that glycosylase action may play a role in protecting cells from the toxic effects of sulfur mustard (Matijasevic et al. 1996). Modulation of other known or putative DNA repair enzymes, such as DNA ligase I or PARP, may provide a useful approach in preventing or reducing sulfur mustard toxicity (Bhat et. al. 2000).

Cell cycle kinetics are involved in the cytotoxic processes following sulfur mustard exposure. Sulfur mustard-induced damage to genomic DNA in cultured human epidermal keratinocytes (HEK), at subvesicating concentrations (<50 μM), resulted in a dose-related reversible block at the G_2/M phase of the cell cycle (Smith et al. 1993a). Okadaic acid and calyculin A, inhibitors of protein phosphatase 2A (PP2A), completely reversed the sulfur mustard-induced G_2/M block (Hart and Schlager 1997). Exposure of human peripheral blood lymphocytes (PBL) to vesicating-equivalent concentrations of sulfur mustard (≥50 μM) resulted in irreversible blockage at the G_1/S interface (Smith et al. 1998). DNA became terminally fragmented. Compounds might be used to hold cells in a selected phase in order to permit DNA repair processes to correct the damaged DNA before normal proliferative events are allowed to proceed. Mimosine, one such inhibitor, was shown to provide limited protection against cytotoxicity of vesicating-equivalent concentrations of sulfur mustard in HEK and HeLa cells (Smith et al. 1998).

Niacinamide (750 mg/kg, intraperitoneal), while not effective as a postexposure treatment, did inhibit microvesicle formation by 50% when given as a pretreatment to cutaneous sulfur mustard exposure in hairless guinea pigs (Yourick et al. 1991). When niacinamide was administered as a 30-minute pretreatment, NAD^+ content in sulfur mustard treated skin biopsies decreased to about 40% of control levels. However, when niacinamide was administered twice, both as a 30-minute pretreatment and as a 2-hour treatment, NAD^+ was maintained at control levels, but microvesicle formation was about the same as in the pretreatment-only case, indicating that maintaining skin NAD^+ content did not absolutely confer protection from microvesication, nor was it a necessary factor for preventing microvesication.

Arginine analogue nitric oxide synthase (NOS) inhibitors, L-nitroarginine methyl ester (L-NAME) (Sawyer 1998; Sawyer et al.1996) and L-thiocitrulline (L-TC) (Sawyer et al. 1998), have been shown to have protective activity against the cytotoxicity of sulfur mustard not related to their NOS-inhibiting activities. L-TC acted rapidly (minutes of preincubation) and was equipotent in protecting either immature (1 day) or mature (5 days) cultures of chick embryo neurons against the toxicity of sulfur mustard (Sawyer et al. 1998), while L-NAME was effective (1 hour pre- to 3 hours post-sulfur mustard exposure) only in mature cultures (Sawyer et al. 1996, 1998). Coadministration of L-TC and L-NAME resulted in synergistic protection only when L-TC was added to the cultures prior to sulfur mustard treatment (Sawyer 1999). These characteristics suggest that they act at different sites to exert their protective effect. Based on these findings, Sawyer (1999) proposed that sulfur mustard initiates its toxicity extremely rapidly through a cell surface-mediated event that that can be blocked by L-TC. A signal may be transduced into the cell that results in an additional event or lesion that manifests itself several hours later, which progresses to cell death unless blocked reversibly by L-NAME (Sawyer 1999).

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 sulfur mustard 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 sulfur mustard.

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.

An acute-duration inhalation MRL was derived for sulfur mustard based on human data. An intermediate-duration inhalation and an acute- and intermediate-duration oral MRLs were derived based on animal data. While additional chronic oral data are needed to derive a chronic-duration oral MRL according to ATSDR guidelines, there is a greater need for additional chronic inhalation and dermal data over oral data in animals, as sulfur mustard hydrolyzes in water, and oral exposure is the least likely of the three routes. Laboratory animals with fur do not provide optimal models for dermal exposure as they do not have sweat glands on most of their body. Further exploration of relevant models including human skin grafts, porcine skin flaps in explant culture, nude mice, and hairless guinea pigs is prudent to study the biochemical events in sulfur mustard toxicity and identify effective therapies.

Questions still remain regarding the mechanisms of toxicity of sulfur mustard. According to Papirmeister (1993), the database would benefit from research leading to greater understanding of the following:

- The involvement of apoptotic and necrotic cell death processes to the cytotoxic and acute skin injury actions of sulfur mustard.
- The importance of DNA repair and the cell cycle in skin cells that undergo apoptosis leading to lesion formation.
- The reason that poly(ADP-ribose) polymerase (PADPRP) inhibitors prevent losses of NAD^+, ATP, and viability in sulfur mustard-treated human peripheral blood lymphocytes (PBL), but fail to prevent sulfur mustard-induced cytotoxicity in human epidermal keratinocytes (HEK) or sulfur mustard-induced acute skin injury.
- Any pathways, other than the PADPRP-mediated NAD^+ loss, by which sulfur mustard-induces inhibition of glycolysis and energy depletion in HEK.
- The mechanism(s) responsible for increasing and maintaining high levels of intracellular calcium in sulfur mustard exposed cells.
- Relationships between sulfur mustard and protein regulation in connection with vesication.

- The contribution of reactive oxygen species to sulfur mustard cytotoxicity.
- The role of inflammation in the development of the acute cutaneous sulfur mustard injury.
- The events within the initial lag period before blistering occurs.
- The identification of therapeutic countermeasures.

3.12.1 Existing Information on Health Effects of Sulfur Mustard

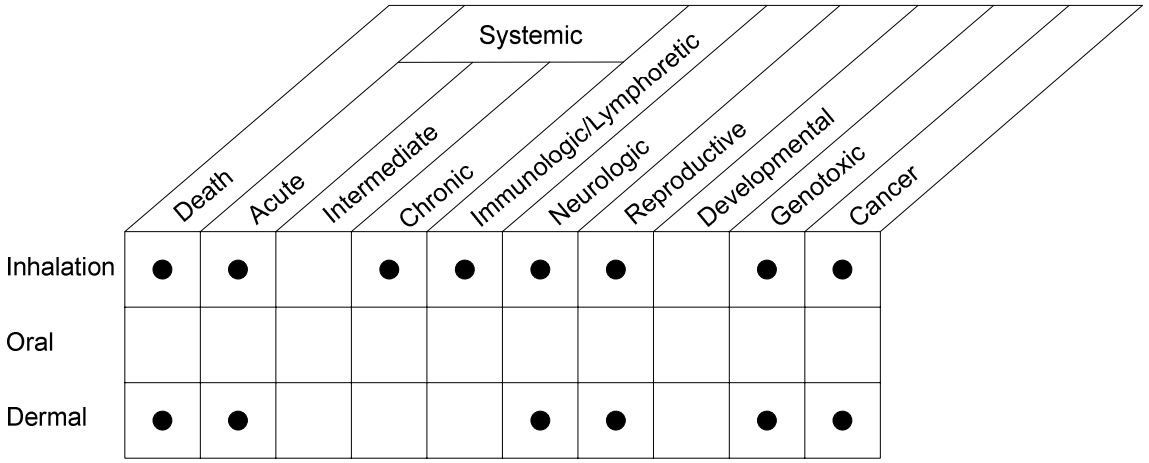
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to sulfur mustard is summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of sulfur mustard Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's Decision Guide for Identifying *Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Data are available for humans regarding respiratory disease and cancer, and the deaths caused by these diseases following acute and chronic inhalation exposure. Very limited animal data are available regarding death, developmental and reproductive effects, and cancer following inhalation exposure. There are no data available on the toxicity of sulfur mustard from oral exposure in humans. Data are available on effects in animals following acute- and intermediate-duration exposures. Limited data are available in humans and animals regarding skin effects from dermal exposure, and cancer in humans from dermal exposure.

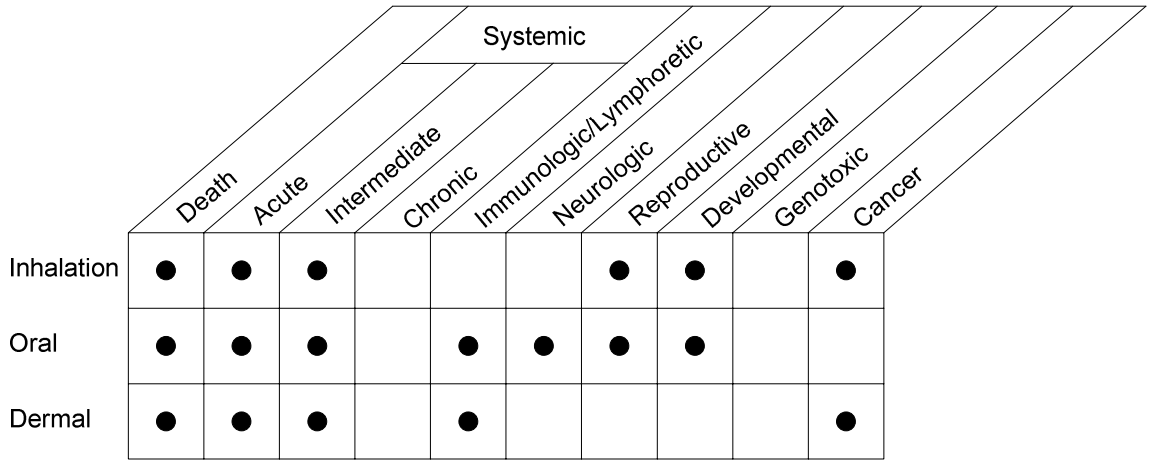
3.12.2 Identification of Data Needs

Acute-Duration Exposure. Sufficient information is available from human exposure data to identify the eyes (Anderson 1942; Guild et al. 1941; Momeni and Aminjavaheri 1994; Momeni et al. 1992; Reed

Figure 3-4. Existing Information on Health Effects of Sulfur Mustard



Human



Animal

● Existing Studies

1918), skin (Franke 1967; Jakubowski et al. 2000; Momeni and Aminjavaheri 1994; NRC 1985; Renshaw 1946; Sinclair 1948, 1950; Smith et al. 1919; Sulzberger et al. 1947; Wulf et al. 1985), and respiratory passages (Beebe 1960; Case and Lea 1955; Momeni and Aminjavaheri 1994; Momeni et al. 1992; Norman 1975) as target organs from acute exposure to sulfur mustard. Data from animal studies also suggest that acute exposure to sulfur mustard in the air is harmful to the eyes (Gates and Moore 1946), gastric mucosa (DOA 1987), skin (McAdams 1956; Venkateswaran et al. 1994a; Young 1947), and respiratory passages (Allon et al. 1993; Heston 1953b; Vijayaraghavan 1997; Winternitz and Finney 1920). Direct application to the skin of animals produced vascular leakage, leukocytic infiltration, and death of basal epidermal cells (Chauhan et al. 1993a, 1993b, 1995; Vogt et al. 1984). Since sulfur mustard has been used in combat, it is known to be lethal from primary (acute pulmonary edema) or secondary effects (respiratory infections) (Case and Lea 1955; Sinclair 1948, 1950; Somani and Babu 1989). While no human definitive oral data are available, effects to the gastric mucosa would be expected as sulfur mustard is a vesicant and direct alkylating agent. Acute inhalation and oral MRLs have been derived. No additional acute-duration testing to identify adverse health effects appears warranted.

Intermediate-Duration Exposure. Intermediate-duration exposure during combat has shown that sulfur mustard can be lethal. Wartime and occupational studies in humans have identified the eyes (Momeni and Aminjavaheri 1994; Momeni et al. 1992; Pechura and Rall 1993), skin (Bullman and Kang 2000; NRC 1985; Sinclair 1948, 1950; Wulf et al. 1985), and respiratory passages (Bullman and Kang 2000; Case and Lea 1955; Easton et al. 1988; Nishimoto et al. 1970; Somani and Babu 1989) as the target organs for sulfur mustard for intermediate-duration exposure. Data from animal studies also suggest that intermediate-duration oral exposure to sulfur mustard is harmful to the gastric mucosa (Sasser et al. 1996a, 1996b). While no human oral data are available, effects to the gastric mucosa would be expected as sulfur mustard is a vesicant and direct alkylating agent. Intermediate-duration inhalation and oral MRLs have been derived. However, further well-conducted intermediate-duration inhalation studies would be useful to support the rather limited available data. The same can be said for dermal data. Further oral studies do not seem warranted since oral exposure is not a likely route of exposure. Male dominant lethal studies in animals with exposure by the inhalation and dermal routes including site of application histological examinations would provide valuable data. It seems likely that, as with the oral route, the application site would be more sensitive to the effects of sulfur mustard than the male reproductive system; however, when considering combat exposure, the genital area was frequently a site affected.

Chronic-Duration Exposure and Cancer. Epidemiological studies of sulfur mustard workers have identified the eyes (Laughlin 1944b, 1944c; Morgenstern et al. 1947), skin (Inada et al. 1978; Klehr 1984; NRC 1985), and respiratory system (Easton et al. 1988; Manning et al. 1981; Morgenstern et al. 1947; Nishimoto et al. 1970; Somani and Babu 1989; Tokuoka et al. 1986; Wada et al. 1968; Weiss and Weiss 1975; Yamada 1963; Yamakido et al. 1996) as the target organs; however, none of these studies has involved the measurement of exposure concentrations, and interpretation of these studies is limited due to potential simultaneous exposure to other toxic agents. Chronic-duration inhalation and oral MRLs were not derived because no chronic bioassays were located. In order to derive these MRLs according to ATSDR guidelines, additional studies would be needed for both exposure routes. However, studies by the inhalation route of exposure should have priority since oral exposure is unlikely.

Factory workers who have been exposed to undetermined levels of sulfur mustard for a number of years have been shown to develop respiratory cancer (Easton et al. 1988; Manning et al. 1981; Morgenstern et al. 1947; Nishimoto et al. 1970; Tokuoka et al. 1986; Wada et al. 1968; Weiss and Weiss 1975; Yamada 1963; Yamakido et al. 1996). There is some evidence that former sulfur mustard factory workers may have an increased risk of developing digestive tract and skin tumors (Inada et al. 1978; Klehr 1984; Yamada 1974). Two animal studies, of low predictive quality due to species strain tendency to develop lung tumors, insufficient animals, and inadequate doses, have also shown increases in tumors from exposure to sulfur mustard in the air (Heston 1953b; McNamara et al. 1975). IARC has classified sulfur mustard as "carcinogenic to humans" (Group 1) based on sufficient evidence in humans, limited evidence in experimental animals, supporting evidence that sulfur mustard is a bifunctional alkylating agent, and positive results in a number of assays for genotoxic effects (IARC 1975, 1987). In order to develop cancer effect levels, appropriate animal studies would be necessary since there are no adequate studies currently available. In the absence of a chronic animal bioassay, several diverse methods (potency relative to benzo(a)pyrene, linear extrapolation from the benchmark dose of forestomach lesions or hyperplasia, potency relative to maximum tolerated dose) have been applied for estimating an upper limit on carcinogenic potency (USACHPPM 1999).

Genotoxicity. Sulfur mustard is known to be highly genotoxic *in vitro*, and further studies would likely not alter this conclusion (Ashby et al. 1991; Auerbach 1947; Ball and Roberts 1971/72; Capizzi et al. 1974; Fahmy and Fahmy 1971, 1972; Fan and Bernstein 1991; Ichinotsubo et al. 1977; Kircher and Brendel 1983; Lin et al. 1996a, 1996b; Ludlum et al. 1994; Ribeiro et al. 1991; Scott et al. 1974; Venitt 1968; Venkateswaran et al. 1994a; Walker and Thatcher 1968).

Reproductive Toxicity. Several human and animal studies suggest that sulfur mustard affects male reproductive function (Azizi et al. 1995; Graef et al. 1948; McNamara et al. 1975; Pour-Jafari and Moushtagi 1992; Rozmiarek et al. 1973; Sasser et al. 1993). Data from animal studies regarding oral exposure to sulfur mustard indicate that the acute- and intermediate oral MRLs derived within this profile would be protective of this system. The mechanism by which sulfur mustard affects reproductive parameters is not known; however, it is reasonable to assume that effects can be produced following any route of exposure providing that enough chemical is absorbed. Additional acute- and intermediate-duration inhalation reproductive studies (including multigeneration) may be needed if the results of a 90-day toxicity study suggest that reproductive organs are targets for sulfur mustard toxicity.

Developmental Toxicity. The only relevant information in humans is that from Pour-Jafari et al. (1994b), who reported an increased incidence of congenital malformations among offspring of Iranian chemical victims (males and females). However, there may have also been exposure to several other chemical agents. In an oral study in animals, fetal toxicity was evidenced by reduced body weight and ossification (DOA 1987). The limited data available suggest that adverse developmental effects occur at doses or exposure levels that produce maternal toxicity. There is no reason to believe that the developmental effects of sulfur mustard are route-specific. Data are lacking regarding the pharmacokinetics of sulfur mustard during pregnancy. Data from animal studies regarding oral exposure to sulfur mustard indicate that the acute-duration oral MRL derived within this profile would be protective of fetal development.

Immunological and Lymphoreticular Toxicity. Sulfur mustard-induced damage to lymphoid tissue was found in war casualties and in animals studies following inhalation, oral, or dermal exposure (Alexander 1947; Cameron 1946; DOA 1987; Venkateswaran et al. 1994a). Sulfur mustard-induced lymphoreticular toxicity does not appear to be route- or species-specific. Data from animal studies regarding inhalation and oral exposure to sulfur mustard indicate that the acute-duration inhalation and oral MRLs derived within this profile would be protective of the lymph system. Additional chronic inhalation studies are required to determine exposure levels for these routes that would limit lymphoreticular toxicity.

Neurotoxicity. There is no evidence that the nervous system is a target for sulfur mustard toxicity. Only minimal animal data are available regarding the neurotoxicity of sulfur mustard (Sasser et al. 1993; Winternitz and Finney 1920). Chronic or latent pain in the exposed skin area experienced by victims of sulfur mustard attacks suggests that sulfur mustard may cause persistent damage to the afferent nerve

system (Thomsen et al. 1998). This effect appears specifically related to dermal exposure and is probably due to a direct effect of sulfur mustard on sensory nerve terminals innervating the skin and would not be expected to occur following inhalation or oral exposure.

Epidemiological and Human Dosimetry Studies. Three types of human epidemiology studies are available: those of men who were exposed briefly during combat in World War I (Beebe 1960; Case and Lea 1955; Norman 1975; Sinclair 1948, 1950), those of subjects exposed for a longer period when producing sulfur mustard in Japanese (Nishimoto et al. 1970, 1983; Tokuoka et al. 1986; Wada et al. 1968; Inada et al. 1978; Yamada 1963; Yamakido et al. 1996), German (Weiss and Weiss 1975), British (Easton et al. 1988; Manning et al. 1981), or American factories (Bullman and Kang 2000), and those of people exposed during the Iran-Iraq War. In none of these studies were the exposure duration and levels quantified. However, in some cases, a relation to dose is apparent as, for example, deaths due to lung cancer increased with greater likelihood of exposure or service years in factories. Currently, the only people with potential exposure to sulfur mustard are those working in military facilities where sulfur mustard is stored and those involved in the destruction of the existing stockpile of sulfur mustard. Monitoring of the former may provide information on potential effects due to long-term exposure. Continued monitoring of sulfur mustard victims of the Iran-Iraq War would provide valuable information on long-term effects caused by acute high-exposure.

Biomarkers of Exposure and Effect.

Exposure. Two closely related metabolites of sulfur mustard that are not detected in normal urine, 1,1'sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methylthio)ethylsulphonyl]-ethane, have been detected in urine collected from Iran-Iraq War casualties of sulfur mustard poisoning (Black and Read 1995b; Black et al. 1991). Sulfur mustard has also been shown to alkylate hemoglobin (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1996, 1997) and albumin (Noort et al. 1999). Both protein adducts have been detected in the blood of Iran-Iraq War victims (Benschop et al. 1997; Noort et al. 1999). Development and validation of standard assays for these urine metabolites and blood protein adducts would be valuable tools for retrospective detection of exposure.

Effect. Various local enzymatic activity and protein alterations have been reported in connection with sulfur mustard exposure, thus providing potential as biomarkers of effect. Additional research providing a further understanding of the mechanisms of sulfur mustard toxicity is required before assay validation.

Absorption, Distribution, Metabolism, and Excretion. There is limited information on the toxicokinetics of sulfur mustard by the inhalation and dermal routes in humans and in animals. Considerable more toxicokinetics information is available for intravenous and intraperitoneal routes of sulfur mustard exposure in animals. These data indicate that it can be absorbed (Cameron et al. 1946; Cullumbine 1946, 1947; Drasch et al. 1987; Hambrook et al. 1993; Klain et al. 1991; Langenberg et al. 1998; Nagy et al. 1946; Papirmeister et al. 1984a, 1984b; Renshaw 1946; Smith et al. 1919) and is excreted in the urine (Black et al. 1992a, 1992b; Davison et al. 1961; Hambrook et al. 1992; Jakubowski et al. 2000; Maisonneuve et al. 1993; Roberts and Warwick 1963; Sandelowsky et al. 1992; Smith et al. 1958; Wils et al. 1985, 1988). Langenberg et al. (1998) detected sulfur mustard DNA adducts in tissues following inhalation exposure in guinea pigs. Metabolic pathways are presumed based on these data. The available information is insufficient to determine whether saturation phenomena play a role in absorption, distribution, or metabolism. As the route of exposure appears to be an important toxicokinetic factor, more studies would be helpful to adequately characterize the rate and extent of sulfur mustard absorption, distribution, and excretion via the dermal and inhalation routes, the most relevant routes of potential exposure.

Comparative Toxicokinetics. Data are available to indicate that the skin, respiratory tract, male reproductive system, and lymph nodes are targets in both humans and animals. Since humans do not have the fur that most laboratory animals do, and since humans have sweat glands over most of their body whereas animals do not, human responses to skin irritants such as sulfur mustard are different from those of animals. The hairless guinea pig model has been used to study the biochemical events in sulfur mustard toxicity. Toxicokinetic studies in animals (rats, mice, and pigs) (Black et al. 1992a, 1992b; Davison et al. 1961; Fidder et al. 1996a; Hambrook et al. 1992; Roberts and Warwick 1963; Sandelowsky et al. 1992; Smith et al. 1958) and humans (Benschop et al. 1997; Black and Read 1995b; Black et al. 1991; Jakubowski et al. 2000; Noort et al. 1999; Wils et al. 1985) indicate that the metabolites are similar across species.

Methods for Reducing Toxic Effects. There are established general decontamination procedures to reduce absorption of sulfur mustard (SBCCOM 2001), but there are no established procedures to reduce body burden or interfere with the mechanism of action of sulfur mustard in humans. Treatments to improve compromised function are primarily supportive. Based on current concepts regarding the mechanisms of toxicity of sulfur mustard, compounds with known biochemical or cellular actions can be identified that may interfere with some or all of pathways of toxicity. Additional studies providing a

more thorough mechanistic understanding, identification of additional toxicity pathways, and validation of the efficacy of existing compounds would be valuable.

Children's Susceptibility. There is qualitative evidence that children are a sensitive group at risk (Momeni et al. 1992; Momeni and Aminjavaheri 1994). Besides two reports of accidental deaths of children exposed to sulfur mustard (Dacre and Goldman 1996; Heully et al. 1956), clinical reports of children exposed during the Iran-Iraq War provide the only non-lethal effects data in children (Momeni et al. 1992; Momeni and Aminjavaheri 1994). The main exposure pathways for children are the same as for adults. The time of onset of sulfur mustard manifestations appears to be shorter, and the lesion severity greater, in children than in adults, possibly due to more delicate skin and epithelial tissues. Children's susceptibility to the effects of sulfur mustard is likely correlated to their understanding of the need for precautionary measures, ability to recognize exposure, and initiate decontamination.

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

One of the major goals of future medical chemical defense research on vesicants is the search for effective prophylactic and therapeutic countermeasures. Screening programs exist for candidate antidotes.

Ongoing studies pertaining to sulfur mustard identified in the Federal Research in Progress database (FEDRIP 2002) are shown in Table 3-4.

Table 3-4. Ongoing Studies on Health Effects of Sulfur Mustard

Investigator	Affiliation	Research description	Study sponsor
Back, DD	Mainstream Engineering Corporation Rockledge, Florida	Highly destructive polymer-contained neutralizing skin protectants: Feasibility of coated topical skin protectant additives using a new class of reactive metal alloys	Army
Hendler, FJ MD, PhD	Department of Veterans Affairs Louisville, Kentucky	Effect of hazardous substances on reproductive capacity and developmental abnormalities	Department of Veterans Affairs Washington, DC
Hinshaw, DB MD	Department of Veterans Affairs Ann Arbor, Michigan	The cytoskeleton and ATP in sulfur mustard-mediated injury to endothelial cells and keratinocytes	Department of Veterans Affairs Washington, DC
Klabunde, KJ	Nantek, Inc. Manhattan, Kansas	Development of reactive topical skin protectants against sulfur mustard and nerve agents	Army
Myer, SB	Tienzyme, Inc. State College, Pennsylvania	Use of fungal peroxidases for neutralization of mustard gas	Army
Richmond, A PhD	Department of Veterans Affairs Nashville, Tennessee	The role of chemokines in wound healing and sepsis:chemical burn (sulfur mustard) model of injury	Department of Veterans Affairs Washington, DC
Sweeney, JF MD	Department of Veterans Affairs Ann Arbor, Michigan	Regulation of polymorphonuclear-leukocyte (PMN) survival and function by proinflammatory agents that are released as a consequence of sulfur mustard mediated injury	Department of Veterans Affairs Washington, DC

Source: FEDRIP 2002

4. CHEMICAL AND PHYSICAL INFORMATION

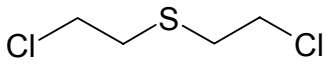
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of sulfur mustard is located in Table 4-1. Sulfur mustard has several synonyms; the most common are “mustard gas”, “H”, and “HD”. The term “mustard gas” may be used interchangeably to identify “sulfur mustard.” “H” refers to undistilled or raw sulfur mustard, which contains a large fraction of impurities (see Table 4-2). “HD” refers to a distilled or purified form of sulfur mustard (see Table 4-3). “HT” is often called sulfur mustard even though it is a mixture of 60% “HD”, <40% Agent T (bis[2-(2-chloroethylthio)ethyl]ether, CAS# 63918-89-8), and a variety of sulfur contaminants and impurities. Most studies on sulfur mustard are based on its distilled or purified form, “HD” (Munro et al. 1999). Other mustard agents, such as “HN” or nitrogen mustard (i.e., bis(2-chloro-ethyl)methylamine hydrochloride; CAS No. 55-86-7) and lewisite (i.e., 2-chlorovinyldichloroarsine; CAS No. 541-25-3) are related to sulfur mustard. Information about “HN”, “HT”, and lewisite are not included in this document.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of sulfur mustard (HD) is located in Table 4-4. Weapons-grade sulfur mustard can contain stabilizers, starting materials, or by-products formed during manufacturing, and products formed from slow reactions during storage (Munro et al. 1999). The typical compositions of HD and H are illustrated in Tables 4-3 and 4-4, respectively (NRC 1999; Rosenblatt et al. 1996). In general, a residual “heel” (i.e., a gel that will not flow) forms with the ageing of sulfur mustard. The heel can amount to more than 10% of the agent and usually contains 14–53% sulfur mustard, 42–86% cyclic sulfonium ions, and metals, such as iron sulfide (NRC 1999).

Table 4-1. Chemical Identity of Sulfur Mustard

Characteristic	Information	Reference
Chemical name	Bis(2-chloroethyl) sulfide	HSDB 2002
Synonym(s)	β,β'-Dichloroethyl sulfide; β,β'-Dichloroethyl sulphide; 1-Chloro-2-(β-chloroethylthio)ethane; 1,1'-Thiobis-(2-chloroethane); 2,2'-Dichlorodiethyl sulfide; 2,2'-Dichlorodiethyl sulphide; 2,2'-Dichloroethyl sulphide; 2,2'-Dichloroethyl sulfide; Bis(β-chloroethyl)sulfide; Bis-(β-chloroethyl)sulphide; Bis(2-chloroethyl)sulphide; Di-2-chloroethyl sulfide; Di-2-chloroethyl sulphide; Dichloro-diethyl-sulphide; Dichlorodiethyl sulfide; Dichloroethyl sulfide; Diethyl sulfide, 2,2'-dichloro; Distilled mustard; Ethane, 1,1'-thiobis(2-chloro-; Gelbkreuz; H; HD; Kampstoff "Lost"; Lost; Mustard, sulfur; Mustard vapor; Mustard gas; Mustard HD; S mustard; S-lost; S-Lost; S-yperite; Schwefel-Lost; Senfgas; Sulfide, bis(2-chloroethyl); Sulfur mustard gas; Sulfur mustard; Yellow Cross Liquid; Yellow Cross Gas	HSDB 2002
Registered trade name(s)	No data	
Chemical formula	$C_4H_8Cl_2S$	Budavari et al. 1996
Chemical structure		Budavari et al. 1996; IARC 1975
Identification numbers:		
CAS registry	505-60-2	HSDB 2002
NIOSH RTECS	WQ0900000	HSDB 2002
EPA hazardous waste	No data	
OHM/TADS	No data	
DOT/UN/NA/IMCO shipping	UN 2810	DOT 2002
HSDB	336	HSDB 2002
NCI	No data	

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

Table 4-2. Typical Composition of Sulfur Mustard (H) from an Old Chemical Munition

Compound	CAS No.	GC/MS peak area percent
Sulfur mustard	505-60-2	62.2
Bis(2-chloroethyl) disulfide	1002-41-1	10.9
1,4-Dithiane	505-29-3	3.2
Bis(2-chloroethyl) trisulfide	19149-77-0	9.6
1,2-Bis(2-chloroethylthio)ethane	3563-36-8	2.6
1,2,3-Trithiolane	—	2.4
1,4-Thioxane	15980-15-1	0.1
1,2,5-Trithiepane	6576-93-8	0.9
1,2,3,4-Tetrathiane	—	1.4
1,2-Dichloroethane	107-06-2	3.2
HD tetrasulfide	—	0.6
Tetrachloroethene	127-18-4	0.3
Sulfur	7704-34-9	0.5
Other	—	1.3

GC/MS = gas chromatography/mass spectrometry

Source: Rosenblatt et al. 1996

Table 4-3. Typical Composition of Sulfur Mustard (HD) in 1-Ton Storage Containers (Aberdeen, Maryland)

Compound	CAS No.	Mole percent
Sulfur mustard	505-60-2	91.38
Q sulfonium	30843-67-5	6.08
2-Chloroethyl 4-chlorobutyl sulfide	114811-35-7	0.86
1,4-Dithiane	505-29-3	0.81
1,2-Dichloroethane	107-06-2	0.35
Bis-3-chloropropyl sulfide	22535-54-2	0.18
2-Chloropropyl 3'-chloropropyl sulfide	—	0.18
2-Chloroethyl 3-chloropropyl sulfide	71784-01-5	0.14
1-Chloropropyl 2-chloroethyl sulfide	—	0.02
1,4-Thioxane	15980-15-1	<0.01

Source: NRC 1999

Table 4-4. Physical and Chemical Properties of Sulfur Mustard

Property	Information	Reference
Molecular weight	159.08	Budavari et al. 1996
Color	Clear	Budavari et al. 1996
	Pale yellow, black if impure	Munro et al. 1999
Physical state	Oily liquid	Budavari et al. 1996
Melting point	13–14 °C	Budavari et al. 1996
Boiling point	217.5 °C	Budavari et al. 1996
Density:		
	1.338 at 13 °C	Budavari et al. 1996
	1.2685 at 25 °C	Rosenblatt et al. 1996
Odor	Weak, sweet, agreeable odor	Budavari et al. 1996
Odor threshold:		
Water	No data	
Air	0.6 mg/m^3	Bowden 1943
Solubility:		
Water	920 mg/L at 22 °C	Rosenblatt et al. 1996
	684 mg/L at 25 °C	Seidell 1941
Organic solvent(s)	Soluble in alcohol, ether, acetone, and benzene; miscible with petroleum ether	HSDB 2002
	Soluble in fat solvents and other common organic solvents	IARC 1975
Partition coefficients:		
Log K_{ow}	2.41	HSDB 2002
	1.37	Rosenblatt et al. 1996
Log K_{oc}	2.43	HSDB 2002
Vapor pressure:		
at 22 °C	0.082 mmHg	Rosenblatt et al. 1996
at 25 °C	0.1059 mmHg	Rosenblatt et al. 1996
Henry's law constant		
	2.4×10^{-5} atm-m^3/mol	Opresko et al. 1998
	1.87×10^{-5} atm-m^3/mol	Rosenblatt et al. 1996
Autoignition temperature	No data	
Flashpoint	221 °F	Sax 1989
Conversion factors:	No data	
Explosive limits	No data	

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

5.1 PRODUCTION

Sulfur mustard is a synthetic organic compound. It was first manufactured in 1822 by the action of ethene on sulfur monochloride or dichloride. Since then, the methods of manufacture have been refined, although they have not been changed substantially. Three main processes have been used. The Germans produced sulfur mustard using the Meyer process, which involved treating ethylene with hypochlorous acid followed by sodium sulfide, yielding β,β'-dihydroxy-methyl sulfide. This in turn was heated with hydrochloric acid, which produced sulfur mustard. In the United States, sulfur mustard was formerly made using the Levenstein process in which ethylene was reacted with sulphur monochloride at 30–35 °C. Sulfur mustard produced by this process contains 62–64% distilled sulfur mustard (or HD) (Munro et al. 1999). This process produces a complex mixture that includes constituents that are more toxic than sulfur mustard itself (Rosenblatt et al. 1975). The most recent process used in the United States involved the formation of bis-(2-hydroxyethyl)-thioether from ethylene oxide and hydrogen sulfide; this was then reacted with hydrochloric acid to form sulfur mustard (Franke 1967; IARC 1975; Rosenblatt et al. 1975). Sulfur mustard produced by this process contains 89% HD (Munro et al. 1999).

Sulfur mustard was manufactured in large quantities during World Wars I and II, but has not been manufactured on an industrial basis in the United States since 1968 (NRC 1994). Stockpiles of sulfur mustard are stored in 1-ton containers and/or chemical munitions at Blue Grass Army Depot in Kentucky, Anniston Army Depot in Alabama, Umatilla Depot Activity in Oregon, Pine Bluff Arsenal in Arkansas, Tooele Army Depot in Utah, Pueblo Army Depot Activity in Colorado, and Aberdeen Proving Grounds in Maryland. Stockpiles of sulfur mustard were also located at the U.S. territory of Johnston Atoll in the North Pacific Ocean. Destruction of sulfur mustard at this location was completed in 2000. Sulfur mustard may also be found at non-stockpile locations in various containers, buried chemical munitions, and at former production facilities. These are currently 45 non-stockpile locations with sulfur mustard across the United States and in the U.S. Virgin Islands as shown in Figure 5-1 (NRC 1996, 2000). Sulfur mustard is probably still being made for laboratory experiments on a small scale.

Information about other mustard agents, such as nitrogen mustard (HN), thickened mustard (HT), and lewisite, are not included in this document (see Chapter 4).

Figure 5-1. Locations of Sulfur Mustard Stockpile and Non-stockpile Sites in the United States[a]

Source: OA 2000, 2002 (★) = Stockpile sites; (O) = Non-stockpile sites

AL
- Anniston Army Depot (★)
- Ft McClellan (O)
- Camp Sibert (O)
- Huntsville Arsenal (O)
- Redstone Arsenal (O)
- Theodore Naval Ammunition Magazine (O) – not shown on map

AK
- Adak (O)
- Chicagoff Harbor (O)
- Gerstle River Test Center (O)

AZ
- Navajo Depot Activity (O)
- Yuma Proving Ground (O)

AR
- Pine Bluff Arsenal (★)

CA
- Ft Ord (O)
- Edwards AFB (O)

CO
- Rocky Mountain Arsenal (O)
- Pueblo Army Depot Activity (★)

FL
- Brooksville Army Air Base (O)
- Drew Field (O)
- MacDill AFB (O)
- Withlacoochee (O)
- Dry Tortuga Keys (O)
- Zephyr Hills Gunner Range (O)

GA
- Ft. Gillem (O)
- Manchester (O)

HI
- Kipapa Ammunition Storage Site (O)
- Schofield Barracks (O)

IL
- Savanna Army Depot Activity (O)

IN
- Camp Atterbury (O)
- Naval Weapons Support Center (O)

KS
- Marysville (O)

KY
- Blue Grass Army Depot (★)

LA
- Ft Polk (O)
- Concord Spur (O)

MD
- Edgewood Area- Aberdeen Proving Grounds (★)

MS
- Camp Shelby (O)
- Columbus Army Airfield (O)
- Horne Island (O)

NC
- Laurinburg-Maxton Army Air Base (O)

NE
- Nebraska Ordnance Plant (O)

NV
- Hawthorne Army Ammunition Plant (O)

NJ
- Raritan Arsenal (O)

OH
- Ravenna Army Ammunition Plant (O)

OR
- Umatilla Depot Activity (★)

SC
- Charleston Army Depot (O)
- Naval Weapons Center (O)–not shown on map

SD
- Black Hills Ordnance Depot (O)

TN
- Defense Depot Memphis (O)

TX
- Ft Hood (O)
- Camp Stanley Storage Activity (O)
- Camp Bullis (O)

UT
- Dugway Proving Grounds (O)
- Defense Depot Ogden (O)
- Tooele Army Depot (★)

VI
- Ft. Segarra (St. Thomas) (O)

Other
- Johnston Atoll (S. Pacific)(★)

Source: NRC 1996, 2000

[a]post office state abbreviations used

5.2 IMPORT/EXPORT

Sulfur mustard is not imported into or exported from the United States.

5.3 USE

The principal use of sulfur mustard was as a vesicant chemical warfare agent. The Germans first used it against the British during World War I during the battle of Flanders, near Ypres, Belgium, in 1917 (Franke 1967; Rosenblatt et al. 1975). It was used by the Allies in 1918 and by the Italians in Ethiopia in 1936. It has also been used recently in the Iran-Iraq War in 1984–1988 and by Iraq against its Kurdish minority in Halabjah in 1988 (Black et al. 1993b; Budiansky 1984).

Attempts have been made to use sulfur mustard as an antineoplastic agent, although this has not met with much success due to its high toxicity. A similar product, nitrogen mustard, has been successfully employed as an anticancer agent (IARC 1975). Sulfur mustard has provided a useful model in biological studies concerning the behavior of alkylating agents (IARC 1975). Sulfur mustard has also been used medicinally to control hyperproliferation of psoratic keratinocytes (SBCCOM 1999).

5.4 DISPOSAL

For the past several decades, the United States has stored its stockpile of sulfur mustard at eight Army facilities under a policy of total containment (Colburn 1978). The total quantity of sulfur mustard (i.e., H, HD, and HT) in the original stockpile was 17,358 tons (34,716,945 pounds) (DOA 2000). The stockpile consists of both munitions and 1-ton containers of bulk agent (see Table 5-1; DOA 2000, 2002; NRC 1994). In addition to sulfur mustard, munitions may contain energetics (e.g., explosives and propellants). Public Law (PL) 99-145 (as amended by PL 100-456) and PL 104-484 (October 23, 1992) requires the Army to destroy the U.S. stockpile of all lethal unitary chemical agents and munitions by December 31, 2004 (DOA 1988; NRC 1994). Some chemical-warfare agents and related material, referred to as non-stockpile chemical material (NSCM), were not included in the stockpile inventory, but were subsequently added to the chemical demilitarization program in HR 101-822, which accompanied

Table 5-1. Original Stockpile Quantities of Sulfur Mustard as Munitions and Bulk Agenta

Chemical munitions or bulk agent	APG	ANAD	BAD	JAP[b]	PBA	PUDA	TEAD[c]	UMDA
HD								
105-mm projectile		68,500		140		1,138,760	5,860	
155-mm projectile		206,420	181,260	66,340		3,504,780		
4.2-inch mortar		452,160		116,294		460,340		
M60 projectile				261,960				
Ton container	3,249,740	185,080		116,294	188,400		11,383,420	
H								
155-mm projectile							639,540	
Ton container								4,679,040
HT								
4.2-inch mortar		1,064,600				118,220	363,020	
Ton container					6,249,100			
Total	3,249,740	1,976,760	181,260	578,705	6,437,500	5,222,100	12,391,840	4,679,040
Percent of total sulfur mustard stockpile	9.4	5.7	0.5	1.7	18.5	15.0	35.7	13.5

[a]Quantities of agent reported in pounds. Original stockpile quantities reflect amounts before the onset of Chemical Stockpile Disposal Program. Quantities do not include non-stockpile amounts of sulfur mustard. Up-to-date information about stockpile destruction is available at http://www-pmcd.apgea.army.mil/.
[b]As of the year 2000, the entire stockpile of sulfur mustard at JAP has been destroyed.
[c]As of November 1, 2002, 44% of the agent and 81% of the munitions at TEAD have been destroyed (exact percentage of sulfur mustard destruction is unspecified)

ANAD = Anniston Army Depot, Alabama; APG = Aberdeen Proving Ground, Maryland; BAD = Blue Grass Army Depot, Kentucky; H = undistilled sulfur mustard; HD = distilled sulfur mustard; HT = 60% sulfur mustard + 40% Agent T; JAP = Johnston Atoll, Pacific Ocean; PBA = Pine Bluff Arsenal, Arkansas; PUDA = Pueblo Depot Activity, Colorado; TEAD = Tooele Depot, Utah; UMDA = Umatilla Depot Activity, Oregon

Source: DOA 2000, 2002

the 1991 Defense Appropriations Act. NSCM includes lethal wastes from past disposal efforts, unserviceable munitions, chemically contaminated containers, chemical-production facilities, newly located chemical munitions, and known sites containing significant quantities of buried chemical weapons and waste (NRC 2000).

As part of the Chemical Stockpile Disposal Program (CSDP) mandated by Congress, the Army currently uses the "baseline system" for destruction of munitions and bulk agents containing sulfur mustard (NRC 1994). The "baseline system" consists of several steps: (1) storage, transportation, and unloading of munitions and containers, (2) disassembly and draining, (3) agent destruction, (4) energetics destruction, (5) metal parts decontamination, and (6) dunnage (i.e., other contaminated materials) disposal (NRC 1994). Munitions are currently stored and monitored in vented igloos; bulk containers are stored in the open or in monitored warehouses. The munitions or bulk sulfur mustard are transported to the on-site disposal facility and unloaded. Munitions are disassembled, drained of sulfur mustard, and separated into streams of bulk liquid agent, metal parts, energetics, and dunnage, all of which contain different amounts of sulfur mustard. Liquid agents from drained munitions and bulk containers are fed into a primary incinerator preheated to an operating temperature of 2,700 °F (1,480 °C). Exhaust gases from the primary incinerator are fed into a secondary incinerator at a temperature of 2,200 °F (1,200 °C) for 2 seconds, after which 99.9999% of the agent is destroyed (DOA 2000). The gaseous effluents then flow into pollution abatement system before release into the atmosphere. Energetic materials are burned in a counterflow rotary kiln and then heated on a discharge conveyor at 1,000 °F (540 °C); the solid waste produced is nonhazardous and may be shipped for land disposal. Discharged gases pass through a secondary incinerator and a pollution abatement system, and then are released to the atmosphere. Metal parts are heated to 1,000 °F for 15 minutes in a fuel-fired metal parts furnace; the heat-treated metal parts are then released as scrap metal. Gases discharged pass through a secondary incinerator and a pollution abatement system, and then are released to the atmosphere. Dunnage generated during the entire process may be either incinerated (with pollution abatement) or shipped for land disposal as hazardous waste. At all steps, monitoring for chemical agents is performed to detect concentrations of the agent well below those that present an immediate threat to personnel or the surrounding population. There are no measurable sulfur mustard effluents leaving the baseline system facilities under normal operating conditions (MacNaughton 2001). At present, Johnston Atoll is the first site to destroy its portion of the chemical agent and munitions stockpile in the United States. Incineration of sulfur mustard is currently underway at Tooele, Utah. Construction of baseline system facilities near Umatilla, Oregon and Anniston, Alabama are completed with operational testing in progress at these facilities. As of late 2002,

a baseline system facility at Pine Bluff, Arkansas is near completion with operational testing to be conducted afterwards.

To address growing public concern over incineration, in 1992, Congress directed the Army to evaluate alternative disposal methods that might be significantly safer and more cost effective than the baseline system (NRC 1994). Two alternatives were accepted by the Army for further development: (1) stand-alone neutralization followed by incineration and (2) neutralization followed by bio-treatment (NRC 1996). Neutralization of sulfur mustard is achieved by hydrolysis with hot water (90 °C) and vigorous mixing. This process reduces the sulfur mustard concentrations to levels <200 ppb and selectively converts 90% of the sulfur mustard to thiodiglycol and hydrochloric acid (Currie et al. 1977; May 1998; NRC 1996). Once the reaction is complete, a base (e.g., sodium hydroxide or lime) is added to neutralize the acid and adjust the pH of the hydrolysate (i.e., product of hydrolysis). The dilute processing of sulfur mustard and the addition of base after completion of the neutralization reaction are designed to minimize the production of unwanted byproducts during reaction (NRC 1996). Hydrolysis has been used effectively to detoxify over 700 tons of sulfur mustard located at a Canadian defense facility in Cornwall, Ontario (Currie et al. 1977). After hydrolysis, the hydrolysate can either be incinerated using the baseline system or biotreated. Biotreatment requires adjusting the pH of the hydrolysate to neutral by adding sodium bicarbonate buffer and some nutrients. Bacteria oxidize thiodiglycol to carbon dioxide, water, and sulfate with high efficiency. During the actual process, approximately 0.8 g of cell mass (dry weight) will be produced for every 1 g of organic carbon removed from solution. The biomass is further oxidized through aerobic digestion, and then dried and disposed of at a commercial water treatment facility. Any volatile organic compounds (VOCs) that are present are condensed and the resulting condensate is removed by direct photodegradation and photooxidation by OH radicals. The treated bioresidue is then filtered, dried, and sent to landfill (May 1998; NRC 1996). A chemical neutralization facility is currently being constructed at Aberdeen, Maryland, where sulfur mustard is stored only as bulk liquid in 1-ton containers (NRC 1994). The waste product from this facility will be transported to Dupont's Chambers Works Plant in Deepwater, New Jersey for final treatment at this chemical waste disposal facility. Chemical neutralization will also be used to destroy the sulfur mustard stockpile at Pueblo, Colorado. In November 2002, the Department of Defense selected neutralization followed by super critical water oxidation to destroy the chemical weapons stockpile at the Blue Grass Army Disposal in Kentucky (DOD 2003).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

Sulfur mustard has been identified in at least 3 of the 1,636 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2003). However, the number of sites evaluated for sulfur mustard is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, all are located within the United States.

Sulfur mustard is not a naturally occurring compound and its primary application is in chemical warfare. The United States has not produced it since 1968. Chemical agents, such as sulfur mustard, are extremely hazardous materials, which is why they were used as weapons. The hazard is increased when the agent is contained in explosively configured munitions, an inherent feature of chemical weapons. Since chemical weapons no longer have any value as a military deterrent, Congress has mandated that all chemical agents and munitions be destroyed by the end of the year 2004 (NRC 1994). However, the destruction of all chemical agents and munitions in the United States containing sulfur mustard is likely to continue for some unspecified time beyond this date. Sulfur mustard is known to be stored at seven Army bases (see Section 5.1) across the continental United States, some of which may also be at NPL sites (DOA 1988). Persons working at or living near Army bases where this material is stored or destroyed are at a greater risk of exposure.

Information about other mustard agents (e.g., nitrogen mustard or HN, thickened mustard or HT, and lewisite), although related to sulfur mustard, is not included in this document (see Chapter 4).

6.2 RELEASES TO THE ENVIRONMENT

During World War I with its use as a chemical warfare agent, sulfur mustard was released directly to the atmosphere in countries outside the United States. From World War I until the 1970s, disposal of chemical weapons, such as sulfur mustard, at sea was accepted practice. Consequently, sulfur mustard is found in ocean waters at several sites around the world. It does not occur naturally, and is no longer produced in the United States. Sulfur mustard that was produced for military applications is now being

Figure 6-1. Frequency of NPL Sites with Sulfur Mustard Contamination

stored in military depots and storage facilities across the United States (see Section 5.1). Both sulfur mustard agent and munitions are currently or will be destroyed on site at these Army facilities. All chemical agents maintained in the Army stockpile are now at least 30 years old and some are more than 50 years old; none were manufactured after 1968 (NRC 1994). There have been almost 1,500 "leaking" munitions identified in the stockpile since 1982, some of which are leaking sulfur mustard (NRC 1994). In September 1993, a 100-gallon spill from a 1-ton container of sulfur mustard was discovered at Tooele Army Depot, Utah (NRC 1994). Other leaks of sulfur mustard have been identified from chemical munitions (e.g., 155-mm projectiles) as recently as October 16, 2002 (DOA 2003). Thus, environmental releases of sulfur mustard may potentially occur near Army bases where this material is stored and destroyed. However, because of the Army's efforts to mitigate exposure of the general population to sulfur mustard (as well as to other stockpile chemical agents), no releases of sulfur mustard have been reported beyond the confines of these storage locations.

6.2.1 Air

Sulfur mustard may be released to air at stockpile and non-stockpile sites across the United States where sulfur mustard is known to be located. However, because of the Army's efforts to mitigate exposure of the general population to sulfur mustard (as well as to other stockpile chemical agents), no releases of sulfur mustard to air have been reported beyond the confinement of these facilities. No known releases of sulfur mustard to the atmosphere have been reported with the destruction of sulfur mustard by incineration (MacNaughton 2001).

Sulfur mustard has not been identified in air at any of the three NPL hazardous waste sites where it was detected in some environmental media (HazDat 2003).

6.2.2 Water

From World War I until the 1970s, disposal of sulfur mustard at sea was standard practice. However, limited information about this practice is available before the mid-1940s. In 1943, sulfur mustard was released into the waters of Bari Harbor, Italy with the sinking of the American freighter, S.S. John Harvey (SIPRI 1971). Since the end of World War II, ocean dumping has occurred in many areas, such as the Baltic Sea (Mazurek et al. 2001); the coastal waters around Japan (Kurata 1980); the Adriatic Sea near

Bari, Italy; and the coastal waters of the United States (Brankowitz 1987). Some of the known ocean dumping sites off the continental United States are summarized in Table 6-1.

Sulfur mustard has not been identified in groundwater or surface water at the three NPL hazardous waste sites where it was detected in some environmental media (HazDat 2003).

6.2.3 Soil

No releases of sulfur mustard to soil have been reported in the literature. However, sulfur mustard is currently stored at several sites around the United States and its territories in stockpile and non-stockpile quantities (see Figure 5-1). Non-stockpile locations include known sites containing significant quantities of buried chemical weapons and wastes. Sulfur mustard is the most frequently identified material at these sites (NRC 2000).

Sulfur mustard has been identified in soil at one site of the three NPL hazardous waste sites where it was detected in some environmental media (HazDat 2003).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

On the basis of its use during warfare and its physical/chemical properties, sulfur mustard should partition to and be transported in the atmosphere following release. The vapor pressure of sulfur mustard is moderate (0.11 mm Hg at 25 °C), but is high enough for sulfur mustard to be in air in the immediate vicinity of liquid droplets (DOA 1996).

On surface soil, Small (1984) reported that volatilization would be the main route of sulfur mustard loss. However, on moist surface soil, hydrolysis would be the main loss pathway. At 25 °C, sulfur mustard deposited on a surface soil will evaporate within 30–50 hours (Munro et al. 1999). Meteorologic conditions such as temperature and wind will greatly affect the persistence of sulfur mustard on soil; with warmer temperatures and stronger winds, persistence of sulfur mustard decreases (Franke 1967). For example, sulfur mustard will vaporize 2–3 times faster at 20 °C than at 5 °C (Franke 1967). The freezing

Table 6-1. Location of Historical Dumping Areas for Sulfur Mustard (H) in Coastal Waters of the United States

Location of munitions loading	Destination	Date	Munition	Quantity
Attu and Adak, Alaska	12 miles off Chichagoff	1947	Bulk agent	Unknown
Charleston, South Carolina	Site “Baker”	August—October 1946	Bombs, projectiles, mines, bulk	Over 7 tons
Colts Neck Naval Pier, Earle, New Jersey	39˚ 39’ N, 70˚ 57’ W	June 15, 1967	Rockets, Bulk	3,890 tons
Colts Neck Naval Pier, Earle, New Jersey	39˚ 33’ N, 71˚ 02’ W	August 7, 1968	Contaminated water	2,975 tons
Edgewood Arsenal, Maryland	38˚ 30’ N, 72˚ 10’ W	June 18, 1962	Projectiles, Bulk	3 tons
Edgewood Arsenal, Maryland	38˚ 30’ N, 71˚ 06’ W	August 6–7, 1964	Bulk, Projectiles	65 tons
Naval Mine Depot, Yorktown, Virginia	Site “Baker”	March 21–25, 1946	Projectiles	13 tons
New Orleans Port of Entry, Braithwaite, Louisiana	Gulf of Mexico	March 1–7, 1946	Projectiles	207 tons
NWS Concord, California	37˚ 40’ N, 125˚ 0’ W	April 8–19, 1958	Bulk	9,030 tons
Theodore Naval Magazine, Mobile, Alabama	Gulf of Mexico	July 13, 1946	Bombs (German)	7 tons

Source: Brankowitz 1987

point of sulfur mustard is between 13 and 15 °C. In temperate regions, sulfur mustard should be a solid for half of the year (Munro et al. 1999). Solidified sulfur mustard is less volatile, of lower water solubility, and is less reactive than liquified sulfur mustard. A study of persistence under winter conditions found that sulfur mustard could be detected after 2 weeks, but was below detection limits (not stated) at 4 weeks (Franke 1967). When snowfall covered samples, high recoveries were demonstrated even after 4 weeks. This study also showed that persistence was affected by the size of droplets. Larger droplets of sulfur mustard increased both stability and recovery (Johnsen and Blanch 1984). Other factors that influence vaporization include pH, moisture content, porosity of the surface, and physical constituents of the soils (Rosenblatt et al. 1975). Because of its low solubility in water (920 mg/L) and ease of hydrolysis once dissolved (see Section 6.3.2.2), sulfur mustard is not transported through soil into groundwater (Munro et al. 1999).

In water, sulfur mustard will volatilize to air, hydrolyze, or remain unchanged. Without turbulence and at low temperatures, large quantities of sulfur mustard will persist under water for long periods of time (Munro et al. 1999). For example, sulfur mustard disposed of in sea water at several locations around the world continues to be brought to the surface where it has injured unsuspecting fisherman (Jorgensen et al. 1985; Kurata 1980; Mazurek et al. 2001). Volatilization of sulfur mustard from water surfaces is expected to be moderate based upon a Henry's law constant of 2.1×10^{-5} atm·m^3/mol (DOA 1996). Using this Henry's law constant and an estimation method (Lyman et al. 1990), volatilization half-lives of sulfur mustard for a model river and model lake are 36 hours and 503 days, respectively. Hydrolysis of sulfur mustard may be slow because of its limited solubility and the fact that sulfur mustard freezes at 14 °C (see Section 6.3.2.2). Sulfur mustard is expected to sink to the bottom of the water column because it is denser than water (1.27 g/cm^3 at 20 °C; see Table 4-1).

Sulfur mustard does not bioconcentrate or biomagnify due to its reactivity. It is also unlikely that it is transported through the vascular systems of plants since it would almost surely undergo hydrolysis in the process (Rosenblatt et al. 1975).

6.3.2 Transformation and Degradation

6.3.2.1 Air

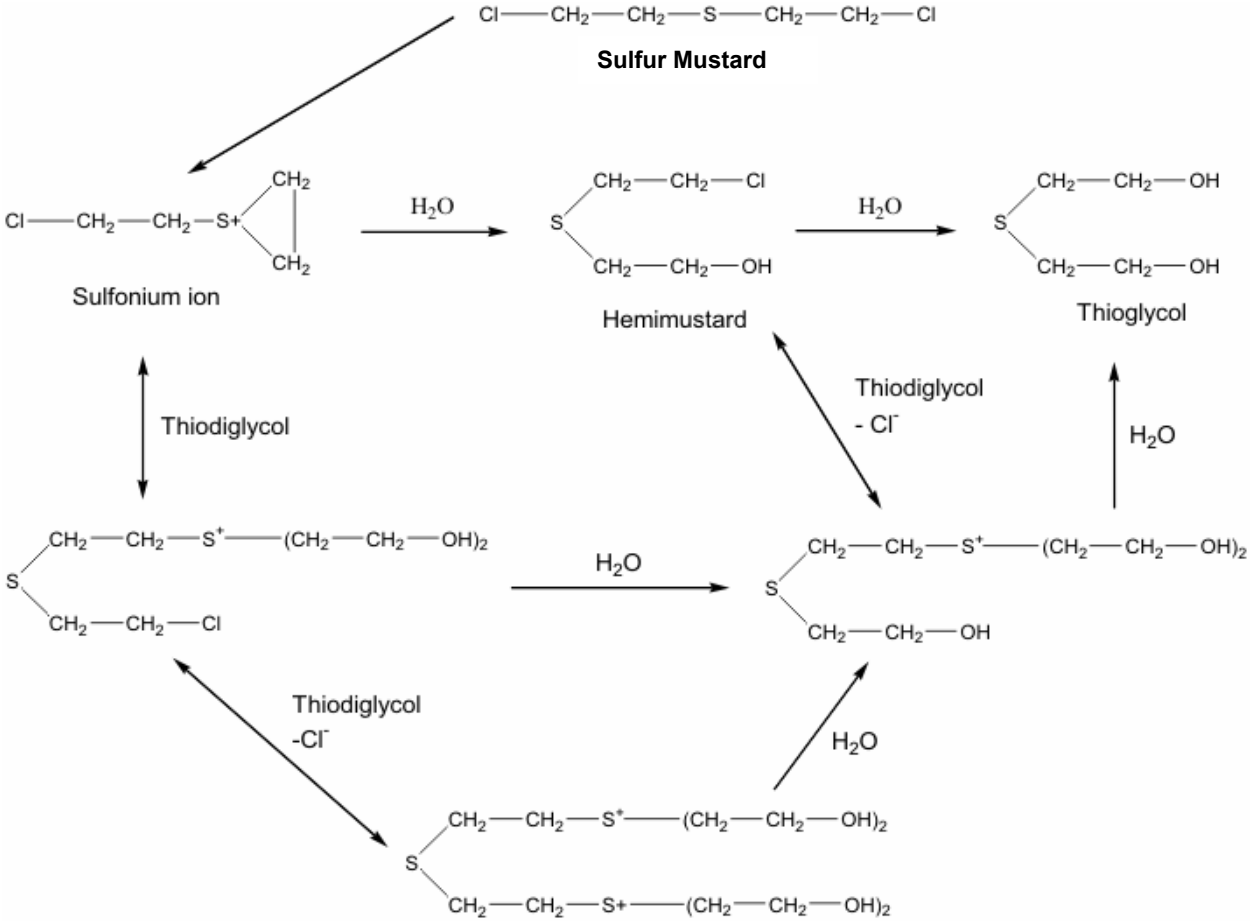
Sulfur mustard does not absorb ultraviolet (UV) radiation above 290 nm (Rewick et al. 1986); thus, photodegradation should not be an important fate process. The rate constant for the vapor-phase reaction of mustard with photochemically-produced hydroxyl radicals has been estimated as 7.82×10^{-12} cm^3/molecule-s at 25 °C using a structure estimation method (Meylan and Howard 1993). This corresponds to an atmospheric half-life of about 2.1 days at an atmospheric concentration of 5×10^5 hydroxyl radicals/m^3 (assumed average concentration in non-smog conditions). Under smog conditions, reaction with nitrate radicals may be important.

6.3.2.2 Water

While hydrolysis of sulfur mustard is relatively rapid in water once dissolved, sulfur mustard dissolution is relatively slow (Bartlett and Swain 1949; Clark 1989; Rosenblatt et al. 1975; Small 1984; Stein 1946). Dissolved sulfur mustard has a hydrolysis half-life of 4–8 minutes at 25 °C in distilled water (Bartlett and Swain 1949). In several studies reviewed by Small (1984), the hydrolysis half-life (first-order rate) of dissolved sulfur mustard ranges from 158 minutes at 0.6 °C to ~1.5 minutes at 40 °C. The hydrolysis products of sulfur mustard are primarily mustard chlorohydrin, thiodiglycol, and hydrochloric acid; others include intermediates such as cyclic sulfonium salts (Rosenblatt et al. 1975, 1996). The hydrolysis of mustard chlorohydrin is somewhat faster than the hydrolysis of sulfur mustard. Consequently, mustard chlorohydrin does not accumulate to high concentrations. Conditions involving relatively small quantities of water give rise to higher concentrations of the cyclic sulfonium salt intermediates, which are rather toxic. Hydrolysis pathways of sulfur mustard in the environment are illustrated in Figure 6-2.

Sufficient levels of chlorine in the water (e.g., salt water) will inhibit the hydrolysis reaction; hydrolysis is decreased by a factor of 2.5 in salt water over fresh water (Clark 1989; Rosenblatt et al. 1975, 1996). Chloride ions react with the cyclic sulfonium intermediates to reform sulfur mustard. Impurities found in sulfur mustard (e.g., polysulfides) might slow the dissolution of the agent, and if they dissolve in water, they will react more slowly with water than sulfur mustard (Rosenblatt et al. 1996). One impurity, 1,2-bis(2-chloroethylthio)ethane, is about 5 times as vesicant as sulfur mustard itself; others, such as 1,8-dichloro-3-oxa-6-thiaoctane, are probably about as toxic as sulfur mustard.

Figure 6-2. Primary Hydrolysis Pathways of Sulfur Mustard in the Environment



Source: Munro et al. 1999

Because sulfur mustard has limited solubility (920 mg/L in water; Rosenblatt et al. 1996), hydrolysis is limited by its slow rate of solution (i.e., $6.77x10^{-8}$ g/cm^2·s at 10 °C). During the dissolution process, the outer surface of a sulfur mustard droplet dissolves and is rapidly hydrolyzed to sulfonium ions and thiodiglycol (see Figure 6-2). These compounds then react with sulfur mustard to form 1,2-bis[(2-chloro-ethyl)thio]ethane and 1,2-dichloroethane or together to form stable sulfonium polymers. Without agitation, sulfonium polymers build up, creating a thick boundary layer, which interferes with the transfer of sulfur mustard into bulk water. Dissolution of bulk sulfur mustard slows because the driving force for diffusion sulfur mustard into the bulk aqueous phase decreases (Rosenblatt et al. 1975, 1996). Without agitation, bulk sulfur mustard may persist in water for up to several years (Small 1984). Epstein et al. (1973) estimated that a 1-ton lump of sulfur mustard would require 5 years to dissolve in water.

The addition of water-soluble organic solvents, such as acetone and ethanol, permits greater concentrations of sulfur mustard to solubilize in water so as to facilitate hydrolysis (Clark 1989). For example, when a small amount of acetone (e.g., 5% solution in water) was used to dissolve sulfur mustard in water at 25 °C, the hydrolysis half-life was 9.0 minutes (first-order rate constant =0.00129 s^{-1}).

Oxidation of sulfur mustard is also known to occur. Reactions with hypochlorite, chlorine water, ozone, and hydrogen peroxide yield mustard sulfoxide, which is extremely stable to hydrolysis and slightly toxic. Further oxidation under more severe conditions forms mustard sulfone, a relatively nontoxic compound. However, in weakly alkaline solution, mustard sulfone is dehydrochlorinated to divinyl sulfone, which is highly toxic (Clark 1989; Price and Bullitt 1947; Rosenblatt 1975).

6.3.2.3 Sediment and Soil

Natural degradation of sulfur mustard in soil is a result of chemical hydrolysis and biodegradation. The major product of chemical hydrolysis is thiodiglycol. Chemical hydrolysis of sulfur mustard and its chlorine derivatives in soil depends on soil type and moisture content, degree of contamination, and temperature. If the moisture content of soil is <50% of its moisture capacity, then chemical hydrolysis in soil does not occur (Medvedeva et al. 2000). With higher temperatures and moisture content, the extent of hydrolysis of sulfur mustard increases, but never to 100% completion. Sulfur mustard is known to degrade faster in alkaline soils (Franke 1967). If sulfur mustard droplets are considerably below the soil surface, then sulfur mustard can persist for several years (Munro et al. 1999; Watson and Griffin 1992).

For example, sulfur mustard has been known persist for weeks to decades in military testing areas and land dumps where large quantities have been deposited underground.

Sulfur mustard can be biodegraded in soil via the thioether oxidation pathway, forming bis-(2-chloro-ethyl)-sulfoxide and corresponding sulfone (U.S. Army Dugway Proving Ground 1985). Recently, Wariishi et al. (2002) demonstrated that fungi (e.g., basidiomycetes) are able to degrade sulfur mustard in soil by directly cleaving the carbon-sulfur bond and by hydrolytic dechlorination. Sulfur mustard can also be biodegraded via reductive dehalogenation and dehydrohalogenation, although these pathways are predicted to be slow.

Degradation of the hydrolysis products of sulfur mustard (e.g., thiodiglycol) proceeds very slowly because these compounds are poorly utilized by microorganisms. For example, in a medially contaminated peaty gley soil characterized by a high rate of microbiological processes, the concentration of thiodiglycol decreased 50% after a year. However in a highly contaminated peaty gley soil, <10% of thiodiglycol degraded in a period of a year (Medvedeva et al. 2000). The reduction in microbial activity is a consequence of the high toxicity of sulfur mustard to soil microorganisms.

6.3.2.4 Other Media

No information was found in the literature regarding transformation and degradation reactions in other media.

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

6.4.1 Air

No information was found in the literature regarding environmental concentrations of sulfur mustard in the ambient atmosphere. Ambient concentrations of sulfur mustard are expected to be zero except near military facilities where former production occurred or where current disposal may be in progress. During World War I, when sulfur mustard was used, the average and maximum atmospheric concentrations in the combat zones were estimated at 3 and 5 ppm, respectively (IARC 1975).

6.4.2 Water

In 1943, sulfur mustard was released into the waters of Bari Harbor, Italy with the sinking of the American freighter, S.S. John Harvey (Mitretek Systems 2002). Since the end of World War II, ocean dumping has occurred in many areas (SIPRI 1971), such as the Baltic Sea (Mazurek et al. 2001); the coastal waters around Japan (Kurata 1980); the Adriatic Sea near Bari, Italy; and the coastal waters of the United States (Brankowitz 1987). Some of the known ocean dumping sites off the continental United States are summarized in Table 6-1. No information was located that describes the concentration of sulfur mustard at these locations.

6.4.3 Sediment and Soil

No information was found in the available literature regarding current soil concentrations of sulfur mustard. For some time after World War I, much of the French soil in the region of battle lines was contaminated, although it is unlikely to have persisted to the present day (IARC 1975). If any sulfur mustard still exists, it would be present only as pockets of liquid, perhaps dissolved in discarded oil, or absorbed on an inert anhydrous soil medium (Rosenblatt et al. 1975). Before 1945, sulfur mustard was produced at the Rocky Mountain Arsenal in Colorado. Only traces of sulfur mustard have been found in soil samples at 3 or 4 locations out of 15,000 sampled during the recent clean-up of this site (Cohn 1999). Soils in Fort McClellan, Alabama are highly polluted with sulfur mustard and its many impurities (Dacre 1994). No additional information was found that quantifies the level of contamination at this site. Other sites where sulfur mustard may be present include non-stockpile sites across the United States (see Figure 5-1). No information was located that quantifies the concentrations of sulfur mustard at these sites.

6.4.4 Other Environmental Media

Normal urinary levels of thiodiglycol, a hydrolysis product of sulfur mustard, are <1 ng/mL, but levels up to *ca.* 16 ng/mL are found in blood (Black and Read 1991). The source of this low backgrounds level is unknown, but sulfur-rich foods in the diet may be one possibility. No other information was found in the available literature regarding concentrations of sulfur mustard in environmental media.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population in the United States is not exposed to sulfur mustard since it is found primarily at seven Army bases in stockpiles (Munro et al. 1999) and at 45 non-stockpile sites across the United States (see Figure 5-1). Populations located near these sites have a potentially greater risk of exposure to sulfur mustard. However, because of the Army's efforts to mitigate exposure of the general population to sulfur mustard (as well as to other stockpile chemical agents), no releases of sulfur mustard have been reported beyond the confines of these facilities. Also, since the entire stockpile of sulfur mustard agent and munitions is presently being destroyed onsite at several locations around the United States, the risk of exposure from accidental leaks and spills of sulfur mustard at these locations is decreasing for the general population. In countries where sulfur mustard was released during warfare (e.g., Belgium, Morocco, Ethiopia, China, and Iran-Iraq), it is possible that conditions have been favorable to allow small quantities to persist (Mitretek Systems 2002). Non-stockpile sites may contain buried munitions or contaminated soils containing sulfur mustard, which may be disturbed with excavation activities. Small quantities of sulfur mustard may persist at these bases. Populations in these areas are at higher risk than those in areas that were never contaminated.

Occupational exposure may occur for fishermen who inadvertently snare lumps of sulfur mustard in their nets. This type of exposure has occurred in areas of historical dumping of sulfur mustard in the seas and ocean. Accidents such as this continue to be reported in the Baltic Sea, Adriatic Sea, Pacific Ocean, and Japanese coastal waters, and have resulted in several hundred deaths over the past 50 years (Brankowitz 1987; Kurata 1980; Mazurek et al. 2001; SIPRI 1971). Individuals involved in activities related to the storage and destruction of this compound are also occupationally exposed. Construction workers may become exposed at Army bases where sulfur mustard was previously released and persisted in the soil or in an excavated munitions dump. Laboratory workers may be exposed to sulfur mustard through their research activities if they do not take the necessary precautions to prevent exposure. Soldiers may be exposed to sulfur mustard with its use as a chemical warfare agent. The most recent report of its use is from the Iran-Iraq War in the 1980s when it was detected in the urine of some soldiers (Vycudilik 1985).

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 3.7 Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children in the United States are not likely to be exposed to sulfur mustard since it is found only at military bases (Munro et al. 1999), and access to these sites is highly restricted.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Since the U.S. stockpile of sulfur mustard is currently found in only at seven storage facilities (Munro et al. 1999), the potential for high exposures is limited to these areas and their surrounding communities. Exposure at or near these Army storage facilities may occur if the munitions or storage containers explode or leak. However, the U.S. Army currently takes corrective and preventive actions to mitigate the risks of exposure to the general population. In addition, the stockpile of chemical weapons containing sulfur mustard is currently being destroyed and is scheduled to be completed by 2004. Thus, the risk of accidental exposure to sulfur mustard is decreasing.

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 sulfur mustard 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 sulfur mustard

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of sulfur mustard are available (Tables 4-1 and 4-4). Experimental determination of properties for sulfur mustard such as log K_{ow}, log K_{oc}, and Henry's law constant values would be useful to determine its environmental fate.

Production, Import/Export, Use, Release, and Disposal. Since 1968, sulfur mustard has not been produced, imported, or exported by the United States. Future production is not expected since international treaties prohibit the manufacture of sulfur mustard. The quantities of sulfur mustard at various locations across the United States are known. The entire stockpile of sulfur mustard munitions and bulk agent is currently in the process of being destroyed. The destruction of the stockpile, mandated by Congress, is to be completed by December 31, 2004. Information on the amounts of sulfur mustard being disposed of by each disposal method is available. Sulfur mustard is not used in the home environment or workplace. Sulfur mustard is also not present in food, and thus, will not be present as a food contaminant.

Environmental Fate. There is limited information on the environmental fate of sulfur mustard. It is known to vaporize and hydrolyze in water (Clark 1989; Rosenblatt et al. 1975; Stein 1946). However, sulfur mustard will persist in the environment in both soil and water. Information of the half-life of sulfur mustard in the environment is known. Additional environmental fate information (e.g., biodegradation) would help to adequately characterize the compound. Information on the fate of sulfur mustard degradation products in the environment would also be useful.

Bioavailability from Environmental Media. Sulfur mustard can be absorbed following inhalation (Drasch et al. 1987; Somani and Babu 1989) and dermal (Cullumbine 1946, 1947; Drasch et al. 1987; Nagy et al. 1946; Renshaw 1946) exposure from air and soil. This was its intended use and it is well studied (see Chapter 3).

Food Chain Bioaccumulation. No information was found regarding the bioconcentration of sulfur mustard by plants, animals, and aquatic organisms, or the biomagnification in terrestrial or aquatic food chains. However, due to the toxicity and metabolism of sulfur mustard, it is unlikely that it will bioconcentrate or biomagnify.

Exposure Levels in Environmental Media. There are limited reports of sulfur mustard being detected in environmental media (e.g., soil and water) at hazardous waste sites, Army chemical weapon stockpile and non-stockpile facilities, ocean disposal sites, or other locations. Additional and up-to-date information is needed on media concentration levels near former and current facilities where sulfur mustard has been produced, stored, or destroyed. This information will be useful in predicting human exposure levels at these locations. Additional data on the amount of sulfur mustard at historical ocean dumping sites would be useful.

Exposure Levels in Humans. No estimates have been for human intake of sulfur mustard from various environmental media. However, since potential exposure to sulfur mustard is currently limited to hazardous waste sites, Army chemical weapon stockpile and non-stockpile facilities, and ocean disposal sites, human intake of sulfur mustard by the general population is expected to be very low or none. Sulfur mustard metabolites have been detected in the urine and blood of exposed humans after its use as a chemical weapon (see Chapter 7). For example, thiodiglycol has been detected in the urine of soldiers after an acute exposure to sulfur mustard (Wils et al. 1985). However, the use of levels in urine or other biomarkers has not been reported in any other exposed populations. More sensitive methods of detection may be useful for assessment of chronic exposure to individuals working at or living near facilities that store or destroy sulfur mustard.

Exposures of Children. Sulfur mustard has been detected in exposed children after its use as a chemical weapon (See Chapter 3) during the Iran-Iraq War (Momeni and Aminjavaheri 1994). More sensitive methods of detection for sulfur mustard may be useful for assessment of chronic exposure to children living near facilities that store or destroy sulfur mustard.

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

Exposure Registries. There are no exposure registries for sulfur mustard. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure

Registry. The compound 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 the exposure to this compound.

6.8.2 Ongoing Studies

The ongoing studies focusing on environmental fate and human exposure of sulfur mustard are presented in Table 6-2 (DTIC 2002; FEDRIP 2002).

Table 6-2. Ongoing Studies on the Environmental Fate of Sulfur Mustard

Investigator	Affiliation	Study	Sponsor
Myer, SB	Tienzyme, Inc., State College, Pennsylvania	Use of fungal peroxidases for neutralization of sulfur mustard	Army
Shaw, RW	University of Florida, Gainesville	Catalytic oxidation of mustard simulants in basic solution	Army Research Office

Sources: DTIC 2002; FEDRIP 2002

7. ANALYTICAL METHODS

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

The most common currently used method of analyzing for the presence of sulfur mustard and its metabolites in biological and environmental samples is gas chromatography/mass spectrometry (GC/MS). Prior to 1987, however, thin-layer chromatography (TLC) with a colorimetric detection system and gas chromatography with either flame ionization detector (FID), electron capture detector (ECD), or flame photometric detector (FPD) were the most frequently used methods. Sample preparation consists primarily of extraction with an organic solvent. Sodium chloride is sometimes added to improve sample stability and prevent sulfur mustard breakdown to thiodiglycol and other metabolites. Depending on the method used, and the possible interfering compounds present, further cleanup and preparative steps may be included. No specific EPA, NIOSH, or AOAC methods were found for this chemical. Table 7-1 summarizes several representative analytical methods for detecting sulfur mustard and its metabolites in biological samples.

Little information was found on the direct detection of sulfur mustard in biological tissues or fluids. However, in two cases of suspected exposure, sodium chloride was first added to the urine samples to stabilize any sulfur mustard that might be present. A semi-quantitative analysis by GC/MS detected low ppb levels of sulfur mustard in these samples compared to none detected in a control sample of a definitely unexposed person (Vycudilik 1985, 1987). The detection limit of the procedure was in the low

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Saturate with sodium chloride; extract with diethyl ether; centrifuge; isolate organic phase and evaporate; redissolve in methylene chloride; clean up with silica gel; centrifuge; evaporate solvent layer; redissolve in methylene chloride	GC/MS (EI)	10 ng/µL injected	20%	Vycudilik 1985
Urine	Hydrolyze sample with helix pomatia (enzymatic hydrolysis); clean up on carbon column; add concentrated hydrochloric acid to convert thio-diglycol to sulfur mustard; headspace analysis® with collection on Tenax; thermally desorb (Thiodiglycol)	GC/MS	1 µg/L (1 ppb)	75%	Wils et al. 1988
Urine	Treat samples with acidic titanium trichloride; final residue dissolved in acetonitrile and toluene	GC-MS-MS	0.1 µg/L (0.1 ppb)	48–56%	Black and Read 1995b
Human fluids and tissues	Homogenize tissue; extract sample with dichloromethane; centrifuge; remove dichloromethane layer and evaporate; redissolve in hexane; clean up on TLC; remove sample spots and complex with gold; extract with toluene	ET-AAS	1.1 mg/L (ppm, body fluids); 0.1 mg/kg (ppm, body tissues)	No data	Drasch et al. 1987

EI = electron impact; ET-AAS = electrothermal atomic adsorption spectroscopy; GC = gas chromatography; GC-MS-MS = gas chromatography-tandem mass spectroscopy; MS = mass spectroscopy; TLC = thin layer chromatography

ppb range with inadequate recoveries of about 20%. Sulfur mustard has also been detected in body tissues and fluids of an alleged victim (Drasch et al. 1987). In this analysis, abdominal fat samples were first qualitatively analyzed by GC/MS.

Sulfur mustard is generally metabolized rapidly in biological systems. The primary method of analyzing for sulfur mustard exposure is by detecting the presence of its hydrolysis metabolites in biological fluids. GC/MS has been used for this purpose. The procedure involves conversion of the most common hydrolysis metabolite, thiodiglycol, to sulfur mustard by heating with concentrated hydrochloric acid (Wils et al. 1985, 1988). The detection limit for this procedure is in the low ppb range (about 1 μg/L) and with inclusion of deuterated thiodiglycol as an internal standard, recoveries of 75% are obtained (Wils et al. 1988). Unfortunately, thiodiglycol (and thiodiglycol sulphoxide) can exist in the urine of both exposed and nonexposed subjects; detection of thiodiglycol in human urine by this procedure at a concentration level of 10–100 μg/L does not prove sulfur mustard poisoning (Wils et al. 1985). Other methods using GC/MS have determined sulfur mustard in urine of exposed rats and guinea pigs by derivatisation of thiodiglycol with heptafluorobutyric anhydride (Jakubowski et al. 1990). Black and co-workers quantified thiodiglycol (and thiodiglycol sulphoxide) in urine of exposed humans using GC/MS after formation of bis(pentafluorobenzoyl) derivatives (Black and Read 1991, 1995a, 1995b; Black et al. 1992a, 1992b, 1994).

Another recent method for sulfur mustard detection in urine is gas chromatography-tandem mass spectrometry (GC-MS-MS) with selected-reaction monitoring. This method was applied to the analysis of urinary metabolites of sulfur mustard derived from hydrolysis (i.e., thiodiglycol and its sulfoxide) and the glutathione pathway after further metabolism involving the enzyme β-lyase (i.e., 1,1-sulphonylbis-[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane). The procedure involves treatment of samples with acidic titanium trichloride to reduce thiodiglycol sulfoxide to thiodiglycol and the two β-lyase metabolites to a single analyte, 1,1-sulphonylbis[2-(2-methylthio)-ethane]. The detection limit for this procedure is in the sub-ppb range (0.1 μg/L) for detection of β-lyase metabolites and in the ppb range (1–12 μg/L) for detection of thiodiglycol. Recoveries, determined in normal urine spiked with 1,1-sulphonylbis[2-(2-methythio)ethane] at a concentration of 1 μg/L, ranged from 48 to 56%. The advantage of this method is that β-lyase metabolites of sulfur mustard have not been observed in normal urine and this method provides an unequivocal biological marker of exposure to sulfur mustard (Black and Read 1995b).

Recently, the detection of DNA adducts formed by the modification of DNA by sulfur mustard in blood offers a promising approach for retrospective detection of exposure. For example, Ludlum et al. (1994) detected an N7-guanine adduct of DNA using high performance liquid chromatography (HPLC) with fluorometric monitoring. In this study, the authors were able to detect one N7-guanine adduct in $3x10^5$ DNA nucleotides. Benschop and co-workers (Benschop et al. 1997; Fidder et al. 1996a) were able to confirm the exposure to sulfur mustard in samples taken in March 1988 from two Iranians. Exposure to sulfur mustard was verified by two independent methods based on immunochemical analysis of the N7-guanine adduct in DNA and GC/MS analysis of the N-terminal valine adduct in globin after a modified Edman degradation. The adduct levels found were considerably higher than the detection limit for the modified Edman procedure (i.e., 0.1 μM sulfur mustard), but just above the detection limit for the immunochemical assay (i.e., 0.07 μM sulfur mustard). In another study, Noort et al. (1996) described the use of liquid chromatography-tandem mass spectrometry (LC-MS-MS) to identify modified sites in human hemoglobin after *in vitro* exposure to sulfur mustard. They note that hemoglobin is efficiently alkylated by sulfur mustard leading to an increase in 104 m/z after hydrolysis. This method is based on cleavage of globin by trypsin and micro-LC-MS analysis of the digests.

7.2 ENVIRONMENTAL SAMPLES

Table 7-2 presents a summary of several common analytical techniques used to analyze for sulfur mustard and its metabolites in environmental samples.

Until recently, GC with FID, ECD, or FPD were the primary methods of analysis for sulfur mustard and its metabolites, with a colorimetric assay utilizing 4-(p-nitrobenzyl) pyridine also frequently used. GC/MS is more commonly used for detecting sulfur mustard and its metabolites in environmental samples. Separation by TLC, followed by detection with a 4-(p-nitrobenzyl) pyridine procedure, has been used qualitatively and quantitatively to detect sulfur mustard in the presence of other vesicant mustards (Sass and Stutz 1981; Stutz and Sass 1969). This technique has proved useful in detecting sulfur mustard in a variety of complex matrices (water, soil, plants) and has a detection limit of 1 μg/sample spot (Sass and Stutz 1981). In addition to being relatively sensitive and selective, it can be scaled up for preparative work and down for small samples. This gives it continued usefulness despite the advent of more sophisticated GC/MS techniques

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect in decalin solvent using double trap system	GC/ECD	0.2 ng/µL injected	99.5–101.5%	Casselman et al. 1973
Air	Collect in diethyl succinate using double trap system	GC/FPD	0.2 ng/µL injected	98–101%	Gibson et al. 1974
Air	Collection in Tenax GC in a glass tube; thermal desorption into GC	GC/FPD	10 ng/m^3	No data	Fowler and Smith 1990
Water	Directly inject sample for thiodiglycol detection; extract with hexane and concentrate for detection of other compounds (metabolites)	GC/FID	50 µg/L (ppb)	No data	D'Agostino et al. 1989
		GC/MS (CI)	No data		D'Agostino et al. 1989
		GC/FTIR	No data		D'Agostino et al. 1989
Water or vapor	Extract with hexane	GC/ECD	160 µg/L (water); 1 µg/L (vapor)	No data	Fisher et al. 1969
Standard solutions and vapors	Dissolve standard of known purity in hexane or chloroform (sulfur mustard and metabolites)	GC/ECD GC/FPD	About 160 µg/L (solution); about 1 µg/L (vapor)	No data	Sass and Steger 1982
Soil	Extract with chloroform; sonicate (sulfur mustard and metabolites)	GC/MS (CI)	5–10 ng/ injection	No data	D'Agostino and Provost 1988b
	No data	GC/MS (EI)	No data		Vycudilik 1985
Soil, plants, water	Separation by TLC	4-(p-nitro-benzyl) pyridine procedure	1 µg/sample spot	No data	Sass and Stutz 1981; Stutz and Sass 1969
Sulfur mustard hydrolysate	Extract with hexane; concentrate	GC/FID	No data	No data	D'Agostino and Provost 1988a

CI = chemical ionization; ECD = electron capture detector; EI = electron impact; FID = flame ionization detector; FPD = flame photometric detector; FTIR = Fourier transform infrared spectroscopy; GC = gas chromatography; MS = mass spectrometry

GC with either FID, ECD, or FPD was the most common technique of the 1970s and early 1980s for determining the presence of sulfur mustard and its metabolites, and is still frequently used. It has been used to detect sulfur mustard in air by passing air through a solvent trap. Aliquots of the solvent are directly injected into the gas chromatograph to detect sulfur mustard (Casselman et al. 1973; Gibson et al. 1974). With both ECD and FPD, recoveries were near 100%, and the detection limit was 0.2 ng/μL injected. Advantages of both were speed, simplicity, and reliability. However, the solvent producing the best results with ECD required ice-bath cooling to prevent solvent and sulfur mustard loss (Casselman et al. 1973). The solvent used with FPD had the advantage of allowing room temperature analysis (Gibson et al. 1974).

GC was used to detect sulfur mustard in water (Fisher et al. 1969) and soil (D'Agostino and Provost 1988a). Using GC/ECD, a minimum detection limit (quantifiable) of 160 μg/L (ppb) for aqueous solutions and 1 μg/L for vapor was obtained. The method used was simple, selective, and precise. The authors proposed that with appropriate sample preparation, it could be used for a variety of media, including soil and biological media (Fisher et al. 1969). Sulfur mustard and metabolites were detected in soil by GC/FID by D'Agostino and Provost (1988a), who also analyzed a hydrolysate remaining from the destruction of munitions grade mustard, but no details on accuracy, precision, or sensitivity were given. A comparison of the various detectors used to analyze for sulfur mustard and its metabolites was conducted and showed ECD to be the most sensitive for detecting sulfur mustard in a mixture of mustard compounds, followed by FPD and FID (Sass and Steger 1982). The detection limit using ECD and FPD was in the mid-ppb range (about 160 μg/L) for solutions and in the low ppb range (about 1 μg/L) for vapors. Beck et al. (2001) found that GC-FPD provided a rapid and sensitive method for analysis of thiodiglycol (TDG) in soil extracts with a detection limit of 1.1 μg/g soil. Pressurized liquid extraction (PLE) with methanol-water (9:1) proved to be the most efficient solvent for TDG extraction with recoveries ranging from 12 to 89% of added TDG for various soil types.

GC/MS has been used to analyze for the presence of sulfur mustard and its metabolites (D'Agostino and Provost 1988b; D'Agostino et al. 1989; Munavalli and Jakubowski 1989; Vycudilik 1985). Tests with pure substances have supported the sensitivity, selectivity, and reliability of this technique, and analysis of pure samples has proved its usefulness. Inclusion of deuterated thiodiglycol as an internal standard increases the precision of GC/MS and makes the technique quantitative as opposed to simply semi-quantitative. Both chemical ionization (CI) and electron impact (EI) have been used to detect sulfur mustard and its characteristic metabolites in samples. Detection of specific mustard metabolites is important in determining sulfur mustard exposure since the chemical can degrade rapidly under certain

environmental conditions. Testing of several EI and CI techniques showed that MS was a sensitive, reliable, and precise detection method for sulfur mustard (Ali-Mattila et al. 1983). This was supported in later studies on sample mixtures of vesicant mustards and degradation products, as well as on water and soil samples (D'Agostino and Provost 1988b, 1992; D'Agostino et al. 1989; Munavalli and Jakubowski 1989; Vycudilik 1985). For example, D'Agostino and Provost (1992) used GC/MS for verification of sulfur mustard and its hydrolysis products in soil. They used sequential hexane and dichloromethane extraction followed by trimethylsilyl derivatization and achieved total recoveries in the 50–90% range for most soil types. Wils et al. (1992) used GC/MS to analyze sulfur mustard in rubber and paint samples in combination with diesel fuel and aromatic white spirit as a background. Sulfur mustard was isolated by extraction with methylene chloride or by dynamic headspace analysis at elevated temperatures. Recoveries of sulfur mustard in rubber and paint ranged from 57 to 86%. Black et al. (1993b) analyzed soil, bomb casing, and sheep wool samples associated with a chemical weapons agent (CWA) incident (obtained from a Kurdish village in the northern part of Iraq in 1988) by GC/MS using headspace analysis, solvent extraction, and thermal desorption methods. Using this technique, the presence of sulfur mustard and 21 related compounds were successfully confirmed in these samples.

Sulfur mustard vapor is typically determined in air by bubbling an air sample through a liquid solvent and analyzing the solvent for absorbed mustard by colorimetry or by GC. However, the colorimetric technique lacks specificity and the solvent entrapment sampling technique possesses a number of drawbacks such as limited analyte-trapping efficiency, high detection limits, and degradation of the analyte (Fowler and Smith 1990). Rapid and accurate methodologies for the detection of sulfur mustard have been developed for use during the demilitarization of mustard stockpiles at U.S. storage sites. These procedures are based largely on the Depot Area Air Monitoring System (DAAMS) technology (Smith et al. 1982). DAAMS procedures have undergone extensive Precision and Accuracy studies (Smith and Fowler 1985) and are methods of choice in current and future demilitarization sites. The sampling and analysis process for DAAMS consists of (1) collection of the airborne sample on the sorbent (Tenax GC) in a glass tube, (2) transfer to a glass tube containing smaller amounts of the same sorbent using an external thermal desorber, and (3) thermal desorption in a specially-modified injection port of a gas chromatograph and subsequent analysis using a flame photometric detector (Fowler and Smith 1990; Posner 1991). The detection limit of sulfur mustard vapor in air by these procedures is about 10 ng/m^3 (or 1.5 parts per trillion).

While GC/MS continues to be the definitive method for assessment of sulfur mustard in environmental matrixes, increasing emphasis has been placed on rapid screening procedures such as liquid

chromatography/mass spectrometry (LC/MS). Although LC/MS methods typically have higher detection limits, these techniques allow for more rapid screening of aqueous samples and extracts, with minimum sample pretreatment and no requirement for dehydration or derivatization (Burrows 1998). Electrospray ionization, thermospray ionization (TSP), and atmospheric pressure chemical ionization (APCI) have shown promise as interfaces for the introduction of the liquid solvent stream containing sulfur mustard and metabolites into the mass spectrometer (Munavalli et al. 1995; Smith and Shih 2001). For example, liquid chromatograph/electrospray-mass spectrometry (LC/ESP-MS) was recently recommended as a rapid screening procedure for verification of the presence of traces of the agent in hydrolysis products in water without derivatization (Borrett et al. 1996). It has also been successfully applied for the direct detection of sulfonium ions formed during the storage and hydrolysis of sulfur mustard (Rohrbaugh and Yang 1997). Black and Read (1997) recently demonstrated a rapid screening procedure for sulfur mustard, which involves separation by liquid chromatography and detection by atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS). Sulfur mustard and its transformation products have been characterized by liquid chromatography/thermospray ionization-mass spectrometry (LC/TSP-MS) in methanol. In both positive and negative modes, a rich complex ion chemistry was observed for the transformation products of sulfur mustard (Munavalli et al. 1995).

Other techniques that have been used to characterize sulfur mustard and its metabolites include capillary electrophoresis (CE) and secondary ion mass spectrometry (SIMS). CE coupled with MS detection is well suited to deal directly with aqueous samples and polar (acidic) degradation products (Hooijschuur et al. 2001). SIMS analysis of solid samples has evidenced the presence of sulfonium ion aggregates resulting from nucleophilic substitution processes (Groenwold et al. 1995). This method also may potentially be able to detect sulfur mustard directly on soil surfaces without the necessity of lengthy extraction procedures (Gresham et al. 2001).

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 sulfur mustard 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 sulfur mustard.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Available information indicates that sensitive, selective, and reliable methods for determining biomarkers of exposure exist for sulfur mustard and its metabolites (Black and Read 1991, 1995a, 1995b; Black et al. 1992a, 1992b, 1993b, 1994; Kientz 1998; Ludlum et al. 1994). Available studies emphasize detection and quantification of the compound and its metabolites. Further studies that attempt to quantify levels in exposed and unexposed populations are useful in assessing the risk associated with sulfur mustard and its metabolites.

Effect. As discussed, sensitive, selective, and reliable methods exist for detecting sulfur mustard and its metabolites in biological tissues and fluids. Available studies do not emphasize quantifying the levels of these compounds and associating the amounts found with specific biomarkers of effect. Further studies associating specific levels in fluids and tissues with known effects are useful in assessing the risk associated with sulfur mustard and its metabolites.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Sensitive, selective, and reliable methods exist for detecting sulfur mustard and its metabolites in air (Casselman et al. 1973; Gibson et al. 1974), water (Fisher et al. 1969; Sass and Stutz 1981; Stutz and Sass 1969), and soil (D'Agostino and Provost 1988a; Sass and Stutz 1981; Stutz and Sass 1969). No information was obtained on the detection of sulfur mustard in other environmental media. The available methods emphasize qualitative and quantitative detection. Further studies to improve the detection of sulfur mustard and its metabolites will aid in assessing the potential risk of sulfur mustard in the environment, especially near hazardous waste facilities and Army storage facilities.

7.3.2 Ongoing Studies

No ongoing studies on the analysis of sulfur mustard in biological and environmental matrixes were located in the Federal Research in Progress (FEDRIP) database.

8. REGULATIONS AND ADVISORIES

Sulfur mustard is on the list of chemicals appearing in "Toxic Chemicals Subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986" (EPA 1987). Available information on regulations and standards is presented in Table 8-1.

ATSDR has derived an acute inhalation MRL of 0.0007 mg/m^3 for sulfur mustard based on a minimal LOAEL of 0.06 mg/m^3 for ocular effects in humans that were exposed to sulfur mustard vapors 8 hours/day, for 3 days (Guild et al. 1941). The LOAEL was duration-adjusted to a time-weighted average (TWA) of 0.02 mg/m^3 and an uncertainty factor of 30 (3 for use of a minimal LOAEL and 10 for human variability) was applied to the TWA minimal LOAEL to derive the MRL (see Appendix A for details).

ATSDR has derived an intermediate-duration inhalation MRL of 0.00002 mg/m^3 for sulfur mustard based on a NOAEL of 0.001 mg/m^3 for ocular effects in dogs that were exposed to sulfur mustard vapors 24 hours/day, 5 days/week, for up to a year (McNamara et al. 1975). The NOAEL was duration-adjusted to a daily TWA of 0.0007 mg/m^3 and an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) was applied to the TWA NOAEL to derive the MRL (see Appendix A for details).

ATSDR has derived an acute oral MRL of 0.0005 mg/kg/day (0.5 µg/kg/day) for sulfur mustard based on a LOAEL of 0.5 mg/kg/day for inflamed mesenteric lymph nodes in rat dams and reduced ossification in fetuses that were exposed for 10 days (DOA 1987). The test material was administered by gavage in oil on gestation days 6–15. An uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) was applied to the LOAEL to derive the MRL (see Appendix A for details).

An intermediate-duration oral MRL for sulfur mustard of 0.00007 mg/kg/day (0.07 µg/kg/day) was based on a TWA LOAEL of 0.02 mg/kg/day for gastrointestinal effects in rats exposed by gavage for a 21-week period (Sasser et al. 1996a). An uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) was applied to the TWA LOAEL to derive the MRL (see Appendix A for details).

Table 8-1. Regulations and Guidelines Applicable to Sulfur Mustard

Agency	Description	Information	References
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 1[a]	IARC 2001
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV-TWA	No data	
NIOSH	REL	No data	
OSHA	PEL (TWA)	No data	
b. Water		No data	
c. Food		No data	
d. Other			
BEA	Chemical Weapons Convention requirements; schedules of chemicals		BEA 2001 15CFR745
DOS	International traffic in arms regulations; United States munitions list; chemical agents		DOS 2001 22CFR121.7
DOT	Hazardous materials table		DOT 2001 49CFR172.101
EPA	CERCLA; reportable quantity	500 pounds	EPA 2001a 40CFR355 Appendix B
	Groundwater protection standards at inactive uranium processing sites		EPA 2001b 40CFR192, Appendix I
	SARA; extremely hazardous substance (TPQ)	500 pounds	EPA 2001a 40CFR355, Appendix B
	RCRA; identification and listing as hazardous waste		EPA 2001c 40CFR261, Appendix VIII
	Toxic chemical release reporting; Community Right-to-Know; effective date	01/01/87	EPA 2001d 40CFR372.65
NTP	Carcinogenic classification	Known to be a human carcinogen	NTP 2001
OSHA	Meets criteria for proposed medical records rule		OSHA 1982
VA	Claims based on chronic effects of exposure		VA 2001 38CFR3.316

Table 8-1. Regulations and Guidelines Applicable to Sulfur Mustard

Agency	Description	Information	References
STATE			
Regulations and Guidelines:			
a. Air			
Colorado	Air contaminant emission notice		BNA 2001
Connecticut	HAP		BNA 2001
Maryland	Toxic air pollutant	Known human carcinogen	BNA 2001
b. Water			
New York	Water regulation		BNA 2001
	TPQ	1 pound	
	RQ	1 pound	
c. Food		No data	
d. Other			
Alabama	Identification and listing of hazardous waste		BNA 2001
Arkansas	Identification and listing of hazardous waste		BNA 2001
California	Chemical known to cause cancer or reproductive toxicity; initial appearance of chemical on list	02/27/87	BNA 2001
	Hazardous substance list		BNA 2001
Colorado	Identification and listing of hazardous waste		BNA 2001
District of Columbia	Identification and listing of hazardous waste		BNA 2001
Delaware	Reportable quantity	1 pound	BNA 2001
Florida	Toxic substances in the workplace		BNA 2001
Georgia	Regulated substance and soil concentration that trigger notification		BNA 2001
Illinois	Identification and listing of hazardous waste		BNA 2001
Kentucky	Extremely hazardous substance (TPQ)	500 pounds	BNA 2001
	Identification and listing of hazardous waste		BNA 2001
Louisiana	Hazardous waste		BNA 2001
Maine	Identification and listing of hazardous waste		BNA 2001
Massachusetts	Containers adequately labeled pursuant to federal law		BNA 2001
Massachusetts	Oil and hazardous material list		BNA 2001
Maryland	Identification and listing of hazardous waste		BNA 2001

Table 8-1. Regulations and Guidelines Applicable to Sulfur Mustard

Agency	Description	Information	References
STATE *(cont.)*			
Michigan	Identification and listing of hazardous waste		BNA 2001
Minnesota	Hazardous constituent		BNA 2001
Nebraska	Hazardous constituent		BNA 2001
New Jersey	Discharge of oil and other hazardous substances		BNA 2001
North Dakota	Identification and listing of hazardous waste		BNA 2001
Ohio	Toxic release inventory rules		BNA 2001
Oregon	Toxic use reduction and hazardous reduction regulations		BNA 2001
South Carolina	Identification and listing of hazardous waste		BNA 2001
Tennessee	Identification and listing of hazardous waste		BNA 2001
Vermont	Hazardous waste management regulation		BNA 2001
Washington	Dangerous waste regulations		BNA 2001
Wisconsin	Identification and listing of hazardous waste		BNA 2001
Wyoming	Identification and listing of hazardous waste		BNA 2001

[a]Group 1: Carcinogenic to humans

ACGIH = American Conference of Governmental Industrial Hygienists; BEA = Bureau of Export Administration; BNA = Bureau of National Affairs; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DOS = Department of State; DOT = Department of Transportation; EPA = Environmental Protection Agency; HAP = hazardous air pollutant; IARC = International Agency for Research on Cancer; NIOSH = National Institute of Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = relative exposure limit; RQ = reportable quantity; SARA = Superfund Amendments and Reauthorization Act; TPQ = threshold planning quantity; TLV = threshold limit values; TWA = time-weighted average; VA = Department of Veteran Affairs

ATSDR has not derived a chronic oral MRL for sulfur mustard because a chronic bioassay was not located.

The acute exposure guideline levels (AEGLs) for sulfur mustard, which were developed by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances, are presented in Table 8-2. A more detailed discussion of these AEGLs is presented in Appendix D.

The Army has recommended sulfur mustard airborne exposure limits for agent workers (WPL) and the general population (GPL) for chronic exposures to provide adequate protection during the limited time of potential exposure prior to the completion of the Chemical Stockpile Demilitarization Program (USACHPPM 2000a, 2003). For the general population, a sulfur mustard GPL of 0.00002 mg/m^3 as a 24-hour TWA, 7 days/week, has been established. For agent workers, the WPL established for sulfur mustard as an 8-hour TWA, 5 days/week, is 0.0004 mg/m^3. In addition, the Army has established a Short Term Exposure Limit (STEL) of 0.003 mg/m^3 and an Immediately Dangerous to Life and Health (IDLH) value of 2.0 mg/m^3 for sulfur mustard agents. Previously established airborne exposure limits for sulfur mustard agents were promulgated by the Centers for Disease Control and Prevention (CDC) in 1988 (DHHS 1988).

The International Agency for Research on Cancer (IARC) has classified sulfur mustard as carcinogenic to humans (Group 1), based on sufficient evidence for carcinogenicity to humans and limited evidence for carcinogenicity to animals (IARC 1975, 1987, 2001).

The U.S. Army has derived health-based environmental screening levels (HBESLs) for sulfur mustard (USACHPPM 1999) as shown in Table 8-3. Ongoing evaluations of alternative approaches for quantitatively estimating noncancer and cancer risk may result in changes to these values (USACHPPM 2000a).

Table 8-2. Acute Exposure Guideline Level (AEGL) Values for Sulfur Mustard (ppm [mg/m3])

Classification	10-minute	30-minute	1-hour	4-hour	8-hour	End point (reference)
AEGL-1	0.06 ppm (0.40)	0.02 ppm (0.13)	0.01 ppm (0.067)	0.003 ppm (0.017)	0.001 ppm (0.008)	Conjunctival injection and minor discomfort with no functional decrement in volunteers (Anderson 1942)
AEGL-2	0.09 ppm (0.60)	0.03 ppm (0.20)	0.02 ppm (0.10)	0.004 ppm (0.025)	0.002 ppm (0.013)	Well-marked generalized conjunctivitis, edema, photophobia, and eye irritation in volunteers (Anderson 1942)
AEGL-3	0.59 ppm (3.9)	0.41 ppm (2.7)	0.32 ppm (2.1)	0.08 ppm (0.53)	0.04 ppm (0.27)	Lethality estimate in mice (Kumar and Vijayaraghavan 1998)

Source: NAC/AEGL 2001

Table 8-3. U.S. Army Toxicity Values for Sulfur Mustard

Parameter	Value	Units
Oral reference dose	0.000007	mg/kg/day
Inhalation reference dose	0.00003	mg/kg/day
Cancer potency oral slope factor	7.7	$(mg/kg/day)^{-1}$
Cancer potency inhalation unit risk	0.085	$(\mu g/m^3)^{-1}$
Cancer potency inhalation slope factor	300	$(mg/kg/day)^{-1}$

Source: USACHPPM 1999

On October 17, 1986, the President signed into law the Superfund Amendments and Reauthorization Act of 1986 (SARA). This act amended the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA), commonly known as "Superfund". The Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) was included under Title III of SARA.

EPA has established a reportable quantity (RQ) for sulfur mustard of 500 pounds under the CERCLA section 103, codified at 40 CFR part 302, in addition to the requirements of 40 CFR part 355, and regulates it as a hazardous constituent of waste under the Resource Conservation and Recovery Act (RCRA, 40 CFR 261).

EPA regulates sulfur mustard under the SARA, subjecting it to reporting requirements. Emergency response plans are required under SARA if the threshold planning quantity (TPQ) of 500 pounds is exceeded.

Under EPCRA, release of sulfur mustard must be reported according to EPA toxic chemical release reporting regulations (40 CFR 372.65).

Sulfur mustard is included as a constituent regulated under the groundwater protection standards for inactive uranium processing sites (40 CFR 192).

OSHA regulates sulfur mustard under the Hazard Communication Standard and as a chemical hazard in laboratories.

The Department of Veterans Affairs regulates compensation based on chronic effects of exposure to sulfur mustard (38 CFR 3.316).

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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.

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.

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$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{LO})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

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

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

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

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

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

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

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

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

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) 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.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates and, possibly membrane permeabilities. The models also utilize biochemical information such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) 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 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 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value - Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

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

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The study of the absorption, distribution and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 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.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 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 agency wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change, as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop E-29, Atlanta, GA 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name: Sulfur mustard [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: August 29, 2003
Profile status: Third Draft
Route: [X] Inhalation [Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 2
Species: Human

Minimal Risk Level: 0.0007 [ ] mg/kg/day [ ] ppm [X] mg/m^3

Reference: Guild WJF, Harrison KP, Fairley A, et al.. 1941. The effect of mustard gas vapor on the eyes. Chemical Board, Physiological Sub-Committee and Panel of Opthalmic Specialists. Porton Report 2297. Chemical Defense Experimental Station, Porton, UK.

Experimental design: Male soldiers wearing respirators (2–6 men/group) were exposed to sulfur mustard vapor concentrations, checked by continuous chemical sampling, ranging from 0.06 to 320 mg/m^3. Continuous exposure durations ranged from 15 seconds to 10 hours, yielding concentration time (Ct) products in the range of 42–144 mg-minute/m^3. Two repeated-exposure tests were also conducted; a group of four men was exposed to 0.22 mg/m^3, 2.5 hours/day, for 2 days, and another group of four men was exposed to 0.06 mg/m^3, 8 hours/day, for 3 days (intermittent Cts of 66 and 86 mg-minute/m^3, respectively). Chamber temperatures for each experiment were not provided, but chamber temperatures were stated to range from 55 to 80 ºF during the testing. Soldiers were in good health and had no previous exposures to sulfur mustard. The subject's eyes were examined for evidence of toxicity subsequent to exposure.

Effects noted in study and corresponding doses: No deaths occurred. For continuous exposures, ocular effects and severity depended on the concentration and duration, or Ct. At the lowest continuous Ct of 42 mg-minute/m^3 (1.4 mg/m^3 for 30 minutes), four of four soldiers showed a slight generalized conjunctival reaction. A slight or just discernable or slight conjunctival reaction was also reported at the lowest concentration of 0.1 mg/m^3 for 8 and 10 hours exposures (Cts of 48 and 60 mg-minute/m^3, respectively). Just discernable angular congestion of the bulbar conjunctiva was reported for Cts ranging from 48 to 75 mg-minute/m^3. Slight to moderate degree of conjunctival congestion was reported for the Ct range of 80-90 mg-minute/m^3. The first casualties (two of two men) [casualty meaning any interference with vision or any lesion of the eyes sufficiently severe to render a soldier, for a time, incapable of carrying out his normal duties] were reported at a Ct of 99 mg-minute/m^3 (16.5 mg/m^3 for 6 minutes). Both subjects showed generalized established conjunctivitis with photophobia. At a Ct of 144 mg-minute/m^3, six of six subjects showed marked generalized conjunctival congestion, with photophobia in one of six subjects. While specific results were not reported for the 2-day repeated exposure (0.22 mg/m^3, 2.5 hours/day, for 2 days), the authors stated that there was no discernable difference in the degree of conjunctival reaction between subjects in this group and subjects exposed to the same concentration for 5 hours (Ct of 66 mg-minute/m^3). A scarcely discernable generalized conjunctival reaction (incidence unspecified) was reported in subjects undergoing the 3-day repeated exposure (0.06 mg/m^3, 8 hours/day, for 3 days; intermittent Cts of 86 mg-minute/m^3). The severity of conjunctivitis for the 3-day intermittent exposure was described as far slighter than the moderate degree of conjunctivitis observed from continuous exposures with Cts ≥80 mg-minute/m^3.

Dose and end point used for MRL derivation: The 3-day repeated exposure experiment is considered to best represent a potential acute exposure, and the time-weighted average concentration of 0.02 mg/m^3 (0.06 mg/m^3 x 8 hours/24 hours) is considered a minimal LOAEL for ocular effects.

[ ] NOAEL [X] LOAEL

Uncertainty factors (UF) and Modifying Factor (MF) used in MRL derivation:

[X] 3 for use of a minimal LOAEL
[X] 10 for human variability

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

Was a conversion used from intermittent to continuous exposure? Yes
$LOAEL_{[ADJ]}$=0.06 mg/m^3 · (8 hours/24 hours) =0.02 mg/m^3

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

Other additional studies or pertinent information that lend support to this MRL: Ocular effects were reported in other chamber tests reported by Reed (1919) and Anderson (1942); however, these studies did not include repeated exposures. Conjunctivitis with photophobia and blepharospasm were reported as the initial signs of exposure in two subjects who underwent chamber tests wearing no respirators and clad only in khaki uniform pants without shirts (Reed 1918). The two subjects were exposed to 1.2 mg/m^3 of sulfur mustard vapor for 20 and 45 minutes (Cts of 24 and 54 mg-minute/m^3), respectively. The variation in sensitivity to sulfur mustard was demonstrated by a latency of 3 hours for the subject exposed to the Ct of 24 mg-minute/m^3, as compared to 6 hours for the subject exposed to the higher Ct. In subsequent chamber tests conducted by Reed (1918), no signs were reported in six subjects exposed to the lowest Ct of 1.0 mg-minute/m^3 (0.1 mg/m^3 for 10 minutes), whereas one of two subjects showed slight, but distinct, conjunctival injection at the next higher Ct of 1.5 mg-minute/m^3 (0.1 mg/m^3 for 15 minutes). In continuous flow tests with human subjects, with only the face and one eye exposed, conjunctivitis was observed at Cts as low as 7 mg-minute/m^3 (0.7 mg/m^3 for 10 minutes); Cts as low as 1.5 mg-minute/m^3 (0.1 mg/m^3 for 15 minutes) were tested (Reed 1918). However, it should be noted that methods for measuring low vapor concentrations of sulfur mustard were not yet validated at the time of these studies. In chamber tests conducted by Anderson (1942), using male soldier wearing respirators, a trace of angular conjunctivitis and "band of fine injection" across the exposed part bulbar conjunctiva were reported at the lowest Ct of 12.5 mg-minute/m^3 (6.25 mg/m^3 for 2 minutes). A "fine injection band" over the exposed sclera was reported at the lowest concentration of 1.7 mg/m^3 (33 min; Ct=56.1 mg-minute/m^3) (Anderson 1942). In addition to chamber testing, numerous reports exist of ocular lesions that occurred in soldiers exposed to sulfur mustard during World War I (Hughes 1942; Philips 1940). In a more recent study of Iranian fighters with a history of sulfur mustard poisoning, delayed ocular lesions from undetermined, presumably acute, exposures included chronic conjunctivitis in 75/85 (32%), keratoconjunctivitis in 7/85 (3%) and blindness in 2/85 (1%) (Balali-Mood 1986). A range of ocular effects, including conjunctivitis, chronic keratitis, and corneal ulcerations, have been reported in dogs and rabbits following acute exposure to sulfur mustard depending on the concentration and duration of exposures (Balali-Mood 1986; Gates and Moore 1946; Laughlin 1944a; Maumenee and Scholts 1948; Reed 1918; Warthin and Weller 1919).

Agency Contact (Chemical Manager): Zemoria A. Rosemond

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name: Sulfur mustard [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: August 29, 2003
Profile status: Third Draft
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to figure: 11
Species: Dog

Minimal Risk Level: 0.00002 [ ] mg/kg/day [ ] ppm [X] mg/m^3

Reference: McNamara BP, Owens EJ, Christensen MK, et al. 1975. Toxicological basis for controlling levels of mustard in the environment. Edgewood Arsenal Special Publication. Aberdeen Proving Ground, Maryland: Department of the Army. EB-SP-74030.

Experimental design: Male and female beagle dogs (6 initially and 4 added), rats (140), A/J mice (140), rabbits (12 initially and 6 added), and guinea pigs (30 initially and 12 added) were exposed to sulfur mustard vapor at a concentration of 0.001 mg/m^3 for 24 hours/day, 5 days/week (time-weighted average concentration of 0.0007 mg/m^3), for varying durations up to 1 year. The same number of animals of each species were exposed to 0.1 mg/m^3 for 6.5 hours followed by exposure to 0.0025 mg/m^3 for the remaining 17.5 hours of the day, 5 days/week, for durations up to a year. The latter exposure is equivalent to a time-weighted average concentration of 0.0206 mg/m^3 [{0.1 mg/m^3 x (6.5 hours/24 hours) + 0.0025 mg/m^3 x (17.5 hours/24 hours)} x (5 days/7 days) = 0.0206 mg/m^3]. Unexposed controls consisted of 10 dogs (6 initially and 4 added), 100 rats, 140 A/J mice (120 initially and 20 added), 22 rabbits (7 initially and 15 added), and 32 guinea pigs (20 initially and 12 added). The treatment protocol was unusual in that new animals were added to replace exposed animals that were sacrificed periodically. At about 7 months after the study was initiated, 100 ICR Swiss albino mice were added to the test chambers, and 50 A/J mice were added about 3 months later. At these same times, the same numbers of each strain were added to the study as additional controls. The animals were observed periodically for clinical signs of toxicity. Body weights were recorded. Red and white blood cell counts, hematocrit, and hemoglobin were measured in dogs and rabbits, but not other species. Clinical chemistry analyses including blood urea nitrogen (BUN), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), and serum alanine aminotransferase (ALT) were conducted in dogs. Gross and microscopic examinations were performed.

Effects noted in study and corresponding doses: No dogs died during the study. Mortality was unrelated to concentration in rabbits and guinea pigs, and comparable to controls in exposed ICR Swiss mice. While no control rats died, death occurred in 3/140 and 16/140 at the low- and high-concentrations, respectively. In the first group of A/J mice, mortality incidence was concentration-related; 4/140, 14/140, and 24/140 in the control, low- and high-concentration groups, respectively. However, in the second group of A/J mice, 3/50 animals in the low-concentration group died, while there were no deaths in the control and high-concentration groups. Because of the lack of correlation between deaths and exposure in the ICR Swiss mice and the second group of A/J mice the authors concluded that deaths were more likely due to conditions of animal storage than treatment. Of 79 rats exposed to the lower concentration and necropsied, 5 developed chronic keratitis. This lesion was not observed in control or high-dose rats. No clinical signs of toxicity were observed in any of the other species exposed to the low concentration. At the high concentration, the only overt signs of toxicity were ocular effects, observed only in dogs. Ocular effects first appearing after 16 weeks of exposure, including corneal opacity, pannus, chronic keratitis, vascularization, pigmentation, and granulation were reported in 3 of 10 high-concentration dogs exposed

for 7.5 or 12 months. The time-weighted average concentration of 0.0206 mg/m^3 is considered a LOAEL for ocular effects in beagle dogs.

Dose and end point used for MRL derivation: The lowest concentration tested in dogs, 0.001 mg/m^3, is a NOAEL for ocular effects (conjunctivitis and chronic keratitis).

[X] NOAEL [ ] LOAEL

Uncertainty factors (UF) and Modifying Factor (MF) used in MRL derivation:

[X] 10 for human variability
[X] 3 for animal-to-human extrapolation

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

Was a conversion used from intermittent to continuous exposure? Yes
$NOAEL_{[ADJ]}$=0.001 mg/m^3 · (24 hours/24 hours) · (5 days/7 days)=0.0007 mg/m^3

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Gates and Moore (1946) reported that the human eye is about four times more sensitive to sulfur mustard than the rabbit eye based on the observation of corneal ulceration produced in rabbits and dogs at Cts of 4 and 2 times the value, respectively, at which this effect occurred in humans. This is consistent with the observation by McNamara et al. (1975) of ocular effects in dogs, but not in rabbits, at the high concentration. Thus, an uncertainty factor of 3 for extrapolation of data from dogs to humans is considered appropriate for derivation of the MRL.

Other additional studies or pertinent information that lend support to this MRL: The intermediate-duration inhalation MRL was based on the same critical endpoint as the acute-duration inhalation MRL. In addition to the supporting information for ocular lesions as provided for the acute-duration MRL, there are numerous reports of eye burns in workers accidentally exposed to large quantities of sulfur mustard vapor, as well as to slow leaks that were not detected by smell (Hughes 1945a; Pechura and Rall 1993; Uhde 1946).

Agency Contact (Chemical Manager): Zemoria A. Rosemond

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name: Sulfur mustard [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: August 29, 2003
Profile status: Third Draft
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 5, 6
Species: Rat

Minimal Risk Level: 0.5 [X] µg/kg/day [ ] ppm [ ] mg/m^3

Reference: DOA. 1987. Teratology studies on lewisite and sulfur mustard agents: Effects of sulfur mustard in rats and rabbits. Fort Detrick, MD: U.S. Army Medical Research and Development Command, U.S. Department of Army. ADA187495.

Experimental design: Sulfur mustard (95.9–96.1% purity) dissolved in sesame oil was administered by intragastric intubation to mated Sprague-Dawley female rats (10–11 weeks old) on gestation days 6 through 15 (10 days). The administered doses were 0, 0.5, 1.0, or 2.0 mg/kg/day and there were 25–27 animals/dose group, of which 20–26/dose group were pregnant. All animals were observed for clinical signs of toxicity prior to and following administration of sulfur mustard. Treated rats were weighed on gestation days 0, 6–15 (exposure days), and on day 20. Necropsy was performed on all rats found dead or in moribund condition. Scheduled necropsy was performed on gestation day 20. Blood samples were collected from maternal animals for hematocrit measurement prior to sacrifice. The animals were examined for gross lesions of major organ systems. The numbers of corpora lutea, implantation sites, resorptions, and live and dead fetuses were determined. Uterine weights were recorded. Live fetuses were removed, weighed, sexed, and examined for gross, soft tissue, and skeletal anomalies.

Effects noted in study and corresponding doses: There were no treatment-related deaths. In rats, a significant dose-related decrease in maternal body weight was observed by gestation day 12 at 0.5 mg/kg/day (4.1–6.6%) and by gestation day 9 in the 1.0 (4.7–9.1%) and 2.0 (6.5–16.0%) mg/kg/day groups. Extragestation weight gain was significantly reduced at ≥0.5 mg/kg/day with dose-related reductions of 25, 38, and 57% at 0.5, 1.0, and 2.0 mg/kg/day, compared to controls. A significantly decreased (16%) gravid uteri weight was measured at the highest dose. Maternal hematocrit values were statistically significantly reduced by 5.4% at 1.0 and 2.0 mg/kg/day. Gastric mucosa inflammation was observed in 2/30 (6.7%) rats at 2.0 mg/kg/day, but not in any of the lower dose or control groups. A significantly increased incidence of inflamed mesenteric lymph nodes was found at ≥0.5 mg/kg/day; the incidences were 0/27 controls, and 11/25 (44%), 16/25 (64%), and 15/27 (56%) rats at 0.5, 1.0, and 2.0 mg/kg/day, respectively.

Fetal body weight was significantly decreased (6–7%) from controls in litters exposed to doses of ≥1.0 mg/kg/day; no clear dose-relation was evident. The sex ratio (percent males) was significantly lower than control at the highest dose (46.2 vs. 51.0%). Placental weight was also significantly reduced (8.4%) at the highest dose. Supernumerary ribs were found in 9/299 (3%) fetuses of one litter in the highest dose group, while this anomaly was not found in any of the fetuses in the lower dose or control groups. The incidence of reduced ossification of the vertebrae and/or sternebrae in all treated groups was significantly higher than controls when individual pup data were compared, but not with litter comparisons, 42/272 (15%) in controls, 51/229 (22%) at 0.5 mg/kg/day, 76/315 (24%) at 1.0 mg/kg/day, and 72/299 (24%) at 2.0 mg/kg/day. All fetal effects in rats occurred at doses that also produced maternal toxicity.

Dose and end point used for MRL derivation: The lowest dose tested in rats, 0.5 mg/kg/day is a LOAEL for inflamed mesenteric lymph nodes in the dams and reduced ossification in the fetuses.

[ ] NOAEL [X] LOAEL

Uncertainty factors (UF) and Modifying Factor (MF) used in MRL derivation:

[X] 10 for LOAEL-to-NOAEL extrapolation
[X] 10 for human variability
[X] 10 for animal-to-human extrapolation

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

Was a conversion used from intermittent to continuous exposure? NA

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

Other additional studies or pertinent information that lend support to this MRL: In support of the critical effect, there is some evidence in humans to indicate that sulfur mustard affects the lymph system. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted in autopsies of victims of the World War II Bari Harbor incident, during which sulfur mustard was released in to the air and water (Alexander 1947). The spleen also demonstrated evidences of gross pathology in 33 of 53 (62%) autopsies (Alexander 1947). In the majority of cases, the spleen was described as shrunken in size with pale color. Microscopically, only 2 of 32 spleens examined showed degeneration or necrosis; pyknosis and karyorrhexis of lymphocytes in some corpuscles were observed in one and slight necrosis of the malpighian follicle, was observed in the other. Additional studies in animals also revealed sulfur mustard-induced damage to the lymph system. Cameron et al. (1946), after observing damage to the cervical lymph nodes and lymphoid tissue throughout the body in rabbits and monkeys that had undergone tracheal cannulation and had been exposed to chamber concentrations of sulfur mustard ranging from 30 to 350 mg/m^3 (5–54 ppm), administered sulfur mustard to animal skin topically or by subcutaneous injection and observed identical changes to the lymph tissue, suggesting that lymphoid tissue damage may be due to systemic absorption. Sulfur mustard produced a significant dose-related decrease in the weight of peripheral lymph nodes (12–44%) when topically applied at single doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994a). A significant decrease in the weight of mesenteric lymph nodes (18%) was noted at the highest dose. Incidence and severity of histological changes in the thymus and spleen were also dose-related. Spleen histopathology included hypocellularity, atrophy of the lymphoid follicles, degeneration of germinal centers, and red pulp infiltrated with macrophages. The cortex and medulla regions of the thymus showed atrophy and hypocellularity. A significant dose-related decrease in the cellularity of the spleen (24–45%) was measured. A dose-related decrease in the cellularity of the thymus was also found, and was significant at the mid- and high doses (36–42%). A significant dose-related reduction in spleen cell number was measured in female mice 7 days after intraperitoneal injection with sulfur mustard (23% at 5 mg/kg and 49% at 10 mg/kg) (Coutelier et al. 1991).

The principal study (DOA 1987) identified the lowest LOAEL of 0.5 mg/kg/day for inflamed mesenteric lymph nodes in rats following acute administration of sulfur mustard. In range-finding experiments, conducted prior to the principal teratology study, in which rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0, or 2.5 mg/kg/day (3–9 animals/dose group of which 2–7/dose group were pregnant) on gestation days 6–15, significant incidences of inflamed mesenteric lymph nodes occurred at ≥0.4 mg/kg/day (DOA 1987). Also in support of the critical dose, another lymphoretic effect, enlarged Peyer's patches, was observed in

rabbits at 0.5 g/kg/day in a range-finding study and at 0.4 g/kg/day in a teratology study (incidence data were not reported) (DOA 1987).

Agency Contact (Chemical Manager): Zemoria A. Rosemond

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name: Sulfur mustard [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: August 29, 2003
Profile status: Third Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to figure: 9
Species: Rat

Minimal Risk Level: 0.07 [X] µg/kg/day [ ] ppm [ ] mg/m^3

Reference: Sasser LB, Cushing JA, Dacre JC. 1996a. Two-generation reproduction study of sulfur mustard in rats. Repro Toxicol 10(4):311-319.

Experimental design: Sulfur mustard (97.3% purity) dissolved in sesame oil was administered intragastrically by intubation to groups of 8-week-old Sprague-Dawley rats (27 females and 20 males/group/generation) at doses of 0, 0.03, 0.1, or 0.4 mg/kg/day. Male and female rats were dosed 5 days/week for 15 weeks that included 13 weeks before and 2 weeks during the mating period. Females were dosed daily (7 days/week) throughout the 21-day gestation period and 4–5 days/week during the 21-day lactation period. Males were dosed 5 days/week during the 21-day gestation period and sacrificed at the birth of their pups. Dams were sacrificed when their pups were weaned. Male and female F1 pups were treated with sulfur mustard until they were mated and the females became pregnant and gave birth. F1 males were sacrificed at the birth of their pups. The dosing of F1 dams continued until pup weaning, at which time, the study was terminated. Animals were weighed weekly. A complete gross necropsy was performed on all rats found dead or in moribund condition. Weights of the testis, prostate, epididymis, ovary, and uterus were recorded. Histopathological evaluations were performed on reproductive organs of the high dose group and control group of the F0 and F1 adults and on the forestomach of animals in all dose groups.

Effects noted in study and corresponding doses: There were no treatment-related deaths. The body weights of the F0 sulfur mustard-exposed rats were not significantly different from controls; however, the growth rate of the high-dose males tended to decline after about 7 weeks of exposure. Body weight gain was significantly lower ($p<0.05$) than control values in F1 rats of both sexes born to high-dose parents beginning 1 or 2 weeks after dosing was started (approximately 20% for males and 15–24% for females). No significant dose-response in body weight occurred at the lower doses. Breeding and reproductive performance in F0 and F1 animals was not affected by treatment. The only statistically significant birth parameter difference was an altered sex ratio (an increase in the fraction of males) of the high-dose F0 offspring. Although not significantly different, litter weights and number of pups per litter tended to decrease in both F1 and F2 animals at the highest exposure level. Except for a slight reduction in absolute ovary weight in high-dose F0 females, absolute and/or relative male and female reproductive organ weights were unaffected by treatment. Microscopic examination of the reproductive organs revealed no evidence of treatment-related effects. Dose-related incidence and severity of lesions of the squamous epithelium of the forestomach characterized by cellular disorganization of the basilar layer, an apparent increase in mitotic activity of the basilar epithelial cells and thickening of the epithelial layer, occurred in both sexes of each treatment group. The incidence of squamous acanthosis (combined F0 and F1 males and females; minimal to marked severity) was 0/94 controls, 69/94 (73%; 27 males/42 females) in the low-dose groups, 90/94 (96%; 39 males/51 females) in the mid-dose groups, and 94/94 in the high-dose groups. Benign neoplasms of the forestomach (squamous papilloma) occurred in 0/94 controls, 0/94 in the low-dose groups, 8/94 (9%) in the mid-dose groups, and 10/94 (11%) in the high-dose groups.

Dose and end point used for MRL derivation: The lowest dose tested, 0.03 mg/kg/day, is a LOAEL for gastric lesions (mild epithelial acanthosis of the forestomach). Although humans do not have forestomachs, the primary mechanism of toxicity of sulfur mustard is epithelial tissue damage from direct contact and, therefore, epithelial acanthosis is considered a suitable critical noncancer end point for deriving an oral MRL. Tissue damage would be expected to occur at the point of contact, even if it were another part of the gastrointestinal tract.

[ ] NOAEL [X] LOAEL

Uncertainty factors (UF) and Modifying Factor (MF) used in MRL derivation:

[X] 10 for LOAEL-to-NOAEL extrapolation
[X] 10 for human variability
[X] 3 for animal-to-human extrapolation*

*Because sulfur mustard is a highly corrosive agent, epithelial lesions at the point of entry into the stomach are likely to occur across species. For this reason, the typical default value of 10 for the uncertainty factor for extrapolation of data from animals to humans is considered to be too high and a lower value of 3 is applied.

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

Was a conversion used from intermittent to continuous exposure? A time-weighted average (TWA) daily dose was calculated as follows. Females were dosed during the lactation period while males were not. Female rats were treated 5 days/week for 15 weeks (75 days), total dose=2.25 mg/kg (75 days x 0.03 mg/kg/day); daily for 3 gestation weeks (21 days), total dose=0.63 mg/kg; and 4 days/week for 3 lactation weeks (12 days), total dose=0.36 mg/kg. The cumulative dose for females over the 21-week period is 3.24 mg/kg (2.25+0.63+0.36 mg/kg). Dividing the cumulative dose of 3.24 by 147 days (21 weeks) yields a TWA dose of 0.02 mg/kg/day. For males, the same TWA daily dose results; however, different time weighting applies [0.03 mg/kg/day x (5 days/7 days)=0.02 mg/kg/day].

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

Other additional studies or pertinent information that lend support to this MRL: Injury to the gastric mucosa (mild epithelial acanthosis of the forestomach) is a portal-of-entry direct contact toxic effect that is consistent with the vesicant properties of sulfur mustard following oral exposure. In support of the critical effect, gastrointestinal effects have been reported in humans following combat exposure to sulfur mustard, in sulfur mustard testing volunteers, and in sulfur mustard factory workers. In all of these cases, exposure was likely by multiple routes including inhalation, oral, and dermal. In 19 of 53 (36%) victims of the World War II Bari Harbor incident autopsied, stomach irritation and inflammation were documented. The lesions varied from simple hyperemia to focal loss of epithelium, necrosis, and ulceration (Alexander 1947). In a review of the clinical manifestations of sulfur mustard exposure in the Iran-Iraq war victims, Pierard et al. (1990) reported that endoscopy frequently revealed acute gastritis. Incidences of gastrointestinal effects of nausea (64%), vomiting (43%), and bleeding (14%) were reported in a group of 14 children and teenagers following exposure to sulfur mustard from air bombs during the Iran-Iraq war (Momeni and Aminjavaheri 1994). Gastrointestinal neoplasms were reported in Japanese sulfur mustard factory workers who were involved with the production of chemical agents during World War II (Yamakido et al. 1985). Sulfur mustard testing volunteers who were wearing respirators and who were exposed to unspecified levels of sulfur mustard vapors and liquids had skin burns, but also complained of nausea, vomiting, anorexia, abdominal pain, diarrhea, headache, and lassitude (Sinclair

1948). These signs could have been primary effects of the sulfur mustard on the rapidly dividing cells of the gastrointestinal epithelium, secondary effects from the skin burns, or psychological effects not related to the sulfur mustard exposure at all.

In addition to the principal study, Sasser et al. (1996a), similar gastric effects, edema, hemorrhage or sloughing of the mucosa, and ulceration) have been identified in rabbits following 14-day exposures at ≥0.4 mg/kg/day (DOA 1987), in rats following 10-day exposures at ≥2.0 mg/kg/day (DOA 1987), and in rats following 13-week exposures at ≥0.1 mg/kg/day (Sasser et al. 1996b). Regarding the relevance of the toxic effects to humans lacking a forestomach, tissue damage at the point of contact would be expected by a vesicant and direct alkylating agent such as sulfur mustard, regardless of the location in the gastrointestinal tract.

Agency Contact (Chemical Manager): Zemoria A. Rosemond

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.

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).

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.

(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/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 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

1 →

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

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
2 → INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 → Systemic	↓	↓	↓	↓	↓		↓
18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
Cancer					11		
					↓		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89-104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79-103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

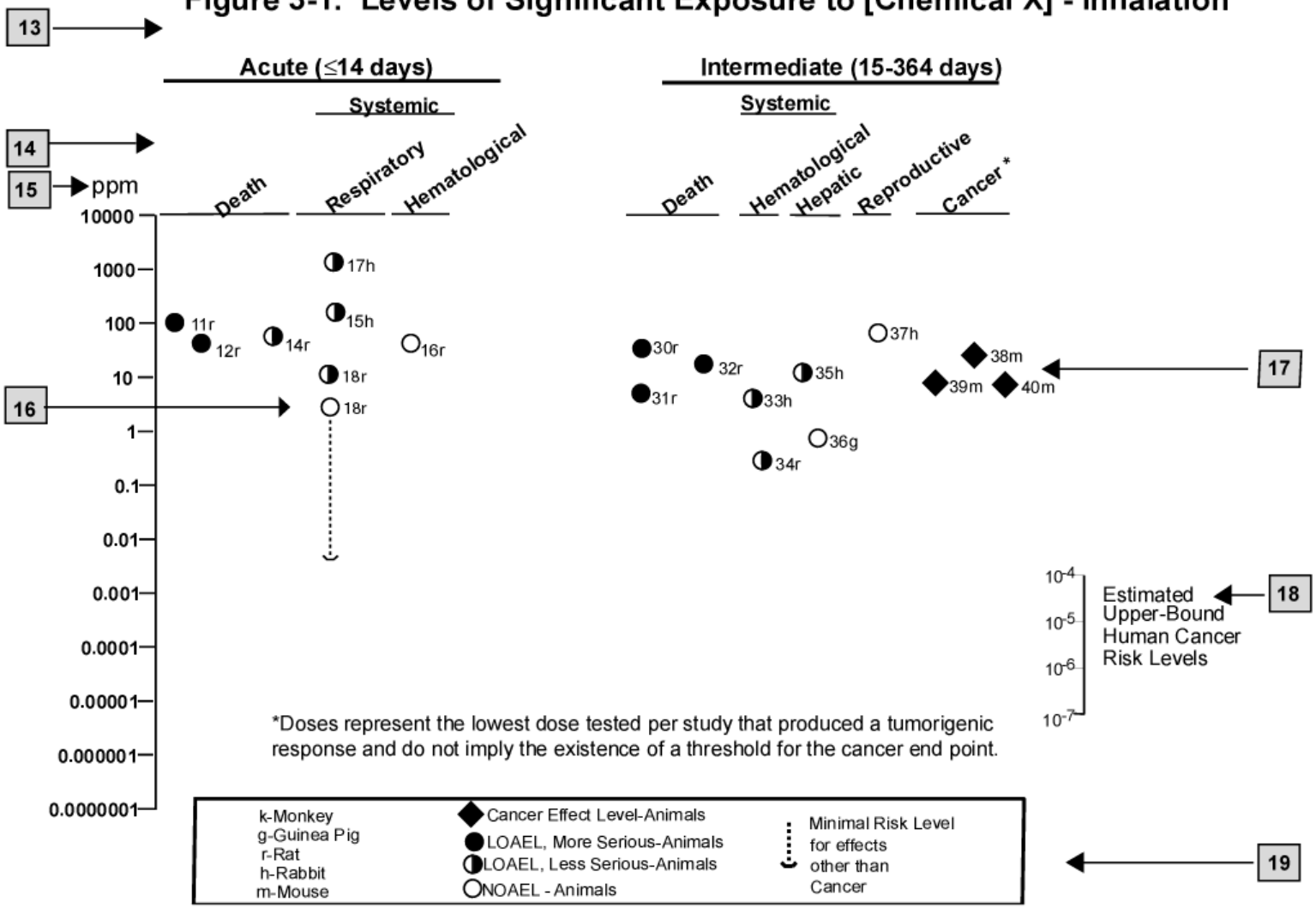
12 →

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

[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 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

ACOEM	American College of Occupational and Environmental Medicine
ACGIH	American Conference of Governmental Industrial Hygienists
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AOEC	Association of Occupational and Environmental Clinics
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
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotranferase
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
Ct	concentration time product
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/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	*Federal Register*
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
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
LCt_{50}	lethal Ct, 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	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
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
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
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
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
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
PID	photo ionization detector

pg	picogram
pmol	picomole
PHS	Public Health Service
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
RTECS	Registry of Toxic Effects of Chemical Substances
RQ	reportable quantity
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

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

APPENDIX D. ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR SULFUR MUSTARD

The National Advisory Committee for the Acute Exposure Guideline Levels for Hazardous Substances has developed acute exposure guideline levels (AEGLs) for sulfur mustard (NAC/AEGL 2001). The AEGLs are threshold exposure limit values for the general public applicable to emergency exposure periods ranging from 10 minutes to 8 hours. For each chemical, three levels of AEGLs, distinguished by varying degrees of severity of toxic effects, are developed: at exposure levels above the AEGL-1, the general population could experience notable discomfort, irritation, or asymptomatic, nonsensory effects; above AEGL-2, the general population could experience irreversible or other serious, long lasting health effects or impaired ability to escape; and above AEGL-3, the general population could experience life-threatening health effects or death. At each AEGL level, values are developed for five exposure periods: 10 minutes, 30 minutes, 1 hour, 4 hours, and 8 hours.

The derivation of the AEGLs for sulfur mustard presented below was excerpted from NRC (2003).

5. DATA ANALYSIS FOR AEGL-l

5.1. Summary of Human Data Relevant to AEGL-l

Walker et al. (1928) reported that four of seven men exposed to sulfur mustard at 0.001 mg/L (1 mg/m^3) for 5-45 min exhibited conjunctivitis, and two exhibited skin burns. It was also reported that, of 17 men exposed at 0.0005 mg/L (0.5 mg/m^3) for 10-45 min (5-22.5 mg·min/m^3), six exhibited conjunctivitis, and one had a skin burn. Three of 13 men exposed for 10-30 min at 0.0001 mg/L (0.1 mg/m^3; Ct of 1-3 mg·min/m^3) showed slight but distinct conjunctivitis. Although not of a severity consistent with an AEGL-2 level, those effects are of greater severity than would be acceptable for AEGL-l development. Guild et al. (1941) also conducted experiments using humans and reported that (1) exposure to Ct values <70 mg·min/m^3 would result in mild conjunctival responses that would not be indicative of a casualty (temporary loss of vision); (2) Ct values of 70-100 mg·min/m^3 would produce some casualties and; (3) Ct values >l00 mg·min/m^3 would be expected to produce disabling ocular effects of several days' duration. Because the subjects wore respiratory protection, effects on the respiratory tract could not be determined.

In experiments with human volunteers exposed to varying concentration-time regimens, Anderson (1942) found that an exposure concentration-time product of 12 mg·min/m^3 was without effects and 30 mg·min/m^3 represented the upper range for mild effects (conjunctival injection and minor discomfort with no functional decrement). Ct products slightly higher than that (e.g., 34-38.1 mg·min/m^3) were,

however, also without appreciable effects, thereby indicating that the response to 30 mg·min/m^3 is consistent with AEGL-l effects.

Odor thresholds of 1 mg·min/m^3 (Bloom 1944), 0.15 mg/m^3 (Ruth 1986) and 0.6 mg/m^3 (Dudley and Wells 1938; Bowden 1943; Fuhr and Krakow 1945) have been reported.

Analysis of the exposure-effect values from the human studies indicated that the 12-mg·min/m^3 value represented a defensible estimate of the threshold for effects consistent with the AEGL-l definition. The 12-mg·min/m^3 exposure was without a symptomatic effect and, therefore, provides the basis for protective AEGL-l values consistent with the AEGL- 1 definition.

5.2. Summary of Animal Data Relevant to AEGL-l

The effects described in the animal studies tend to be of a greater severity than those associated with AEGL-l (i.e., signs of severe ocular irritation, body weight loss, respiratory depression, evidence, evidence of respiratory tract histopathology, etc.). There were no definitive exposure-response data in animals that were considered appropriate for the development of AEGL-l values.

5.3. Derivation of AEGL-l

The most tenable AEGL-l values were developed using data reported by Anderson (1942) in which three to four human volunteers were exposed to agent HD at varying concentration-time regimens. In an analysis of those data, Anderson found that an exposure concentration-time product of 30 mg·min/m^3 represented the upper range for mild effects (conjunctival injection and minor discomfort with no functional decrement) and that 12 mg·min/m^3 represented a threshold for such effects. The 12 mg·min/m^3 represents a defensible estimate of the threshold for AEGL-1 effects. The 12-mg·min/m^3 exposure resulted in only minor conjunctival injection and no sensation of irritation. Ocular effects appear to be the most sensitive indicator of sulfur mustard exposure and toxicity, thereby justifying ocular irritation as an appropriate end point for development of AEGL values. All of the data considered were from human subjects, and, therefore, the uncertainty factor (UF) application to the 12-mg·min/m^3 value was limited to 3 for protection of sensitive individuals. The adjustment is considered appropriate for acute exposures to chemicals whose mechanism of action primarily involves surface contact irritation of ocular and/or respiratory tract rather than systemic activity that involves absorption and distribution of the parent chemical or a biotransformation product to a target tissue. In addition, Anderson (1942) noted that there was little variability in the ocular responses among the individuals participating in the study. That the AEGL-1 values are based on a sensitive end point is also reflected in that they are below reported odor thresholds (0.6 mg/m^3 and 1 mg·min/m^3).

Because exposure-response data were unavailable for all of the AEGL- specific exposure durations, temporal extrapolation was used in the development of AEGL-1 values for the AEGL-specific time periods. The concentration-exposure time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent *n* ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analyses of available data regarding AEGL-1 type effects reported by Reed (1918), Reed et al. (1918), Guild et al. (1941), and Anderson (1942) indicate that for the exposure periods up to several hours, the concentration-exposure time relationship is a near-linear function (i.e., Haber's law where $n = 1$ for $C^n \times t = k$) as shown by *n* values of 1.11 and 0.96 for various data sets consistent with AEGL-l effects (Appendix B). Therefore, an empirically derived, chemical-specific estimate of $n = 1$ was used, rather than a default value, based on the ten Berge (1986) analyses. The derivation of the exponent (*n*) utilized human response data where 75-100% of the responders showed a mild response that would be consistent with the definition of AEGL-l effects. In addition, the data provided by Anderson (1942) were indicative of a linear concentration-time relationship. The AEGL-l values developed using the 12-$mg \cdot min/m^3$ exposure value reported by Anderson (1942) are shown in Table 2-9. The AEGL-l values are below the odor threshold for sulfur mustard (0.6 mg/m^3 and 1 $mg \cdot min/m^3$).

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Quantitative data regarding the human experience and AEGL-2 level effects are limited to responses ranging from signs of mild ocular irritation to ocular irritation that impairs normal visual function. Reed (1918) reported that 20-45 min exposure of himself and a volunteer at 1.2 mg/m^3 resulted in severe ocular irritation and dermal lesions. In a report of a subsequent experiment, Reed et al. (1918) noted that exposure of human volunteers at 0.1- 4.3 mg/m^3 for 5 -45 min produced ocular irritation and skin burns (0.5 mg/m^3 for 30 min) and very severe conjunctivitis, photophobia, skin bums, and nasopharyngeal exfoliation (1.0 mg/m^3 for 45 min). The analytical techniques used in these experiments were suspect; actual exposures were likely 30-40% higher. The report by Guild et al. (1941) of human exposure experiments did not provide findings of effects consistent with the AEGL-2 definition. Anderson (1942) reported on a series of human exposures resulting in varying degrees of ocular responses ranging from nonsymptomatic ocular injection to ocular irritation that required medical treatments and was considered severe enough to impair normal function.

6.2. Summary of Animal Data Relevant to AEGL-2

With the exception of a study reported by Warthin and Weller (1919) regarding the effects in rabbits following acute exposure, there is little exposure-response data for animals consistent with AEGL-2-severity effects. Weller and Warthin reported severe ocular effects and dermal burns in rabbits exposed for 12 h to

sulfur mustard at 130 mg/m^3. That study, however, was compromised by the use of single animals and lacks detail. Kumar and Vijayaraghavan (1998) reported alterations in purine catabolism exposed for 1 h to sulfur mustard at 21.2- 84.6 mg/m^3 but those exposures also represented 0.5, 1.0, and 2.0 LC^{50} responses. Statistically significant reductions in body weights were also observed for the mice at 14 d following a 1-h exposure to concentrations at 16.9- 42.3 mg/m^3; however at least some of the exposures were also associated with lethality. Dogs, rats, mice, and guinea pigs exposed continuously to sulfur mustard at 0.001 mg/m^3 or discontinuously (6.5 h/d, 5 d/wk) at 0.1 mg/m^3 for up to 52 wk did not exhibit effects consistent with the AEGL-2 definition (McNamara et al. 1975).

Table **2-9 AEGL-l Values for Sulfur Mustard (ppm [mg/m^3]) [a]**

10-min	30-min	1-h	4-h	8-h
0.06 (0.40)	0.02 (0.13)	0.01 (0.067)	0.003 (0.017)	0.001 (0.008)

[a]The AEGL-1 values are at or below the odor threshold for sulfur mustard.

6.3. Derivation of AEGL-2

The AEGL-2 values for sulfur mustard were developed using data from Anderson (1942). The study utilized three or four human volunteers' exposed to varying concentrations of sulfur mustard (1. 7-15.6mg/m^3) for time periods varying from 2 to 33 min. Anderson considered a Ct value of 60 mg·min/m^3 as the lowest concentration-time product for which ocular effects could be characterized as military casualties and that personnel exposed might be ineffective for up to (but no more than) 7 d. Effects included irritation, soreness, and widespread conjunctivitis, frequently accompanied by chemosis and photophobia. The 60-mg·min/m^3 exposure was used as the basis for developing the AEGL-2 values because it is representative of an acute exposure causing an effect severe enough to impair normal visual function and, although not irreversible, would certainly result in potential for additional injury. The ocular irritation and damage were also considered appropriate as a threshold estimate for AEGL-2 effects, because the eyes are generally considered the most sensitive indicator of sulfur mustard exposure, and irritation would likely occur in the absence of vesication effects and severe pulmonary effects. The fact that the AEGL-2 is based on human data precludes the use of an interspecies UF. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). The factor was limited to 3 under the assumption that the primary mechanism of action of sulfur mustard involves a direct effect on the ocular surface and that the response will not vary greatly among individuals (as noted by Anderson [1942]). A modifying factor of 3 was applied to accommodate potential onset of long-term ocular or respiratory effects. It was justified by the absence of long-term follow-up in the subjects of the Anderson (1942) study to confirm or deny development of permanent ocular or respiratory tract damage. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16 x 3.16 = 10. Further reduction by the

application of additional modifying factors was not warranted because of the use of a sensitive indicator representing an AEGL-2 effect of marginal severity. As is the case for AEGL-1 values, time scaling was conducted using an *n* of 1 for all time points (Appendix B). The resulting AEGL-2 values are shown in Table 2-10, and their derivation is presented in Appendix A. Similar to the AEGL-l values, all of the AEGL-2 values are at or below the reported odor thresholds (0.6 mg/m^3 and 1 $mg \cdot min/m^3$).

TABLE 2-10 AEGL-2 Values for Sulfur Mustard (ppm [mg/m^3])[a]

10-min	30-min	1-h	4-h	8-h
0.09 (0.60)	0.03 (0.20)	0.02 (0.10)	0.004 (0.025)	0.002 (0.013)

[a]The AEGL-2 values are at or below odor threshold for sulfur mustard.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Human lethality data are limited to an inhalation LCt_{50} estimate of 1,500 $mg \cdot min/m^3$ and percutaneous LCt^{50} estimate of 10,000 $mg \cdot min/m^3$ estimated from animal data (DA 1974). The NRC (1997) concluded that an estimated LCt^{50} for humans of 900 $mg \cdot min/m^3$ developed by the U. S. Army based on an average of animal LCt^{50} data was scientifically valid but was developed in reference to healthy male military personnel and does *not* apply to civilians.

7.2. Summary of Animal Data Relevant to AEGL-3

Various lethality values have been reported for laboratory species exposed to sulfur mustard. Vijayaraghavan (1997) reported a 1-h LC^{50} of 42.5 mg/m^3 for mice (head-only exposure). In a follow-up study reported by Kumar and Vijayaraghavan (1998), 1-h exposure of mice at 21.2 mg/m^3 did not result in lethality. Lethality estimates were based on deaths occurring up to 14 d after exposure. Langenberg et al. (1998) reported a 5-min LCt_{50} of 800 $mg \cdot min/m3$ for rabbits (deaths determined up to 96 h after exposure). These studies utilized up-to-date exposure and analytical systems and provided lethality estimates based on adequate numbers of animals evaluated at post exposure time frames appropriate for the known latency in sulfur-mustard-induced lethality.

7.3. Derivation of AEGL-3

As noted in Section 3.1.4, the lethality data from earlier reports were not verifiable but are not inconsistent with those from later studies. The 1-h LC_{50} values for rats and mice derived from the 840 and 860 $mg \cdot min/m^3$ 60-min LCt5o values reported by Fuhr and Krakow (1945) are similar to the lower confidence limit of the mouse 1-h LC_{50} reported by Vijayaraghavan (1997) (i.e., 14.0, 14.3,

and 13.5 mg/m^3, respectively; the corresponding Ct values are 840,858, and 810 mg·min/m^3). The values are also similar to a 1-h LC_{50} of 13.3 mg/m^3 for guinea pigs extrapolated (assuming $C^1 \times t = k$) from the 5-min LCt_{50} of 800 mg·min/m^3 reported by Langenberg et al. (1998). However, the values from the earlier studies are not verifiable. In the inhalation toxicity study by Vijayaraghavan (1997), mice were exposed head only) for 60 min to sulfur mustard at concentrations of 0.0, 8.5, 16.9, 21.3, 26.8, 42.3 or 84.7 mg/m^3. The study investigator derived a 60-min LC_{50} of 42.5 mg/m^3 based on lethality at 14 d post exposure (95% confidence interval: 13.5-133.4 mg/m^3). In a follow-up study (Kumar and Vijayaraghavan 1998), there was no mortality in mice exposed at 0.5 LC_{50} mg/m^3). Therefore, the 1-h exposure at 21.2 mg/m^3 was selected as an estimate of the lethality threshold in mice.

When compared with the human exposure-effect data, the 21.2-mg/m^3 concentration (Ct of 1,272 mg·min/m^3 for a 60-min exposure) is not an exposure that has been associated with lethality in humans (see Section 2.1). An intraspecies UF of 3 was applied for protection of sensitive individuals. This adjustment was considered appropriate for acute exposures to chemicals whose mechanism of action primarily involves surface contact irritation of ocular and/or respiratory tract tissue rather than systemic activity that involves absorption and distribution of the parent chemical or a biotransformation product to a target tissue. An interspecies UP was limited to 3 because available data do not suggest that humans are notably more sensitive than animals regarding lethality from inhalation exposure to sulfur mustard. The mechanism of pulmonary injury leading to lethality appears to be a function of the direct contact of an alkylating agent with epithelial tissue. This mechanism is likely to be more similar than different across mammalian species. Furthermore, the AEGL-3 values resulting from the aforementioned complement of UFs (total UF adjustment was 10; see Section 6.3) are equivalent to exposures known to cause only mild ocular effects in humans. The modifying factor of 3 utilized in the development of AEGL-2 values to account for uncertainties regarding the latency and persistence of the irritant effects of low-level exposure to sulfur mustard was not applied for AEGL-3 because lethality of the mice was assessed at 14 d post exposure in the key studies by Vijayaraghavan (1997) and Kumar and Vijayaraghavan (1998).

For derivation of the AEGL-3 values, there was uncertainty regarding the validity of applying linear extrapolation based on ocular effects to concentration-time extrapolations for lethality. As reported by ten Berge et al. (1986), the concentration-time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5. Therefore, in the absence of chemical-specific lethality data, time scaling was performed using exponential extrapolation ($n = 3$) for shorter time periods and linear extrapolation ($n = 1$) for longer time periods, thereby providing a somewhat more conservative (i.e., protective) estimate of the AEGL-3 values than would be obtained using an n value based on ocular irritation. The AEGL-3 values were derived by scaling from the 1-h LC_{50} of 21.2 mg/m^3 reported by

Kumar and Vijayaraghavan (1998) using C^n x t = k where n = l or 3 (Appendix A). The concentration-time constant, k, was 1,272 mg·min/m^3 where n = 1 and 571,687.68 mg·min/m^3 where n = 3. The AEGL-3 values are shown In Table 2-11, and their derivation is presented in Appendix A. The 4-h and 8-h AEGL-3 values are at or below reported odor thresholds.

TABLE 2-11 AEGL-3 Values for Sulfur Mustard (ppm [mg/m^3])

10 min	30 min	1 h	4 h	8 h
0.59 (3.9)	0.41 (2.7)	0.32 (2.1)	0.08 (0.53)	0.04 (0.27)

Note: The 4 –h and 8 – h AEGL-3 values are below the odor threshold for sulfur mustard.

When comparing the Ct values generated by the draft AEGL-3 numbers the human exposure data, any further reduction appears indefensible. The Ct values resulting from theAEGL-3 numbers (i.e., 39-130 mg·min/m^3) are similar to cumulative exposures shown to cause only ocular irritation in humans (Guild et al. 1941; Anderson 1942) and are similar to the ECt_{50} of 100 mg·min/m^3 for severe ocular effects (for soldiers) determined by Reutter and Wade (1994) and the NRC (1997). Furthermore, the AEGL-3 values are nearly similar to those developed using the human lethality estimate of 900 mg·min/m^3 (Reutter and Wade 1994) that was derived from multiple-species animal data and reviewed by the NRC (1997). Assuming a 3-fold reduction for estimation of a lethality threshold ([900 mg·min/m^3]/3 = 300 mg·min/m^3) and another 3-fold reduction for consideration of sensitive populations ([300 mg·min/m^3]/3 = 100 mg·min/m^3), the resulting AEGL-3 values from the Reutter and Wade (1994) and NRC (1997) reports would be 4.8, 3.3, 1.7, 0.42, and 0.21 mg/m^3 for 10 min, 30 min, and 1, 4, 8 h, respectively. These highly derivative estimates are comparable to, and supportive of, AEGL-3 estimates derived from the experimental data of Kumar and Vijayaraghavan (1998) (see Table 2-11).

8. SUMMARY OF AEGLs

8.1. AEGL Values and Toxicity End Points

Human data are available from several independent sources that define exposure-response for AEGL-l and AEGL-2 effects. Although a definitive demarcation of the exposure-response for sensitive populations was not provided by those data, the human data eliminated the uncertainties inherent in the use of data from animal studies. Both the AEGL-l and AEGL-2 values were based on effect end points consistent with the respective AEGL definitions (i.e., threshold for barely discernible ocular irritation [AEGL-l] and threshold for ocular irritation indicative of functional impairment [AEGL-2]). Areas of uncertainty were associated with the sensitive responders and the relationship between ocular effects and the onset of respiratory effects. Human data from which to develop AEGL-3 values were

unavailable. The AEGL-3 was based on an estimated lethality threshold from studies in mice (Vijayaraghavan 1997; Kumar and Vijayaraghavan 1998). When compared with human exposure-response data and lethality estimates, the mouse lethality data were considered a defensible approach to AEGL-3 derivation. AEGL-3 values based on a human lethality estimate of 900 $mg \cdot min/m^3$ (Reutter and Wade 1994; NRC 1997) were very similar to those developed using the animal data of Vijayaraghavan (1997) and Kumar and Vijayaraghavan (1998). An estimate of theoretical excess cancer risk based upon a geometric mean of inhalation slope factors developed using various data sets and procedures revealed that exposure concentrations representing a theoretical 10^{-4} lifetime risk were similar to the AEGL-3 exposure concentration values. The exposures for theoretical excess lifetime cancer risk at 10^{-5} and 10^{-6} levels would be correspondingly reduced. The use of excess cancer risk estimates in setting AEGL values is precluded by the uncertainties involved in assessing excess cancer risk following a single acute exposure of 8-h or less duration, by the relatively small population exposed in an emergency release situation, and by the potential risks associated with evacuations.

The AEGL values for sulfur mustard are summarized in Table 2-12. Extrapolation to exposure durations of less than 10 min is not recommended in the absence of careful evaluation of existing data and comparison of any derivative values with those data.

8.2. Comparison with Other Standards and Guidelines

Comparison of the draft AEGL values with other existing standards and guidelines is shown in Table 2-13. No other standards or guidelines from other agencies or programs (e.g., NIOSH, ERPG, ACGIH, MAK, MAC, and OSHA) were available.

8.3. Data Adequacy and Research Needs

The AEGL-1 values are based on human data and are considered estimates for exposures that would cause no significant health effects or sensations of irritation beyond minimal conjunctivitis.

TABLE 2-12 Summary of AEGL Values for Sulfur Mustard[a]

AEGL Level	10 min	30 min	1 h	4 h	8 h
AEGL-1[a] (Nondisabling)	0.06 ppm (0.40 mg/m^3)	0.02 ppm (0.13 mg/m^3)	0.01 ppm (0.067 mg/m^3)	0.003 ppm (0.017 mg/m^3)	0.001 ppm (0.008 mg/m^3)
AEGL-2[a] (Disabling)	0.09 ppm (0.60 mg/m^3)	0.03 ppm (0.20 mg/m^3)	0.02 ppm (0.10 mg/m^3)	0.004 ppm (0.025 mg/m^3)	0.002 ppm (0.013 mg/m^3)
AEGL-3[a]	0.59 ppm (3.9 mg/m^3)	0.41 ppm (2.7 mg/m^3)	0.32 ppm (2.1 mg/m^3)	0.08 ppm (0.53 mg/m^3)	0.04 ppm (0.27 mg/m^3)

A AEGL-1 and AEGL-2 values, and the 4- and 8-h AEGL-3 values are at or below the odor threshold for sulfur mustard.

The ocular irritation on which the AEGL-l and AEGL-2 values are based is the most sensitive response to sulfur mustard vapor. The AEGL-2 values provide Ct exposures that are well below those known to induce severe ocular effects in normal humans (i.e., 70-90 mg·min/m^3). AEGL-3 values provide Ct values (39-130 mg·min/m^3) that are at levels known to cause moderate to severe ocular irritation and possible respiratory tract irritation in human subjects Anderson 1942; Guild et al. 1941) but no life-threatening effects or death. Although the overall database for acute inhalation exposure to sulfur mustard is not extensive, the AEGL values are supported by the available data.

The absence of multiple-species lethality data for acute exposures limits a thorough understanding of variability. Data providing definitive demarcation of the threshold for serious and/or irreversible effects would provide a more complete picture of responses resulting from acute inhalation exposure to sulfur mustard. That is especially relevant to assessing the potential for serious respiratory tract damage or permanent ocular pathology following acute exposure. Although sulfur mustard is a genotoxic chemical capable of inducing tumors in animals and humans, the carcinogenic potential of acute inhalation exposures has not been defined.

TABLE 2-13 Comparison of AEGL Values for Sulfur Mustard with Other Extant Standards and Guidelines

Guideline	10 min	30 min	1 h	4 h	8 h	Other
AEGL-1	0.40 mg/m^3 (0.06 ppm)	0.13 mg/m^3 (0.02 ppm)	0.067 mg/m^3 0.01 ppm)	0.017 mg/m^3 (0.003 ppm)	0.008 mg/m^3 0.001 ppm)	
AEGL-2	0.60 mg/m^3 (0.09 ppm)	0.20 mg/m^3 (0.03 ppm)	0.10 mg/m^3 (0.02 ppm)	0.025 mg/m^3 (0.004 ppm)	0.013 mg/m^3 (0.002 ppm)	
AEGL-3	3.9 mg/m^3 (0.59 ppm)	2.7 mg/m^3 (0.41 ppm)	2.1 mg/m^3 (0.32 ppm)	0.53 mg/m^3 (0.08 ppm)	0.27 mg/m^3 (0.04 ppm)	
Department of the Army/Civilian Occupational WPL[a]					0.003mg/m^3 (0.0005 ppm)	
Department of the Army/Civilian GPL[b]						0.0001 mg/m^3 (1.5×10^{-5} ppm)
CDC-CSEPP (Thacker, 1994)[c]						2.0 $mg \cdot min/m^3$ (0.3 ppm)

[a]Worker Population Exposure Limit (DA 1991, 1997; DHHS 1988), 8-h TWA, 5 d/wk
[b]General Population Limit (no observable effects), 24-h TWA, 7 d/wk
[c]Recommended acute effects levels for determining emergency evacuation distances in the Chemical Stockpile Emergency Preparedness Program (CSEPP); no set exposure time.

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APPENDIX A

Derivations of AEGL Values

Derivation of AEGL-l

Key Study: Anderson (1942)

Toxicity End point: Exposure concentration-time product of 12 mg·min/m^3 represented the threshold for ocular effects (conjunctival injection and minor discomfort with no functional decrement) for human volunteers exposed to agent HD at varying exposure regimens. The eye is generally considered to be the most sensitive organ/tissue relative to agent HD exposure.

Scaling: The concentration-time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analysis of available data indicated n to be near unity (Appendix B), hence, $C^1 \times t = k$.

Uncertainty factors: Total adjustment of 3. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that the response will not vary greatly among individuals. In

addition, subjects in the Anderson (1942) study exhibited little variability in ocular response.

Because the AEGL-l is based on human data, the interspecies UF is 1.

10-min AEGL-l: C^1 x 10 min = 12 mg·min/m^3
C = 1.2 mg/m^3
10-min AEGL-1 = (1.2 mg/m^3)/3 = 0.40 mg/m^3
(0.06 ppm)

30-min AEGL-l: C^1 x 30 min= 12mg·min/m^3
C = 0.4 mg/m^3
30-min AEGL-1 = (0.4 mg/m^3)/3 = 0.13 mg/m^3
(0.02 ppm)

l-h AEGL-l: C^1 x 60 min = 12 mg·min/m^3
C= 0.2 mg/m^3
1-h AEGL-1 = (0.2 mg/m^3)/3 = 0.067 mg/m^3
(0.01 ppm)

4-h AEGL-l: C^1 x 240 min = 12 mg·min/m^3
C= 0.05 mg/m^3
4-h AEGL-1 = (0.05 mg/m^3)/3 = 0.017 mg/m^3
(0.003 ppm)

8-h AEGL-l: C^1 x 480 min = 12 mg·min/m^3
C = 0.025 mg/m^3
8-h AEGL-1 = (0.025 mg/m^3)/3 = 0.008 mg/m^3
(0.001ppm)

Derivation of AEGL-2

Key study: Anderson (1942)

Toxicity end point: A concentration-time product of 60 mg·min/m^3 was considered the lowest exposure causing ocular effects (we1l-marked, generalized conjunctivitis, edema, photophobia, and irritation) resulting in effective performance decrement and characterized as a military casualty requiring treatment for up to 1 wk.

Scaling: The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n x\ t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al.

1986). Analysis of available data indicated *n* to be near unity (Appendix B), hence, $C^1 \times t = k$.

Uncertainty Factors: Total adjustment of 10. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Because the AEGL-l is based on human data, the interspecies UF is 1. A modifying factor of 3 was applied to accommodate potential onset of long-term ocular or respiratory effects.

Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16 x 3.16 = 10.

10-min AEGL-2: $C^1 \times 10 \text{ min} = 60 \text{ mg}^{\cdot}\text{min/m}^3$
$C = 6\text{mg}$
10-min AEGL-2 = $(6 \text{ mg/m}^3)/10 = 0.60 \text{ mg/m}^3$
(0.09 ppm)

30-min AEGL-2: $C^1 \times 30 \text{ min} = 60 \text{ mg}^{\cdot}\text{min/m}^3$
$C = 2.00$ mg
30-min AEGL-2 = $(2.00 \text{ mg/m}^3)/10 = 0.20 \text{ mg/m}^3$
(0.03 ppm)

1.-h AEGL-2: $C^1 \times 60 \text{ min} = 60 \text{ mg}^{\cdot}\text{min/m}^3$
$C = 1.00 \text{ mg/m}^3$
1-h AEGL-2 = $(1.00 \text{ mg/m}^3)/10 = 0.10$
(0.02 ppm)

4 –h AEGL-2: $C^1 \times 240 \text{ min} = 60 \text{ mg}^{\cdot}\text{min/m}^3$
$C = 0.25 \text{ mg/m}^3$
4-h AEGL-2 = $(0.25 \text{ mg/m}^3)/10 = 0.025 \text{ mg/m}^3$
(0.004 ppm)

8-h AEGL-2: $C^1 \times 480 \text{ min} = 60 \text{ mg}^{\cdot}\text{min/m}^3$
$C = 0.125 \text{ mg/m}^3$
8-h AEGL-2 = $(0.125 \text{ mg/m}^3)/10 = 0.013 \text{ mg/m}^3$
(0.002 ppm)

Derivation of AEGL-3

Key study: Kumar and Vijayaraghavan (1998)

Toxicity end point: Estimated lethality threshold of 21.2 mg/m^3 for 1 h based on no deaths in mice exposed to that concentration, which is 0.5 of the 1-h LC_{50} in mice reported by Vijayaraghavan (1997).

Scaling: The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analysis of available data pertaining to ocular effects indicated n to be near unity (Appendix B). However, there was uncertainty regarding the validity of applying linear extrapolation based on ocular effects to concentration-time extrapolations for lethality. Therefore, in the absence of chemical-specific lethality data, time scaling was performed using exponential extrapolation (n = 3) for shorter time periods (<1 h) and linear extrapolation (n = 1) for longer time periods (> 1 h), thereby providing a somewhat more conservative (i.e., protective) estimate of the AEGL-3 values than would be obtained using an n value based on ocular irritation. The concentration-time constant, k, was 1272 mg^n·min/m^3 where n = 1 and 571,687.68 mg^n·min/m^3 where n = 3.

Uncertainty factors: Total UF was 10. A UF for interspecies was limited to 3 because human data are available showing that exposures to the AEGL-3 values are more likely to produce only severe ocular irritation and possible minor or moderate irritation of the upper respiratory tract. Intraspecies variability was limited to 3 because lethality appears to be a function of extreme pulmonary damage resulting from direct contact of the agent with epithelial surfaces. No modifying factor was applied because the basis of lethality estimate was from studies utilizing a 14-d observation period to assess the lethal response from a 1-h exposure.

Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16 x 3.16 = 10.

10-min AEGL-3: $C^3 \times 10$ min = 571,687.68 mg^n·min/m^3
C^3 = 57,168.76 mg^n·min/m^3
C = 38.52 mg/m^3
10-min AEGL-3 = (38.52 mg/m^3)/10 = 3.9 mg/m^3
(0.59 ppm)

30-min AEGL-3: $C^3 \times 30$ min = 571,687.68 mg^n·min/m^3
C^3 = 19,056.26 mg^n·min/m^3
C= 26.7 mg/m^3

30-min AEGL-3 = (26.7 mg/m^3)/10 = 2.7 mg/m^3
(0.41 ppm)

1 –h AEGL-3: C^1 x 60 min = 1,272 mg·min/m^3
C= 21.2 mg/m^3
1-h AEGL-3 = (21.2 mg/m^3)/10 = 2.1 mg/m^3
(0.32 ppm)

4 –h AEGL-3: C^1 x 240 min = 1,272 mg·min/m^3
C = 5.3 mg/m^3
4-h AEGL-3 = (5.3 mg/m^3)/10= 0.53 mg/m^3
(0.08 ppm)

8-h AEGL-3: C^1 x 480 min = 1,272 mg·min/m^3
C = 2.65 mg/m^3
8-h AEGL-3 = (2.65 mg/m3)/10 = 0.27 mg/m^3
(0.04 ppm)

APPENDIX B

Determination of Temporal Scaling Factor *(n)* for AEGL Derivations

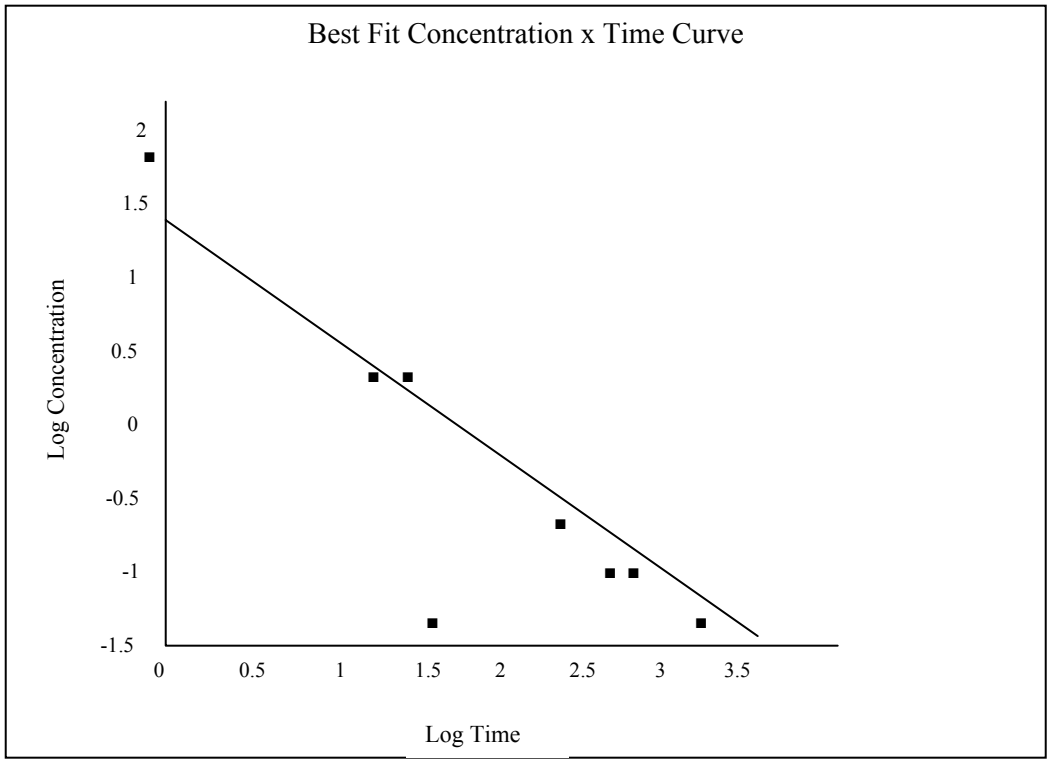
Derivation of *n* for C^n x *t* = *k;* data points indicative of a 100% response for mild ocular irritation following exposure to sulfur mustard agent HD) at various concentrations and times (Reed 1918; Reed et al., 1918; Guild et a1. 1941; Anderson 1942)

Time	Concentration	Log Time	Log Concentration
1	72	0.0000	1.8573
30	1.4	1.4771	0.1461
30	0.06	1.4771	-1.2218
45	1.4	1.6532	0.6198
210	0.24	2.3222	-0.6198
480	0.1	2.6812	-1.0000
600	0.1	2.7782	-1.0000
1,440	0.06	3.1584	-1.2218

Regression output:

Intercept	1.3852
Slope	-0.9002
R squared	0.7434
Correlation	-0.8622
Degrees of Freedom	6
Observations	8

$n = 1.11$
$k = 34.58$



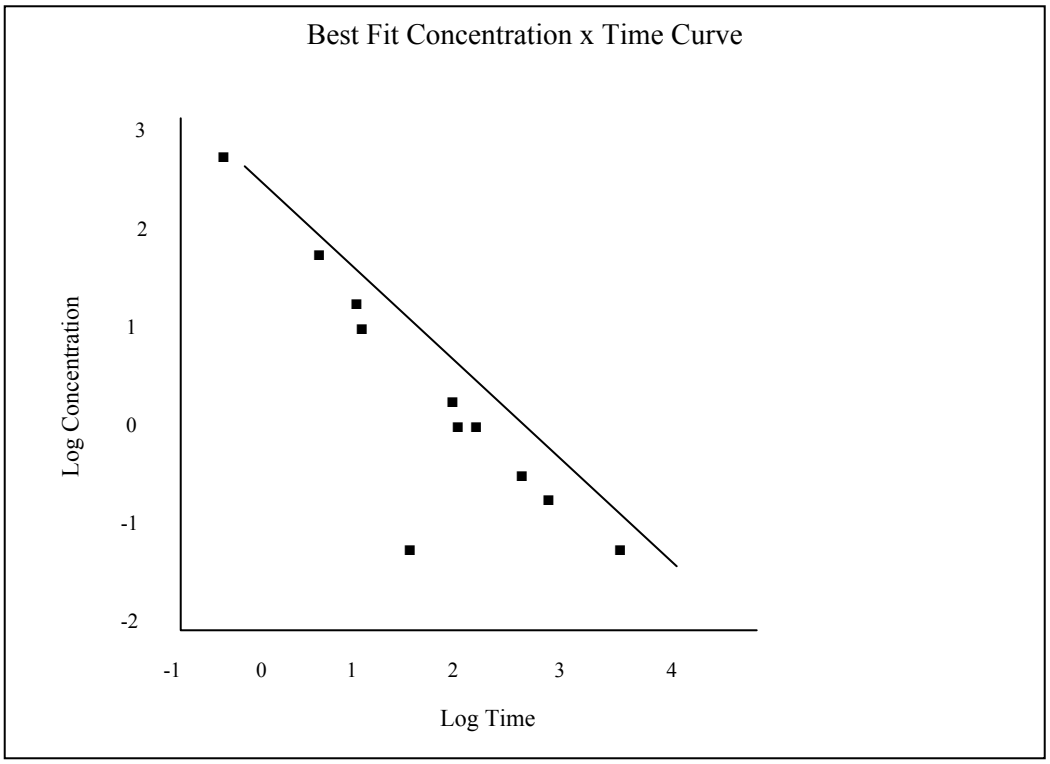
Derivation of n for $C^n \times t = k$; data points indicative of a 75-100% *response* for mild ocular irritation following exposure to sulfur mustard (agent HD) at various concentrations and times (Reed 1918; Reed et al. 1918; Guild et al. 1941; Anderson 1942)

Time	Concentration	Log Time	Log Concentration
1	72	0.0000	1.8573
30	1.4	1.4771	0.1461
30	0.06	1.477	-1.2218
45	1.4	1.6532	0.1461
210	0.24	2.3222	-0.6198
480	0.1	2.6812	-1.0000
600	0.1	2.7782	-1.0000
1,440	0.06	3.1584	-1.2218
33	1.7	1.5185	0.2304
3	12.7	0.4771	1.1038
3	30	0.4771	1.4771
2.5	30	0.3979	1.4771
2	30	0.3010	1.4771
0.25	320	-0.6021	2.5051

Regression Output:

Intercept	1.7240
Slope	-1.0356
R squared	0.8891
Correlation	-0.9429
Degrees of freedom	12
Observations	14

n = 0.96
k = 46.05



APPENDIX C

Carcinogenicity Assessment for Acute Exposure to Sulfur Mustard (Agent HD)

The cancer assessment for acute inhalation exposure to sulfur mustard was conducted following the NRC methodology for EEGLs, SPEGLs, and CEGLs (NRC 1986). The virtually safe dose (VSD) was determined from an inhalation slope factor of 14 $(mg/kg/d)^{-1}$ for the general population (USACHPPM 2000). The slope factor was a geometric mean of slope factors developed using various data sets and procedures and was considered the most tenable quantitative assessment for potential cancer risk from inhalation exposure to sulfur mustard. The corresponding Inhalation Unit Risk was 0.0041 $(\mu g/m^3)^{-1}$ or 4.1 $(mg/m^3)^{-1}$ (USACHPPM2000). The VSD was calculated as follows:

VSD = Risk Level/Unit Risk

$$\frac{\text{VSD} = 1 \times 10^{-4} \text{ risk} = 2.5 \times 10^{-5} \text{ mg/m}^3}{(4.1 \text{ mg/m}^3)^{-1}}$$

Assuming the carcinogenic effect to be a linear function of cumulative dose (d), a single-day exposure is equivalent to d x 25,600 d (average lifetime).

$$\begin{aligned}\text{24-h exposure} &= \text{VSD} \times 25{,}600 \\ &= (2.5 \times 10^{-5} \text{ mg/m}^3) \times 25{,}600 \\ &= 0.64 \text{ mg/m}^3\end{aligned}$$

Adjustment to allow for uncertainties in assessing potential cancer risks under short term exposures under the multistage model (Crump and Howe 1984).

$$\frac{\text{24- hr exposure}}{6} \quad \frac{0.64 \text{ mg/m}^3}{6} = 0.1 \text{ mg/m}^3$$

If the exposure is limited to a fraction (*f*) of a 24-h period, the fractional exposure becomes l/*f* x 24 h (NRC 1985). For a 1 x 10^{-4}, 1 x 10^{-5}, and 1 x 10^{-6} risk, the fractional exposures are shown below.

Exposure Duration	10^{-4}	10^{-5}	10^{-6}
24-h	0.1 mg/m^3 (0.02 ppm)	0.01 mg/m^3 (0.002 ppm)	0.001 mg/m^3 (0.002 ppm)
8-h	0.3 mg/m^3 (0.05 ppm)	0.03 mg/m^3 (0.005 ppm)	0.003 mg/m^3 (0.0005 ppm)
4-h	0.6 mg/m^3 (0.09 ppm)	0.06 mg/m^3 (0.009 ppm)	0.006 mg/m^3 (0.0009 ppm)
1-h	2.4 mg/m^3 (0.36 ppm)	0.24 mg/m^3 (0.036 ppm)	0.024 mg/m^3 (0.0036 ppm)
30-min	4.8 mg/m^3 (0.72 ppm)	0.48 mg/m^3 (0.072 ppm)	0.048 mg/m^3 (0.0072 ppm)
10-min	14.1 mg/m^3 (2.16 ppm)	1.41 mg/m^3 (0.22 ppm)	0.141 mg/m^3 (0.022 ppm)

Because the derivation of the cancer slope factor requires conversion of animal doses to human equivalent doses, no reduction of exposure levels is applied to account for interspecies variability. With the exception of the 10-min, 30-min, and 1-h values for 10^{-4} risk and the 10- min 10^{-5} risk, these exposures are at or below the odor threshold for sulfur mustard. A cancer risk assessment based on a geometric mean of inhalation slope factors developed using various data sets and procedures indicated an excess cancer risk of 1 in 10,000 (10^{-4}) may be associated with exposures similar to the AEGL-3 values. The use of excess cancer risk

estimates in setting AEGL values is precluded by the uncertainties involved in assessing excess cancer risk following a single acute exposure of 8-h or less duration, by the relatively small population exposed in an emergency release situation, and by the potential risks associated with evacuations.

APPENDIX D

DERIVATION SUMMARY FOR ACUTE EXPOSURE GUIDELINES LEVELS

Sulfur Mustard (CAS NO.505-60-2)

AEGL-1				
10 min	30 min	1 h	4 h	8 h
0.40 mg/m^3 (0.06 ppm)	0.13 mg/m^3 (0.02 ppm)	0.067 mg/m^3 (0.01 ppm)	0.017 mg/m^3 (0.003 ppm)	0.008 mg/m^3 (0.001 ppm)
Key Reference: Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India)				
Test species/strain/gender/number: 3-4 human volunteers				
Exposure route/concentrations/durations: Vapor exposure to varying concentrations (1.7 – 15.6 mg/m^3) for varying durations (2-33 min)				
Effects: Mild ocular effects (mild injection to notable conjunctivitis)				
End point/concentration/rationale: Concentration-time threshold of 12 mg·min/m^3 for ocular effects (conjunctival injection with minor discomfort and no functional decrement.				
Uncertainty factors/rationale: Interspecies: 1 (human subjects) Intraspecies: A factor of 3 was applies for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that the response will not vary greatly among individuals. Furthermore, little variability was observed in the tested subjects regarding ocular responses.				
Modifying factor: None applied				
Animal to human dosimetric adjustment: Not applicable				
Time scaling: $C^n \times t = k$, where $n = 1$ based on analysis of available human exposure data for ocular effects.				
Data adequacy: The key study was conducted using human volunteers thus avoiding uncertainties associated with animal studies. Ocular irritation is considered the most sensitive end point for assessing the effects of acute exposure to sulfur mustard and the available data were sufficient for developing AEGL-1 values.				

AEGL-2				
10 min	30 min	1 h	4 h	8 h
0.60 mg/m^3 (0.09 ppm)	0.20 mg/m^3 (0.03 ppm)	0.10 mg/m^3 (0.02 ppm)	0.025 mg/m^3 (0.004 ppm)	0.013 mg/m^3 (0.002 ppm)
Key Reference: Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India)				
Test species/strain/gender/number: 3-4 human volunteers				
Exposure route/concentrations/durations: Vapor exposure to varying concentrations (1.7 – 15.6 mg/m^3) for varying durations (2-33 min)				
Effects: Ocular effects ranging from mild injection to notable conjunctivitis, photophobia, lacrimation, blepharospasm				
End point/concentration/rationale: Exposure-concentration time product of 60 mg·min/m^3 representing exposure at which ocular irritation (Well marked, generalized conjunctivitis, edema, photophobia, and irritation) will occur resulting in performance decrement and necessitating medical treatment.				
Uncertainty factors/rationale: Interspecies: 1 (human subjects) Intraspecies: A factor of 3 was applies for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that the response will not vary greatly among individuals. Furthermore, little variability was observed in the tested subjects regarding ocular responses.				
Modifying factor: A modifying factor of 3 was applied to accommodate uncertainties regarding the onset of potential long-term ocular effects of respiratory effects.				
Animal to human dosimetric adjustment: Not applicable				
Time scaling: $C^n \times t = k$, where $n = 1$ based on analysis of available human exposure data for ocular effects.				
Data adequacy: The key study was conducted using human volunteers thus avoiding uncertainties associated with animal studies. The AEGL-2 values are based on ocular effects that may be considered severe enough to impair vision. The data were considered sufficient for developing AEGL-2 values.				

AEGL-3				
10 min	30 min	1 h	4 h	8 h
3.9 mg/m^3 (0.59 ppm)	2.7 mg/m^3 (0.41 ppm)	2.1 mg/m^3 (0.32 ppm)	0.053 mg/m^3 (0.08 ppm)	0.27 mg/m^3 (0.04 ppm)
Key Reference: Kumar, O., and R. Vijayaraghavan. 1998. Effect of sulfur mustard inhalation exposure on some urinary variables in mice. J. Appl. Toxicol. 18:257-259.				
Test species/strain/gender/number: Swiss mice/femal/4 per exposure group.				
Exposure route/concentrations/durations: Head-only inhalation exposure for 1 h to sulfur mustard (>99% purity) at 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0, and 2.0LC_{50}). Subjects were sacrificed at 6, 24, or 48 h or 7 d after exposure. Three groups of 10 mice were exposed at each concentration and observed for up to 14 d.				
Effects: Lethality assessed up to 14 d post exposure				
End point/concentration/rationale: No mortality in mice at 14 d following 10h exposure at 21.2 mg/m^3. The exposure was considered an estimate of the lethality threshold in mice.				
Uncertainty factors/rationale: Total uncertainty factor: 10 Interspecies: A factor of 3 was applied to account for possible interspecies variability in the lethal response to sulfur mustard. Application of any additional uncertainty factors or modifying factors was not warranted because the AEGL-3 values are equivalent to exposures in humans that are known to produce only ocular and respiratory tract irritation. Intraspecies: Intraspecies variability was limited to 3 because lethality appears to be a function of extreme pulmonary damage resulting from direct contact of the agent with epithelial surfaces.				
Modifying factor: No modifying factor was applied because the basis of lethality estimate was from a study utilizing a 14-d observation period to assess the lethal response from a 1-h exposure.				
Animal to human dosimetric adjustment: Insufficient data				
Time scaling: $C^n \times t = k$, where $n = 1$ or 3. The concentration-time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). In the absence of chemical-specific lethality data, time scaling was performed using exponential extrapolation ($n = 3$) for shorter time periods and linear extrapolation ($n = 1$) for longer time periods, thereby providing a somewhat more conservative (i.e., protective) estimate of the AEGL-3 values than would be obtained using an n value of 1 based on ocular irritation.				
Data adequacy: Uncertainties exist regarding a definitive lethality threshold for single acute exposures to sulfur mustard. However, the key study appeared to be well-designed and properly conducted and is considered sufficient for developing AEGL-3 values.				