AD _____ 1

TERATOLOGY STUDIES ON LEWISITE AND SULFUR MUSTARD AGENTS:
EFFECTS OF SULFUR MUSTARD IN RATS AND RABBITS

FINAL REPORT: PART 2, APPENDICES

P. L. Hackett, R. L. Rommereim, F. G. Burton, R. L. Buschbom, and L. B. Sasser

September 1987

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<p>Sulfur mustard (HD) was administered to rats and rabbits by intragastric intubation. Rats were dosed daily from 6 through 15 days of gestation (dg) with 0. 0.5, 1.0 or 2.0 mg of HD/kg; rabbits were dosed with 0, 0.4, 0.6 or 0.8 mg/kg on 6 through 19 dg. Maternal animals were weighed periodically and, at necropsy, were examined for gross lesions of major organs and reproductive performance; live fetuses were weighed and examined for external, internal and skeletal defects. In rats, reductions in body weights were observed in maternal animals and their female fetuses at the lowest administered dose (0.5 mg/kg), but the incidence of fetal malformations was not increased. In rabbits the highest administered dose (0.8 mg/kg) induced maternal mortality and depressed body weight measures but did not affect fetal development. These results suggest that orally administered HD is not teratogenic in rats and rabbits since fetal effects were observed only at dose levels that induced frank maternal toxicity. Estimations of dose ranges for "no observable effects levels" in rats and rabbits, respectively, were: <0.5 and <0.4 mg/kg in maternal animals and <0.5 and >0.8 mg/kg in their fetuses.</p>					
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APPENDIX A
PURITY AND STABILITY ANALYSES

Purity Analysis Report -- Sulfur Mustard (HD)

Empirical Formula: $C_4H_8Cl_2S$

Molecular Weight: 159.08

Provided by: USAMRICD, APG, MD, 4/25/84

Lot #: None given

Storage Conditions: The material is stored in sealed Wheaton bottles in the storage container (refrigerator) at 6°C.

Analysis Procedure: Gas chromatography using a 30 meter capillary glass column, narrow (0.25 mm) bore, DB-5 bonded phase (J&W Scientific, Inc.); split injection.

Analysis Date: 7/25/84

Notebook Reference: 50113-10,11

Gas Chromatographic Analysis: Purity by the area percent method. HD retention time 11.43-11.98 min. This procedure heavily loads the column; the HD peak may not be linear in this range as a result of the current generated in the flame ionization detector exceeding the electrometer's range. As a result, the amount of impurities may be overestimated.

Gas Chromatographic Results: Results of two runs are shown in the table. Using the area percent method, the HD is 95.87-96.10% pure. There are two impurities with concentrations in the range of 0.87 to 1.17% and six impurities with concentrations in the range of 0.10 to 0.35%.

Conclusions from the Analysis: We have identified the major peak as HD; however, this has not been independently verified using a standard. The determined purity reasonably agrees with the purity given by USAMRICD (97.9-98.7 wt.% pure). Provided the major peak is HD, this lot of HD is acceptable for use as test material.

Chemist

Date

Table 1. Major peaks ($\geq 0.1\%$) in sulfur mustard (HD)

<u>Retention Time</u>	<u>Sample # 1</u>	<u>Sample # 2</u>
1.53	0.186	0.114
6.99	1.165	0.969
11.98	96.093	95.876
12.25	0.272	0.269
13.68	0.285	0.290
13.81	0.287	0.298
14.75	0.874	0.944
16.81	0.158	0.113
24.14	0.158	0.349

100 150 200
 400 100 150 200

BLANK 7/25/84

OUT SPD 0.50
 ZERO 10.0
 ATTN 2+ 0
 FID SIGNAL +0
 SLP SENS 0.10
 AREA REJ 10
 FLOW A 0.0 29.6
 FLOW B 0.0 24.3

START

TIME2 0 0

33.47

40.23

48.16

HP RUN # 15
 INPUT 1462
 AREA %

RT	AREA	AREA %
33.25	7126	54.589
33.47	1126	8.626
33.57	2119	16.233
40.23	2655	20.339
48.16	28	0.214

71

START

NEAT HD
7/25/84

NEHY HD - 7/25/84

150

HP RUN # 17
INPUT 1462
AREA %

RT	AREA	AREA %
1.29	2611	0.087
1.47	1183	0.039
1.54	3433	0.114
1.85	33	0.001
2.20	78	0.003
3.03	1458	0.049
3.22	512	0.017
3.53	101	0.003
4.70	22	0.001
6.98	29110	0.969
7.25	49	0.002
8.77	100	0.003
11.43	2881000	95.876
11.62	639	0.021
11.76	1294	0.043

73

151

DISTILLED MUSTARD STABILITY STUDY

To determine the stability of the gavage dosing solutions used for the range-finding study over a period of time, aliquots of the dosing solutions were saved in the refrigerated storage container until the end of the study. From each concentration and preparation date a one-ml aliquot was drawn and mixed with a known amount of 2,4-dichlorotoluene in isooctane. These solutions were transferred to automatic liquid sampler (ALS) vials (2 per sample) and analyzed by gas chromatography [30 meter capillary column, narrow (0.25 mm bore), DB-5 bonded phase (J&W Scientific, Inc.), by split injection]. The ratio of the area of the distilled mustard (HD) to the area of the internal standard was determined and compared to the target concentration of the HD in the sesame oil. The data were fit to a linear regression curve using a Hewlett Packard 25 calculator and the coefficient of determination determined.

The HD samples used in this determination were prepared 7/20/84 and 7/31/84 (0.06, 0.12, 0.24, and 0.48 mg/ml) and 8/20/84 (0.60 and 0.75 mg/ml). The sample aliquots were removed from the storage container, brought to room temperature, and mixed with the 2,4-dichlorotoluene aliquot on 8/28/84. Two full chromatographic runs were made on the set of samples 9/6/84 - 9/8/84 and 9/9/84 - 9/13/84. As a result, each sample had been analyzed four times.

The plotted data is shown in the attached figure. The data from 7/20/84 are significantly lower than the data obtained for the 7/31/84 data set. However, the higher points (from 7/31/84) do lay directly on the line formed by the highest concentrations which were prepared at a later date (8/20/84), which could indicate that the solutions prepared 7/31/84 were slightly more concentrated (approximately 10%), rather than that significant degradation had occurred in the sample. Proportionately lower values for the 0.06 and 0.12 mg/ml samples is most likely due to the problem the gas chromatograph has with accurate integration of these small areas (a few hundred area units) rather than any actual degradation occurring within the sample.

Linear regression analysis gives a slope of 3.75, an intercept of -0.02, and a coefficient of determination (r^2) of 0.994. Since samples prepared a month apart and analyzed at the same time fall on the same line, and this line has an intercept close to zero and a high coefficient of determination, we conclude that no significant degradation occurs in the sample (particularly over the maximum 10 days of dosing used for each prepared sample) and thus the procedure of making up the dosing solutions in advance of dosing is acceptable.

FC Burton
9/28/84

7/20/84	-	x
7/31/84	-	⊙
8/20/84	-	△

PLH 64A

HD/150 HENH 01/14H

2.0

1.5

75

1.0

0.5

0.06

0.12

0.24

0.48

0.60

0.75

HD TARGET CONCENTRATION, mg/ml

Question: Is sulfur mustard (HD) stable in sesame oil over a 10-day period?

We have studied the stability of HD in sesame oil using the samples prepared for the rat teratology study. This was done by using a 1-ml aliquot of each of the doses (0.15, 0.30, and 0.60 mg/ml) remaining at the end of each dosing day. The 1-ml aliquot was mixed with a 1-ml aliquot of isooctane containing 144.45 micrograms 2,4-dichlorotoluene as the internal standard, and divided between two automatic liquid sampler (ALS) vials which were then securely capped. Each sample (a total of six per day) was then analyzed by gas chromatography. The ratio of the area of the HD to the area of the internal standard was determined. This ratio was then plotted against the number of days since the sample had been prepared and a straight line fitted to the data by the method of least squares. Degradation of the HD would be indicated by a negative slope to the curve.

Since the dosing solutions were prepared at two different times, there are a total of six sets of data (two times and three doses). The raw data is summarized in Table A. Note that in the Study No. 1 there are high values in the third or fourth day. Since these might be due to a handling error, the data sets were analyzed both with and without the set of data which is shown in parentheses.

The data was submitted to a statistician to determine, by regression analysis, whether the data indicated a decay with time (i.e., the slope was significantly different than zero). The results of the analysis are attached. Based on this, there is no reason to suspect that the distilled mustard in sesame oil degrades significantly over nine days when stored at 6°C until used.

As a test of the stability of the mixture of distilled mustard and sesame oil at room temperature, the samples above were analyzed on the day that they were mixed with the internal standard and on the succeeding two days. The resulting ratios were compared for the three-day period for the data from Study No. 2. In 20 cases the ratio increased over the initial value, in 14 cases it remained the same, and in 19 cases it decreased from the initial value. It thus appears that the sample stored at room temperature (containing the internal standard) does not change significantly over a two-day interval.

F C Burton
3 NOV 84

Table A. Ratio of HD to Internal Standard from the GC Analyses to Determine the HD Stability.

<u>Days after preparation</u>	<u>Study No. 1</u>			<u>Study No. 2</u>		
	<u>0.15 Level</u>	<u>0.30 Level</u>	<u>0.60 Level</u>	<u>0.15 Level</u>	<u>0.30 Level</u>	<u>0.60 Level</u>
3	(0.50)	1.11	(2.19)	0.54	1.12	2.08
	(0.63)	1.16	(2.56)	0.54	1.11	2.09
4	0.55	(1.19)	2.30	0.52	1.07	2.27
	0.55	(1.18)	2.30	0.54	1.08	2.27
5	0.55	1.09	2.38	0.51	1.07	2.09
	0.55	1.08	2.38	0.51	1.06	2.22
6	0.54	1.08	2.20	0.54	1.12	2.16
	0.54	1.09	2.20	0.54	1.10	2.22
7	0.55	1.07	2.22	0.52	1.05	2.03
	0.56	1.09	2.19	0.52	1.08	2.06
8	0.52	1.10	2.25	0.54	1.04	2.26
	0.53	1.11	2.28	0.54	1.05	2.26
9	0.58	1.12	2.34			
	0.57	1.12	2.35			

DATE: 31 October 1984

TO: Fred Burton

FROM: Dave Carlile

SUBJECT: HD Stability Study. Regression analysis and interpretation.

Parameter estimates for slopes and intercepts from regressions of HD values vs. days after preparation are shown in Table 1. For all combinations of study number and level (where level refers to 0.15, 0.30 or 0.60) except one (Study No. 1, Level 0.30), the slope did not differ significantly from zero. Assessment of significance or non-significance can be made by looking at the last column in Table 1. If the value is less than or equal to 0.05, then the slope differs significantly ($\alpha=0.05$) from zero. When data from day 4 was excluded from the regressions, the slope for Study 1, Level 0.30 was not significantly different from zero. These results suggest that no change in HD values occurred over the course of this study.

DIST. DWC file/lb.

Table 1. Summary of parameter estimates (intercept and slope) of regressions of HD values versus days after preparation.

STUDY NO.	LEVEL	INTERCEPT	SLOPE	r-SQUARE	OBSERVED SIGNIFICANCE LEVEL TESTING FOR SLOPE = 0
1	0.15	0.555	-0.0005	0.001	0.9002
	0.30	1.158	-0.0008	0.170	0.1428
	0.60	2.368	-0.0120	0.058	0.4061
2	0.15	0.530	0	0.000	1.0000
	0.30	1.132	-0.0100	0.378	0.0335
	0.60	2.108	0.0087	0.027	0.6073
1*	0.15	0.537	0.0018	0.042	0.5241
	0.30	1.113	-0.0018	0.024	0.6270
	0.60	2.304	-0.0033	0.007	0.7972

*Regressions done with values in parentheses from raw data sheet excluded.

Analysis of HD/sesame oil solution for initial rabbit range-finding dosing

I have assembled all GC data for this study; it is summarized below. In each case, it was analyzed on the day that the sample was prepared, on the last day that it was used, and once between those dates. In all cases the internal standard used was 2,4-dichlorotoluene in isooctane at a concentration of 1.4445 mg/ml. A one-ml aliquot of this solution was mixed with a one-ml aliquot of the prepared HD in sesame oil, split into two ALS vials, and analyzed by gas chromatography. The ratio of the area under the HD peak to the area under the internal standard peak was then determined; these are the figures reported below.

HD Conc. mg/ml	HD/ISTD Ratio					
	First Preparation			Second Preparation		
	11/21/84	12/3/84	12/6/84	12/3/84	12/9/84	12/10/84
2.0	0.76	0.77	0.76	0.83	0.69	0.76
	0.77	0.77	0.76	0.69	0.69	0.75
4.0	1.47	1.39	1.37	1.48	1.45	1.53
	1.41	1.39	1.38	1.45	1.45	1.53
8.0	2.87	2.78	2.77	2.68	2.67	2.98
	2.87	2.83	2.81	2.66	2.69	2.97
10.0	3.67	3.14	3.12	3.98	3.96	3.67
	3.64	3.16	3.16	3.98	3.99	3.69

A couple things are obvious from the data. The 2.0 mg/ml data for 12/3 did not duplicate well -- the chromatograms look good so I am unable to determine why this discrepancy exists. The 10.0 mg/ml sample prepared 11/21 appears to degrade to a lower value with time. This is much less obvious in the second preparation at the same concentration. In the latter case (and others where some variability appears to exist), a portion of the problem may be attributable to the difficulty of accurately adding 1 ml of the HD/sesame oil to precisely 1 ml of the isooctane/ISTD.

The data contained in this table were fit to a linear regression curve using a Hewlett Packard 25 calculator and the coefficient of determination determined. Linear regression analysis for this present data gives a slope of 3.53 (compared to the previous 3.75), an intercept of 0.03 (compared to the previous -0.02), and a coefficient of determination (r^2) of .973 (compared to the previous 0.994). Deleting the four values

from the 10.0 mg/ml sample in the first preparation that appear to have partially degraded, the the determined values are 3.70, -0.03, and 0.99 respectively.

While some variability does exist, and it appears that one sample may have degraded approximately 14%, there appears to be no overall degradation with time and the solutions do represent the concentrations which were required for dosing.

F. G. Burton
12/18/84

ANALYSIS OF HD/SESAME OIL SOLUTIONS FOR RABBIT TERATOLOGY STUDY

Purpose of the Study

This report summarizes the results of the gas chromatographic analyses of distilled mustard (HD) in sesame oil solutions prepared for the rabbit gavaging studies to determine the amounts of HD in those solutions. This will give an estimate of the amounts of HD that were present and will allow a determination of whether any degradation occurs over the storage period of the sample.

Analytical Procedures

Samples were prepared for analysis in the following way. One-ml aliquots of the internal standard (ISTD), 1.4445 mg/ml of 2,4-dichlorotoluene in isooctane, were placed in test tubes. A one-ml aliquot of the HD/sesame oil gavaging solution was then taken from the gavaging bottle and thoroughly mixed with the ISTD solution. This solution was then split into two automatic liquid sample (ALS) vials and analyzed by gas chromatography. For certain of the samples, two one-ml aliquots were taken from a single gavaging solution to estimate variability between samples.

To quantitate the results of the gas chromatography, the chromatograms were inspected and the ratio of the area of the HD to that of the ISTD was determined. The resulting data were fit to a linear regression curve using a Hewlett Packard 25 calculator and the slope, the intercept, and the coefficient of determination (r^2) determined.

Results and Discussion

The determined ratios of the areas of HD to the areas of the ISTD for the rabbit teratology study are given in Table 1. Data obtained from the linear regression curves are given in Table 2. In this table the data for this study are compared with the results obtained from earlier studies in both rats and rabbits. (Since the rat data was obtained using an ISTD solution which was precisely 1/10th the concentration of that used in the rabbit study, the slope values for the rabbit data included an additional factor of 10.)

For unknown reasons the slope of the curve for the first preparation for the rabbit teratology study is significantly lower than that of any of the other studies. There is a possibility that, due to a preparation error, the dosing solutions for the first half of the rabbit teratology study contained only 84 to 89% of the desired amount of distilled mustard. A second possibility, since the gas chromatograph had been sitting idle and cooled down until just before this analysis, is that insufficient time had been allowed for returning the GC to operating temperatures and conditions and thus the response factors for the HD and internal standard were not in their normal ratio.

Inspection of the data does not give any indication that the gavage solutions show significant degradation of the distilled mustard with time. This is consistent with previous studies.

F.C. Burton
5/8/85

Table 1. HD/ISTD Ratio of Analyzed Gavage Solutions for Rabbit Teratology

Target HD Conc., mg/ml	First Preparation				Second Preparation				
	1/18	2/1		1/27	1/25		2/2	2/4	
1.6	0.51	0.54	0.51	0.51	0.57	0.53	0.58	0.57	0.59
	0.51	0.53	0.52	0.51	0.57	0.53	0.58	0.57	0.57
2.4	0.77	0.76	0.77	0.76	0.90	0.79	0.85	0.88	0.90
	0.77	0.76	0.77	0.76	0.91	0.79	0.86	0.90	0.90
3.2	0.97	1.07	0.99	1.07	1.09	1.12	1.18	1.17	1.19
	0.99	1.07	0.98	1.07	1.09	1.14	1.19	1.17	1.19

Table 2. Summary of Data from Curve Fitting for all HD Studies

<u>Study</u>	<u>Intercept</u>	<u>Slope</u>	<u>r²</u>
Rat Rangefinding (combined data from three preparations)	-0.02	3.75	0.994
Rat Teratology			
First Preparation	-0.04	3.89	0.992
Second Preparation	-0.01	3.64	0.994
Rabbit Rangefinding			
First Preparation	-0.013	3.56	0.992
Second Preparation	-0.056	3.79	0.982
Rabbit Teratology			
First Preparation	0.006	3.18	0.984
Second Preparation	-0.018	3.67	0.979

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ANALYSIS OF SESAME OIL FOR PEROXIDE

Analyte: Peroxide
Matrix: Sesame oil
Procedure: Titration

Date Written: 5 July 1984

1. Principle of the Method

Peroxides (and similar substances) that oxidize aqueous iodide under the conditions of the test are determined in sesame oil by titration with sodium thiosulfate.

2. Interferences

Any compound found in sesame oil that will oxidize aqueous iodide will be included as part of the peroxide number determined by this test. Variation in the procedure may affect the results.

3. Collection and storage of Samples

The sesame oil to be used (gavage vehicle) on the program will be purchased in large (preferably quart or gallon) containers involving the minimum number of lots. It will be stored at 5°C or lower. Samples to be analyzed for peroxide will be removed from the container shortly before use.

4. Apparatus

- a. Corning, Model 125, digital pH meter or equivalent
- b. 250-ml beakers
- c. Pasteur pipettes
- d. Orion combination redox electrode (#97-78) or equivalent.

5. Reagents

- a. Glacial acetic acid:chloroform (60:40 v/v)
- b. Saturated (aqueous KI solution)
- c. 0.005 N sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$); VWR #AL69980-4 or equivalent (standardize monthly or use fresh bottle)
- d. 4 M KCl saturated with AgCl (electrode filling solution).

6. Procedure

- a. Weigh 5.00 ± 0.05 g of sesame oil used in the gavage study into a 250-ml Erlenmeyer flask.
- b. Add 30 ml of glacial acetic acid:chloroform and stir until the sesame oil has dissolved completely.
- c. Add 0.5 ml saturated KI solution, stir thoroughly and allow to stand for 1 minute.
- d. At the end of 1 minute, add 30 ml distilled water.
- e. Titrate the solution with standard 0.005 N sodium thiosulfate solution, stirring vigorously to ensure thorough mixing.
- f. Titrations should be run in triplicate.
- g. A blank titration of reagents should be run on the day the oil sample is analyzed. The blank should not titrate more than 1.0 ml of 0.005 N sodium thiosulfate standard solution.

7. Calculations

The peroxide number, expressed in milliequivalents of peroxide per kilogram of oil (meq/kg), is calculated as follows:

$$\text{Peroxide Number} = \frac{(V - B) \times N \times 1,000}{W}$$

where:

V = volume (in ml) of thiosulfate solution required for titration of oil sample

B = volume (in ml) of thiosulfate solution required for titration of reagent blank

N = normality of sodium thiosulfate solution

W = weight of oil sample in grams.

8. Program Requirements

- a. Sesame oil with a peroxide number equal to, or greater than, 10 meq/kg will be considered rancid for purposes of the program and will be replaced immediately.
- b. Analyses for peroxides in sesame oil will be made monthly.

9. Reporting the Results

- a. Reports will accompany the first Monthly Report after the analysis, using the form given in Figure 1.
- b. When reporting follow-up analyses, the dates and results of all previous analyses of the batch will be included for comparison.
- c. Conclusions and actions taken as a result will be noted and the report will be signed and dated by the Technician and responsible Chemist.

10. References

- a. Gunston, F. D. 1984. Reaction of Oxygen and Unsaturated Fatty Acid, Volume 61, No. 2, pp. 441-447. JAOCs.
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ANALYSIS OF SESAME OIL GAVAGE VEHICLE FOR PEROXIDE

Compound:
Brand:
Source:
Date of Purchase:
Lot Number:

<u>Assay Date</u>	<u>BNW Notebook Reference</u>	<u>meq/kg Peroxide</u>	<u>Container Number</u>
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Conclusions and Actions taken:

Technician_____	Date_____
Chemist_____	Date_____

FIGURE 1. Example of Report Form for Peroxide Assay of Sesame Oil

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Rev. Letter	Date Implemented	Comment	Signature/Date	
			Task Leader	Section Manager

STANDARDIZATION OF DILUTE SODIUM THIOSULFATEI. INTRODUCTION

It is necessary to standardize sodium thiosulfate for use in the bromobenzene gavage study. This method measures the reduction of iodate to iodine using a starch-iodide indicator and is adapted from "Fundamentals of Analytical Chemistry". This is adapted for .005 N thiosulfate, however, it could be used as is for concentrations ranging from 0.001N to 0.01N and adapted for other concentrations.

II. THEORY

Iodate ion reacts rapidly and quantitatively with iodide in slightly acid solution to give iodine.

The iodine then reacts with the thiosulfate ion to yield iodide and tetrathionate giving the reaction:

APPENDIX B

The thiosulfate is added slowly to prevent decomposition. The starch indicator gives a ~~NEGROPSY OBSERVATIONS~~ presence of iodine. Starch indicator is added in the final stages of the reaction because it is partially decomposed in the presence of a large excess of iodine (see VII).

III. APPARATUS

250 ml volumetric flasks
125 ml erlenmeyer flasks
10 ml burette
5 ml pipette, class A

IV. REAGENTS

Potassium iodate, pure primary standard
Saturated (aqueous) potassium iodide solution
1 N HCl
Starch solution
Sodium thiosulfate

V. PROCEDURE

- A. Dry potassium iodate overnight in 150°C oven.
- B. Cool potassium iodate in a dessicator.

- C. In triplicate, weigh approximately 50 mg, record weights and transfer to 3 x 250 ml volumetric flasks.
- D. Dissolve in and bring to volume with deionized water.
- E. Fill a 10 ml burette with .005 N sodium thiosulfate.
- F. Pipette 5 ml of each potassium iodate solution into individual 125 ml erlenmeyer flasks.
- G. Add approximately 0.5 ml saturated aqueous potassium iodide solution.
- H. Add 10 ml 1N HCl.
- I. Titrate with sodium thiosulfate solution until pale yellow, add approximately 5 ml starch solution (the solution will turn blue). Continue titrating until all the blue color disappears.
- J. Run a blank containing everything except the potassium iodate.

VI. CALCULATIONS

A. The equivalent weight of KIO_3 is 35.67 g/eq

$$\text{B. Normality of } \text{KIO}_3 = \frac{\text{wt } \text{KIO}_3}{35.67 \text{ g/eq}} \times \frac{1}{0.2500 \text{ l}}$$

where wt of KIO_3 is in grams

$$\text{C. Normality of thiosulfate} = \frac{\text{Norm } \text{KIO}_3 \times \text{ml } \text{IO}_3}{(\text{ml thiosulfate titrated} - \text{ml Blank})}$$

1. Criteria for Acceptance/Rejection:

- a. Average the 3 titrations and use the mean. The standard deviation should be no greater than ± 0.0005 if it is, repeat standardization.
- b. The standard sodium thiosulfate should be free from turbidity and any bacterial growth. Discard if inadequate.

VII. REFERENCES

Douglas A. Skoog and Donald M. West. Fundamentals of Analytical Chemistry. Holt Rinehart & Winston. Standardization of thiosulfate solutions, page 488, 1963.

APPENDIX B
NECROPSY OBSERVATIONS

TABLE B1. OBSERVATIONS AT NECROPSY OF RATS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

DOSE mg/Kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATION
Vehicle	9	20	10	AN ^b
	24	20	10	AN
	27	20	10	AN
	30	20	10	AN
	45	20	10	AN
	49	20	10	AN
	50	20	10	AN
	51	20	10	AN
	55	20	10	Ovarian cyst
<hr/>				
0.2	2	20	10	AN
	5	20	10	AN
	11	20	10	AN
	16	20	10	AN
	21	20	10	AN
	32	20	10	AN
	43	20	10	AN
	57	20	10	AN
<hr/>				
0.4	1	20	10	AN
	3	20	10	Inflamed mesenteric lymph nodes; small lung lesion ^c
	23	20	10	AN
	26	20	10	AN
	37	20	10	Hemorrhagic mesenteric lymph nodes
	38	20	10	Inflamed mesenteric lymph nodes; esophageal and lung lesion ^c
	39	20	10	AN
	54	20	10	Inflamed mesenteric lymph nodes
	56	20	10	AN
<hr/>				
0.8	4	20	10	Hemorrhagic mesenteric lymph nodes
	8	20	10	Inflamed mesenteric lymph nodes; small lung lesion ^c
	13	20	10	Inflamed mesenteric lymph nodes
	29	20	10	Inflamed mesenteric lymph nodes
	35	20	10	Inflamed mesenteric, enlarged lumbar lymph nodes; slight renal cavitation
	40	20	10	Inflamed mesenteric lymph nodes
	47	20	10	Inflamed mesenteric lymph nodes; small lung lesion ^c
	48	20	10	Inflamed mesenteric lymph nodes
	58	20	10	Inflamed mesenteric lymph nodes

^aDays after sperm detection; scheduled sacrifice on 20 dg

^bApparently normal (AN)

^cProbable cause of lung lesion: CO₂ euthanasia

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TABLE B1 (CONTINUED). OBSERVATIONS AT NECROPSY OF RATS IN THE DOSE RANGE STUDY OF SULFUR MUSTARD

<u>DOSE</u> <u>mg/Kg</u>	<u>ANIMAL</u> <u>NUMBER</u>	<u>DAY OF</u> <u>DEATH^a</u>	<u>NUMBER</u> <u>OF DOSES</u>	<u>OBSERVATION</u>
1.6	7	20	10	Inflamed mesenteric and enlarged lumbar lymph nodes
	14	20	10	AN ^b
	25	20	10	AN
	31	20	10	Hemorrhagic mesenteric lymph nodes
	36	20	10	Inflamed mesenteric lymph nodes
	41	20	10	Inflamed mesenteric lymph nodes
	46	20	10	Inflamed mesenteric lymph nodes
	52	20	10	Inflamed mesenteric lymph nodes
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2.0	10	20	10	Inflamed mesenteric lymph nodes; inflamed renal papillae
	28	20	10	Bilateral renal cavitation; esophageal blister (mesenteric); petechial hemorrhage and sloughing of gastric mucosa
	34	20	10	Inflamed mesenteric lymph nodes; cartilaginous mass near hyoid bone; lung lesion
<hr/>				
2.5	12 ^a	12	6	Died; pericardial and gastrointestinal inflammation; inflamed thymus, lymph nodes and Peyer's patches; gastric mucosa sloughing; inflamed renal papillae
	19	20	10	Inflamed mesenteric lymph nodes
	53	20	10	Petechial hemorrhage and thickened gastric mucosa; inflamed mesenteric lymph nodes; pericardium adhered to heart

^aDays after sperm detection; scheduled sacrifice on 20 dg

^bApparently normal (AN)

TABLE B2. OBSERVATIONS AT NECROPSY OF RATS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

<u>DOSE</u> <u>mg/kg</u>	<u>ANIMAL</u> <u>NUMBER</u>	<u>DAY OF</u> <u>DEATH^a</u>	<u>NUMBER</u> <u>OF DOSES</u>	<u>OBSERVATIONS</u>
Vehicle	69	20	10	Renal cavitation
	77	20	10	AN ^b
	88	20	10	AN
	92	20	10	Perforated esophagus; pericardial effusion
	94	20	10	AN
	98	20	10	Esophageal cyst; pericardial effusion
	110	20	10	AN
	113	20	10	AN
	117	20	10	AN
	133	20	10	AN
	145	16	10	Euthanized; perforated esophagus
	148	20	10	AN
	150	20	10	AN
	160	20	10	AN
	161	20	10	AN
	166	20	10	AN
	184	20	10	AN
	191	20	10	AN
	198	20	10	AN
	201	20	10	AN
	208	20	10	AN
	217	20	10	Small red foci on large intestine
	221	20	10	AN
	255	20	10	AN
	256	20	10	AN
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0.5	62	20	10	AN
	66	20	10	Inflamed mesenteric lymph nodes
	70	20	10	Perforated esophagus; infected mass in left axilla; pericardial effusion
	78	20	10	Inflamed mesenteric lymph nodes
	83	20	10	AN
	85	20	10	Inflamed mesenteric lymph nodes
	87	20	10	AN
	90	20	10	Inflamed mesenteric lymph nodes
	108	20	10	AN
	109	20	10	Inflamed mesenteric lymph nodes
	116	20	10	AN
	118	20	10	Inflamed mesenteric lymph nodes
	123	20	10	Inflamed mesenteric lymph nodes
	136	10	4	Died; perforated esophagus
	137	20	10	Inflamed mesenteric lymph nodes

^aDays after detection of sperm; scheduled sacrifice on 20 dg
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^bApparently normal (AN)

TABLE B2 (CONTINUED). OBSERVATIONS AT NECROPSY OF RATS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATIONS
0.5	144	20	10	AN ^b
	154	20	10	Inflamed mesenteric lymph nodes
	168	20	10	AN
	171	20	10	AN
	183	15	10	Died; perforated lung
	185	20	10	Pericardial effusion
	189	20	10	Inflamed mesenteric lymph nodes
	195	20	10	AN
	210	20	10	AN
	252	20	10	Inflamed mesenteric lymph nodes
1.0	60	20	10	Inflamed mesenteric lymph nodes
	67	20	10	Inflamed mesenteric lymph nodes
	71	20	10	Inflamed mesenteric lymph nodes
	75	20	10	Inflamed mesenteric lymph nodes
	76	20	10	Inflamed mesenteric lymph nodes
	89	20	10	AN
	91	20	10	Inflamed mesenteric lymph nodes
	96	20	10	Inflamed mesenteric lymph nodes
	100	20	10	Jejunal evagination
	101	20	10	AN
	111	20	10	AN
	112	20	10	AN
	119	20	10	Inflamed mesenteric lymph nodes
	120	20	10	Inflamed mesenteric lymph nodes
	128	9	3	Died; perforated esophagus
	146	20	10	Inflamed mesenteric lymph nodes
	170	20	10	Inflamed mesenteric lymph nodes
	186	20	10	AN
	188	20	10	Inflamed mesenteric lymph nodes
	194	20	10	Inflamed mesenteric lymph nodes
	204	20	10	Inflamed mesenteric lymph nodes
	220	20	10	AN
	235	20	10	Inflamed mesenteric lymph nodes; small inflamed site on gastric mucosa
	245	20	10	Inflamed mesenteric lymph nodes
	248	20	10	AN

^aDays after detection of sperm; scheduled sacrifice on 20 dg

^bApparently normal (AN)

TABLE B2 (CONTINUED). OBSERVATIONS AT NECROPSY OF RATS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

<u>DOSE</u> <u>mg/kg</u>	<u>ANIMAL</u> <u>NUMBER</u>	<u>DAY OF</u> <u>DEATH^a</u>	<u>NUMBER</u> <u>OF DOSES</u>	<u>OBSERVATIONS</u>
2.0	63	20	10	Inflamed mesenteric lymph nodes
	65	15	10	Died; blood vessel ruptured during dosing
	74	20	10	Inflamed mesenteric lymph nodes
	102	20	10	Inflamed mesenteric lymph nodes
	105	20	10	Inflamed mesenteric lymph nodes
	114	20	10	AN ^b
	122	20	10	Inflamed mesenteric lymph nodes; evagination in small intestine
	130	20	10	Inflamed mesenteric lymph nodes
	131	20	10	AN
	135	8	2	Died; perforated esophagus
	141	20	10	Inflamed mesenteric lymph nodes
	142	20	10	AN
	143	20	10	AN
	149	20	10	Left eye darkened
	151	20	10	AN
	152	20	10	AN
	153	20	10	Inflamed mesenteric lymph nodes, gastric mucosa
	158	20	10	AN
	159	20	10	Inflamed mesenteric lymph nodes
	164	20	10	Inflamed mesenteric lymph nodes
	174	20	10	AN
	182	20	10	Inflamed mesenteric lymph nodes
	197	20	10	Inflamed mesenteric lymph nodes
	211	20	10	AN
	216	20	10	Inflamed mesenteric lymph nodes
	240	20	10	Inflamed mesenteric lymph nodes
	251	20	10	Inflamed mesenteric lymph nodes

^aDays after detection of sperm; scheduled sacrifice on 20 dg

^bApparently normal (AN)

TABLE B3. OBSERVATIONS AT NECROPSY OF RABBITS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATION
Vehicle	6308	30	14	Enlarged mesenteric lymph nodes
	6434	10	5	Died; gastric hairball, lung adhesion; probable cause of death was stress
	6450	30	14	Cecal wall thickened
	6464	30	14	AN ^b
	6530	30	14	AN
	6531	30	14	Small, cream-colored lesion within left liver lobe; cecal wall thickened
	6663	30	14	Slight pulmonary adhesion
	6704	30	14	Inflamed mesenteric lymph nodes; small growth near cardiac sphincter.
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0.5	6435	30	14	AN
	6609	6	1	Died- lung dose; lungs hemorrhagic; trachea filled with blood
	6629	30	14	Inflamed mesenteric lymph nodes
	6631	30	14	Enlarged Peyer's patches
	6633	30	14	AN
	6785	30	14	Enlarged Peyer's patches
	8819	30	14	AN
	8932	30	14	AN
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1.0	6311	30	14	White areas on 4 of 7 placentas
	6367	6	1	Died-lung dose; oil droplets in thorax
	6465	30	14	AN
	6470	14	9	Euthanized (dyspnea and cyanosis); focal hemorrhage and ulceration of gastric mucosa; adhesions and fluid in thorax; hemorrhagic foci in lungs.
	6646	9	4	Euthanized (severe dyspnea); gastric mucosa hemorrhagic; adhesions and fluid in thorax; pericardium fluid- filled; heart appears enlarged
	6755	21	10	Died; diarrhea; bloody nasal and vaginal discharge (abortion just initiated); hemorrhagic foci throughout stomach, mucosa thin in midregion, thickened in pylorus; adhesions and fluid in thorax; right lung congested and firm; Peyer's patches enlarged
	6757	30	14	White areas on 3 of 12 placentas
	6782	30	14	Mesenteric lymph nodes enlarged

^aDays after insemination; scheduled sacrifice on 30 dg

^bApparently normal (AN)

TABLE B3 (CONTINUED). OBSERVATIONS AT NECROPSY OF RABBITS
IN THE DOSE RANGE STUDY OF SULFUR MUSTARD

<u>DOSE</u> <u>mg/kg</u>	<u>ANIMAL</u> <u>NUMBER</u>	<u>DAY OF</u> <u>DEATH^a</u>	<u>NUMBER</u> <u>OF DOSES</u>	<u>OBSERVATION</u>
2.0	6370	7	2	Died (stress); hemorrhage, edema and thickening of gastric mucosa; mucosal inflammation in small intestine; cecal blood vessels enlarged
	6607	30	14	Suspected abortion on 24 dg; uterus regressed; 8 nidation sites
	6620	30	14	Enlarged mesenteric lymph nodes; cystic areas on cecal wall
	6632	24	14	Died (diarrhea, vaginal bleeding, aborted); GIT filled with bloody fluid, gas; gastric wall smooth, no convoluted areas; liver pale; heart flaccid; few hemorrhagic foci in lungs
	6636	13	7	Died; bloody fluid in abdomen, thorax; small hemorrhagic area in stomach; GIT filled with gas; lungs pale; membranes edematous on thoracic side of diaphragm
	6644	30	14	AN ^D
	6664	10	4	Died (stress); hemorrhage and ulceration of gastric mucosa; hair ball, gas in stomach; gas in colon; purulent material in small intestine
	6679	24	14	Euthanized (dyspnea, diarrhea, cachexia, vaginal bleeding; abortion in progress); gastric hemorrhage and ulceration near pyloric sphincter; mucosa of small intestine and cecum inflamed; foci of infection in cecum; right thorax filled with purulent exudate; heart is flaccid

TABLE B3 (CONTINUED). OBSERVATIONS AT NECROPSY OF RABBITS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATION
2.5	6330	7	1	Died—lung dose; oil in lungs; blood in right thorax; hemorrhage, inflammation of gastric mucosa
	6471	24	14	Died; hairball in stomach; gastric walls thin, petechial hemorrhage in cardiac region; cecum inflamed, contains green fluid; lungs slightly inflamed
	6540	16	10	Died; hairball in stomach, stomach lining smooth; diarrhea; fecal material in vagina and bladder; cecum hemorrhagic and thickened; inflamed areas in small intestine, Peyer's patches inflamed; pale, small lesion on liver; lungs inflamed
	6622	22	10	Died; diarrhea; lethargic; GIT distended with gas; hairball in stomach; 5 mm ulcer in mid section of stomach; small intestine filled with yellow fluid and purulent material; mesenteric lymph nodes inflamed and enlarged; right median lung lobe consolidated, darkened with purulent foci
	6638	14	8	Died; abdomen filled with bloody fluid; hemorrhagic areas in small intestine; cecum filled with gas and green fluid; stomach contains gas and bloody fluid; gastric mucosa hemorrhagic; caudal end of right diaphragmatic lobe of lung is greenish-white
	6707	30	14	Enlarged mesenteric lymph nodes and Peyer's patches
	8959	30	14	Enlarged Peyer's patches; cecal wall thickened

^aDays after insemination; scheduled sacrifice on 30 dg

TABLE B4. OBSERVATIONS AT NECROPSY OF RABBITS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATION
Vehicle	7008	30	14	Few dark red foci on left lung lobe ^b
	7011	30	14	AN ^c
	7063	30	14	AN
	7188	30	14	AN
	7228	11	6	Died (convulsions, stress); no oil in lungs; trachea, esophagus intact; blood in nose, right lung and trachea; oil in stomach
	7234	8	2	Euthanized (stress); dyspnea prior to dosing and continuing for 5 hrs although animal was not dosed; no signs of injury or foreign material in lungs.
	7237	30	14	AN
	7272	30	14	Slight adhesion on right caudal lung lobe
	7299	30	14	AN
	7317	30	14	AN
	7330	30	14	Enlarged Peyer's patches
	7354	19	14	Died; perforated right lung; oil in thorax
	7382	30	14	AN
	7456	30	14	Red foci on left lung ^b
	7470	30	14	AN
	7475	30	14	AN
	7495	6	1	Died (stress); cyanosis and convulsions following dosing; blood in nose; esophagus, trachea and lungs normal; oil in stomach
	8644	30	14	AN
	8675	30	14	AN
0.4	6740	30	14	Excess pericardial fluid; right lung lobe mottled; enlarged Peyer's patches
	6888	30	14	Small adhesion on right lung
	6927	30	14	Enlarged mesenteric lymph nodes
	7149	14	9	Died; convulsions following two attempts at dosing; oil in stomach; puncture in left caudate lung lobe; stomach filled with dark fluid, pyloric region thickened, edematous, ulcerated
	7187	30	14	AN
	7316	30	14	Enlarged Peyer's patches
	7391	30	14	AN
	7392	30	14	Enlarged Peyer's patches
	7401	30	14	AN
	7469	30	14	AN
	7476	30	14	AN

^aDays after insemination; scheduled sacrifice on 30 dg

^bProbable cause of lung lesions: CO₂ euthanasia

PLH-654A

^cApparently normal (AN)

TABLE B4 (CONTINUED). OBSERVATIONS AT NECROPSY OF RABBITS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATION
0.4	7496	14	8	Died; dyspnea and cyanosis after dosing but appeared normal in pm; died during the night; oil in thorax; severe lung adhesions; esophageal and gastric walls thin; brown serous fluid in abdomen; Peyer's patches enlarged
	7595	9	3	Euthanized; dyspnea from aspirated blood; damage to nasopharynx during dosing; blood in lungs and stomach; gastric mucosa inflamed around pylorus
	7614	30	14	Enlarged Peyer's patches
	8674	30	14	Enlarged Peyer's patches; right lung red
	8734	30	14	AN ^b
	8737	30	14	AN
	8764	30	14	AN
0.6	7053	30	14	AN
	7137	30	14	Nasal discharge
	7255	30	14	Adhesions, inflammation of right lung; purulent exudate in right uterine horn
	7267	30	14	AN
	7281	30	14	AN
	7282	30	14	Lungs slightly inflamed; purulent material in small intestine
	7283	30	14	Enlarged Peyer's patches
	7331	30	14	AN
	7356	30	14	AN
	7412	30	14	AN
	7464	30	14	Dark red foci, white lesions in right lung; enlarged Peyer's patches
	7490	30	14	Right lung slightly red
	7618	30	14	Adhesions, mottling of right lung; inflamed area of small intestine
	8633	23	14	Died (toxemia); vaginal bleeding; all fetuses were resorbing
	8729	30	14	AN
	8738	30	14	Pulmonary-pericardial adhesions; purulent exudate
	8739	30	14	AN
	8743	8	3	Euthanized; severe dyspnea; small, firm, dark area in right lung, adhesions; slightly enlarged Peyer's patches; large intestine filled with gas

^aDays after insemination; scheduled sacrifice on 30 dg

^bApparently normal (AN)

TABLE B4 (CONTINUED). OBSERVATIONS AT NECROPSY OF RABBITS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	NUMBER OF DOSES	OBSERVATION
0.8	6717	30	14	Enlarged Peyer's patches
	6749	30	14	Gastric ulcer, purulent material in small intestine; abdominal adhesions
	6831	9	3	Euthanized; dyspnea; lungs mottled, hemorrhage in right lung (no oil observed); petechial hemorrhage of gastric mucosa; inflamed vasculature of small intestine; inflamed Peyer's patches
	6857	30	14	AN ^b
	6901	30	14	AN
	7013	30	14	AN
	7136	30	14	Enlarged Peyer's patches
	7227	29	14	Died; extended anorexia; gastric ulcer near pyloric sphincter
	7252	30	14	AN
	7257	30	14	AN
	7271	30	14	AN
	7314	30	14	Lung adhesions; left kidney pale and mottled; small gall bladder; enlarged Peyer's patches
	7353	12	6	Died; anorectic; brown, serous fluid in abdomen; perforated gastric ulcer in cardiac region; stomach wall thickened and edematous in pyloric region; small intestine contains gas and yellow fluid
	7418	30	14	AN
	7467	30	14	Purulent material in small intestine
	8643	30	14	AN
	8741	30	14	Lungs slightly red; purulent material in small intestine
	8744	30	14	AN

^aDays after insemination; scheduled sacrifice on 30 dg

^bApparently normal (AN)

APPENDIX C
REPRODUCTIVE MEASURES

TABLE C1. REPRODUCTIVE STATUS OF RATS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

DOSE mg/Kg	ANIMAL NUMBER	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS		FETUSES ^a	
				EARLY	LATE	DEAD	LIVE
Vehicle	9	17	18	1	0	0	17
	24	16	15	1	0	0	14
	27	18	17	1	0	0	16
	30	16	16	0	0	0	16
	45	0	0	-	-	-	-
	49	25	14	0	0	0	14
	50	21	13	2	0	0	11
	51	0	0	-	-	-	-
	55	20	19	0	0	0	19
<hr/>							
0.2	2	26	9	0	0	0	9
	5	21	15	1	0	0	14
	11	16	16	1	0	0	15
	16	0	0	-	-	-	-
	21	19	9	0	1	0	8
	32	27	15	3	0	0	12
	43	0	0	-	-	-	-
	57	0	0	-	-	-	-
<hr/>							
0.4	1	0	0	-	-	-	-
	3	18	14	0	0	0	14
	23	27	8	3	0	0	5
	26	15	6	0	0	0	6
	37	18	19	2	0	0	17
	38	16	16	0	0	0	16
	39	13	13	0	0	0	13
	54	18	10	0	0	0	10
	56	0	0	-	-	-	-
<hr/>							
0.8	4	0	0	-	-	-	-
	8	16	9	1	0	0	8
	13	0	0	-	-	-	-
	29	15	15	1	0	0	14
	35	19	20	0	0	0	20
	40	17	15	0	0	0	15
	47	14	14	0	0	0	14
	48	17	15	1	0	0	14
	58	18	15	1	0	0	14

^aFetuses or embryos, depending on development time

TABLE C1 (CONTINUED). REPRODUCTIVE STATUS OF RATS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

<u>DOSE</u> <u>mg/Kg</u>	<u>ANIMAL</u> <u>NUMBER</u>	<u>CORPORA</u> <u>LUTEA</u>	<u>IMPLAN-</u> <u>TATIONS</u>	<u>RESORPTIONS</u>		<u>FETUSES^a</u>	
				<u>EARLY</u>	<u>LATE</u>	<u>DEAD</u>	<u>LIVE</u>
1.6	7	16	15	2	0	0	13
	14	0	0	-	-	-	-
	25	15	12	0	1	0	11
	31	16	13	1	2	0	10
	36	17	17	0	0	0	17
	41	18	18	2	0	0	16
	46	18	17	1	0	0	16
	52	13	10	0	0	0	10

2.0	10	16	16	0	0	0	16
	28	13	13	0	0	0	13
	34	0	0	-	-	-	-

2.5	12 ^b	16	16	6	0	0	10
	19	14	14	0	0	0	14
	53	0	0	-	-	-	-

^aFetuses or embryos, depending on development time

^bDied on 12 dg

TABLE C2. REPRODUCTIVE STATUS OF RATS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS		FETUSES ^a	
				EARLY	LATE	DEAD	LIVE
Vehicle	69	14	13	0	0	0	13
	77	16	16	1	0	0	15
	88	0	0	-	-	-	-
	92	17	9	0	0	0	9
	94	17	17	0	0	0	17
	98	16	15	0	0	0	15
	110	14	9	0	0	0	9
	113	13	13	0	0	0	13
	117	17	16	2	0	0	14
	133	17	18	1	0	0	17
	145 ^b	14	14	1	-	-	13
	148	19	18	1	0	0	17
	150	21	16	0	0	0	16
	160	17	15	1	0	0	14
	161	15	15	0	0	0	15
	166	17	17	1	0	0	16
	184	20	9	0	0	0	9
	191	0	0	-	-	-	-
	198	17	17	1	0	0	16
	201	0	0	-	-	-	-
	208	16	6	0	0	0	6
	217	19	16	1	0	0	15
	221	13	13	0	0	0	13
	255	0	0	-	-	-	-
	256	17	14	0	1	0	13
<hr/>							
0.5	62	16	13	0	0	0	13
	66	17	12	0	0	0	12
	70	17	12	0	0	0	12
	78	17	17	0	1	0	16
	83	0	0	-	-	-	-
	85	17	17	2	1	0	14
	87	15	13	0	0	0	13
	90	15	15	1	0	0	14
	108	14	15	1	0	0	14
	109	18	16	0	0	0	16
	116	0	0	-	-	-	-
	118	16	15	1	0	0	14
	123	17	16	0	0	0	16
	136 ^c	18	17	0	-	-	17
	137	24	17	0	0	0	17

^aFetuses or embryos, depending on development time

^bEuthanized on 16 dg

^cDied on 10 dg

TABLE C2 (CONTINUED). REPRODUCTIVE STATUS OF RATS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS		FETUSES ^a	
				EARLY	LATE	DEAD	LIVE
0.5	144	13	10	5	0	0	5
	154	15	15	1	0	0	14
	168	16	16	4	0	0	12
	171	0	0	-	-	-	-
	183 ^b	17	17	0	-	-	17
	185	0	0	-	-	-	-
	189	13	13	0	0	0	13
	195	21	17	0	0	0	17
	210	0	0	-	-	-	-
	252	12	10	1	0	0	9

1.0	60	20	18	0	0	0	18
	67	16	15	2	0	0	13
	71	13	14	4	0	0	10
	75	18	18	0	0	0	18
	76	17	13	0	0	0	13
	89	0	0	-	-	-	-
	91	20	17	1	0	0	16
	96	15	15	1	0	0	14
	100	16	14	0	0	0	14
	101	17	16	1	0	0	15
	111	15	15	0	0	0	15
	112	13	8	0	0	0	8
	119	17	13	0	0	0	13
	120	17	11	0	0	0	11
	128 ^c	15	14	0	-	-	14
	146	19	16	2	0	0	14
	170	19	17	0	0	0	17
	186	18	17	0	0	0	17
	188	14	12	1	0	0	11
	194	20	18	4	1	0	13
	204	18	18	0	0	0	18
	220	19	16	1	0	0	15
	235	17	15	1	0	0	14
	245	16	15	0	0	0	15
	248	0	0	-	-	-	-

^aFetuses or embryos, depending on development time

^bDied on 15 dg

^cDied on 9 dg

TABLE C2 (CONTINUED). REPRODUCTIVE STATUS OF RATS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS		FETUSES ^a	
				EARLY	LATE	DEAD	LIVE
2.0	63	17	16	0	0	0	16
	65 ^b	12	10	0	-	-	10
	74	14	13	0	0	0	13
	102	16	17	5	0	0	12
	105	15	12	2	0	0	10
	114	18	17	2	0	0	15
	122	19	15	0	0	0	15
	130	13	12	0	0	0	12
	131	15	15	2	0	0	13
	135 ^c	15	15	-	-	-	15
	141	18	15	2	0	0	13
	142	15	15	0	0	0	15
	143	0	0	-	-	-	-
	149	16	10	5	0	0	5
	151	14	14	2	0	0	12
	152	18	14	0	0	0	14
	153	17	16	1	0	0	15
	158	16	11	0	0	0	11
	159	21	17	1	0	0	16
	164	11	9	0	0	0	9
	174	16	9	0	0	0	9
	182	20	17	2	0	0	15
	197	15	15	2	0	0	13
	211	19	10	0	0	0	10
	216	18	16	2	0	0	14
	240	11	9	0	0	0	9
	251	13	13	0	0	0	13

^aFetuses or embryos, depending on development time

^bDied on 15 dg

^cDied on 8 dg

TABLE C3. REPRODUCTIVE STATUS OF RABBITS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS			FETUSES ^b	
					EARLY	MID	LATE	DEAD	LIVE
Vehicle	6308	30	11	8	0	0	0	0	8
	6434	10	11	11	0	-	-	-	11
	6450	30	15	13	0	0	2	0	11
	6464	30	6	5	0	0	0	0	5
	6530	30	13	10	0	0	1	0	9
	6531	30	0	0	-	-	-	-	-
	6663	30	6	5	0	0	0	0	5
	6704	30	5	2	0	0	0	0	2

0.5	6435	30	1	0	-	-	-	-	-
	6609	6	15	0 ^c	-	-	-	-	-
	6629	30	14	11	1	1	0	0	9
	6631	30	1	1	1	0	0	0	0
	6633	30	0	0	-	-	-	-	-
	6785	30	8	5	0	0	0	0	5
	8819	30	3	0	-	-	-	-	-
	8932	30	14	9	0	0	0	0	9

1.0	6311	30	12	10	0	2	1	0	7
	6367	6	7	0	-	-	-	-	-
	6465	30	- ^d	8	0	0	1	0	7
	6470	14	19	0	-	-	-	-	-
	6646	9	14	14	0	-	-	-	14
	6755	21	13	13	2	0	0	-	11
	6757	30	15	15	0	1	2	0	12
	6782	30	13	12	1	0	1	0	10

2.0	6370	7	8	8	0	-	-	-	8
	6607 ^e	30	8	8	-	-	-	-	-
	6620	30	10	10	5	0	0	0	5
	6632 ^f	24	11	11	-	-	-	4	2
	6636	13	8	2	0	0	-	-	2
	6644	30	13	10	0	2	1	0	7
	6664	10	7	3	0	-	-	-	3
	6679	24	0 ^g	16	0	0	16	-	0

^aDays after insemination; scheduled sacrifice on 30 dg

^bFetuses or embryos, depending on development time

^cImplantation may not have been evident at this time

^dNumber not determined

^eAborted; nidation sites determined

^fAborted 4 fetuses; 2 live fetuses found in uterus

^gCorpora lutea appeared to have regressed

TABLE C3 (CONTINUED). REPRODUCTIVE STATUS OF RABBITS IN THE
DOSE RANGE STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS			FETUSES ^b	
					EARLY	MID	LATE	DEAD	LIVE
2.5	6330	7	12	12	0	-	-	-	12
	6471	24	16	0	-	-	-	-	-
	6540	16	0	0	-	-	-	-	-
	6622	22	0	0	-	-	-	-	-
	6638	14	11	12	0	0	-	-	12
	6707	30	0	0	-	-	-	-	-
	8959	30	6	0	-	-	-	-	-

^aDays after insemination; scheduled sacrifice on 30 dg

^bFetuses or embryos, depending on development time

TABLE C4. REPRODUCTIVE STATUS OF RABBITS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS			FETUSES ^b	
					EARLY	MID	LATE	DEAD	LIVE
Vehicle	7008	30	0	0	-	-	-	-	-
	7011	30	13	9	0	1	0	0	8
	7063	30	0	0	-	-	-	-	-
	7188	30	0	0	-	-	-	-	-
	7228	11	14	14	0	0	-	-	14
	7234	8	11	11	-	-	-	-	8
	7237	30	12	9	1	0	0	0	8
	7272	30	9	7	1	0	0	0	6
	7299	30	14	13	0	0	0	0	13
	7317	30	5	5	0	0	0	0	5
	7330	30	10	5	0	0	0	0	5
	7354	19	12	6	0	0	-	-	6
	7382	30	14	8	2	1	1	0	4
	7456	30	10 ^c	12	0	2	1	0	9
	7470	30	11	9	0	0	0	0	9
	7475	30	9	8	0	1	1	0	6
	7495	6	10	0 ^d	-	-	-	-	-
	8644	30	12	11	0	0	0	0	11
	8675	30	0	0	-	-	-	-	-
0.4	6740	30	10	10	0	1	0	0	9
	6888	30	9	8	0	3	0	0	5
	6927	30	12	8	0	0	1	0	7
	7149	14	9	0	-	-	-	-	-
	7187	30	3	0	-	-	-	-	-
	7316	30	12	7	0	0	0	0	7
	7391	30	13	13	0	0	4	0	9
	7392	30	6	0	-	-	-	-	-
	7401	30	3	0	-	-	-	-	-
	7469	30	0	0	-	-	-	-	-
	7476	30	10	7	1	0	0	0	6
	7496	14	15	10	0	0	-	-	10
	7595	9	12	11	-	-	-	-	11
	7614	30	14	11	1	3	0	0	7
	8674	30	7	5	0	0	0	0	5
	8734	30	6	0	-	-	-	-	-
	8737	30	6	4	0	0	0	0	4
	8764	30	13	11	0	0	0	0	11

^aDays after insemination; scheduled sacrifice on 30 dg

^bFetuses or embryos, depending on development time

^cThree additional corpora lutea appeared to be regressing

^dImplantation may not have been evident at this time

TABLE C4 (CONTINUED). REPRODUCTIVE STATUS OF RABBITS IN THE
TERATOLOGY STUDY OF SULFUR MUSTARD

DOSE mg/kg	ANIMAL NUMBER	DAY OF DEATH ^a	CORPORA LUTEA	IMPLAN- TATIONS	RESORPTIONS			FETUSES ^b	
					EARLY	MID	LATE	DEAD	LIVE
0.6	7053	30	12	7	0	0	0	0	7
	7137	30	12	9	0	0	2	0	7
	7255	30	12	11	3	0	2	0	6
	7267	30	7	0	-	-	-	-	-
	7281	30	10	10	0	0	0	0	10
	7282	30	12	10	0	0	1	0	9
	7283	30	10	7	1	0	1	0	5
	7331	30	0	0	-	-	-	-	-
	7356	30	8	7	0	0	1	0	6
	7412	30	16	9	1	0	1	0	7
	7464	30	9	8	0	0	0	0	8
	7490	30	11	8	0	0	0	0	8
	7618	30	11	10	0	7	0	0	3
	8633	23	12	7	1	0	6	-	0
	8729	30	10	10	1	0	0	0	9
	8738	30	6	7	7	0	0	0	0
	8739	30	12	12	0	0	0	0	12
	8743	8	9	9	-	-	-	-	9
0.8	6717	30	0	0	-	-	-	-	-
	6749	30	10	10	10	0	0	0	0
	6831	9	13	9	-	-	-	-	9
	6857	30	14	7	0	0	0	0	7
	6901	30	9	9	0	0	2	0	7
	7013	30	0	0	-	-	-	-	-
	7136	30	9	2	0	0	0	0	2
	7227	29	0	0	-	-	-	-	-
	7252	30	0	0	-	-	-	-	-
	7257	30	11	11	0	0	0	0	11
	7271	30	10	9	0	0	2	0	7
	7314	30	6	0	-	-	-	-	-
	7353	12	10	11	0	-	-	-	11
	7418	30	0	0	-	-	-	-	-
	7467	30	0	0	-	-	-	-	-
	8643	30	11	11	1	1	0	0	9
	8741	30	8	7	0	0	0	0	7
	8744	30	2	0	-	-	-	-	-

^aDays after insemination; scheduled sacrifice on 30 dg

^bFetuses or embryos, depending on development time

APPENDIX D
QUALITY ASSURANCE STATEMENT

TERATOLOGY STUDIES OF SULFUR MUSTARD IN RATS AND RABBITS

(Final Report)

Quality Assurance Statement

Listed below are phases and/or procedures included in the study described in this report which were reviewed specifically for this study by the Quality Assurance Unit during the period, 7/2/84 - 4/10/87, 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.)

PHASES/PROCEDURES REVIEWED	REVIEW DATES	DATE FINDINGS SUBMITTED IN WRITING TO STUDY DIRECTOR/MANAGEMENT
Health Screen	7/02/84	3/06/85
Animal Identification	7/17/84	3/06/85
Body Weights	7/17/84	3/06/85
Dosing	7/26/84	3/06/85
Necropsy	8/12/84	3/06/85
Dose Preparation	9/28/84	3/29/85
Vaginal Lavage	9/28/84	-
Animal Identification	9/28/84	3/29/85
Body Weights	9/28/84	3/29/85
Dosing	10/02/84	3/29/85
Necropsy	10/16/84	10/16/84
Artificial Insemination	11/20/84	8/26/85
Dosing	12/01/84	8/26/85
Necropsy	12/21/84	8/26/85
Artificial Insemination	1/14/85	8/26/85
Dose Preparation	1/23/85	8/26/85
Dosing	1/29/85	8/26/85
Necropsy	2/14/85	8/26/85
Data	3/05/85	3/06/85
Data	3/26-27/85	3/29/85
Data	4/18, 5/10, 20/85	8/26/85
Draft Final Report	8/12, 15-16/85	8/26/85
Final Report	3/31-4/2/87	*

* Results of the review have been discussed with the study director and will be reported in writing to management.

Eileen M. Crow

Quality Assurance Specialist

6/15/87

Date

R. A. Gelman

Quality Assurance Specialist

6/15/87

Date

APPENDIX E
STUDY DATES FOR SULFUR MUSTARD

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APPENDIX E. STUDY DATES FOR SULFUR MUSTARD

DOSE RANGE STUDY IN RATS I

Animals arrived	6/26/84
Detection of copulation (0 dg)	7/17/84 - 7/22/84
Administration of dose (6 dg- 15 dg)	7/23/84 - 8/7/84
Sacrifice (20 dg)	8/6/84 - 8/12/84
Sample/data evaluation	8/12/84 - 9/12/84

DOSE RANGE STUDY IN RATS II

Animals arrived	6/26/84
Detection of copulation (0 dg)	8/15/84
Administration of dose (6 dg- 15 dg)	8/21/84 - 8/30/84
Sacrifice (20 dg)	9/4/84
Sample/data evaluation	9/4/84 - 9/12/84

TERATOLOGY STUDY IN RATS

Animals arrived	9/4/84
Detection of copulation (0 dg)	9/25/84 - 9/28/84
Administration of dose (6 dg- 15 dg)	10/1/84 - 10/13/84
Sacrifice (20 dg)	10/15/84 - 10/18/84
Sample/data evaluation	10/18/84 - 11/21/84

DOSE RANGE STUDY IN RABBITS

Animals arrived	10/29/84
Artificial insemination (0 dg)	11/20/84 - 11/21/84
Administration of dose (6 dg- 19 dg)	11/26/84 - 12/10/84
Sacrifice (30 dg)	12/20/84 - 12/21/84
Sample/data evaluation	12/21/84 - 1/10/85

TERATOLOGY STUDY IN RABBITS

Animals arrived	12/21/84
Artificial insemination (0 dg)	1/14/85 - 1/16/85
Administration of dose (6 dg- 19 dg)	1/20/85 - 2/4/85
Sacrifice (30 dg)	2/13/85 - 2/15/85
Sample/data evaluation	2/15/85 - 3/14/85

SUBMISSION OF DRAFT FINAL REPORT

July, 1985

APPENDIX F
PERSONNEL LIST

PERSONNEL LIST

Function	Name
Principal Investigators	P.L. Hackett L.B. Sasser
Facility Manager	F.G. Burton
Solution Preparation and Analyses	F.G. Burton M. McCulloch G.J. Powers
Animal Exposures and Evaluation	C.A. Bolt J.E. Evanoff P.L. Hackett D.G. Jones D.L. Lundstrom D.L. Matuszewsky M. Orgill R.L. Rommereim R.C. Zangar
Animal Resource Center	E.L. Wierman
Health Evaluation	S.E. Rowe
Statistical Analyses	R.F. Buschbom

P.L. Hackett

P.L. Hackett

6-15-87

Date

L.B. Sasser

L.B. Sasser

6-15-87

Date

APPENDIX G
DISTRIBUTION

DISTRIBUTION

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Center (DTIC)
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Cameron Station
Alexandria, VA 22304-6145

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