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United States Environmental Protection Agency
Office of Pollution Prevention and Toxics

SULFUR MUSTARD (AGENT HD)
CAS Reg. No. 505-60-2

PROPOSED ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

"PUBLIC DRAFT"

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee to develop Acute Exposure Guideline Levels (AEGLs) has been established to identify, review and interpret relevant toxicologic and other scientific data, and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent ceiling exposure values for the general public and are applicable to emergency exposure periods ranging from less than 1 hour to 8-hours. Three AEGLs will be developed for each of four exposure periods (30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm or mg/m^3) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience notable discomfort. Airborne concentrations below AEGL-1 represent exposure levels that could produce mild odor, taste, or other sensory irritation.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience irreversible or other serious, long-lasting effects or impaired ability to escape. Airborne concentrations below the AEGL-2 but at or above AEGL-1 represent exposure levels that may cause notable discomfort.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience life-threatening effects or death. Airborne concentrations below AEGL-3 but at or above AEGL-2 represent exposure levels that may cause irreversible or other serious, long-lasting effects or impaired ability to escape.

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EXECUTIVE SUMMARY

Sulfur mustard (Agent HD) is an alkylating chemical vesicant developed as a warfare agent that affects any epithelial surface it contacts. The active component is *bis*(2-chloroethyl)sulfide (CAS No. 505-60-2). Although the chemical is a liquid at ordinary ambient temperatures, its volatility results in rapid generation of vapors with a garlic-like odor. Due to its low aqueous solubility, it is persistent in the environment. Odor thresholds of 1 mg- min/m^3 and 0.6 mg/m^3 have been reported.

Exposure to sulfur mustard vapor may result in irritation and damage to the eyes, respiratory tract and skin. The toxic effects of sulfur mustard are temperature and humidity dependent; for a given exposure, the effects may be greater with increasing temperature and humidity. An exposure-dependent latency period of hours to days is documented for the toxic effects of sulfur mustard and is relevant for all routes of exposure but may be less for ocular and upper respiratory tract effects than for dermal and systemic responses. Both human and animal data indicate that the eyes are the most sensitive organ/tissue although deaths resulting from sulfur mustard exposure are likely the result of respiratory tract involvement. Because the toxic effects of sulfur mustard (at least for short time periods) appear to be a linear function of exposure duration and exposure concentration, most of the available exposure-response data are expressed as cumulative exposures (Ct).

Minor ocular irritation (conjunctival injection in the absence of irritation) is reported to occur in humans following exposures to 12-30 mg-min/m^3 and more severe effects at 60-75 mg-min/m^3 (conjunctivitis, irritation, photophobia) and 100 mg-min/m^3 (severe ocular irritation). Exposure estimates for human lethality range from 900-1500 mg-min/m^3.

Animal lethality following acute exposure to sulfur mustard occurs at cumulative exposures ranging from approximately 600-1500 mg-min/m^3. Nonlethal effects were similar to those observed in humans and included effects on the eyes, respiratory tract, and skin. Long-term exposure of dogs, rats, and guinea pigs to concentrations of 0.03 mg/m^3 produced only minor signs of ocular and respiratory tract irritation. One-hour exposure of mice to concentrations up to 16.9 mg/m^3 resulted in notable but not serious effects on respiratory parameters and acute exposures of rabbits (20 minutes to 12 hours) to concentrations ranging from 58 - 389 mg/m^3 (Ct $\geq$2,300 mg-min/m^3) resulted in severe respiratory tract damage.

Because exposure-response data were unavailable for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL values for the AEGL-specific time periods. The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5. Analysis of available data regarding AEGL-1 type effects reported by Reed (1918), Reed et al. (1918), Guild et al. (1941), and Anderson (1942) indicate that, for exposure periods up to several hours, the concentration-exposure time relationship is a near-linear function (i.e., Haber's Law where $n = 1$ for $C^n \times t = k$) as shown by n values of 1.11 and 0.96 for various data sets analyzed that were consistent with AEGL-1 effects. Therefore, an empirically derived, chemical-specific estimate of $n = 1$ was used for derivation of most of the AEGL values rather than a default value based upon the ten Berge (1986) analysis. Due to uncertainty regarding linear extrapolation to a time duration notably shorter than that for which empirically derived

lethality data were available , the 10-minute AEGL-3 values utilized exponential time scaling where *n* was 3.

The AEGL-1 values were based upon data from Anderson (1942) who found that an exposure concentration-time product of 12 mg-min/m^3 represented a threshold for ocular effects (conjunctival injection and minor discomfort with no functional decrement) in human volunteers acutely exposed to sulfur mustard. Uncertainty factor adjustment was limited to a factor of 3 for protection of sensitive individuals. This adjustment was considered appropriate for acute exposures to chemicals whose mechanism of action primarily involves surface contact irritation of ocular and/or respiratory tract tissue rather than systemic activity that involves absorption and distribution of the parent chemical or a biotransformation product to a target tissue. Anderson (1942) noted that there was little variability in the ocular responses among the subjects in his study, thereby providing additional justification for the intraspecies uncertainty factor of 3.

The AEGL-2 values for sulfur mustard were also developed using the data from Anderson (1942). Anderson reported that a Ct value of approximately 60 mg-min/m^3 represented the lowest concentration-time product for which ocular effects could be characterized as military casualties. The 60 mg-min/m^3 exposure was used as the basis for developing the AEGL-2 values because it represented an acute exposure causing an effect severe enough to impair escape and, although not irreversible, would certainly result in potential for additional injury. Anderson (1942) characterized the 60 mg-min/m^3 Ct as representing the lower margin of the concentration-effect zone that would result in ineffective military performance (necessary to complete a mission), and that may require treatment for up to one week. The ocular irritation and damage were also considered appropriate as a threshold estimate for AEGL-2 effects because the eyes are generally considered the most sensitive indicator of sulfur mustard exposure and would likely occur in the absence of vesication effects and severe pulmonary effects. The fact that the AEGL-2 is based upon human data precludes the use of an interspecies uncertainty factor. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to three under the assumption that the primary mechanism of action of sulfur mustard involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Anderson also noted little variability in the ocular responses among the subjects in his study. A modifying factor of 3 was applied to accommodate potential onset of long-term ocular or respiratory effects. This was justified by the fact that there was no long-term follow-up reported by Anderson with which to confirm or deny the development of permanent ocular or respiratory tract damage. The total uncertainty/modifying factor adjustment was 10*.

For development of the AEGL-3, a 1-hour exposure of mice to 21.2 mg/m^3 was used as an estimated lethality threshold (Kumar and Vijayaraghavan, 1998). This value is also near the lower bound of the 95% confidence interval for the 1-hour mouse LC_{50} of 42.5 mg/m^3 reported by Vijayaraghavan (1997). The intraspecies variability was limited to 3 because the lethality resulting from acute inhalation exposure to sulfur mustard appears to be a function of pulmonary damage resulting from direct contact of the agent with epithelial surfaces and would not likely exhibit an order-of-magnitude variability among individuals. An uncertainty factor of 3 was also applied to account for possible interspecies variability in the lethal response to sulfur mustard. The resulting total uncertainty factor adjustment was 10*. The modifying factor of 3 utilized for AEGL-2 development to account for uncertainties regarding the latency and persistence of the irritant effects of low-level exposure to sulfur mustard was not applied for AEGL-3 because lethality of the mice was assessed at 14 days post exposure in a study by Vijayaraghavan (1997). Application

of any additional uncertainty factors or modifying factors was not warranted because the proposed AEGL-3 values are equivalent to exposures in humans that are known to produce only ocular and respiratory tract irritation.

The AEGL values for sulfur mustard are based upon noncancer endpoints. Sulfur mustard is genotoxic and has induced carcinogenic responses in humans following single high exposures and following multiple exposures that were sufficient to produce adverse effects. Carcinogenic responses, however, are not known to occur with asymptomatic exposures. Limitations on the currently available data do not allow for a definitive quantitative cancer risk assessment, especially for an acute, once-in-a-lifetime, exposure.

The overall confidence in the AEGL values for sulfur mustard is medium. The AEGL-1 and AEGL-2 values are based upon human exposure data and are considered to be defensible estimates for exposures representing thresholds for the respective AEGL effect levels. The ocular irritation upon which the AEGL-1 and AEGL-2 values are based is the most sensitive response to sulfur mustard vapor. The AEGL-3 values provide Ct products (approximately 60-130 mg-min/m^3) that are known to cause only moderate to severe ocular irritation and possible respiratory tract irritation in human subjects but not life-threatening health effects or death. Although, the overall database for acute inhalation exposure to sulfur mustard is not extensive, the EGL values appear to be supported by the available data and in some cases, similar values obtained using somewhat differing approaches.

* The total adjustment is 10 because the factors of 3 each represent a logarithmic mean (3.16) of 10, therefore 3.16 x 3.16 = 10.

SUMMARY OF PROPOSED AEGL VALUES FOR SULFUR MUSTARD

Classification	10-min.	30-min.	1-hour	4-hour	8-hour	Endpoint (Reference)
AEGL-1	0.06 ppm 0.40 mg/m^3	0.02 ppm 0.13 mg/m^3	0.01 ppm 0.067 mg/m^3	0.003 ppm 0.017 mg/m^3	0.001 ppm 0.008 mg/m^3	Conjunctival injection and minor discomfort with no functional decrement in human volunteers (Anderson, 1942)
AEGL-2	0.09 ppm 0.60 mg/m^3	0.03 ppm 0.20 mg/m^3	0.02 ppm 0.10 mg/m^3	0.004 ppm 0.025 mg/m^3	0.002 ppm 0.013 mg/m^3	Well marked, generalized conjunctivitis, edema, photophobia, and eye irritation in human volunteers (Anderson, 1942)
AEGL-3	0.91 ppm 6.1 mg/m^3	0.63 ppm 4.2 mg/m^3	0.32 ppm 2.1 mg/m^3	0.08 ppm 0.53 mg/m^3	0.04 ppm 0.27 mg/m^3	Lethality estimate in mice (Kumar and Vijayaraghavan, 1998)

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1. INTRODUCTION

Sulfur mustard (Agent HD) is an alkylating chemical vesicant developed as a warfare agent that affects any epithelial surface it contacts. The active component is *bis*(2-chloroethyl)sulfide (CAS No. 505-60-2). Although the chemical is a liquid at ordinary ambient temperatures, its volatility results in rapid generation of vapors (see review by Watson and Griffin, 1992). Ambient temperature and humidity govern the degree of "casualty effect." Under hot and humid conditions, much lower mustard concentrations generate debilitating effects. Sulfur mustard has a garlic-like odor and, due to its low aqueous solubility, is persistent in the environment. Watson and Griffin (1992) have summarized information on the distribution of unitary chemical weapon stockpiles in the United States.

TABLE 1. PHYSICOCHEMICAL DATA FOR SULFUR MUSTARD

Synonyms	Agent HD; sulfur mustard; dichloroethyl sulfide; yperite; mustard gas; BIS(2-chloroethyl) sulfide; sulfide, BIS (2-chloroethyl); 1,1'-thiobis[2-chloroethane] ; yellow cross; LOST	DA, 1996; Budavari et al., 1989; Büscher, 1932
CAS Registry No.	505-60-2	Budavari et al., 1989
Chemical formula	$C_4H_8Cl_2S$	Budavari et al., 1989
Molecular weight	159.08	DA, 1996
Physical state	oily liquid	DA, 1996
Vapor pressure	0.072 mm Hg at 20°C 0.11 mm Hg at 25°C	DA, 1996
Density	5.4	DA, 1996
Boiling/melting point	215-217 °C/ 13-14 °C	DA, 1996; Budavari et al., 1989
Solubility	Sparingly soluble in water; soluble in organic solvents	DA, 1996; Budavari et al., 1989
Conversion factors in air	1 ppm = 6.49 mg/m^3 1 mg/m^3 = 0.15 ppm	
Odor threshold	1 mg-min/m^3 0.6 mg/m^3	Bloom et al. (1944) Dudley and Wells (1938); Bowden (1943); Fuhr and Krakow (1945)

2. HUMAN TOXICITY DATA

2.1 Acute Lethality

Either inhalation or percutaneous exposure to sulfur mustard vapor may result in lethality, although inhalation exposure is the more sensitive route. Estimates of human LCt_{50} values for agent vapor inhalation are several times lower than the estimated human percutaneous LCt_{50} (Robinson, 1967; DA, 1974). This contention is supported by animal LCt_{50} data (Robinson, 1967; DA, 1974; Watson and Griffin, 1992). Human lethality data are available only as estimates

attained by extrapolation from animal data. The estimated human LCt_{50} values in current use by the U.S. Department of the Army are 1,500 mg-min/m^3 and 10,000 mg-min/m^3, respectively, for inhalation and percutaneous vapor exposure (DA, 1974; NRC, 1997).

Although lacking quantitative exposure terms, Warthin and Weller (1919) provided qualitative clinical information regarding two fatalities resulting from sulfur mustard exposures during manufacture of the agent. Both men were wearing gas masks so ocular involvement was inconsequential but the exposure concentrations were high enough to result in severe burns. Within hours, both victims exhibited lesions about the lips and necrotic lesions in the mouth and nasopharyngeal region. By seven to eight days, there was evidence of more severe respiratory involvement as demonstrated by moist rales and physical signs indicative of bronchopneumonia. One victim died eight days after the accident and the other four weeks after the exposure.

Between 1919 and 1923, site remediation and scrap metal recovery operations at a vast (25 square miles) "gas dump" at Breloh, near Munster in what is now Lower Saxony, resulted in numerous cases of occupational exposure to warfare agents either manufactured or captured by German forces during World War I (Büscher, 1932). Thousands of tons of German and captured "gas" munitions as well as tank cars and storage buildings containing sulfur mustard and other chemical warfare agents were involved. Summary reports for the years 1920-1923 by the site primary care physician documents "two or three" fatalities among workmen who had received concentrated sulfur mustard vapor exposures to the skin, eyes, and respiratory tract in combination. In these cases, "death came very soon" (Büscher, 1932). Büscher (1932) was not equipped to gather source term information for any of these fatal episodes.

Estimated lowest lethal doses of 150 mg/m^3 (10 minutes) and 70 mg/m^3 (30 minutes) have been reported (Back et al., 1972; Inada et al., 1978). However, these values are not based on definitive exposure values or controlled exposure conditions.

Available hospital records from World War I and sketchy casualty reports from the Iran-Iraq conflict indicate mortality rates of 1-3% from acute sulfur mustard exposure (Blewett, 1986; Dunn, 1986). Actual battlefield concentrations to which victims were exposed have not been reported, but may well have been in excess of 1500 mg/m^3 (Watson and Griffin, 1992).

Human lethalities were reported by a number of European physicians asked to provide humanitarian treatment for war gas casualties arising from the Iran-Iraq conflict. Eisenmenger et al. (1991) treated sulfur-mustard exposed Iranian patients in a German hospital; one patient admitted in a semiconscious state with serious exfoliative lesions five days post exposure died during treatment. Other Iranian soldiers exhibiting the characteristic burns, edema, and damage to the respiratory tract associated with battlefield exposures to sulfur mustard died from various combinations of respiratory insufficiency and infection between 5 and 36 days post-exposure (1 on Day 7, 3 on Days 12-15, 1 on Day 36; N=5)(D'Halluin and Roels, 1984; Mandl and Frielinger, 1984). Sulfur mustard agent is a known immunosuppressant (IOM, 1993). No exposure terms for any of these wartime cases were available, however.

In an effort to establish updated toxicity estimates for humans, the U.S. Army Chemical Defense Equipment Process Action Team (CDEPAT, 1994) developed a revised estimated LCt_{50} of 900 mg-min/m^3 for human inhalation exposure from an average of animal LCt_{50} data. The National Research Council Committee on Toxicology (Subcommittee on Toxicity Values for Selected

Nerve and Vesicant Agents) concluded that the 900 mg-min/m^3 estimate was scientifically valid (NRC, 1997). CDEPAT developed this estimate based upon an average of animal LCt_{50} data.

2.2 Nonlethal Toxicity

Clinical presentation in humans following acute exposure to sulfur mustard vapor may involve dermal, ocular, and respiratory tract effects, all of which are preceded by a latency period dependent upon the exposure concentration and exposure duration (Eisenmenger et al., 1991). Systemic effects (nausea, vomiting, abdominal pain, headache, weight loss, hematopoietic effects) may also occur as a result of gastrointestinal involvement or deep penetration dermal involvement (Büscher, 1932). The eye appears to be the most frequently affected and most sensitive organ and also has one of the shortest latency periods (Warthin and Weller, 1919; Papirmeister et al., 1991). Latency periods vary with changes in exposure parameters but tend to be several hours to days for dermal effects, 2-8 hours for ocular effects, and several hours for upper respiratory tract effects (up to several days for progression to full severity respiratory tract involvement). Studies involving controlled exposure to human volunteers as well as studies on war casualties and occupational exposures are available; the latter providing clinical information but lacking quantitative exposure data.

Controlled, human clinical trials conducted by Büscher (1932) to better define treatment regimens were confined to "drop" tests of sulfur mustard on various skin sites, with observation of the time course under differing decontamination protocols. Inhalation exposures occurred to Breloh "gas dump" site workers as a consequence of munition explosions, inhalation of smoke plumes generated during primitive "bonfire" heat cleaning of contaminated metal scrap, off-gassing of contaminated clothing in warm rooms, and the use of contaminated wood scraps as heating fuel in winter quarters. Büscher (1932) described the clinical course of respiratory effects and their treatment, but does not present dose-response data.

Reed (1918) conducted preliminary experiments in which he and another volunteer participated in exposure chamber experiments at a sulfur mustard concentration of 0.0012 mg/L (1.2 mg/m^3); mustard was generated as a spray in absolute ethanol for 45 minutes in a 10,000 L chamber. The subjects were clad in ordinary khaki uniforms, without blouses, and with no facial protection. A slight odor was initially detected but the olfactory response accommodated within three minutes for one subject and eight minutes for the other. Slight irritation of the mucosa of the nose and nasopharyngeal regions occurred at eight minutes and progressed in severity such that, at 20 minutes, one individual previously determined to be sensitive to HD on the basis of skin tests withdrew from the exposure chamber. At 25 minutes, the remaining subject experienced heavy eyelids and "huskiness" of the voice but no coughing or sneezing. At three to six hours after the 45-minute or 20-minute exposure, respectively, a sudden and severe conjunctivitis developed that was accompanied by photophobia and blepharospasm. By 12 hours post exposure, vision was severely impaired, and severe pain and rhinitis was experienced for 30 hours. These effects were somewhat less severe in the subject originally classified as more sensitive. Conjunctival injection did not resolve for over a month. At three days post exposure, intense pruritus and erythema developed over the neck, shoulders, upper arms and trunk that began abating after seven days. Ocular hypersensitivity and exercise-induced dermal wheals occurred for weeks after the exposure.

Reed (1918) then conducted additional experiments using lower sulfur mustard concentrations. In these experiments, one to six volunteers were exposed to various low concentrations of sulfur mustard (0.0001 - 0.0043 mg/L, nominal; equivalent to 0.1-4.3 mg/m^3) for time periods of 5 - 45 minutes. The exposure atmospheres were generated by slowly spraying sulfur mustard in absolute alcohol and continual mixing of the air with an electric fan. Subsequent investigations revealed that the actual exposure concentrations were $\leq$60-70% of nominal, although Reed (1918) freely admitted that "it is impossible to state what the actual concentration was" due to analytical limitations of the time. It is assumed from context that these volunteers were clothed similarly to those in initial trials (e.g., khaki uniforms without blouses) and wore no facial protection during the period of exposure. Of the 22 men participating in this series (see Table 2), a majority had been previously exposed to sulfur mustard, and 12 had sustained "one or more burns" either experimentally or accidentally (Reed, 1918). The most prominent effect of the controlled atmospheric exposures was ocular irritation (conjunctival injection, conjunctivitis, photophobia) which varied with exposure concentration, duration, and among individuals. The results of these experiments are summarized in Table 2.

Additional research was conducted by Reed et al (1918) which utilized improved methods (hydrogen ion method) for measurement of exposure concentrations. To minimize hydrolysis, the HD was again delivered in absolute alcohol.

Walker et al. (1928) reported that of seven men exposed to sulfur mustard at 0.001 mg/L (1 mg/m^3) for 5-45 minutes, four showed conjunctivitis and two exhibited skin burns. It was also reported that of seventeen men exposed to 0.0005 mg/L (0.5 mg/m^3) for 10-45 minutes, six exhibited conjunctivitis , one had a skin burn, and three of 13 men exposed for 10-30 minutes to 0.0001 mg/L (0.1 mg/m^3) showed slight but distinct conjunctivitis.

Guild et al. (1941) conducted experiments using human volunteers exposed to sulfur mustard at varying acute exposure regimens. The sulfur mustard vapor was generated by heat volatilization in the 100 m^3 exposure chamber. The subjects were male soldiers and officers, and one civilian who had not had previous exposure to sulfur mustard. All subjects wore paint or "dope" spray respirators "to protect the lungs" (Guild et al, 1941). For each of the tests, 2-6 individuals were exposed. Guild et al. concluded that Ct is constant for ocular effects for exposure periods of 2 minutes to 20 hours and for a range of sulfur mustard concentrations of 0.07 - 65 mg/m^3. Based upon the results of the experiments, it was reported that exposure to Ct values <70 $mg\text{-}min/m^3$ would result in mild conjunctival responses that would not be indicative of a casualty (defined by the authors as temporary loss of vision), Ct values

TABLE 2. EFFECTS OF ACUTE EXPOSURE TO SULFUR MUSTARD (AGENT HD) IN HUMAN VOLUNTEERS (REED, 1918) [a]			
Nominal Conc. (mg/m^3)	**Exposure Duration (min)**	**No. of Subjects**	**Results**
0.1	10	6	No detectable effect
0.1	15	2	½ Slight conjunctival injection
0.1	30	5	1/5 Marked bilateral conjunctival injection 1/5 Slight conjunctival injection
0.5	10	5	2/5 Conjunctival injection
0.5	15	3	1/3 Slight conjunctival injection
0.5	30	8	1/8 Conjunctivitis, rhinitis 1/8 Severe conjunctivitis, marked skin burn 1/8 Marked conjunctivitis, slight facial burn
0.5	45	1	No effect
1.0	5	1	1/1 Marked conjunctivitis, photophobia, rhinitis, laryngitis, pulmonary congestion
1.0	10	2	½ Slight conjunctivitis
1.0	15	2	No effect
1.0	20	1	1/1 Severe conjunctivitis, severe skin burns
1.0	45	1	1/1 Very severe conjunctivitis, photophobia, skin burns, mucosal exfoliation in nasopharynx
2.6	5	1	No effect
4.3	10	1	1/1 Marked conjunctivitis, no pain

[a] Unprotected face assumed from context

of 70-100 mg-min/m^3 would produce some casualties, and Ct values > 100 mg-min/m^3 would be expected to produce disabling ocular effects of several days' duration. In the military context of this study, Guild et al (1941) defined "disablement" as "injury sufficient to prevent troops from taking an active part in operations for 1-2 weeks." Because the subjects wore respiratory protection, effects on the respiratory tract could not be determined and were not reported.

In a study reported by Anderson (1942) and performed as a follow-up to the Guild et al (1941) recommendation to replicate the earlier Guild experimental design under tropical conditions, three to four human volunteers were exposed to each of several concentration-time regimens of agent HD "under Indian hot weather conditions." Sulfur mustard vapor was generated by heat volatilization in a 50 m^3 exposure chamber; mixing was accomplished by use of an electric fan in the chamber. Subjects included both British and Indian troops without respiratory protection, and who wore tropical service dress of drill shorts and open-necked cotton shirts. To minimize off-gassing exposure, subjects bathed and dressed in clean clothing upon completion of each experiment. Eyes of each subject were examined prior to the first expermimental exposure; the author noted that a certain degree of fine conjunctival injection was a normal baseline condition for a large proportion of persons living in India at that time. Allowance was thus made for this baseline condition in assessing post-exposure effects to sulfur mustard vapor. Effects on the respiratory tract were not reported.

Anderson (1942) determined HD concentrations by use of the gold-benzidine method and analyzed in a "Spekker photoelectric absorptiometer". In an analysis of these data and cross-comparison with the temperate-zone results of Guild et al (1941), Anderson determined that comparable eye effects of a particular degree of severity are usually produced at a lower Ct under tropical conditions. Anderson found that an exposure concentration-time product of 30 mg-min/m^3 represented the upper range for mild effects with no disability (conjunctival injection and minor discomfort with no functional decrement). Ct products slightly higher than this (e.g., 34-38.1 mg-min/m^3) were, however, also without appreciable casualty effects. A concentration-time product of 12 mg-min/m^3 value was noted by Anderson (1942) as representing the limit for ocular effects as characterized by conjunctival injection in the complete absence of irritation. Ct values of 60-75 mg-min/m^3 were considered a danger zone for widespread conjunctivitis frequently accompanied by chemosis, photophobia, and irritation. At Ct values of 75-90 mg-min/m^3, more severe ocular effects would be expected to the extent that several weeks' treatment would be necessitated in a high proportion of those so exposed. At Ct values $\geq$>100 mg-min/m^3, a 100% casualty rate (as determined by militarily disabling ocular effects) would be expected. The results of these experiments are summarized in Table 3.

Please note that the longest reported period of follow-up in the Anderson (1942) study was 36 days post-exposure for a case requiring infirmary treatment and exhibiting conjunctivitis, photophobia, and injection with corneal injury. By discharge on Day 36, both eyes were reported as "normal." It is observed that, during the 1940's, it was common practice to employ minimal long-term medical follow-up in studies of military personnel experimentally exposed to chemical warfare agents (IOM, 1993). Short-term casualty effects were the primary focus of military investigators at the time.

The penetration of sulfur mustard vapor into human skin was studied by Nagy et al. (1946) in an attempt to more fully understand the relationship between dermal penetration rate and severity of toxicity. Using a carefully designed and tested technique and human volunteers, the penetration rate of sulfur mustard for human skin was determined. The application times studied using human skin were 3, 6, and 10-minute exposures. A saturated atmosphere (under an application cup) of sulfur mustard was applied to a 1.3 cm^2 area of the flexor aspect of the forearm; lesions were evaluated at 48 hours after the exposure. It was found that an increase in temperature (from 21-23°C to 30-31°C) produced an increase in the penetration rate from 1.4 $\mu g/cm^2$/min to 2.7 $\mu g/cm^2$/min.

TABLE 3. EFFECTS OF ACUTE EXPOSURE TO SULFUR MUSTARD (AGENT HD) IN HUMAN VOLUNTEERS (ANDERSON, 1942) [a]

Mean Conc. (mg/m^3)	Exposure Duration (min)	No. of Subjects	Cumulative Exposure Ct (mg-min/m^3)	Results
6.25	2	4	12.5	3/4-- Band of fine injection across exposed bulbar conjuntiva; 1/4--trace angular conjunctivitis. All non-casualties
7.0	3.3	4	23.1	3/4Obvious band of injection across exposed bulbar conjuntiva; 1/4--angular conjunctivitis. All non-casualties

TABLE 3. EFFECTS OF ACUTE EXPOSURE TO SULFUR MUSTARD (AGENT HD) IN HUMAN VOLUNTEERS (ANDERSON, 1942) [a]				
Mean Conc. (mg/m^3)	**Exposure Duration (min)**	**No. of Subjects**	**Cumulative Exposure Ct ($mg\text{-}min/m^3$)**	**Results**
10.0	2.75	3	27.5	2/3–Mild injection band over exposed sclera; 1/3--band of injection with slight discomfort. All non-casualties
6.8	5	3	34.0	3/3–Well-marked injection of conjunctivae; slight edema in 1/3; all complaining of eye soreness. Injection visible in 1/3 at 14 days post-exposure. All non-casualties.
12.7	3	3	38.1	3/3–Band of conjunctival injection over exposed sclera; no discomfort. All non-casualties.
12.6	3.3	3	41.8	3/3–Effects slightly more marked than in previous expt; mild discomfort in 1/3. All non-casualties.
11.0	4	3	44.0	3/3--Moderate injection of exposed bulbar conjunctiva and lower lids (to a lesser degree); 1/3--slight edema; 1/3--complained of sore eyes in first 24 hrs. All non-casualties.
7.6	6	4	45.6	3/4–Widespread conjunctivitis involving lids and bulb. 1/4--exhibiting trace chemosis; 1/4--slight photophobia on Days 2 and 3; 1/4--moderate band of injection. All complaining of discomfort.
13.0	3.75	3	48.8	3/3–Widespread moderate injection of conjunctiva; 1/3--slight discomfort; 1/3--transient edema. All non-casualties.
10.5	4.75	3	49.8	3/3–Well-marked injection of lids and exposed conjunctiva; 2/3--discomfort. All non-casualties.
2.5	20	3	50.0	3/3--band of moderate injection over exposed part of sclera; 2/3--slight soreness
10.6	5	2	53.0	2/2--widely generalized conjunctival injection visible after 14 days; 1/3--complaining of sore eyes. All non-casualties.
15.6	3.5	1	54.6	Band of injection across exposed part of sclera; slight conjunctival injection; soreness in one eye. Non-casualty.

TABLE 3. EFFECTS OF ACUTE EXPOSURE TO SULFUR MUSTARD (AGENT HD) IN HUMAN VOLUNTEERS (ANDERSON, 1942) [a]				
Mean Conc. (mg/m^3)	**Exposure Duration (min)**	**No. of Subjects**	**Cumulative Exposure Ct ($mg\text{-}min/m^3$)**	**Results**
5.8	9.5	4	55.1	1/4--casualty; wide and intense redness over entire conjunctiva, slight photophobia, moderate chemosis and blepharospasm. 3/4--just short of casualty with widespread conjunctival injection, slight edema and mild photophobia in first 24 hrs, sore eyes for 2-3 days.
14.0	4.0	3	56.0	3/3--well-marked and widespread conjunctival injection, discomfort. All non-casualties.
1.7	33	3	56.1	3/3--fine injection band over exposed sclera. All non-casualties.
2.9	20	3	58.0	3/3--moderate and generalized conjunctival congestion; 1/3-mild discomfort. All non-casualties.
4.5	13.5	3	60.7	3/3--band of moderate injection over exposed sclera; 1/3--reported headache on Day 1 and later developed generalized urticaria. All non eye casualties.
13.7	4.75	3	65.0	2/3--widespread conjunctival injection, slight edema and mild discomfort; 1/3--severe injection of conjunctiva, well developed edema, very near casualty, severe urticarial reaction first day post-exposure, positive reaction to 1:25,000 sulfur mustard after 1 month.
5	14	3	70	2/3--well marked and generalized conjunctivitis with edema, photophobia, lacrimation and blepharospasm. Sore eyes and frontal headache, casualties up to 1 week. 1/3--intense congestion of entire conjunctiva, lacrimation, chemosis and photophobia

TABLE 3. EFFECTS OF ACUTE EXPOSURE TO SULFUR MUSTARD (AGENT HD) IN HUMAN VOLUNTEERS (ANDERSON, 1942) [a]				
Mean Conc. (mg/m^3)	**Exposure Duration (min)**	**No. of Subjects**	**Cumulative Exposure Ct ($mg\text{-}min/m^3$)**	**Results**
15.6	4.5	2	70.2	½--injection of lids, well-marked band of injection across exposed sclera, soreness up to Day 3, non casualty. ½ --severe conjunctival injection, slight hazing of cornea with photophobia and soreness up to Day 3 post-exposure, lacrimation and slight interference with vision, casualty requiring 4-5 days treatment.
4.7	15	3	70.5	3/3--lids injected, well-marked and generalized conjunctivitis with edema, photophobia and eye soreness; 1/3-- headache. All near casualties requiring 3-5 days treatment

[a] No respiratory protection worn during exposure periods

Moore and Rockman (1950) studied variability in hypersensitivity reactions to sulfur mustard using human volunteers. A single drop (4.5 ± 0.22 mm^3) of various dilutions of purified sulfur mustard (1:500 to 1:8000 in petroleum ether) was applied to each subject's volar forearm. The test area was examined at 24, 48, and 72 hours and a description of the reaction was recorded. About 25% of those given two exposures to sulfur mustard with a week interval exhibited a flare response at the first site even when the second application was at a different site (e.g., opposite arm). Although a conversion of this exposure regimen to an ambient concentration equivalent was not feasible, the results of the study provide evidence of possible sensitization to sulfur mustard dermal exposure. Similar findings are reported in Büscher (1932) and IOM (1993).

Warm, moist, anatomical areas such as the axillae and groin are especially susceptible to sulfur mustard vapor injury (IOM, 1993).

Eisenmenger et al. (1991) reported on the clinical and morphologic findings from eleven Iranian patients exposed to sulfur mustard during the Iran-Iraq conflict and treated in a German hospital. Quantitative exposure data are lacking for these case reports but the information provides a clinical picture of the progression of sulfur mustard lesions. Upon admittance to the hospital (4-6 or 17 days after exposure), all patients exhibited conjunctivitis with some also exhibiting erosions and slight corneal opacity, and reddened, blistered skin. The severity of respiratory tract involvement tended be concentration-dependent, with only upper respiratory tract involvement at lower concentrations. The most serious respiratory effects were observed at 14 days post exposure. One patient admitted in a semiconscious state with serious exfoliative lesions five days post-exposure died, and several others would likely have died without medical intervention. No follow-up study was performed on these patients. Although lacking quantitative data useful for developing AEGL values, this clinical report provides qualitative information regarding human

exposure to sulfur mustard and indicates that effects observed in humans are similar to those observed in animals

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

2.3 Epidemiologic Studies

Emad and Rezaian (1997) conducted a cross-sectional clinical study of late pulmonary sequelae exhibited by 197 Iranian military veterans 10 years after receiving a single, high-concentration sulfur mustard exposure in 1986 during the Iran-Iraq conflict. A control group consisted of 86 nonexposed veterans. In 1986, exposure to sulfur mustard had been initially confirmed at hospital admission by urine and vesicular fluid analysis (by the method of Heyndrickx et al, 1984), and by presentation with respiratory symptoms that included rhinorrhea, sore throat, hoarseness, cough, chest tightness, and dyspnea. Participants were screened for asthma and prior exposures to environmental agents known to cause interstitial lung disease or extrinsic allergic alveolitis. Additionally, participants had not been allowed to have jobs that might have created interference with the study (e.g., woodworking, milling, welding, farming, sculpturing, painting, fire fighting, baking) since 1986. The incidences of asthma (10.65%), chronic bronchitis (58.88%), bronchiectasis (8.62%), airway narrowing due to scar or granulation tissue (9.64%), and pulmonary fibrosis (12.18%) in the sulfur mustard-exposed group were all greater than that found in the control group (0% in all categories except for one case of bronchitis [1%]). The investigators concluded that the exposure to the clinically significant sulfur mustard concentrations created greater potential for development of chronic destructive pulmonary sequelae. The authors further conclude that the relatively low incidence of pulmonary fibrosis results from the fact that the largest proportion of mustard agent was absorbed in the upper airways rather than the alveoli. Further, no bronchial carcinoma or lung malignancy has been observed to date in this group of veterans (Emad and Rezaian, 1997).

2.4 Developmental/Reproductive Toxicity

There are no data currently available regarding the developmental/reproductive effects of inhaled sulfur mustard toxicity in humans.

2.5 Genotoxicity

IARC (1975), Fox and Scott (1980), ATSDR (1992), Papirmeister et al. (1991), and Watson and Griffin (1992) have summarized the available evidence concerning the genotoxicity of sulfur mustard. Because sulfur mustard is a strong DNA alkylating agent, genotoxic effects occur through cross-link formation, inhibition of DNA synthesis and repair, point mutations due to replication or repair errors, chromosome breaks, and chromatid aberrations. Some of these conditions have been observed in humans following exposure to sulfur mustard, others have occurred in various test systems including bacteria, yeast, insects, and mammalian cell cultures.

Retrospective studies have been conducted on Japanese workers who had been employed at a chemical agent manufacturing plant from 1929 to 1945. Although sulfur mustard was the main product of the facility, lewisite, diphenylarsine, hydrocyanic acid, phosgene, and chloroacetophenone were also produced there (Inada et al., 1978), and it is not known to what degree these other chemicals contributed to the observed effects. In one study of these workers, Yanagida et al. (1988) found that the frequency of mutations to hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) deficiency in 28 exposed individuals was significantly elevated when compared with two control groups matched for age and smoking status. One control group consisted of healthy men and the other of individuals with bronchitis. The data also showed that the mutations were significantly more frequent in those workers who had longer exposures. A chromosome study of 16 former workers of this same factory indicated a significantly higher incidence of sister chromatid exchanges (SCE) in peripheral lymphocytes when compared with a control group ($p < 0.03$) (Shakil et al., 1993). Two individuals with chronic myelocytic leukemia had an almost three-fold higher SCE rate than controls and also a high (12.1%) incidence of chromosome abnormalities (Shakil et al., 1993). In an evaluation of the p53 mutations found in lung tumors of these workers, Takeshima et al. (1994) found that the mutations were similar to those in lung tumors of tobacco smokers (the factory workers were also tobacco smokers), however, the prominence of G: C to A: T transitions and the occurrence of double mutations in two of twelve cases suggested that exposures in the chemical agent manufacturing plant did contribute to the development of the lung cancers.

Yamakido et al. (1985) studied the potential genotoxicity of sulfur mustard in children of workers previously exposed at a Japanese poison gas factory. This study utilized general health exams in conjunction with one-dimensional electrophoretic analysis of blood protein variants to identify gene mutations. Although variants were detected, the investigators considered the results inconclusive as to the potential genotoxicity of sulfur mustard in humans due to the small size of the population sampled.

Wulf et al. (1985) reported significant ($p < 0.001$) increases in sister chromatid exchanges in lymphocytes of eleven fisherman who had accidentally been exposed to sulfur mustard in sufficiently high concentrations to cause signs of acute toxicity. These fisherman had received contact exposure to sulfur mustard from nets deployed in areas where WW II-era munitions had been dumped at sea.

Cytometric analysis of DNA damage was shown for cultured human epithelial cells exposed to sulfur mustard (Emison and Smith, 1997). The cell cycle was found to be blocked at the G1-S interface at concentrations equivalent to a vesicating in vivo dose (>100 μM) and is blocked in the G2 phase at concentrations below a vesicating equivalent concentration. At concentrations of 3

μM, the cell cycle was initially blocked at G2/M but the cells recover normal cell cycle progression. Quantitation of DNA strand breaks was possible at concentrations equivalent to both vesicating and nonvesicating exposures.

2.6 Carcinogenicity

Studies evaluating workers occupationally exposed to sulfur mustard indicate elevated risks of respiratory tract and skin tumors after long-term exposure. Genotoxicity and animal carcinogenicity data, as well as information characterizing the alkylating properties of sulfur mustard, provide supporting evidence for the carcinogenicity of this chemical warfare agent in humans.

The International Agency for Research on Cancer (IARC) classified sulfur mustard as a Group 1 compound (carcinogenic to humans) (IARC, 1987), and the National Toxicological Program (NTP) first categorized "mustard gas" as a substance “known to be a human carcinogen” in its *First Annual Report on Carcinogens, 1980*. Mustard gas is still listed in the same category in the *Eighth Report On Carcinogens, 1998* (http://ntp-server.niehs.nih.gov/NewHomeRoc/CurrentLists.html). The State of Maryland also considers "mustard gas" as a "known human carcinogen" (a Class I.A. Toxic Air Pollutant as defined by the Code of Maryland Regulations, CMR Title 26 Subtitle 11, as amended).

IARC (1975), Waters et al. (1983), Watson et al. (1989), and the IOM (1993) summarized the epidemiological evidence concerning the potential carcinogenicity of sulfur mustard in humans. These data are primarily from studies of soldiers exposed during World War I and from studies of workers at chemical warfare agent manufacturing facilities.

Individual case studies of WW I veterans include those of Case and Lea (1955) and Beebe (1960). Case and Lea (1955) reported that the mortality ratio (2.07)of 1267 WW I United Kingdom veterans indicated a highly significant elevated risk for respiratory tract neoplasms ($p < 0.01$). A similar tumor incidence rate and mortality ratio (2.01) were found in a population of veterans who had never been exposed to mustard but who were suffering from bronchitis. Case and Lea (1955) concluded that the evidence did not support the view that sulfur mustard was a direct carcinogen. Beebe (1960) evaluated the occurrence of respiratory tract cancers among a group of 2718 American soldiers exposed to sulfur mustard during World War I and found that the ratio of observed to expected cases was 1.47 (based on U.S. mortality rates) compared with 1.15 for wounded soldiers not exposed to sulfur mustard, and 0.81 for soldiers who had pneumonia, but who had not been exposed to mustard. Norman (1975) evaluated the same group of soldiers after a 10-year follow-up period (study completed in 1965) and found that the exposed men had a 40% excess of lung cancer mortality, with an estimated relative risk of 1.3 (95% confidence limits of 0.9-1.9) compared with a control group consisting of wounded soldiers without exposure to mustard. The latency period was estimated to be 22-37 years. Norman (1975) further concluded that there was no evidence in this limited data set that mustard exposure and cigarette smoking had a synergistic effect on lung cancer mortality.

Retrospective studies of Japanese workers who had been employed at a chemical warfare agent manufacturing plant from 1929 to 1945 have revealed that these individuals have an increased risk of developing respiratory tract cancers (see Yamakido et al., 1996, for most recent review). Although sulfur mustard was the main product of the facility, lewisite, diphenylarsine, hydrocyanic

acid, phosgene, and chloroacetophenone were also produced (Inada et al., 1978). The concentration of mustard in the workplace was estimated to be as high as 50-70 mg/m^3 (Nakamura, 1956), and workers frequently exhibited signs of mustard toxicity during the period of agent manufacture; these signs included acute conjunctivitis, acute rhinitis, acute bronchitis, and acute dermatitis with blister formation. Studies completed in the 1950's documented individual cases of bronchial and laryngeal carcinoma in this population of workers (Yamada et al., 1953, 1957, 1963) and an elevated incidence of deaths due to cancers of the respiratory tract and oropharynx (16.3% vs 0.4% in non-exposed inhabitants from the same geographic area). Elevated mortality rates among the former factory workers due to respiratory tract cancer was later confirmed by Wada et al. (1968). Neoplasms occurred in the tongue, pharynx, sphenoidal sinus, larynx, trachea, and bronchi; only one occurred peripherally in the lung. The median length of employment at the chemical warfare agent manufacturing facility was 7.4 years, and the median interval between first employment and death from cancer of the respiratory tract was 24.4 years (Wada et al., 1968).

Additional studies of this population of workers were conducted by Nishimoto et al. (1988) who incorporated histopathological and mortality data gathered between 1952 and 1986. For 1632 of these workers, the overall standardized mortality ratio (SMR) for respiratory tract tumors was 3.9 (70 observed vs. 17.8 expected, $p < 0.001$, based on data for the Japanese male population) and the overall SMR for all malignant tumors was 1.2 (173 observed vs. 142 expected, $p < 0.01$). Age-adjusted SMRs for total malignancies, respiratory tract tumors, and gastrointestinal tract tumors showed significantly higher SMRs for the age-groups from 40 to 80 years. Nishimoto et al. (1988) also found that the SMR was about 2.7 for individuals who had worked at the factory 0.5 to 5 years, but 7.17 for individuals who had been employed for more than 5 years. The SMR was not significantly elevated for individuals who had worked at the factory for 7 months or less.

Data on this same group of workers followed up to 1992 has been summarized by Yamakido et al. (1996). The results do not differ substantially from those of Nishimoto et al. (1983, 1988).

Of 488 former workers who received dermatological examination, 115 had abnormal pigmentation and 22 had skin tumors of which 8 were cases of Bowen's disease (intra-epidermal squamous cell carcinoma) (Inada et al., 1978). Hyperkeratotic skin lesions such as Bowen's disease, basal cell carcinomas, and hyperkeratotic papular eruptions, were present in 14 cases out of 109 engaged only in mustard production and in 1 case out of 16 engaged only in lewisite production. No abnormalities were observed in 77 former factory workers who had no exposure to chemical agents (Inada et al., 1978). It was also observed that the longer an individual had been exposed to mustard, the more marked the skin lesions tended to become (Inada et al., 1978).

The studies of Yamakido et al. (1996), Nishimoto et al. (1988), Yamada (1974) and Inada et al., (1978) provide strong evidence for a causal link between chemical agent exposure and cancer of the respiratory tract; however, because the workers were potentially exposed to lewisite as well, it is not possible to state conclusively that the cancers were due solely to sulfur mustard. Furthermore, it should be noted that several possible confounding factors, such as tobacco smoking habits, pre-existing health conditions, and post-exposure occupational histories of the workers, were not evaluated. In addition, the SMR may not provide a good estimate of cancer risk, because it does not take into account the impact of medical intervention and social/economic factors that can affect survival rates.

Weiss and Weiss (1975) conducted studies evaluating the health of 271 workers employed for varying lengths of time between 1935-1945 at a munitions depot where the production, testing and destruction of sulfur and nitrogen mustard (as well as bromoacetone, phosgene, chloropicrin and organic arsenicals) had occurred. Ninety percent of the group had chronic health problems and 114 had died by the end of 1974. Thirty-five percent died from cancer, of which 38% were bronchial cancers. The total number of deaths from cancer was significant ($p < 0.01$) and the number of bronchial cancers was also significant (11 observed vs. 5 expected for the population of the geographic region where the facility was located). The number of cancers of the gastrointestinal tract was 35% greater than expected. The average tumor induction time was 21.6 years. IARC (1975) noted that the study was limited to workers with available medical records, which "raises the possibility that the proportion with cancer may have been inflated, since medical records or autopsy records would more likely have been preserved for workers with cancer". Furthermore, IARC (1975) does not mention whether Weiss and Weiss (1975) accounted for smoking habits and other confounding factors.

According to Klehr (1984), German workers involved in the dismantling of a sulfur mustard facility developed multiple skin lesions including basal cell carcinomas, Bowen's disease, Bowen's carcinomas, and carcinoma spinocellulare. The incidence rate for all tumors (including skin tumors) was 34% in 53 workers evaluated.

Manning et al. (1981) evaluated the incidence of cancer among former workers of a British mustard manufacturing facility (1939-1945). As of 1974, the number of deaths from all neoplasms combined (45) was slightly greater than that expected from national death rates, but the increase was not statistically significant. In follow-up investigations of this cohort, Easton et al. (1988) evaluated the mortality records of 3354 workers and found greater numbers of cancer deaths when compared to national mortality rates. Significant increases were observed in deaths from cancer of the larynx, pharynx, and all other buccal cavity and upper respiratory sites combined. There were also elevated numbers of deaths from lung cancer compared with those expected ($p < 0.001$). It was also reported that the risks of developing cancer of the lung and pharynx were significantly related to the duration of employment. Significant excess mortality was also observed for cancers of the esophagus and stomach but there was no correlation with time since first exposure or duration of exposure.

Manning et al. (1981) concluded that it was very likely that the observed cancers of the pharynx, larynx and other upper respiratory sites were due to exposure to sulfur mustard because the excesses were too large to be accounted for by confounding factors (the effects of smoking, however, were not evaluated), increased with increasing duration of employment, and were limited to the period more than 10 years after first employment. Evidence for a causal relationship between sulfur mustard exposure and other cancers, including lung cancer, was not considered to be as strong.

Although a large number of American military personnel were exposed to sulfur mustard in chamber and field tests conducted during World War II, the morbidity and mortality records of this cohort have not been adequately evaluated to document long-term health risks (IOM, 1993).

Evaluations of available human and lab animal data sets have resulted in numerous estimates of a slope factor for sulfur mustard (McNamara et al, 1975; NRC, 1999; Rosenblatt, 1987; USACHPPM and the Toxicology and Risk Analysis Section, 1999; USEPA, 1991; Watson et al,

1989). The slope factor range documented in this literature is 1.6 to 7.7 per (mg/kg)/day for oral cancer potency. Values of inhalation unit risk may be calculated from these slope factors; the resulting estimates vary according to the exposure assumptions made.

2.7 Summary

Human data regarding nonlethal effects of sulfur mustard are available from studies using volunteer subjects. Qualitative descriptions of the clinical presentation of injury following exposure to sulfur mustard vapor are also available for war casualties and occupational exposures. Lethality data for humans are not available but LCt_{50} values have been estimated based upon extrapolation from animal data.

The available data suggest that the location and severity of damage resulting from exposure to sulfur mustard are concentration-dependent and a function of the highly reactive nature of sulfur mustard (Papirmeister et al., 1991). Ocular surfaces appear to be a sensitive, rapidly responding target (Reed, 1918; Reed et al., 1918; Anderson, 1942) . At low exposures, sulfur mustard-induced injury appears to be limited to the upper respiratory tract (Eisenmenger et al., 1991) and eyes (Reed, 1918; Reed et al., 1918; Anderson, 1942). Anderson (1942) considered Ct values of 60-75 mg-min/m^3 as representing exposures that would result in conjunctivitis, photophobia, and ocular irritation, while Ct values of 75-90 mg-min/m^3 would cause a high proportion of casualties as determined by more severe ocular damage requiring several weeks of treatment. At higher concentrations, the pulmonary regions are also affected (Eisenmenger et al., 1991). For all targets, there is a latency period between initial exposure and development of effects. The eyes and respiratory tract appear to have the shortest latency period; usually a matter of hours depending on the severity of exposure.

3. ANIMAL TOXICITY DATA

3.1 Acute Lethality

3.1.1 Rats

Fuhr and Krakow (1945) reported 2, 30, and 60-minute LCt_{50} values of 1512, 990, and 840 mg·min/m^3, respectively, for rats. However, data are unavailable for verifying these values or the analytical techniques utilized in their development.

3.1.2 Mice

Fuhr and Krakow (1945) also reported 2, 30, and 60-minute LCt_{50} values of 4140, 1320, and 860 mg·min/m^3, respectively, for mice. As for rats, these data are unavailable for verification.

In a head-only inhalation study, groups of four adult female Swiss mice (24-26 g) were exposed to sulfur mustard (>99% purity) at concentrations of 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 60 minutes (Vijayaraghavan, 1997). A group of mice exposed to filtered air for 60 minutes served as controls and mice exposed to acetone vapor served as vehicle controls. Respiratory patterns of the mice were monitored for seven days and the animals were observed for up to 14 days post exposure. Sulfur mustard vapor was generated using a known quantity of sulfur mustard diluted with acetone pumped into a compressed air nebulizer. Pressure in the nebulizer was adjusted for complete evaporation of the acetone diluent. A constant air flow of 20 L/min was maintained in the 50 cm x 10 cm exposure chamber (constructed of PTFE). The chamber air

was sampled at a rate of 50 mL/minute for five minutes and analyzed by gas chromatography (flame ionization detector). The primary focus of this study was assessment of changes in respiratory patterns and to this end an RD_{50} of 27.4 mg/m^3 (RD_{50} is the exposure concentration necessary to evoke a 50% decrease in respiratory rate) was determined along with other effects on respiration described in Section 3.2.3. The study author noted that mice started dying six days after exposure to "higher concentrations" and provided a 60-minute LC_{50} of 42.5 mg/m^3. No exposure-response data or other details regarding lethality were provided except that the confidence interval for the LC_{50} was very large (13.5 - 133.4 mg/m^3) due to the fact that sensory irritation and decreased respiratory frequency of mice in the higher exposure groups affected the actual intake and absorption of the sulfur mustard (most likely only for the latter half of the exposure period as the mice did not exhibit notable decrement in respiratory function during the first 15-20 minutes of exposure).

Kumar and Vijayaraghavan (1998) provided additional information regarding the lethal response of mice exposed to sulfur mustard. Groups of 30 female albino mice were exposed (head only) for one hour to sulfur mustard at concentrations of 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0 and 2.0 LC_{50}) and sacrificed at 6, 24, or 48 hours, or 7 days after exposure. Three groups of 10 mice were exposed at each concentration. The exposure system was as previously described by Vijayaraghavan (1997). No mice died during the exposure and none of the mice in the lowest exposure group died prior to scheduled termination. Within seven days, however, five mice of the the 42.3 mg/m^3 group and eight mice in the 84.6 mg/m^3 died. It was not stated when these mice expired and, because groups of mice were terminated at three time points prior to seven days post exposure, it not possible to determine the overall 7-day mortality rate.

3.1.3 Guinea pigs

Langenberg et al. (1998) provided data on the lethality of inhaled sulfur mustard in guinea pigs. In this study examining both the toxicity and toxicokinetics of sulfur mustard, male hairless guinea pigs (8 per group) were exposed to sulfur mustard via nose-only inhalation or by percutaneous exposure to vapors. The investigators reported the 96-hr LCt_{50} for 5-minute exposure to be 800 mg-min/m^3 (95% confidence interval of 700-920 mg-min/m^3). No percutaneous exposure lethality values were provided due to difficulties with the exposure system when exposing the guinea pigs to concentrations consistent with percutaneous LCt_{50} values (10,000 mg-min/m^3) previously reported in the literature. The vapor generating system and exposure system were modified from those used in nerve agent studies. Modifications included replacement of portions of the chamber so that they would be inert to sulfur mustard and an increase in chamber temperature (thermostat controlled at 25-30°C) to accommodate the lower vapor pressure of sulfur mustard.

3.1.4 Summary of Acute Lethality Data in Animals

The available acute lethality data in animals are summarized in Table 4. Lethality data from earlier reports was not verifiable but is not totally inconsistent with that from later studies. For example, the 1-hr LC_{50} values for rats and mice derived from the 840 and 860 mg-min/m^3 60-min LCt_{50} values reported by Fuhr and Krakow (1945) are similar to the lower confidence limit of the mouse 1-hr LC_{50} (13.5 mg/m^3) reported by Vijayaraghavan (1997) (i.e., 14.0, 14.3, and 13.5 mg/m^3, respectively). These value are also similar to a 1-hr LC_{50} of 13.3 mg/m^3 for guinea pigs

that can be extrapolated (assuming $C^1 \times t = k$) from the 5-min LCt_{50} of 800 mg-min/m^3 for reported by Langenberg et al., (1998).

TABLE 4. ACUTE LETHALITY OF SULFUR MUSTARD IN LABORATORY SPECIES

Species	Lethality Value	Concentration (mg/m^3) and exposure duration (min)	Reference
Rat	2-min LCt_{50}: 1512 mg-min/m^3 30-min LCt_{50}: 990 mg-min/m^3 60-min LCt_{50}: 840 mg-min/m^3	756 mg/m^3 (2 min) 33 mg/m^3 (30 min) 14 mg/m^3 (60 min)	Fuhr and Krakow, 1945 (not verified)
Mouse	2-min LCt_{50}: 4140 mg-min/m^3 30-min LCt_{50}: 1320 mg-min/m^3 60-min LCt_{50}: 860 mg-min/m^3	2070 mg/m^3 (2 min) 44 mg/m^3 (30 min) 14.3 mg/m^3 (60 min)	Fuhr and Krakow, 1945 (not verified)
Mouse	60-min LC_{50}: 42.5 mg/m^3	42.5 mg/m^3 (60 min)	Vijayaraghavan, 1997
Guinea pig	5-min LCt_{50}: 800 mg-min/m^3	160 mg/m^3 (5 min)	Langenberg et al., 1998

3.2 Nonlethal Toxicity

3.2.1 Dogs

McNamara (1975) conducted long-term inhalation studies of sulfur mustard in several species including dogs. In these experiments groups of dogs (gender and strain not specified) were exposed continuously to 0.001 mg sulfur mustard/m^3 or discontinuously (6.5 hrs/day, 5 days/week) to 0.03 mg/m^3 for up to 52 weeks (the latter group actually received 0.1 mg/m^3 6.5 hrs/day and 0.0025 mg/m^3 for the remaining 17.5 hrs/day for a time-weighted average exposure of 0.03 mg/m^3 over a 24-hour period; the study author referred to the groups as 0.1 mg/m^3). Ocular effects consisting of corneal opacities, pannus, chronic keratitis, vascularization, pigmentation and granulation, were the only overt signs of toxicity observed in the course of the study and only for dogs in the 0.03 mg/m^3 exposure group. Clinical chemistry analysis revealed only a slight increase in serum glutamic oxaloacetic transaminase (SGOT) activity in the high dose dogs that was of no biologic consequence. Three of ten dogs exposed to 0.03 mg/m^3 exhibited chronic keratitis and conjunctivitis that was considered to be treatment related following prolonged exposure (7.5 or 12 months) to the sulfur mustard. Additionally, there was no evidence of respiratory sensitization in the sulfur mustard-exposed dogs. Although not providing data that are directly applicable to the development of AEGL values, the results of this long-term exposure study may be useful as reference points with which to assess the validity of AEGLs.

3.2.2 Rats

McNamara (1975) also conducted long-term inhalation studies of sulfur mustard in Sprague Dawley-Wistar rats. In these experiments groups of rats (gender not specified) were exposed continuously to 0.001 mg sulfur mustard/m^3 or discontinuously (6.5 hrs/day, 5 days/week) to 0.03 mg/m^3 (see Section 3.2.1) for up to 52 weeks. Of the 79 rats exposed to 0.03 mg/m^3, there were no compound-related overt signs of toxicity effects. Necropsy revealed keratitis, possibly compound-related, in five of the rats. Necropsy revealed squamous cell carcinomas (skin) considered as definitely treatment related in four rats and squamous or basal cell carcinomas considered as possibly treatment related in five rats (see Section 3.5).

Anderson et al. (1996) reported on the pathologic changes in adult male rats following 50-minute intratracheal administration of sulfur mustard (0.35 mg/100 μL absolute ethanol). The administered sulfur mustard was selected based upon preliminary studies (data not provided) indicating that such an exposure would produce consistent but nonlethal damage at 24 hours post exposure. Controls were treated similarly but without the involvement of sulfur mustard. During exposure, the rats were anesthetized with Ketamine and they were euthanized at 0, 1, 4, 6, 12, 18, or 24 hours post exposure. At six hours post exposure, gross pathology assessments revealed multifocal, petechial hemorrhages on the pleural surface of the lungs. Atelectasis and edema of the accessory lobe, and necrosis and sloughing of tracheal and bronchial epithelia were observed at 6-12 hours post exposure. Analysis revealed that most histologically defined lesions were confined to the trachea , bronchi, and larger bronchioles rather than in the pulmonary region. There were no findings in the control group and little or no effects were observed in the sulfur mustard-treated rats during the first four hours after exposure. A latent phase of 4-6 hours following sulfur mustard exposure was required for development of histologic lesions (epithelial necrosis and sloughing). Lymphoid necrosis, loss of lymphocytes, and damage to tracheal cartilage were observed at 12 hours post exposure. At 24 hours post exposure, peribronchiolar and perivascular edema were detected but small bronchioles and alveoli appeared to be unaffected but did contain some cellular debris and inflammatory cells. Ultrastructural examination revealed an increased number of alveolar macrophages in some foci of mild edema at six hours. At 12 hours post exposure, injury to Type 1 pneumocytes was observed, and edematous material, cellular debris, extravasated erythrocytes, and fibrin were seen in scattered alveoli. Evidence of hyperplasia and hypertrophy of Type II pneumocytes was observed at 18-24 hours post exposure. An actual administered concentration of sulfur mustard was not provided and there were no provisions in the experimental apparatus for actual measurement of the test material. The results of this study are consistent with the pattern of respiratory tract injury observed in humans following low level exposure to sulfur mustard. Although not providing data that are directly applicable to the development of AEGL values, the results of this long-term exposure study may be useful as reference points with which to assess the validity of AEGLs.

3.2.3 Mice

In the long-term inhalation study by McNamara (1975), groups of A/J mice were exposed to sulfur mustard at 0.001 mg /m^3 continuously or discontinuously (6.5 hrs/day, 5 days/week) at 0.03 mg/m^3 (see Section 3.2.1) for up to 52 weeks. There were no overt signs of toxicity in the exposed mice during the treatment period. Deaths did occur among the mice but the investigators attributed these to adverse temperature extremes in the animal quarters and not to cumulative Ct for sulfur mustard. No clinical chemistry analyses were performed on the mice. There were no treatment-related tumors were noted in mice exposed to sulfur mustard at 0.03 mg/m^3 (see Section 3.5). Although not providing data that are directly applicable to the development of AEGL values, the results of this long-term exposure study may be useful as reference points with which to assess the validity of AEGLs.

Groups of four adult female Swiss mice (24-26 g) were exposed to sulfur mustard (>99% purity) at concentrations of 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 60 minutes (Vijayaraghavan, 1997). A group of mice exposed to filtered air for 60 minutes served as untreated controls and mice exposed to acetone vapor served as vehicle controls. In this head-only exposure study, respiratory patterns of the mice were monitored for seven days and the animals were observed for up to 14 days post exposure. Sulfur mustard vapor was generated using a known quantity of sulfur mustard diluted with acetone pumped into a compressed air nebulizer. Pressure in the nebulizer was adjusted for complete evaporation of the acetone diluent. A constant air flow of 20 L/min was maintained in the 50 cm x 10 cm exposure chamber. The chamber air was sampled at a rate of 50 mL/min for five minutes and analyzed by gas chromatography (flame ionization detector). At 15-20 minutes into the exposure, the rats exposed to sulfur mustard exhibited signs of sensory irritation and respiratory rate progressively decreased up to 30 minutes into the exposure after which no further decrement was detected. The RD_{50} was calculated to be 27.4 mg/m^3. By post-exposure Day 1, there was a concentration-dependent decreased respiratory rate over the 7-day monitoring period that was statistically significant ($p < 0.05$) for the 21.3, 26.8, and 42.3 mg/m^3 groups relative to unexposed controls. Decreases were as much as 40-60 % of controls in these three exposure groups. Respiratory rate was also notably decreased (64.8% of controls) in the 16.9 mg/m^3 group but the change was not statistically significant. Although exposure-response data were not provided, lethality was reported for mice in the "higher exposure" groups up to six days post exposure.

Kumar and Vijayaraghavan (1998) provided additional information regarding nonlethal responses of mice to inhaled sulfur mustard. Groups of 30 female albino mice were exposed (head only) for one hour to sulfur mustard at concentrations of 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0 and 2.0 LC_{50}) and sacrificed at 6, 24, or 48 hours, or 7 days after exposure. The exposure system was as previously described by Vijayaraghavan (1997). Even at the highest exposure, no mice died during exposure although the mice did exhibit sensory irritation resulting in pauses between inspiration and expiration, and decreased ventilatory frequency. Effects of the sulfur mustard exposure on blood uric acid and urinary uric acid were also examined as an index of purine catabolism. Exposure to sulfur mustard at all concentrations tested resulted in significant increases in blood uric acid and urinary uric acid at all time points measured (except 6-hr time point for the low-dose group). The greatest increase appeared to be at 24 hours and generally decreased, although not to control levels, by seven days. The increased blood uric acid was postulated as being the result of catabolism of apurinated bases resulting from DNA adduct formation by sulfur mustard.

3.2.4 Rabbits

In an early study by Warthin and Weller (1919), rabbits (no information provided regarding gender, age, weight, or strain) were exposed to sulfur mustard at various concentrations and for various periods of time. The sulfur mustard concentrations were determined based on changes in weight of the sulfur mustard sample and the air flow, and were simply expressed as ratios. The exposure regimen for eight rabbits and their respective responses are summarized in Table 5. The study report authors summarized the following: 1) respiratory lesions are proportional to the concentration and the length of exposure, 2) effects are mild following 10-15 minute exposures at dilutions of 1:110,000 (58 mg/m^3) or 1 to several minutes at higher concentrations, 3) nasal irritation is almost immediate followed by moderate ocular effects (photophobia, lacrimation) within 2-3 hours and respiratory involvement at 2-3 hours, 4) for prolonged or high-concentration exposures, pronounced respiratory effects occur somewhat later than ocular effects, 5) there is a concentration and time dependent effect on severity of gross and histopathologic lesions such that long exposures or exposures to high concentrations will result in deeper tissue damage, damage of pulmonary in addition to nasopharyngeal regions, and may increase susceptibility to secondary infection.

Rabbits exposed continuously to sulfur mustard at 0.001 mg /m^3 or discontinuously (6.5 hrs/day, 5 days/week) at 0.03 mg/m^3 (see Section 3.2.1) for up to 52 weeks exhibited no overt signs of toxicity (McNamara, 1975). Ocular sensitization tests were also performed on rabbits, the results for which were negative.

The effect of sulfur mustard vapor on rabbit eyes was examined by Laughlin (1944). In this study, rabbits were exposed to sulfur mustard (200-1200 mg·min/m^3) for 30 or 60 minutes and observed for 24 hours. Further details regarding experimental protocol are unavailable. Laughlin provided the following observations: redness and conjunctival edema but no corneal damage at 200 mg·min/m^3; some corneal opacity but no conjunctival discharge at 400 mg·min/m^3; excessive lacrimation with no purulent discharge at 600 mg·min/m^3; purulent discharge at 800 mg·min/m^3; severe conjunctival edema at 1200 mg·min/m^3. It was also reported that for ocular effects a Ct over a 2-minute period resulted in a more severe effect than the same Ct delivered over a 30-minute or 60-minute period and that when the exposure duration was extended to seven hours the severity of the effect was diminished (i.e., the 7-hr Ct needed to be twice the 30- or 60-minute Ct to obtain an equivalent effect). These observations imply that the concentration becomes less important over time and that there may be some form of a detoxification/recovery mechanism regarding ocular effects (Laughlin, 1944; McNamara, 1975).

TABLE 5. EFFECTS ON RABBITS OF ACUTE INHALATION EXPOSURE TO SULFUR MUSTARD		
Rabbit No.	**Exposure***	**Effects**
32	58 mg/m^3 (1:110,000); 40 min	Signs of mild ocular and nasal irritation during exposure; increasing severity of conjunctival erythema and lacrimation up to sacrifice at 12 hrs. Pulmonary congestion and edema
33	389 mg/m^3 (1:15,000); 20 min	Mild irritation during exposure; increased lacrimation and marked erythema of nostrils, mouth, ears, conjunctiva, and some dermal areas up to sacrifice at 36 hrs. Evidence of edema and necrosis in nasal passages.
30	389 mg/m^3 (1:15,000); 30 min	Signs of ocular irritation within 5 min after exposure; increased severity of ocular involvement progressing to extreme conjunctival edema and corneal ulceration; evidence of respiratory involvement by Day 2; no increase in severity at time of sacrifice (4.25 days). Marked congestion and edema in all areas of respiratory tract.
31	214 mg/m^3 (1:30,000); 35 min	Minor nasal and ocular irritation immediately following exposure period that increased in severity up to sacrifice at 30 hrs. Congestion in all areas of respiratory tract.
46	130 mg/m^3 (1:50,000); 6 hrs	Signs of irritation during exposure; dead at 60 hrs post exposure (likely due to *Staphylococcus* infection)
45	130 mg/m^3 (1:50,000); 6 hrs	Similar effects and cause of death as noted for rabbit # 46.
43	130 mg/m^3 (1:50,000); 12 hrs	Signs of ocular and nasal irritation, and lethargy during exposure; dead at 54 hrs post exposure. Marked respiratory tract involvement and secondary infection in larynx and trachea.
44	130 mg/m^3 (1:50,000); 12 hrs	Severe ocular effects and generalized dermal burns; congestion and necrosis in respiratory tract; congestion in other organs; secondary *Staphylococcus* infection involvement; sacrificed at 92 hrs post exposure.

* Values in parentheses are the dilutions as reported by Warthin and Weller (1919)

3.2.5 Guinea pigs

In the long-term inhalation study by McNamara et al (1975), guinea pigs were used to assess the sensitization potential of sulfur mustard. For this phase of the study, the guinea pigs were exposed to sulfur mustard at 0.001 mg /m^3 continuously or discontinuously (6.5 hrs/day, 5 days/week) at 0.03 mg/m^3 (see Section 3.2.1) for up to 52 weeks. Groups of six animals were removed after 1, 2, 4, 8, 32, and 52 weeks of exposure. There was no evidence of sensitization in any of these group following challenge with a 7.9 μg dermal application of sulfur mustard in olive oil. This challenge had been previously shown to induce erythema, edema and necrosis in sensitized animals. Dermal application of 31.6 μg or 63.2 μg sulfur mustard (shown to induce a response in normal animals) to these same guinea pigs produced responses similar to that of controls indicating that a tolerance had not been developed. Respiratory patterns were also examined during the sensitization tests and found to be unaffected by the treatment. No other treatment-related effects were reported for the guinea pigs.

The effects of sulfur mustard injected intratracheally (0.3 mg/kg; equivalent to approximately 0.6 mg sulfur mustard/m^3 based upon a body weight of 0.84 kg and ventilatory rate of 0.40 m^3/day) into male Hartley guinea pigs were studied by Calvet et al. (1994). In this study, guinea pigs (five per group) received a single intratracheal injection. Lung mechanics, airway responsiveness, microvascular permeability, and neutral endopeptidase activity in tracheal epithelium were assessed five hours and 14 days after administration of the test article. At five hours post injection, there was a 3-fold increase in respiratory system resistance ($p<0.05$) and a 2-fold increase in microvascular permeability ($p<0.05$). Histopathologic findings included shedding of tracheal epithelium columnar cells and peribronchial edema. At 14 days post injection, the guinea pigs exhibited airway hyperactivity to inhaled substance P and histamine.

3.2.6. Summary of Nonlethal Toxicity Data in Animals

Overall, the available animal data suggest that test species exhibit signs of toxicity that are qualitatively similar to humans when acutely exposed to sulfur mustard vapor. Ocular and respiratory tract irritation and the fact that these are primary targets are plainly evident in studies using dogs, rats, mice, rabbits, and guinea pigs. Long-term exposure of dogs, rats, and guinea pigs to concentrations of 0.03 mg/m^3 produced only minor signs of ocular and respiratory tract irritation although similar exposure in mice were tumorigenic. One-hour exposure of mice to concentrations up to 16.9 mg/m^3 resulted in notable but not serious effects on respiratory parameters and acute exposures of rabbits (20 minutes to 12 hours) to concentrations ranging from 58-389 mg/m^3 ($Ct \geq 2{,}300$ mg-min/m^3) resulted in severe respiratory tract damage.

3.3 Developmental/Reproductive Toxicity

In the McNamara et al (1975) study, groups of 10 female rats were exposed to sulfur mustard at 0.001 or 0.1 mg/m^3 during the first, second or third week of gestation or for the entire gestation period. No increase in fetal abnormalities was observed and fetal mortality rate was also within normal limits.

3.4 Genotoxicity

The potential genotoxicity of sulfur mustard was also examined by McNamara et al (1975). Groups of 10 female rats were bred to males that had been exposed to sulfur mustard at 0.001 or 0.1 mg/m^3 for 1, 2, 4, 8, 12, 24, 36, or 52 weeks. Based upon number of live or dead fetuses and implantation sites, there was no evidence of dominant lethal mutagenesis.

3.5 Carcinogenicity

McNamara et al (1975) provided evidence of the tumorigenic potential of long-term exposure to sulfur mustard in Sprague Dawley-Wistar rats. Seventy male and 70 female rats were discontinuously exposed to sulfur mustard at 0.001 mg /m^3 (24 hr/day, 5 days/wk) or at 0.1 mg/m^3 for 6.5 hr followed by 0.0025 mg/m^3 for 17.5 hr for each day of exposure, 5 days/week, for up to 12 months. Fifty individuals of each gender were maintained as controls. Results of this toxicity study are shown in Table 6.

The USEPA (1991) emphasized that the studies of McNamara et al (1975) contain deficiencies that make a quantitative analysis difficult. The McNamara et al (1975) studies were conducted in

1970, do not conform to current standards of experimental protocol, and likely contain bias in the assignment of animals to test categories. In addition, many of the exposures were very brief and included only a few animals, many of which were sacrificed (and some were replaced) before their capacity to develop late-appearing tumors could be fully tested. Despite these shortcomings, the USEPA (1991) noted that the McNamara et al (1975) data are the best available for directly estimating the carcinogenic potency of sulfur mustard.

Additionally, a study specifically addressing carcinogenic potential was also conducted in which groups of rats were exposed for varying times up to 21 months to the same sulfur mustard concentrations as used in the toxicity study. These animals were then observed for varying periods of time before being sacrificed. The results of this study are shown in Table 7.

TABLE 6. RAT SKIN TUMOR DATA[a] FROM MCNAMARA ET AL (1975) TOXICITY STUDY

Gender	Exposure Groups		
	Control	Low exposure[b]	High exposure[c]
Males	0/11	0/10	4/11
Females	0/8	0/19	5/18
Both genders	0/19	0/29	9/29

From U.S.EPA, 1991.

[a] Includes only data for rats living longer than the time for first tumor appearance (12 months exposure plus 70 days post-exposure)

[b] 0.001 mg/m^3 for 24 hr/day, 5 days/wk

[c] 0.1 mg/m^3 for 6.5 hr followed by 0.0025 mg/m^3 for 17.5 hr per day, 5 days/wk

McNamara (1975) also conducted carcinogenicity studies in ICR Swiss albino as well as strain A/J mice, dogs, rabbits, and guinea pigs exposed to the same sulfur mustard concentration protocols as in the previously described "toxicity study" for varying exposure durations up to 1 year. No exposure-related tumors were observed in any of these species.

A recent comparative analysis evaluated the tumorigenicity of sulfur mustard relative to alkylating compounds used in chemotherapy or treatment of other diseases (Nicholson and Watson, 1993). By considering all possible combinations of experiments and several reference compounds, sulfur mustard tumorigenicity was determined to be comparable with nitrogen mustard (HN2 and HN2-HCl)) tumorigenicity in laboratory rodents. Additional relative potency comparisons were made for the therapeutic nitrogen mustards melphalan and chlorambucil, and the alkylating carcinogenic compound bis(chloromethyl) ether. Comparisons of laboratory rodent data indicated that sulfur mustard and nitrogen mustard had tumorigenic potencies comparable with melphalan and bis(chloromethyl) ether; the tumorigenic potencies of sulfur and nitrogen mustard were possibly greater than that of chlorambucil (Nicholson and Watson, 1993).

TABLE 7. RAT SKIN TUMOR DATA FROM MCNAMARA ET AL (1975) CANCER STUDY, BY INCREASING LIFETIME DAILY EXPOSURE

Exposure Duration (weeks)	Exposure Concentration[a]	Lifetime[b] average daily exposure ($\mu g/m^3$)	Incidence of skin carcinomas

Control	0	0.0	0/27
1	Low	0.0096	0/5
2	Low	0.0192	0/5
4	Low	0.0385	0/5
8	Low	0.0769	0/4
12	Low	0.115	0/5
26	Low	0.250	0/4
1	High	0.279	0/5
39	Low	0.375	0/3
52	Low	0.500	0/17
2	High	0.558	0/5
4	High	1.12	0/6
8	High	2.23	0/4
12	High	3.35	4/5
26	High	7.25	4/5
39	High	10.9	4/4
52	High	14.5	10/23

From USEPA, 1991

[a]Low exposure was 0.001 mg/m^3 24 hr/day, 5 days/week; high exposure was 0.1 mg/m^3 for 6.5 hr followed by 0.0025 mg/m^3 for the remaining 17.5 hr daily, 5 days/week.

[b]A 2-yr lifetime was assumed

A tentative quantitative assessment of cancer risk for a single acute exposure is presented in Appendix D. This assessment follow the NRC methodology for EEGLs, SPEGLs and CEGLs (NRC, 1986).

4. SPECIAL CONSIDERATIONS

4.1 Metabolism and Disposition

A thorough understanding of the metabolism and disposition of sulfur mustard is not likely to be pivotal in the quantitative assessment of human health risk from acute exposures. One of the most important aspects of the disposition of sulfur mustard is that its lipophilic nature allows for toxicologically significant quantities to penetrate the skin (Papirmeister et al., 1991). Additionally, its extreme cytotoxicity is not dependent upon metabolism and disposition nor is its toxic potential to primary targets significantly ameliorated via detoxification processes. The stratum corneum of the skin offers the greatest barrier to penetration by sulfur mustard and it is the absence of this layer that make the eyes and respiratory tract so susceptible to toxic insult.

Papirmeister et al. (1991) have reviewed available studies regarding the absorption and distribution of sulfur mustard. Although only a relatively small amount of sulfur mustard is absorbed following percutaneous application, experiments with radio-labeled material have shown distribution to most tissues within short periods of time (e.g.,15 minutes). Henriques et al. (1943)

estimated that about 12% of a dose absorbed into the skin actually reacts with tissue components and that it is this portion of the dose that is responsible for the vesicant effects.

The toxicokinetics of sulfur mustard and its DNA adduct, N7-hydroxyethylthioethyl guanine (SM-7-gua), were studied by Langenberg et al. (1998) in hairless guinea pigs exposed via nose-only inhalation, percutaneous exposure to vapors or intravenous injection of sulfur mustard. The time-course for sulfur mustard in the blood of guinea pigs following a single intravenous injection of 1 or 0.3 LD_{50} (96-hr i.v. LD_{50} = 8.2 mg/kg) showed a rapid disappearance (>1,000-fold reduction) within 10 minutes and maintained this level or slightly less to 360 minutes. Overall, the toxicokinetics of intravenously administered sulfur mustard was biphasic and exhibited a very rapid distribution phase and a slow elimination phase. Significant partitioning of sulfur mustard into the lungs, liver, spleen, and bone marrow was also observed. At time points from 0.05 to 48 hours after i.v. administration, the concentration of SM-7-gua adducts (expressed per 10^7 nucleotides) was significantly greatest in the lung (10-400) but also detected (2-30) in all tissues examined (liver, spleen, bone marrow, small intestine, blood). Results of inhalation toxicokinetic studies using the hairless guinea pigs exposed nose-only to 1 LCt_{50} for 5 minutes revealed sulfur mustard concentrations in the blood to be below detection limits (5 pg/ml). SM-7-gua adducts could not be detected in the spleen, bone marrow, or small intestine but very low levels (0.7 adducts/10^7 nucleotides) were detected in the lung at 10 minutes and 48 hours after exposure. Adducts (50-80 adducts/10^7 nucleotides) were, however, detected in the nasal, nasopharynx, larynx, trachea, and carina of the respiratory tract at 4 hours after exposure. Based on these blood concentration data and adduct distribution the authors concluded that, during acute inhalation exposure in guinea pigs, most of the sulfur mustard reacts with upper airway tissues. For species with a less complex nasal system (such as humans), more sulfur mustard could conceivably reach the lungs.

4.2 Mechanism of Toxicity

The principal mechanism of toxicity for sulfur mustard may be attributed to its capacity as an alkylating agent and consequent ability to react with DNA, RNA, and other macromolecules (reviewed by Watson and Griffin, 1992). Endothelial cells are a major target for sulfur mustard (Dabrowska et al., 1996). Because of the fundamental nature of these targets, the actual mechanism of toxicity may be complex. Cross-linking with DNA (Lohs et al., 1975; Gross et al., 1985; Lin et al., 1996) and inhibition of enzymes such as hexokinase (Dixon and Needham, 1946) have been reported, and sulfur mustard has been shown to be especially toxic to proliferating cells (Vogt et al., 1984; Gross et al., 1985). Additionally, mechanisms such as the cell membrane modifications in the absence of DNA damage have been described (Levy, 1934).

An hypothesis for the skin lesion/blistering effects of sulfur mustard has been provided by the U.S. Army Medical Research Institute of Chemical Defense (Papirmeister et al., 1985; Gross et al., 1985). This hypothesis contends that a depletion of NAD+ arising from efforts to repair extensive DNA damage results in inhibition of glycolysis. The inhibition of glycolysis stimulates the hexose monophosphate shunt which causes a release of proteases that are instrumental in the skin damage associated with sulfur mustard exposure. More recently, Petrali and MeGee (1997) reported results from investigations using several animal models, cultured isolated human cells, and *in vitro* organotypic skin models. Histopathologic and ultrastructural analysis indicated that basal cells of the stratum basale layer is an early target of sulfur mustard and that resulting injury that is evident by 4-6 hours after exposure represents a progressive and irreversible cell injury and death.

Additionally, there appears to be a disabling of anchoring hemidesmosome filaments resulting in microvesicle formation, and interaction with various membrane proteins such that there is a loss of immunospecificity.

Using a chromogenic peptide substrate assay, Cowan et al. (1993) found that sulfur mustard enhanced proteolytic activity. A time-dependent and temperature-dependent proteolysis was observed for *in vitro* experiments using human peripheral blood lymphocytes. A similar response was also seen for *in vivo* exposures using the hairless guinea pig.

In vitro experiments conducted by Smith et al. (1990, 1997) using primary human epidermal keratinocytes, provided results showing a concentration-dependent interference with cell cycling. At concentrations equivalent to those that would produce vesication, the cell cycle was blocked at the G1-S interface while at sub-vesicant concentrations, the cell cycle was blocked in the G2 phase.

Using bovine pulmonary artery endothelial cells, Dabrowski et al. (1996) showed that sulfur mustard ($\leq$250 μM) induced apoptosis within 5 hours. At concentrations $\geq$500 μM both apoptotic and necrotic cell death occurred after 5-6 hours. Necrosis was accompanied by a significant depletion of intracellular ATP.

Most sulfur mustard-induced fatalities have been due to respiratory tract involvement. The mechanism of sulfur mustard-induced pulmonary damage was studied by Anderson et al. (1997) using lavage fluid from rats in which sulfur mustard (0.35 mg) was intratracheally intubated for 50 minutes. At 1, 4, or 24 hours after the treatment, the rats were euthanized and the lungs lavaged with physiologic saline. Lactate dehydrogenase and gamma glutamyltransferase were increased ($p \leq 0.05$) at all time points and total protein was increased ($p < 0.001$) at 4 and 24 hours. The investigators contended that these indices were useful indicators of early pulmonary injury following low-dose exposure to sulfur mustard.

4.3 Structure-Activity Relationships

There are no structure-activity data would be instrumental in the development of AEGL values for sulfur mustard.

4.4 Other Relevant Information

There are several important aspects of sulfur mustard toxicology that impact the toxic response and that are relevant to assessing human health risk. These include the latency period between initial exposure and development of effects, the effect of temperature and humidity, variable sensitivity among the tissues and sites affected, and the sensitization potential for vesicating effects. Firstly, it is well documented (summarized by Papirmeister et al., 1991) that a latency period exists between the initial exposure to sulfur mustard and the development of toxic effects. This pertains not only to onset of effects but also to development of full severity of effects. The ocular response appears to have the shortest latent period, sometimes as short as minutes, whereas dermal and respiratory effects following acute exposure may take days for full development. It is also known that higher ambient temperature and greater humidity enhance the dermal response to sulfur mustard (Nagy et al., 1946; Renshaw, 1947; Papirmeister et al., 1991). Although the mechanism is unknown, increased temperature and humidity decrease the dose required for a given response and increase the severity of the response. In this respect, moisture (in addition to skin characteristics) is relevant to the greater sensitivity of certain anatomical areas (e.g., axial, interdigital and popliteal areas, scrotum, perineum). The eyes and respiratory tract are generally considered the most sensitive organs/tissues (eyes somewhat more so) for acute exposures to sulfur mustard. Both involve latency periods and a wide range of severity of effects depending primarily upon the exposure concentration but injury to the respiratory tract is considered more relevant regarding lethal responses. Sensitization to sulfur mustard-induced dermal effects appears to be associated with repeated exposures and, according to McNamara et al. (1975), after detectable insult (i.e., overt clinical signs). There tends to be a greater sensitivity to high exposures and not greater severity in response to lower exposures or greater likelihood of a response to lower exposures (Sulzberger et al., 1945).

4.4.1 Species Variability

All of the species tested exhibit qualitatively similar responses to sulfur mustard vapor and affirm that the eyes and respiratory tract are the most sensitive targets. Available lethality data (LC_{50} and LCt_{50}) are remarkably similar across species (see Section 3.1.4).

4.4.2 Concurrent Exposure Issues

It would be reasonable to assume that concurrent exposure to any chemicals for which the eyes and respiratory tract are primary targets would impact on the response to sulfur mustard.

5. RATIONALE AND PROPOSED AEGL-1

5.1 Summary of Human Data Relevant to AEGL-1

Walker et al. (1928) reported that four of seven men exposed to sulfur mustard at 0.001 mg/L (1 mg/m^3) for 5-45 minutes exhibited conjunctivitis and two exhibited skin burns. It was also reported that of seventeen men exposed to 0.0005 mg/L (0.5 mg/m^3) for 10-45 minutes (5-22.5 mg-min/m^3) six exhibited conjunctivitis and one had a skin burn and three of 13 men exposed for 10-30 minutes to 0.0001 mg/L (0.1 mg/m^3; Ct of 1-3 mg-min/m^3) showed slight but distinct conjunctivitis. Although not of a severity consistent with an AEGL-2 level, these effects are of greater severity than would be acceptable for AEGL-1 development. Guild et al. (1941) also conducted experiments using humans and reported that: 1) exposure to Ct values <70 mg-min/m^3

would result in mild conjunctival responses that would not be indicative of a casualty (temporary loss of vision), 2) Ct values of 70-100 mg-min/m^3 would produce some casualties and, 3) Ct values > 100 mg-min/m^3 would be expected to produce disabling ocular effects of several days' duration. Because the subjects wore respiratory protection, effects on the respiratory tract could not be determined.

In experiments with human volunteers exposed to varying concentration-time regimens, Anderson (1941) found that an exposure concentration-time product of 30 mg · min/m^3 represented the upper range for mild effects (conjunctival injection and minor discomfort with no functional decrement). Ct products slightly higher than this (e.g., 34-38.1 mg · min/m^3) were, however, also without appreciable effects thereby indicating the response to 30 mg · min/m^3 to be consistent with AEGL-1 effects.

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

Analysis of the exposure-effect values from the human studies indicated that the 30 mg · min/m^3 values represented a defensible estimate of the threshold for effects consistent with the AEGL-1 definition. The 12 mg · min/m^3 exposure was without a symptomatic effect consistent with AEGL-1 effects of discomfort or irritation and, therefore, considered too low as the basis for AEGL-1 derivation.

5.2 Summary of Animal Data Relevant to AEGL-1

The effects described in animal studies tended to be a of greater severity than that associated with AEGL-1 (i.e., signs of severe ocular irritation, body weight loss, respiratory depression, evidence of respiratory tract histopathology, etc.). There were no definitive exposure-response data in animals that were considered appropriate for development of AEGL-1 values.

5.3 Derivation of AEGL-1

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

Because exposure-response data were unavailable for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL-1 values for the AEGL-specific time periods. The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al., 1986). Analysis of available data regarding AEGL-1 type effects reported by Reed (1918), Reed et al. (1918), Guild et al. (1941), and Anderson (1942) indicate that for the exposure periods up to several hours, the concentration-exposure time relationship is a near-linear function (i.e., Haber's Law where $n = 1$ for $C^n \times t = k$) as shown by n values of 1.11 and 0.96 for various data sets consistent with AEGL-1 effects (Appendix B). Therefore, an empirically derived, chemical-specific estimate of $n = 1$ was used rather than a default value based upon the ten Berge (1986) analysis. The derivation of the exponent (n) utilized human response data where 75-100% of the responders showed a mild response that would be consistent with the definition of AEGL-1 effects. Additionally, the data provided by Anderson (1942) were also indicative of a linear concentration-time relationship. The AEGL-1 values developed using the 12 mg-min/m^3 exposure value reported by Anderson (1942) are shown in Table 8.

For comparative purposes, AEGL-1 values were also developed using logistic and probit modeling that utilized multiple data sets. These models were used to determine a 99% response at 30 minutes, 1 hour, and 4 hours and were based upon the mild response data drawn from the literature and shown in Appendix C. AEGL-1 values developed using exposure values generated by these models are consistent with those developed using the data of Anderson (1942) and affirm that the proposed AEGL-1 values are defensible and protective of human health relative to AEGL-1 level effects. The AEGL-1 values result in cumulative exposures far below the Ct limits determined by Anderson (1942) and Guild et al. (1941) as causing even mild ocular irritation.

TABLE 8. AEGL-1 VALUES FOR SULFUR MUSTARD

AEGL Level	10-min	30-min	1-hr	4-hr	8-hr
AEGL-1	0.06 ppm 0.40 mg/m^3	0.02 ppm 0.13 mg/m^3	0.01 pm 0.067 mg/m^3	0.003 ppm 0.017 mg/m^3	0.001 ppm 0.008 mg/m^3

6. RATIONALE AND PROPOSED AEGL-2

6.1 Summary of Human Data Relevant to AEGL-2

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

6.2 Summary of Animal Data Relevant to AEGL-2

With the exception of a study reported by Warthin and Weller (1919) regarding the effects in rabbits following acute exposure, there is little exposure-response data consistent with AEGL-2 severity effects in animals. Weller and Warthin reported severe ocular effects and dermal burns in rabbits exposed for 12 hours to sulfur mustard at 130 mg/m^3. This study, however, is compromised by the use of single animals and lack of details. Kumar and Vijayaraghavan (1998) reported alterations in purine catabolism in rats exposed for 1 hour to 21.2 - 84.6 mg sulfur mustard/m^3 but these exposures also represented 0.5, 1.0 and 2.0 LC_{50} responses. Statistically significant reductions in body weights were also observed for the mice at 14 days following a 1-hour exposure to concentrations of 16.9-42.3 mg/m^3; at least some of these exposures, however, were also associated with lethality. Dogs, rats, mice, and guinea pigs exposed continuously to 0.001 mg sulfur mustard/m^3 or discontinuously (6.5 hrs/day, 5 days/week) to 0.03 mg/m^3 for up to 52 weeks did not exhibit effects consistent with AEGL-2 definition (McNamara, 1975).

6.3 Derivation of AEGL-2

The AEGL-2 values for sulfur mustard were also developed using the data from Anderson (1942). This study utilized 3-4 human volunteers exposed to varying concentrations of sulfur mustard (1.7-15.6 mg/m^3) for time periods varying from 2-33 minutes. Anderson considered a Ct value of 60 mg-min/m^3 as the lowest concentration-time product for which ocular effects could be characterized as military casualties and that such personnel might be ineffective for up to (but no more than) seven days. These effects included irritation, soreness, and widespread conjunctivitis frequently accompanied by chemosis and photophobia. The 60 mg-min/m^3 exposure was used as the basis for developing the AEGL-2 values because it is representative of an acute exposure causing an effect severe enough to impair escape and, although not irreversible, would certainly result in potential for additional injury. The ocular irritation and damage were also considered appropriate as a threshold estimate for AEGL-2 effects because the eyes are generally considered the most sensitive indicator of sulfur mustard exposure and would likely occur in the absence of vesication effects and severe pulmonary effects. The fact that the AEGL-2 is based upon human data precludes the use of an interspecies uncertainty factor. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to three under the assumption that the primary mechanism of action of sulfur mustard involves a direct effect on the ocular surface and that this response will not vary greatly among individuals (this was also noted by Anderson). A modifying factor of 3 was applied to accomodate potential onset of long-term ocular or respiratory effects. This was justified by the absence of long-term follow-up in the subjects of the Anderson (1942) study with which to confirm or deny development of permanent ocular or respiratory tract damage. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16 x 3.16 = 10. Further reduction by the application of additional modifying factors was not warranted due to the use of a sensitive indicator representing an AEGL-2 effect of marginal severity. As for AEGL-1 values, time scaling was conducted using an *n* of 1 for all time points. The resulting AEGL-2 values are shown in Table 9 and their derivation is presented in Appendix A.

TABLE 9. AEGL-2 VALUES FOR SULFUR MUSTARD

AEGL Level	10-min	30-min	1-hr	4-hr	8-hr

AEGL-2	0.09 ppm 0.60 mg/m^3	0.03 ppm 0.20 mg/m^3	0.02 ppm 0.10 mg/m^3	0.004 ppm 0.025 mg/m^3	0.002 ppm 0.013 mg/m^3

7. RATIONALE AND PROPOSED AEGL-3

7.1 Summary of Human Data Relevant to AEGL-3

Human lethality data are limited to an inhalation LCt_{50} estimate of 1,500 mg-min/m^3 and percutaneous LCt_{50} estimate of 10,000 mg-min/m^3 estimated from animal data (DA, 1974). The NRC (1997) concluded that an estimated LCt_{50} for humans of 900 mg-min/m^3 developed by the U.S. Army based upon an average of animal LCt_{50} data was scientifically valid.

7.2 Summary of Animal Data Relevant to AEGL-3

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

7.3 Derivation of AEGL-3

As previously noted in Section 3.1.4, the lethality data from earlier reports was not verifiable but is not totally inconsistent with that from later studies. The 1-hr LC_{50} values for rats and mice derived from the 840 and 860 mg-min/m^3 60-min LCt_{50} values reported by Fuhr and Krakow (1945) are similar to the lower confidence limit of the mouse 1-hr LC_{50} reported by Vijayaraghavan (1997) (i.e., 14.0, 14.3, and 13.5 mg/m^3, respectively). These values are also similar to a 1-hr LC_{50} of 13.3 mg/m^3 for guinea pigs that can be extrapolated (assuming $C^1 \times t = k$) from the 5-min LCt_{50} of 800 mg-min/m^3 reported by Langenberg et al., (1998). However, the values from the earlier studies are not verifiable. In the inhalation toxicity study by Vijayaraghavan (1997), mice were exposed (head only) for 60 minutes to sulfur mustard at concentrations of 0.0, 8.5, 16.9, 21.3, 26.8, 42.3 or 84.7 mg/m^3. The study investigator derived a 60-min LC_{50} of 42.5 mg/m^3 based upon lethality at 14 days post exposure (95% confidence interval: 13.5- 133.4 mg/m^3). In a follow-up study (Kumar and Vijayaraghavan , 1998), there was no mortality in mice exposed to 0.5 LC_{50} (21.2 mg/m^3). Therefore, the 1-hour exposure to 21.2 mg/m^3 was selected as an estimate of the lethality threshold in mice.

When compared to the human exposure-effect data, the 21.2 mg/m^3 concentration (Ct of 1,272 mg-min/m^3 for a 60-minute exposure) is not an exposure that has been associated with lethality in humans (see Section 2.1). An uncertainty factor for intraspecies variability was limited to 3 because the lethality resulting from acute inhalation exposure to sulfur mustard appears to be a function of pulmonary damage resulting from direct contact of the agent with epithelial surfaces. An uncertainty factor of 3 was limited to 3 because available data do not suggest that humans are notably more sensitive than animals regarding lethality from inhalation exposure to sulfur

mustard. Furthermore, the AEGL-3 values resulting from the aforementioned complement of uncertainty factors (total uncertainty factor adjustment was 10; see Section 6.3) are equivalent to exposures known to cause only mild ocular effects in humans. The modifying factor of 3 utilized in the development of AEGL-2 values development to account for uncertainties regarding the latency and persistence of the irritant effects of low-level exposure to sulfur mustard was not applied for AEGL-3 because lethality of the mice was assessed at 14 days post exposure in the key studies by Vijayaraghavan (1997) and Kumar and Vijayaraghavan (1998).

For derivation of the 10-minute AEGL-3 value, there was uncertainty regarding linear extrapolation to a time duration notably shorter than that for which empirically derived lethality data were available. Because of this, time scaling was performed using exponential extrapolation (i.e., where *n* of 3 rather than 1), thereby providing a somewhat more conservative (i.e., protective) estimate of the 10-minute value than would be obtained using a linear function ($n = 1$). The 10-minute value was derived by scaling the 30-minute AEGL-3 value (4.2 mg/m^3) using $C^3 \times t = k$ (Appendix A). The AEGL-3 values are shown in Table 10 and their derivation presented in Appendix A

When comparing the Ct values generated by the draft AEGL-3 numbers to the human exposure data, any further reduction appears indefensible. The Ct values resulting from the AEGL-3 numbers (i.e., 60-130 mg-min/m^3) are similar to cumulative exposures shown to cause only ocular irritation in humans (Guild et al., 1941; Anderson, 1942) and are similar to the ECt_{50} of 100 mg-min/m^3 for severe ocular effects (for soldiers) as determined by CDEPAT (1994) and the NRC (NRC, 1997). Furthermore, these AEGL-3 values are nearly identical to those developed using the human lethality estimate of 900 mg-min/m^3 (CDEPAT, 1994) and reviewed by the NRC (1997). Assuming a 3-fold reduction for estimation of a lethality threshold (900 mg-min/m^3 ÷ 3 = 300 mg-min/m^3) and another 3-fold reduction for consideration of sensitive subpopulations (300 mg-min/m^3 ÷ 3 = 100 mg-min/m^3), the resulting AEGL-3 values from the CDEPAT (1994) and NRC (1997) reports would be 9.9, 3.3, 1.7, 0.42, and 0.21 mg/m^3, respectively, for 30 minutes, 1, 4, and 8 hours.

TABLE 10. AEGL-3 VALUES FOR SULFUR MUSTARD

AEGL Level	10-min	30-min	1-hr	4-hr	8-hr
AEGL-3	0.91 ppm 6.1 mg/m^3	0.63 ppm 4.2 mg/m^3	0.32 ppm 2.1 mg/m^3	0.08 ppm 0.53 mg/m^3	0.04 ppm 0.27 mg/m^3

8. SUMMARY OF PROPOSED AEGLS

8.1 AEGL Values and Toxicity Endpoints

Human data were available from several independent sources that adequately defined the exposure-response for AEGL-1 and AEGL-2 effects. Although a definitive demarcation of the exposure-response for sensitive subpopulations was not provided by these data, the human data eliminated the uncertainties inherent in the use of data from animal studies. Both the AEGL-1 and AEGL-2 values were based upon effect endpoints consistent with the respective AEGL definitions (i.e., threshold for barely discernible ocular irritation [AEGL-1] and threshold for ocular irritation indicative of functional impairment [AEGL-2]). Areas of uncertainty were associated with the sensitive responders and the relationship between ocular effects and the onset

of respiratory effects. Human data were unavailable with which to develop AEGL-3 values. The AEGL-3 was based upon an estimated lethality threshold from two recent studies in mice (Vijayaraghavan, 1997; Kumar and Vijayaraghavan, 1998). When compared to human exposure-response data and lethality estimates, the use of the mouse lethality data appeared to represent a defensible approach to AEGL-3 derivation. Development of AEGL-3 values based upon the U.S. Army human lethality estimate of 900 mg-min/m^3 (CDEPAT, 1994; NRC, 1997) were very similar to those developed using the animal data of Vijayaraghavan (1997) and Kumar and Vijayaraghavan (1998).

8.2 Comparison with Other Standards and Criteria

Comparison of the draft AEGL values with other existing standards and recommendations is shown in Table 11.

8.4 Data Deficiencies

The absence of lethality data for acute exposures is a notable deficiency, especially with regard to species variability. Data providing a more definitive demarcation of the threshold for serious and/or irreversible effects would also provide a more complete picture of toxic responses resulting from acute inhalation exposure to sulfur mustard. This is especially relevant to assessing the potential for serious respiratory tract effects or long-lasting ocular effects following an acute exposure. Although sulfur mustard is a genotoxic chemical capable of inducing tumorigenic responses in animals and humans, the carcinogenic potential of acute inhalation exposures has not been well defined.

TABLE 11. COMPARISON OF AEGL VALUES FOR SULFUR MUSTARD WITH OTHER EXTANT STANDARDS AND GUIDELINES					
Exposure Duration	**Standard or Guideline**				
	AEGL-1	**AEGL-2**	**AEGL-3**	**U.S.Army/Civil Occup.TWA[a]**	**CDC-CSEPP (Thacker, 1994)[b]**
10 min	0.40 mg/m^3	0.60 mg/m^3	6.1 mg/m^3		
30 min	0.13 mg/m^3	0.60 mg/m^3	4.2 mg/m^3		
1 hour	0.067 mg/m^3	0.10 mg/m^3	2.1 mg/m^3		
4 hours	0.017 mg/m^3	0.025 mg/m^3	0.53 mg/m^3		
8 hours	0.008 mg/m^3	0.013 mg/m^3	0.27 mg/m^3		
				0.003 mg/m^3	2.0 $mg\text{-}min/m^3$

[a] (DA, 1991, 1997; DHHS, 1988)

[b] Recommended acute effects levels for determining emergency evacuation distances in the Chemical Stockpile Emergency Preparedness Program (CSEPP)

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APPENDIX A

DERIVATION OF AEGL VALUES

DERIVATION OF AEGL-1 VALUES

Key study: Anderson (1942)

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

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

Uncertainty factors: Total adjustment of 3

A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to three under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Additionally, subjects in the Anderson (1942) study exhibited little variability in ocular response.

Because the AEGL-1 is based upon human data, the interspecies uncertainty factor is 1.

10-min AEGL-1

C^1 mg/m^3 x 10 min = 12 mg·min/m^3

C = 1.2 mg/m^3

10-min AEGL-1 = 1.2 mg/m^3 ÷ 3 = 0.40 mg/m^3 (0.06 ppm)

30-min AEGL-1

C^1 mg/m^3 x 30 min = 12 mg·min/m^3

C = 0.4 mg/m^3

30-min AEGL-1 = 0.4 mg/m^3 ÷ 3 = 0.13 mg/m^3 (0.02 ppm)

1-hr AEGL-1

C^1 mg/m^3 x 60 min = 12 mg·min/m^3

C = 0.2 mg/m^3

1-hr AEGL-1 = 0.2 mg/m^3 ÷ 3 = 0.067 mg/m^3 (0.01 ppm)

4-hr AEGL-1

C^1 mg/m^3 x 240 min = 12 mg·min/m^3

C = 0.05 mg/m^3

4-hr AEGL-1 = 0.05 mg/m^3 ÷ 3 = 0.017 mg/m^3 (0.003 ppm)

8-hr AEGL-1

C^1 mg/m^3 x 480 min = 12 mg·min/m^3

C = 0.025 mg/m^3

8-hr AEGL-1 = 0.025 mg/m^3 ÷ 3 = 0.008 mg/m^3 (0.001ppm)

DERIVATION OF AEGL-2 VALUES

Key study: Anderson (1942)

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

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

Uncertainty factors: Total adjustment of 10. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to three under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Because the AEGL-1 is based upon human data, the interspecies uncertainty factor is 1. A modifying factor of 3 was applied to accomodate potential onset of long-term ocular or respiratory effects. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16 x 3.16 = 10.

10-min AEGL-2

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

30-min AEGL-2

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

1-hr AEGL-2

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

4-hr AEGL-2

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

8-hr AEGL-2

$$C^1 \times 480 \text{ min} = 60 \text{ mg-min/m}^3$$

$C = 0.125 \text{ mg/m}^3$

8-hr AEGL-2 $= 0.125 \text{ mg/m}^3 \div 10 = 0.013 \text{ mg/m}^3$ (0.002 ppm)

DERIVATION OF AEGL-3 VALUES

Key study: Kumar and Vijayaraghavan (1998)

Toxicity endpoint: Estimated lethality threshold of 21.2 mg/m^3 for one hour based upon no deaths in mice exposed to this concentration which is 0.5 of the 1-hr LC_{50} in mice reported by Vijayaraghavan (1997).

Scaling: The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al., 1986). Analysis of available data indicated n to be near unity (Appendix B). Therefore; (21.2 mg/m^3)^1 x 60 min = 1,272 mg-min/m^3

The 10-minute AEGL value was exponentially scaled (where n=3) from the 30-minute AEGL-3 value. This approach was applied due to uncertainties regarding extrapolation from 1-hour exposures to the much shorter 10-minute time frame and the lack of lethality data for such short exposure durations. This approach resulted in a somewhat lower value than would be derived using linear scaling (n=1).

Uncertainty factors: Total uncertainty factor application: 10.

An uncertainty factor for interspecies was limited to 3 because human data are available showing that exposures to the proposed AEGL-3 values is more likely to produce only severe ocular irritation and possible minor or moderate irritation of the upper respiratory tract. Intraspecies variability was limited to 3 because lethality appears to be a function of extreme pulmonary damage resulting from direct contact of the agent with epithelial surfaces. No modifying factor was applied because the basis of lethality estimate was from a studies utilizing a 14-day observation period with which to assess the lethal response from a 1-hour exposure. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16 x 3.16 = 10.

<u>10-min AEGL-3</u>

$$(4.2 \text{ mg/m}^3)^3 \times 30 \text{ min} = 2{,}222.6 \text{ mg-min/m}^3$$
$$C^3 \times 10 = 2{,}222.6 \text{ mg-min/m}^3$$
$$C = 6.05 \text{ mg/m}^3 \text{ (0.91 ppm)}$$

(No uncertainty factor adjustment as it is already incorporated in the 30-min AEGL-3 of 4.2 mg/m^3)

<u>30-min AEGL-3</u>

$$C^1 \times 30 \text{ min} = 1{,}272 \text{ mg-min/m}^3$$
$$C = 42.4 \text{ mg/m}^3$$
$$\text{30-min AEGL-3} = 42.4 \text{ mg/m}^3 \div 10 = 4.2 \text{ mg/m}^3 \text{ (0.63 ppm)}$$

1-hr AEGL-3

$C^1 \times 1 \text{ hr} = 1{,}272 \text{ mg-min/m}^3$

$C = 21.2 \text{ mg/m}^3$

1-hr AEGL-3 $= 21.2 \text{ mg/m}^3 \div 10 = 2.1 \text{ mg/m}^3$ (0.32 ppm)

4-hr AEGL-3

$C^1 \times 4 \text{ hrs} = 1{,}272 \text{ mg-min/m}^3$

$C = 5.3 \text{ mg/m}^3$

4-hr AEGL-1 $= 5.3 \text{ mg/m}^3 \div 10 = 0.53 \text{ mg/m}^3$ (0.08 ppm)

8-hr AEGL-3

$C^1 \times 8 \text{ hrs} = 1{,}272 \text{ mg-min/m}^3$

$C = 2.65 \text{ mg/m}^3$

4-hr AEGL-1 $= 2.65 \text{ mg/m}^3 \div 10 = 0.27 \text{ mg/m}^3$ (0.04 ppm)

APPENDIX B

DETERMINATION OF TEMPORAL SCALING FACTOR (*N*) FOR AEGL DERIVATIONS

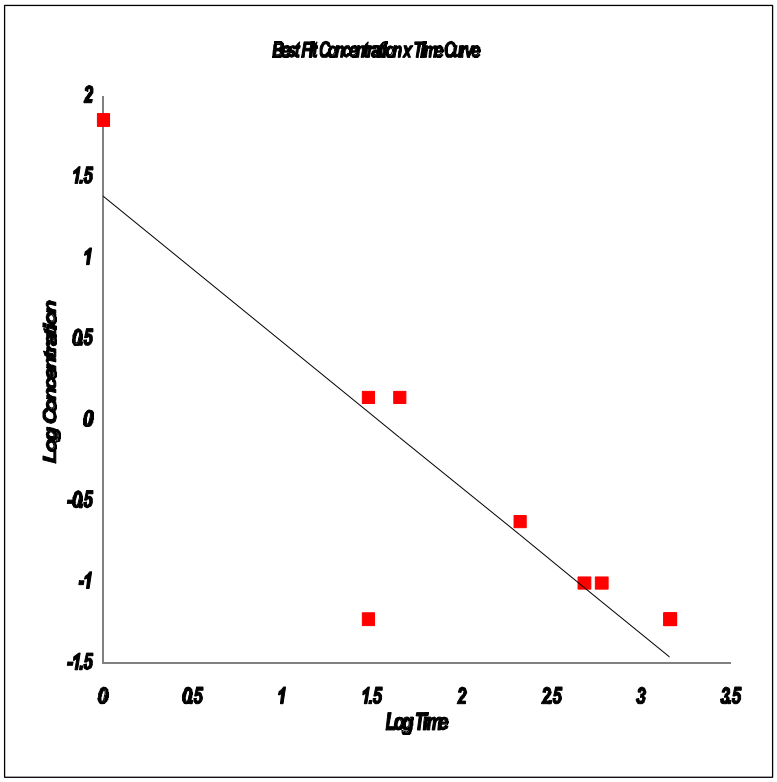
Derivation of n for $C^n \times t = k$; data points indicative of a 100% response for mild ocular irritation following exposure to sulfur mustard (Agent HD) at various concentrations and times (Reed, 1918; Reed et al., 1918; Guild et al., 1941; Anderson, 1942)

Time	Conc.	Log Time	Log Conc.
1	72	0.0000	1.8573
30	1.4	1.4771	0.1461
30	0.06	1.4771	-1.2218
45	1.4	1.6532	0.1461
210	0.24	2.3222	-0.6198
480	0.1	2.6812	-1.0000
600	0.1	2.7782	-1.0000
1440	0.06	3.1584	-1.2218
n =	1.11		
k =	34.58		

Regression Output:

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

Minutes	Conc.	Hours	Conc.
30	1.14	0.5	45.30
60	0.61	1.0	24.27
240	0.17	4.0	6.97
480	0.09	8.0	3.73



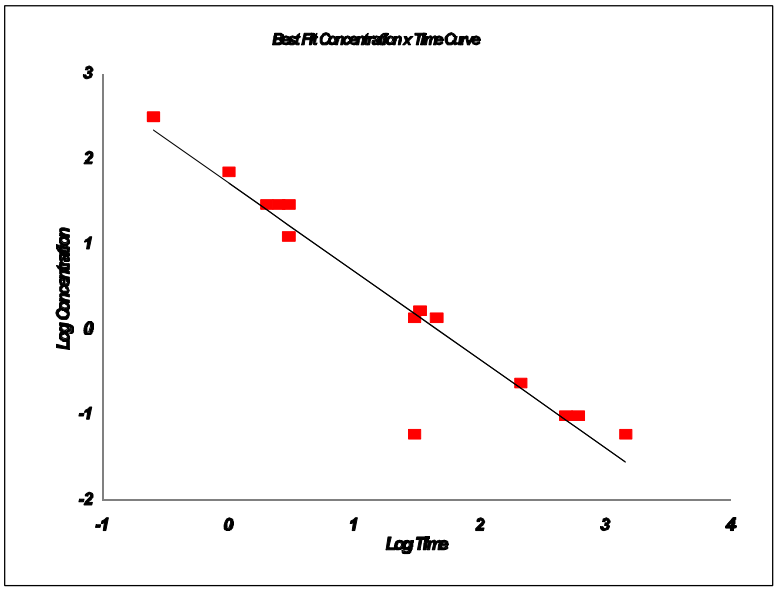
Derivation of n for $C^n \times t = k$; data points indicative of a 75-100% response for mild ocular irritation following exposure to sulfur mustard (Agent HD) at various concentrations and times (Reed, 1918; Reed et al., 1918; Guild et al., 1941; Anderson, 1942)

Time	Conc.	Log Time	Log Conc.
1	72	0.0000	1.8573
30	1.4	1.4771	0.1461
30	0.06	1.4771	-1.2218
45	1.4	1.6532	0.1461
210	0.24	2.3222	-0.6198
480	0.1	2.6812	-1.0000
600	0.1	2.7782	-1.0000
1440	0.06	3.1584	-1.2218
33	1.7	1.5185	0.2304
3	12.7	0.4771	1.1038
3	30	0.4771	1.4771
2.5	30	0.3979	1.4771
2	30	0.3010	1.4771
0.25	320	-0.6021	2.5051

Regression Output:	
Intercept	1.7240
Slope	-1.0365
R Squared	0.8891
Correlation	-0.9429
Degrees of Freedom	12
Observations	14

n = 0.96
k = 46.05

Minutes	Conc.	Hours	Conc.
30	1.56	0.5	108.65
60	0.76	1.0	52.97
240	0.18	4.0	12.59
480	0.09	8.0	6.14


Best Fit Concentration x Time Curve
Log Concentration
Log Time
3
2
1
0
-1
-2
-1
0
1
2
3
4

APPENDIX C
AEGL Derivations Using Alternate Approaches

AEGL-1 values were also developed using logistic and probit modeling that utilized multiple data sets. These models were used to determine a 99% response at 30 minutes, 1 hour, and 4 hours and were based upon the mild response data drawn from the available study reports. The 10-minute AEGL-1 values were derived based upon linear extrapolation from the 30-minute values using $C^1 \times t = k$. AEGL-1 values developed using exposure values generated by these models affirm that the proposed AEGL-1 values are defensible and protective of human health relative to AEGL-1 level effects.

TABLE C-1. AEGL-1 VALUES (MG/M^3) DEVELOPED USING DIFFERENT DATA SETS[a]

10-min	30-min	1-hr	4-hrs	Criteria	Reference
1.05	0.35	0.22	0.004	Probit procedure; 99% response; UF=3[a]; *n*=1[b]	Reed, 1918; Reed et al., 1918; Guild et al., 1941; Anderson, 1942
0.6	0.20	0.13	0.009	Logistic procedure; 90% response; UF= 3[a]; *n*=1	Reed, 1918; Reed et al., 1918; Guild et al., 1941; Anderson, 1942

[a] Uncertainty factor applied to protect sensitive individuals.

[b] *n* is the exponent in the equation, $C^n \times t = k$, used for extrapolation of exposures to different time periods; empirically derived for agent HD (see Appendix B).

APPENDIX D

CARCINOGENICITY ASSESSMENT FOR SULFUR MUSTARD (AGENT HD)

CARCINOGENICITY ASSESSMENT FOR ACUTE EXPOSURE TO SULFUR MUSTARD (AGENT HD)

The cancer assessment for acute inhalation exposure to sulfur mustard ws conducted following the NRC methodology for EEGLs, SPEGLs and CEGLs (NRC, 1986).

The virtually safe dose (VSD) was determined from an inhalation Slope Factor of 14 $(mg/kg/day)^{-1}$ for the general population. This slope factor was a geometric mean of slope factors developed using various data sets and procedures, and was consdiered the most tenable quantitative assessment for potential cancer risk from inhalation exposure to sulfur mustard. The corresponding Inhalation Unit Risk was 0.0041 $(\mu g/m^3)^{-1}$ or 4.1 $(mg/m^3)^{-1}$. The VSD was calculated as:

$$\text{VSD} = \text{Risk Level /Unit Risk}$$

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

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

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

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

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

If the exposure is limited to a fraction (f) of a 24-hr period, the fractional exposure becomes 1/f x 24 hrs (NRC, 1985). For a 1 x 10^{-4} risk:

24-hr exposure = 0.1 mg/m^3 (0.02 ppm)
8-hr = 0.3 mg/m^3 (0.05 ppm)
4-hr = 0.6 mg/m^3 (0.09 ppm)
1-h r= 2.4 mg/m^3 (0.36 ppm)
30 min = 4.8 mg/m^3 (0.72 ppm)
10 min = 14.1 mg/m^3 (2.16 ppm)

Because the derivation of the cancer slope factor requires conversion of animal doses to human equivalent doses, no reduction of exposure levels is applied to account for interspecies variability. For 10^{-5} and 10^{-6} risk levels, the 10^{-4} values are reduced by 10-fold or 100-fold, respectively.

ACUTE EXPOSURE GUIDELINES FOR SULFUR MUSTARD
(CAS NO. 505-60-2)

AEGL-1 VALUES				
10 min	**30 min**	**1 hr**	**4 hrs**	**8 hrs**
0.06 ppm **0.40 mg/m^3**	**0.02 ppm** **0.13 mg/m^3**	**0.01 ppm** **0.067 mg/m^3**	**0.003 ppm** **0.017 mg/m^3**	**0.001 ppm** **0.008 mg/m^3**

Key Reference: Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India).

Test Species/Strain/Number: 3-4 human volunteers

Exposure Route/Concentrations/Durations: Inhalation exposure to varying concentrations (1.7- 15.6 mg/m^3) for varying durations (2-33 minutes)

Effects: Mild ocular effects (mild injection to notable conjunctivitis)

Endpoint/Concentration/Rationale: Concentration-time threshold of 12 mg-min/m^3 for ocular effects (conjunctival injection with minor discomfort and no functional decrement)

Uncertainty Factors/Rationale:
Interspecies: 1 (human subjects)
Intraspecies: A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to three under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Furthermore, little variability was observed in the tested subjects regarding ocular responses.

Modifying Factor: None applied

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: $C^n \times t = k$, where $n = 1$ based on analysis of available human exposure data for ocular effects.

Confidence and Support for AEGL Levels: The key study was conducted using human volunteers thus avoiding uncertainties associated with animal studies. Ocular irritation is considered the most sensitive endpoint for assessing the effects of acute exposure to sulfur mustard. The AEGL-1 values are considered to be adequately protective of human health and the confidence rating for the AEGL-1 values is considered to be medium.

ACUTE EXPOSURE GUIDELINES FOR SULFUR MUSTARD
(CAS NO. 505-60-2)

AEGL-2 VALUES				
10 min	**30 min**	**1 hr**	**4 hrs**	**8 hrs**
0.09 ppm **0.60 mg/m^3**	**0.03 ppm** **0.20 mg/m^3**	**0.02 ppm** **0.10 mg/m^3**	**0.004 ppm** **0.025 mg/m^3**	**0.002 ppm** **0.013 mg/m^3**

Key Reference: Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India).

Test Species/Strain/Sex/Number: 3-4 human volunteers

Exposure Route/Concentrations/Durations: Inhalation exposure to varying concentrations (1.7- 15.6 mg/m^3) for varying durations (2-33 minutes)

Effects: Ocular effects ranging from mild injection to notable conjunctivitis, photophobia, lacrimation, blepharospasm

Endpoint/Concentration/Rationale: Exposure-concentration time product of 60 mg-min/m^3 representing the threshold for ocular irritation (well marked, generalized conjunctivitis, edema, photophobia, and irritation) resulting in performance decrement and necessitating medical treatment.

Uncertainty Factors/Rationale:
Interspecies: 1 (human subjects)
Intraspecies: A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to three under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Furthermore, little variability was observed in the tested subjects regarding ocular responses.

Modifying Factor: A modifying factor of 3 was applied to accommodate uncertainties regarding the onset of potential long-term ocular effects or respiratory effects.

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: $C^n \times t = k$, where $n = 1$ based on analysis of available human exposure data for ocular effects.

Confidence and Support for AEGL Levels: The key study was conducted using human volunteers thus avoiding uncertainties associated with animal studies. The AEGL-2 values are based upon ocular effects that may be considered severe enough to impair vision and escape. Confidence in the AEGL-2 values is medium.

ACUTE EXPOSURE GUIDELINES FOR SULFUR MUSTARD
(CAS NO. 505-60-2)

AEGL-3 VALUES				
10 min	**30 min**	**1 hr**	**4 hrs**	**8 hrs**
0.91 ppm **6.05 mg/m^3**	**0.63 ppm** **4.2 mg/m^3**	**0.32 ppm** **2.1 mg/m^3**	**0.08 ppm** **0.53 mg/m^3**	**0.04 ppm** **0.27 mg/m^3**

KeyReference: Kumar, O., Vjayaraghavan, R. 1998. Effect of sulphur mustard inhalation exposure on some urinary variables in mice. J. Appl. Toxicol. 18: 257-259.

Test Species/Strain/Sex/Number: Swiss mice/female/4 per exposure group

Exposure Route/Concentrations/Durations: Head-only inhalation exposure for 1 hr to sulfur mustard (>99% purity) at 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0 and 2.0 LC_{50}) and sacrificed at 6, 24, or 48 hours, or 7 days after exposure. Three groups of 10 mice were exposed at each concentration.; observed for up to 14 days

Effects: Lethality assessed up to 14 days post exposure

Endpoint/Concentration/Rationale: No mortality in mice at 14 days following 1-hr exposure to 21.2 mg/m^3. This exposure was considered an estimate of the lethality threshold in mice.

Uncertainty Factors/Rationale:
Total uncertainty factor: 10
Interspecies: An uncertainty factor of 3 was also applied to account for possible interspecies variability in the lethal response to sulfur mustard. Application of any additional uncertainty factors or modifying factors was not warranted because the proposed AEGL-3 values are equivalent to exposures in humans that are known to produce only ocular and respiratory tract irritation.
Intraspecies: Intraspecies variability was limited to 3 because lethality appears to be a function of extreme pulmonary damage resulting from direct contact of the agent with epithelial surfaces.

Modifying Factor: No modifying factor was applied because the basis of lethality estimate was from a study utilizing a 14-day observation period with which to assess the lethal response from a 1-hour exposure.

Animal to Human Dosimetric Adjustment: Insufficient data

Time Scaling: $C^n \times t = k$ where n = 1 based upon analysis of human exposure data for ocular effects. For development of the 10-minute AEGL-3 value, exponential scaling (where n=3) was applied due to uncertainty regarding linear extrapolation to a time duration notably shorter than that for which empirically derived lethality data were available.

Confidence and Support for AEGL Levels: The confidence in the precision of the AEGL-3 values is low to medium due uncertainites regarding a definitive lethality threshold. The key study appeared to be a well-designed and properly conducted. Based upon the available human data and the approach used for AEGL development, the resulting AEGL-3 values are considered to represent a conservative estimate for the threshold for lethal responses to acute sulfur mustard exposure.