REPORT NO. PNL-6408 PT. 2

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

FINAL REPORT: PART 2, APPENDICES

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DECEMBER 31, 1987

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012

Army Project Order No. 83PP3810

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SECURITY CLASSIFICATION OF THIS PAGE

# REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp. Date Jun 30, 1986

| | |
|---|---|
| 1a. REPORT SECURITY CLASSIFICATION<br>Unclassified | 1b. RESTRICTIVE MARKINGS<br>--- |
| 2a. SECURITY CLASSIFICATION AUTHORITY<br>--- | 3. DISTRIBUTION/AVAILABILITY OF REPORT<br>Approved for public release; distribution unlimited |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE<br>--- | |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S)<br>DE-AC06076RLO-1830 | 5. MONITORING ORGANIZATION REPORT NUMBER(S)<br>--- |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Pacific Northwest Laboratory<br>6b. OFFICE SYMBOL (If applicable)<br>--- | 7a. NAME OF MONITORING ORGANIZATION<br>--- |
| 6c. ADDRESS (City, State, and ZIP Code)<br>P.O. Box 999<br>Richland, WA 99352-0999 | 7b. ADDRESS (City, State, and ZIP Code)<br>--- |
| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION<br>U.S. Army Medical R&D Command<br>8b. OFFICE SYMBOL (If applicable)<br>--- | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER<br>Army Project Order #83PP3810 |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick<br>Frederick, MD 21701-5012 | 10. SOURCE OF FUNDING NUMBERS<br>PROGRAM ELEMENT NO. 63764A; PROJECT NO. 3M56376 4D995; TASK NO. AA; WORK UNIT ACCESSION NO 033 |

11. TITLE (Include Security Classification) Teratology Studies on Lewisite and Sulfur Mustard Agents: Effects of Lewisite in Rats and Rabbits Part 2, Appendicies

12. PERSONAL AUTHOR(S) P.L. Hackett, L.B. Sasser, R.L. Rommereim, J.A. Cushing, R.L. Buschbom and D.R. Kalkwarf

| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| Final | FROM 5/15/83 TO 2/28/87 | 1987, December 31 | |

16. SUPPLEMENTARY NOTATION Project Officer: Robert A. Finch, PhD. US Army Biomedical Research Development Laboratory Fort Detrick, Frederick, MD 21701-5010. Sulfur mustard results are contained in Final Report dated September 21, 1987.

| 17. COSATI CODES | | |
|---|---|---|
| FIELD | GROUP | SUB-GROUP |
| 06 | 20 | |
| 06 | 10 | |

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Lewisite, Teratology, Rats and Rabbits

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Lewisite 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, 2.0 and 2.5 mg/kg in the dose-range study and with 0, 0.5, 1.0 and 1.5 mg/kg in the teratology study; rabbits were dosed from 6 through 19 dg with 0, 0.5, 1.0, 1.5 and 2.0 mg/kg and 0, 0.07, 0.2 and 0.6 mg/kg in the dose-range and teratology studies, respectively. Maternal animals were weighed periodically, and, at necropsy (20 dg in rats and 30 dg in rabbits), 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, a dose level of 1.5 mg/kg did not induce toxic or teratogenic responses in maternal animals or their fetuses. At 2.0 mg/kg, 10% maternal mortality, trends in decreased maternal and fetal body weights and a significant reduction in the number of viable fetuses were evident. In rabbit studies, maternal mortality occurred in all but one of the lewisite treatment groups and

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|---|---|
| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT<br>☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS | 21 ABSTRACT SECURITY CLASSIFICATION<br>Unclassified |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>Virginia M. Miller | 22b TELEPHONE (Include Area Code)<br>(301) 663-7325<br>22c OFFICE SYMBOL<br>SGRD-RMI-S |

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted.
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SECURITY CLASSIFICATION OF THIS PAGE

ranged from 13% to 100% at dose levels of 0.07 and 1.5 mg/kg, respectively. This mortality rate limited the sample size and impaired the detection of statistical significance among treatments. However, at the lowest dose level of the teratology study (0.07 mg/kg), maternal mortality was the only indicator of lewisite toxicity; at the highest dose (0.6 mg/kg), significant findings included 86% maternal mortality, a decrease in maternal body weight gains and an increase in the incidence of fetal stunting, although only a tendency in decreased fetal body weights was observed. These results suggest that maternal mortality was the most important factor in predicting the induction of maternal and fetal effects and, therefore, a "no observable effect level" in maternal animals and their fetuses would be between 1.5 and 2.0 mg/kg in rats and less than 0.07 mg/kg in rabbits.

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# APPENDIX A

# ANALYTICAL METHODS

# ASSAYS FOR SULFUR MUSTARD AND LEWISITE IN SESAME OIL

D.R.Kalkwarf

Methods were developed for the assay of either sulfur mustard or lewisite in sesame oil by gas chromatography, using a capillary column and flame-ionization detection. The method for sulfur mustard can be used at concentrations as low as 0.01 mg/mL, whereas the method for lewisite can be used at concentrations as low as 0.01 mg/mL.

The gas-chromatographic assay of sulfur mustard in sesame oil 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 and the following temperature program was used:
80° C, 5min; (5°/min) to 140° C; (20°/min) to 300°, 300° C, 20min

The procedure consisted of diluting 0.500mL of sample with 0.500mL of 18.7ng/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 7672A automatic sample changer were used with a J & W DB-5 column.

Using this method, samples were analyzed immediately after preparation and after storage in a refigerator for time periods of up to 31 days. Less than 10% of the sulfur mustard was found to have decomposed during those time periods.

The gas-chromatographic assay of lewisite was further complicated by its low decomposition temperature, 190° C. The problem was to find conditions that would allow the sesame oil to migrate through the column without decomposing the lewisite on the way. No direct way was found; however, it was found that lewisite could be derivatized in sesame oil by addition of 2-mercaptoethanol (2-ME) at room temperature. This reaction can be written:

$$ClCH{=}CHAsCl_2 + 2\,HSCH_2CH_2OH \dashrightarrow ClCH{=}CHAs(SCH_2CH_2OH)_2 + 2HCl$$

The product of this reaction proved to be so stable that the same column-injection temperature and temperature program as in the sulfur-mustard assay could be used. The same chromatographic instumentation and column were also used.

The procedure consisted of diluting 0.500mL of sample with 0.500mL of a solution containing 25ng of naphthalene and 5720 ng of 2-ME/ uL methanol contained in a 1.5-mL automatic sampler vial with a Teflon-lined crimped-top cap.The naphthalene was used as an internal standard for the assay.

Using this method, samples were analyzed immediately after preparation and after storage in a refigerator for 8 days. Less than 10% of the lewisite was found to have decomposed during that time period.

# DEVELOPMENT OF A GAS-CHROMATOGRAPHIC ASSAY FOR LEWISITE

**Purpose:** To establish conditions for assaying sesame-oil solutions for Lewisite (L) with the Hewlett-Packard 5840A gas chromatograph.

## Procedure

On 860116, L Sasser prepared 3mL of solution labeled CSM-2-85-HEX-1 containing 0.945mg/mL of L in hexane. This solution was stored in the refrigerator of the CSM facility. On 860120, I received 0.5mL of this solution from M. Karagianes and treated it as follows. The 0.5mL was pipeted into a 1.5-mL sample-changer vial together with 0.5mL of 37.4ng/uL 2,4-dichlorotoluene (DCT) in isooctane. The vial was labeled CSM-2-85-HEX-1A, and it contained 472ng/uL of L and 18.7ng/uL of DCT as an internal standard. This solution was used to prepare 3 further dilutions as follows.

A 0.5-mL portion was diluted to 1.00mL with 37.4ng/uL DCT and labeled CSM-2-85-HEX-1B. It contained 236ng/uL of L and 0.5(37.4) +0.5(18.7)= 28.0ng/uL of DCT.

A 250-uL portion of HEX-1A was diluted to 1.00mL with 0.5mL of 37.4ng/uL DCT and 250uL of hexane; and this solution was labeled CSM-2-85-HEX-1C. It contained 118ng/uL of L and 0.5(37.4)+0.25(18.7)= 23.4ng/uL of DCT.

A 0.125-mL portion of HEX-1A was diluted to 1.00mL with 0.5mL of 37.4ng/uL DCT and 0.375mL of hexane; and this solution was labeled CSM-2-85-HEX-1D. It contained 59.0ng/uL of L and 0.5(37.4) + 0,125(18.75)=21.1ng/uL of DCT.

The rest of solution CSM-2-85-HEX-1A was decontaminated by treatment with 2.5M NaOH.

The 3 dilutions of HEX-1A were analyzed with the Hewlett-Packard 5840A gas chromatograph using a 28-m DB-1 capillary column with flame ionization detector and the following temperature program: 100°C,1 min (5°/min) 200°C. A Hewlett-Packard 7672A sample changer was also used.

## Results

The assay results are listed below. They are based on gas-chromatographic records (GCR's) filed in the Mustard Assay Book stored in Room 208, 329 Building. The chromatographic peak with a retention time of 8.6 min was attributed to L, whereas the peak with a retention time of 10.6 min was attributed to DCT. These results are summarized by the values for the relative sensitivity of L with respect to DCT, $S = (A_L/A_{DCT})([DCT]/[L])$, where A designates peak areas and [] designates concentrations. This sensitivity appeared to decrease with decreasing concentration.

| Sample | GCR | [L] (ng/uL) | [DCT] (ng/uL) | $A_L$ (counts) | $A_{DCT}$ (counts) | S |
|---|---|---|---|---|---|---|
| HEX-1D | HP-F134 | 59.0 | 21.1 | 174 | 4433 | 0.014 |
| HEX-1C | HP-F135 | 118 | 23.4 | 1513 | 4917 | 0.061 |
| HEX-1B | HP-F136 | 236 | 28.0 | 5195 | 5628 | 0.110 |

Date: January 8, 1987

To: Pat Hackett

From: Don Kalkwarf

Information About the Identity and Purity of the Lewisite Used in Your Study.

I understand that lewisite from lot CSM-2-85 was used in all of your experiments. I measured the ultraviolet absorption spectrum of that material dissolved in isooctane and concluded that it contained lewisite and no other uv-absorbing compound. The spectrum showed a strong absorption line at 215 nm with no other pronounced spectral features, as has been reported in the literature[1,2]. The absorptivity of the material at 215 nm, which probably measures the number of vinyl groups in solution, was 0.036 $ppm^{-1}cm^{-1}$ This value is close to that found by Mohler and Sorge, 0.048 $ppm^{-1}cm^{-1}$[2] but less than that reported by Rewick et al.[1]. Since the latter authors were unable to assay their lewisite by gas chromatography, they did not know what or how many components were in their sample. The report by Mohler was not available at PNL but has been requested from another library. Common impurities in lewisite that would be expected to absorb uv-light are lewisite oxide and the various cis-trans isomers of bis(2-chlorovinyl)chloroarsine and tris(2-chlorovinyl)arsine[3]. Since I did not have authentic samples of any of these compounds, I can only conclude is that CSM-2-85 contained the same substance or mixture of substances as the material examined by Mohler and Sorge.

I did analyze solutions of CSM-2-85 by gas chromatography following derivatization of the lewisite with 2-mercaptoethanol. The only peaks observed were due to the thiolated lewisite, the excess derivatizing agent and an internal standard compound, 1-chloronaphthalene, which we added to correct for variations in the volumes injected on the chromatographic column. It is doubtful, however, that lewisite oxide or the other common impurities would be observed since they would not be expected to react with the derivatizing agent, and probably share the instability at high temperatures shown by lewisite itself. The injection port of the gas chromatograph was run at 200°C , which is 10 degrees higher that the decomposition temperature of lewisite. I concluded that CSM-2-85 contains only lewisite with possibly some heat-labile lewisite derivatives.

(1) Rewick, R.T., M.L.Schumacher and D.L.Haynes. 1986. "The UV Absorption Spectra of Chemical Agents and Simulants". Appl.Spectroscopy 40:152-156.

(2) Mohler, H. and J.Sorge. 1939. "Chemical Warfare Materials. XII. Light Absorption by Nose and Throat, Lung and Skin Poisons In Ultraviolet of Short Wave Length". Helv.Chim Acta 22:235-239.

(3) D.H.Rosenblatt, T.A.Miller, J.C.Dacre, T.Muril and D.R.Cogley. 1975. Problem Definition Studies on Potential Environmental Pollutants Physical/Chemical Toxicological and Biological Properties of 16 Substances, AD A030428, Technical Report 7509, US Army Medical and Bioengineering Research and Development Laboratory, Fort Dietrick, Frederick, MD.

# APPENDIX B

# NECROPSY OBSERVATIONS

TABLE B1. OBSERVATIONS AT NECROPSY OF RATS IN THE DOSE RANGE STUDY OF LEWISITE[a].

| LEWISITE DOSE | ANIMAL NUMBER | DAY OF NECROPSY[b] | NUMBER OF DOSES | OBSERVATIONS |
|---|---|---|---|---|
| Vehicle | 331 | 20 | 10 | Pregnant; perforated lung[c] |
| | 356 | 20 | 10 | Pregnant; no CL observed; one implant detected by uterine stain[c]. |
| 0.5 mg/kg | 348 | 20 | 10 | Pregnant; bilateral hydronephrosis |
| 1.0 mg/kg | 299 | 7 | 1 | Not pregnant; euthanized for dyspnea; backflow noted during dosing; GIT filled with gas and yellow fluid; PCOD[d] – lung dose |
| 2.0 mg/kg | 339 | 9 | 4 | Pregnant; euthanized for dyspnea; pericardium filled with blood; GIT filled with gas; PCOD – dosing trauma |
| | 347 | 12 | 6 | Pregnant; died; GIT filled with gas, yellow and bloody fluid; gastric inflammation and edema; PCOD – lewisite toxicity |
| | 352 | 16 | 10 | Pregnant; died; perforated esophagus; PCOD – dosing trauma |
| 2.5 mg/kg | 317 | 7 | 2 | Pregnant; euthanized for dyspnea; backflow noted during dosing; stomach filled with gas; inflammation of gastric mucosa and muscularis; PCOD – lung dose |
| | 355 | 14 | 9 | Pregnant; died; GIT filled with gas, yellow and blood fluid; gastric inflammation and focal hemorrhage; PCOD – lewisite toxicity |
| | 329 | 16 | 9 | Pregnant; died; GIT filled with gas, yellow and bloody fluid; PCOD – lewisite toxicity |
| | 296 | 20 | 10 | Pregnant; lung lesion; GIT filled with gas |
| | 272 | 20 | 10 | Not pregnant; small (~1 mm) gastric ulcer; GIT filled with gas |

[a]All other animals in the study were apparently normal at scheduled sacrifice.
[b]Days from detection of copulation; scheduled sacrifice at 20 days
[c]Data omitted from maternal and fetal summaries
[d]Probable cause of death (PCOD)

TABLE B2. OBSERVATIONS AT NECROPSY OF RATS IN THE TERATOLOGY STUDY OF LEWISITE[a]

| DOSE mg/kg | ANIMAL NUMBER | DAY OF NECROPSY[b] | NUMBER OF DOSES | OBSERVATIONS |
|---|---|---|---|---|
| Vehicle | 394 | 10 | 5 | Not pregnant, died; mottled lung, enlarged heart and thymus, small intestines filled with blood; PCOD[c] - dosing trauma |
| 1.0 | 396 | 20 | 10 | Pregnant; cream-colored mottling of median and right liver lobes |
| | 433 | 13 | 8 | Pregnant, euthanized for dyspnea; perforated esophagus, mottled lung, oil in thorax; PCOD - dosing trauma |
| | 501 | 10 | 8 | Pregnant, died; mottled lungs; other organs are apparently normal; PCOD - lung dose |
| 1.5 | 517 | 20 | 10 | Not pregnant; bilateral hydronephrosis |

[a]All other animals in the study were apparently normal at scheduled sacrifice.
[b]Days from detection of copulation; scheduled sacrifice at 20 days
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| Vehicle | 499 | 30 | 14 | Pregnant, AN[b] |
| | 515 | 30 | 14 | Not pregnant, AN |
| | 521 | 10 | 5 | Pregnant, died; oil in thorax; right lung lobe perforated; PCOD[c] - dosing error |
| | 597 | 30 | 14 | Pregnant, left lung consolidated and adhered to pleural lining; lesion in right lung; lung dose probable on 14 dg. |
| | 629 | 30 | 14 | Not pregnant; AN |
| | 694 | 30 | 14 | Not pregnant; AN |
| | 739 | 30 | 14 | Pregnant, AN |
| | 763 | 30 | 14 | Not pregnant, AN |
| 0.5 | 497 | 30 | 14 | Pregnant, gastric mucosa inflammed |
| | 520 | 30 | 14 | Pregnant, excessive amount of peritoneal fluid; gastric mucosa inflamed |
| | 599 | 7 | 1 | Pregnant, died; oil in thorax; right lung lobe hemorrhegic; PCOD - lung dose |
| | 665 | 16 | 11 | Pregnant, died; fluid in thorax, right lung lobe hemorrhagic, necrotic; hemorrhage in pyloric and cardiac regions of stomach; PCOD - lung dose |
| | 738 | 30 | 14 | Pregnant, excessive fluid in pericardial sac; gastric mucosa inflamed |
| | 759 | 10 | 4 | Pregnant, died; oil in thorax; right lung lobe necrotic, hemorrhegic; inflammation of pyloric region of stomech; PCOD - lung dose |
| | 798 | 10 | 5 | Pregnant, died; oil in thorax; left lung lobe hemorrhagic; PCOD - lung dose |
| | 1053 | 14 | 8 | Pregnant, died; oil in thorax; right lung lobes dark; pyloric and cardiac regions of stomech have gray-black areas; PCOD - lung dose |
| 1.0 | 500 | 15 | 9 | Pregnant, died; cardiac, pyloric regions of stomach are hemorrhagic; edema in cardiac region; PCOD - lewisite toxicity |
| | 518 | 22 | 14 | Pregnant; died; gastric mucosa hemorrhagic; duodenum inflamed and necrotic; hemorrhagic foci in cecum PCOD - lewisite toxicity |

[a]Days after insemination; scheduled sacrifice on 30 dg
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 1.0 | 519 | 30 | 14 | Pregnant, excessive amount of fluid in the peritoneum; gastric mucosa inflamed |
| | 525 | 11 | 5 | Pregnant, died; inflammation of pyloric and cardiac regions of stomach; hemorrhagic area in left lung; PCOD - lewisite toxicity |
| | 558 | 15 | 9 | Pregnant, died; pyloric and cardiac regions of stomach are hemorrhagic; PCOD - lewisite toxicity |
| | 559 | 13 | 8 | Pregnant, died; severe hemorrhage of pyloric and cardiac regions of stomach and duodenal mucosa; PCOD - lewisite toxicity |
| | 737 | 9 | 4 | Pregnant, died; oil in thorax; hemorrhagic pyloric and inflammed cardiac regions of stomach; PCOD - lung dose |
| | 740 | 15 | 9 | Pregnant, died; hemorrhage of pyloric and cardiac regions of stomach; PCOD - lewisite toxicity |
| 1.5 | 276 | 10 | 5 | Pregnant; died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 435 | 12 | 7 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 541 | 11 | 5 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 667 | 7 | 2 | Pregnant, died; blood in thorax; cardiac region of stomach is hemorrhagic; PCOD - lung dose |
| | 695 | 11 | 5 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 781 | 6 | 1 | Pregnant, died; oil in thorax; stomach AN[b]; PCOD - lung dose |

[a]Days after insemination; scheduled sacrifice on 30 dg
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 1.5 | 783 | 10 | 4 | Pregnant, died; oil in thorax; hemorrhagic area in left lung; hemorrhage of pyloric and cardiac mucosa of stomach; PCOD[b] - lung dose |
| | 1196 | 8 | 3 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| 2.0 | 113 | 9 | 4 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewite toxicity |
| | 434 | 11 | 5 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 505 | 8 | 3 | Pregnant, died; severe edema, hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 524 | 9 | 4 | Pregnant, died; hemorrhage of pyloric mucosa of stomach; PCOD - lewisite toxicity |
| | 600 | 8 | 3 | Pregnant, died; hemorrhage of gastric mucosa; lung is mottled and hemorrhagic; PCOD - lewisite toxicity |
| | 631 | 11 | 5 | Pregnant, died; hemorrhage of gastric mucosa; PCOD - lewisite toxicity |
| | 663 | 9 | 4 | Pregnant, died; hemorrhage of pyloric mucosa of stomach; PCOD - lewisite toxicity |
| | 732 | 7 | 2 | Pregnant, died; hemorrhage of pyloric mucosa of stomach; lungs slightly mottled; clear fluid in thorax; PCOD - lewisite toxicity |

[a]Days after insemination; scheduled sacrifice on 30 dg
[b]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| Vehicle | 1560 | 30 | 14 | Not pregnant, AN[b] |
| | 1732 | 30 | 14 | Not pregnant, AN |
| | 1739 | 30 | 14 | Aborted prior to sacrifice on 30 dg, gastric hairball, dermal abcess under chin |
| | 1780 | 30 | 14 | Pregnant, AN |
| | 3010 | 30 | 14 | Not pregnant, infected area in fallopian tubes adjacent to ovaries |
| | 3068 | 30 | 14 | Parturition in progress at sacrifice, AN |
| | 3114 | 30 | 14 | Pregnant, AN |
| | 3177 | 30 | 14 | Pregnant, AN |
| | 3188 | 30 | 14 | Not pregnant, AN |
| | 3520 | 30 | 14 | Not pregnant, 4 cm umbilical hernia filled with clear fluid, abdominal wall intact; all other tissues AN |
| | 3760 | 13 | 8 | Pregnant, died, no cause revealed in autopsy, PCOD[c] - stress or trauma |
| | 3761 | 30 | 14 | Aborted on 26 and 27 dg; except foruterus, AN at sacrifice |
| | 3766 | 30 | 14 | Pregnant, AN |
| | 3773 | 30 | 14 | Pregnant, AN |
| | 3774 | 30 | 14 | Pregnant, infection, adhesions of right lung |
| | 5905 | 30 | 14 | Not pregnant, AN |
| | 5912 | 30 | 14 | Pregnant, AN |
| | 5923 | 30 | 14 | Not pregnant, AN |
| | 5952 | 30 | 14 | Pregnant, AN |
| 0.07 | 3009 | 30 | 14 | Not pregnant, AN |
| | 3065 | 30 | 14 | Pregnant, AN |
| | 3116 | 30 | 14 | Pregnant, AN |
| | 3150 | 27 | 14 | Pregnant, died; clear liquid, mucous, traces of blood in stomach; right thorax filled with bloody, serous fluid; left thorax filled with blood and purulent material; left lung has focis of infection and "raised" ulcers; thick, white, purulent material in pericardium; PCOD - lung dose |
| | 3152 | 30 | 14 | Not pregnant, AN |
| | 3176 | 30 | 14 | Premature parturition or abortion; remainder of tissues AN |
| | 3207 | 16 | 10 | Not pregnant; died; inflamed mucosa in cardiac region of stomach; inflamed vasculature in duodenum and jejunum; right lung lobes dark red and severely consolidated; left lung AN; PCOD - lung dose |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 0.07 | 3208 | 19 | 13 | Pregnant; died; inflamed gastric mucosa; inflamed jejunal vasculature; liver mottled and yellow; thorax contains blood; right lung lobe red and consolidated; PCOD[c] - lung dose |
| | 3538 | 18 | 12 | Not pregnant; died; cardiac region of stomach was slightly hemorrhagic; thorax is AN; slight consolidation in upper left lung; PCOD - lewisite toxicity |
| | 3627 | 30 | 14 | Pregnant, AN[b] |
| | 3765 | 28 | 14 | Pregnant; died; stomach AN; lungs AN, slight inflammation of thymus; abortion in progress, one fetus in vagina; PCOD - abortion or toxemia of pregnancy |
| | 5709 | 30 | 14 | Not pregnant; AN |
| | 5759 | 17 | 11 | Pregnant; euthanized for dyspnea; mucosa of cardiac region of stomach apparently sloughed, wall is thin and fragile; left lung lobes dark red; area of consolidation at the caudal end of the right diaphragmatic lung lobe; thorax contains blood; PCOD - lewisite toxicity |
| | 5760 | 17 | 11 | Not pregnant; euthanized for dyspnea; gastrointestinal tract AN, traces of oil and food in stomach; thorax filled with blood; lungs inflammed; membranes adhered to lung, pericardium and wall of thorax. PCOD - dosing error or trauma. |
| | 5903 | 30 | 14 | Pregnant; bilateral ocular discharge; pale area surrounded by hemorrhagic area in left lung lobes. |
| | 5920 | 30 | 14 | Not pregnant; multiple firm nodules containing purulent material within lung lobes |
| | 5921 | 30 | 14 | Pregnant; AN |
| | 5927 | 30 | 14 | Pregnant; thorax contains bloody, purulent fluid; right lung lobes covered with purulent material, contain firm nodules filled with purulent material |
| 0.2 | 1654 | 22 | 14 | Pregnant; died; gastric mucosa edematous, slightly hemorrhagic and necrotic; right lung contains firm nodules, thoracic cavity AN; traces of blood in vagina; left uterine horn inflamed, all fetuses resorbing in right horn; PCOD - toxicity/septicemia |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 0.2 | 3105 | 8 | 3 | Pregnant; died; right thoracic cavity filled with blood; right lungs hemorrhegic and edhered to thoracic wall; all other organs AN; PCOD - lung dose |
| | 3185 | 30 | 14 | Pregnant; AN |
| | 3202 | 30 | 14 | Pregnant; AN |
| | 3245 | 14 | 9 | Pregnant; died; thoracic cavity AN; slight consolidation in left lungs; gastrointestinal tract AN; food in stomach; PCOD - stress/trauma |
| | 3522 | 10 | 5 | Pregnant; died; gastric mucosa inflamed; small, hemorrhagic foci near pylorus; thoracic cavity and lungs AN; PCOD - stress/trauma |
| | 3771 | 30 | 14 | Not pregnant; nodule containing purulent material on distal end of right lung lobe; remainder of tissues AN |
| | 3772 | 14 | 8 | Pregnant; died; post-dose dyspnea on previous day; puncture in lower right lung lobe; right lung adhered to pleura; pericardium edematous; perforated ulcer along lesser curvature of stomach; petechial hemorrhage along greater curvature; PCOD - dosing trauma |
| | 5908 | 20 | 14 | Not pregnant; died; thoracic cavity AN; upper right lung lobes red and consolidated; gastric mucosa is hemorrhegic, stomech contains black fluid; multiple purulent areas in cecal pouch, edematous lining; no formed feces in colon; PCOD - lewisite toxicity/septicemia |
| | 5911 | 15 | 9 | Pregnant; died; thoracic cavity filled with blood; punture in lower right lung lobe; gastric mucosa inflammed; small petechial hemorrhages in cardiec region of stomach; PCOD - dosing trauma |
| | 5913 | 30 | 14 | Not pregnant; AN |
| | 5930 | 30 | 14 | Pregnant; AN |
| | 5936 | 20 | 14 | Pregnant; died; thoracic cavity AN; right lung lobes consolidated; necrosis, hemorrhege in pyloric and cardiac regions of stomach; stomach filled with dark liquid and oil; no formed feces in colon; PCOD - lewisite toxicity |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 0.2 | 5938 | 18 | 12 | Not pregnant; died; thoracic cavity AN[b]; some consolidation in right and left cephalic lung lobes; severe hemorrhage in ~40% of the gastric mucosa; stomach contains small amount of fluid and oil; formed feces in colon; PCOD[c] – lewisite toxicity |
| | 5939 | 22 | 14 | Pregnant; died; veginal bleeding noted on 19 dg during dosing; all fetuses resorbed/aborted; gastric mucosa is hemorrhagic; stomach filled with fluid, hair, traces of blood; small intestine is empty; cecum is inflamed; colon contains a few fecal pellets; right thorax filled with dark liquid; right lung lobes red, consolidated, coated with dark-colored material; PCOD – lewisite toxicity |
| | 5940 | 30 | 14 | Pregnant; AN |
| | 5951 | 20 | 14 | Not pregnant; died; some hemorrhage of gastric mucosa in pyloric and cardiac regions; stomach contains pale green liquid and oil; thoracic cavity AN; right lung is hemorrhegic, consolidated and covered with brown material; PCOD – lewisite toxicity/septicemia |
| | 5955 | 30 | 14 | Pregnant; AN |
| 0.6 | 1486 | 19 | 13 | Pregnant; died; thoracic cavity AN; upper right lung lobe consolidated; pleural adhesion on right lung; hemorrhagic areas in cardiac and pyloric regions of stomach; stomach filled with clear, green liquid and oil; PCOD – lewisite toxicity |
| | 1864 | 8 | 3 | Pregnant; died; punture in caudal tip of right diaphragmatic lung lobe; oil in thoracic cavity; gastric mucosa slightly inflammed; PCOD – dosing trauma |
| | 3006 | 15 | 10 | Pregnant; died; thoracic cavity and lungs AN; small hemorrhagic foci in pyloric region of stomach; intestines filled with blood; no formed feces in colon; PCOD – lewisite toxicity |
| | 3007 | 13 | 7 | Pregnant; died; thoracic cavity and lungs AN except for slight consolidation in lower right lung lobe; ~90% of the gastric mucosa in the pyloric and cardiac regions is hemorrhagic; PCOD – lewisite toxicity |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 0.6 | 3070 | 9 | 3 | Pregnant; died; thoracic cavity and lungs AN[b]; gastric mucosa edematous and hemorrhagic with 0.75 cm perforated ulcer; food in stomach; PCOD[c] - lewisite toxicity |
| | 3184 | 20 | 14 | Pregnant; died; thoracic cavity and lungs AN; necrosis, hemorrhage in pyloric region of stomach; cecum filled with necrotic tissue, vasculature is inflamed; PCOD - lewisite toxicity |
| | 3210 | 19 | 13 | Not pregnant; died; thoracic cavity and lungs AN; entire gastric mucose is severely hemorrhagic with areas of blisters/pustules; inflamed cecal vasculature; PCOD - lewisite toxicity |
| | 3250 | 16 | 10 | Not pregnant; died; thoracic cavity, trachea contain blood, thoracic membranes are edematous; right lung lobes are slightly red along edges; left lung AN; no evidence of punture in lungs or trachea; 0.5 cm, black ulceration in stomach wall; PCOD - lewisite toxicity |
| | 3759 | 16 | 10 | Pregnant; died; thoracic cavity, trachea filled with blood; inflammation, edema, adhesions of pericardial membranes; right lung lobes red and adhered to edematous pleural membranes; no evidence of puncture in thorax, lungs or trachea; cardiac region of stomach extremely thin-walled with 2 perforations, multiple hemorrhagic areas; inflamed duodenum; PCOD - lewisite toxicity |
| | 3763 | 30 | 14 | Pregnant; AN |
| | 3767 | 30 | 14 | Not pregnant; AN |
| | 3768 | 30 | 14 | Pregnant; AN |
| | 5761 | 13 | 7 | Pregnant; died; thoracic cavity, lungs AN; all stomach lining hemorrhagic, necrotic; inflamed, hemorrhagic area in duodenum adjacent to pyloric sphincter; PCOD - lewisite toxicity |
| | 5902 | 30 | 14 | Pregnant; AN |
| | 5906 | 11 | 10 | Pregnant; died;blood around nose and mouth; right lung lobes hemorrhagic; gastric mucosa hemorrhagic; PCOD - lung dose |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | NUMBER OF DOSES | OBSERVATION |
|---|---|---|---|---|
| 0.6 | 5914 | 24 | 14 | Pregnant; died; blood stains around vagina, aborted tissue observed at death; thoracic cavity, lungs AN[b]; hemorrhagic areas in stomach and cecum; PCOD[c] - lewisite toxicity/septicemia |
| | 5915 | 18 | 13 | Pregnant; died; thoracic cavity, lungs AN; severe necrosis and hemorrhage in pyloric and cardiac regions of stomach; PCOD - lewisite toxicity |
| | 5924 | 8 | 3 | Pregnant; died; thoracic cavity filled with blood; right lung lobes hemorrhagic, small puncture in tip of diaphragmatic lobe; 2 cm hemorrhagic area in gastric mucosa; PCOD - dosing trauma |
| | 5937 | 30 | 14 | Not pregnant; hemorrhagic area with purulent center in left lung lobe |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Apparently normal (AN)
[c]Probable cause of death (PCOD)

# APPENDIX C

# REPRODUCTIVE MEASURES

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLAN-TATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES[b] DEAD | FETUSES[b] LIVE |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 282 | 20 | 19 | 14 | 1 | 0 | 0 | 0 | 13 |
| | 286 | 20 | 17 | 17 | 0 | 0 | 0 | 0 | 17 |
| | 297 | 20 | 0 | - | - | - | - | - | - |
| | 301 | 20 | 0 | - | - | - | - | - | - |
| | 302 | 20 | 17 | 10 | 2 | 0 | 0 | 0 | 8 |
| | 321 | 20 | 18 | 17 | 0 | 0 | 0 | 0 | 17 |
| | 331 | 20 | 22 | 17 | 0 | 0 | 0 | 0 | 17[c] |
| | 346 | 20 | 20 | 18 | 1 | 0 | 0 | 0 | 17 |
| | 356[c] | 20 | 0 | 1[d] | - | - | - | - | - |
| | 358 | 20 | 16 | 16 | 1 | 0 | 0 | 0 | 15 |
| 0.5 mg/kg | 269 | 20 | 17 | 17 | 3 | 0 | 0 | 0 | 14 |
| | 275 | 20 | 17 | 14 | 10 | 0 | 0 | 0 | 4 |
| | 278 | 20 | 0 | - | - | - | - | - | - |
| | 284 | 20 | 16 | 16 | 2 | 0 | 0 | 0 | 14 |
| | 307 | 20 | 18 | 14 | 2 | 0 | 0 | 0 | 12 |
| | 318 | 20 | 17 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 323 | 20 | 8 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 337 | 20 | 15 | 15 | 1 | 0 | 0 | 0 | 14 |
| | 348 | 20 | 18 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 351 | 20 | 15 | 13 | 0 | 0 | 0 | 0 | 13 |
| 1.0 mg/kg | 298 | 20 | 21 | 11 | 0 | 0 | 0 | 0 | 11 |
| | 299 | 7 | 17 | 0 | - | - | - | - | - |
| | 304 | 20 | 20 | 13 | 0 | 0 | 0 | 0 | 13 |
| | 305 | 20 | 0 | - | - | - | - | - | - |
| | 309 | 20 | 16 | 16 | 1 | 0 | 0 | 0 | 15 |
| | 310 | 20 | 16 | 13 | 1 | 0 | 0 | 0 | 12 |
| | 326 | 20 | 22 | 20 | 3 | 0 | 0 | 0 | 17 |
| | 334 | 20 | 18 | 15 | 0 | 0 | 0 | 0 | 15 |
| | 335 | 20 | 21 | 19 | 1 | 1 | 0 | 0 | 17 |
| | 344 | 20 | 21 | 18 | 0 | 0 | 0 | 0 | 18 |
| 2.0 mg/kg | 273 | 20 | 10 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 287 | 20 | 16 | 15 | 0 | 14 | 1 | 0 | 0 |
| | 288 | 20 | 0 | - | - | - | - | - | - |
| | 295 | 20 | 22 | 8 | 0 | 5 | 0 | 0 | 3 |
| | 312 | 20 | 18 | 15 | 2 | 0 | 0 | 0 | 13 |
| | 339 | 9 | 17 | 16 | - | - | - | - | 16 |
| | 341 | 20 | 24 | 16 | 3 | 2 | 0 | 0 | 11 |
| | 347 | 12 | 15 | 15 | 15 | - | - | - | 0 |
| | 350 | 20 | 16 | 15 | 0 | 0 | 0 | 0 | 15 |
| | 352 | 16 | 21 | 16 | 1 | 0 | - | - | 15 |

[a]Days after detection of copulation
[b]Fetus or embryo, depending on the stage of development
[c]Perforated lung observed at necropsy; data were omitted
[d]Detected by uterine staining

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLANTATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES[b] DEAD | FETUSES[b] LIVE |
|---|---|---|---|---|---|---|---|---|---|
| 2.5 mg/kg | 272 | 20 | 0 | - | - | - | - | - | - |
| | 274 | 20 | 6 | 2 | 2 | 0 | 0 | 0 | 0 |
| | 296 | 20 | 17 | 2 | 0 | 0 | 0 | 0 | 2 |
| | 308 | 20 | 16 | 15 | 2 | 1 | 0 | 0 | 12 |
| | 314 | 20 | 0 | - | - | - | - | - | - |
| | 315 | 20 | 12 | 0 | - | - | - | - | - |
| | 317 | 7 | 15 | 15 | - | - | - | - | 15 |
| | 329 | 16 | 29 | 16 | 0 | 0 | - | - | 16 |
| | 345 | 20 | 15 | 15 | 1 | 0 | 0 | 0 | 14 |
| | 355 | 14 | 18 | 18 | 1 | - | - | - | 17 |
| | 360 | 20 | 15 | 14 | 0 | 0 | 0 | 0 | 14 |

[a]Days after detection of copulation

[b]Fetus or embryo, depending on the stage of development

TABLE C2. REPRODUCTIVE STATUS OF RATS IN THE TERATOLOGY STUDY OF LEWISITE.

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLAN-TATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES DEAD | FETUSES LIVE |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 365 | 20 | 6 | 5 | 2 | 0 | 0 | 0 | 3 |
| | 369 | 20 | 20 | 18 | 0 | 0 | 0 | 0 | 18 |
| | 375 | 20 | 16 | 16 | 3 | 0 | 0 | 0 | 13 |
| | 384 | 20 | 20 | 17 | 3 | 0 | 0 | 0 | 14 |
| | 394 | 10 | 0 | - | - | - | - | - | - |
| | 406 | 20 | 0 | - | - | - | - | - | - |
| | 408 | 20 | 21 | 14 | 1 | 0 | 0 | 0 | 13 |
| | 418 | 20 | 20 | 19 | 1 | 0 | 0 | 0 | 18 |
| | 422 | 20 | 10 | 8 | 0 | 0 | 0 | 0 | 8 |
| | 432 | 20 | 0 | - | - | - | - | - | - |
| | 435 | 20 | 8 | 8 | 0 | 0 | 0 | 0 | 8 |
| | 445 | 20 | 18 | 13 | 3 | 0 | 0 | 0 | 10 |
| | 448 | 20 | 9 | 8 | 0 | 0 | 0 | 0 | 8 |
| | 453 | 20 | 22 | 15 | 2 | 3 | 0 | 0 | 10 |
| | 472 | 20 | 19 | 2 | 0 | 0 | 0 | 0 | 2 |
| | 502 | 20 | 17 | 16 | 0 | 1 | 0 | 0 | 15 |
| | 515 | 20 | 18 | 18 | 0 | 1 | 0 | 0 | 17 |
| | 519 | 20 | 0 | - | - | - | - | - | - |
| | 533 | 20 | 0 | - | - | - | - | - | - |
| | 538 | 20 | 16 | 15 | 0 | 0 | 0 | 0 | 15 |
| | 539 | 20 | 16 | 16 | 2 | 0 | 0 | 0 | 14 |
| | 544 | 20 | 15 | 15 | 0 | 0 | 0 | 0 | 15 |
| | 551 | 20 | 18 | 18 | 0 | 0 | 0 | 0 | 18 |
| | 559 | 20 | 18 | 17 | 1 | 0 | 0 | 0 | 16 |
| | 566 | 20 | 0 | - | - | - | - | - | - |
| 0.5 | 362 | 20 | 20 | 19 | 1 | 1 | 0 | 0 | 17 |
| | 372 | 20 | 20 | 18 | 0 | 1 | 0 | 0 | 17 |
| | 376 | 20 | 12 | 6 | 0 | 0 | 0 | 0 | 6 |
| | 377 | 20 | 12 | 4 | 2 | 0 | 0 | 0 | 2 |
| | 383 | 20 | 16 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 387 | 20 | 0 | - | - | - | - | - | - |
| | 397 | 20 | 0 | - | - | - | - | - | - |
| | 407 | 20 | 18 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 413 | 20 | 14 | 16 | 2 | 0 | 0 | 0 | 14 |
| | 419 | 20 | 17 | 17 | 0 | 0 | 0 | 0 | 17 |
| | 427 | 20 | 0 | - | - | - | - | - | - |
| | 440 | 20 | 21 | 19 | 0 | 0 | 0 | 0 | 19 |
| | 449 | 20 | 18 | 18 | 0 | 0 | 0 | 0 | 18 |
| | 467 | 20 | 20 | 17 | 2 | 0 | 0 | 0 | 15 |
| | 479 | 20 | 16 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 493 | 20 | 17 | 17 | 0 | 0 | 0 | 0 | 17 |
| | 511 | 20 | 19 | 18 | 2 | 0 | 0 | 0 | 16 |

[a]Days after detection of copulation; scheduled sacrifice on 20 days

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLANTATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES DEAD | FETUSES LIVE |
|---|---|---|---|---|---|---|---|---|---|
| 0.5 | 518 | 20 | 0 | - | - | - | - | - | - |
| | 534 | 20 | 0 | - | - | - | - | - | - |
| | 536 | 20 | 17 | 12 | 2 | 0 | 0 | 0 | 10 |
| | 543 | 20 | 17 | 17 | 0 | 1 | 0 | 0 | 16 |
| | 547 | 20 | 16 | 2 | 0 | 0 | 0 | 0 | 2 |
| | 561 | 20 | 20 | 19 | 0 | 1 | 0 | 0 | 18 |
| | 565 | 20 | 17 | 13 | 4 | 0 | 0 | 0 | 9 |
| | 568 | 20 | 14 | 7 | 2 | 0 | 0 | 0 | 5 |
| 1.0 | 364 | 20 | 16 | 10 | 0 | 0 | 0 | 0 | 10 |
| | 373 | 20 | 19 | 17 | 0 | 0 | 0 | 0 | 17 |
| | 385 | 20 | 0 | - | - | - | - | - | - |
| | 396 | 20 | 7 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 410 | 20 | 0 | - | - | - | - | - | - |
| | 412 | 20 | 12 | 13 | 0 | 1 | 0 | 0 | 12 |
| | 416 | 20 | 19 | 19 | 0 | 0 | 0 | 0 | 19 |
| | 421 | 20 | 19 | 17 | 1 | 0 | 0 | 0 | 16 |
| | 424 | 20 | 19 | 16 | 1 | 0 | 0 | 0 | 15 |
| | 426 | 20 | 16 | 8 | 2 | 0 | 0 | 0 | 6 |
| | 428 | 20 | 16 | 14 | 0 | 1 | 0 | 0 | 13 |
| | 433 | 13 | 16 | 15 | 0 | - | - | - | 15[b] |
| | 470 | 20 | 0 | - | - | - | - | - | - |
| | 475 | 20 | 16 | 13 | 0 | 0 | 0 | 0 | 13 |
| | 476 | 20 | 0 | - | - | - | - | - | - |
| | 478 | 20 | 19 | 19 | 1 | 0 | 0 | 0 | 18 |
| | 481 | 20 | 17 | 18 | 0 | 0 | 0 | 0 | 18 |
| | 484 | 20 | 0 | - | - | - | - | - | - |
| | 501 | 10 | 16 | 16 | - | - | - | - | 16[b] |
| | 510 | 20 | 0 | - | - | - | - | - | - |
| | 532 | 20 | 16 | 3 | 2 | 0 | 0 | 0 | 1 |
| | 540 | 20 | 0 | - | - | - | - | - | - |
| | 546 | 20 | 16 | 16 | 2 | 0 | 0 | 0 | 14 |
| | 550 | 20 | 0 | - | - | - | - | - | - |
| | 563 | 20 | 17 | 16 | 0 | 0 | 0 | 0 | 16 |
| 1.5 | 366 | 20 | 19 | 17 | 0 | 0 | 0 | 0 | 17 |
| | 367 | 20 | 15 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 368 | 20 | 17 | 17 | 2 | 0 | 0 | 0 | 15 |
| | 370 | 20 | 0 | - | - | - | - | - | - |
| | 381 | 20 | 6 | 2 | 1 | 0 | 0 | 0 | 1 |
| | 390 | 20 | 12 | 13 | 1 | 0 | 0 | 0 | 12 |
| | 423 | 20 | 0 | - | - | - | - | - | - |
| | 429 | 20 | 15 | 15 | 0 | 0 | 1 | 0 | 14 |

[a]Days after the detection of copulation; scheduled sacrifice at 20 days
[b]Live embryos at this stage of development

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLAN-TATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES DEAD | FETUSES LIVE |
|---|---|---|---|---|---|---|---|---|---|
| 1.5 | 436 | 20 | 16 | 13 | 0 | 0 | 0 | 0 | 13 |
| | 437 | 20 | 22 | 21 | 2 | 0 | 0 | 0 | 19 |
| | 452 | 20 | 0 | - | - | - | - | - | - |
| | 455 | 20 | 18 | 15 | 1 | 0 | 0 | 0 | 14 |
| | 477 | 20 | 16 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 483 | 20 | 19 | 16 | 0 | 0 | 0 | 0 | 16 |
| | 487 | 20 | 0 | - | - | - | - | - | - |
| | 489 | 20 | 21 | 19 | 3 | 1 | 0 | 0 | 15 |
| | 496 | 20 | 16 | 12 | 2 | 0 | 0 | 0 | 10 |
| | 499 | 20 | 0 | - | - | - | - | - | - |
| | 513 | 20 | 19 | 18 | 0 | 0 | 0 | 0 | 18 |
| | 514 | 20 | 0 | - | - | - | - | - | - |
| | 517 | 20 | 0 | - | - | - | - | - | - |
| | 524 | 20 | 20 | 18 | 1 | 0 | 0 | 0 | 17 |
| | 530 | 20 | 17 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 545 | 20 | 19 | 17 | 3 | 0 | 0 | 0 | 14 |
| | 567 | 20 | 19 | 19 | 0 | 0 | 0 | 0 | 19 |

[a]Days after the detection of copulation; scheduled sacrifice on 20 days

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLANTATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES[b] DEAD | FETUSES[b] LIVE |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 499 | 30 | 9 | 8 | 0 | 0 | 1 | 0 | 7 |
| | 515 | 30 | 2 | 0 | - | - | - | - | - |
| | 521 | 10 | 8 | 6 | 0 | 0 | 0 | 0 | 6 |
| | 597 | 30 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| | 629 | 30 | 1 | 0 | - | - | - | - | - |
| | 694 | 30 | 1 | 0 | - | - | - | - | - |
| | 739 | 30 | 11 | 11 | 0 | 0 | 0 | 0 | 11 |
| | 763 | 30 | 0 | - | - | - | - | - | - |
| 0.5 | 497 | 30 | 12 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 520 | 30 | 17 | 16 | 0 | 0 | 2 | 1 | 13 |
| | 599 | 7 | 14 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 665 | 16 | 8 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 738 | 30 | 9 | 9 | 0 | 0 | 1 | 0 | 8 |
| | 759 | 10 | 12 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 798 | 10 | 16 | 9 | 0 | 0 | 0 | 0 | 9 |
| | 1053 | 14 | 10 | 10 | 0 | 0 | 0 | 0 | 10 |
| 1.0 | 500 | 15 | 13 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 518 | 22 | 16 | 13 | 0 | 0 | 0 | 0 | -[c] |
| | 519 | 30 | 12 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 525 | 11 | 8 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 558 | 15 | 12 | 11 | 0 | 0 | 0 | 0 | 11 |
| | 559 | 13 | 10 | 9 | 0 | 0 | 0 | 0 | 9 |
| | 737 | 9 | 11 | 9 | 0 | 0 | 0 | 0 | 9 |
| | 740 | 15 | 13 | 9 | 0 | 0 | 0 | 0 | 9 |
| 1.5 | 276 | 10 | 15 | 8 | 8 | 0 | 0 | 0 | 0 |
| | 435 | 12 | 12 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 541 | 11 | 9 | 8 | 0 | 0 | 0 | 0 | 8 |
| | 667 | 7 | 10 | 6 | 0 | 0 | 0 | 0 | 6 |
| | 695 | 11 | 13 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 781 | 6 | 13 | 12 | 0 | 0 | 0 | 0 | 12 |
| | 783 | 10 | 11 | 11 | 0 | 0 | 0 | 0 | 11 |
| | 1196 | 8 | 10 | 10 | 0 | 0 | 0 | 0 | 10 |

[a]Days after insemination; scheduled sacrifice on 30 dg
[b]Fetuses or embryos, depending on development time
[c]At necropsy, the conceptus was autolyzed

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLAN-TATIONS | RESORPTIONS EARLY | RESORPTIONS MID | RESORPTIONS LATE | FETUSES[b] DEAD | FETUSES[b] LIVE |
|---|---|---|---|---|---|---|---|---|---|
| 2.0 | 113 | 9 | 13 | 13 | 0 | 0 | 0 | 0 | 13 |
| | 434 | 11 | 13 | 12 | 12 | 0 | 0 | 0 | 0 |
| | 505 | 8 | 13 | 6 | 0 | 0 | 0 | 0 | 6 |
| | 524 | 9 | 13 | 8 | 0 | 0 | 0 | 0 | 8 |
| | 600 | 8 | 12 | 10 | 0 | 0 | 0 | 0 | 10 |
| | 631 | 11 | 9 | 8 | 8 | 0 | 0 | 0 | 0 |
| | 663 | 9 | 8 | 1 | 0 | 0 | 0 | 0 | 1 |
| | 732 | 7 | 18 | 9 | 0 | 0 | 0 | 0 | 9 |

[a]Days after insemination; scheduled sacrifice on 30 dg
[b]Fetuses or embryos, depending on development time

TABLE C4. REPRODUCTIVE STATUS OF RABBITS IN THE TERATOLOGY STUDY OF LEWISITE

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLAN-TATIONS | RESORPTIONS EARLY | RESORPTIONS LATE | FETUSES[b] DEAD | FETUSES[b] LIVE |
|---|---|---|---|---|---|---|---|---|
| Vehicle | 1560 | 30 | 0 | - | - | - | - | - |
| | 1732 | 30 | 0 | - | - | - | - | - |
| | 1739[c] | 30 | 5 | 7 | 0 | 0 | 7 | 0 |
| | 1780 | 30 | 13 | 6 | 0 | 1 | 0 | 5 |
| | 3010 | 30 | 0 | - | - | - | - | - |
| | 3068[d] | 30 | 13 | 11 | 0 | 0 | 0 | 11 |
| | 3114 | 30 | 3 | 2 | 0 | 0 | 0 | 2 |
| | 3177 | 30 | 12 | 11 | 1 | 0 | 0 | 10 |
| | 3188 | 30 | 0 | - | - | - | - | - |
| | 3520 | 30 | 0 | - | - | - | - | - |
| | 3760 | 13 | 14 | 5 | 0 | - | - | 5 |
| | 3761[e] | 30 | 14 | 7 | 3 | 4 | - | 0 |
| | 3766 | 30 | 15 | 15 | 1 | 0 | 0 | 14 |
| | 3773 | 30 | 10 | 5 | 0 | 0 | 0 | 5 |
| | 3774 | 30 | 4 | 4 | 0 | 0 | 0 | 4 |
| | 5905 | 30 | 0 | - | - | - | - | - |
| | 5912 | 30 | 12 | 9 | 4 | 1 | 0 | 4 |
| | 5923 | 30 | 0 | - | - | - | - | - |
| | 5952 | 30 | 14 | 9 | 0 | 1 | 0 | 8 |
| 0.07 | 3009 | 30 | 0 | - | - | - | - | - |
| | 3065 | 30 | 15 | 12 | 0 | 3 | 0 | 9 |
| | 3116 | 30 | 5 | 3 | 0 | 0 | 0 | 3 |
| | 3150 | 27 | 15 | 12 | 0 | 0 | - | 12 |
| | 3152 | 30 | 0 | - | - | - | - | - |
| | 3176[f] | 30 | 5 | 4 | 3 | 0 | 1 | 0 |
| | 3207 | 16 | 9 | 0 | - | - | - | - |
| | 3208 | 19 | 12 | 12 | 0 | 0 | - | 12 |
| | 3538 | 18 | 10 | 0 | - | - | - | - |
| | 3627 | 30 | 13 | 11 | 1 | 0 | 0 | 10 |
| | 3765[g] | 28 | 10 | 9 | 0 | 9 | - | 0 |
| | 5709 | 30 | 0 | - | - | - | - | - |
| | 5759 | 17 | 10 | 9 | 0 | 0 | - | 9 |
| | 5760 | 17 | 6 | 0 | - | - | - | - |
| | 5903 | 30 | 0[h] | 3 | 3 | 0 | 0 | 0 |
| | 5920 | 30 | 0 | - | - | - | - | - |
| | 5921 | 30 | 1 | 1 | 0 | 0 | 0 | 1 |
| | 5927 | 30 | 11 | 11 | 0 | 7 | 4 | 0 |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Fetuses or embryos, depending on development time
[c]Aborted; 1 fetus found; number of nidation sites determined
[d]Parturition in progress; 1 kit in vaginal canal
[e]Aborted on 26 and 27 dg; number of nidation sites determined
[f]Aborted prior to sacrifice
[g]Evidence of abortion after death; 1 kit in vaginal canal
[h]Corpora lutea had regressed; implantation sites visualized with uterine stain

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

| DOSE mg/kg | ANIMAL NUMBER | DAY OF DEATH[a] | CORPORA LUTEA | IMPLAN-TATIONS | RESORPTIONS EARLY | RESORPTIONS LATE | FETUSES[b] DEAD | FETUSES[b] LIVE |
|---|---|---|---|---|---|---|---|---|
| 0.2 | 1654 | 22 | 12 | 5 | 0 | 5 | - | 0 |
| | 3105 | 8 | 11 | 2 | 0 | - | - | 2 |
| | 3185 | 30 | 14 | 14 | 0 | 0 | 0 | 14 |
| | 3202 | 30 | 12 | 13 | 0 | 2 | 0 | 11 |
| | 3245 | 14 | 22 | 2 | 0 | - | - | 2 |
| | 3522 | 10 | 12 | 12 | 0 | - | - | 12 |
| | 3771 | 30 | 0 | - | - | - | - | - |
| | 3772 | 14 | 18 | 14 | 0 | - | - | 14 |
| | 5908 | 20 | 0 | - | - | - | - | - |
| | 5911 | 15 | 9 | 6 | 0 | - | - | 6 |
| | 5913 | 30 | 1 | 0 | - | - | - | - |
| | 5930 | 30 | 2 | 2 | 2 | 0 | 0 | 0 |
| | 5936 | 20 | 14 | 14 | 0 | 5 | - | 9 |
| | 5938 | 18 | 0 | - | - | - | - | - |
| | 5939[c] | 22 | 5 | 5 | 0 | 5 | - | 0 |
| | 5940 | 30 | 8 | 9 | 0 | 0 | 0 | 9 |
| | 5951 | 20 | 0 | - | - | - | - | - |
| | 5955 | 30 | 6 | 5 | 0 | 0 | 0 | 5 |
| 0.6 | 1486 | 19 | 13 | 10 | 0 | 10 | - | 0 |
| | 1864 | 8 | 13 | 6 | 0 | - | - | 6 |
| | 3006 | 15 | 13 | 9 | 1 | - | - | 8 |
| | 3007 | 13 | 16 | 11 | 0 | - | - | 11 |
| | 3070 | 9 | 18 | 4 | 0 | - | - | 4 |
| | 3184 | 20 | 14 | 8 | 0 | 4 | - | 4 |
| | 3210 | 19 | 0 | - | - | - | - | - |
| | 3250 | 16 | 11 | 0 | - | - | - | - |
| | 3759 | 16 | 12 | 9 | 1 | 0 | - | 8 |
| | 3763 | 30 | 7 | 3 | 1 | 1 | 0 | 1 |
| | 3767 | 30 | 0 | - | - | - | - | - |
| | 3768 | 30 | 13 | 8 | 0 | 3 | 0 | 5 |
| | 5761 | 13 | 7 | 7 | 0 | - | - | 7 |
| | 5902 | 30 | 15 | 10 | 0 | 0 | 0 | 10 |
| | 5906 | 11 | 16 | 14 | 4 | - | - | 10 |
| | 5914[d] | 24 | 17 | 13 | 0 | 13 | - | 0 |
| | 5915 | 18 | 9 | 9 | 0 | 0 | - | 9 |
| | 5924 | 8 | 18 | 6 | 6 | - | - | 0 |
| | 5937 | 30 | 1 | 0 | - | - | - | - |

[a]Days after insemination; scheduled sacrifice on 30 days
[b]Fetuses or embryos, depending on development time
[c]Aborted during dosing on 19 dg
[d]Aborted just prior to, or at death

# APPENDIX D

# GOOD LABORATORY PRACTICES

DOSE RANGE AND TERATOLOGY STUDIES OF LEWISITE IN RATS AND RABBITS

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 during the period, 3/1/85 - 10/1/86, 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 |
|---|---|---|
| Mating | 3/14/85 | 3/20/85 |
| Body Weights | 3/19/85 | 4/12/85 |
| Dosing | 3/19/85 | 4/12/85 |
| Dose Preparation | 3/22/85 | 4/12/85 |
| Data | 11/27/85 | 12/4/85 |
| Animal Identification | 2/13/86 | 3/3/86 |
| Body Weights | 2/13/86 | 3/3/86 |
| Health Screen | 2/19/86 | 3/10/86 |
| Body Weights | 2/20/86 | 3/10/86 |
| Mating | 2/20/86 | 3/10/86 |
| Dosing | 3/6/86 | 3/10/86 |
| Necropsy | 3/11/86 | 3/20/86 |
| Dosing | 6/20/86 | 6/20/86 |
| Body Weights | 6/20/86 | 6/20/86 |
| Artificial Insemination | 6/20/86 | 7/9/86 |
| Clinical Observations | 6/20/86 | 6/20/86 |
| Necropsy | 6/10/86 | 7/10/86 |
| Vehicle Analysis | 7/11-12/86 | 7/22/86 |
| Animal Receipt | 7/25/86 | 7/31/86 |
| Body Weights | 8/19/86 | 8/26/86 |
| Artificial Insemination | 8/19/86 | 8/26/86 |
| Necropsy | 9/18/86 | 9/19/86 |
| Data | 3/23-26/87 | 12/17/87 |
| Final Report | 3/23-26/87&12/10,11&17/87 | 12/17/87 |

R A Gelman
Quality Assurance Specialist

12/17/87
Date

## PERSONNEL LIST

| Function | Name |
|---|---|
| Principal Investigators | P.L. Hackett<br>L.B. Sasser |
| Facility Manager | M.T. Karagianes |
| Solution Preparation and Analysis | D.R. Kalkwarf<br>C.W. Lindenmeier<br>L.B. Sasser<br>C. Veverka, Jr. |
| Animal Exposures and Evaluation | T.A. Breier<br>J.A. Cushing<br>J.J. Evanoff<br>P.L. Hackett<br>C.W. Lindenmeier<br>M. Orgill<br>R.L. Rommereim<br>D.W. Shea<br>M.L. Sours<br>B.J. Willemsen<br>R.C. Zangar |
| Animal Resource Center | E.L. Wierman |
| Health Evaluation | S.E. Rowe |
| Statistical Analyses | R.F. Buschbom |

P.L. Hackett — 12/21/87
P.L. Hackett — Date

L.B. Sasser — 12-21-87
L.B. Sasser — Date

# APPENDIX E

# STUDY DATES

# SCHEDULE OF EVENTS FOR LEWISITE STUDIES

| | |
|---|---|
| **DOSE RANGE STUDY IN RATS** | |
| Animals arrived | 2/12/85 |
| Detection of copulation (0 dg) | 3/12/85 - 3/15/85 |
| Adminstration of dose (6 dg-15 dg) | 3/18/85 - 3/30/85 |
| Sacrifice (20 dg) | 4/1/85 - 4/4/85 |
| Sample/data evaluation | 4/4/85 - 5/4/85 |
| **TERATOLOGY STUDY IN RATS** | |
| Animals arrived | 2/4/86 |
| Detection of copulation (0 dg) | 2/18/86 - 2/21/86 |
| Adminstration of dose (6 dg-15 dg) | 2/24/86 - 3/8/86 |
| Sacrifice (20 dg) | 3/10/86 - 3/13/86 |
| Sample/data evaluation | 3/10/86 - 4/15/86 |
| **DOSE RANGE STUDY IN RABBITS** | |
| Animals arrived | 5/16/86 |
| Artificial insemination (0 dg) | 6/10/86 - 6/11/86 |
| Adminstration of dose (6 dg-19 dg) | 6/16/86 - 6/30/86 |
| Sacrifice (30 dg) | 7/10/86 - 7/11/86 |
| Sample/data evaluation | 7/11/86 - 8/1/86 |
| **TERATOLOGY STUDY IN RABBITS** | |
| Animals arrived | 7/25/86 |
| Artificial insemination (0 dg) | 8/18/86 - 8/20/86 |
| Adminstration of dose (6 dg-19 dg) | 8/24/86 - 9/8/86 |
| Sacrifice (30 dg) | 9/17/86 - 9/19/86 |
| Sample/data evaluation | 9/19/86 - 11/3/86 |
| **SUBMISSION OF DRAFT FINAL REPORT** | January, 1987 |

# APPENDIX F

# DISTRIBUTION

## DISTRIBUTION

OFFSITE

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