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

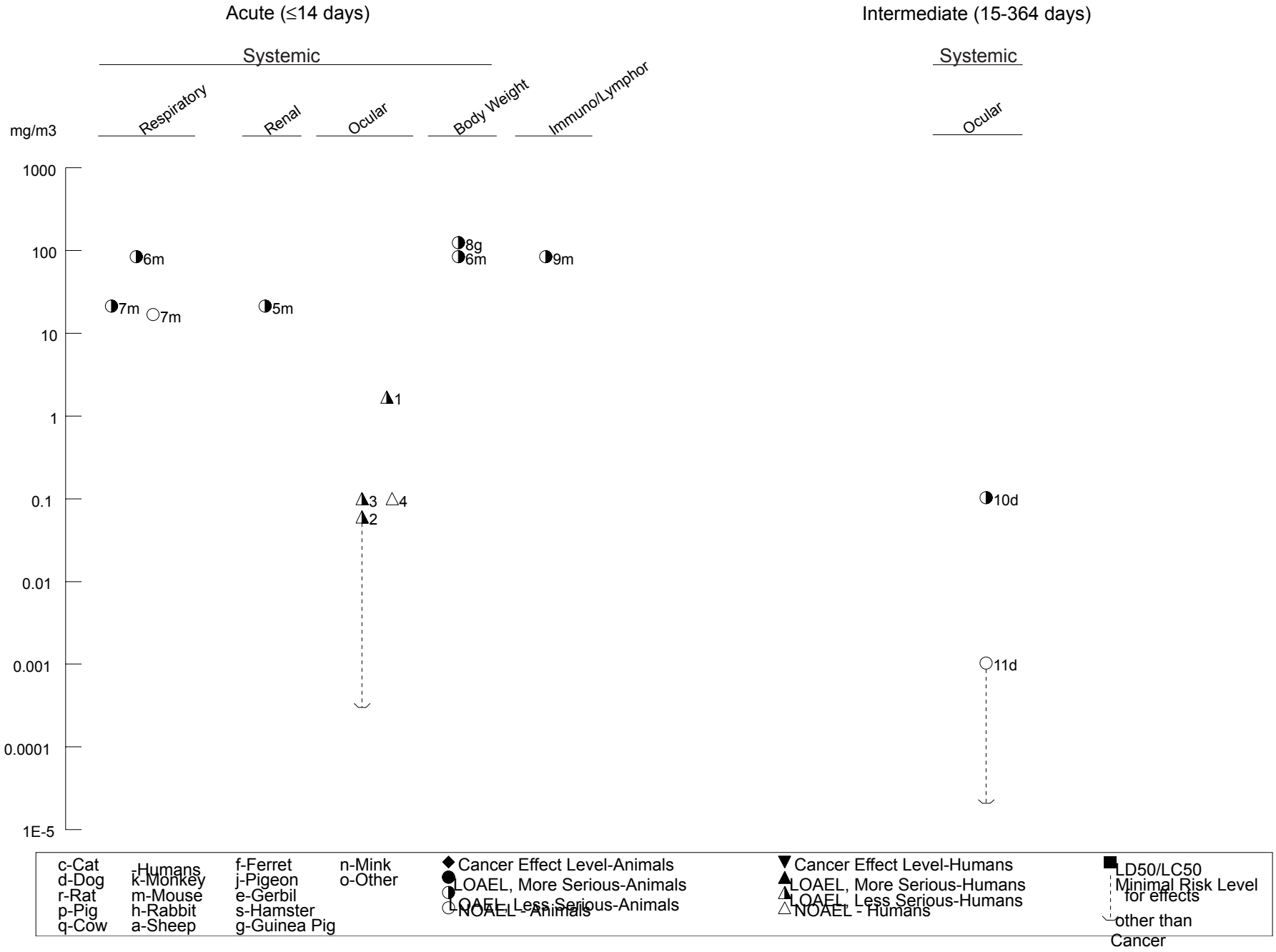
a The number corresponds to entries in Figure 3-1.

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)

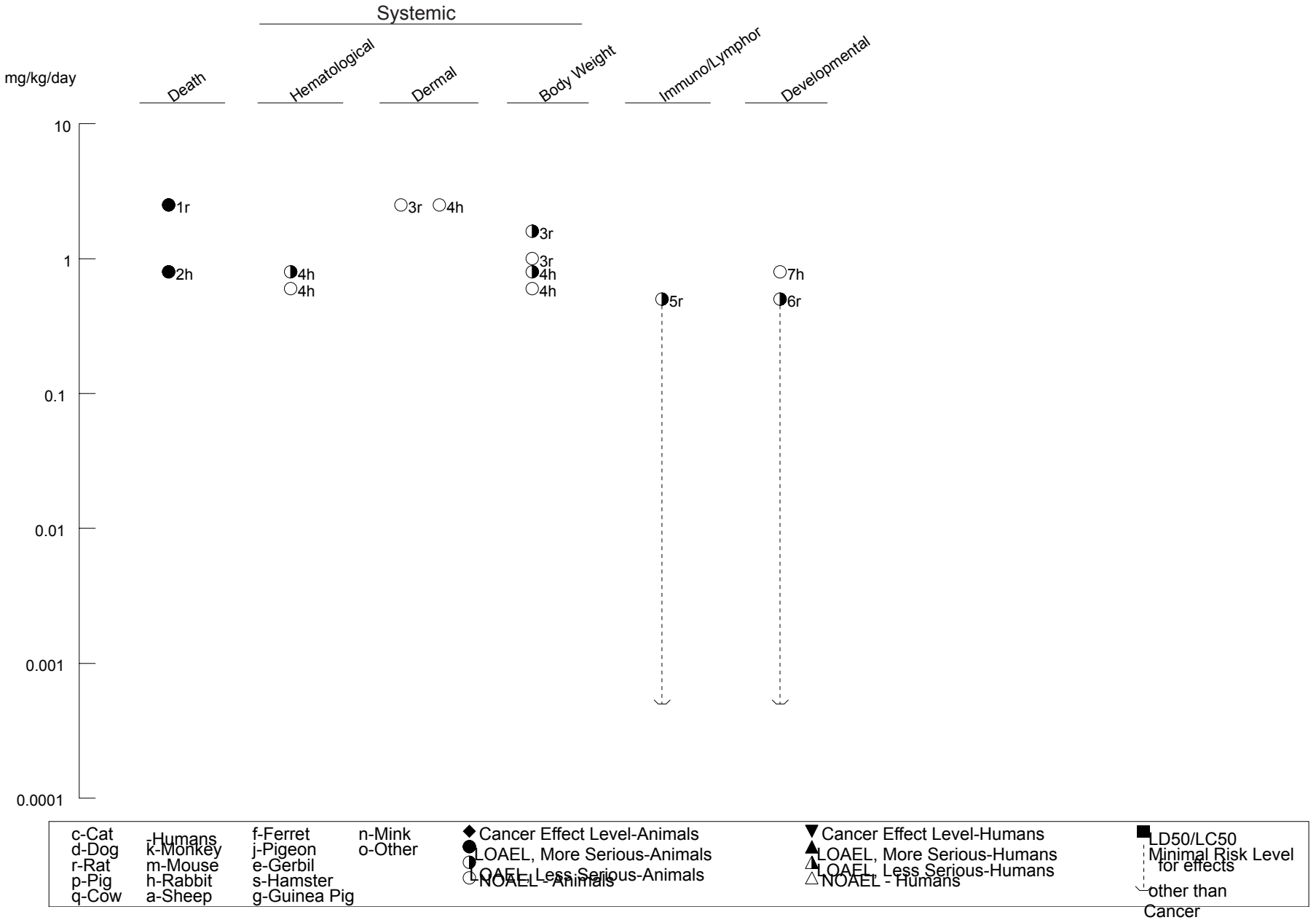
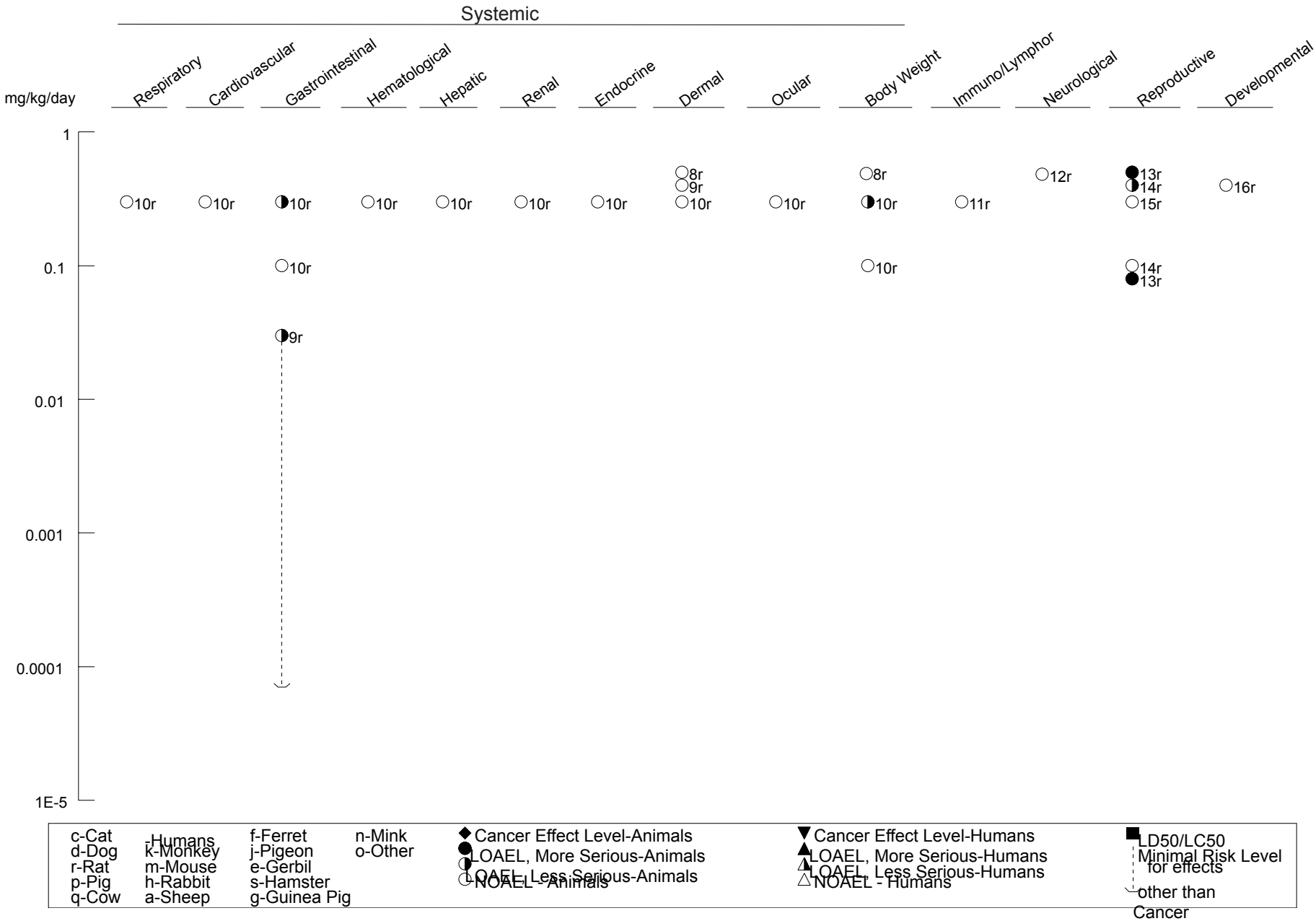


Figure 3-2. Levels of Significant Exposure to Sulfur Mustard - Oral (*Continued*)

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.

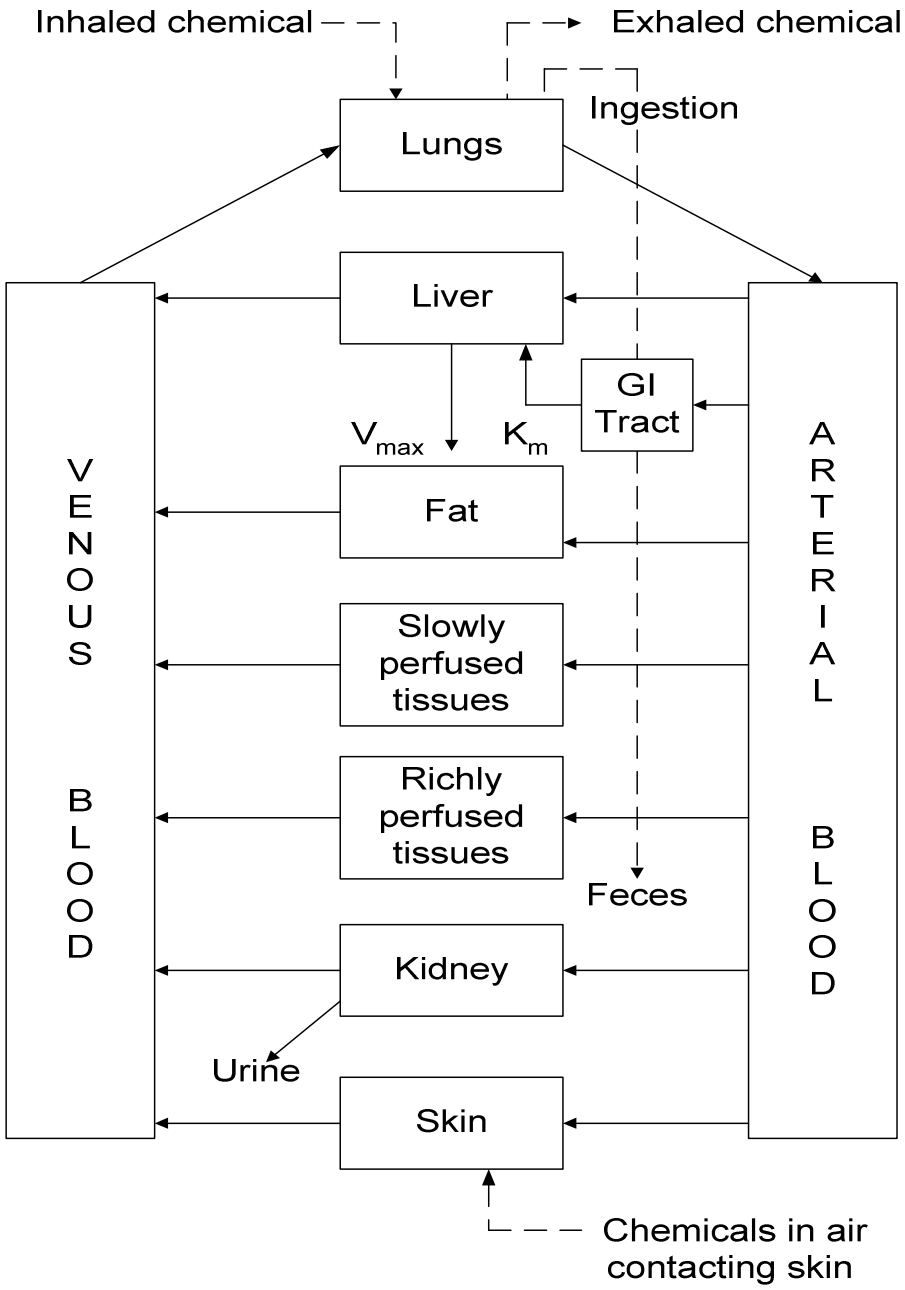
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically 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

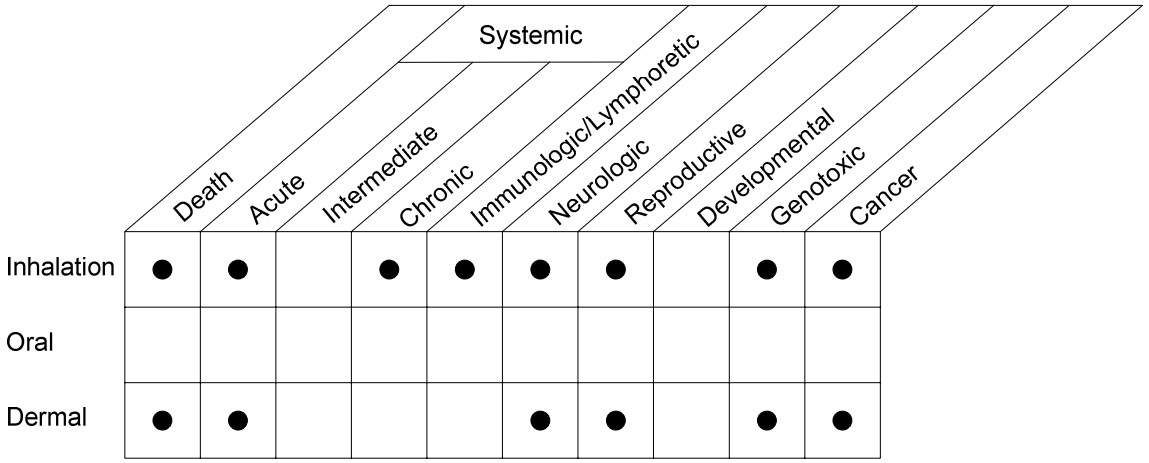
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to sulfur mustard is summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of sulfur mustard Each dot in the figure indicates that 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.

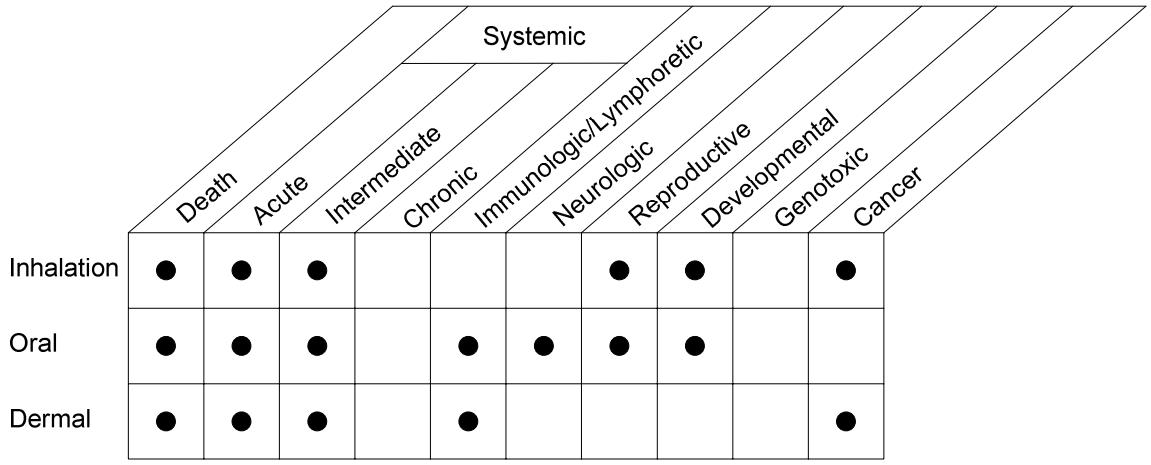
3.12.2 Identification of Data Needs

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