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Toxicology Studies on Lewisite and Sulfur Mustard Agents: Subchronic Toxicity of Sulfur Mustard (HD) in Rats

Final Report

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Occupational health standards have not been established for sulfur mustard [bis(2-chlorethyl)-sulfide], a strong alkylating agent with known mutagenic properties. Seventy-two Sprague-Dawley rats of each sex, 6-7 weeks old, were divided into six groups (12/group/ sex) and gavaged with either 0, 0.003 , 0.01 , 0.03 , 0.1 or 0.3 mg/kg of sulfur mustard in sesame oil 5 days/week for 13 weeks. No dose-related mortality was observed. A significant decrease ($P < 0.05$) in body weight was observed in both sexes of rats only in the 0.3 mg/kg group. Hematological evaluations and clinical chemistry measurements found no consistent treatment-related effects at the doses studied. The only treatment-related lesion associated with gavage exposure upon histopathologic evaluation was epithelial hyperplasia of the forestomach of both sexes at 0.3 mg/kg and males at 0.1 mg/kg. The hyperplastic change was minimal and characterized by cellular disorganization

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of the basilar layer, an apparent increase in mitotic activity of the basilar epithelial cells, and thickening of the epithelial layer due to the apparent increase in cellularity. The estimated NOEL for HD in this 90-day study is 0.1 mg/kg/day when administered orally.

FOREWORD

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J. B. Jones 6/30/84

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EXECUTIVE SUMMARY

Chemical warfare agents present an obvious risk to individuals suffering acute exposure, but they may also present long-term environmental or occupational health hazards for workers in operations involving these chemical agents. Occupational health standards have not been established for sulfur mustard [bis(2-chloroethyl)-sulfide] a strong alkylating agent with known mutagenic and suspected carcinogenic properties. Sulfur mustard is used in a number of research laboratories, stored in depot sites throughout the country and occasionally transported to distant sites. The destruction of current stockpiles of sulfur mustard by the U.S. Army in the near future could create additional environmental and occupational risk. To establish a data base for setting environmental and occupational standards, we have conducted studies to evaluate the toxicity, mutagenicity, and reproductive effects of sulfur mustard using *in vitro* and *in vivo* study systems. This report presents the results of a 13-week subchronic study in male and female rats exposed to sulfur mustard by intragastric incubation.

Solutions of sulfur mustard were prepared for administration by diluting the neat agent with sesame oil. Seventy-two Sprague-Dawley rats of each sex, 6-7 weeks old, were divided into six groups (12/group/sex) and gavaged with either 0, 0.003, 0.01, 0.03, 0.1 or 0.3 mg/kg of sulfur mustard 5 days/week for 13 weeks. A constant dosage volume of 1.67 ml/kg of body weight was given.

Body weights were measured weekly throughout the study and animals were observed twice each day of dosing for mortality and moribundity. Opthamology evaluations were performed at the beginning and termination of the study. Hematological evaluations were performed at 6 weeks and at the termination of the study. Serum BUN, creatinine, total protein, SGPT and SGOT were evaluated at terminal sacrifice. A complete gross necropsy was performed and a histopathologic evaluation of collected tissue was made.

No dose-related mortality was observed. A significant decrease in body weight was observed in both sexes of the 0.3 mg/kg dose group. Hematological evaluation and serum chemistry measurements found no consistent treatment-related effects at the doses studied. The only treatment-related lesion associated with the gavage exposure upon histopathologic evaluation was epithelial hyperplasia of the forestomach of both sexes at 0.3 mg/kg. The forestomach of one 0.1 mg/kg male was also ulcerated. The hyperplastic change was minimal and was characterized by cellular disorganization of the basilar layer, and apparent increase in mitotic activity of the basilar epithelial cells, and thickening of the epithelial layer due to the apparent increase in cellularity. The estimated NOEL for HD in this 90-day study is 0.1 mg/kg/day when administered orally.

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INTRODUCTION

Chemical warfare agents present an obvious risk to individuals suffering acute exposures and may also present certain long-term environmental or occupational health hazards for workers in operations involving these chemical agents. These materials are used in a number of research laboratories, stored in depot sites throughout the country and occasionally transported to distant sites. In addition, stockpiles of agents are scheduled for destruction by the U.S. Army in the near future, creating an additional potential for environmental and occupational exposure. Although considerable information is known concerning the acute effects of these agents, little information is available on the long-term hazards of these materials. Segments of the population that may be particularly sensitive to their toxicity include the chronically ill, the young and old, and the unborn. It is therefore necessary that potentially toxic and mutagenic chemicals be identified, and that a database be established for the development of hazard evaluations and occupational health standards for these chemicals. This database will be used by the U.S. Army to derive occupational exposure criteria for sulfur mustard.

The two general categories of vesicants are typified by lewisite [dichloro(2-chlorovinyl)arsine] and sulfur mustard (HD) [bis(2-chloroethyl) sulfide] (Cassarett and Doull, 1986). Contact with these chemicals produces severe skin burns. Recently, a renewed interest in these chemicals was generated by the release of a United Nations report that contained substantial evidence that Iraq was manufacturing and using these agents as chemical warfare agents (Marshall, 1984).

The mustard compounds (both sulfur and nitrogen) are biochemically related to a group of cytotoxic alkylating agents, including the ethylenimines, sulfonic esters, epoxides and n-alkyl-n-nitroso compounds (Wheeler, 1962). These chemicals react rapidly with certain functional groups of proteins (OH, NH_2, and SH) to alter their metabolic activity. In aqueous solutions, both sulfur and nitrogen mustard hydrolyze to form cyclic sulfonium or immunium forms, respectively, which, in turn, will react with nucleophilic sites. The sulfur mustard reaction proceeds more rapidly to the reaction with nucleophiles than does nitrogen mustard and is independent of the concentration of nucleophiles present (Fox and Scott, 1980). The cytotoxic, mutagenic, and carcinogenic properties of mustard compounds have been studied extensively (Fox and Scott, 1980), but most of these data relate to nitrogen mustard because sulfur mustard is a more toxic and chemically reactive vesicant.

Relevant chemical and physical properties of sulfur mustard are summarized in Table 1. In aqueous solutions, sulfur mustard rapidly hydrolyzes to form a cyclic sulfonium salt, β-chloroethyl-ethylenesulfonium chloride. This salt reacts with water to form β-chloroethyl β-hydroxyethyl sulfide and hydrochloric acid. Subsequent hydrolysis of the sulfide, presumably through the intermediation of a second sulfonium salt, forms

thiodiglycol (Anslow et al., 1948). These workers have investigated the intravenous (ip) and subcutaneous (sc) toxicity of these derivatives of sulfur mustard and a number of other intermediates isolated from hydrolysates of sulfur mustard. They found that two of the derivatives, β-chloroethyl β-hydroxyethyl sulfide and thiodiglycol, were relatively nontoxic.

TABLE 1. Relevant Chemical and Physical Properties of Sulfur Mustard, Bis(2-Chloroethyl)Sulfide[a]

CAS #:	505-60-2
RETCS #:	WQ0900000
Structural formula:	$Cl-CH_2-CH_2$ \ S / $Cl-CH_2-CH_2$
Molecular weight:	159.1 g
Density at 25°C:	1.3 g/ml
State:	Colorless, oily liquid
Vapor pressure at 20°C:	0.072 mm
Decomposition temperature:	149-177°C
Solubility in water at 25°C:	0.68 g/L
Hydrolysis	
Rate ($T_{\frac{1}{2}}$ at 25°C, pH 7):	8.5 min
Products:	Thiodiglycol, chloride

[a]Rosenblatt et al., 1975 and Windholz, 1983.

Few values are available in the literature for the LD_{50} of sulfur mustard. Table 2 includes LD_{50} data for sulfur mustard administered by various routes to mice, rats and rabbits. Haskin (1948) reported that extensive edema occurred at the site of administration of nitrogen mustard (ip and sc) and that diarrhea, dyspnea, and anorexia were common observations. Death occurred in rats within 3 to 4 days after administration at dose levels of 1.8 to 3.1 mg/kg and within 5 to 19 days of administered doses of 1 to 1.2 mg/kg.

Relatively little is known concerning the effects of HD on development and reproduction. Chronic inhalation exposure of male rats to sulfur mustard (0.1 mg/m³) was reported to produce significant dominant lethal effects, but exposure of pregnant females to the same concentrations for a shorter time interval failed to induce fetal malformations (Rozmiarek et al., 1973). McNamara et al. (1975) subsequently reported from these same data that there were no differences between the control and experimental groups and no evidence of mutagenesis. It is difficult to resolve the apparent conflict

between the conclusions of these two reports, but the fetal mortality values presented in the McNamara report suggest at least a trend for a significant dominant lethal effect. Complete control data are missing from the report and statistical evaluation of the results is not presented, but percentage fetal death at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001 and 0.1 mg/m³ exposure groups, respectively.

TABLE 2. LD_{50} Values[a] of Various Routes of Administration for Sulfur and Nitrogen Mustard

Chemical	Route of Administration[b]	LD_{50} (mg/kg) Rat	Rabbit	Mouse
Sulfur Mustard	iv	0.7	1.1	8.6
	sc	1.5	2.0	2.0
Nitrogen Mustard	iv	1.1	---	---
	sc	---	---	1-4
	ip	1.8-2.5	---	4.4
	Oral	---	---	10-20

[a]Registry of Toxic Effects of Chemical Substances, 1987.
[b]iv = intravenous; sc = subcutaneous; ip = intraperitoneal.

The teratogenic potential of HD was studied in rats exposed to two concentrations of inhaled HD (0.001 and 0.1 mg/m³) during each of the 3 weeks of gestation or throughout the entire gestation period (McNarmara et al., 1975). No evidence of dose-related fetal mortality or gross abnormalities was noted. Teratology studies (following the segment II teratology protocol) were recently conducted in rats and rabbits by Hackett et al. (1987). Rats were exposed to 0.5 to 2.0 mg/kg HD by gastric intubation from 6 to 15 days of gestation (dg) and were killed on dg 20. No evidence of a teratogenic response to HD was observed since fetal effects occurred only at doses exhibiting signs of maternal toxicity. Likewise, fetal development of rabbits exposed to 0.4-0.8 mg/kg HD between 6 and 19 dg was not affected even though maternal mortality was induced at the highest dose. These results suggest that HD is not teratogenic in rats and rabbits since fetal effects were observed only at dose levels that induced frank maternal toxicity.

Comprehensive information is not available to evaluate the potential risk to reproduction from long-term occupational exposure to sulfur mustard. The purposes of this subchronic study were to define a No-Observed-Effect-Level (NOEL), determine the target organ or organs with greatest susceptibility to the chemical agent and provide data to establish dose

levels for subsequent multi-generation reproductive and dominant lethality studies.

MATERIALS AND METHODS

SULFUR MUSTARD

Procurement and Characterization

The sulfur mustard used in these studies was 2,2',dichlorodiethyl sulfide, also known as bis(2-choroethyl)sulfide or distilled mustard (HD).

The sulfur mustard was supplied by the U.S. Army Medical Research Institute for Chemical Defense (USAMRICD), Chemical Surety/Safety Office, Aberdeen Proving Ground, Edgewood Arsenal MD from lot No. HD-U-4244-CTF-N-1, previously designated Lot No. ICD-HD-1. The material was prepared August 31, 1981 and analyzed for purity September 4, 1984 by Captain William Beaudry and Linda Szafdraniec (Research Directorate Chemical Research) by nuclear magnetic resonance. Purity, calculated on a weight basis, was 97.3%. There were two impurities with concentrations of 1.2% (assumed to be dithiane) and 1.5% (identity unknown). Material from this lot has been proposed as the standard analytical reference for USAMRDC and USAMRDC has agreed to retain aliquots of this material to comply with the requirements of Good Laboratory Practices (GLP).

A shipment of 25 ml of HD (in two ampules) was delivered on March 7, 1985 by a team from the U.S. Army Technical Escort Unit. The ampules were inspected and found to be intact. Subsequently the HD was transferred from the ampules into 30-ml Wheaton bottles, sealed and stored in secondary unbreakable containers in a refrigerated storage container at approximately 6°C.

Selection and Characterization of Diluent

Sulfur mustard is relatively insoluble (680 mg/L) and also is rapidly hydrolyzed in water, therefore sesame oil was employed as the diluent for dosing solutions in this study. This selection was not only based on the chemical and physical properties of the compound, but also on the lack of a toxic response of the vehicle when introduced into the stomach of the animal. Corn oil is commonly the vehicle used for the administration of water-insoluble compounds; however, Hackett et al. (1987) concluded from data in the literature that corn oil may not be appropriate for reproductive studies because of its high steroid content and recommended using sesame oil in their studies of the teratology of sulfur mustard. Sesame oil contains no preservatives, appears to be stable when stored under proper conditions, is relatively low in steroids and is readily available.

The sesame oil (Hain Pure Food Company, Los Angeles, CA) used in this study was purchased locally in one quart bottles and numbered according to lot and bottle. Peroxide analyses of each lot of sesame oil was performed at the beginning of the study or when purchased and periodically throughout the study to provide a measure of oxidation as an indication rancidity of the oil. The method measures the ability of the oil to oxidize aqueous iodide. Only oil in which the peroxide content was less than 10 meq/kg was used in the study.

The results of the peroxide analyses of the sesame oil used are given in Table 3. The amount of peroxide in the sesame oil was well within the acceptable limits of 10 meq/kg set forth in the protocol.

TABLE 3. Analysis of Sesame Oil for Peroxide

Lot No.	Date Purchased	Assay Date	Container Identification	Peroxide meq/kg ± SD
50774-2	7/1/85	7/2/85	1	4.90 ± 0.15
		8/15/85	6	8.44 ± 0.04
50774-19	8/20/85	8/28/85	8	4.72 ± 0.10
		9/24/85	12	4.73 ± 0.04
11421/B	9/19/85	9/24/85	13	4.91 ± 0.00

Preparation of Solutions for Administration

The HD dosing solutions administered to the animals were prepared in advance and stored in a refrigerator at approximately 6°C for no longer than one week. Hackett et al. (1987) found that HD in sesame oil was stable up to 1 week when stored under these conditions. The general procedure was to determine in advance the amount of neat HD needed, based on the volumes to be prepared and the final concentrations desired. This volume was then removed from the bottle of neat HD and thoroughly mixed into a known volume of sesame oil. Aliquots of this intermediate concentration were then diluted further to give the final concentration needed for the dosing solutions. Aliquots of the final solutions were placed in Wheaton bottles with teflon-lined sepa and aluminum caps. Each Wheaton bottle contained sufficient volume of HD-sesame oil for 1 day's use. The bottles were labeled with the name and the concentration of the agent (HD) and placed into a secondary unbreakable container which was identified by chemical name, concentration, lot number and date prepared.

Analytical Procedures

Methods were developed for the assay of HD in sesame oil by gas chromatography, using a capillary column and flame-ionization detection. The assay was complicated by the high boiling points of some components in sesame oil. As a result, the temperature of the capillary-column inlet had to be maintained at 200°C. The procedure consisted of diluting 0.50 ml of the HD-sesame oil sample with 0.50 ml of 18.7 ng/ul 2,4-dichlorotoluene (DCT) in isooctane, contained in a 1.5-ml automatic sampler vial with a Teflon-lined crimped-top cap. The DCT was used as an internal standard for the assay. A Hewlett-Packard 5840A gas chromatograph and 7672 automatic sample changer

were used with a capillary DB-5 column (J & W Scientific, Folsom, CA). The method can detect as low as 0.01 mg/ml.

Results of samples analyzed using this method are presented in Table 4; means are reported when duplicate samples were available. Theoretical and analyzed values were essentially the same especially at the higher concentrations. Some deviation between theoretical and analyzed values was seen at the low concentration. This may have resulted from a lack of precision of the method or could be the result of degradation by the sesame oil as the percentage of oil increased. When samples were repeatedly analyzed at several times, no evidence of degradation was seen up to 52 days (Table 4).

ANIMAL MAINTENANCE

Four week old male and female rats of Sprague-Dawley derivation were obtained from Charles River Laboratories, Inc., Portage, MI facility and quarantined in isolation for about 3 weeks until a health evaluation could be completed. The CD rat was selected because it has been used in a number of previous reproductive studies at PNL including gavage studies of sulfur mustard and background information is available for estimating doses. During quarantine the rats were group housed, separated by sex, in stainless-steel wire bottom cages placed on automatic flush racks with an automatic watering system.

The environmental conditions specified for the animal rooms were temperatures of 72±3°F, relative humidity of 50±15%, and a lighting cycle of 12 hours on and 12 hours off. Purina Certified Rodent Chow (#5002) was purchased and drinking water were provided *ad libitum*. Drinking water supplied to the animal rooms was passed through a reverse-osmotic purification unit containing two particle filters and a carbon filter.

Near the end of quarantine seven rats, 4 males and 3 females, were subjected to a health evaluation and tested for antibodies to viral pathogens. No significant pathogens or lesions were found. Pre-study hematology was evaluated on blood samples collected from 19 additional rats, 13 males and 6 females.

Following isolation the rats were weighed and assigned to the appropriate treatment groups by sex and weight by means of a formal randomization statistical package (See Statistical Methods). Each animal was assigned an individual identification number by means of a metal ear tag. The animals were individually housed as described above and cage cards were used to indicate the animal number and treatment group. On the first day of the study excess animals were discarded.

Experiment Design

The experimental design for the 90-day subchronic study is described below in Table 5. Twelve 7-week old weanling rats of each sex were assigned to one of five treatment groups or to the vehicle control group. Solutions

Table 4. Sulfur Mustard (HD) Dose Levels and Solution Concentration for Samples Analyzed from the 90-Day Subchronic Study

Date Prepared	Date Analyzed	Dose Level (mg/kg)	HD Concentration (ug/ml) Theoretical	HD Concentration (ug/ml) Analyze[a]
9/20/85	9/27/85	0.3	180	180
		0.1	60	62
		0.03	18	16
		0.01	6	4.8
		0.003	1.8	1.8
9/27/85	10/4/85	0.3	180	180
		0.1	60	59
		0.03	18	16
		0.01	6	4.4
		0.003	1.8	0.8
10/4/85	10/4/85	0.3	180	180
		0.1	60	56
		0.03	18	16
		0.01	6	4.4
		0.003	1.8	0.8
10/4/85	10/14/85	0.3	180	169 ± 3
		0.1	60	53 ± 2
		0.03	18	14 ± 1
		0.01	6	4.3 ± 3
		0.003	1.8	0.8
10/4/85	11/14/85	0.3	180	173
		0.1	60	44
		0.03	18	15
		0.01	6	4.6
		0.003	1.8	ND[b]
10/4/85	11/21/85	0.3	180	178 ± 5
		0.1	60	56 ± 3
		0.03	18	15 ± 0
		0.01	6	4.3 ± 0.5
		0.003	1.8	0.9 ± 0.1

[a]Mean ± SE when duplicate values were available.
[b]Not detected.

TABLE 5. Experimental Design

	Number of Animals per Level	Number of Sexes	Number of Dose Levels	Total
HD Exposure Groups	12	2	5	120
Vehicle control	12	2	1	24
Health screen	--	--	--	7
Pre-study hematology	--	--	--	19
Total				170

administered to the animals by intragastric intubation 5 days per week for approximately 13 weeks or approximately 65 dosing days. The animals were weighed each week and the weekly doses for individual animals were calculated from their body weight. A constant dosage volume of 1.67 ml/kg of body weight was given.

An upper dose of 0.3 mg/kg was selected based on data obtained from a short-term teratology study (Hackett et al. 1987). Each of the remaining dose levels was set using a sequential three-fold reduction which resulted in dose levels of 0.3, 0.1, 0.03, 0.01 and 0.003 mg/kg of body weight.

Oral exposure was specified by the sponsor for this study. The expected routes of environmental exposure are inhalation, dermal exposure, or ingestion, either direct or from swallowing inhaled material. Oral exposure was selected over inhalation, dermal application and subcutaneous or intraparietal injection for a number of reasons. It was considered impractical to expose by inhalation because of the potential hazards to personnel, technical aspects of generating the agent and the cost of a long-term inhalation exposure. Direct application to the animal was not desirous because of hazards incurred while handling the animals and the possible development of lesions after long-term exposure which could affect the translocation of material to the body. Injection of the material was ruled out because of the potential of local lesions from multiple injections of the agent.

TOXICOLOGIC EVALUATIONS

Individual animals were weighed immediately prior to initiation of chemical treatment, at weekly intervals throughout the study, prior to necropsy of moribund or dead animals and at terminal sacrifice. All animals were observed twice daily, morning and afternoon, of each day of dosing for mortality and moribundity. Daily observations were also made on each non-dosing day. All animals were evaluated weekly for clinical signs of toxicity.

Opthamology evaluations were performed at the beginning and termination of the study.

Hematological and Clinical Chemistry Evaluation

The general provisions of the National Toxicology Program (NTP) July 1984 General Statement of Work were utilized in the hematology and clinical chemistry evaluations. Blood were collected from the abdominal aorta of 17 selected pre-study rats and of all surviving animals at terminal sacrifice. At the 6th week of the study blood specimens were obtained from the retro-orbital plexus of all study animals for hematological evaluation.

An Othro Elt-8/ds multiparameter hematologic evaluation was determined for the following parameters: platelets, leukocytes, red blood cell (RBC) count, hemoglobin concentration, volume of packed red cells (VPRC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Blood smears were stained with Wright/Giemsa stain in a Gam-Rad automated stainer. Leukocyte differential counts were based on classifying a minimum of 100 leukocytes. Reticulocytes, stained supravitally with New Methylene Blue, were enumerated using the Miller disc method.

Clinical chemistry was evaluated only at terminal sacrifice. Blood was collected without anticoagulants from the abdominal aorta of the anesthetized rat. Following centrifugation of the blood sample, the serum was removed and assayed immediately for bilalary urea nitrogen (BUN), creatinine, total protein, serum glutamic pyruvic transaminase (SGPT) and glutamic oxaloacetic transaminase (SGOT) using an Abbot VP Chemistry unit.

Necropsy and Histological Evaluations

A complete gross necropsy was performed on all rats found dead or in moribund condition and those killed at the scheduled terminal sacrifice. Live animals were euthanitized with 70% CO_2 within 24 hours of the last two consecutive dosings and immediately necropsied. Weights were taken of liver, thymus, right kidney, right testis, heart, brain and adrenals. Tissues were stored in 10% neutral buffered formalin (NBF).

To standardize the degree of distension of pulmonary alveoli with fixative, the lungs were fixed by inserting a blunted needle into the laryngeal lumen through which the fixative was infused (10% NBF).

Tissues from 12 male and 12 female rats of the 0.3 mg/kg and control groups were examined microscopically. Target organs identified in the 0.3 mg/kg group were examined successively in the next lower doses until no effect was found. Histopathologic diagnoses were tabulated in the Microscopic Pathology Incidence Table.

STATISTICAL METHODS

The PNL derived computer software program (DRANDBLK) for randomizing animals into experimental groups is based on a single blocking factor for animal weight. Animal weights for a given study are ordered from lightest to heaviest; blocks of animal weights are then randomly assigned to the treatment groups and the control group. Block sizes are governed by the number of test groups.

Analysis of variance was used to analyze weight, hematology and clinical chemistry data (SAS, 1985). If the results of the analyses were significant, Tukey's Studentized Range Test was used to delineate intergroup differences among means (Tukey, 1953). An orthogonal contrast was used to test for a trend in the results repeated over time on the same animal, a randomization test was used to test for differences among growth curves (Zerbe, 1979). This test is a nonparametric statistical test that is based on the absolute area between growth curves and allows for correlation of body-weight measurements over time.

RESULTS

All but 3 of the 144 treatment animals survived to scheduled sacrifice. None of the deaths were attributed to chemical exposure; all appeared to be due to trauma (perforated lung or hard palate) occurring immediately prior to death from dosing. Lesions, including a mass adhered to the atrium and a lung lesion, were observed at the scheduled sacrifice of one rat from the 0.3-mg/kg group. This animal was excluded from all summaries because of evidence of dosing trauma.

Body weights of male and female rats are presented in Tables 6 and 7, respectively. The rats readily adapted to the exposure environment as control animals maintained a typical growth rate during the study (Figure 1). Growth curves for both sexes of the 0.3 mg/kg group began to deviate from the control group at 2 weeks of exposure and continued at a decreased rate throughout the study. In comparing weekly weights of control and treated animals, the 0.3 mg/kg females weighed significantly ($P < 0.05$) less than controls at week 4 and during the last 5 weeks of exposure whereas the males showed more variability, weighing significantly less than controls at weeks 3, 4, 7, 8, 9, and 12. There was no evidence of a dose-response at lower doses.

Weekly clinical observations of behavior, respiration, skin and haircoat, and fecal material revealed no significant changes. Ocular damage was observed in a number of animals as a result of orbital bleeding for hematological evaluation at 6 weeks, but this affect was not attributed to treatment. There was no evidence of a dose-response at lower doses.

Hematology data are summarized in Tables 8 and 9. Evidence of treatment-related changes in white cell indices were not observed, although total white cells, lymphocytes and neutrophils (in the case of females) of all exposure groups were greater at 6 weeks compared to pre-study or 13 week values (Table 9). This inconsistency may have been the result of the method of blood sampling; 6 week samples were collected from the retro-orbital plexus, whereas the pre-study and 13 week samples were collected from the descending aorta at terminal sacrifice. Platelets were not significantly altered in either sex by HD exposure (Table 8). Significant differences were not observed for red blood indices after 6 or 13 weeks of exposure, except for the mean corpuscular hemoglobin concentration (MCHC) of the 0.003 and 0.01 mg/kg male groups at 6 weeks (Table 9). These changes are not dose-related and probably are not biologically significant.

Serum protein concentrations were significantly decreased only in females given 0.3 mg/kg/day HD (Table 10). Neither BUN nor creatinine were statistically altered at any treatment level. No statistically significant dose-related differences were found in SGOT or SGPT activity in either sex, although some lower values were noted.

Terminal body weights and organ weights expressed on an absolute and relative weight basis, are shown in Table 11. Body weights of both female and male rats of the 0.3 mg/kg group decreased significantly ($P < 0.05$) compared to the control group. No statistically significant dose-related differences were

TABLE 6. Body Weights (g) of Male Rats Exposed to Sulfur Mustard (Mean ± SE)[a]

Week	Dose (mg/kg/day) 0	0.003	0.01	0.03	0.1	0.3
0	193 ± 2.4	193 ± 2.6	193 ± 2.6	193 ± 2.7	193 ± 2.6	193 ± 2.8
1	242 ± 2.7	238 ± 3.8	239 ± 3.5	239 ± 3.5	238 ± 3.0	234 ± 3.4
2	279 ± 3.0	278 ± 4.3	281 ± 4.4	280 ± 4.6	279 ± 4.6	268 ± 4.0
3	314 ± 2.9	314 ± 5.0	318 ± 4.5	320 ± 5.0	315 ± 4.8	289 ± 4.5*
4	345 ± 3.2	350 ± 5.6	354 ± 4.7	354 ± 5.8	347 ± 5.7	323 ± 5.5*
5	370 ± 4.3	374 ± 4.9	380 ± 4.7	384 ± 7.7	372 ± 5.6	346 ± 6.2
6	389 ± 4.4	396 ± 5.5	403 ± 5.8	406 ± 8.8	390 ± 6.5	363 ± 6.2
7	405 ± 4.7	412 ± 5.0	417 ± 6.1	423 ± 9.0	405 ± 7.9	375 ± 6.7*
8	424 ± 5.3	432 ± 5.8	440 ± 6.9	445 ± 10.1	421 ± 8.3	391 ± 7.4*
9	441 ± 6.4	450 ± 6.2	458 ± 7.8[b]	462 ± 12.0	440 ± 8.8	404 ± 7.5*
10	451 ± 6.3[b]	463 ± 6.1	469 ± 8.8[b]	478 ± 12.5	450 ± 9.3	417 ± 7.8
11	462 ± 7.4[b]	474 ± 6.9	482 ± 9.7[b]	492 ± 13.6	461 ± 9.5	424 ± 7.5
12	472 ± 7.1[b]	484 ± 7.4	494 ± 10.7[b]	502 ± 13.7	471 ± 9.3	430 ± 7.9*
13	479 ± 7.6[b]	496 ± 7.2	501 ± 10.4[b]	510 ± 14.5	477 ± 9.5	438 ± 9.6
14	481 ± 7.3[b]	501 ± 6.3*	509 ± 11.0[b]	520 ± 14.9	483 ± 10.6	441 ± 9.4

[a] N=12 unless otherwise noted.
[b] N=11.
* Significantly different from control value by Tukey's Test ($P < 0.05$).

TABLE 7. Body Weights (g) of Female Rats Exposed to Sulfur Mustard (Mean ± SE)[a]

Week	Dose (mg/kg/day)					
	0	0.003	0.01	0.03	0.1	0.3
0	143 ± 2.3	143 ± 2.1	143 ± 2.3	143 ± 2.3	143 ± 2.3	143 ± 2.3
1	162 ± 3.1	162 ± 1.8	162 ± 3.3	161 ± 2.9	162 ± 3.1	159 ± 2.4
2	180 ± 4.0	181 ± 2.0	174 ± 4.5	178 ± 3.7	178 ± 3.3	168 ± 3.0
3	196 ± 4.6	196 ± 2.0	192 ± 4.7	193 ± 4.2	191 ± 4.1	180 ± 3.9
4	209 ± 3.7	207 ± 2.7	202 ± 5.8	206 ± 4.7	204 ± 4.3	191 ± 4.2*
5	222 ± 4.7	215 ± 3.1	211 ± 6.2	214 ± 5.7	217 ± 5.4	205 ± 5.0
6	228 ± 5.1	226 ± 3.1	221 ± 5.8	224 ± 5.7	217 ± 6.1	210 ± 5.1
7	233 ± 5.4	229 ± 3.1	222 ± 6.3	232 ± 6.2	225 ± 6.2	214 ± 5.1
8	242 ± 5.6	238 ± 3.2	226 ± 5.7	236 ± 7.0	230 ± 6.4	219 ± 5.2
9	248 ± 6.1	244 ± 4.2	233 ± 6.9	242 ± 7.2	235 ± 6.8	224 ± 6.0
10	255 ± 6.3	253 ± 4.4	238 ± 6.4	247 ± 7.4	242 ± 7.0	226 ± 6.1*
11	259 ± 6.1	258 ± 4.9	243 ± 6.8	254 ± 7.8	246 ± 6.8	231 ± 6.1*
12	264 ± 6.4	262 ± 5.0	246 ± 6.8	259 ± 7.4	248 ± 7.4	234 ± 6.1*
13	271 ± 7.3	267 ± 5.3	250 ± 6.6*	263 ± 7.5	252 ± 7.6	240 ± 7.1[b]*
14	275 ± 7.4	270 ± 5.7	253 ± 6.9*	267 ± 8.1	254 ± 7.4	240 ± 6.2[b]*

[a] N=12 unless otherwise noted.
[b] N=11.
* Significantly different from control value by Tukey's Test ($P < 0.05$).

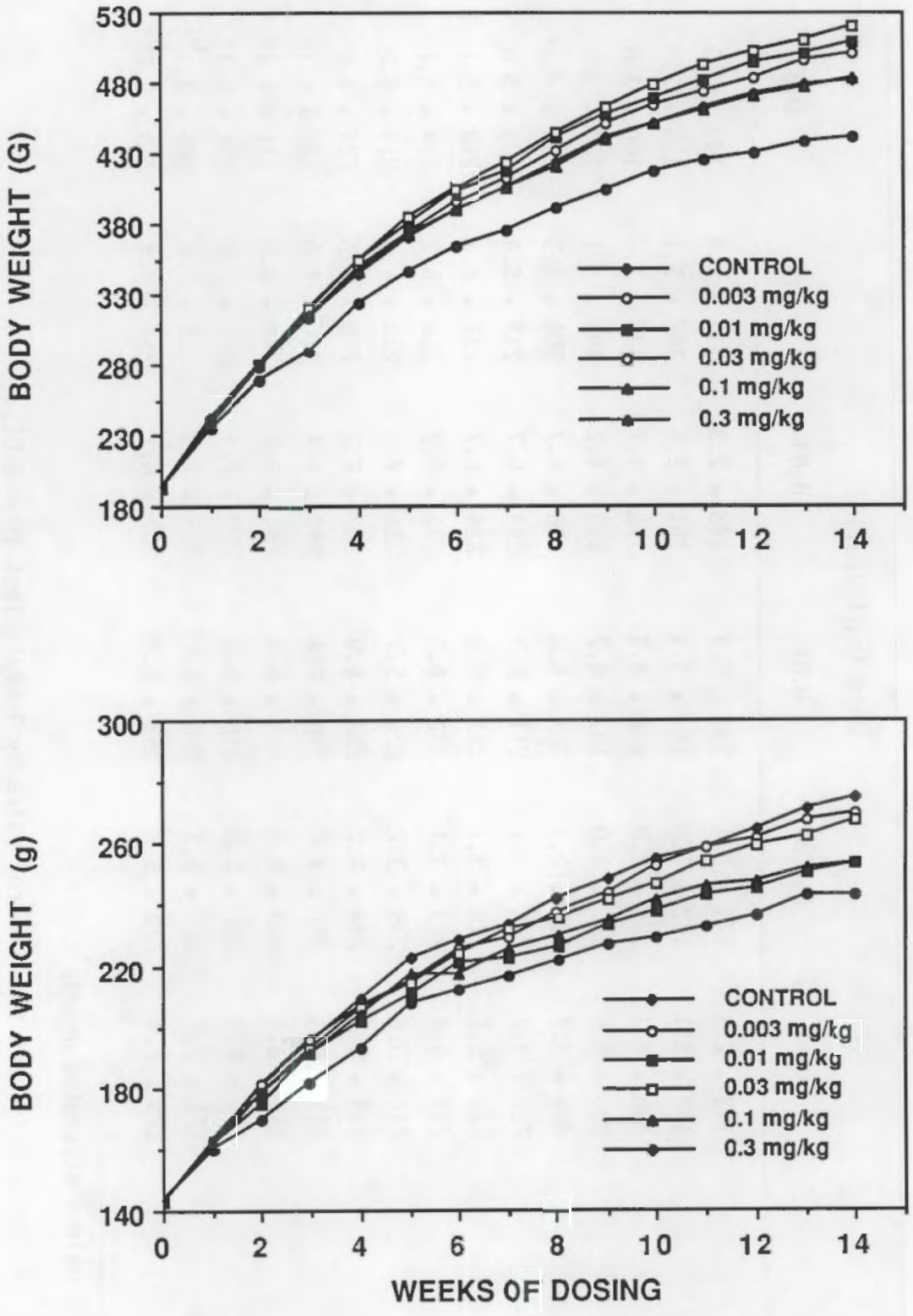

Figure 1. Body weight of male (Upper graph) and female (lower graph) rats exposed to HD for 13 weeks.

TABLE 8. The Effect of Sulfur Mustard on White Cell Indices and Platelets in Male and Female Rats Prior to and at 6 and 13 Weeks of Exposures (mean ± SE)

Dose mg/kg/day	N	WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Platelets
		x 10^3/ul					
				MALE			
				Pre-Treatment			
0	13	4.90 ± 0.58	0.55 ± 0.08	4.27 ± 0.53	0.071 ± 0.025	0.008 ± 0.005	795 ± 50
				Week Six			
0	12	12.01 ± 0.89	0.67 ± 0.11	11.02 ± 0.89	0.198 ± 0.052	0.114 ± 0.040	754 ± 23
0.003	12	10.95 ± 1.00	0.99 ± 0.12	9.75 ± 0.97	0.147 ± 0.033	0.060 ± 0.300	738 ± 16
0.01	12	10.45 ± 0.76	1.59 ± 0.72	8.68 ± 1.02	0.153 ± 0.045	0.031 ± 0.016	710 ± 24
0.03	12	11.57 ± 0.98	1.62 ± 0.39	9.52 ± 0.73	0.357 ± 0.074	0.072 ± 0.022	752 ± 38
0.1	12	11.13 ± 0.76	1.04 ± 0.17	9.60 ± 0.66	0.368 ± 0.087	0.123 ± 0.037	721 ± 17
0.3	12	11.52 ± 0.92	1.52 ± 0.28	9.62 ± 0.81	0.230 ± 0.084	0.100 ± 0.035	785 ± 30
				Week Thirteen			
0	11	6.69 ± 0.67	0.83 ± 0.14	5.49 ± 0.61	0.258 ± 0.052	0.112 ± 0.040	871 ± 29
0.003	12	7.11 ± 0.69	1.65 ± 0.25	5.19 ± 0.65	0.222 ± 0.043	0.041 ± 0.018	802 ± 19
0.01	10	6.62 ± 0.83	1.18 ± 0.29	5.11 ± 0.59	0.247 ± 0.069	0.083 ± 0.029	797 ± 20
0.03	12	6.68 ± 0.48	1.30 ± 0.21	5.04 ± 0.33	0.250 ± 0.052	0.087 ± 0.024	877 ± 52
0.1	12	5.92 ± 0.49	1.04 ± 0.24	4.59 ± 0.41	0.211 ± 0.038	0.079 ± 0.024	811 ± 17
0.3	12	5.75 ± 0.61	1.02 ± 0.25	4.45 ± 0.44	0.205 ± 0.049	0.060 ± 0.012	881 ± 23
				FEMALE			
				Pre-Treatment			
0	6	3.35 ± 0.25	0.44 ± 0.12	2.89 ± 0.22	0.012 ± 0.012	0.007 ± 0.006	814 ± 31
				Week Six			
0	12	10.27 ± 0.88	1.39 ± 0.30	8.44 ± 0.77	0.368 ± 0.079	0.083 ± 0.020	735 ± 31
0.003	12	9.07 ± 0.45	1.12 ± 0.45	7.66 ± 0.62	0.151 ± 0.028	0.137 ± 0.066	707 ± 56
0.01	12	8.16 ± 0.63	0.76 ± 0.20	7.22 ± 0.47	0.109 ± 0.020	0.079 ± 0.031	790 ± 38
0.03	12	8.92 ± 0.50	1.40 ± 0.21	7.22 ± 0.43	0.207 ± 0.004	0.104 ± 0.034	760 ± 19
0.1	12	8.97 ± 0.80	1.33 ± 0.39	7.39 ± 0.61	0.196 ± 0.061	0.047 ± 0.033	793 ± 52
0.3	12	8.42 ± 1.05	1.20 ± 0.34	7.02 ± 0.77	0.157 ± 0.053	0.050 ± 0.018	757 ± 25
				Week Thirteen			
0	12	3.72 ± 0.46	0.46 ± 0.08	3.08 ± 0.32	0.159 ± 0.24	0.031 ± 0.007	806 ± 31
0.003	12	3.82 ± 0.28	0.45 ± 0.05	3.13 ± 0.24	0.223 ± 0.060	0.024 ± 0.010	832 ± 32
0.01	12	4.38 ± 0.37	0.74 ± 0.12	3.41 ± 0.36	0.200 ± 0.026	0.031 ± 0.009	837 ± 27
0.03	12	3.97 ± 0.35	0.70 ± 0.16	3.10 ± 0.27	0.139 ± 0.028	0.032 ± 0.012	783 ± 24
0.1	12	3.33 ± 0.33	0.58 ± 0.11	2.60 ± 0.24	0.111 ± 0.022	0.043 ± 0.013	843 ± 23
0.3	12	3.80 ± 0.54	0.70 ± 0.15	2.90 ± 0.50	0.135 ± 0.034	0.063 ± 0.021	904 ± 47

TABLE 9. The Effect of Sulfur Mustard on Red Cell Indices in Male and Female Rats Prior to and at 6 and 13 weeks of Exposure (Mean ± SE)

Dose mg/kg	N	RBC (10^6/ul)	Hemoglobin (g/dl)	VPRC (ml/dl)	MCV (u^3)	MCH (uug)	MCHC (%)	Reticulocytes (x 10^3/ul)
					MALE			
					Pre-Treatment			
0	13	6.41 ± 0.06	12.9 ± 0.11	38.6 ± 0.02	60.2 ± 0.45	20.2 ± 0.19	33.5 ± 0.14	241 ± 12
					Week 6			
0	12	7.70 ± 0.08	13.7 ± 0.15	41.1 ± 0.42	53.4 ± 0.47	17.8 ± 0.17	33.4 ± 0.19	117 ± 13
0.003	12	7.81 ± 0.19	13.8 ± 0.28	42.2 ± 0.88	54.2 ± 0.55	17.7 ± 0.19	32.6 ± 0.14*	108 ± 10
0.1	12	7.80 ± 0.12	13.8 ± 0.16	42.5 ± 0.47	54.6 ± 0.53	17.8 ± 0.19	32.6 ± 0.14*	112 ± 15
0.03	12	7.61 ± 0.15	13.6 ± 0.28	41.2 ± 0.73	54.3 ± 0.40	17.8 ± 0.16	32.9 ± 0.23	101 ± 15
0.1	12	8.03 ± 0.06	14.2 ± 0.37	42.9 ± 0.37	53.4 ± 0.40	17.7 ± 0.14	33.2 ± 0.11	120 ± 8
0.3	12	7.85 ± 0.10	14.2 ± 0.15	42.6 ± 0.42	54.2 ± 0.50	18.1 ± 0.15	33.3 ± 0.10	92 ± 11
					Week Thirteen			
0	11	7.91 ± 0.07	15.0 ± 0.17	42.3 ± 0.38	53.4 ± 0.49	18.9 ± 0.19	35.4 ± 0.17	71 ± 13
0.003	12	8.02 ± 0.10	15.0 ± 0.13	42.5 ± 0.40	53.0 ± 0.58	18.7 ± 0.24	35.3 ± 0.15	58 ± 9
0.1	10	8.16 ± 0.11	15.3 ± 0.12	42.7 ± 0.42	52.5 ± 0.72	18.8 ± 0.25	35.8 ± 0.15	73 ± 9
0.03	12	7.97 ± 0.11	14.9 ± 0.24	42.2 ± 0.67	53.0 ± 0.51	18.7 ± 0.19	35.4 ± 0.15	81 ± 14
0.1	12	8.04 ± 0.13	15.0 ± 0.20	42.3 ± 0.61	52.7 ± 0.55	18.7 ± 0.16	35.6 ± 0.19	96 ± 20
0.3	12	8.06 ± 0.14	15.4 ± 0.26	43.3 ± 0.76	53.8 ± 0.42	19.1 ± 0.16	35.5 ± 0.16	70 ± 10
					FEMALE			
					Pre-treatment			
0	6	6.41 ± 0.005	12.7 ± 0.20	38.6 ± 0.50	60.2 ± 0.65	19.8 ± 0.23	32.9 ± 0.15	206 ± 21
					Week Six			
0	12	7.19 ± 0.07	12.8 ± 0.14	39.1 ± 0.43	54.2 ± 0.64	17.8 ± 0.12	32.8 ± 0.11	97 ± 8
0.003	12	7.30 ± 0.15	12.8 ± 0.43	40.1 ± 0.73	55.0 ± 0.37	17.4 ± 0.38	31.7 ± 0.66	105 ± 14
0.01	12	7.45 ± 0.85	13.2 ± 0.16	40.7 ± 0.56	54.6 ± 0.29	17.7 ± 0.37	32.4 ± 0.27	116 ± 5
0.03	12	7.33 ± 0.11	13.0 ± 0.21	40.3 ± 0.60	55.1 ± 0.26	17.7 ± 0.10	32.2 ± 0.08	94 ± 10
0.1	12	7.28 ± 0.09	13.0 ± 0.21	40.0 ± 0.66	54.8 ± 0.50	17.9 ± 0.16	32.5 ± 0.07	85 ± 11
0.3	12	7.43 ± 0.07	13.0 ± 0.14	40.4 ± 0.47	54.3 ± 0.26	17.6 ± 0.15	32.3 ± 0.24	125 ± 8
					Week Thirteen			
0	12	7.36 ± 0.06	14.5 ± 0.17	40.8 ± 0.34	55.5 ± 0.31	19.7 ± 0.20	35.4 ± 0.20	57 ± 8
0.003	12	7.33 ± 0.15	14.6 ± 0.24	41.1 ± 0.70	56.2 ± 0.57	20.0 ± 0.19	35.6 ± 0.09	74 ± 10
0.01	12	7.10 ± 0.08	13.9 ± 0.13	39.0 ± 0.39	54.8 ± 0.39	19.5 ± 0.12	35.5 ± 0.11	78 ± 11
0.03	12	7.42 ± 0.13	14.7 ± 0.27	41.3 ± 0.75	55.7 ± 0.33	19.8 ± 0.07	35.5 ± 0.11	62 ± 11
0.1	12	7.45 ± 0.09	14.7 ± 0.19	41.4 ± 0.66	55.6 ± 0.62	19.7 ± 0.16	35.4 ± 0.12	77 ± 12
0.3	12	7.06 ± 0.16	13.7 ± 0.35	38.6 ± 0.98	54.7 ± 0.29	19.4 ± 0.43	35.6 ± 0.52	85 ± 10

*Significantly different from control value by Tukey's test ($P < 0.05$).

TABLE 10. The Effect of Sulfur Mustard on Serum Concentrations of Protein, BUN, Creatinine, SGOT and SGPT of Rats at 13 Weeks of Exposure (mean ± SE)

Dose (mg/kg/day) (IU)	N	Serum Protein (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)	SGOT (IU)	SGPT (IU)
			FEMALE			
0	12	7.24 ± 0.21	20.9 ± 0.9	0.68 ± 0.05	122 ± 17	43.8 ± 4.6
0.003	12	7.15 ± 0.16	19.0 ± 0.8	0.68 ± 0.05	187 ± 79	91.8 ± 40.7
0.01	12	6.95 ± 0.10	20.3 ± 0.8	0.65 ± 0.05	141 ± 33	56.2 ± 15.5
0.03	12	7.38 ± 0.20	20.4 ± 1.1	0.69 ± 0.05	153 ± 44	61.7 ± 9.6
0.1	12	7.43 ± 0.15	20.9 ± 1.0	0.74 ± 0.05	134 ± 24	52.8 ± 7.3
0.3	11	6.64 ± 0.09*	20.3 ± 1.9	0.88 ± 0.13	107 ± 7	30.8 ± 2.0
			MALE			
0	11	6.58 ± 0.18	20.0 ± 1.0	0.73 ± 0.05	111 ± 11	32.5 ± 2.4
0.003	12	6.51 ± 0.19	19.0 ± 0.6	0.69 ± 0.03	107 ± 8	32.0 ± 1.8
0.01	12	6.23 ± 0.17	20.9 ± 1.0	0.74 ± 0.04	112 ± 4	35.2 ± 2.3
0.03	11	6.54 ± 0.22	21.5 ± 1.0	0.77 ± 0.04	115 ± 12	34.0 ± 3.0
0.1	12	6.33 ± 0.09	20.2 ± 0.9	0.68 ± 0.02	112 ± 20	40.2 ± 10.0
0.3	12	6.31 ± 0.19	18.4 ± 0.7	0.70 ± 0.04	97 ± 9	36.5 ± 4.2

*Significantly different from control value by Tukey's test ($P < 0.05$).

TABLE 11. Final Body Weights and Organ Weights of Rats Orally Exposed to Sulfur Mustard for 13 Weeks (Mean ± SE).

Dose mg/kg/day	N	Body Weight (g)	Adrenal		Brain		Heart		Kidney		Liver		Thymus		Testes	
			mg	mg/100 g	g	g/100 g	g	g/100 g	g	g/100 g	g	g/100 g	mg	mg/100 g	g	g/100 g
MALES																
0	11	483.5 ±8.2	52[a] ±4	11[a] ±1	2.05 ±0.025	0.425 ±0.008	1.32 ±0.03	0.273 ±0.008	1.60 ±0.04	0.332 ±0.010	16.06 ±0.46	3.32 ±0.067	245 ±21	51 ±4	1.60 ±0.05	0.333 ±0.12
0.003	12	503.9 ±6.5	45 ±3	9 ±1	2.02 ±0.027	0.401 ±0.003	1.30 ±0.059	0.258 ±0.012	1.60 ±0.046	0.316 ±0.007	16.61 ±0.33	3.30 ±0.057	234 ±19	46 ±4	1.64 ±0.025	0.326 ±0.004
0.01	11	509.8 ±9.9	43 ±2	9 ±1	2.10 ±0.028	0.414 ±0.012	1.37 ±0.045	0.269 ±0.007	1.63 ±0.042	0.320 ±0.006	16.32 ±0.72	3.20 ±0.116	254 ±16	50 ±3	1.67 ±0.035	0.328 ±0.008
0.03	12	521.5 ±15.6	46 ±3	9 ±1	2.02 ±0.018	0.390 ±0.011	1.36 ±0.039	0.262 ±0.003	1.62 ±0.053	0.311 ±0.006	17.61 ±0.68	3.37 ±0.055	442 ±177	88 ±36	1.66 ±0.019	0.322 ±0.010
0.1	12	480.1 ±10.0	47 ±2	10 ±0	2.06 ±0.035	0.430 ±0.009	1.26 ±0.025	0.263 ±0.007	1.57[b] ±0.050	0.328[b] ±0.008	15.77 ±0.70	3.28 ±0.098	231 ±21	48 ±4	1.62 ±0.093	0.336 ±0.019
0.3	12	436.1* ±9.7	46[b] ±2	10[b] ±1	2.01 ±0.024	0.463 ±0.010	1.21 ±0.041	0.279 ±0.008	1.48 ±0.024	0.340 ±0.005	14.74 ±0.56	3.37 ±0.073	200 ±20	46 ±5	1.56 ±0.084	0.362 ±0.024
FEMALES																
0	12	274.3 ±6.8	56 ±4	20 ±1	1.83 ±0.02	0.671 ±0.014	0.859 ±0.02	0.314 ±0.008	0.913 ±0.03	0.333 ±0.008	9.32 ±0.25	3.40 ±0.049	204 ±9	75 ±5		
0.003	12	272.5 ±5.4	60 ±3	22 ±1	1.83 ±0.03	0.676 ±0.020	0.871 ±0.02	0.320 ±0.004	0.979 ±0.02	0.360 ±0.009	9.25 ±0.25	3.40 ±0.075	221 ±12	81 ±5		
0.01	12	253.8 ±6.2	55 ±2	22 ±1	1.93 ±0.083	0.765 ±0.37	0.823 ±0.017	0.326 ±0.007	0.916 ±0.014	0.363 ±0.008	9.06 ±0.14	3.59 ±0.074	197 ±12	77 ±4		
0.03	12	267.3 ±7.8	53 ±3	20 ±1	1.82 ±0.023	0.687 ±0.021	0.854 ±0.025	0.320 ±0.003	0.986 ±0.036	0.369 ±0.008	9.27 ±0.40	3.46 ±0.084	387 ±116	141 ±39		
0.1	12	255.0 ±7.2	54 ±3	21 ±1	1.82 ±0.040	0.718 ±0.026	0.842 ±0.025	0.332 ±0.010	0.908 ±0.045	0.358 ±0.018	8.83 ±0.25	3.47 ±0.074	213 ±23	84 ±10		
0.3	11	242.5* ±6.0	48 ±3	20 ±1	1.81 ±0.021	0.749 ±0.015	0.751 ±0.027	0.309 ±0.006	0.905 ±0.041	0.373 ±0.012	8.50 ±0.24	3.51 ±0.070	177 ±14	73 ±6		

* Significantly different from control means by Tukey's Test ($P < 0.05$).
[a] $n = 9$
[b] $n = 11$

found for organ weights when expressed on an absolute or relative weight basis. Absolute organ weights of the 0.3 mg/kg female and male animals, although not statistically different, were slightly decreased compared to control groups. An exception to this generality was brain weights, which tended to increase on a relative basis at the high exposure level.

Microscopic examination of stomach sections revealed lesions involving epithelial hyperplasia of the forestomach of 5 animals of each sex at 0.3 mg/kg and of 1 male at 0.1 mg/kg. The hyperplastic change was minimal and characterized by cellular disorganization of the basilar layer, an apparent increase in mitotic activity of the basilar epithelial cells, and thickening of the epithelial layer due to the apparent increase in cellularity (Figure 2). Lesions were not apparent in any female of the 0.1 mg/kg group nor in either sex of the 0.03 mg/kg group; therefore, the forestomachs of the 0.003 or 0.01 treatment group were not examined.

Mandibular lymphoid hyperplasia was noted in over 50% of control (14/24) and high dose (12/22) animals. The incidence was not dose related nor were there differences due to sex. The cause is unknown. Prestudy serology was negative for tested pathogen and salivary glands or other correlating lesions were not observed.

Relatively few other changes were noted and none appeared to be dose related. A number of changes, such as the chronic focal inflammation of the lung, alveolar histiocytic cellular infiltrate, pericardial and epicardial inflammation and mediastinal inflammation, may have been due to oral gavaging. Most others were considered spontaneous lesions and were evenly distributed throughout the groups.

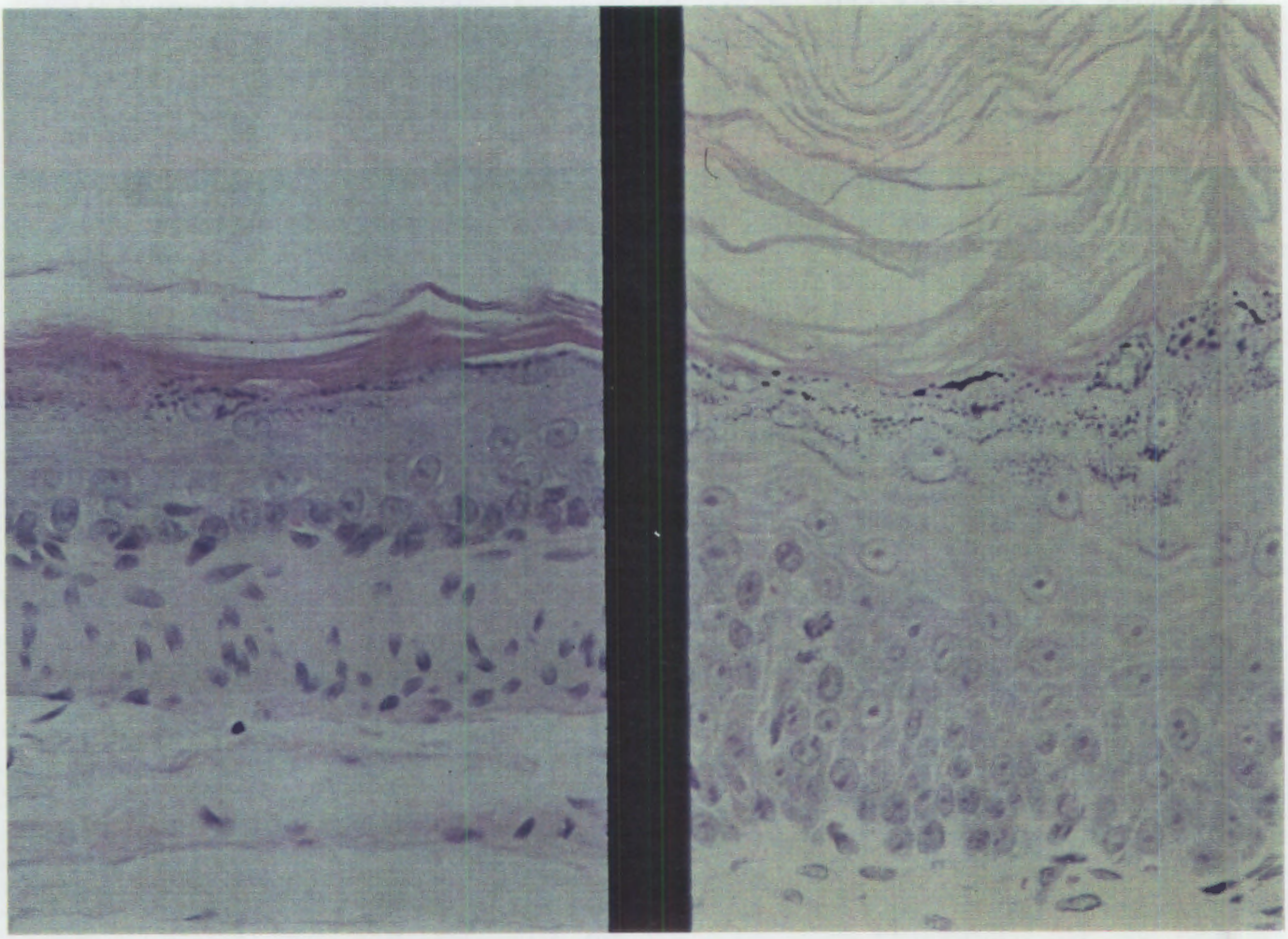

Figure 2. Forestomach of control (left) and HD-exposed (0.3 mg/kg, right) male rats after a 13 weeks subchronic study. Note thickening of the keratin layer, thickened layer of stratified squamous epithelium and thickened basilar epithelial layer in exposed tissue.

DISCUSSION

Results from this study suggest that the primary toxic effect in the rat was localized in the forestomach at the site of the HD application. Lesions were present in approximately 40% of the rats of the highest dose group (only one of 24 had lesions in the next lower dose). Similar lesions were not observed for the control group. Reduction in body weight was also observed in both sexes only at the high exposure level. No other dose-related adverse effects were observed in the study. Since the only detectable effect in the 0.1 mg/kg group was a forestomach ulcer in one of 24 animals (not different from controls), the estimated NOEL for this 13-week study is 0.1 mg/kg/day. However, in a recently completed two-generation reproduction study, dose-related forestomach lesions were found in rats at doses of 0.03, 0.1 and 0.4 mg/kg/day after intragastic administration of HD for 17 to 22 weeks (Sasser et al., 1989).

Body weights of the 0.3 mg/kg/day exposure group were significantly reduced at various times throughout the study and at terminal sacrifice compared to control animals, whereas only a slight nonsignificant decrease in tissue weights occurred. Reduction in body weight without accompanying changes in organ weights suggests that part of the weight loss was associated with food reduction during the exposure.

The forestomach appears to be more sensitive to HD than the glandular stomach as little pathology was associated with the glandular region. The reason for this apparent sensitivity has yet to be determined, but the sensitivity may merely reflect a higher concentration of HD at the initial site of deposition or may truly represent a particularly sensitive tissue. We found no evidence of dose-related pathology in the intestine. However, Hackett et al. (1987) found inflammation, petechial hemorrhage, thickening, and sloughing of gastric mucosa 5 days after rats were given 2.5 mg/kg/day for 14 days, and noted inflammation in the intestine of one rat treated orally with HD. The forestomach has been shown to be more sensitive than the glandular stomach to acute and repeated gavaging of ethyl acrylate (Ghanayem, et al. 1985a, 1985b, 1986); indeed forestomach lesions and tumors are reported in rats for a number of chemical irritants administered orally. A relationship between forestomach lesions and squamous cell carcinomas has been reported for butylated hydroxyanisole (BHA) given in the feed (Ito et al., 1982, 1983) and for aristoloic acid given by gavage (Mengs, 1983). Induction of squamous cell carcinomas of the forestomach of the rat was reported after oral gavage with methyl bromide (Danse et al., 1984; Boorman et al., 1986). In recent reviews on this subject, as many as 60 compounds were found which demonstrated carcinogenic activity of the forestomach (Kroes and Webster, 1986; Webster and Kroes, 1988).

Ito et al. (1982) postulated that increased proliferation of cells provides a favorable environment for the development of cancer, but we have found no evidence that the forestomach lesions observed in this study were precancerous. Induction of forestomach carcinoma is no doubt both time- and dose-dependent and our study was not of sufficient length to determine the malignant nature of the lesions. However, Japanese mustard factory workers,

malignant nature of the lesions. However, Japanese mustard factory workers, who were involved in the production of chemical agents during World War II and who have been studied for a number of years because of the presence of respiratory tract cancer (Wada et al., 1968; Norman, 1975; Manning et al., 1981), are now showing evidence of increases in gastrointestinal neoplasms (Yamakido and Shigenobu, 1985). These workers were occupationally exposed to unknown quantities of HD, lewisite and other agents in plants operated from 1929-1945 (Wada et al., 1968).

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STUDY DATES

Subchronic Study

Animal arrival	10/07/86
Health evaluation	11/05/86
Exposure period	11/10/86 to 02/12/87
Hematology evaluation	12/17/86 to 12/18/86 02/11/87 to 02/13/87
Opthamology evaluation	10/03/86 01/30/87
Necropsy	02/11/87 to 02/13/87

Data are property of the U.S. Army and will be archived under the army's direction in approved facilities.

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L.B. Sasser

6/30/89
Date

90-Day Subchronic Gavage Sulfur Mustard (HD) Study in Rats

Quality Assurance Statement

Listed below are the phases and/or procedures included in the study described in this report which were reviewed by the Quality Assurance Unit specifically for this study and the dates the reviews were performed and findings reported to management. (All findings were reported to the study director or his designee at the time of the review.)

Phase/Procedure Reviewed	Review Date	Date Findings Submitted in Writing to Study Director/Management
Vehicle Analysis	6/26/85	6/27/85
Animal Identification	6/26/85	6/27/85
Health Screen	7/1, 8/85	7/11/85
Body Weights	7/2, 8/85	7/11/85
Vehicle Analysis	7/2/85	7/11/85
Dose Preparation	7/8/85	7/11/85
Dosing	7/16/85	7/16/85
Data	7/18, 8/4-5/86	8/7/86
Data	8/28-29/85	9/10/85
Necropsy	10/9/85	10/10/85
Data	11/27, 12/31/85	1/30/86
Final Report	9/11, 18, 19, 25/89	10/8/89

Quality Assurance Auditor — Date

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