

# Toxicity of Vesicant Agents Scheduled for Destruction by the Chemical Stockpile Disposal Program

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The vesicant agents of the unitary chemical munitions stockpile include various formulations of sulfur mustard [*bis*-(2-chloroethyl) sulfide; agents H, HD, and HT] and small quantities of the organic arsenical Lewisite [dichloro(2-chlorovinyl) arsine; agent L]. These agents can be dispersed in liquid, aerosol, or vapor form and are capable of producing severe chemical burns upon direct contact with tissue. Moist tissues such as the eyes, respiratory tract, and axillary areas are particularly affected. Available data summarizing acute dose response in humans and laboratory animals are summarized. Vesicant agents are also capable of generating delayed effects such as chronic bronchitis, carcinogenesis, or keratitis/keratopathy of the eye under appropriate conditions of exposure and dose. These effects may not become manifest until years following exposure. Risk analysis derived from carcinogenesis data indicates that sulfur mustard possesses a carcinogenic potency similar to that of benzo[*a*]pyrene. Because mustard agents are alkylating compounds, they destroy individual cells by reaction with cellular proteins, enzymes, RNA, and DNA. Once begun, tissue reaction is irreversible. Mustard agents are mutagenic; data for cellular and laboratory animal assays are presented. Reproductive effects have not been demonstrated in the offspring of laboratory rats. Acute Lewisite exposure has been implicated in cases of Bowen's disease, an intraepidermal squamous cell carcinoma. Lewisite is not known to generate reproductive or teratogenic effects.

## Introduction

This paper is the second in a journal series of review articles synthesizing pertinent toxicological data on chemical warfare agents scheduled for destruction by the Department of the Army (DoA) in the 1990s. The first paper in this series (1) summarized recommended treatment/decontamination protocols for the organophosphate nerve agents VX [S-(diisopropylaminoethyl)methylphosphonothiolate, *o*-ethyl ester], GA [Tabun; *N,N*-dimethylphosphoroamidocyanidate, ethyl ester], and GB [Sarin; methylphosphonofluoridate, isopropyl ester] as well as the vesicant (blister) agents H, HD, HT [various forms of sulfur mustard, *bis*(2-chloroethyl)sulfide], and Lewisite [an organic arsenical, dichloro (2-chlorovinyl)-arsine]. The current paper focuses on the acute and delayed toxicity of vesicant agents, some of which are known carcinogens.

The Department of Defense Authorization Act of 1986 (PL 99-145) directed and authorized the Secretary of Defense to destroy the United States' stockpile of lethal unitary chemical munitions and agents by September 30, 1994. The Act was amended in 1988 to permit operations

testing of a commercial-scale incinerator design at Johnston Island in the South Pacific and to allow for unitary munition disposal completion by April 30, 1997. (The current target completion date is 1999.) Chemical and physical properties of these agents are detailed in Carnes (2) and summarized in Table 1. All but approximately 6% of the U.S. stockpile of unitary munitions and bulk agent is currently stored at eight separate locations in the continental U.S. as bombs, cartridges, mines, projectiles, spray tanks, and ton containers (Fig. 1). The remainder is either stored on Johnston Island or was transported from a military site near Clausen, Germany, to Johnston Island in 1990. The Department of the Army's current method of choice for agent destruction is high-temperature (1130°–1400°C) incineration (4).

The process of "reverse assembly" and munition disposal that precedes agent incineration is thoroughly addressed in the final programmatic environmental impact statement [FPEIS (3)] commissioned by the Chemical Stockpile Disposal Program (CSDP) activity of the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA). The process is also summarized in recent papers by Carnes (2) and Carnes and Watson (5). The analysis contained within the FPEIS led to the February, 1988, decision by then-Undersecretary of the Army, James R. Ambrose, to proceed with on-site incineration disposal pending completion of site-specific analyses.

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**Table 1. Physical and chemical characteristics of vesicant agents.**

Agent	Common name	CAS no. <sup>a</sup>	Chemical name	Chemical formula	Vapor pressure at 25°C	Liquid density at 25°C	Freezing point	Color
H	Mustard	505-60-2	<i>bis</i> (2-Chloroethyl) sulfide	C <sub>4</sub> H <sub>8</sub> Cl <sub>2</sub> S	0.08 mm Hg <sup>b</sup>	1.27 g/cm <sup>3</sup>	8–12°C	Amber to dark brown
HD	Mustard (distilled)	505-60-2	<i>bis</i> (2-Chloroethyl) sulfide	C <sub>4</sub> H <sub>8</sub> Cl <sub>2</sub> S	0.11 mm Hg	1.27 g/cm <sup>3</sup>	14°C	Clear to pale yellow to black
HT	Mustard		60% HD and 40% T <sup>c</sup>	T = C <sub>8</sub> H <sub>16</sub> Cl <sub>2</sub> OS <sub>2</sub>	0.104 mm HG	1.27 g/cm <sup>3</sup>	1°C	Amber to dark brown
L	Lewisite	541-25-3	Dichloro(2-chloro-vinyl)arsine	C <sub>2</sub> H <sub>2</sub> AsCl <sub>3</sub>	0.58 mm Hg	1.89 g/cm <sup>3</sup> <sup>d</sup>	-18°C <sup>e</sup>	Amber to dark brown to black

<sup>a</sup>Chemical Abstracts Service number.

<sup>b</sup>At 20°C.

<sup>c</sup>Agent T is *bis*(2-chloroethylthio)ethyl ester; it is CAS no. 63918-89-8.

<sup>d</sup>Varies with purity of sample.

<sup>e</sup>Varies ± 0.1°C, depending on purity and isomers present.

The evaluation of vesicant toxicity data that follows is intended to provide background and insight to host community emergency planners and health professionals as they prepare for the advent of the disposal program as well as access to previously unavailable “grey literature” for members of the international community interested in vesicant control and disposal. Recent world events, such as the reunification of Germany and the Gulf War, have raised concerns regarding public access to former military sites

where chemical munitions were manufactured, stored, or armed. The current review will provide information relevant to any decommissioning decisions involving vesicants.

## Vesicant Agent Characteristics

The active ingredient in H and HD and a major component (60%) of HT is the same chemical compound, *bis*(2-

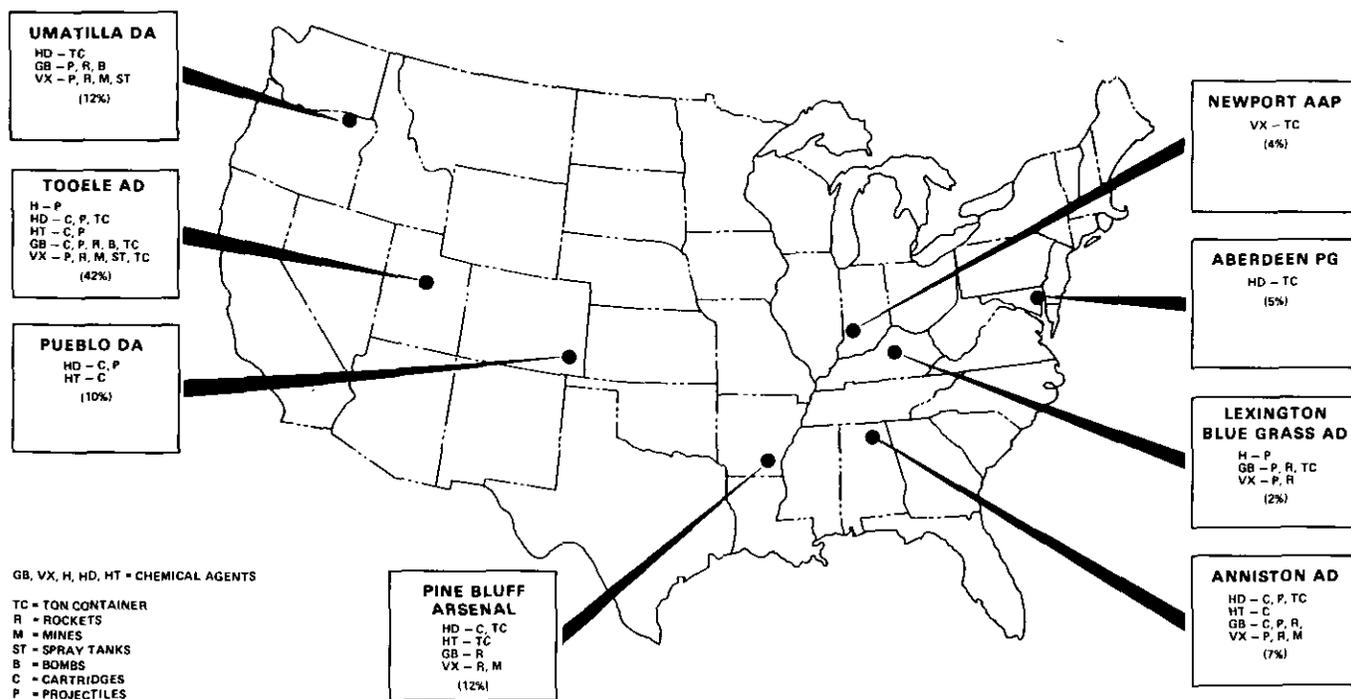


FIGURE 1. Distribution of the unitary chemical weapons stockpile throughout the continental United States [small quantities of GA and Lewisite are also stored at Tooele Army Depot (3)].

chloroethyl)sulfide (CAS no. 505-60-2; Table 1). Many names, such as mustard gas, sulfur mustard, mustard, yperite, etc., have been applied to this agent. Throughout this analysis we will use the terms mustard or mustard agent as a synonym for sulfur mustard agents. Nitrogen mustard is a different chemical agent and is not part of the unitary stockpile scheduled for disposal. The term "mustard gas" is a misnomer because the chemical is a liquid at ordinary environmental temperatures. The chemical warfare agent H is an agent containing 70% sulfur mustard plus 30% sulfur impurities and is manufactured by the unstable Levinstein process. The chemical warfare agent HD is purified sulfur mustard from which impurities have been removed by distillation and washing. Agents H and HD will not be considered separately in the ensuing toxicological discussion. Some physical properties of sulfur mustard are listed in Table 1.

Mustard has an odor like garlic. It has significant volatility at ordinary temperatures, so that mustard vapor would be in the air immediately surrounding droplets of liquid mustard. Thus, the hazard of human contact is not only with droplets of liquid agent, but also with agent vapors. Because of its low aqueous solubility, mustard agent is very persistent in the environment.

HT is a product of a reaction that yields about 60% HD (described above), <40% T (*bis*[2-(2-chloroethylthio)ethyl]ether; CAS no. 63918-89-8), plus a variety of sulfur contaminants and impurities. It is very similar in appearance and biological activity to H/HD, but it possesses greater toxicity and stability due to the presence of T, which lowers the freezing point and adds toxic properties to the mixture. Thus, HT is considered to be more active than H/HD. Agent HT is also liquid at room temperature but is soluble only in organic solvents. Its poor water solubility makes it a persistent contaminant of soils and surfaces other than rubber, which it readily permeates. Hydrolysis in water occurs only after prolonged boiling, while caustic alkalis hydrolyze HT readily. See Table 1 for a more complete physical description of HT.

Lewisite (Agent L) is an arsenical vesicant (dichloro[2-chlorovinyl]arsine); CAS no. 541-25-3 (Table 1). This agent is approximately 10 times more volatile than HD and can be used as a "moderate irritant" vapor over great distances. Lewisite is liquid at room temperature and is only slightly soluble in water. It is considered of intermediate persistency in soils because of its low water solubility (6). It decomposes upon application of heat and may degrade considerably upon shell detonation; it is reasonably stable when stored free of water contamination (6).

## Acute Toxicity

Mustard agents are much less potent than nerve agents under comparable conditions of exposure. The human skin LD<sub>50</sub> for VX, an organophosphate nerve agent, is 0.04 mg/kg; the comparable dose for H/HD is 100 mg/kg, or a 2500-fold difference (6, 7). Available hospital records from World War I and sketchy casualty reports from the recent Iran-Iraq conflict indicate mortality rates of 1-3% from acute sulfur mustard exposure (8,9). Actual battlefield con-

centrations to which victims were exposed have not been reported but may well have been in excess of 1500 mg-min/m<sup>3</sup>, the sulfur mustard LC<sub>50</sub> for unprotected adult personnel (6). Exposure estimates from a World War II Japanese poison gas factory have suggested that mustard air concentrations between 50 and 70 mg/m<sup>3</sup> are acutely irritating and can produce most of the acute signs of mustard poisoning (10).

Warfare use of vesicants decreases the opponents' ability to fight by producing chemical burns on tissues that come into contact with either vapors or liquid droplets/aerosols. Exposed skin surfaces, eyes, nose, throat, bronchial, and upper gastrointestinal tract are all at risk. The moist surfaces of perspiring skin, conjunctiva of the eye, airway mucosa or mucous membranes preferentially absorb mustard agent and distribute it over a larger area. Thus, the unprotected eye is considered the most sensitive organ to the action of H-agents, and ambient temperature/humidity govern the degree of "casualty effect." Under hot, humid conditions when large areas of skin are likely to be wet with perspiration, much lower mustard concentrations generate debilitating effects (Table 2).

After initial tissue damage, various debilitating effects follow, such as the development of large, painful blisters that arise on exposed skin. An individual exposed to blistering concentrations of agent is incapacitated, often for weeks, before returning to normal activity. A description of acute clinical signs produced by individual vesicants follows.

## Agents H/HD

**Mode of Action.** In terms of chemical reactivity, sulfur mustard is a classic alkylating agent and readily reacts with components of DNA, RNA, and proteins (19,25-28). The chemical modifications that various biological molecules undergo through alkylation can result in severe disorganization of their normal biological function. Mustard is considered a cell poison (29) and is particularly toxic to mitotic cells. Cytostasis, mutation, and slow cell death can also result (30). It was known from early biological studies of mustard that this agent produced many cytological abnormalities (31). Because of the similarity of cellular lesions produced by mustard and X-rays, the mustards and other similarly acting chemicals are sometimes termed "radiomimetic," that is, imitating the effect of radiation (32,33). Thus, intestinal epithelial damage leading to diarrhea, depression of proliferation of white cell precursors in the bone marrow leading to depressed white blood cell counts, and injury to respiratory epithelium can all be features of mustard poisoning, as they are of radiation injury (30). The skin epithelium is an important target because of its proliferating basal cell layer (30). It should not be concluded, however, that mustard is only effective against proliferating cells; at sufficient concentration, it produces cellular necrosis in any exposed cells (26,30).

From a biochemical point of view, it would be important to know the exact molecular sites that mustard attacks, as this knowledge might allow development of rational therapy at the molecular level. Unfortunately, the problem

**Table 2. Acute toxic effects of vesicant agents H/HD, HT, Lewisite, and T.**

Exposure route	H/HD	HT	Lewisite	T	References
<b>Inhalation LC<sub>50</sub>, mg-min/m<sup>3</sup></b>					
Human (estimated)	1,500 <sup>a</sup>		1,200–1,500	~400	U.S. DoA (6); Robinson (11)
Monkey	800				U.S. DoA (6)
Goat	1,900				U.S. DoA (6)
Dog	600	100–200			U.S. DoA (6); Robinson (11)
Cat	700				U.S. DoA (6); Robinson (11)
Rabbit	1,025	3,000–6,000			U.S. DoA (6)
Guinea pig	1,700	3,000–6,000			U.S. DoA (6)
Rat	800–1,512 <sup>b</sup>		1,500 (9 min)		U.S. DoA (6,12)
Mouse	860–1,380 <sup>b</sup>	1,100 (10 days) 820 (15 days)	150 (10 min)		U.S. DoA (6,12) U.S. DoA (6)
<b>Inhalation LC<sub>50</sub>, mg-min/m<sup>3</sup> (head exposed, body protected)</b>					
Mouse			1,400–1,600 (10 min)		U.S. DoA (6)
<b>Percutaneous LC<sub>50</sub> (vapor), mg-min/m<sup>3</sup> (head protected, body exposed)</b>					
Human (estimated)	10,000		100,000		U.S. DoA (6)
Monkey	13,000				U.S. DoA (6)
Dog	7,700		40,000 (10 min) 30,000 (30–60 min)		U.S. DoA (6) U.S. DoA (6)
Cat	8,700		30,000 (30–45 min)		U.S. DoA (6)
Rabbit	5,000		15,000 (10 min)		U.S. DoA (6)
Guinea pig	~20,000		20,000–25,000 (10–40 min)		U.S. DoA (6)
Rat	~3,000		20,000 (9–25 min)		U.S. DoA (6)
Mouse	3,400		300–7,000 (10 min)		U.S. DoA (6)
<b>Percutaneous LD<sub>50</sub> (liquid), mg/kg body weight (head protected, body exposed)</b>					
Mouse			15		U.S. DoA (6)
Rat			15–24		U.S. DoA (6)
Rabbit			5–6		U.S. DoA (6)
Guinea pig			12		U.S. DoA (6)
Dog			~70,38		U.S. DoA (6)
Goat			10–24		U.S. DoA (6)
<b>Percutaneous + inhalation LC<sub>50</sub>, mg-min/m<sup>3</sup> (no protection)</b>					
Mouse			900–2500 (10 min) 500 (9–14 min)		U.S. DoA (6) U.S. DoA (6)
Rat			1,500 (9–25 min) 580 (60–180 min)		U.S. DoA (6) U.S. DoA (6)
Guinea pig			20,000 1,000 (9–14 min)		U.S. DoA (12) U.S. DoA (6)
Rabbit			470 (60–180 min) 1,200 (7.5–13 min)		U.S. DoA (6) U.S. DoA (6)
Goat			1,500 (60–310 min)		U.S. DoA (6)
Dog			1,250 (100–255 min) 1,400 (7.5–15 min)		U.S. DoA (6) U.S. DoA (6)
<b>Skin LD<sub>50</sub>, mg/kg body weight (applied as liquid)</b>					
Human (estimated)	100				U.S. DoA (6)
Farm animals (unspecified)			10,15		NRDC (13); U.S. DoA (12)
Dog			15,38		Cameron et al. (14), U.S. DoA (12)
Rabbit	100		4,6		U.S. DoA (6), Danielli et al. (15), Cameron et al. (14)
Guinea pig			12		NRDC (13)
Rat	9,18		15–24		Young (16), U.S. DoA (6,12), Cameron et al. (14)
Mouse	15 (96-hr mortality) 194 (24-hr mortality)				Vojvodic et al. (17) Vojvodic et al. (17)
Goat	92		15		U.S. DoA (6), NRDC (13)
			15		Cameron et al. (14)
<b>Intravenous LD<sub>50</sub>, mg/kg body weight</b>					
Dog	0.2		2.0		U.S. DoA (6); NRDC (13)
Rabbit	~1.1–4.5		0.5, 2.0		U.S. DoA (6); Cameron et al. (14)
Rat	0.7–3.3				Anslow et al. (18)
Mouse	3.3, 8.6				Anslow et al. (18)
<b>Oral LD<sub>50</sub>, mg/kg body weight</b>					
Rat	17		50		U.S. DoA (6,12)

*continued*

Table 2. Continued.

Exposure route	H/HD	HT	Lewisite	T	References
Incapacitating dose, IC <sub>50</sub> , mg-min/m <sup>3</sup>					
Human, percutaneous (masked)	2,000 (70–80°F) <sup>c</sup> –1,000 (90°F)	None established	>1,500		U.S. DoA (6); McNamara et al. (19)
Human, eyes	200	None established	<300		U.S. DoA (6)
Minimum effective dose, ED					
Human skin (irritation)			95 µg/man		NRDC (13)
Human skin (blisters)	50 mg-min/m <sup>3d</sup>	~3.5 mg/man 4 mg/man	~200 mg-min/m <sup>3</sup> (30 min)	4 mg/man	U.S. DoA (6); Robinson (11)
Human eyes (marginal)	12–70 mg-min/m <sup>3</sup>				U.S. DoA (6)
Conjunctivitis	30 mg-min/m <sup>3</sup> (60 min)				Dahl et al. (20)
Reddening, no incapacitation	70 mg-min/m <sup>3</sup>				U.S. DoA (6)
Reddening, mild incapacitation	90 mg-min/m <sup>3</sup>				U.S. DoA (6)
Rabbit skin			~25 mg-min/m <sup>3</sup> (30 min)		U.S. DoA (6)
Rabbit eyes		Similar to HD	~1 mg-min/m <sup>3</sup> (30 min)		U.S. DoA (6)
Dog skin			~50 mg-min/m <sup>3</sup> (30 min)		U.S. DoA (6)
Dog eyes			~20 mg-min/m <sup>3</sup> (30 min)		U.S. DoA (6)
No effect dose, mg/min/m <sup>3</sup>					
Human eyes	<12				McNamara et al. (19)
Human (estimated)	2(≥90°F)				McNamara et al. (19)
Severe systemic effects, mg/kg body weight					
Human skin (estimated)			13.4 8.1		Windholz et al. (21) Sollman (22)
Inhalation, lowest lethal dose, mg/m <sup>3</sup>					
Human	150 (10 min) 70 (30 min)		48 (30 min)		Back et al. (23) Inada et al. (10)
Skin absorption, lethal, mg/kg body weight					
Human	64		37.6 53.7		WHO (24); Sollman (22) Windholz et al. (21)

<sup>a</sup>Because HD exposures are cumulative, the lethal dose is not changed with variations in time of exposure, within reasonable limits.

<sup>b</sup>Ranges of LC<sub>50</sub> values are summarized for all exposure times reported.

<sup>c</sup>Incapacitating dose varies significantly with amount of perspiration on skin surface, which is in turn dependent on ambient temperature and humidity levels.

<sup>d</sup>Mild to moderate erythema is produced at ambient temperature of 90°F.

becomes very complex because such a wide variety of cellular targets are available for reaction with mustard. For instance, cross-linking damage to DNA can account for many of the deleterious cellular effects (26,32,33). Other cellular macromolecules are also susceptible to mustard attack. Levy (34) found evidence of cell membrane modification by doses of mustard too small to effectively alter DNA. Certain enzymes, notably hexokinase, were inhibited when incubated *in vitro* with very low doses of mustard, although the majority of enzymes so tested were not affected (25). An interesting biochemical hypothesis to account for the generation of mustard-induced skin lesions has been proposed by workers at the U.S. Army Medical Research Institute of Chemical Defense (27,32). This hypothesis links initial chemical binding of mustard to DNA through a complex series of steps to release intracellular enzymes that are responsible for the skin damage and blistering produced by the agent.

Briefly, the sequence of steps envisioned is that the initial DNA damage produced by mustard results in

activation of a repair enzyme that uses NAD<sup>+</sup> as a cofactor. If the damage to DNA is extensive enough, the enzyme's activity could result in depletion of cellular NAD<sup>+</sup>, thus inhibiting glycolysis. This in turn could result in stimulation of the hexose monophosphate shunt, which has been found to be associated with enhanced synthesis and release of proteases. These proteases are hypothesized to be the immediate cause of the skin damage associated with mustard exposure. It should be emphasized that this complex sequence is a theoretical construct: certain segments of it have been verified by experimentation, but whether it serves to explain what happens in mustard-damaged skin is unknown.

Because H contains less active ingredients than HD (see Table 1), it is expected to have a slightly less blistering effect than HD (35) but to otherwise have the same biological properties. Acute effects (8,29,36) after exposure to toxicologically active concentrations (50 to >100 mg-min/m<sup>3</sup>) of mustard can be characterized by the following: a) a latency period of several hours before chemical burns

become manifest (at high concentrations, immediate irritation may be produced). Because agent contact does not produce immediate symptoms, exposed individuals often do not promptly decontaminate or request medical assistance; *b*) inflamed and painful eyes, swollen eyelids, and temporary blindness. The eye is affected at lower vapor concentrations than any other tissue; *c*) a variety of dose-dependent effects on the respiratory tract, including throat discomfort, continuous hoarse coughing, nasal discharges, copious mucus production, and bronchial inflammation. Secondary infection and potentially fatal bronchopneumonia can result; *d*) general irritation of the skin, first manifested as an itching rash, which then develops (at higher exposures) into large, painful blisters that may require weeks to heal; *e*) at high doses, mustard can depress immune system response and render the exposed individual more susceptible to infections; and *f*) acute lethality of 1–3%.

Table 2 summarizes available information regarding the acute toxic signs generated by certain doses of HD. The various lethal doses listed for humans are estimates based upon extrapolations from other species. As previously described, even under wartime exposure conditions, HD is not a notably lethal agent. Significant human exposures to HD during operations of the Chemical Stockpile Disposal Program are conceivable during a major, unplanned release, such as a plane crash into a storage bunker (37). These events are not very probable and have been calculated to occur with a probability approximating  $1 \times 10^{-4}$  or less over the life of the disposal program for the entire stockpile (37). Exposures expected under such circumstances could occur by inhalation of HD vapors or skin contact with vapors or liquid. Any form of percutaneous dose summarized in Table 2 is probably not comparable to expected exposures during normal stockpile disposal operations. Dermal exposure to liquid HD droplets (e.g., see skin  $LD_{50}$  values) for the general public is also unlikely since a low-probability explosive release would be required to generate skin doses to off-site populations (5).

Intravenous  $LD_{50}$  values indicate that among-species variation spans an approximate 40-fold range. For rabbits and rats, intravenous  $LD_{50}$  values range between 1 and 4 mg/kg body weight. Mice seem somewhat more resistant than rabbits and rats, while dogs are more sensitive.

From inspection of Table 2, it can be noted that the inhalation  $LCt_{50}$  dose approximates 1000–1500 mg-min/m<sup>3</sup> and is similar for a number of animal species. The human  $LCt_{50}$  estimate is extrapolated from animal toxicity data. Because the effects of HD are cumulative (i.e., very limited detoxification), the lethal dose is not significantly changed, within reasonable limits, with variations in exposure time (6). What these "reasonable" limits are (minutes or hours) was not stated. McNamara and his colleagues (19) note that the effect of the same HD dose is reduced when given over a longer period of time; this finding suggests that some degree of biological detoxification takes place. The lowest reported lethal doses for H/HD in humans are 150 mg/m<sup>3</sup> for 10 min (23) and 70 mg/m<sup>3</sup> for 30 min (10) (Table 2).

Two types of lethal dermal exposures to HD are denoted

in Table 2. In the first, experimental animals were exposed in a special chamber in which only the body was in contact with HD vapor or liquid, while the head remained outside the chamber (i.e., no inhalation exposure). It seems possible that, because of inherently low HD volatility, condensation might occur in the vapor exposure experiments. At least some of the total dose may have been received as a liquid (droplet) application upon skin. In the second type of dermal exposure tested, data for direct skin application (with no protection from inhalation exposure) of liquid agent are presented. In all species for which data are available for this comparison, the  $LD_{50}$  dose (on a milligram per kilogram basis) for direct skin application of liquid agent is an approximate order of magnitude larger than that for intravenous injection. This result would be expected because HD would react with the outer layers of skin cells, leaving only a fraction of the total applied dose to reach capillaries and be absorbed systemically.

Clinical signs (8,29,33,36) associated with nonlethal HD poisoning are described below. The following observations are not definitive with regard to timing of manifestations, etc., and should be considered representative of the constellation of effects that can arise. The biological activity of mustard agent is characterized by a latent period followed by severe inflammation, blistering, and local necrosis (cell death). Medema (29) states that there is an observed effect within 1 hr of exposure only when gross contact with liquid agent occurs.

Toxicological effects are local at the point of agent contact with skin, eyes, or respiratory tract. The first effect to manifest itself is usually eye irritation (watering, reddening, pain, swelling of the eyelids, etc.), taking place 2–3 hr following exposure. In the period 4–16 hr after exposure, eye effects become more severe, nasal discharge occurs, nausea and vomiting may begin and recur for several hours, and diarrhea may develop. Skin rashes also begin to manifest themselves at this time. Twenty-four hours after exposure, eyes can be swollen almost shut and very painful, exposed skin is swollen and reddened, there is hoarse coughing, and the throat may be raw and irritated. During the next 24 hr, skin erythema may progress into (sometimes large) blisters, and eye irritation begins to subside (although inflammation persists for several days). In cases of severe exposure, damage to the respiratory tract becomes evident at this time; expectoration is copious, with mucus and occasional sloughing of tracheal mucosa. Secondary infection of the respiratory tract can occur (e.g., bronchopneumonia), with attendant fever. In less severe exposures, involvement of the respiratory tract may be manifested by any or all of the following: rhinitis, laryngitis, tracheitis, and bronchitis. From the experience of World War I soldiers exposed to mustard, damage to the throat and other portions of the upper respiratory tract presented the greatest potential for lethal consequences due to the development of secondary infection in the absence of antibiotic therapy (36,38). Recovery of individuals with respiratory damage to a state where moderate activity was possible could take from 4 to 8 weeks (38).

The severity of skin lesions experienced by exposed individuals is influenced by a number of factors indepen-

dent of the exposure concentration, including individual differences in skin sensitivity, ambient temperature, amount of sweat on the skin, etc. (6,36). With milder exposures or as the first stage of a severe exposure, skin damage takes the form of an itching erythema. With more severe exposures, erythematous areas begin to fill with fluid, and a blister arises (sometimes with a diameter of 3–4 in), reaching a maximum size in approximately 24 hr. After several days, the blisters usually break. Blisters are relatively painless for several days, but after 5–6 days the pain becomes severe upon exposure to air or on contact; sensitivity of the blistered area can persist for 2–3 weeks. Ulceration of the blister may or may not develop; in most severe burns, a blister does not develop, but the initial burn progresses to an ulcer, which may take 5–7 weeks to heal. As might be expected, mustard agent burns are susceptible to infection, and boils can develop in and around the affected area, although the fluid from the blister itself does not cause a secondary blister to develop. Any preexisting skin damage such as cuts, abrasions or sunburn would likely enhance the effect of mustard at the wound site.

Table 2 presents mustard dosages that can produce the described symptoms. Concentrations of mustard barely perceptible by odor can produce eye damage while not affecting the skin or the respiratory tract (6). Dahl and his colleagues (20) note that vapor concentrations of 0.5  $\mu\text{g}/\text{L}$  (e.g., 0.5  $\text{mg}/\text{m}^3$ ) for exposure periods of 1 hr (i.e., 30  $\text{mg}\cdot\text{min}/\text{m}^3$ ) are sufficient to produce conjunctivitis in man. Mustard agent also rapidly penetrates the cornea; 10 min is cited in Dahl et al. (20) and Geeraets et al. (39). Because of this rapid penetration and subsequent disappearance from the corneal surface, attempts to irrigate the eye must take place promptly after exposure. Compounding this difficulty is the fact that there is a latent period before eye effects begin to appear (20). Mild exposures (20–70  $\text{mg}/\text{min}/\text{m}^3$ ) to HD vapor may produce lacrimation and swelling, whereas more severe exposures (100  $\text{mg}\cdot\text{min}/\text{m}^3$ ) can produce blepharospasm, blurring of vision, edema of the conjunctiva and eyelids, iritis, and a mucous discharge (20,36,39). Pain is usually associated with these ocular effects. Following this acute phase, there occurs a gradual regeneration of damaged tissues, so that the eyes may become normal within weeks after exposure (20). After severe injury, however, heavy corneal vascularization may occur, and corneal erosion and ulceration may develop over several months (39). A later phase is mustard-induced keratitis or keratopathy, which may occur 8–40 years after exposure and result in vision loss (20,26). This point will be further discussed as a delayed effect. The no-effect dose for the eyes of 12  $\text{mg}\cdot\text{min}/\text{m}^3$  (Table 2) seems reasonably consistent with other toxicological information presented in Table 2. The incapacitating eye dose (200  $\text{mg}\cdot\text{min}/\text{m}^3$ ) (Table 2) is probably somewhat subjective, depending on the individual.

Vapor, mist, and/or liquid droplets of mustard can produce skin damage as well as eye damage. Basal cells of the epidermis are rapidly killed by the agent. Separation of the epidermis from the underlying dermis follows, and the resulting vascular permeability produces edema. Some degree of inflammatory response also occurs (27,30). Vas-

cular leakage accounts for the large blisters produced by mustard poisoning. Doses of HD that can produce various degrees of human skin damage reported by Papirmeister et al. (27) are a) 0.1–1.0  $\mu\text{g}/\text{cm}^2$  for erythema/edema, b) 1–2.5  $\mu\text{g}/\text{cm}^2$  for edema, vesication, and c) > 2.5  $\mu\text{g}/\text{cm}^2$  for central necrosis and vesication on the circumference of the necrotic area. Nagy et al. (40) estimate that 6  $\mu\text{g}/\text{cm}^2$  of mustard agent produces vesication in 50% of exposed sites. Thus, small amounts of liquid mustard applied to human skin can produce damage.

In their studies of mustard agent effects on skin of human volunteers, Nagy et al. (40) reported that there was an approximate linear relationship between the amount of mustard that penetrated the skin and the time of exposure, at least over the range of 0–30 min. The approximate rate of penetration of mustard into human skin at 21°–23°C was 1.5  $\mu\text{g}/\text{cm}^2$  skin area/min. No difference in the rate of mustard penetration between the skin of whites and blacks was observed (40). Nagy et al. (40) also compared the skin penetration of mustard at two ambient temperatures (22°C and 31°C); penetration was greater (amount of mustard penetrating at higher temperature is approximately 1.6 times that penetrating at the lower temperature) at 31°C, but no temperature-dependent difference in the ability of mustard to generate blisters was observed. As previously noted, the amount of moisture on the skin surface also has a decided effect upon the degree of damage produced by HD (6,40).

The minimal effective dose (50  $\text{mg}/\text{min}/\text{m}^3$ ) for human skin exposure (Table 2) refers to development of a mild to moderate erythema. The time to erythema appearance varies with exposure concentration and time as well as skin moisture; in general, mild skin damage appears relatively late after exposure and heals earlier than the more extensive damage from blistering concentrations (36). After healing, areas of skin affected by mustard usually undergo hyperpigmentation, may become hypersensitive to mechanical irritation, and can become permanently scarred (36). Large individual differences regarding skin damage susceptibility exist; there can be a 100-fold difference in skin reaction between a resistant and sensitive person (36). Note that the groin region (often moist) is considered 10 times more sensitive to mustard than other body regions (29). The level of skin damage that becomes incapacitating is, as with the eyes, somewhat objective; the incapacitating skin dose noted in Table 2 is 1000–2000  $\text{mg}\cdot\text{min}/\text{m}^3$  for an individual protected by a respirator.

Blewett's article (8) includes a table (reproduced here as Table 3) presenting the distribution of agent-induced injuries among body parts of World War I soldiers exposed to battlefield concentrations of sulfur mustard. Note that each of the 6980 casualties observed had an average of 3.5 separate mustard injuries. Data indicate that the regions affected in the highest percentage of victims were the eyes (86%), respiratory tract (75%), scrotum (42%), face (27%), and anus (24%). Thus, the most vulnerable areas to the vesicant action of sulfur mustard are moist body parts in general.

Acute systemic reactions to mustard are likely to occur

**Table 3. Distribution of mustard gas injuries on bodies of World War I casualties.<sup>a</sup>**

Body part	Reported injuries, %
Eyes	86.1
Respiratory tract	75.3
Scrotum	42.1
Face	26.6
Anus	23.9
Back	12.9
Armpits	12.5
Neck	12.0
Arms	11.7
Chest	11.5
Legs	11.4
Buttocks	9.8
Abdomen	6.4
Thighs	6.0
Hands	4.3
Feet	1.5

<sup>a</sup>Percentage of mustard gas injuries to various body parts in 6980 World War I casualties (8,41).

with severe exposures (i.e.,  $\geq 1000$  mg-min/m<sup>3</sup>) (6). Some of the toxic signs may include loss of appetite, malaise, nausea, vomiting, depression, and fever (6,33,35) and may appear before or concurrently with skin manifestations. Recovery from vomiting may occur within 24–36 hr, although the other manifestations may continue for longer periods (6). These and collateral reactions, such as anorexia, leukopenia, thrombocytopenia, epigastric pain, and anemia were noted in Iranian soldiers exposed to battlefield concentrations of sulfur mustard during the Iran–Iraq conflict (42). Two-year follow-up of severely exposed Iranian soldiers reported central nervous system involvement (43).

Victims of the Bari incident of World War II (in which naval personnel swam through a floating mixture of mustard agent and fuel oil in the Adriatic Sea to escape sinking ships) experienced a systemic, shocklike syndrome that was not amenable to usual medical therapy (55). In British and French warfare agent factories during World War I (1916–1918), a form of systemic poisoning was also noted in mustard workers who exhibited symptoms of listlessness, depression, headaches, indigestion, eyelid spasms, and breathlessness (38). The concentration of mustard to which these factory workers were exposed was unreported but was sufficiently high to cause worker deaths in some shell-filling plants.

From a military standpoint, one of mustard's most useful properties is its persistence (6,8,29). Droplets of the agent released, for example, in an explosive accident could deposit on numerous surfaces and slowly evaporate, thus posing a risk from agent inhalation as well as a dermal contact hazard. Indeed, this very set of conditions was observed in World War I after mustard shelling (8). One reason for sulfur mustard's persistence is its characteristic freezing at moderate temperatures (13°–15° C) (6); droplets or bulk quantities would thus be expected to remain where initially deposited during cool weather or under winter/arctic conditions. In addition, mustard agents do not readily dissolve in aqueous solution (water solubility of 0.68–0.92 g/L at 25°C for H/HD; HT is consid-

ered insoluble) (6). Thus, bulk quantities of mustard agent spilled or splashed onto soil would not degrade in a matter of days (Table 4).

Reports exist of burns to military personnel who came in contact with soil contaminated by HD 3 years previously as well as decades-long persistence of HD in military land dumps (46). In all cases of such lengthy persistence, the source was spilled or leaked mustard in bulk quantities: a) An incident at Edgewood Arsenal (now the Edgewood Area of Aberdeen Proving Ground), probably around 1921, reported by Walker et al. (47) "men digging in an area where there had been no new mustard for at least three years . . . were definitely burned. The mustard contaminated the soil due to leakage, but the total amount in the soil was not known. It was probably very great." b) Epstein et al. (48) cite a source that reported that mustard dumped at Edgewood Arsenal in 1941 was still detectable in 1971. The area involved was known to have been used as a dump for munitions for several years. c) One positive detection of HD in surface soil was reported from a closed training area at Fort McClellan in January 1973 (49). This occurred several months after last known agent presence in the area, which had been used for storage. Spills of agent had been previously reported. d) During the recent Iran–Iraq conflict, samples of air from within bomb craters 14–15 days after enemy attack contained "detectable" to 2.5 mg/m<sup>3</sup> mustard vapor concentrations, even though the craters had undergone decontamination and excess water was present (9).

Persistence of mustard sprayed on snow has been reported to range from 14 to 56 days, with little migration from the contaminated surface into the snowpack (50). Simulated snowfall (5 cm new snow) after initial HD deposition increased persistence, probably by means of reduced volatilization and dissolution (51). Observation of sulfur mustard spray degradation on various soil types (50 g/m<sup>2</sup> on "sand, cultivatable soil, uncultivable soil and gravelly soil") under ambient conditions demonstrated that sand exhibited the longest persistence (68 hr) and gravelly soil the least persistence (27 hr) (45). It is thus quite possible for heavily contaminated sites to be a source of damaging acute exposure for days or weeks after release, depending on the magnitude of the original contamination and environmental conditions.

## Agents HT/T

Agent HT is a product of one manufacturing process for making mustard. HT contains about 60% HD, < 40%

**Table 4. Persistence times ( $\tau$ , hours) predicted for HD droplets on soil under various weather conditions (45,46).<sup>a</sup>**

Temperature °C	Calm, dry, hr <sup>b,c</sup>	Windy, dry, hr	Light rain, hr <sup>d</sup>	Heavy rain, hr <sup>d</sup>
0	1530	1743	2215	1122
25	41.5	47.3	51.2	30.5

<sup>a</sup>Time required for agent to degrade to 0.033 mg/m<sup>2</sup> (i.e., 1500-fold degradation from initial concentration of 50 g/m<sup>2</sup>).

<sup>b</sup>Calm indicates wind speed <3 m/sec.

<sup>c</sup>Dry indicates rainfall intensity <0.05 mm/hr (0.047 in/day).

<sup>d</sup>Light rain indicates an intensity between 0.05 mm/hr and 0.3 mm/hr (0.28 in/day).

agent T, and a variety of sulfur contaminants and impurities. The acute effects of HD have been described above. Agent T (*bis*(2(2-chloroethylthio)ethyl)ether; see Table 1) lowers the freezing point and thereby increases stability. It also possesses significant toxic properties of its own.

Agent T has been considered a mustard with relatively weak vesicant action because of an observed delay in symptom onset (52). However, the estimated human lethal dose for inhalation ( $LC_{50}$  of 40 mg-min/m<sup>3</sup>) from exposure to T is much less than that for agent HD ( $LC_{50}$  of 1500 mg-min/m<sup>3</sup>) (11) (Table 2). Thus, agent T, far from being a mere additive, contributes considerable biological activity to the HT mixture.

Available data characterizing HT toxicity have been confined to studies performed by the U.S. Army, which are summarized in chemical agent data sheets (6). Results of animal and human acute toxicity testing are presented in Table 2. Most biological effects observed after animal exposure to HT are similar to those induced by HD, although induction following HT exposure is more rapid and/or severe. This greater activity is a result of the presence of stable agent T in the mixture; the more volatile HD dissipates and leaves a reactive blend containing a higher concentration of T.

### Agent L (Lewisite)

Lewisite [dichloro(2-chlorovinyl) arsine] is considered not only a lethal vesicant but also a systemic poison when absorbed into the bloodstream. The liver, gall bladder, and bile duct are particularly vulnerable, although damage to the kidneys and urinary tract is also possible at high skin doses (14). Experimental subcutaneous exposure in rabbits targets the liver, lung, and kidneys (53). Lewisite inhalation and ingestion severely damage the mucous membranes of the airways, mouth, stomach, and intestine (14). Like the mustard agents, Lewisite is also a cellular poison, but in a somewhat different manner. Rather than indiscriminantly destroying proteins, Lewisite directly affects cellular enzyme systems.

Lethal exposures in humans and experimental animals can occur via inhalation, skin or eye contact, or ingestion (Table 2). Mustard and Lewisite exhibit approximately equal inhalation toxicity (1200–1500 mg-min/m<sup>3</sup>); however, Lewisite is faster acting and more toxic via direct skin contact. According to some estimates, a 2-mL skin dose of liquid Lewisite to an adult (i.e., 37.6 mg/kg) can be fatal (21,22). Without treatment, death from such a dose can occur in a matter of hours. One hypothesis contends that immediate death is due to "Lewisite shock," or loss of blood plasma resulting from the increased permeability of capillaries damaged by circulating Lewisite (14). Severely burned victims of house fires, vehicular accidents, etc., suffer similar loss of blood plasma ("burn shock"). Smaller, but still lethal, doses of Lewisite reduce liver function and result in death among experimental animal populations after some delay, but usually within a week after exposure (14). The threshold for onset of severe systemic effects in humans is approximately 10 mg/kg to the skin (range of 9.1 to 13.4 mg/kg) (21,22).

Lewisite exposure is further characterized by immediate onset of pain, in direct contrast to the delayed pain reaction of mustard agents (54,55). So it is likely that, unless unconscious, anyone exposed to agent L would seek and receive some degree of decontamination and/or treatment. Decontamination by copious flushing with water or mild solutions of sodium bicarbonate or detergent needs to be particularly swift in the case of ocular exposure, in which permanent blindness from corneal necrosis and secondary scarring may result if decontamination is not accomplished within 60 sec (6). Inflammation of the iris can also result from sublethal exposures to the eye.

It is not known if Lewisite is persistent. However, arsenic is an elemental poison and any residual hydrolysis, combustion, or decontamination product is likely to contain an arsenical compound.

### Delayed Toxicity

In addition to acute effects, there is also the possibility of delayed or latent effects arising some time after vesicant agent exposure. For the sake of discussion and data organization, we will use the term "delayed toxicity" or "delayed effects" generically to encompass any adverse biological effects that are not acute. The reader should understand that such categorization includes effects that might be manifested after a period of seeming inactivity, or that arise following long-term exposures to low concentrations of a given vesicant agent (i.e., chronic exposure).

### Agents H/HD

Because of the ability to react irreversibly with a variety of biological molecules, the resultant biological damage could have immediate consequences or manifest itself after a considerable interval. Delayed effects from HD exposure include keratitis or keratopathy of the eye; respiratory diseases other than respiratory cancer; carcinogenesis; mutagenesis, particularly in relation to reproductive effects; and a generic category that includes other, less well-defined effects. Before more detailed discussions of these points, a brief summary of major findings is presented.

a) Among the population who sustained eye damage from mustard exposure in World War I, some lesions that had apparently healed relapsed decades later and resulted in eventual vision loss. b) Individuals poisoned by sulfur mustard in World War I were at an increased risk of developing chronic bronchitis. c) Epidemiological studies established a direct relationship between cancer of the respiratory tract and occupational mustard agent exposures. d) Evidence exists that these same groups of occupationally exposed workers (in wartime poison-gas factories) are also at increased risk of developing skin cancer. e) In addition, individuals whose skin has been blistered by mustard are vulnerable to subsequent mechanical injury at the same skin sites. f) Mustard can induce cancer in experimental animals and mutations in a variety of biological test systems. g) Some biological

assays have also demonstrated heritable genetic effects, implying that mustard produces damages in parental germ cells. *h*) Evidence for human reproductive effects following mustard exposure is equivocal.

**Toxicity to the Eye.** Eye damage suffered by most soldiers exposed to mustard in World War I was temporary in nature, and no permanent effects were observed at the time (26). In a smaller number of soldiers, where the eye was probably exposed to higher vapor concentrations or liquid droplets, a permanent, relapsing keratitis (delayed keratopathy) developed (26,39). This chronic condition is characterized by recurring erosion and ulceration of the cornea, eventual vision impairment and, in some cases, blindness (26,39). The latent period for this delayed effect has been observed to range from 8 to 40 years after apparent recovery from the initial acute injury (20). In some cases the condition has been observed to relapse for decades (26).

One case involving a single acute injury to an adult victim who received immediate medical attention after direct exposure to sulfur mustard (unreported whether vapor or liquid droplets) illustrates the potential for delayed toxicity to the eye (39). In spite of copious eye irrigation with physiologic saline within a few minutes after exposure, as well as antibiotic and atropine sulfate therapy, corneal damage was apparent and did not completely resolve with time. Two years after the event, the cornea was reported to be opaque, and the victim's vision was reduced to light perception only. Therapeutic corneal transplantation was recommended. This may be an exceptional case, as the dose to the eye could have been very large, or the individual could have been hypersensitive or experienced an infection or additional, unrelated injury. Balanced against this must be the experience of exposed World War I soldiers, many of whom experienced severe acute eye effects but did not develop any permanent damage (26).

**Nonmalignant Respiratory and Skin Damage.** Evidence from occupational and wartime exposures indicates that, under appropriate conditions, mustard agent can induce long-term respiratory damage. Reported ailments range from asthmalike conditions to a severe chronic emphysematous bronchitis, and secondary infections such as bronchopneumonia and tuberculosis (33,36). Among groups of World War I soldiers exposed to mustard, results of subsequent epidemiological studies can be summarized as follows.

In a study of British World War I pensioners, the history of respiratory disease in war veterans previously exposed to battlefield concentrations of mustard agent was compared to that of pensioners with chronic bronchitis and amputees (56). Neither the chronic bronchitis nor the amputee group had experienced a mustard exposure. When compared with the amputee group and the general male population, significant excess mortality from chronic bronchitis, tuberculosis, and pneumonia was noted in both the group exposed to mustard and the group exhibiting chronic bronchitis. The difference in mortality was most notable in pensioners less than 50 years of age (56).

An investigation of U.S. World War I veterans compared

former soldiers who underwent mustard exposure during the war with nongassed veterans who had a diagnosed pneumonia infection during the influenza outbreak of 1918 and veterans who had been wounded in the extremities, but who had not been a victim of pneumonia or mustard exposure (57). The mustard group exhibited significantly greater mortality from tuberculosis and pneumonia than either reference group. During the entire follow-up period (1919-1955), mortality from respiratory diseases (exclusive of tuberculosis and neoplasms) was 3.5, 2.7, and 1.9% for the mustard agent, pneumonia, and control cohort, respectively. The mortality from respiratory diseases was elevated in the mustard agent group. (The statistical significance of this elevation is unclear in the report. Beebe (57) states that the comparison between the mustard agent and wounded control rosters is "well outside the expected range of chance.") It was also found that of those in the mustard cohort dying of respiratory disease, there was a significant ( $p < 0.01$ ) excess of pneumonia deaths (2.9%) when compared to the wounded group (1.4%). When respiratory tuberculosis was examined as a cause of death, mortality in the mustard gas group (3.6%) was significantly ( $p < 0.01$ ) elevated compared to the pneumonia (2.9%) and wounded (2.1%) cohorts. In the follow-up period (1929-1938), the rate of respiratory death was highest in the mustard group (1.63 per 1000 men per year) when compared to rates of 0.70 and 0.54 for the pneumonia and control groups, respectively.

Weapons plant workers exposed to toxic vesicant concentrations under wartime conditions have been the subject of far more thorough investigation. Again, however, there is little characterization of workplace atmospheres or dose-response relationships. Between 1929 and 1945, the Japanese Army operated a chemical warfare agent manufacturing facility on Okuno-jima, an island of the Inland Sea (58-63). At peak capacity, this facility produced Lewisite (50 tons/month), mustard (450 tons/month), hydrocyanic acid (50 tons/month), diphenylcyanarsine (sneezing gas: 50 tons/month), chloroacetophenone (tear gas; 25 tons/month), and phosgene (unreported tons/month). During the period of maximum production (1937-1942), approximately 1000 individuals were employed throughout the facility. Interview data indicate that, given the minimal level of industrial hygiene practice in use at the time, multiple-agent exposures were common and agent-specific exposure occurred rarely (10,58). In the mustard production areas, atmospheric concentrations of mustard (estimated at 50-70 mg/m<sup>3</sup>) (10) were sufficient to produce most symptoms of acute mustard toxicity in workers (Table 2). Mustard production workers experienced numerous skin lesions (57 cases out of 109 workers engaged only in the production of mustard), the severity of which could be positively correlated with the years of employment associated with mustard manufacture [mean of 9 years (10)]. German workers exposed to sulfur and nitrogen mustard during the dismantling of a chemical warfare agent factory have experienced subsequent (latent period, if any, unspecified) skin tumors and necrotic skin ulcerations that spread and were resistant to therapy (64). A high proportion of the Japanese factory workers

also had a productive cough, irregular fevers, long-standing chronic bronchitis, emphysematous changes, and pleural adhesions (58,65). A study of 156 death certificates from former workers in the Japanese weapons factory noted that 54% died of respiratory diseases (58). Wada and his colleagues did not report an analysis of tobacco smoking history.

It seems clear from these studies that high-level exposures to mustard agent can produce permanent respiratory damage, which can take the form of a chronic bronchitis and which can also predispose affected individuals to other respiratory infections (e.g., pneumonia, tuberculosis). It must also be noted, however, that doses of agent capable of producing these respiratory effects are not well defined. An estimate of atmospheric mustard concentration in areas of falling gas shells during World War I is 19–33 mg/m<sup>3</sup> (66). In the case of Japanese workers, wartime worker safety provisions were minimal at best, and acute toxic effects from exposure to mustard and other chemical warfare agents were frequently reported (59). Some workers died as a result of acute gas poisoning during plant operation. Another factor to be considered is that occupationally exposed individuals experienced daily doses to mustard as well as other warfare agents over a period of years.

Appropriate enforcement of industrial hygiene practices can make a significant difference in the frequency and severity of delayed respiratory effects seen among chemical warfare agent factory workers. Manning et al. (67) retrospectively studied a group of workers (*N* = 428) from a British facility that manufactured mustard agent during World War II. The only significantly increased cause of respiratory mortality compared to controls was that resulting from pneumonia. The observed to expected ratio from pneumonia was 2.0 (*p* < 0.05) for the group of workers studied. This elevated pneumonia incidence, however, was of borderline statistical significance, and the significance disappeared if untraced members of the cohort were assumed to have survived throughout the study period. Chronic bronchitis was not found to be significantly elevated among observed British workers. The authors attribute the mortality differences between the Japanese and British workers to differences in the degree and quality of industrial hygiene measures practiced at the two weapons factories.

Application of the findings of these studies to the Chemical Stockpile Disposal Program indicates that respiratory injury with delayed effects could occur only in the event of a major mustard release generating atmospheric concentrations comparable to battlefield or war gas factory levels. At Aberdeen Proving Ground (APG) (Fig 1.), where sulfur mustard in ton containers is the only unitary munition, the probability of such an occurrence during on-site incineration disposal is less than  $1 \times 10^{-5}$  for the entire period of APG stockpile disposal (3).

Epidemiological evidence suggests that exposures associated with development of significant respiratory effects were either occupational exposures of long duration to (at least) irritating levels of mustard, or exposures of unprotected soldiers in battlefield situations. The maximum

atmospheric concentrations of mustard agent permitted during normal incinerator plant operation (workplace time-weighted average [TWA] of  $3 \times 10^{-3}$  mg/m<sup>3</sup>; general population level TWA of  $1 \times 10^{-4}$  mg/m<sup>3</sup>; see Table 5) are between 10<sup>3</sup> and 10<sup>5</sup> times lower than those levels associated with acute or delayed respiratory and skin damage.

**Carcinogenesis.** The carcinogenicity of sulfur mustard in mammalian systems has been previously summarized (63) and will not be detailed here. Briefly, the record of human cancer induction is based on retrospective studies of populations exposed to acutely toxic concentrations on the battlefield (World War I and II) or in weapons plants operating under wartime conditions of inadequate ventilation and industrial hygiene practices during the years immediately before and during World War II (56–59,62,65,68–71). Study of Okuno-jima worker death certificates through 1962 revealed a high incidence of respiratory tract cancer (14%) and digestive tract cancer (9.6%) (58). The remaining deaths were largely caused (39.7%) by respiratory disease (tuberculosis or other pulmonary infections) thought to be secondary to epithelial damage induced by vesicant gas inhalation.

Later follow-up divided the worker population into exposure groups based on job title (60). Examination of death certificates and autopsy reports through 1979 found that "Those . . . who were engaged in manufacture of yperite [mustard] and Lewisite gases had a high mortality due to diseases of the respiratory tract, particularly malignant tumors" (60). Smoking had been previously ruled out as a factor. Retired workers were also observed to exhibit impaired immunity (61).

Estimates of possible exposure concentrations in factories and on the battlefield range from 50 to 70 mg/m<sup>3</sup>. It can be expected that mustard-exposed survivors of the recent Iran–Iraq conflict (1980–1988) will be subjects of additional studies within the next decades.

In 1975, the International Agency for Research on Cancer (IARC) concluded that available data were sufficient to support classification of mustard agent as a "group I" carcinogen (68). This category includes compounds for which a causal relationship between exposure and subsequent human cancer induction can be adequately substantiated (72,73). Other respiratory carcinogens in IARC's group I include arsenic, asbestos, and vinyl chloride.

The conditions of exposure inherent to available human retrospective studies do not permit an estimate of dose response for sulfur mustard carcinogenicity. Animal carcinogenesis studies can provide more carefully defined exposure parameters than available epidemiological studies; nevertheless, the problem of species extrapolation

**Table 5. Maximum vesicant agent control limits recommended by the Surgeon General's working group.<sup>a</sup>**

Agent	Workplace (8 hr), mg/m <sup>3</sup>	General population (72-hr TWA), mg/m <sup>3</sup>
H/HD/HT	$3 \times 10^{-3}$	$1 \times 10^{-4}$
Lewisite	$3 \times 10^{-3}$	$3 \times 10^{-3}$

TWA, time-weighted average.

<sup>a</sup>Values recommended by Surgeon General's working group after review of pertinent data. See Carnes and Watson (5) for details.

Table 6. Delayed/latent effects observed for the vesicant agents H/HD, HT, Lewisite, and T.

Exposure regimen (duration)	H/HD response (dose)	Lewisite response	T response	References
<b>Carcinogenicity</b>				
Mouse, inhalation (15-min exposure)	Pulmonary tumors (~1590mg/m <sup>3</sup> ) <sup>a</sup>			Heston and Levillain (79)
Mouse, SC injection (6 weeks)	Fibrosarcomas at injection site (~6 mg/kg body weight)			Heston (74)
Mouse, IV (6 days)	Pulmonary tumors (~3-4 mg/kg body weight)			Heston (74)
Rat, inhalation (≥3-month exposure)	Skin tumors (0.1 mg/m <sup>3</sup> ) <sup>b</sup>			McNamara et al. (19)
Human, inhalation and skin deposition	Respiratory tract tumors, skin cancers (unknown) <sup>c</sup>			Inada et al. (10); IARC (68)
Mouse, skin (278 days)	Negative (2 mg total)			Fell and Allsopp (31)
Mouse, skin	Negative (dose unknown)			Bereblum and Shubik (76)
<b>Mutagenicity</b>				
<i>S. typhimurium</i> , Ames test	Positive <sup>d</sup> (1-50 µg/plate)	Negative <sup>e</sup> (0.001-5 µg/plate)		Stewart et al. (80,81)
<i>Neurospora crassa</i> (30-min exposure)	Specific locus mutation (200 µmole/L)			Dickey et al. (82)
<i>Saccharomyces cerevisiae</i>	DNA damage (500 µmole/L)			Kircher and Brendel (83)
<i>Drosophila melanogaster</i> ; parenteral injection	Specific locus mutation (45 pmole/fly)			Fahmy and Fahmy (84)
<i>D. melanogaster</i> ; vapor (5-min exposure)			Production of sex-linked lethal mutations	Auerbach and Robson (85)
<i>D. melanogaster</i> ; vapor (6-30 min)		Negative		Auerbach and Robson (85)
<i>D. melanogaster</i> ; vapor (15-min exposure)	Cytogenetic damage visible mutations, deletions, inversions (dose unknown) <sup>f</sup>			Auerbach and Robson (85)
<i>D. melanogaster</i> ; vapor (15-min exposure)	Sex chromosome loss and nondisjunction (dose unknown) <sup>f</sup>			Auerbach and Robson (85)
<i>D. melanogaster</i> ; parenteral injection	Sex chromosome loss and nondisjunction (75 pmole/fly)			Fahmy and Fahmy (86)
<i>D. melanogaster</i> ; vapor (15-min exposure)	Heritable translocation (dose unknown) <sup>f</sup>			Auerbach and Robson (85)
Mouse, ascites cells, IP injection (1 hr) <sup>g</sup>	DNA damage (5 mg/kg body weight)			Brookes and Lawley (87)
Mouse, L cells (10 min-24 hr)	DNA damage (1 mg/L)			Reid and Walker (88)
Mouse leukocytes, SC (injection) <sup>h,i</sup>	Somatic cell mutation (100 mg/kg body weight)			Capizzi et al. (89)
Mouse leukocytes <sup>i</sup>	Somatic cell mutation (1 µg/L)			Capizzi et al. (89)
Mouse leukocytes <sup>i</sup>	Chromosomal aberrations (20 µg/L)			Capizzi et al. (89)
Hamster fibroblasts (20 min)	Chromosomal aberrations (8 µg/L)			Savage and Breckon (90)
Human cells, HeLa <sup>i</sup>	DNA damage (2 mg/L)			Ball and Roberts (91)
<i>Vicia faba</i> (broad bean, root meristem)		Negative		Loveless (92)
CHO cells, HGPRT mutation assay (1 hr)	Sporadically positive (0.15-0.45 mg/L)	Negative (24-414 µg/L)		Jostes et al. (93,94)
Chromosome aberrations, CHO cells (1 hr)	Positive (80-159 µg/L)	Positive (24-414 µg/L)		Jostes et al. (93,94)

continued

Table 6. Continued.

Exposure regimen (duration)	H/HD response (dose)	Lewisite response	T response	References
<i>In vitro</i> sister chromatid exchange assay, CHO cells (1 hr)	Positive (10–40 µg/L)	Negative (40–207 µg/L)		Jostes et al. (93,94)
<i>D. melanogaster</i> vapor (15-min exposure)	Dominant lethal mutations (dose unknown) <sup>f</sup>			Auerbach and Robson (85)
Rat, inhalation (≥2weeks)	Dominant lethal mutation <sup>l</sup> Positive (male) (0.1 mg/m <sup>3</sup> ) <sup>k</sup>			Rozimarek et al. (95)
Rat, intragastric <sup>l</sup>	Dominant lethal mutations Positive (male) (0.50 mg/kg) <sup>m</sup> Negative (female)			Sasser et al. (96) Sasser et al. (96)
Teratogenicity				
Rat, intragastric <sup>n</sup>	Negative <sup>o</sup>	Negative <sup>o</sup>		Hackett et al. (97,112)
Rabbit, intragastric <sup>n</sup>	Negative <sup>o</sup>	Negative <sup>o</sup>		Hackett et al. (97,112)
Rat, inhalation (1–52 weeks)	Negative			McNamara et al. (19)
Reproductive effects				
Rat, inhalation	Negative (0.1 mg/m <sup>3</sup> ) <sup>b</sup>			McNamara et al. (19)
Rat, two-generation reproduction, intragastric <sup>p</sup>	Negative	Negative		Sasser et al. (98,99)
Subchronic effects				
Rat, subchronic toxicity, intragastric	Decreased body weight; forestomach epithelial hyperplasia <sup>q,r</sup>	Forestomach lesions; <sup>s</sup> changes in serum chemistry and hematology		Sasser et al. (100,101)

<sup>a</sup>Dose is estimated assuming complete volatilization of HD.

<sup>b</sup>Exposure to 0.1 mg/m<sup>3</sup> of HD was for 6.5 hr/day, 5 days/week; for the remainder of each 24-hr day, animals were exposed to 0.0025 mg/m<sup>3</sup> of HD vapor.

<sup>c</sup>Exposures were either in war situations or to workers in a mustard gas manufacturing plant during wartime production. The duration of exposure for these workers was years.

<sup>d</sup>Without metabolic activation.

<sup>e</sup>Tested with and without metabolic activation.

<sup>f</sup>Difficult to estimate dose received. A 1:10 mixture of mustard gas in cyclohexane was sprayed by an atomizer at 10-sec intervals in an air stream that flowed at 2 L/min. This air stream flowed through the exposure chamber.

<sup>g</sup>Host-mediated assay.

<sup>h</sup>Host-mediated assay. Murine leukemia L5178Y cells were grown as ascites in mice.

<sup>i</sup>Duration of exposure was not given.

<sup>j</sup>Male rats were exposed 6 hr/day, 5 days per week to 0.1 mg/m<sup>3</sup> of HD by inhalation for 1–52 weeks. They were mated to unexposed females and dominant lethality determined.

<sup>k</sup>Estimated total dose was 630 µg/kg body weight.

<sup>l</sup>HD tested at doses of 0.08, 0.20, and 0.50 mg/kg body weight.

<sup>m</sup>Significant dominant lethal effects observed in offspring of male rats exposed to HD (most consistent effects observed at 0.50 mg/kg dose of HD). Significant increase in abnormal parental sperm observed in this dose group. F<sub>1</sub> effects include increased early fetal resorptions and preimplantation losses in addition to decreased total live embryo implants.

<sup>n</sup>See text for dosages. Treatment times were gestational days 6–15 in rats and 6–19 in rabbits.

<sup>o</sup>No clear evidence of teratogenic effects by the agent; certain trends seen could be ascribed to maternal toxicity.

<sup>p</sup>HD tested at doses of 0.03, 0.1, and 0.4 mg/kg body weight; L tested at doses of 0.10, 0.25, and 0.60 mg/kg/day. Pregnant females were dosed 7 days/week. Males of this mating group were sacrificed at birth of the pups. Females giving birth continued to receive the agent during lactation. Pups were weaned at 21 days of age, and the dams were sacrificed. Pups continued to receive agent for 13 weeks and were mated as above, repeating the dosing schedule. Study concluded with sacrifice of the second-generation pups and their dams at weaning.

<sup>q</sup>Rats (6–7 weeks old) received 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg body weight of HD 5 days/week for 13 weeks. In the L study, 6 to 7-week-old rats received 0.01, 0.10, 0.50, 1.0, and 2.0 mg/kg body weight of agent 5 days/week for 13 weeks.

<sup>r</sup>Effects noted only in highest (0.3 mg/kg) dose group. All other parameters studied were not different from controls.

<sup>s</sup>See text for discussion.

requires consideration. Tumorigenesis of sulfur mustard in laboratory species of mice and rats has been confirmed via inhalation and injection exposure (19,74,75). Evidence is summarized in Table 6. Tumors were not observed in guinea pigs, rabbits, and dogs exposed to atmospheric

concentrations of 0.001 mg/m<sup>3</sup> or 0.1 mg/m<sup>3</sup> for periods up to 1 year (19). The laboratory rat (Sprague-Dawley Wistar) was the only species observed to develop significantly elevated tumorigenicity at 0.1 mg/m<sup>3</sup>. Exposure protocols and animal strains tested for sulfur mustard tumori-

genesis are detailed in Watson *et al.* (63).

Berenblum and Shubik (76) tested mustard in an initiation-promotion study on mouse skin and found it was not active as an initiator. The test promoter was croton oil. Doses of mustard actually received by mice in this study were not well defined because the mustard was applied as a droplet on the end of a glass rod that had been dipped into a solution of 0.1% mustard in paraffin oil. This experiment is interesting, however, in that it demonstrated that a low concentration of mustard was not an initiator when contrasted to various components of coal tar applied at similar concentrations.

These animal data have been evaluated to derive an estimate of carcinogenic potency associated with sulfur mustard exposure as well as to address the issue of species extrapolation (63). Comparisons of tumorigenicity in the same and related species for sulfur mustard and the well-characterized industrial carcinogen benzo[*a*]pyrene (BaP) indicate that these two compounds are of approximately equivalent carcinogenic potency in test animals. This finding can be used to estimate the potential carcinogenic risk of chronic inhalation of air containing mustard agent (potential agent incinerator emissions) or ingestion of contaminated foodstuffs (potential plume deposition following an unplanned release). The calculated lifetime cancer risk for chronic exposure to control limit concentrations (Table 5) at maximal assumptions of hypothetical exposure during the period of incinerator operation is as follows: *a*) for incinerator workers maximally exposed to the mustard 8-hr TWA of  $3 \times 10^{-3}$  mg/m<sup>3</sup>, calculated excess lifetime risk approximates  $3 \times 10^{-4}$ ; *b*) for the general public maximally exposed to the mustard 72-hr TWA of  $1 \times 10^{-4}$  mg/m<sup>3</sup>, calculated excess lifetime risk approximates  $3 \times 10^{-5}$  (5,63). Note that maximum estimates of excess lifetime cancer risks for "fenceline individuals" potentially exposed to engineering estimates of sulfur mustard at  $1.3$  to  $3.7 \times 10^{-8}$  mg/m<sup>3</sup> along the boundary of the Aberdeen Proving Ground during planned mustard incinerator operation ranges between  $4 \times 10^{-9}$  and  $1 \times 10^{-8}$  (63). Federal agencies do not routinely regulate compounds for which the excess lifetime cancer risk  $\leq 10^{-6}$  (77). For comparison, the present U.S. lifetime cancer incidence from all causes approximates  $2.5 \times 10^{-1V}$  (78).

Concentrations of mustard to which the general population would be exposed during an unplanned release might be large enough to produce acute health effects. The question arises as to whether such an exposure might lead to an increased risk of respiratory cancer. The most relevant exposure experience for comparison is that of World War I soldiers. Medical data available for these veterans suggest there was some increased respiratory cancer mortality (56,57,78). However, the increased risk over that of the control population was not large and not statistically significant. Battlefield doses were not known, nor was the incidence of secondary respiratory infection or the effect of their subsequent life experience (e.g., poor nutrition during the Great Depression, occupational exposures, etc.) Only limited information on smoking was available in these studies. Occupational exposures that

proved to be carcinogenic are not comparable to the acute exposures expected during a single accidental release; doses that war gas factory workers received were to a variety of toxic compounds and were clearly large enough and extended over a sufficient period of time (months to years) to induce repeated acute effects.

**Mutagenesis.** Mustard agent induces mutagenesis in a wide variety of test organisms (see Table 6). The content of Table 6 is not intended to be encyclopedic; many other studies could be cited in a summary of known mutagenic activity for this agent. Mutations in *Drosophila* have included dominant lethal and phenotypic mutations as well as recessive sex-linked; autosomal, and phenotypic lethal mutations (102). Chromosomal aberrations following mustard exposure include deletions, inversions, duplications, and translocation. Mustard agent has also been demonstrated to produce various kinds of chromosomal structural damage in plant and animal cells; structural aberrations, chromosome stickiness, and chromosomal breakage have all been observed (102). There is no doubt as to the mutagenicity of sulfur mustard.

Evidence of elevated sister chromatid exchanges (SCE) (compared to controls) has been noted in lymphocytes of fishermen inadvertently exposed to mustard (103) when they dredged up leaking mustard shells discarded in the North Sea after World War II. The time between exposure and first SCE analysis varied between 4 and 11 days. The SCE count was still significantly elevated above matched controls 3 weeks after exposure. The specific dose of mustard that individuals received cannot be quantified, but each person experienced acute toxicity in the form of skin blisters, painful irritation of the eyes, and transient blindness. This symptomology suggests that victims experienced high doses of mustard.

A correlation between mutagenic activity of a compound and that compound's carcinogenic potency has been observed in a number of experimental assays (104). In the case of mustard agent, sufficient evidence already exists to classify it as a human carcinogen under appropriate exposure conditions. The fact that it is a mutagen supports its classification as a carcinogen.

**Reproductive Effects and Teratogenesis.** Reproductive effects studies have focused on occupational populations exposed in chemical warfare agent factories for a number of reasons: these individuals were exposed to the highest agent doses for the longest duration, and the exigencies of wartime resulted in the presence of male and female workers. Thus, analysis of data was possible for both genders.

Yamakido *et al.* (70) studied a group ( $N = 325$ ) of former workers from the Okuno-jima agent factory, their spouses ( $N = 226$ ), and their offspring ( $N = 456$ ) for possible genetic effects. The workers and their families were divided into three groups: agent production workers who were exposed primarily to mustard and Lewisite and who were thought to have received the largest exposures; other factory workers, not engaged in agent production and who probably received exposure to moderate agent concentrations (compared to the first and last groups); and workers not directly involved with mustard or Lewisite

(e.g., office workers, transportation, etc.) who were thought to have received the lowest agent exposures.

In the first group, 18% were females, whereas there were 17% and 64% females in the second and third groups, respectively. The gender distribution in the offspring studied was about equally divided between male and female. A general health examination was carried out on all individuals under study, as well as biochemical analyses (i.e., starch gel electrophoresis of plasma and erythrocyte proteins) of blood samples from offspring.

The general health examination of offspring did not reveal evidence of any diseases that could be ascribed to genetic effects or any abnormality that was present in significantly different proportions from that exhibited in parental groups. Blood analyses detected evidence of genetic variants in a small number of the children examined. Specifically, six kinds of plasma protein variants were detected in 11 children, while 11 variants of erythrocyte proteins were found in 25 individuals. Examination of the parents demonstrated that the specific genetic variation was also present in one or both parents, so that each offspring's variant could not be uniquely ascribed to a mutation induced in a parental germ cell by mustard exposure. Yamakido et al. (70) concluded that no evidence of mustard agent-induced mutations could be detected in their study group.

Lohs (33) briefly summarizes a study of potential reproductive effects in a population of German chemical warfare agent factory workers (105). These male workers were exposed to sulfur and nitrogen mustard under wartime conditions in World War II, although no details of possible exposure parameters, length of employment, manufacture of other agent, etc., were provided. Evidence for dominant, sex-linked, lethal mutations was detected in connection with an increase in the sex ratio among the offspring of 134 fathers employed in agent production. Impairment of various stages (unspecified) of spermatogenesis was also noted. It is difficult to know how much importance should be attached to these observations. The confounding fact that workers were simultaneously exposed to unreported concentrations of nitrogen mustard (a potent mutagen) as well as sulfur mustard makes interpretation of the results uncertain.

A few animal studies have directly investigated the potential of mustard agent to induce reproductive or teratogenic effects. The potential reproductive-fetotoxic activity of low-level exposures to HD was studied in male rats (19) exposed to atmospheres containing either 0.001 mg/m<sup>3</sup> or 0.1 mg/m<sup>3</sup> mustard for varying time intervals ranging from 1 to 52 weeks. Following termination of agent exposure, male rats were bred to unexposed female rats and pregnancy outcomes were monitored. The index of dominant lethal mutagenesis in the F<sub>1</sub> generation was the percentage of dead fetuses. The percentage of fetal death in the controls (12 month) and the ranges in the two exposed groups (animals were tested at 1, 2, 4, 8, 12, 24, 36, and 52 weeks) were as follows: 4.12% (control); 1.18–8.60% (low mustard exposure); and 1.72–21.05% (high mustard exposure). The highest percentage of fetal deaths (21.05%) in the offspring of the high-exposure group occurred in the

litters bred from males that underwent 12 weeks of exposure. Other elevated values were seen in the highest exposure group after 4 weeks (10.1%), 24 weeks (10.3%), and 52 weeks (12.5%). No statistical evaluation of these differences are published in the report. McNamara et al. (19) concluded that there was no evidence for mutagenesis and that no differences between control and experimental groups were observed. Perhaps McNamara's conclusions were based on statistical analyses that were not explicitly stated; the current analysis considers that the elevated fetal mortality in the high-exposure group suggests a possible connection between agent exposure in the male and fetal death.

Conclusions differing from that of McNamara et al. (19) were reached by Rozimarek et al. (95) in their evaluation of the same data set. Rozimarek and his colleagues concluded that significant dominant lethal mutagenesis was observed in the high-exposure (0.1 mg/m<sup>3</sup>) group. The dominant lethal mutation rate attained a maximum of 9.4% after 12 weeks of exposure and did not alter following successively greater exposure periods. The reason for this difference in interpretation between authors is unclear. Experimental results seem to at least suggest some effect of HD exposure on mammalian male fertility.

Further studies by McNamara et al. (95) investigated the effect of low-level HD exposures on fetal toxicity when pregnant female rats were directly exposed to HD. Animals were exposed to the same two HD air concentrations as in the male study (i.e., 0.001 and 0.1 mg/m<sup>3</sup>) during the first, second, or third weeks of gestation, or during the entire pregnancy. No change in fetal mortality was observed when the exposed group was compared to control groups.

Teratology studies in rats and rabbits were conducted more recently (97). Pregnant rats were exposed to mustard doses of 0.2, 0.4, 0.8, 1.6, 2.0, and 2.5 mg/kg body weight by gastric intubation from days 6 to 15 of gestation and sacrificed on day 20 in a range-finding study. On the basis of this preliminary study, HD doses of 0.5, 1.0, and 2.0 mg/kg were used in the teratology study, over the same gestational period, and with the same sacrifice schedule. There was a significant ( $p \leq 0.05$ ) decrease in body weight (compared to controls) of pregnant rats in the 1.0 and 2.0 mg/kg dose group by 9 days of gestation. Similar findings were noted at 12 days of gestation in the 0.5 mg/kg groups. Thus, some evidence of maternal toxicity was seen at all dose levels. An increase in litter resorptions was noted in the treatment groups, but this effect was not statistically significant. There was a significant decrease in fetal body weights in the 1.0 and 2.0 mg/kg dose groups. No significant level of increase in major fetal malformations was seen in any dose group, but the number of minor anomalies (e.g., misaligned sternbrae [embryonic segments which eventually develop into the sternum]) was significantly increased in the highest dose group. The authors concluded that the fetal findings observed could be attributed to the evident maternal toxicity produced by HD.

Pregnant rabbits were also used in the same studies to detect potential HD teratogenicity (97). In the range-finding study, rabbits received 0.5, 1.0, 2.0, or 2.5 mg/kg

body weight of HD for days 6–19 of gestation and were sacrificed on day 30 of gestation. Doses of 1 and 2 mg/kg produced maternal mortality, so the teratology study was limited to doses of 0.4, 0.6, and 0.8 mg/kg of HD. There was no evidence of significant effects on intrauterine growth or fetal growth and development at these doses. A higher percentage of resorptions in the two highest dose groups was noted. The major finding was evidence of maternal toxicity, as evidenced by a significantly depressed weight gain in the 0.8 mg/kg dose group from 11 to 20 days of gestation. The authors concluded that both rabbit and rat studies indicated that HD was not teratogenic, since the effects observed were at doses which produced overt signs of maternal toxicity.

Results of further studies of possible reproductive effects carried out at the Pacific Northwest Laboratory (Richland, Washington) are summarized in Table 6 (96). In the dominant lethal mutation study, male or female rats received HD by gavage for 5 days/week, for 10 weeks. Exposed females were then mated to either unexposed or exposed males during a 3-week, post-exposure mating period. To evaluate male dominant lethal effects, exposed male rats were mated to unexposed females during a 10-week mating period following exposure. No evidence of a significant female dominant lethal effect was observed, but a significant male dominant lethal effect was noted, particularly at the highest dose (0.50 mg/kg body weight) used. Furthermore, sperm abnormalities (abnormal sperm heads) were observed to be significantly ( $p < 0.05$ ) elevated in this dose group. Thus, HD has been demonstrated to produce reproductive effects in male rats under the particular set of exposure conditions used in this study.

Sasser et al. (98) carried out a two-generation study in rats gavaged with 0.03, 0.1, or 0.4 mg/kg/day, according to the dosing protocol detailed in Table 6. Males who had mated with females were sacrificed at the birth of their pups. Dams who had given birth were sacrificed when the pups were weaned. Male and female  $F_1$  pups received HD until they were mated, the females became pregnant and gave birth. At this point,  $F_1$  males (fathers) were sacrificed and  $F_1$  dams continued on the dosage schedule until weaning, at which point the study was terminated. Thus, two generations of rats received chronic exposure to HD, with each generation going through a mating cycle. Similarly, two generations of pups were born to parents who had received HD. All animals in this study were examined for evidence of adverse effects on reproductive performance, fertility, or reproductive organ weights. In addition, gross and microscopic examinations of reproductive organs were carried out on all groups. There was no evidence of adverse reproductive effects at the HD doses tested. However, there was a significant inhibition of growth (i.e., reduced body weight gain) in the rats of both sexes (the  $F_1$  generation) born to parents who had received the highest dose (0.4 mg/kg/day) of HD. The authors conclude that the HD exposures did not affect reproductive performance or fertility.

In addition to the rat studies, dominant lethal effects have also been observed in fruit flies (85) (Table 6). The exposure necessary to produce this effect is not clearly

stated by the investigators, and the extrapolation between insects and mammalian species is not straightforward.

In summary, evidence from both human and animal studies regarding the reproductive toxicity of mustard is generally negative, except for the evidence of dominant lethal mutations in exposed male rats (96). In some cases, effects are noted, but other similar studies are negative. The evidence from the German chemical warfare agent factory worker populations suggests an adverse effect, but it is not clear that this is due solely to sulfur mustard exposure. We do not consider this positive result to be of more importance than the negative findings in the more fully characterized Japanese worker population. In any case, the human occupational exposures in the few available studies were almost certainly to high levels of agents for long durations; in addition, workers were essentially without protective equipment. Unplanned release scenarios that might occur during chemical stockpile disposal are not comparable (37). As a result, reproductive effects from mustard exposure are unlikely for occupational or general populations during the sulfur mustard disposal process.

**Other Effects.** A variety of other delayed effects from mustard exposure, some rather ill defined, are also presented in the literature (106). Among workers in mustard agent factories before and during World War II, the following delayed effects were observed: periodontitis leading to tooth decay; osteoporosis; premature aging; elevated pH of stomach fluid; liver injury (rare); and unspecified injuries of the central nervous system. The types of exposures (multiple agents? concentration? protective measures?) and durations that were associated with these effects are not stated and have not been documented elsewhere (58,67).

Healed skin lesions may exhibit evidence of permanent damage. There is often some alteration of pigmentation at the site of damage and the affected areas are often unusually sensitive to subsequent mechanical injury. A mild contusion or abrasion after what appears to be complete healing may produce one or more blisters (36).

McNamara et al. (19) studied a number of physiological parameters in laboratory animals (rabbits, guinea pigs, and dogs) during their chronic exposure experiments at air concentrations of 0.1 mg/m<sup>3</sup> or 0.001 mg/m<sup>3</sup> of HD for 5 days/week, for periods of 1–52 weeks. Experimental animals were closely monitored for any toxic signs resulting from exposure. No overt toxic signs were detected in any experimental animals exposed to 0.001 mg/m<sup>3</sup> for up to 52 weeks. Corneal opacity, chronic keratitis of the eye, and excess vascularization, pigmentation, and granulation of the eye were noted in dogs exposed for more than 16 weeks to 0.1 mg/m<sup>3</sup>; no other animal species tested exhibited overt toxic signs on exposure to this concentration. Hematologic parameters such as red blood cell count, hematocrit, white blood cell count, and serum enzymes in exposed rabbits and dogs were not significantly different from those of control animals. There was a tendency toward elevated concentrations of the blood enzyme serum glutamic-oxalic transaminase (SGOT) in dogs after 12–28 weeks of exposure to 0.1 mg/m<sup>3</sup> of HD, although no

statistical analysis of this trend was reported. This finding could be an indicator of liver and/or heart tissue injury. McNamara et al. (19) also observed that the serum albumin/globulin ratios of dogs exposed to either concentration of HD for 52 weeks was unchanged when compared to control values. None of the species exposed to either concentration of HD displayed any evidence of skin, eye, or respiratory tract sensitization. An antigen challenge in a rabbit exposed to 0.1 mg/m<sup>3</sup> of HD for 1 year produced an essentially normal response (19).

A study of subchronic HD toxicity in rats was recently reported by Sasser et al. (100) (Table 6). In this study, rats received a 13-week exposure to various HD doses by gavage and were monitored for various parameters/toxicological signs including body weight, evidence of moribundity, hematological parameters including selected serum enzymes (serum glutamic-pyruvic transaminase [SGPT] and SGOT, which are considered indicators of liver function), and an ophthalmologic examination at the beginning and end of the study. At sacrifice, a gross necropsy and microscopic exam of selected tissues was made. There was no evidence of adverse toxic effects other than the conditions noted in Table 6. Given the known cellular toxicity of HD, it is perhaps not surprising that forestomach hyperplasia was observed, as this would be the tissue to receive immediate contact with HD during intragastric dosing.

## Agents HT/T

No data specific to delayed toxicity of agent HT were identified during preparation of this review. Because HT is a mixture of about 60% distilled mustard (HD) and up to 40% agent T (described in Table 1), it is expected that delayed effects of HT would encompass those of both mustard formulations H/HD as well as agent T.

Agent T is highly mutagenic (107), with a demonstrated ability to produce sex-linked lethal mutations in *Drosophila melanogaster* on an order comparable to that of mustard agent and X-rays. A standard C1B test mated untreated adult females with adult males exposed to 5 min of volatilized, neat, agent T (85). The 8.5% lethal mutation rate observed in the resulting F<sub>1</sub> progeny indicates a significant induction of lethal mutations on the X-chromosome of the spermatozoa (normal percentage of F<sub>1</sub> lethals in *D. melanogaster* ranges between 0.1 and 0.4%) (107). Further experiments by Auerbach and Robson (52) suggest that agent T may have the capacity to induce chromosomal rearrangements in *D. melanogaster*. However, the corroborative data for this effect are considered suggestive rather than conclusive. While results from a single insect species cannot be considered an absolute indication of mutagenic activity in human systems, the data represent reason for caution.

## Agent L (Lewisite)

The literature on potential delayed or latent effects of Lewisite exposure is somewhat limited, at least in comparison to HD. There is some relevant experience regard-

ing human exposures to Lewisite in combination with other agents (i.e., workers in chemical warfare agent factories). Probably the most complete data has emerged from recent work sponsored by the U.S. Army Medical Research and Development Command in Fort Detrick, Maryland (99,101). The following discussion relies heavily on these results, in addition to a few studies of World War II vintage.

The ability of Lewisite to produce sex-linked lethal mutations and chromosomal rearrangements among the F<sub>1</sub> generation of exposed adult *D. melanogaster* was tested in the Pharmacology Department of the University of Edinburgh during World War II and reported primarily in the late 1940s (52,108). All results were negative. The mutagenicity of Lewisite has also been investigated in the Ames assay (bacterial mutation) and in mammalian cells (81,94) (Table 6). Neither the bacterial nor mammalian cell assay provide convincing evidence of mutagenicity. A confounding factor is the powerful cytotoxicity of Lewisite. The higher doses of Lewisite (Table 6) resulted in extensive cell killing, and mutants could only be detected among the surviving fraction. Besides mutation at a specific locus, cytogenic assays were used in the study of Lewisite effects on mammalian cells. Although evidence for a weakly positive SCE response to Lewisite was observed, the SCE rate was not significantly different from control values (94). Chromosomal aberrations were significantly increased in Chinese hamster ovary cells treated with Lewisite. This result indicates Lewisite can produce chromosome damage under appropriate experimental conditions (94). How this might apply to Lewisite exposures among humans is unclear.

The few cases of long-term follow-up indicate that carcinogenicity may be an effect of acute Lewisite exposures. A former infantryman in the German Occupation Army of France during World War II received an accidental exposure to liquid Lewisite on the skin of his lower right leg in 1940 (109). Immediately upon exposure, intense pain ensued and a blistered lesion formed; the wound never healed. In 1948, the ulcerated lesion was diagnosed as malignant, surgically removed, and later treated with X-rays. By 1978, the ulcerated area involved the inner third of the victim's lower leg and was histologically diagnosed as Bowen's disease, an intraepidermal squamous cell carcinoma. At the time of last report (109), the patient was 77 years old, in otherwise good health, and receiving palliative treatment 38 years after a single exposure. No metastasis was noted.

Other human evidence is less direct. The Okuno-jima chemical warfare agent factory operated by the Japanese Army from 1929 through 1945 produced Lewisite, as well as sulfur mustard and several other irritant compounds for wartime use against personnel. Operations details are provided in the sulfur mustard text above and in Watson et al. (63), Tanaka (62), Wada et al. (58,59), and Nishimoto et al. (60,61). Several cases of Bowen's disease were also noted among former workers of the Okuno-jima facility (10). It is unclear whether these cases were induced by single-agent exposure or by combined exposure from the arsenic in Lewisite and diphenylcyanarsine plus mustard

agent. As previously discussed, protective clothing, ventilation, monitoring, and sanitation were poor or nonexistent, and many workers suffered severe exposures. Because each worker was exposed to both agents, it is not possible to completely distinguish Lewisite from mustard agent as a carcinogen in these studies. Furthermore, there are no quantitative estimates of dose or exposure rates, although they must have been high under the wartime conditions described. However, there are sufficient data to suggest that Lewisite may be a carcinogen at elevated, sublethal exposures that cause blistering and pain.

Teratogenic properties and reproductive toxicity of high-level Lewisite exposure are suspected but have not been substantiated. The active arsenical group in Lewisite is thought to react with proteins and could thus affect developing offspring (110,111). Several experiments designed to examine aspects of reproduction have been recently completed by the U.S. Army Biomedical Engineering Research and Development Laboratory (USABRDL) (99,112). Examination of maternal and fetal effects was accomplished by exposing pregnant rats and rabbits to Lewisite by gastric intubation at a daily dose range of 0–1.5 mg/kg for rats and 0–0.6 mg/kg for rabbits. In the rat study, no evidence of teratology or maternal toxicity was obtained. Rabbits were found to be much more sensitive to the toxic effects of Lewisite. A very significantly elevated maternal mortality was observed in the Lewisite-treated groups of rabbits. Furthermore, maternal weight gain was significantly depressed at the 0.6 mg/kg dose level. Because of the toxicity of Lewisite, the number of surviving litters was smaller than anticipated, so statistical comparisons among treatment and control groups were less reliable than had been hoped for. Placental weights and fetal body weights showed a trend (not statistically significant) toward lower values in the Lewisite-treated rabbits. The incidence of major malformations was not elevated in any of the groups of rabbits exposed to the various Lewisite doses. However, there was a significant ( $p \leq 0.05$ ) increase in incidence of, otherwise normal, stunted fetuses (defined as having body weight  $\leq 2$  standard deviations of the mean body weight) in dams treated with the 0.6 mg/kg Lewisite dose. In this latter group, the incidence of fetuses with supernumerary ribs and reduced pelvis ossification was also significantly elevated. The conclusion from these studies was that the effects observed in the fetuses were likely to be due to maternal toxicity.

Sasser et al. (99) have studied the reproductive effects of Lewisite exposure in a two-generation reproductive study in rats (Table 6). There were no adverse effects of Lewisite exposure on reproductive performance, fertility, or reproductive organ weights observed in this study. Minor (but statistically significant) decrease in growth among females (both generations) was noted. Histopathologic study of various tissues did not identify a target organ for the site of Lewisite action. The authors again note the strong toxicity of Lewisite which deterred them from being able to use higher doses in this study.

The effect of Lewisite on rats was also studied in a 90-day subchronic toxicity study (101) (Table 6). A variety of

toxicity measures were monitored, including body weight, ophthalmology, hematology, various serum proteins and enzymes, as well as histopathological evaluations of collected tissues. No effects on body weight were observed. Significant ( $p < 0.05$ ) decreases in total serum protein, serum creatinine, and the serum enzymes SGOT and SGPT were observed in male animals in the highest dose (2.0 mg/kg) group at 13 weeks. The SGOT effect was observed in all the other Lewisite dose groups in the male animals. In females, there were significant increases in lymphocytes and platelet counts in the highest dose group. The lymphocyte increase was observed at 6 weeks (the only time other than 13 weeks when hematology was performed) but not at 13 weeks. The platelet count was elevated at 13 weeks. The investigators were not able to interpret the significance, if any, of these various changes in hematologic and enzymatic parameters.

Of possibly more importance was the consistent finding of forestomach lesions in both rat genders given the highest Lewisite dose (2 mg/kg). This finding was observed in 80% of the males and 40% of the females at this dose group, and in 10% of the males in the 1 mg/kg dose group. These lesions were described as involving necrosis of the stratified squamous epithelium accompanied by infiltration of neutrophils and macrophages, hemorrhage, edema, and fibroblast proliferation. In a few instances, the epithelium adjacent to ulcerated areas was hyperplastic. In addition to the forestomach lesions, acute inflammation of the glandular stomach was seen in 10% of the males and 30% of the females in the high dose group. This lesion was described as very mild. The lesions seen in the forestomach and glandular stomach probably are consistent with the irritant action of Lewisite when administered by gavage. The investigators found no evidence that the forestomach lesions were precancerous. They were also careful to point out that this was a subchronic toxicity study, and the length of treatment was insufficient to determine carcinogenicity.

## Conclusions

Available data characterizing dose response to vesicant (blister) agent exposure are summarized from unclassified, internal Army reports as well as the open literature. Acute and delayed toxicity of various sulfur mustard formulations (H, HD, and HT) and Lewisite are now documented in a readily accessible form useful for emergency planning.

Historical military data from World War I and the recent Iran–Iraq conflict indicate that acute lethality rates following battlefield exposures to sulfur mustard agents (estimated 1500 mg-min/m<sup>3</sup>) range between 1 and 3%. Mustard “gas” was responsible for 0.5% of World War I battlefield deaths (i.e., 600 of the 126,000 American deaths).

Vesicants are cellular poisons in target tissues. Sulfur mustard is an alkylating agent; individual cells are destroyed by the chemical reaction of mustard with cellular proteins, enzymes, and nucleic acids. Lewisite, an organic arsenical, also produces cell death, but by altering critical cellular enzyme systems.

Following exposure to any formulation of sulfur mustard agent, humans usually undergo a latency period of several hours before signs of toxicity begin to appear. These signs include eye inflammation (occurs at lower doses than any other effect and is therefore the most sensitive indicator of mustard agent exposure), skin irritation (rash or blisters), and irritation of the respiratory tract. Moist tissues are particularly vulnerable. Recovery from these toxic effects can take days or weeks. The more serious acute effects are certainly disabling, although usually not permanently so; special care and resources are required to prevent subsequent infection of the skin, respiratory tract, and eyes. The difference between a lethal percutaneous dose (estimated  $LCt_{50}$  of 10,000 mg-min/m<sup>3</sup>) and a lethal inhalation dose (estimated  $LCt_{50}$  of 1500 mg-min/m<sup>3</sup>) is approximately 10-fold; respiratory protection even in the absence of any other protective clothing is thus critical in an environment where mustard exposure is likely.

Dose response to H and HD is temperature dependent, in part due to the relatively high freezing point of sulfur mustard (8°–14°C). Percutaneous response at > 14°C ambient is a function of skin temperature and moisture, which are largely controlled by ambient temperature. Between 21° and 27°C ambient, 2000 mg-min/m<sup>3</sup> is necessary to generate an incapacitating percutaneous dose to a masked individual; at 32°C ambient, only 1000 mg-min/m<sup>3</sup> is required for the same response.

Mustard agent exposure can also produce delayed or latent effects. Apparent healing of eye damage after acute, high-level exposure can be followed by delayed keratopathy over the course of years, although this effect is infrequent. Following sufficiently severe exposure, respiratory tract damage can result in chronic bronchitis and emphysema. Epidemiological evidence and results of animal studies both indicate that sulfur mustard agents can induce cancer. World War I veterans and workers in armament factories who were exposed to intensely irritating levels of this agent under wartime conditions developed respiratory tract and epithelial malignancies. Because of its highly reactive chemical nature, mustard agent can react with DNA to produce mutations in microbial, insect, and mammalian cell culture lines, insect colonies, and the offspring of male rats undergoing intragastric exposure to HD at 0.5 mg/kg (dominant lethal mutations observed as early fetal resorptions, etc., in the F<sub>1</sub> generation were associated with abnormal parental sperm). Evidence from epidemiological studies of armament factory workers has yet to reliably demonstrate that exposure to sulfur mustard agents produces reproductive effects in humans. Recent, two-generation intragastric exposure of male and female rats to HD resulted in no observed effects on reproductive performance, fertility, or reproductive organ weights of males and females.

Toxicity of agents HT and Lewisite are not as well characterized as sulfur mustard, but it is clear that these agents possess generally similar vesicant properties. Agent HT is more stable and more acutely lethal than HD. It is considered carcinogenic because of the presence of HD and mutagenic because both HD and T (*bis*[2(2-

chloroethylthio)ethyl]ether) react with nucleic acids. Lewisite does not exhibit the latency period displayed by mustard agent and is noted for causing immediate pain upon contact with the skin and eyes (a sensitive indicator of exposure). There are major differences between lethal inhalation doses (estimated  $LCt_{50}$  of 1200 to 1500 mg-min/m<sup>3</sup>) and percutaneous doses (estimated  $LCt_{50}$  of 100,000 mg-min/m<sup>3</sup>) for masked individuals exposed to agent L. Lewisite is also known to be a systemic poison (liver and kidneys) at sufficiently large doses. Among military personnel and armament factory workers who received large dermal exposures, agent L has been associated with induction of Bowen's disease, a relatively slow-growing and usually nonfatal intraepidermal squamous cell carcinoma. Recent mammalian assays, which included a two-generation rat study, did not demonstrate teratogenicity or reproductive effects at the doses tested. However, the high cytotoxic potency of Lewisite may have precluded such observations.

No acute vesicant effects are expected at the recommended inhalation exposure control limits documented in Table 5. However, because current Federal regulatory thinking considers that carcinogenesis exhibits a linear, nonthreshold dose response, any level of exposure poses a degree of calculated cancer risk.

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