

Toxicology Research Laboratory

UIC The University of Illinois
at Chicago

Department of Pharmacology (M/C 868)
1940 W. Taylor St.
Chicago, Illinois 60612-7353

20100915246

Contract No.: DAMD17-92-C-2001
Task Order No.: UIC-11A
UIC/TRL Study No.: 168

Title Page

Draft Report for Task Order No. UIC-11A

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Sponsor: US Army Medical Materiel
Development Activity

Test Article: Halofantrine HCl

Contract No.: DAMD17-92-C-2001

Study Director

Barry S. Levine, D.Sc., D.A.B.T.

In-Life Phase Completed On

October 26, 1994

Performing Laboratory

TOXICOLOGY RESEARCH LABORATORY (TRL)
University of Illinois at Chicago (UIC)
Department of Pharmacology
1940 W. Taylor St.
Chicago, IL 60612-7353

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

DRAFT**REPORT DOCUMENTATION PAGE**Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION			1b. RESTRICTIVE MARKINGS			
2a. SECURITY CLASSIFICATION AUTHORITY Unclassified			3. DISTRIBUTION/AVAILABILITY OF REPORT			
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			Unlimited			
4. PERFORMING ORGANIZATION REPORT NUMBER(S) UIC-11A (UIC/TRL Study No. 168)			5. MONITORING ORGANIZATION REPORT NUMBER(S)			
6a. NAME OF PERFORMING ORGANIZATION Toxicology Research Laboratory University of Illinois at Chicago		6b. OFFICE SYMBOL (If applicable)		7a. NAME OF MONITORING ORGANIZATION U.S. Army Medical Materiel Development Activity		
6c. ADDRESS (City, State, and ZIP Code) Department of Pharmacology (M/C 868) 1940 W. Taylor Street Chicago, IL 60612-7353			7b. ADDRESS (City, State, and ZIP Code) ATTN: MCMR-RMA-RD Fort Detrick Frederick, MD 21702-5014			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Materiel Development Activity		8b. OFFICE SYMBOL (If applicable) MCMR-UMP		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-92-C-2001		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21702-5009			10. SOURCE OF FUNDING NUMBERS			
PROGRAM ELEMENT NO. 63807A		PROJECT NO. 30463807		TASK NO. QC	WORK UNIT ACCESSION NO. 073	
11. TITLE (Include Security Classification) Four Week Oral (Gavage) Dose Range-Finding Study of Halofantrine Hydrochloride in Mice						
12. PERSONAL AUTHOR(S) Levine, Barry S., Wheeler, Clyde W. and Morrissey, Robert L. (PAI)						
13a. TYPE OF REPORT Study		13b. TIME COVERED FROM 8/26/94 TO _____		14. DATE OF REPORT (Year, Month, Day)		15. PAGE COUNT
16. SUPPLEMENTARY NOTATION						
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)			
FIELD	GROUP	SUB-GROUP	Halofantrine Hydrochloride			
			Toxicity			
			Mice			
19. ABSTRACT (Continue on reverse if necessary and identify by block number) This study evaluated the toxicity of halofantrine hydrochloride in B6C3F1 mice following four weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 4, 20 and 100 mg/kg/day. Clinical signs of toxicity (rough coat, hunched posture, decreased activity and lethargy) and decreased body weight gains were limited to high dose animals. During week 3, one high dose male was found dead and the other four high dose animals were sacrificed moribund. Splenic lymphocytic necrosis, observed in all high dose males and three of five high dose females, and moderate splenic lymphocytic depletion, were considered possible contributing factors to their deaths. Splenic granulopoiesis secondary to the splenic lymphocytic necrosis, supported by neutrophilia and splenomegaly, was observed in high dose females. Marginal leukopenia, consisting of decreased numbers of mature neutrophils and lymphocytes, was seen in mid dose males but not females and may be indicative of the initial insult producing splenic lymphocytic depletion in the high dose animals. Dose-related, mild, microcytic, apparent iron-deficiency anemia was seen in high dose females and to a lesser extent in mid dose animals and low dose females. Thrombocytosis in high dose females may have been secondary to the anemia. Increased serum ALT and cholesterol levels in high dose females and increased serum ALT in mid dose males, not accompanied by corresponding histologic changes, suggests that halofantrine may be marginally hepatotoxic. Decreases in serum alkaline phosphatase levels were also observed in high dose females, and may have been related to reductions in food intake. The purpose of the study was to select dose levels for a three month toxicity study in mice. Because marginal halofantrine-induced toxicity was seen in low dose females, the following dose level ranges are suggested: 1 - 2, 4 - 8 and 15 - 30 mg/kg/day.						
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS				21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Barry S. Levine			22b. TELEPHONE (Include Area Code) (312) 996-5543		22c. OFFICE SYMBOL N/A	

D R A F T

Signature Page

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Test Article.: Halofantrine HCl (WR171669)

Sponsor: US Army Medical Materiel
Development Activity
Fort Detrick
Frederick, MD 21702-5009

Sponsor
Representative: George J. Schieferstein, Ph.D.

Testing Facility: TOXICOLOGY RESEARCH LABORATORY (TRL)
University of Illinois at Chicago (UIC)
Department of Pharmacology
1940 W. Taylor St.
Chicago, IL 60612-7353

Barry S. Levine, D.Sc., D.A.B.T.
Study Director

Date

Clyde W. Wheeler, PhD.
Toxicologist

Date

Study Initiation: August 26, 1994
Dosing Initiation: September 28, 1994
In-Life Completion: October 26, 1994

TABLE OF CONTENTS

DRAFT

TITLE PAGE1
SIGNATURE PAGE2
TABLE OF CONTENTS3
1. SUMMARY5
2. INTRODUCTION5
3. MATERIALS AND METHODS5
 3.1 Test Article5
 3.2 Animals6
 3.3 Experimental Design6
 3.4 Statistical Analyses9
4. RESULTS9
 4.1 Mortality and Clinical Signs/Observations9
 4.2 Body Weight9
 4.3 Food Consumption10
 4.4 Clinical Pathology10
 4.5 Organ Weights11
 4.6 Pathology11
5. DISCUSSION/CONCLUSION11
6. REFERENCES12
7. PERSONNEL13
8. ARCHIVES13

TABLES

1 Summary of Toxic Responses14
2 Summary of Clinical Signs15
3 Summary of Body Weights16
4 Summary of Weight Gains18

DRAFT

TABLE OF CONTENTS (contd.)

TABLES (contd.)

5	Summary of Daily Food Consumption	20
6	Summary of Clinical Chemistry Tests	22
7	Summary of Hematology Tests	24
8	Organ Weight Summary (% Brain Weight)	28
9	Summary of Gross and Microscopic Lesions	30

FIGURES

1	Summary of Male Body Weights	31
2	Summary of Female Body Weights	32

APPENDICES

1	Clinical Pathology Methodology	1-1
2	Individual Observations (Clinical Signs)	2-1
3	Individual Body Weight and Body Weight Gain Data	3-1
4	Individual Food Consumption Data	4-1
5	Individual Clinical Chemistry Data	5-1
6	Individual Hematology Data	6-1
7	Individual Organ Weight Data	7-1
8	Pathology Report	8-1
9	Protocol and Protocol Amendments	9-1
10	Study Deviations	10-1

DRAFT

1. SUMMARY

This study evaluated the toxicity of halofantrine hydrochloride in B6C3F1 mice following four weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 4, 20 and 100 mg/kg/day. The study results are summarized in Table 1. Clinical signs of toxicity (rough coat, hunched posture, decreased activity and lethargy) and decreased body weight gains were limited to high dose animals. During week 3, one high dose male was found dead and the other four high dose animals were sacrificed moribund. Splenic lymphocytic necrosis, observed in all high dose males and three of five high dose females, and moderate splenic lymphocytic depletion, were considered possible contributing factors to their deaths. Splenic granulopoiesis secondary to the splenic lymphocytic necrosis, supported by neutrophilia and splenomegaly, was observed in high dose females. Marginal leukopenia, consisting of decreased numbers of mature neutrophils and lymphocytes, was seen in mid dose males but not females and may be indicative of the initial insult producing splenic lymphocytic depletion in the high dose animals. Dose-related, mild, microcytic, apparent iron-deficiency anemia was seen in high dose females and to a lesser extent in mid dose animals and low dose females. Thrombocytosis in high dose females may have been secondary to the anemia. Increased serum ALT and cholesterol levels in high dose females and increased serum ALT in mid dose males, not accompanied by corresponding histologic changes, suggests that halofantrine may be marginally hepatotoxic. Decreases in serum alkaline phosphatase levels were also observed in high dose females, and may have been related to reductions in food intake. The purpose of the study was to select dose levels for a three month toxicity study in mice. Because marginal halofantrine-induced toxicity was seen in low dose females, the following dose level ranges are suggested: 1 - 2, 4 - 8 and 15 - 30 mg/kg/day.

2. INTRODUCTION

This non-GLP study was conducted to select dose levels for a 13 week oral toxicity study. The study was conducted in accordance with the specifications of the Sponsor. The B6C3F1 mice used in the study are a standard and accepted rodent species for regulatory toxicology studies, and were specified by the Sponsor. Oral administration is the intended clinical route and was also specified by the Sponsor. All methods and procedures were conducted within the spirit of the Quality Assurance Programs of the Toxicology Research Laboratory, University of Illinois at Chicago and Pathology Associates, Inc., designed to conform with FDA Good Laboratory Practices Regulations. No unforeseen circumstances affected the integrity of the study. Dosing was initiated on September 28, 1994 and the in-life portion was terminated on October 26, 1994.

3. MATERIALS AND METHODS

3.1 Test Article

Halofantrine HCl (WR171669) (Bottle No. BM01792), a white powder, was received on September 20, 1994 from Herner & Co., and was assigned an in-house chemical number (1950614). It was stored at ambient temperature and humidity. The Certificate of Analysis accompanying the test article indicated that the purity was 100% (analysis performed at Laboratorios Julian de Mexico, Smithkline Beckman Co.).

DRAFT

3.2 Animals

Male and female B6C3F1 Virus Antibody Free (VAF) mice were obtained from Charles River Breeding Laboratories (Portage, MI) on September 21, 1994. The animals were approximately 7 weeks old upon arrival at the UIC AAALAC-accredited animal facility (date of birth August 5, 1994). Each animal was given a study-unique quarantine/pretest number following placement in cages. The animals were singly housed in polycarbonate cages with Anderson bed-o-cob bedding (Heinhold, Kankakee, IL) in a temperature (65 - 78°F) and humidity (30 - 70%) controlled room with a 14 hour light/10 hour dark cycle. The cage size, 395 cm² and 12.5 cm height, was adequate to house mice at the upper weight range as described in the *Guide for the Care and Use of Laboratory Animals*, DHEW (NIH) No. 86.23. All animals were routinely transferred to clean cages once weekly.

libitum Certified Rodent Chow No. 5002 (PMI Feeds Inc., St. Louis, MO) was provided *ad libitum* from arrival until termination. Tap water from an automatic watering system in which the room distribution lines were flushed daily was provided *ad libitum*. The water was not treated with additional chlorine or HCl. There were no known contaminants in the feed or water which were expected to influence the study. The results of the bimonthly comprehensive chemical analyses of Chicago water performed by the City of Chicago are documented in files maintained by Quality Assurance.

3.3 Experimental Design

All animals were examined daily during the seven day quarantine/pretest period, and were approved for use by the Clinical Veterinarian prior to being placed on test. Near the end of the quarantine/pretest period, 20 animals of each sex were randomized by sex into the groups shown in the following table using a computer-generated randomization program, stratified on the basis of body weight.

<u>Treatment Group</u>	<u>Dose Level (mg/kg/day)</u>	<u>Number of Males</u>	<u>Number of Females</u>
1	0	5	5
2	4	5	5
3	20	5	5
4	100	5	5

Dose levels were selected on the basis of Sponsor-supplied subchronic toxicity data in rats and following discussions with the Sponsor.

During the test animal selection process, each animal was assigned an animal number unique to it within the population making up the study. This number appeared as an ear tag and also appeared on a cage card visible on the front of each cage. The cage card additionally contained the study number, test article identification, sex, treatment group number and dose level. Cage cards were color-coded as a function of treatment group.

D R A F T

Dosage formulations were prepared once weekly and were administered daily by gavage, at a dosing volume of 10 ml/kg/day, 7 days a week. Suspensions were prepared on the basis of the weight of the hydrochloride salt of halofantrine. The 0.5% methylcellulose vehicle was prepared at least weekly by placing the required amount of deionized water in a beaker and then adding the required amount of methylcellulose which was weighed on an analytical balance (0.5 g of methylcellulose per 100 ml of deionized water). The mixture was stirred until homogeneous and then refrigerated. The lot number of methylcellulose used for the 4 week study will be the same for the 13 week study (Sigma Chemical Co., Lot No. 123H0589).

A stock test article dosing suspension, which was also the high dose formulation, was prepared by triturating the appropriate amount of halofantrine HCl with approximately one-third to one-half of the required 0.5% methylcellulose vehicle in a mortar. The mixture was transferred to a graduated cylinder, the mortar was rinsed with vehicle and added to the graduated cylinder, and the final volume was brought to mark with vehicle. The entire mixture was then thoroughly stirred. The mid and low dose level suspensions were prepared by diluting and thoroughly mixing an appropriate volume of the high dose formulation with additional vehicle. All suspensions were stored at 2 - 8°C. Approximately 10 ml aliquots from each weekly dosing suspension set were retained and frozen at -20°C for possible analysis.

The test article was administered by gavage once daily for 28 days commencing on September 28, 1994. Control animals received the test article vehicle. All animals received the vehicle by gavage for 4 days during week -1 to acclimate them to the procedure. All animals were dosed up to and including the day prior to their scheduled necropsy. Dosing volume was 10 ml/kg/day, adjusted on the basis of each animal's most recent body weight. The actual volume (ml) administered was documented in the raw data. The mice weighed 21.7 - 25.5 g (males) and 18.7 - 21.1 g (females) on day 0 and were approximately eight weeks old at initiation of treatment.

Non-fasted body weights were recorded on day -2, on day 0, weekly thereafter and at scheduled termination. Clinical signs were observed and recorded for all animals once daily, approximately 1 - 2 hours after dosing. The general behavior, posture, locomotion, breathing pattern and coat were observed for all animals. The animals were also observed immediately prior to dosing and in the afternoon for moribundity/mortality. Physical examinations (clinical observations) which included examination of eyes and all orifices were conducted once weekly commencing in week -1. Food consumption was measured for all animals weekly commencing with week -1.

Hematology and clinical chemistry parameters were measured in all surviving animals at necropsy (day 28). The non-fasted animals were anesthetized by carbon dioxide inhalation (80% CO₂:20% O₂), and approximately 0.5 - 0.75 ml of blood were collected from the orbital sinus to measure the following parameters. The samples were processed in the same random order as collected.

DRAFT

Hematology

*Erythrocyte count and morphology	Mean corpuscular volume (MCV)
Hematocrit	Mean corpuscular hemoglobin (MCH)
Hemoglobin	Mean corpuscular hemoglobin concentration (MCHC)
Leukocyte count, total and differential	Platelet count
	Reticulocyte count

*Includes nucleated RBCs.

Clinical Chemistry

The clinical chemistry tests were prioritized as shown on the basis of the sample volume obtained.

- | | |
|------------------------------------|-------------------------|
| (1) Alanine aminotransferase (ALT) | (4) Glucose |
| (2) Alkaline phosphatase | (5) Urea nitrogen (BUN) |
| (3) Cholesterol | (6) Triglycerides |

Animals which were found dead or moribund sacrificed were necropsied on that day. Surviving animals were sacrificed and necropsied following four weeks of treatment (day 28). Euthanasia was accomplished by carbon dioxide asphyxiation (80% CO₂:20% O₂), and an extensive necropsy was performed under the direction and supervision of the pathologist. Terminal body weights were collected prior to routine sacrifice. The necropsy procedure was a thorough and systematic examination and dissection of the animal viscera and carcass, and collection and fixation of the following tissues/organs in 10% neutral buffered formalin. The ear with its attached identification tag was saved with the wet tissues.

Adrenal glands	Pancreas
*Brain	Pituitary
Cecum	Prostate
Colon	Salivary gland (submaxillary)
Duodenum	Sciatic nerve
Epididymides	Skeletal muscle
Esophagus	Skin (abdominal) with mammary gland
Eyes with hardierian glands	Spinal cord (thoracic)
Femur with marrow	*Spleen
Gallbladder	Stomach
Gross lesions	*Testes
*Heart	Thymus
Ileum	Thyroid gland+Parathyroids
Jejunum	Tongue

(tissue list continued on next page)

D R A F T

*Kidneys	Trachea
*Liver	Ureter
*Lungs/Bronchi	Urinary bladder
Lymph node (mesenteric)	Uterus
Ovaries	Vagina

*Weighed at scheduled necropsy. Paired organs were weighed as a unit.

The following tissues were examined microscopically in all animals in all groups.

Brain (fore-, mid-, hind-)	Liver
Gross lesions	Ovaries
Heart	Spleen
Kidneys	Testes

3.4 Statistical Analyses

For each sex, Analysis of Variance tests were conducted on body weight, weight gains, food consumption, hematology, clinical chemistry and organ weight data. Organ weight analysis considered weights relative to brain weight. If a significant F ratio was obtained ($p \leq 0.05$), Dunnett's t test was used for pair-wise comparisons to the control group. In addition to the written report, individual data in "ASCII" form and summary data tables of parameters and variability were transmitted to the Sponsor on magnetic media (computer diskette).

4. RESULTS

4.1 Mortality and Clinical Signs/Observations

Summaries of clinical signs are presented in Table 2. Individual clinical signs and the daily incidence of clinical signs are contained in Appendix 2.

One high dose male was found dead on day 14 and four high dose males were sacrificed moribund on days 14 and 15. Beginning on day 13, treatment-related clinical signs (1 - 2 hrs post-dosing) were observed in high dose males and included rough coat, hunched posture and decreased activity. On days 14 and 15, two high dose males were also observed to be lethargic prior to being sacrificed moribund. Beginning on day 20, one high dose female (animal no. 289) had rough coat and was later observed as having decreased activity and hunched posture by days 21 and 22, respectively. Clinical signs of toxicity were not seen in any other animals.

4.2 Body Weight

Summaries of body weights and summaries of weight gains are presented in Tables 3 and 4, respectively. Individual body weights and weight gains are contained in

Appendix 3. In addition, summaries of body weights are graphically depicted in Figures 1 (males) and 2 (females).

During the second week of treatment, the high dose males lost 4.6 g of body weight (mean), approximately 18% of their previous week's mean body weight. On day 28, a significant decrease in body weight gain was observed in high dose females resulting in an overall significant decrease in total body weight gain. During the third week of treatment, statistically insignificant decreases in body weight gain were also observed in high dose females. Body weights were not affected in other treatment groups. A sporadic increase was seen in week 3 in mid dose males, which was not considered biologically significant.

4.3 Food Consumption

Summaries of food consumption are in Table 5. Individual food consumption data are shown in Appendix 4.

During the fourth week of treatment, a decrease in food consumption was seen in high dose females. Food intake was not affected in other treatment groups including the high dose males which died on test.

4.4 Clinical Pathology

Summaries of clinical chemistry tests are presented in Table 6. Individual clinical chemistry data are in Appendix 5. Summaries of hematology tests are presented in Table 7. Individual hematology data are in Appendix 6.

If not significant, why mention it.

At necropsy (day 28), mild hepatotoxicity in high dose females was suggested by statistically significant increases in serum ALT and cholesterol levels. Although not statistically significant, possibly due to intra-animal variability, similar increases in serum ALT levels were also seen in mid dose males. Decreased alkaline phosphatase levels were only seen in high dose females.

Mild, dose-related changes in RBC parameters were observed in high dose females and to a lesser extent in the lower dose levels. On day 28, decreases in RBC count (statistically insignificant), hemoglobin, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were seen in high dose females. Slight, but statistically significant decreases in MCV and MCH were also seen in mid dose animals and low dose females. These microcytic, anemic changes without corresponding compensatory responses are suggestive of an iron-deficiency anemia (Jain, 1986).

Leukocytosis consisting of increased numbers of mature neutrophils and monocytes was seen in high dose females. Paradoxically, marginal leukopenia characterized by decreased numbers of mature neutrophils and lymphocytes was observed in mid dose males. Slight, but significant thrombocytosis was seen in high dose females, but not in the lower dose levels, and may have been in response to the apparent iron-deficiency anemia.

DRAFT

4.5 Organ Weights

Organ weight summaries expressed as % brain weight are presented in Table 8. Individual organ weight data are contained in Appendix 7.

At necropsy (day 28), splenomegaly was seen in high dose females. This was not seen in the lower dose levels, and no other changes in organ weights were seen.

4.6 Pathology

The Pathology Report is contained in Appendix 8. A summary of gross and microscopic lesions is shown in Table 9.

One high dose male (animal no. 282) was found dead on day 14 and the other four high dose males were sacrificed moribund on day 14 or day 15 (two animals each day). At necropsy or tissue trimming, four of five high dose males were observed as having reduced spleen size. Splenic lesions consisting of lymphocytic necrosis and depletion were seen in the high dose males, and were considered possible contributing factors in their deaths. Splenic lymphocytic necrosis consisted of multiple foci of cell debris in the white pulp (lymphoid follicle) regions and was seen in all high dose males (mean group severity score = 2.00; maximum = 4.00) and 3 of 5 high dose females (mean group severity score = 1.20). Lymphocytic depletion, a notable reduction in the relative amount of white pulp in the spleen, was also observed in the one high dose male found dead (animal no. 282, severity score = 3.00). Splenic granulopoiesis was observed in 3 of 5 high dose females (mean group severity score = 1.00), but not in high dose males. This change was characterized by the presence of colonies of granulocytic precursors in the subcapsular regions of the red pulp of the spleen.

No other microscopic changes were considered to be related to halofantrine HCl treatment.

5. DISCUSSION/CONCLUSION

This study evaluated the toxicity of halofantrine HCl in B6C3F1 mice following four weeks of **daily oral (gavage)** administration. The results are summarized in Table 1. The apparent treatment-related deaths (found dead or moribund sacrifice) of the five high dose males in week 3 were most-likely associated with halofantrine HCl-induced splenic lesions (reduced spleen size, lymphocytic necrosis and lymphocytic depletion). In the second week of treatment, high dose males demonstrated significant body weight loss without an accompanying decrease in food consumption. Within 1 - 2 days of their demise on day 14 or 15, rough coat, hunched posture, decreased activity and/or lethargy were seen in the high dose males. Clinical signs of toxicity were not observed in high dose females until day 20 and were limited to rough coat, hunched posture and decreased activity in one high dose female. In high dose females, statistically significant decreases in body weight gain accompanied by decreased food intake were only seen during the last week of treatment. Neither clinical signs nor effects on body weight or food consumption were seen in the lower dose levels.

D R A F T

Increased serum ALT and cholesterol levels in high dose females and increased serum ALT in mid dose males, not accompanied by corresponding histologic changes, suggests that halofantrine may be marginally hepatotoxic. Decreased serum alkaline phosphatase levels in high dose females may have been related to their reduction in food intake in week 4 as fasting results in this phenomenon.

Treatment-related anemia, as indicated by decreases in RBC count, hemoglobin, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), was observed in high dose females and to a lesser extent (decreased MCV and MCH) in mid dose animals and low dose females. The decreased RBC parameters without compensatory responses (i.e. reticulocytosis) is consistent with iron-deficiency anemia (Jain, 1986). These changes in RBC parameters were marginal in the lower dose levels. Females appear to be more sensitive than males to halofantrine-induced effects on RBC production. The thrombocytosis observed in high dose females may have been secondary to the anemia.

Splenic lymphocytic necrosis was seen in 3 of 5 high dose females and all high dose males, and was accompanied by a reduction in splenic size in 4 high dose males. Moderate splenic lymphocytic depletion was also seen in the high dose male which was found dead on day 14, but not in the other four males which were moribund sacrificed or in high dose females. Splenic granulopoiesis observed in high dose females was considered secondary to the lymphocytic depletion, and was supported by splenomegaly and leukocytosis (increased numbers of mature neutrophils and monocytes). The high dose males were more sensitive to the halofantrine-induced splenic toxicity than the high dose females, which resulted in their deaths, while the high dose females initiated compensatory responses (splenic granulopoiesis and neutrophilia). The increased sensitivity of males compared to females is further supported by the apparent leukopenia (decreased numbers of mature neutrophils and lymphocytes) seen in mid dose males at necropsy, but not in mid dose females. This leukopenia may be indicative of the initial sequence of events leading to the splenic lesions in the high dose animals.

The purpose of this study was to select dose levels for a subsequent three month toxicity study in mice. It is anticipated that significant toxicity at the high dose, marginal or no toxicity at the mid dose, and no toxicity at the low dose would occur. Because marginal halofantrine-induced toxicity (RBC changes) were seen in low dose females, the following dose level ranges are suggested: 1 - 2, 4 - 8 and 15 - 30 mg/kg/day.

6. REFERENCES

Jain, N.M. (1986). Blood loss or hemorrhagic anemias. In *Schalm's Veterinary Hematology*. Lea & Febiger, Philadelphia, p. 581.

D R A F T

7. PERSONNEL

Study Director	Barry S. Levine, D.Sc., D.A.B.T.
Toxicologist	Clyde W. Wheeler, Ph.D.
Pathologist	Robert L. Morrissey, D.V.M., Ph.D., D.A.C.V.P.
Clinical Veterinarian	James E. Artwohl, D.V.M., M.S., D.A.C.L.A.M.
Clinical Pathology	Maria Lang, A.H.T., C.V.T.
Lab Supervisor	Soudabeh Soura, B.S.
Lead Technician	Teresa O'Neill, B.S.

Report preparation was assisted by Rae-Jean T. Ballentine, B.S.

8. ARCHIVES

The raw data, specimens, test article reserves, and final report are archived at the Toxicology Research Laboratory (TRL), University of Illinois at Chicago (UIC), Department of Pharmacology, 1940 W. Taylor St., Chicago, IL 60612-7353.

DRAFT

Table 1

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Summary of Toxic Responses

Dose (mg/kg/day)	1	4	20	100
Mice/Sex	5	5	5	5
Deaths ^a	0	0	NE	5M
Clinical Signs	-	NE	NE	Rough coat (5M/1F) Hunched posture (5M/1F) Decreased activity (5M/1F) Lethargic (2M)
Body Weights/Gains	-	NE	NE	↓
Food Consumption	-	NE	NE	↓ (F)
Clinical Chemistry ^a	-	NE	↑ ALT (M?)	↑ ALT (F) ↑ CHOL (F) ↓ ALKP (F)
Hematology ^b	-	↓ MCV (F) ↓ MCH (F)	↓ MCV ↓ MCH ↓ LEUK (M) ↓ MNEUTR (M) ↓ LYMPH (M)	↓ RBC (F?) ↑ PLT (F) ↓ HGB (F) ↑ LEUK (F) ↓ HCT (F) ↑ MNEUTR (F) ↓ MCV (F) ↑ MONO (F) ↓ MCH (F)
Organ Weights (% brain weight)	-	NE	NE	↑ Spleen (F)
Gross Lesions	-	NE	NE	↓ Splenic size (4M)
Histopathology	-	NE	NE	SPLEEN - Lymphocytic necrosis (5M/3F) - Lymphocytic depletion (1M) - Granulopoiesis (3F)
CONCLUSIONS	<p>The primary treatment-related effects of halofantrine were seen in the spleen and RBC formation. During week 3, one high dose male was found dead and the other four high dose animals were sacrificed moribund. Splenic lymphocytic necrosis, observed in all high dose males and three of five high dose females, and moderate splenic lymphocytic depletion, were considered possible contributing factors to their deaths. Splenic granulopoiesis secondary to the splenic lymphocytic necrosis, supported by neutrophilia and splenomegaly, was observed in high dose females. Marginal leukopenia, consisting of decreased numbers of mature neutrophils and lymphocytes, was seen in mid dose males but not females and may be indicative of the initial insult producing splenic lymphocytic depletion in the high dose animals. Dose-related, mild, microcytic, apparent iron-deficiency anemia was seen in high dose females and to a lesser extent in mid dose animals and low dose females. Thrombocytosis seen in high dose females may have been secondary to the anemia. Increased serum ALT and cholesterol levels in high dose females and increased serum ALT in mid dose males, not accompanied by corresponding histologic changes, suggests that halofantrine may be marginally hepatotoxic. Decreases in serum alkaline phosphatase levels were also observed in high dose females, and may have been related to reductions in food intake. The purpose of the study was to select dose levels for a three month toxicity study in mice. Because marginal halofantrine-induced toxicity was seen in low dose females, the following dose level ranges are suggested: 1 - 2, 4 - 8 and 15 - 30 mg/kg/day.</p>			

^aALT = alanine aminotransferase, CHOL = cholesterol, ALKP = alkaline phosphatase.

^bRBCs = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, PLT = platelets, LEUK = leukocytes, MNEUTR = mature neutrophils, LYMPH = lymphocytes, MONO = monocytes.

? = Possible or marginal effect

NE = No effect

M = Male, F = Female

Table 2

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF CLINICAL SIGNS

STUDY: 168

SEX: MALE

	DOSE:(mg/kg)			
	0	4	20	100
	GROUP:			
	1-M	2-M	3-M	4-M
Animal Found Dead	0	0	0	1
Sacrificed Moribund	0	0	0	4
Decreased Activity	0	0	0	5
Hunched Posture	0	0	0	5
Lethargic	0	0	0	2
Rough Coat	0	0	0	5
Total Number of Animals	5	5	5	5

STUDY: 168

SEX: FEMALE

	DOSE:(mg/kg)			
	0	4	20	100
	GROUP:			
	1-F	2-F	3-F	4-F
Decreased Activity	0	0	0	1
Hunched Posture	0	0	0	1
Rough Coat	0	0	0	1
Total Number of Animals	5	5	5	5

Table 3.1

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF BODY WEIGHTS (Grams)

STUDY: 168

SEX: MALE

PERIOD	DOSE: (mg/kg) GROUP:	0	4	20	100
		1-M	2-M	3-M	4-M
DAY -2	MEAN	23.1	23.3	23.0	23.2
	S.D.	1.19	1.08	1.26	0.96
	N	5	5	5	5
DAY 0	MEAN	23.3	23.5	23.8	23.4
	S.D.	1.16	0.95	0.79	1.36
	N	5	5	5	5
DAY 7	MEAN	24.3	24.8	25.1	25.0
	S.D.	1.14	1.02	0.70	1.37
	N	5	5	5	5
DAY 14	MEAN	25.5	25.4	25.4	20.4*
	S.D.	1.44	0.94	1.06	1.49
	N	5	5	5	5
DAY 20	MEAN	26.1	26.4	26.6	--
	S.D.	1.32	0.98	1.32	--
	N	5	5	5	0
DAY 28	MEAN	27.2	27.0	27.4	--
	S.D.	1.68	0.82	1.60	--
	N	5	5	5	0

* P less than .05

Analysis of Variance using DUNNETT'S Procedure

-- = Data Unavailable

Table 3.2

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF BODY WEIGHTS (Grams)

STUDY: 168

SEX: FEMALE

PERIOD	DOSE: (mg/kg) GROUP:	0	4	20	100
		1-F	2-F	3-F	4-F
DAY -2	MEAN	19.3	19.3	19.3	19.3
	S.D.	0.44	0.64	0.48	0.53
	N	5	5	5	5
DAY 0	MEAN	20.0	19.8	19.7	19.6
	S.D.	0.60	0.62	0.54	0.51
	N	5	5	5	5
DAY 7	MEAN	21.4	20.9	21.1	20.9
	S.D.	0.76	0.69	0.72	0.58
	N	5	5	5	5
DAY 14	MEAN	22.0	21.5	21.8	21.3
	S.D.	0.75	0.70	0.53	1.10
	N	5	5	5	5
DAY 20	MEAN	23.2	22.5	22.5	21.7
	S.D.	0.77	0.73	0.72	1.89
	N	5	5	5	5
DAY 28	MEAN	23.9	23.6	23.5	21.2*
	S.D.	0.69	0.65	0.58	1.68
	N	5	5	5	5

* P less than .05

Analysis of Variance using DUNNETT'S Procedure

Table 4.1

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF WEIGHT GAINS (Grams)

STUDY: 168

SEX: MALE

PERIOD ^a	DOSE: (mg/kg) GROUP:	0	4	20	100
		1-M	2-M	3-M	4-M
DAY 7 ^b	MEAN	1.0	1.3	1.4	1.6
	S.D.	0.80	0.43	0.49	0.33
	N	5	5	5	5
DAY 14	MEAN	1.2	0.6	0.2	-4.6*
	S.D.	0.44	0.38	0.50	2.22
	N	5	5	5	5
DAY 20	MEAN	0.6	1.0	1.3**	--
	S.D.	0.36	0.11	0.34	--
	N	5	5	5	0
DAY 28	MEAN	1.1	0.6	0.8	--
	S.D.	0.37	0.25	0.38	--
	N	5	5	5	0
TOTAL GAIN	MEAN	3.9	3.5	3.6	--
	S.D.	1.42	0.30	1.14	--
	N	5	5	5	0

* P less than .05

Analysis of Variance using DUNNETT'S Procedure

-- = Data Unavailable

^aSuccessive periods^bBaseline is day 0

Table 4.2

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF WEIGHT GAINS (Grams)

STUDY: 168

SEX: FEMALE

PERIOD ^a	DOSE: (mg/kg) GROUP:	0	4	20	100
		1-F	2-F	3-F	4-F
DAY 7 ^b	MEAN	1.3	1.0	1.5	1.3
	S.D.	0.43	0.27	0.48	0.18
	N	5	5	5	5
DAY 14	MEAN	0.7	0.7	0.6	0.3
	S.D.	0.21	0.49	0.30	0.60
	N	5	5	5	5
DAY 20	MEAN	1.2	1.0	0.7	0.4
	S.D.	0.48	0.33	0.55	0.90
	N	5	5	5	5
DAY 28	MEAN	0.7	1.1	1.0	-0.5*
	S.D.	0.28	0.22	0.22	0.58
	N	5	5	5	5
TOTAL GAIN	MEAN	3.9	3.8	3.8	1.6*
	S.D.	0.27	0.34	0.28	1.23
	N	5	5	5	5

* P less than .05

Analysis of Variance using DUNNETT'S Procedure

^a Successive periods^b Baseline is day 0

Table 5.1

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF DAILY MEAN FOOD CONSUMPTION (Grams)

STUDY: 168

SEX: MALE

PERIOD ^a	DOSE:(mg/kg) GROUP:	DOSE:			
		0 1-M	4 2-M	20 3-M	100 4-M
DAY 0 ^b	INTAKE (g)	6.5	5.9	6.3	7.2
	S.D.	4.19	4.30	3.34	2.02
	N	5	5	5	5
DAY 7	INTAKE (g)	3.8	3.9	3.8	3.8
	S.D.	0.30	0.19	0.44	0.69
	N	5	5	5	5
DAY 14	INTAKE (g)	7.7	6.5	5.7	5.5
	S.D.	2.13	2.14	1.92	2.50
	N	5	5	5	5
DAY 20	INTAKE (g)	3.8	3.6	3.9	--
	S.D.	0.90	0.29	0.22	0.00
	N	5	5	5	0
DAY 28	INTAKE (g)	4.0	4.0	4.2	--
	S.D.	0.61	0.28	0.12	0.00
	N	5	5	5	0

* P less than .05

Analysis of Variance using DUNNETT'S Procedure

-- = Data Unavailable

^aInclusive intervals^bBaseline is day -6

Table 5.2

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF DAILY MEAN FOOD CONSUMPTION (Grams)

STUDY: 168		SEX: FEMALE			
PERIOD ^a	DOSE:(mg/kg) GROUP:	0 1-F	4 2-F	20 3-F	100 4-F
^b					
DAY 0	INTAKE (g)	7.0	5.2	8.3	5.7
	S.D.	4.25	1.18	4.62	2.00
	N	5	5	5	5
DAY 7	INTAKE (g)	3.9	4.6	4.6	4.2
	S.D.	0.98	0.82	0.68	1.23
	N	5	5	5	5
DAY 14	INTAKE (g)	8.1	6.7	8.0	6.3
	S.D.	2.97	2.45	2.50	1.09
	N	5	5	5	5
DAY 20	INTAKE (g)	4.1	4.4	3.8	3.1
	S.D.	0.80	0.92	0.83	1.09
	N	5	5	5	5
DAY 28	INTAKE (g)	4.8	5.2	4.1	3.0*
	S.D.	0.98	1.70	0.61	0.59
	N	5	5	5	5

* p less than .05

Analysis of Variance using DUNNETT'S Procedure

^aInclusive intervals^bBaseline is day -6

Table 6.1

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

SUMMARY OF CLINICAL CHEMISTRY TESTS
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s): UNITS:	ALT IU/L	ALKP IU/L	CHOL mg/dL	TRIG mg/dL	BUN mg/dL	GLU mg/dL
Group: 1-M : 0 (mg/kg/day)						
MEAN	71	152	102	219	29.2	167
SD	28.5	7.6	5.9	84.1	3.51	27.7
N	5	5	5	5	5	5
Group: 2-M : 4 (mg/kg/day)						
MEAN	102	146	98	225	32.2	164
SD	53.2	18.7	5.8	51.7	3.68	14.7
N	5	5	5	5	5	5
Group: 3-M : 20 (mg/kg/day)						
MEAN	128	160	104	228	37.3	151
SD	76.2	15.4	15.2	108.7	6.85	21.7
N	5	5	5	5	5	5
Group: 4-M : 100 (mg/kg/day)						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0

? Range of no/male values

NA-Not Applicable

LABCAT CC4.31

28-NOV-1994

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICESUMMARY OF CLINICAL CHEMISTRY TESTS
PERIOD: Day 28STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(S): UNITS:	ALT IU/L	ALKP IU/L	CHOL mg/dL	TRIG mg/dL	BUN mg/dL	GLU mg/dL
Group: 1-F : 0 (mg/kg/day)						
MEAN	41	186	89	161	31.2	157
SD	13.4	8.0	8.0	64.6	7.11	26.8
N	5	5	5	5	5	5
Group: 2-F : 4 (mg/kg/day)						
MEAN	41	190	88	151	29.9	169
SD	10.4	21.3	5.4	49.9	3.63	32.2
N	5	5	5	5	5	5
Group: 3-F : 20 (mg/kg/day)						
MEAN	58	195	89	135	23.6	146
SD	15.7	14.6	6.3	27.5	8.34	3.9
N	5	5	5	5	5	5
Group: 4-F : 100 (mg/kg/day)						
MEAN	169*	83*	131*	103	22.6	131
SD	82.0	16.5	23.5	34.3	4.21	11.7
N	5	4	4	4	4	4

*-Significant Difference from Control P < .05

LABCAT CC4.31

28-NOV-1994

Table 7.1

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

SUMMARY OF HEMATOLOGY TESTS
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	RBC	HGB	HCT	MCV	MCH	MCHC	RETICS	NRBC	PLT
UNITS:	10 ⁶ /mm ³	g/dL	%	fL	pg	g/dL	% RBCs	#/100 WBC	10 ³ /mm ³
Group: 1-M : 0 (mg/kg/day)									
MEAN	10.06	17.1	50.5	50.2	17.0	33.8	0.6	0.0	1117
SD	0.286	0.57	1.46	0.13	0.15	0.40	0.22	0.00	115.2
N	5	5	5	5	5	5	5	5	5
Group: 2-M : 4 (mg/kg/day)									
MEAN	9.53	16.1	47.8	50.2	16.9	33.7	0.6	0.0	1222
SD	0.370	0.44	1.54	0.66	0.27	0.27	0.35	0.00	42.8
N	5	5	5	5	5	5	5	5	5
Group: 3-M : 20 (mg/kg/day)									
MEAN	9.80	16.2	48.1	49.1*	16.6*	33.7	0.4	0.0	1013
SD	0.638	1.04	3.28	0.33	0.21	0.41	0.16	0.00	192.9
N	5	5	5	5	5	5	5	5	5
Group: 4-M : 100 (mg/kg/day)									
MEAN	NA	NA	NA	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0	0	0	0

WBC corrected for NRBC = or > 10

*-Significant Difference from Control P < .05

NA-Not Applicable

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

SUMMARY OF HEMATOLOGY TESTS
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	WBC M.	Neutrop I.	Neutrop	Lymphocyte	Monocytes	Eosinophil	Basophils	Atypical L
UNITS:	10 ³ /mm ³	10 ³ /mm ³	10 ³ /mm ³	10 ³ /mm ³	10 ³ /mm ³	10 ³ /mm ³	10 ³ /mm ³	10 ³ /mm ³
Group: 1-M : 0 (mg/kg/day)								
MEAN	8.1	1.2	0.0	6.6	0.2	0.1	0.0	0.0
SD	1.58	0.31	0.00	1.36	0.10	0.09	0.00	0.00
N	5	5	5	5	5	5	5	5
Group: 2-M : 4 (mg/kg/day)								
MEAN	7.3	1.1	0.0	6.0	0.1	0.1	0.0	0.0
SD	1.06	0.35	0.00	0.71	0.13	0.08	0.00	0.00
N	5	5	5	5	5	5	5	5
Group: 3-M : 20 (mg/kg/day)								
MEAN	4.2*	0.6*	0.0	3.4*	0.0	0.0	0.0	0.0
SD	2.40	0.30	0.00	2.12	0.05	0.09	0.00	0.00
N	5	5	5	5	5	5	5	5
Group: 4-M : 100 (mg/kg/day)								
MEAN	NA	NA	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0	0	0

WBC corrected for NRBC = or > 10

*-Significant Difference from Control P < .05

NA-Not Applicable

LABCAT HE4.31

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICESUMMARY OF HEMATOLOGY TESTS
PERIOD: Day 28STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	RBC	HGB	HCT	MCV	MCH	MCHC	RETICS	NRBC	PLT
UNITS:	10 ⁶ /mm ³	g/dL	%	fL	pg	g/dL	% RBCs	#/100 WBC	10 ³ /mm ³
Group: 1-F : 0 (mg/kg/day)									
MEAN	9.37	16.3	48.0	51.2	17.4	34.0	0.8	0.0	1010
SD	0.414	0.65	2.02	0.25	0.15	0.24	0.25	0.00	93.0
N	5	5	5	5	5	5	5	5	5
Group: 2-F : 4 (mg/kg/day)									
MEAN	9.69	16.5	48.4	50.0*	17.1*	34.1	0.6	0.0	955
SD	0.485	0.71	1.64	0.89	0.21	0.55	0.25	0.00	137.6
N	5	5	5	5	5	5	5	5	5
Group: 3-F : 20 (mg/kg/day)									
MEAN	9.81	16.6	48.8	49.7*	16.9*	34.0	0.5	0.0	816
SD	0.540	0.77	2.70	0.26	0.19	0.46	0.26	0.00	169.6
N	5	5	5	5	5	5	5	5	5
Group: 4-F : 100 (mg/kg/day)									
MEAN	8.83	13.9*	40.2*	45.5*	15.7*	34.6	0.5	0.0	1456*
SD	0.375	0.68	1.58	0.52	0.27	0.51	0.40	0.00	144.4
N	5	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

*-Significant Difference from Control P < .05

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF HEMATOLOGY TESTS
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	WBC	M. Neutrop	I. Neutrop	Lymphocyte	Monocytes	Eosinophil	Basophils	Atypical L
UNITS:	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$	$10^3/\text{mm}^3$
Group: 1-F : 0 (mg/kg/day)								
MEAN	6.6	0.8	0.0	5.8	0.1	0.1	0.0	0.0
SD	2.37	0.55	0.00	1.97	0.04	0.05	0.00	0.00
N	5	5	5	5	5	5	5	5
Group: 2-F : 4 (mg/kg/day)								
MEAN	6.0	0.9	0.0	4.9	0.1	0.1	0.0	0.0
SD	2.07	0.47	0.00	1.57	0.08	0.11	0.00	0.00
N	5	5	5	5	5	5	5	5
Group: 3-F : 20 (mg/kg/day)								
MEAN	7.7	0.8	0.0	6.7	0.1	0.1	0.0	0.0
SD	2.13	0.30	0.00	1.74	0.13	0.08	0.00	0.00
N	5	5	5	5	5	5	5	5
Group: 4-F : 100 (mg/kg/day)								
MEAN	14.6*	8.3*	0.0	5.5	0.8*	0.1	0.0	0.0
SD	6.75	5.74	0.00	1.24	0.36	0.09	0.00	0.00
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

*-Significant Difference from Control P < .05

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

ORGAN WEIGHT SUMMARY (% BRAIN WEIGHT)

STUDY: 168
SEX: MALE

ALL FATES DAYS: 28-28 ALL BALANCES
ANALYSIS OF VARIANCE USING DUNNETT'S PROCEDURE

GROUP:	(1) 1-M	(2) 2-M	(3) 3-M	(4) 4-M
Heart (% BRAIN WEIGHT)				
MEAN	33.94	32.09	35.50	0.00
SD	1.460	2.755	1.961	NA
N	5	5	5	0
Kidneys (% BRAIN WEIGHT)				
MEAN	106.90	108.04	106.37	0.00
SD	10.302	6.331	4.262	NA
N	5	5	5	0
Liver (% BRAIN WEIGHT)				
MEAN	342.35	344.62	349.61	0.00
SD	33.558	11.573	28.448	NA
N	5	5	5	0
Lungs/Bronchi (% BRAIN WEIGHT)				
MEAN	56.51	53.18	58.28	0.00
SD	10.944	6.984	12.603	NA
N	5	5	5	0
Spleen (% BRAIN WEIGHT)				
MEAN	15.85	13.83	15.13	0.00
SD	2.051	0.980	1.798	NA
N	5	5	5	0
Testes (% BRAIN WEIGHT)				
MEAN	49.95	46.58	47.01	0.00
SD	2.109	3.064	3.677	NA
N	5	5	5	0

(1)-0 mg/kg/day
(2)-4 mg/kg/day
(3)-20 mg/kg/day

(4)-100 mg/kg/day
NA-Not Applicable

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

ORGAN WEIGHT SUMMARY (% BRAIN WEIGHT)

STUDY: 168
SEX: FEMALE

ALL FATES DAYS: 28-28 ALL BALANCES
ANALYSIS OF VARIANCE USING DUNNETT'S PROCEDURE

GROUP:	(5)	(6)	(7)	(8)
	1-F	2-F	3-F	4-F
Heart (% BRAIN WEIGHT)				
MEAN	29.17	27.82	27.36	24.89
SD	3.149	2.166	1.202	2.300
N	5	5	5	5
Kidneys (% BRAIN WEIGHT)				
MEAN	74.98	75.18	73.56	77.93
SD	3.437	5.141	5.415	4.019
N	5	5	5	5
Liver (% BRAIN WEIGHT)				
MEAN	306.35	293.87	287.51	324.20
SD	16.797	14.749	23.411	7.879
N	5	5	5	5
Lungs/Bronchi (% BRAIN WEIGHT)				
MEAN	52.31	58.28	60.72	47.22
SD	9.641	12.110	10.358	3.828
N	5	5	5	5
Spleen (% BRAIN WEIGHT)				
MEAN	21.84	19.53	19.94	28.00*
SD	2.066	2.615	1.506	4.518
N	5	5	5	5

(5)-0 mg/kg/day
(6)-4 mg/kg/day
(7)-20 mg/kg/day

(8)-100 mg/kg/day
* - Significant difference P<.05

DRAFT

Table 9

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Summary of Gross and Microscopic Lesions

GROSS LESIONS		Dose (mg/kg/day)			
ORGAN - Lesion	Sex	0	4	20	100
SPLEEN - Reduced size	M	0/5	0/5	0/5	4/5
	F	0/5	0/5	0/5	0/5

MICROSCOPIC LESIONS ^{a,b}		Dose (mg/kg/day)			
ORGAN - Lesion	Sex	0	4	20	100
SPLEEN - Lymphocytic necrosis	M	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	5/5 (2.00)
	F	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	3/5 (1.20)
- Lymphocytic depletion	M	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	1/5 (0.60)
	F	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)
- Granulopoiesis	M	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)
	F	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	3/5 (1.00)

^aIncidences (mean group severity) - Group mean severity was calculated by dividing the sum of all severity scores for a finding by the number of tissues examined.

^bLesion severity was scored as follows:

1 = Minimal 3 = Moderate
 2 = Mild 4 = Marked

For additional information see Pathology Report in Appendix 10.

Figure 1
Summary of Male Body Weights
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

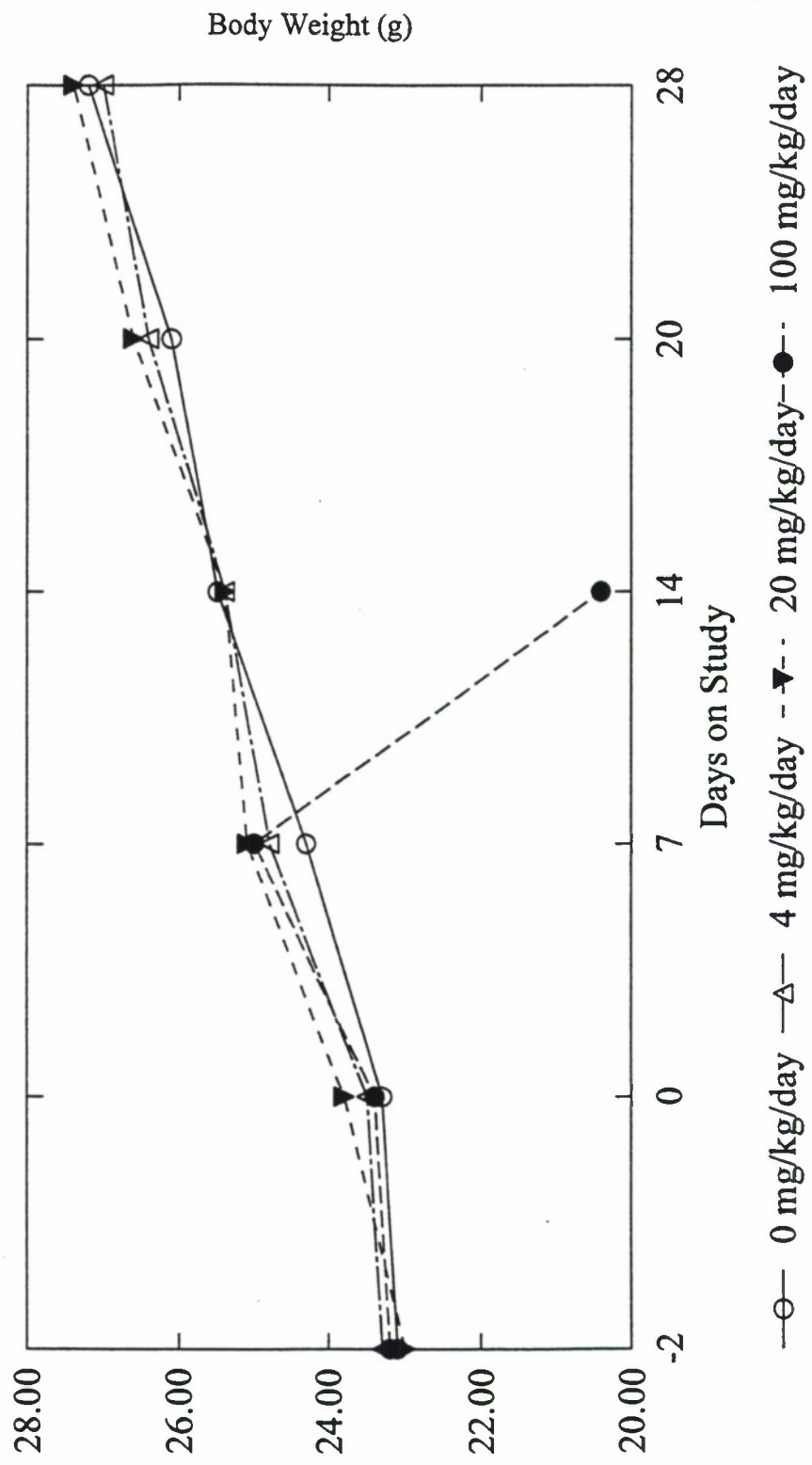
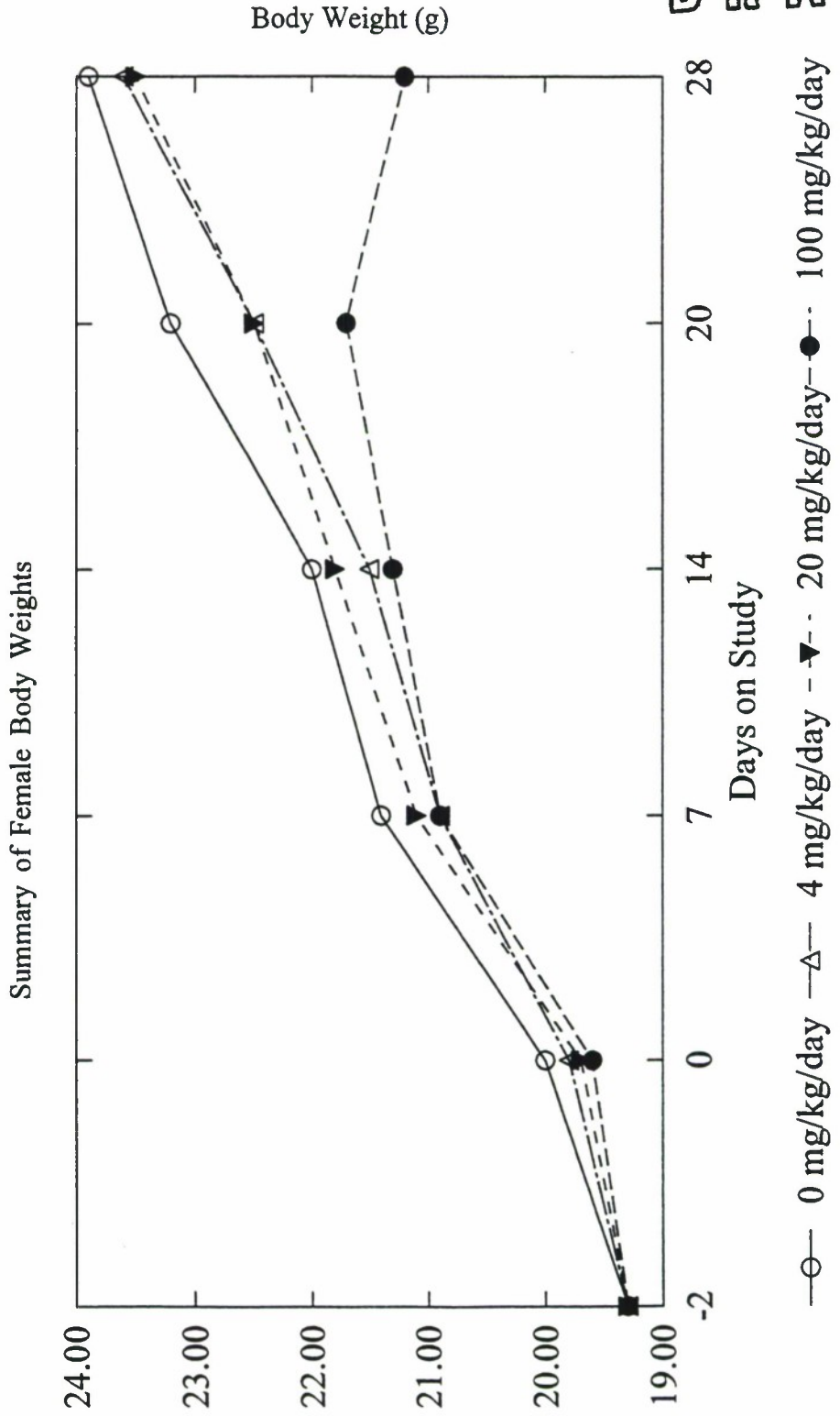


Figure 2
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE



DRAFT

APPENDIX 1

Clinical Pathology Methodology

CLINICAL CHEMISTRY

D R A F T

Alanine Aminotransferase (ALT/GPT)

Modified Wroblewski & La Due procedure
Ciba-Corning 550 Express Clinical Chemistry System
Henry, R.J., Chiamori, N., Golub, O.J. and Berkman, S.
Am. J. Clin. Path., 34, 381, 1960.

Alkaline Phosphatase

Modified Bessey-Lowry procedure
Ciba-Corning 550 Express Clinical Chemistry System
Neumann, H. and Von Vreedendaal
M. Clin. Chem. Acta., 17, 183, 1967.

Cholesterol

Cholesterol esterase-oxidase method
Ciba-Corning 550 Express Clinical Chemistry System
Rosechlow, P., et. al
Z.F. Klin. Chem. V. Klin. Biochem. 12, 226, 1974.

Glucose

Hexokinase method
Ciba-Corning 550 Express Clinical Chemistry System
Bondar, J.L. and Mead, D.C.
Clin. Chem. 20, 586, 1974.

Urea Nitrogen (BUN)

Modified urease technique
Ciba-Corning 550 Express Clinical Chemistry System
Talke, H. and Schubert, G.E.
Klin. Wchnschr. 43, 174, 1965.

Triglycerides

Tetrazolium salt reduction method
Ciba-Corning 550 Express Clinical Chemistry System
Klotzsch, S., et. al.
Advances Automated Analysis, Vol. 1, Mediad Inc., Tarrytown, N.Y., p. 111, 1973.

Erythrocyte Count

Electronic counting procedure
Sysmex K1000 Hematology Analyzer

Hemoglobin

Cyanomethemoglobin method
Sysmex K1000 Hematology Analyzer

Hematocrit

Indirect method; calculated value based on volume of red cells and volume of blood

Mean Corpuscular Volume (MCV)

Indirect method; calculated value based on hematocrit and red blood cell count

Mean Corpuscular Hemoglobin (MCH)

Indirect method; calculated value based on erythrocyte count and hemoglobin

Mean Corpuscular Hemoglobin Concentration (MCHC)

Indirect method; calculated value based on hematocrit and hemoglobin

Reticulocyte Count

New methylene blue staining procedure
Brecher, G., Am. J. Clin. Path., 19, 895, 1949.

Platelet Count

Electronic counting procedure
Sysmex K1000 Hematology Analyzer

Leukocyte Count

Electronic counting procedure
Sysmex K1000 Hematology Analyzer

Leukocyte Differential Count

Neutrophils - Immature (bands)
Neutrophils - Mature (segs)
Monocytes
Basophils
Lymphocytes
Eosinophils
Wright stain procedure
Schalm, O.W., Jain, N.C. and Carroll, E.J. Veterinary Hematology, Color Plates Chapter, 3rd Edition, Lee and Febiger, 1975.

Nucleated RBCs

Wright stain procedure
Schalm, O.W., Jain, N.C. and Carroll, E.J. Veterinary Hematology, Color Plates Chapter, 3rd Edition, Lee and Febiger, 1975.

RBC Morphology

Wright stain procedure
Schalm, O.W., Jain, N.C. and Carroll, E.J. Veterinary Hematology, Color Plates Chapter, 3rd Edition, Lee and Febiger, 1975.

DRAFT

APPENDIX 2

Individual Observations (Clinical Signs)

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
DAY 0-DAY 28

GROUP: 1-M
DOSE: 0 (mg/kg)

SEX: MALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
251	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
252	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
253	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
254	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
255	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
DAY 0-DAY 28

GROUP: 1-F
DOSE: 0 (mg/kg)

SEX: FEMALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
256	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
257	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
258	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
259	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
260	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
DAY 0-DAY 28

GROUP: 2-M
DOSE: 4 (mg/kg)

SEX: MALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
261	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
262	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
263	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
264	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
265	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
DAY 0-DAY 28

GROUP: 2-F
DOSE: 4 (mg/kg)

SEX: FEMALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
266	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
267	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
268	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
269	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
270	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
DAY 0-DAY 28

GROUP: 3-M
DOSE: 20(mg/kg)

SEX: MALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
271	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
272	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
273	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
274	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
275	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
DAY 0-DAY 28

GROUP: 3-F
DOSE: 20(mg/kg)

SEX: FEMALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
276	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
277	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
278	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
279	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
280	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
 DAY 0-DAY 28

GROUP: 4-M
 DOSE: 100(mg/kg)

SEX: MALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
281	Decreased Activity Decreased Activity Hunched Posture Lethargic Normal Normal Rough Coat Sacrificed Moribund			DAY 2 DAY 14 DAY 14-DAY 15 DAY 15 DAY 0-DAY 1 DAY 3-DAY 13 DAY 14-DAY 15 DAY 15
282	Decreased Activity Animal Found Dead Hunched Posture Normal Rough Coat			DAY 13 DAY 14 DAY 13 DAY 0-DAY 12 DAY 13
283	Decreased Activity Hunched Posture Normal Rough Coat Sacrificed Moribund			DAY 13 DAY 13 DAY 0-DAY 12 DAY 13 DAY 14
284	Decreased Activity Hunched Posture Normal Rough Coat Sacrificed Moribund			DAY 13 DAY 13 DAY 0-DAY 12 DAY 13 DAY 14
285	Decreased Activity Hunched Posture Lethargic Normal Rough Coat Sacrificed Moribund			DAY 13 DAY 13-DAY 15 DAY 14-DAY 15 DAY 0-DAY 12 DAY 14-DAY 15 DAY 15

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

D R A F T

INDIVIDUAL CLINICAL SIGNS

STUDY: 168
 DAY 0-DAY 28

GROUP: 4-F
 DOSE: 100 (mg/kg)

SEX: FEMALE

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	TIME OCCURRED
286	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
287	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
288	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28
289	Decreased Activity Decreased Activity Hunched Posture Normal Rough Coat Scheduled Sacrifice			DAY 21-DAY 23 DAY 27 DAY 22-DAY 27 DAY 0-DAY 19 DAY 20-DAY 27 DAY 28
290	Normal Scheduled Sacrifice			DAY 0-DAY 27 DAY 28

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-M	2-M	3-M	4-M
DAY 0					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 1					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 2					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	4 80%
Decreased Activity		0	0	0	1 20%
DAY 3					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 4					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 5					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 6					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 7					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 8					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 9					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-M	2-M	3-M	4-M
DAY 10					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 11					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 12					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 13					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	1 20%
Decreased Activity		0	0	0	4 80%
Hunched Posture		0	0	0	4 80%
Rough Coat		0	0	0	3 60%
DAY 14					
No. Observed		5	5	5	5
Animal Found Dead		0	0	0	1 20%
Sacrificed Moribund		0	0	0	2 40%
Normal		5 100%	5 100%	5 100%	0
Decreased Activity		0	0	0	1 20%
Hunched Posture		0	0	0	2 40%
Lethargic		0	0	0	1 20%
Rough Coat		0	0	0	2 40%
DAY 15					
No. Observed		5	5	5	2
Sacrificed Moribund		0	0	0	2 100%
Normal		5 100%	5 100%	5 100%	0
Hunched Posture		0	0	0	2 100%
Lethargic		0	0	0	2 100%
Rough Coat		0	0	0	2 100%
DAY 16					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-M	2-M	3-M	4-M
DAY 17					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 18					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 19					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 20					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 21					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 22					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 23					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 24					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 25					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 26					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-M	2-M	3-M	4-M
DAY 27					
No. Observed		5	5	5	0
Normal		5 100%	5 100%	5 100%	0
DAY 28					
No. Observed		5	5	5	0
Scheduled Sacrifice		5 100%	5 100%	5 100%	0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-F	2-F	3-F	4-F
DAY 0					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 1					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 2					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 3					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 4					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 5					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 6					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 7					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 8					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 9					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-F	2-F	3-F	4-F
DAY 10					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 11					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 12					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 13					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 14					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 15					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 16					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 17					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 18					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%
DAY 19					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	5 100%

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: FEMALE

PERIOD		DOSE:(mg/kg) GROUP:	0 1-F	4 2-F	20 3-F	100 4-F
Day 20	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Rough Coat		0	0	0	1 20%
DAY 21						
	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Decreased Activity		0	0	0	1 20%
	Rough Coat		0	0	0	1 20%
DAY 22						
	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Decreased Activity		0	0	0	1 20%
	Hunched Posture		0	0	0	1 20%
	Rough Coat		0	0	0	1 20%
DAY 23						
	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Decreased Activity		0	0	0	1 20%
	Hunched Posture		0	0	0	1 20%
	Rough Coat		0	0	0	1 20%
DAY 24						
	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Hunched Posture		0	0	0	1 20%
	Rough Coat		0	0	0	1 20%
DAY 25						
	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Hunched Posture		0	0	0	1 20%
	Rough Coat		0	0	0	1 20%
DAY 26						
	No. Observed		5	5	5	5
	Normal		5 100%	5 100%	5 100%	4 80%
	Hunched Posture		0	0	0	1 20%
	Rough Coat		0	0	0	1 20%

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 168

SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0	4	20	100
		1-F	2-F	3-F	4-F
DAY 27					
No. Observed		5	5	5	5
Normal		5 100%	5 100%	5 100%	4 80%
Decreased Activity		0	0	0	1 20%
Hunched Posture		0	0	0	1 20%
Rough Coat		0	0	0	1 20%
DAY 28					
No. Observed		5	5	5	5
Scheduled Sacrifice		5 100%	5 100%	5 100%	5 100%

DRAFT

APPENDIX 3

Individual Body Weight and Body Weight Gain Data

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 1-M
 DOSE: 0 (mg/kg)

SEX: MALE

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
251	22.2	22.3	22.9	23.9	24.4	25.1
252	24.3	24.5	24.5	25.7	26.6	27.7
253	23.4	24.0	25.9	27.8	28.0	29.7
254	21.5	21.9	23.6	24.9	25.9	27.0
255	24.0	24.0	24.7	25.4	25.7	26.6
MEAN	23.1	23.3	24.3	25.5	26.1	27.2
S.D.	1.19	1.16	1.14	1.44	1.32	1.68
N	5	5	5	5	5	5

--: Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 1-F
 DOSE: 0 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
256	19.4	21.1	22.4	23.2	24.4	25.0
257	19.4	19.7	21.1	21.6	23.3	23.7
258	18.6	19.7	20.6	21.3	22.9	23.4
259	19.8	19.9	21.9	22.3	23.1	24.1
260	19.2	19.8	20.8	21.7	22.3	23.3
MEAN	19.3	20.0	21.4	22.0	23.2	23.9
S.D.	0.44	0.60	0.76	0.75	0.77	0.69
N	5	5	5	5	5	5

--: Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 2-M
 DOSE: 4 (mg/kg)

SEX: MALE

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
261	22.9	23.5	25.4	25.5	26.4	27.0
262	24.8	24.6	25.9	26.3	27.2	27.8
263	23.5	23.9	25.3	26.1	27.2	27.7
264	23.4	23.5	24.2	25.3	26.4	26.7
265	21.8	22.0	23.4	23.9	24.8	25.8
MEAN	23.3	23.5	24.8	25.4	26.4	27.0
S.D.	1.08	0.95	1.02	0.94	0.98	0.82
N	5	5	5	5	5	5

---: Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
RANGE-FINDING STUDY OF HALOFANTRINE
HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 2-F
DOSE: 4 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
266	19.4	19.9	21.0	21.5	22.2	23.2
267	18.6	19.6	20.6	21.2	22.7	23.6
268	18.7	19.1	20.4	20.6	21.6	22.8
269	20.1	20.8	22.0	22.5	23.6	24.5
270	19.7	19.7	20.3	21.8	22.5	23.9
MEAN	19.3	19.8	20.9	21.5	22.5	23.6
S.D.	0.64	0.62	0.69	0.70	0.73	0.65
N	5	5	5	5	5	5

--: Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 3-M
 DOSE: 20 (mg/kg)

SEX: MALE

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
271	24.5	24.5	25.5	25.8	27.1	27.7
272	21.1	22.6	24.3	24.6	25.7	26.0
273	22.7	23.7	24.5	24.2	25.4	26.0
274	23.1	23.5	25.5	25.4	26.3	27.4
275	23.6	24.5	25.9	26.9	28.7	29.9
MEAN	23.0	23.8	25.1	25.4	26.6	27.4
S.D.	1.26	0.79	0.70	1.06	1.32	1.60
N	5	5	5	5	5	5

--: Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 3-F

SEX: FEMALE

DOSE: 20 (mg/kg)

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
276	19.3	19.4	20.9	21.5	22.1	23.4
277	18.7	19.2	20.0	21.0	22.2	23.1
278	18.9	19.6	21.4	22.2	22.6	23.5
279	19.9	20.6	21.8	22.3	23.7	24.4
280	19.5	19.6	21.6	21.8	21.9	22.9
MEAN	19.3	19.7	21.1	21.8	22.5	23.5
S.D.	0.48	0.54	0.72	0.53	0.72	0.58
N	5	5	5	5	5	5

--- Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 4-M

SEX: MALE

DOSE: 100 (mg/kg)

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
281	22.6	23.2	24.7	22.4	d	d
282	23.4	23.5	25.4	18.5	c	c
283	24.4	25.5	26.8	20.7	d	d
284	23.6	23.1	25.1	19.4	d	d
285	21.9	21.7	23.0	20.8	d	d
MEAN	23.2	23.4	25.0	20.4	--	--
S.D.	0.96	1.36	1.37	1.49	--	--
N	5	5	5	5	0	0

--: Data Unavailable c: Animal Found Dead d: Sacrificed Moribund

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY: 168

GROUP: 4-F

SEX: FEMALE

DOSE: 100 (mg/kg)

ANIMAL #	DAY -2	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
286	19.7	19.8	21.3	22.5	23.0	22.9
287	19.3	19.8	21.2	21.3	22.5	21.8
288	18.7	19.6	21.1	21.7	22.7	21.3
289	18.8	18.7	19.9	19.5	18.4	18.4
290	19.9	20.0	21.1	21.3	21.8	21.6
MEAN	19.3	19.6	20.9	21.3	21.7	21.2
S.D.	0.53	0.51	0.58	1.10	1.89	1.68
N	5	5	5	5	5	5

---: Data Unavailable

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams) ^a

STUDY: 168

GROUP: 1-M
 DOSE: 0 (mg/kg)

SEX: MALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
251	0.6	1.0	0.5	0.7	2.8
252	0.0	1.2	0.9	1.1	3.2
253	1.9	1.9	0.2	1.7	5.7
254	1.7	1.3	1.0	1.1	5.1
255	0.7	0.7	0.3	0.9	2.6
MEAN	1.0	1.2	0.6	1.1	3.9
S.D.	0.80	0.44	0.36	0.37	1.42
N	5	5	5	5	5

--: Data Unavailable b: Scheduled Sacrifice

^a Successive periods

^c Baseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams)^a

STUDY: 168

GROUP: 1-F
 DOSE: 0 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
256	1.3	0.8	1.2	0.6	3.9
257	1.4	0.5	1.7	0.4	4.0
258	0.9	0.7	1.6	0.5	3.7
259	2.0	0.4	0.8	1.0	4.2
260	1.0	0.9	0.6	1.0	3.5
MEAN	1.3	0.7	1.2	0.7	3.9
S.D.	0.43	0.21	0.48	0.28	0.27
N	5	5	5	5	5

--: Data Unavailable b: Scheduled Sacrifice

^aSuccessive periods

^cBaseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams) ^a

STUDY: 168

GROUP: 2-M
 DOSE: 4 (mg/kg)

SEX: MALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
261	1.9	0.1	0.9	0.6	3.5
262	1.3	0.4	0.9	0.6	3.2
263	1.4	0.8	1.1	0.5	3.8
264	0.7	1.1	1.1	0.3	3.2
265	1.4	0.5	0.9	1.0	3.8
MEAN	1.3	0.6	1.0	0.6	3.5
S.D.	0.43	0.38	0.11	0.25	0.30
N	5	5	5	5	5

--: Data Unavailable

b: Scheduled Sacrifice

^a Successive periods

^b Baseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams) ^a

STUDY: 168

GROUP: 2-F
 DOSE: 4 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
266	1.1	0.5	0.7	1.0	3.3
267	1.0	0.6	1.5	0.9	4.0
268	1.3	0.2	1.0	1.2	3.7
269	1.2	0.5	1.1	0.9	3.7
270	0.6	1.5	0.7	1.4	4.2
MEAN	1.0	0.7	1.0	1.1	3.8
S.D.	0.27	0.49	0.33	0.22	0.34
N	5	5	5	5	5

--: Data Unavailable b: Scheduled Sacrifice

^aSuccessive periods

^cBaseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams) ^a

STUDY: 168

GROUP: 3-M
 DOSE: 20 (mg/kg)

SEX: MALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
271	1.0	0.3	1.3	0.6	3.2
272	1.7	0.3	1.1	0.3	3.4
273	0.8	-0.3	1.2	0.6	2.3
274	2.0	-0.1	0.9	1.1	3.9
275	1.4	1.0	1.8	1.2	5.4
MEAN	1.4	0.2	1.3	0.8	3.6
S.D.	0.49	0.50	0.34	0.38	1.14
N	5	5	5	5	5

--: Data Unavailable b: Scheduled Sacrifice

^a Successive periods

^b Baseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams)^a

STUDY: 168

GROUP: 3-F
 DOSE: 20 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
276	1.5	0.6	0.6	1.3	4.0
277	0.8	1.0	1.2	0.9	3.9
278	1.8	0.8	0.4	0.9	3.9
279	1.2	0.5	1.4	0.7	3.8
280	2.0	0.2	0.1	1.0	3.3
MEAN	1.5	0.6	0.7	1.0	3.8
S.D.	0.48	0.30	0.55	0.22	0.28
N	5	5	5	5	5

--: Data Unavailable b: Scheduled Sacrifice

^aSuccessive periods

^bBaseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams) ^a

STUDY: 168

GROUP: 4-M
 DOSE: 100 (mg/kg)

SEX: MALE

ANIMAL #	DAY 7 ^b	DAY 14	DAY 20	DAY 28	TOTAL GAIN
----------	--------------------	--------	--------	--------	------------

281	1.5	-2.3	d	d	--
282	1.9	-6.9	c	c	--
283	1.3	-6.1	d	d	--
284	2.0	-5.7	d	d	--
285	1.3	-2.2	d	d	--

MEAN	1.6	-4.6	--	--	--
S.D.	0.33	2.22	--	--	--
N	5	5	0	0	0

--: Data Unavailable

c: Animal Found Dead

d: Sacrificed Moribund

^a Successive periods

^b Baseline is day 0

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL WEIGHT GAIN (Grams) ^a

STUDY: 168

GROUP: 4-F
 DOSE: 100 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY 7 ^c	DAY 14	DAY 20	DAY 28	TOTAL GAIN
286	1.5	1.2	0.5	-0.1	3.1
287	1.4	0.1	1.2	-0.7	2.0
288	1.5	0.6	1.0	-1.4	1.7
289	1.2	-0.4	-1.1	0.0	-0.3
290	1.1	0.2	0.5	-0.2	1.6
MEAN	1.3	0.3	0.4	-0.5	1.6
S.D.	0.18	0.60	0.90	0.58	1.23
N	5	5	5	5	5

--: Data Unavailable b: Scheduled Sacrifice

^a Successive periods

^b Baseline is day 0

DRAFT

APPENDIX 4

Individual Food Consumption Data

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams)^a

STUDY: 168

GROUP: 1-M
 DOSE: 0 (mg/kg)

SEX: MALE

ANIMAL #	DAY 0 ^b	DAY 7	DAY 14	DAY 20	DAY 28
251	7.3	3.5	6.3	3.1	3.7
252	13.3	3.5	8.7	3.2	3.3
253	3.3	3.8	4.8	4.4	4.7
254	5.3	4.1	8.9	5.1	4.6
255	3.1	4.1	10.0	3.2	3.8

MEAN	6.5	3.8	7.7	3.8	4.0
S.D.	4.19	0.30	2.13	0.90	0.61
N	5	5	5	5	5

--: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams) ^a

STUDY: 168

GROUP: 1-F
 DOSE: 0 (mg/kg)

SEX: FEMALE

ANIMAL # DAY 0 ^b DAY 7 DAY 14 DAY 20 DAY 28

256	13.9	4.5	11.7	3.5	4.4
257	5.0	5.1	8.4	5.4	6.1
258	5.7	3.7	9.1	4.2	5.5
259	7.6	3.8	7.8	3.7	3.6
260	2.7	2.5	3.5	3.5	4.6
MEAN	7.0	3.9	8.1	4.1	4.8
S.D.	4.25	0.98	2.97	0.80	0.98
N	5	5	5	5	5

--: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams)^a

STUDY: 168

GROUP: 2-M
 DOSE: ^b 4 (mg/kg)

SEX: MALE

ANIMAL #	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
261	3.8	4.0	4.7	4.1	4.4
262	3.5	3.9	9.6	3.4	3.9
263	2.7	3.6	4.3	3.7	3.7
264	13.2	4.0	6.8	3.4	3.8
265	6.2	4.1	7.2	3.6	4.1
MEAN	5.9	3.9	6.5	3.6	4.0
S.D.	4.30	0.19	2.14	0.29	0.28
N	5	5	5	5	5

--: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams) ^a

STUDY: 168

GROUP: 2-F
 DOSE: ^b 4 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY 0	DAY 7	DAY 14	DAY 20	DAY 28
266	6.1	4.8	10.1	4.8	5.1
267	6.5	5.5	6.3	4.4	3.6
268	4.3	5.3	7.5	5.4	8.1
269	3.7	3.6	3.3	2.9	4.5
270	5.3	4.0	6.5	4.4	4.9
MEAN	5.2	4.6	6.7	4.4	5.2
S.D.	1.18	0.82	2.45	0.92	1.70
N	5	5	5	5	5

---: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams)^a

STUDY: 168

GROUP: 3-M
 DOSE: 20 (mg/kg)^b

SEX: MALE

ANIMAL #	DAY 0 ^b	DAY 7	DAY 14	DAY 20	DAY 28
271	4.6	3.6	4.4	3.7	4.2
272	5.3	3.1	6.5	4.0	4.2
273	11.5	4.2	4.4	3.7	4.1
274	2.7	4.1	4.4	4.0	4.1
275	7.2	3.8	8.7	4.2	4.4
MEAN	6.3	3.8	5.7	3.9	4.2
S.D.	3.34	0.44	1.92	0.22	0.12
N	5	5	5	5	5

--: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams)^a

STUDY: 168

GROUP: 3-F
 DOSE:^b 20 (mg/kg)

SEX: FEMALE

ANIMAL #	DAY 0 ^b	DAY 7	DAY 14	DAY 20	DAY 28
276	10.3	3.9	10.2	3.2	3.9
277	2.5	4.0	4.5	3.1	3.9
278	10.4	5.0	9.8	5.0	5.1
279	4.7	5.5	9.3	4.3	4.3
280	13.8	4.7	6.3	3.3	3.5
MEAN	8.3	4.6	8.0	3.8	4.1
S.D.	4.62	0.68	2.50	0.83	0.61
N	5	5	5	5	5

---: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams)^a

STUDY: 168

GROUP: 4-M
 DOSE: 100 (mg/kg)^b

SEX: MALE

ANIMAL # DAY 0 DAY 7 DAY 14 DAY 20 DAY 28

281	7.3	3.6	6.3	d	d
282	7.5	4.4	9.5	c	c
283	10.1	3.1	4.3	d	d
284	6.5	4.6	3.8	d	d
285	4.5	3.2	3.5	d	d
MEAN	7.2	3.8	5.5	--	--
S.D.	2.02	0.69	2.50	--	--
N	5	5	5	0	0

--: Data Unavailable c: Animal Found Dead d: Sacrificed Moribund

^aInclusive intervals

^bBaseline is day -6

FOUR WEEK ORAL (GAVAGE) DOSE
 RANGE-FINDING STUDY OF HALOFANTRINE
 HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL DAILY FOOD CONSUMPTION (Grams)^a

STUDY: 168

GROUP: 4-F
 DOSE:^b 100 (mg/kg)

SEX: FEMALE

ANIMAL # DAY 0 DAY 7 DAY 14 DAY 20 DAY 28

286	6.9	5.5	7.1	3.6	2.9
287	2.7	2.6	5.1	3.8	3.7
288	7.2	3.6	5.8	3.0	3.1
289	7.2	5.4	7.8	1.3	2.1
290	4.7	4.1	5.9	4.0	3.3
MEAN	5.7	4.2	6.3	3.1	3.0
S.D.	2.00	1.23	1.09	1.09	0.59
N	5	5	5	5	5

--: Data Unavailable

^aInclusive intervals

^bBaseline is day -6

DRAFT

APPENDIX 5
Individual Clinical Chemistry Data

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Clinical Chemistry Test Directory

STUDY: 168

NO.	ABBR. UNITS	DESCRIPTION PRECISION	CALCULATED	OPERAND A	OPERAND B	---LOWER LIMIT---		---UPPER LIMIT---	
						MALE	FEMALE	MALE	FEMALE
1.	ALT IU/L	Alanine Aminotransferase Integer	NO			30	30	100	100
2.	ALKP IU/L	Alkaline Phosphatase Integer	NO			100	150	200	250
3.	CHOL mg/dL	Cholesterol Integer	NO			60	60	125	125
4.	TRIG mg/dL	Triglycerides Integer	NO			150	150	350	350
5.	BUN mg/dL	Blood Urea Nitrogen 0.0	NO			25.0	25.0	37.0	37.0
6.	GLU mg/dL	Glucose Integer	NO			100	100	200	200

(END OF REPORT)

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICEIND. ANIMAL CLINICAL CHEMISTRY REPORT BY GROUP
PERIOD: Day 28STUDY ID: 168
STUDY NO: 168

SEX: MALE

Animal ID	ALT IU/L	ALKP IU/L	CHOL mg/dL	TRIG mg/dL	BUN mg/dL	GLU mg/dL
GROUP: 1-M:0 (mg/kg/day)						
251	40	160	101	126	25.0	193
252	93	156	99	207	29.5	156
253	100	154	110	331	33.6	124
254	41	151	106	275	31.4	183
255	79	140	95	157	26.5	180
MEAN	71	152	102	219	29.2	167
SD	28.5	7.6	5.9	84.1	3.51	27.7
N	5	5	5	5	5	5

GROUP: 2-M:4 (mg/kg/day)						
261	66	147	104	225	30.1	169
262	108	169	103	175	28.3	164
263	116	143	100	204	30.5	186
264	41	155	92	211	37.2	156
265	180	118	92	312	34.8	147
MEAN	102	146	98	225	32.2	164
SD	53.2	18.7	5.8	51.7	3.68	14.7
N	5	5	5	5	5	5

GROUP: 3-M:20 (mg/kg/day)						
271	97	157	104	286	36.5	157
272	103	174	98	135	40.5	132
273	62	136	102	293	26.5	160
274	118	159	88	89	38.1	179
275	259	173	129	336	45.0	126
MEAN	128	160	104	228	37.3	151
SD	76.2	15.4	15.2	108.7	6.85	21.7
N	5	5	5	5	5	5

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

IND. ANIMAL CLINICAL CHEMISTRY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

Animal ID	ALT IU/L	ALKP IU/L	CHOL mg/dL	TRIG mg/dL	BUN mg/dL	GLU mg/dL
GROUP: 4-M:100 (mg/kg/day)						
281	--	--	--	--	--	--
282	--	--	--	--	--	--
283	--	--	--	--	--	--
284	--	--	--	--	--	--
285	--	--	--	--	--	--
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0

(--) - Data Unavailable

NA - Not Applicable

LABCAT CC4.31

28-NOV-1994

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

IND. ANIMAL CLINICAL CHEMISTRY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

Animal ID	ALT IU/L	ALKP IU/L	CHOL mg/dL	TRIG mg/dL	BUN mg/dL	GLU mg/dL
GROUP: 1-F:0 (mg/kg/day)						
256	42	174	102	254	29.0	148
257	48	191	86	178	41.6	143
258	28	195	86	169	32.7	135
259	29	186	92	119	30.7	203
260	60	183	81	84	21.9	158
MEAN	41	186	89	161	31.2	157
SD	13.4	8.0	8.0	64.6	7.11	26.8
N	5	5	5	5	5	5

GROUP: 2-F:4 (mg/kg/day)						
266	50	208	87	143	34.9	226
267	34	161	89	155	27.4	153
268	43	198	81	72	25.5	162
269	51	175	96	186	31.3	148
270	27	209	88	200	30.3	157
MEAN	41	190	88	151	29.9	169
SD	10.4	21.3	5.4	49.9	3.63	32.2
N	5	5	5	5	5	5

GROUP: 3-F:20 (mg/kg/day)						
276	42	216	99	153	17.1	150
277	62	193	88	138	36.6	147
278	43	202	91	126	20.2	142
279	79	185	82	164	27.0	141
280	65	179	87	93	17.0	148
MEAN	58	195	89	135	23.6	146
SD	15.7	14.6	6.3	27.5	8.34	3.9
N	5	5	5	5	5	5

LABCAT CC4.31

28-NOV-1994

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

IND. ANIMAL CLINICAL CHEMISTRY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

Animal ID	ALT IU/L	ALKP IU/L	CHOL mg/dL	TRIG mg/dL	BUN mg/dL	GLU mg/dL
GROUP: 4-F:100 (mg/kg/day)						
286	191	104	132	108	28.4	148
287	122	80	110	63	18.9	122
288	189	85	117	96	23.0	125
289	63	64	163	146	20.2	129
290	281	QNS	QNS	QNS	QNS	QNS
MEAN	169	83	131	103	22.6	131
SD	82.0	16.5	23.5	34.3	4.21	11.7
N	5	4	4	4	4	4

QNS - Quantity Not Sufficient

LABCAT CC4.31

28-NOV-1994

DRAFT

APPENDIX 6

Individual Hematology Data

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Hematology Test Directory

STUDY: 168

NO.	ABBR. UNITS	DESCRIPTION PRECISION	CALCULATED	OPERAND A	OPERAND B	---LOWER LIMIT---		---UPPER LIMIT---	
						MALE	FEMALE	MALE	FEMALE
1.	RBC 10 ⁶ /mm ³	Erythrocytes 0.00	NO			9.00	8.00	12.00	11.00
2.	HGB g/dL	Hemoglobin 0.0	NO			15.0	14.0	19.0	18.0
3.	HCT %	Hematocrit 0.0	NO			45.0	43.0	55.0	53.0
4.	MCV fL	Mean Corpuscular Volume 0.0	NO			45.0	45.0	55.0	55.0
5.	MCH pg	Mean Corpuscular Hemoglobin 0.0	NO			15.0	15.0	20.0	20.0
6.	MCHC g/dL	Mean Corpus. Hemo. Conc. 0.0	NO			30.0	30.0	37.0	37.0
7.	RETICS % RBCs	Reticulocytes 0.0	NO			0.0	0.0	2.0	2.0
8.	PLT 10 ³ /mm ³	Platelets Integer	NO			800	800	1300	1300
9.	WBC 10 ³ /mm ³	Leukocytes 0.0	NO			5.0	3.0	13.0	10.0

(END OF REPORT)

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Hematology Test Directory - WBC Differentials

NO.	ABBR. UNITS	DESCRIPTION PRECISION	CALCULATED	OPERAND A	OPERAND B	---LOWER LIMIT---		---UPPER LIMIT---	
						MALE	FEMALE	MALE	FEMALE
1.	NRBC #/100 WBC	Nucleated Red Cells Integer	NO			0	0	1	1
2.	M. NEUTROP 10 ³ /mm ³	Mature Neutrophils 0.0	NO			0.5	0.5	3.0	3.0
3.	I. NEUTROP 10 ³ /mm ³	Immature Neutrophils 0.0	NO			0.0	0.0	0.5	0.5
4.	LYMPHOCYTE 10 ³ /mm ³	Lymphocytes 0.0	NO			3.0	3.0	9.0	7.0
5.	MONOCYTES 10 ³ /mm ³	Monocytes 0.0	NO			0.0	0.0	0.5	0.5
6.	EOSINOPHIL 10 ³ /mm ³	Eosinophils 0.0	NO			0.0	0.0	0.5	0.5
7.	BASOPHILS 10 ³ /mm ³	Basophils 0.0	NO			0.0	0.0	0.5	0.5
8.	ATYPICAL L 10 ³ /mm ³	Atypical Lymphocytes 0.0	NO			0.0	0.0	0.5	0.5

(END OF REPORT)

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

Animal ID	RBC 10 ⁶ /mm ³	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RETICS % RBCs	NRBC #/100 WBC
GROUP: 1-M:0 (mg/kg/day)								
251	10.14	17.3	50.8	50.1	17.1	34.1	0.8	0
252	10.14	17.0	51.1	50.4	16.8	33.3	0.6	0
253	10.46	17.9	52.5	50.2	17.1	34.1	0.6	0
254	9.75	16.6	48.8	50.1	17.0	34.0	0.6	0
255	9.82	16.5	49.4	50.3	16.8	33.4	0.2	0
MEAN	10.06	17.1	50.5	50.2	17.0	33.8	0.6	0.0
SD	0.286	0.57	1.46	0.13	0.15	0.40	0.22	0.00
N	5	5	5	5	5	5	5	5

GROUP: 2-M:4 (mg/kg/day)								
261	9.72	16.2	48.7	50.1	16.7	33.3	1.0	0
262	9.97	16.8	49.9	50.1	16.9	33.7	0.8	0
263	9.46	15.9	46.8	49.5	16.8	34.0	0.3	0
264	9.51	16.1	47.6	50.1	16.9	33.8	0.2	0
265	8.97	15.6	46.0	51.3	17.4	33.9	0.8	0
MEAN	9.53	16.1	47.8	50.2	16.9	33.7	0.6	0.0
SD	0.370	0.44	1.54	0.66	0.27	0.27	0.35	0.00
N	5	5	5	5	5	5	5	5

GROUP: 3-M:20 (mg/kg/day)								
271	10.19	16.5	49.8	48.9	16.2	33.1	0.7	0
272	9.22	15.3	45.5	49.3	16.6	33.6	0.3	0
273	9.40	15.7	46.0	48.9	16.7	34.1	0.4	0
274	9.46	15.7	46.1	48.7	16.6	34.1	0.3	0
275	10.73	17.9	53.1	49.5	16.7	33.7	0.4	0
MEAN	9.80	16.2	48.1	49.1	16.6	33.7	0.4	0.0
SD	0.638	1.04	3.28	0.33	0.21	0.41	0.16	0.00
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

Animal ID	RBC 10 ⁶ /mm ³	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RETICS % RBCs	NRBC #/100 WBC
GROUP: 4-M:100 (mg/kg/day)								
281	--	--	--	--	--	--	--	--
282	--	--	--	--	--	--	--	--
283	--	--	--	--	--	--	--	--
284	--	--	--	--	--	--	--	--
285	--	--	--	--	--	--	--	--
MEAN	NA	NA	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0	0	0

WBC corrected for NRBC = or > 10
(--)- Data Unavailable

NA - Not Applicable

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

Animal ID	RBC 10 ⁶ /mm ³	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RETICS % RBCs	NRBC #/100 WBC
GROUP: 1-F:0 (mg/kg/day)								
256	9.30	16.4	48.0	51.6	17.6	34.2	0.8	0
257	9.44	16.5	48.1	51.0	17.5	34.3	0.4	0
258	9.96	17.1	50.8	51.0	17.2	33.7	1.1	0
259	8.80	15.3	45.1	51.3	17.4	33.9	0.8	0
260	9.33	16.2	47.8	51.2	17.4	33.9	0.8	0
MEAN	9.37	16.3	48.0	51.2	17.4	34.0	0.8	0.0
SD	0.414	0.65	2.02	0.25	0.15	0.24	0.25	0.00
N	5	5	5	5	5	5	5	5

GROUP: 2-F:4 (mg/kg/day)								
266	9.63	16.2	48.6	50.5	16.8	33.3	1.0	0
267	9.49	16.3	47.9	50.5	17.2	34.0	0.6	0
268	9.28	16.1	47.1	50.8	17.3	34.2	0.6	0
269	9.54	16.3	47.4	49.7	17.1	34.4	0.6	0
270	10.53	17.8	51.2	48.6	16.9	34.8	0.3	0
MEAN	9.69	16.5	48.4	50.0	17.1	34.1	0.6	0.0
SD	0.485	0.71	1.64	0.89	0.21	0.55	0.25	0.00
N	5	5	5	5	5	5	5	5

GROUP: 3-F:20 (mg/kg/day)								
276	9.82	16.5	48.9	49.8	16.8	33.7	0.7	0
277	9.59	16.2	47.8	49.8	16.9	33.9	0.6	0
278	10.35	17.3	51.8	50.0	16.7	33.4	0.3	0
279	9.02	15.5	44.8	49.7	17.2	34.6	0.8	0
280	10.26	17.3	50.6	49.3	16.9	34.2	0.2	0
MEAN	9.81	16.6	48.8	49.7	16.9	34.0	0.5	0.0
SD	0.540	0.77	2.70	0.26	0.19	0.46	0.26	0.00
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

Animal ID	RBC 10 ⁶ /mm ³	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RETICS % RBCs	NRBC #/100 WBC
GROUP: 4-F:100 (mg/kg/day)								
286	8.82	14.2	40.8	46.3	16.1	34.8	0.5	0
287	8.93	13.9	40.7	45.6	15.6	34.2	1.2	0
288	9.23	14.3	41.4	44.9	15.5	34.5	0.5	0
289	8.97	14.3	40.5	45.2	15.9	35.3	0.1	0
290	8.22	12.7	37.4	45.5	15.5	34.0	0.4	0
MEAN	8.83	13.9	40.2	45.5	15.7	34.6	0.5	0.0
SD	0.375	0.68	1.58	0.52	0.27	0.51	0.40	0.00
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 1-M : 0 (mg/kg/day)

SEX: MALE

Animal ID	Day 28
251	Polychromasia, Slight
252	Polychromasia, Slight
253	Polychromasia, Slight
254	Polychromasia, Slight
255	Polychromasia, Slight

LABCAT HE4.31

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 2-M : 4 (mg/kg/day)

SEX: MALE

Animal ID	Day 28
261	Normal Red Blood Cells
262	Polychromasia,Slight
263	Normal Red Blood Cells
264	Normal Red Blood Cells
265	Polychromasia,Slight

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 3-M : 20 (mg/kg/day)

SEX: MALE

Animal ID	Day 28
271	Polychromasia,Slight
272	Polychromasia,Slight
273	Normal Red Blood Cells
274	Normal Red Blood Cells
275	Polychromasia,Slight

LABCAT HE4.31

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 4-M : 100 (mg/kg/day)

SEX: MALE

Animal ID	Day 28
281	--
282	--
283	--
284	--
285	--

(--) - Data Unavailable

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 1-F : 0 (mg/kg/day)

SEX: FEMALE

Animal ID	Day 28
256	Polychromasia,Slight
257	Normal Red Blood Cells
258	Normal Red Blood Cells
259	Polychromasia,Slight Macrocytes,Slight
260	Polychromasia,Slight

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 2-F : 4 (mg/kg/day)

SEX: FEMALE

Animal ID	Day 28
266	Polychromasia, Moderate;Macrocytes, Moderate
267	Normal Red Blood Cells
268	Normal Red Blood Cells
269	Polychromasia,Slight
270	Normal Red Blood Cells

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 3-F : 20 (mg/kg/day)

SEX: FEMALE

Animal ID	Day 28
276	Polychromasia,Slight
277	Polychromasia,Slight
278	Polychromasia,Slight
279	Normal Red Blood Cells
280	Polychromasia,Slight

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

RBC MORPHOLOGY OBSERVATIONS

STUDY ID: 168
STUDY NO: 168

GROUP: 4-F : 100 (mg/kg/day)

SEX: FEMALE

Animal ID	Day 28
286	Normal Red Blood Cells
287	Normal Red Blood Cells
288	Normal Red Blood Cells
289	Normal Red Blood Cells
290	Polychromasia, Slight

LBCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICEINDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28STUDY ID: 168
STUDY NO: 168

SEX: MALE

Animal ID	WBC 10 ³ /mm ³	M. Neutrop 10 ³ /mm ³	I. Neutrop 10 ³ /mm ³	Lymphocyte 10 ³ /mm ³	Monocytes 10 ³ /mm ³	Eosinophil 10 ³ /mm ³	Basophils 10 ³ /mm ³	PLT 10 ³ /mm ³
GROUP: 1-M:0 (mg/kg/day)								
251	8.5	1.2	0.0	7.2	0.1	0.0	0.0	1167
252	6.1	1.0	0.0	4.8	0.3	0.1	0.0	1109
253	6.9	0.9	0.0	5.9	0.1	0.0	0.0	929
254	10.1	1.2	0.0	8.4	0.3	0.2	0.0	1237
255	8.7	1.7	0.0	6.9	0.2	0.0	0.0	1145
MEAN	8.1	1.2	0.0	6.6	0.2	0.1	0.0	1117
SD	1.58	0.31	0.00	1.36	0.10	0.09	0.00	115.2
N	5	5	5	5	5	5	5	5

GROUP: 2-M:4 (mg/kg/day)								
261	8.2	1.1	0.0	6.8	0.1	0.2	0.0	1264
262	6.8	1.3	0.0	5.4	0.0	0.1	0.0	1231
263	8.2	1.4	0.0	6.6	0.2	0.0	0.0	1243
264	5.7	0.5	0.0	5.2	0.0	0.1	0.0	1151
265	7.5	1.1	0.0	6.0	0.3	0.2	0.0	1219
MEAN	7.3	1.1	0.0	6.0	0.1	0.1	0.0	1222
SD	1.06	0.35	0.00	0.71	0.13	0.08	0.00	42.8
N	5	5	5	5	5	5	5	5

GROUP: 3-M:20 (mg/kg/day)								
271	5.8	0.6	0.0	4.8	0.1	0.2	0.0	970
272	2.0	0.3	0.0	1.7	0.0	0.0	0.0	1151
273	7.6	1.1	0.0	6.5	0.0	0.0	0.0	1108
274	2.6	0.7	0.0	1.8	0.1	0.0	0.0	1144
275	3.0	0.5	0.0	2.4	0.0	0.0	0.0	694
MEAN	4.2	0.6	0.0	3.4	0.0	0.0	0.0	1013
SD	2.40	0.30	0.00	2.12	0.05	0.09	0.00	192.9
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: MALE

Animal ID	WBC 10 ³ /mm ³	M. Neutrop 10 ³ /mm ³	I. Neutrop 10 ³ /mm ³	Lymphocyte 10 ³ /mm ³	Monocytes 10 ³ /mm ³	Eosinophil 10 ³ /mm ³	Basophils 10 ³ /mm ³	PLT 10 ³ /mm ³
GROUP: 4-M:100 (mg/kg/day)								
281	--	--	--	--	--	--	--	--
282	--	--	--	--	--	--	--	--
283	--	--	--	--	--	--	--	--
284	--	--	--	--	--	--	--	--
285	--	--	--	--	--	--	--	--
MEAN	NA	NA	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0	0	0

WBC corrected for NRBC = or > 10
(--)- Data Unavailable

NA - Not Applicable

LABCAT HE4.31

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

Animal ID	WBC 10 ³ /mm ³	M. Neutrop 10 ³ /mm ³	I. Neutrop 10 ³ /mm ³	Lymphocyte 10 ³ /mm ³	Monocytes 10 ³ /mm ³	Eosinophil 10 ³ /mm ³	Basophils 10 ³ /mm ³	PLT 10 ³ /mm ³
GROUP: 1-F:0 (mg/kg/day)								
256	7.6	0.5	0.0	7.0	0.1	0.1	0.0	963
257	5.9	0.5	0.0	5.3	0.1	0.1	0.0	1050
258	5.7	0.3	0.0	5.4	0.1	0.0	0.0	888
259	3.8	0.8	0.0	3.0	0.0	0.0	0.0	1015
260	10.1	1.7	0.0	8.2	0.1	0.1	0.0	1136
MEAN	6.6	0.8	0.0	5.8	0.1	0.1	0.0	1010
SO	2.37	0.55	0.00	1.97	0.04	0.05	0.00	93.0
N	5	5	5	5	5	5	5	5

GROUP: 2-F:4 (mg/kg/day)								
266	3.0	0.4	0.0	2.6	0.1	0.0	0.0	795
267	8.7	1.6	0.0	6.9	0.0	0.3	0.0	1123
268	6.8	1.0	0.0	5.5	0.2	0.1	0.0	1071
269	5.6	1.1	0.0	4.4	0.1	0.1	0.0	915
270	6.0	0.6	0.0	5.0	0.2	0.1	0.0	872
MEAN	6.0	0.9	0.0	4.9	0.1	0.1	0.0	955
SO	2.07	0.47	0.00	1.57	0.08	0.11	0.00	137.6
N	5	5	5	5	5	5	5	5

GROUP: 3-F:20 (mg/kg/day)								
276	10.0	1.1	0.0	8.8	0.1	0.0	0.0	939
277	4.5	0.4	0.0	4.1	0.0	0.0	0.0	616
278	7.1	0.7	0.0	6.3	0.0	0.1	0.0	681
279	7.8	0.9	0.0	6.6	0.2	0.1	0.0	1020
280	9.2	1.1	0.0	7.6	0.3	0.2	0.0	826
MEAN	7.7	0.8	0.0	6.7	0.1	0.1	0.0	816
SO	2.13	0.30	0.00	1.74	0.13	0.08	0.00	169.6
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

D R A F T

INDIVIDUAL ANIMAL HEMATOLOGY REPORT BY GROUP
PERIOD: Day 28

STUDY ID: 168
STUDY NO: 168

SEX: FEMALE

Animal ID	WBC 10 ³ /mm ³	M. Neutrop 10 ³ /mm ³	I. Neutrop 10 ³ /mm ³	Lymphocyte 10 ³ /mm ³	Monocytes 10 ³ /mm ³	Eosinophil 10 ³ /mm ³	Basophils 10 ³ /mm ³	PLT 10 ³ /mm ³
GROUP: 4-F:100 (mg/kg/day)								
286	12.2	5.1	0.0	6.3	0.7	0.0	0.0	1481
287	9.8	3.5	0.0	5.5	0.7	0.1	0.0	1322
288	15.4	10.0	0.0	4.5	0.8	0.2	0.0	1692
289	25.9	17.6	0.0	7.0	1.3	0.0	0.0	1370
290	9.6	5.3	0.0	4.0	0.3	0.0	0.0	1414
MEAN	14.6	8.3	0.0	5.5	0.8	0.1	0.0	1456
SD	6.75	5.74	0.00	1.24	0.36	0.09	0.00	144.4
N	5	5	5	5	5	5	5	5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 1-M : 0 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
251	Nucleated Red Cells	0	
	M. Neutrophils	14.0	1.2
	I. Neutrophils	0.0	0.0
	Lymphocytes	85.0	7.2
	Monocytes	1.0	0.1
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		8.5	
252	Nucleated Red Cells	0	
	M. Neutrophils	16.0	1.0
	I. Neutrophils	0.0	0.0
	Lymphocytes	78.0	4.8
	Monocytes	5.0	0.3
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		6.1	
253	Nucleated Red Cells	0	
	M. Neutrophils	13.0	0.9
	I. Neutrophils	0.0	0.0
	Lymphocytes	86.0	5.9
	Monocytes	1.0	0.1
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		6.9	
254	Nucleated Red Cells	0	
	M. Neutrophils	12.0	1.2
	I. Neutrophils	0.0	0.0
	Lymphocytes	83.0	8.4
	Monocytes	3.0	0.3
	Eosinophils	2.0	0.2
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		10.1	

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 1-M : 0 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
255	Nucleated Red Cells	0	
	M. Neutrophils	19.0	1.7
	I. Neutrophils	0.0	0.0
	Lymphocytes	79.0	6.9
	Monocytes	2.0	0.2
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		8.7

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 2-M : 4 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
261	Nucleated Red Cells	0	
	M. Neutrophils	14.0	1.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	83.0	6.8
	Monocytes	1.0	0.1
	Eosinophils	2.0	0.2
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		8.2
262	Nucleated Red Cells	0	
	M. Neutrophils	19.0	1.3
	I. Neutrophils	0.0	0.0
	Lymphocytes	80.0	5.4
	Monocytes	0.0	0.0
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		6.8
263	Nucleated Red Cells	0	
	M. Neutrophils	17.0	1.4
	I. Neutrophils	0.0	0.0
	Lymphocytes	80.0	6.6
	Monocytes	3.0	0.2
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		8.2
264	Nucleated Red Cells	0	
	M. Neutrophils	8.0	0.5
	I. Neutrophils	0.0	0.0
	Lymphocytes	91.0	5.2
	Monocytes	0.0	0.0
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		5.7

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 2-M : 4 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
265	Nucleated Red Cells	0	
	M. Neutrophils	14.0	1.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	80.0	6.0
	Monocytes	4.0	0.3
	Eosinophils	2.0	0.2
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		7.5

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 3-M : 20 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
271	Nucleated Red Cells	0	
	M. Neutrophils	11.0	0.6
	I. Neutrophils	0.0	0.0
	Lymphocytes	83.0	4.8
	Monocytes	2.0	0.1
	Eosinophils	4.0	0.2
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		5.8
272	Nucleated Red Cells	0	
	M. Neutrophils	14.0	0.3
	I. Neutrophils	0.0	0.0
	Lymphocytes	85.0	1.7
	Monocytes	1.0	0.0
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		2.0
273	Nucleated Red Cells	0	
	M. Neutrophils	14.0	1.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	86.0	6.5
	Monocytes	0.0	0.0
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		7.6
274	Nucleated Red Cells	0	
	M. Neutrophils	28.0	0.7
	I. Neutrophils	0.0	0.0
	Lymphocytes	70.0	1.8
	Monocytes	2.0	0.1
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		2.6

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 3-M : 20 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
275	Nucleated Red Cells	0	
	M. Neutrophils	17.0	0.5
	I. Neutrophils	0.0	0.0
	Lymphocytes	81.0	2.4
	Monocytes	1.0	0.0
	Eosinophils	1.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		3.0

WBC corrected for NRBC = or > 10

(--) - Data Unavailable

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 4-M : 100 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
281	Nucleated Red Cells	0	
	M. Neutrophils	0	--
	I. Neutrophils	0	--
	Lymphocytes	0	--
	Monocytes	0	--
	Eosinophils	0	--
	Basophils	0	--
	Atypical Lymphocytes	0	--
WBC		--	
282	Nucleated Red Cells	0	
	M. Neutrophils	0	--
	I. Neutrophils	0	--
	Lymphocytes	0	--
	Monocytes	0	--
	Eosinophils	0	--
	Basophils	0	--
	Atypical Lymphocytes	0	--
WBC		--	
283	Nucleated Red Cells	0	
	M. Neutrophils	0	--
	I. Neutrophils	0	--
	Lymphocytes	0	--
	Monocytes	0	--
	Eosinophils	0	--
	Basophils	0	--
	Atypical Lymphocytes	0	--
WBC		--	
284	Nucleated Red Cells	0	
	M. Neutrophils	0	--
	I. Neutrophils	0	--
	Lymphocytes	0	--
	Monocytes	0	--
	Eosinophils	0	--
	Basophils	0	--
	Atypical Lymphocytes	0	--
WBC		--	

WBC corrected for NRBC = or > 10

(--) - Data Unavailable

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 4-M : 100 (mg/kg/day)

SEX: MALE

Animal ID		Day 28	
		REL	ABS
285	Nucleated Red Cells	0	
	M. Neutrophils	0	--
	I. Neutrophils	0	--
	Lymphocytes	0	--
	Monocytes	0	--
	Eosinophils	0	--
	Basophils	0	--
	Atypical Lymphocytes	0	--
	WBC		--

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 1-F : 0 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
256	Nucleated Red Cells	0	
	M. Neutrophils	6.0	0.5
	I. Neutrophils	0.0	0.0
	Lymphocytes	92.0	7.0
	Monocytes	1.0	0.1
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		7.6
257	Nucleated Red Cells	0	
	M. Neutrophils	9.0	0.5
	I. Neutrophils	0.0	0.0
	Lymphocytes	89.0	5.3
	Monocytes	1.0	0.1
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		5.9
258	Nucleated Red Cells	0	
	M. Neutrophils	5.0	0.3
	I. Neutrophils	0.0	0.0
	Lymphocytes	94.0	5.4
	Monocytes	1.0	0.1
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		5.7
259	Nucleated Red Cells	0	
	M. Neutrophils	21.0	0.8
	I. Neutrophils	0.0	0.0
	Lymphocytes	79.0	3.0
	Monocytes	0.0	0.0
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		3.8

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 1-F : 0 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
260	Nucleated Red Cells	0	
	M. Neutrophils	17.0	1.7
	I. Neutrophils	0.0	0.0
	Lymphocytes	81.0	8.2
	Monocytes	1.0	0.1
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		10.1

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

DRAFT

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 2-F : 4 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
266	Nucleated Red Cells	0	
	M. Neutrophils	13.0	0.4
	I. Neutrophils	0.0	0.0
	Lymphocytes	85.0	2.6
	Monocytes	2.0	0.1
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		3.0	
267	Nucleated Red Cells	0	
	M. Neutrophils	18.0	1.6
	I. Neutrophils	0.0	0.0
	Lymphocytes	79.0	6.9
	Monocytes	0.0	0.0
	Eosinophils	3.0	0.3
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		8.7	
268	Nucleated Red Cells	0	
	M. Neutrophils	15.0	1.0
	I. Neutrophils	0.0	0.0
	Lymphocytes	81.0	5.5
	Monocytes	3.0	0.2
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		6.8	
269	Nucleated Red Cells	0	
	M. Neutrophils	19.0	1.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	78.0	4.4
	Monocytes	2.0	0.1
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		5.6	

WBC corrected for NRBC = or > 10

LABCAT HE4.31

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 2-F : 4 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
270	Nucleated Red Cells	0	
	M. Neutrophils	10.0	0.6
	I. Neutrophils	0.0	0.0
	Lymphocytes	84.0	5.0
	Monocytes	4.0	0.2
	Eosinophils	2.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		6.0

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 3-F : 20 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
276	Nucleated Red Cells	0	
	M. Neutrophils	11.0	1.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	88.0	8.8
	Monocytes	1.0	0.1
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		10.0	
277	Nucleated Red Cells	0	
	M. Neutrophils	9.0	0.4
	I. Neutrophils	0.0	0.0
	Lymphocytes	90.0	4.1
	Monocytes	1.0	0.0
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		4.5	
278	Nucleated Red Cells	0	
	M. Neutrophils	10.0	0.7
	I. Neutrophils	0.0	0.0
	Lymphocytes	89.0	6.3
	Monocytes	0.0	0.0
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		7.1	
279	Nucleated Red Cells	0	
	M. Neutrophils	12.0	0.9
	I. Neutrophils	0.0	0.0
	Lymphocytes	85.0	6.6
	Monocytes	2.0	0.2
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
WBC		7.8	

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 3-F : 20 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
280	Nucleated Red Cells	0	
	M. Neutrophils	12.0	1.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	83.0	7.6
	Monocytes	3.0	0.3
	Eosinophils	2.0	0.2
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		9.2

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 4-F : 100 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
286	Nucleated Red Cells	0	
	M. Neutrophils	42.0	5.1
	I. Neutrophils	0.0	0.0
	Lymphocytes	52.0	6.3
	Monocytes	6.0	0.7
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		12.2
287	Nucleated Red Cells	0	
	M. Neutrophils	36.0	3.5
	I. Neutrophils	0.0	0.0
	Lymphocytes	56.0	5.5
	Monocytes	7.0	0.7
	Eosinophils	1.0	0.1
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		9.8
288	Nucleated Red Cells	0	
	M. Neutrophils	65.0	10.0
	I. Neutrophils	0.0	0.0
	Lymphocytes	29.0	4.5
	Monocytes	5.0	0.8
	Eosinophils	1.0	0.2
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		15.4
289	Nucleated Red Cells	0	
	M. Neutrophils	68.0	17.6
	I. Neutrophils	0.0	0.0
	Lymphocytes	27.0	7.0
	Monocytes	5.0	1.3
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		25.9

WBC corrected for NRBC = or > 10

LABCAT HE4.31

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

WHITE DIFFERENTIAL DATA

STUDY ID: 168
STUDY NO: 168

GROUP: 4-F : 100 (mg/kg/day)

SEX: FEMALE

Animal ID		Day 28	
		REL	ABS
290	Nucleated Red Cells	0	
	M. Neutrophils	55.0	5.3
	I. Neutrophils	0.0	0.0
	Lymphocytes	42.0	4.0
	Monocytes	3.0	0.3
	Eosinophils	0.0	0.0
	Basophils	0.0	0.0
	Atypical Lymphocytes	0.0	0.0
	WBC		9.6

WBC corrected for NRBC = or > 10

LABCAT HE4.31

DRAFT

APPENDIX 7

Individual Organ Weight Data

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: MALE

GROUP: 1-M - 0 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	251	252	253	254	255
BODY WEIGHT (G)	25.1	27.7	29.7	27.0	26.6
Brain (G)	0.464	0.484	0.478	0.458	0.462
Heart (G)	0.160	0.162	0.163	0.164	0.147
% BRAIN WEIGHT	34.48	33.47	34.10	35.81	31.82
Kidneys (G)	0.457	0.488	0.582	0.457	0.525
% BRAIN WEIGHT	98.49	100.83	121.76	99.78	113.64
Liver (G)	1.415	1.569	1.830	1.706	1.512
% BRAIN WEIGHT	304.96	324.17	382.85	372.49	327.27
Lungs/Bronchi (G)	0.206	0.340	0.313	0.243	0.228
% BRAIN WEIGHT	44.40	70.25	65.48	53.06	49.35
Spleen (G)	0.077	0.074	0.082	0.081	0.058
% BRAIN WEIGHT	16.59	15.29	17.15	17.69	12.55
Testes (G)	0.239	0.240	0.241	0.213	0.239
% BRAIN WEIGHT	51.51	49.59	50.42	46.51	51.73

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: FEMALE

GROUP: 1-F - 0 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	256	257	258	259	260
BODY WEIGHT (G)	25.0	23.7	23.4	24.1	23.3
Brain (G)	0.463	0.474	0.490	0.465	0.457
Heart (G)	0.140	0.125	0.132	0.131	0.156
% BRAIN WEIGHT	30.24	26.37	26.94	28.17	34.14
Kidneys (G)	0.368	0.342	0.380	0.345	0.327
% BRAIN WEIGHT	79.48	72.15	77.55	74.19	71.55
Liver (G)	1.539	1.477	1.483	1.343	1.354
% BRAIN WEIGHT	332.40	311.60	302.65	288.82	296.28
Lungs/Bronchi (G)	0.279	0.227	0.318	0.208	0.200
% BRAIN WEIGHT	60.26	47.89	64.90	44.73	43.76
Spleen (G)	0.107	0.093	0.098	0.102	0.112
% BRAIN WEIGHT	23.11	19.62	20.00	21.94	24.51

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: MALE

GROUP: 2-M - 4 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	261	262	263	264	265
BODY WEIGHT (G)	27.0	27.8	27.7	26.7	25.8
Brain (G)	0.494	0.484	0.474	0.478	0.456
Heart (G)	0.144	0.165	0.157	0.167	0.133
% BRAIN WEIGHT	29.15	34.09	33.12	34.94	29.17
Kidneys (G)	0.559	0.563	0.494	0.486	0.478
% BRAIN WEIGHT	113.16	116.32	104.22	101.67	104.82
Liver (G)	1.700	1.694	1.637	1.559	1.630
% BRAIN WEIGHT	344.13	350.00	345.36	326.15	357.46
Lungs/Bronchi (G)	0.279	0.257	0.195	0.277	0.261
% BRAIN WEIGHT	56.48	53.10	41.14	57.95	57.24
Spleen (G)	0.071	0.069	0.062	0.060	0.068
% BRAIN WEIGHT	14.37	14.26	13.08	12.55	14.91
Testes (G)	0.221	0.248	0.213	0.230	0.200
% BRAIN WEIGHT	44.74	51.24	44.94	48.12	43.86

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: FEMALE

GROUP: 2-F - 4 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	266	267	268	269	270
BODY WEIGHT (G)	23.2	23.6	22.8	24.5	23.9
Brain (G)	0.472	0.461	0.462	0.479	0.487
Heart (G)	0.134	0.125	0.121	0.150	0.127
% BRAIN WEIGHT	28.39	27.11	26.19	31.32	26.08
Kidneys (G)	0.396	0.326	0.342	0.359	0.352
% BRAIN WEIGHT	83.90	70.72	74.03	74.95	72.28
Liver (G)	1.368	1.471	1.299	1.372	1.426
% BRAIN WEIGHT	289.83	319.09	281.17	286.43	292.81
Lungs/Bronchi (G)	0.337	0.197	0.260	0.247	0.338
% BRAIN WEIGHT	71.40	42.73	56.28	51.57	69.40
Spleen (G)	0.103	0.099	0.095	0.087	0.076
% BRAIN WEIGHT	21.82	21.48	20.56	18.16	15.61

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: MALE

GROUP: 3-M - 20 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	271	272	273	274	275
BODY WEIGHT (G)	27.7	26.0	26.0	27.4	29.9
Brain (G)	0.467	0.475	0.457	0.489	0.483
Heart (G)	0.178	0.169	0.149	0.175	0.171
% BRAIN WEIGHT	38.12	35.58	32.60	35.79	35.40
Kidneys (G)	0.514	0.494	0.491	0.490	0.532
% BRAIN WEIGHT	110.06	104.00	107.44	100.20	110.14
Liver (G)	1.690	1.507	1.607	1.604	1.880
% BRAIN WEIGHT	361.88	317.26	351.64	328.02	389.23
Lungs/Bronchi (G)	0.302	0.216	0.269	0.229	0.365
% BRAIN WEIGHT	64.67	45.47	58.86	46.83	75.57
Spleen (G)	0.074	0.064	0.062	0.073	0.086
% BRAIN WEIGHT	15.85	13.47	13.57	14.93	17.81
Testes (G)	0.243	0.209	0.218	0.237	0.207
% BRAIN WEIGHT	52.03	44.00	47.70	48.47	42.86

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: FEMALE

GROUP: 3-F - 20 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	276	277	278	279	280
BODY WEIGHT (G)	23.4	23.1	23.5	24.4	22.9
Brain (G)	0.487	0.468	0.478	0.474	0.472
Heart (G)	0.135	0.124	0.127	0.139	0.126
% BRAIN WEIGHT	27.72	26.50	26.57	29.32	26.69
Kidneys (G)	0.359	0.331	0.326	0.391	0.343
% BRAIN WEIGHT	73.72	70.73	68.20	82.49	72.67
Liver (G)	1.434	1.260	1.341	1.540	1.267
% BRAIN WEIGHT	294.46	269.23	280.54	324.89	268.43
Lungs/Bronchi (G)	0.246	0.318	0.328	0.229	0.322
% BRAIN WEIGHT	50.51	67.95	68.62	48.31	68.22
Spleen (G)	0.091	0.104	0.099	0.091	0.089
% BRAIN WEIGHT	18.69	22.22	20.71	19.20	18.86

DRAFT

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INDIVIDUAL ORGAN WEIGHTS

STUDY: 168
SEX: FEMALE

GROUP: 4-F - 100 mg/kg/day
ALL FATES DAYS: 28-28 ALL BALANCES

ANIMAL ID: BALANCE NO.:	286	287	288	289	290
BODY WEIGHT (G)	22.9	21.8	21.3	18.4	21.6
Brain (G)	0.469	0.440	0.438	0.437	0.477
Heart (G)	0.123	0.124	0.103	0.098	0.115
% BRAIN WEIGHT	26.23	28.18	23.52	22.43	24.11
Kidneys (G)	0.354	0.360	0.336	0.320	0.393
% BRAIN WEIGHT	75.48	81.82	76.71	73.23	82.39
Liver (G)	1.562	1.376	1.414	1.442	1.538
% BRAIN WEIGHT	333.05	312.73	322.83	329.98	322.43
Lungs/Bronchi (G)	0.207	0.218	0.194	0.198	0.252
% BRAIN WEIGHT	44.14	49.55	44.29	45.31	52.83
Spleen (G)	0.115	0.116	0.110	0.124	0.170
% BRAIN WEIGHT	24.52	26.36	25.11	28.38	35.64

DRAFT

APPENDIX 8
Pathology Report

DRAFT

DRAFT PATHOLOGY REPORT FOR
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
UIC/TRL STUDY NUMBER 168

PREPARED
BY
PATHOLOGY ASSOCIATES, INC.
2201 WEST CAMPBELL PARK DRIVE, SUITE 327
CHICAGO, IL 60612

FOR
TOXICOLOGY RESEARCH LABORATORY (M/C 868)
DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF ILLINOIS AT CHICAGO
COLLEGE OF MEDICINE
1940 WEST TAYLOR STREET
CHICAGO, IL 60612-7353

DECEMBER 13, 1994

TABLE OF CONTENTS

DRAFT

SECTION	TITLE	PAGE
I	Pathology Narrative	3
	Summary of Experimental Design (Table I)	6
	Protocol-Required Tissues, Necropsy (Table II)	6
	Protocol-Required Tissues, Histopathology (Table III)	7
	Report Codes Table	8
II	Project Summary Table	9
	Males	10
	Females	11
III	Severity Summary Table	12
	Males	13
	Females	14
IV	Tabulated Animal Data	15
	Males	16
	Females	20
V	Correlation of Gross and Microscopic (Micro) Findings	24
	Males	25
	Females	29

D R A F T

SECTION I
PATHOLOGY NARRATIVE

DRAFT PATHOLOGY REPORT

D R A F T

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

INTRODUCTION

This pathology report, submitted by Pathology Associates, Inc. (PAI) to Toxicology Research Laboratory (TRL), University of Illinois at Chicago, represents the histopathology findings for the study designated as "Four Week Oral (Gavage) Dose Range-Finding Study of Halofantrine Hydrochloride in Mice", UIC/TRL Study Number 168.

EXPERIMENTAL DESIGN AND METHODS

Four groups, each composed of 5 male and 5 female B6C3F1 (Virus Antibody Free) mice, received control or test article formulations by oral gavage. Dose groups, dose level, and animals per group are detailed in the Summary of Experimental Design (Table I). All animals received vehicle or test substance daily for at least 4 weeks.

Unscheduled necropsies were performed by TRL personnel. All animals that survived until the scheduled terminal sacrifice were sacrificed. Necropsies were performed according to TRL Standard Operating Procedures. Tissues required by the protocol for collection and fixation at necropsy were examined and fixed in 10% neutral buffered formalin (see Table II, Protocol-Required Tissues, Necropsy). Tissues required for histopathologic evaluation were trimmed, processed, and slides were prepared in accordance with PAI Standard Operating Procedures. These tissues (see Table III, Protocol-Required Tissues, Histopathology) included brain, heart, kidneys, liver, ovaries, spleen, testes, and gross lesions. These tissues were then evaluated by light microscopy.

Microscopic findings for all groups are summarized in the Project Summary Tables (Section II). The mean group severity scores are found in the Severity Summary Tables (Section III). The mean group severity scores were determined by dividing the sum of all severity scores for a finding by the number of tissues examined. Microscopic findings in the protocol-required tissues for individual animals are presented in the Tabulated Animal Data Tables (Section IV). The correlation of the necropsy findings and histopathology findings are reported in the Correlation of Gross and Microscopic (Micro) Findings (Section V). The codes used as entries in these tables are explained in the Report Codes Table.

RESULTS AND DISCUSSION

The Results and Discussion section is divided into three parts: Necropsy Findings, Diagnostic Terms, and Histopathology Findings. The Necropsy Findings portion gives lesions seen at necropsy that were test article-related. The Diagnostic Terms portion lists and clarifies diagnostic terminology that may be unclear. Terms listed in the Diagnostic Terms portion of this section were not necessarily considered to be test article-related. The Histopathology Findings portion of this section reports the results and provides discussion of the histopathologic evaluation of the tissues.

DRAFT

Necropsy Findings

All 5 male mice given 100 mg/kg/day of halofantrine HCl were either found dead or sacrificed in a moribund state on day 14 or 15 of the study. Reduced spleen size was observed at necropsy or trimming in male (4 of 5) mice given 100 mg/kg/day of halofantrine hydrochloride.

Diagnostic Terms

The morphologic characteristics of observations and lesions which require comment are presented in subsequent paragraphs to aid in the interpretation of the data.

Spleen

Lymphocytic necrosis was characterized by multiple foci of cell debris in white pulp (lymphoid follicle) regions of spleen. Lymphocytic depletion was diagnosed when the relative amount of white pulp was notably reduced. Granulopoiesis consisted of colonies of granulocytic precursors in subcapsular regions of the red pulp. Erythropoiesis was represented by an increased number of erythrocyte precursors which occurred as colonies in red pulp.

The remainder of the diagnoses used in this study were considered to be self-explanatory and were not discussed in this section.

Histopathology Findings

Spleen

Lymphocytic necrosis occurred in male (5 of 5) and female (3 of 5) mice given 100 mg/kg/day of halofantrine hydrochloride, but not in those given 0, 4, or 20 mg/kg/day of halofantrine hydrochloride. The mean group severity scores were 2.00 and 1.20 for male and female high dose mice, respectively. Also, lymphocytic depletion was observed in 1 of 5 males given 100 mg/kg/day of halofantrine hydrochloride, but not in those given 0, 4, or 20 mg/kg/day of halofantrine hydrochloride. The splenic lesions may have contributed to the death or moribund state of the 5 high dose males.

Splenic granulopoiesis only occurred in female mice (3 of 5) given 100 mg/kg/day of halofantrine hydrochloride, and was interpreted as secondary to the concurrent splenic necrosis. The mean group severity score was 1.00.

Splenic erythropoiesis was observed in both treated and untreated female mice, and was interpreted as an incidental finding.

Other Lesions

All other lesions were considered to be incidental changes not related to the test article.

CONCLUSIONS

Under the conditions of this study, administration of 100 mg/kg/day of halofantrine hydrochloride for four weeks resulted in splenic lymphocytic necrosis, lymphocytic depletion, and granulopoiesis. Based on pathology findings, the no-effect treatment level was 20 mg/kg/day.

Robert L. Morrissey, DVM, Ph.D.
Diplomate, ACVP

Date

TABLE I

DRAFT

SUMMARY OF EXPERIMENTAL DESIGN

Treatment Group	Dose Level (mg/kg/day)	Number of Males	Number of Females
1	0	5	5
2	4	5	5
3	20	5	5
4	100	5	5

TABLE II

PROTOCOL-REQUIRED TISSUES, NECROPSY

Adrenal glands	Pituitary gland
Brain	Prostate
Cecum	Salivary gland (submaxillary)
Colon	Sciatic nerve
Duodenum	Skeletal muscle
Epididymides	Skin (abdominal) with mammary gland
Esophagus	Spinal cord (thoracic)
Eyes with harderian glands	Spleen
Femur with marrow	Stomach
Gallbladder	Testes
Gross lesions	Thymus
Heart	Thyroid gland (with parathyroids)
Ileum	Tongue
Jejunum	Trachea
Kidneys	Ureter
Liver	Urinary bladder
Lungs with bronchi	Uterus
Lymph node (mesenteric)	Vagina
Ovaries	
Pancreas	

TABLE III
PROTOCOL-REQUIRED TISSUES, HISTOPATHOLOGY

DRAFT

Brain
Heart
Kidneys
Liver
Ovaries
Spleen
Testes
Gross lesions

L-113 T

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

Report Codes Table

A. Codes applying to organs

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
P	Paired organ missing
U	Tissues unsuitable for complete evaluation
S	Tissues not applicable to animal
R	Recut
*	Tissues not required by protocol

B. Codes applying to microscopic diagnoses

1	minimal
2	mild
3	moderate
4	marked
()	focal
[]	locally extensive
< >	multifocal
P	Present
B	Neoplasm, benign
M	Neoplasm, malignant without metastasis
C	Neoplasm, malignant with metastasis
X	Metastatic site (+)
I	Bilateral
L	Unilateral
-	No data entered

D R A F T

SECTION II
PROJECT SUMMARY TABLE

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

PROJECT SUMMARY

STUDY ID : TRL168
 FATE: ALL

STUDY NUMBER: TRL168

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:	1M		2M		3M		4M	
	(1)		(2)		(3)		(4)	
NUMBER OF ANIMALS:	5		5		5		5	
	#	%	#	%	#	%	#	%
BRAIN	# EX	5	5	5	5	5	5	
LIVER	# EX	5	5	5	5	5	5	
Fatty change		0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0	
SPLEEN	# EX	5	5	5	5	5	5	
Necrosis, lymphocyte		0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	5 100.0	
Depletion, lymphocyte		0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0	
HEART	# EX	5	5	5	5	5	5	
KIDNEY	# EX	5	5	5	5	5	5	
TESTES	# EX	5	5	5	5	5	5	

Incidence Calculated by No. of Tissues Scored

(1) - 0 mg/kg/day

(2) - 4 mg/kg/day

(3) - 20 mg/kg/day

(4) - 100 mg/kg/day

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

PROJECT SUMMARY

STUDY ID : TRL168

STUDY NUMBER: TRL168

FATE: ALL

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:	1F (1)	2F (2)	3F (3)	4F (4)
NUMBER OF ANIMALS:	5	5	5	5
	# %	# %	# %	# %
BRAIN	# EX 5	5	5	5
LIVER	# EX 5	5	5	5
Inflammation, chronic	0 0.0	1 20.0	0 0.0	1 20.0
Granulopoiesis	0 0.0	0 0.0	0 0.0	1 20.0
SPLEEN	# EX 5	5	5	5
Necrosis, lymphocyte	0 0.0	0 0.0	0 0.0	3 60.0
Erythropoiesis	3 60.0	4 80.0	3 60.0	5 100.0
Granulopoiesis	0 0.0	0 0.0	0 0.0	3 60.0
HEART	# EX 5	5	5	5
KIDNEY	# EX 5	5	5	5
OVARY	# EX 5	5	5	5

Incidence Calculated by No. of Tissues Scored

(3) - 20 mg/kg/day

(1) - 0 mg/kg/day

(4) - 100 mg/kg/day

(2) - 4 mg/kg/day

DRAFT

SECTION III
SEVERITY SUMMARY TABLE

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

SEVERITY SUMMARY

STUDY ID : TRL168

STUDY NUMBER: TRL168

FATE: ALL

SEX: MALE

GROUP:	1M	2M	3M	4M
	(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:	5	5	5	5
BRAIN	# EX 5	# SEV 5	# SEV 5	# SEV 5
LIVER	# EX 5	# SEV 5	# SEV 5	# SEV 5
Fatty change	0 0.00	0 0.00	0 0.00	1 0.20
SPLEEN	# EX 5	# SEV 5	# SEV 5	# SEV 5
Necrosis, lymphocyte	0 0.00	0 0.00	0 0.00	5 2.00
Depletion, lymphocyte	0 0.00	0 0.00	0 0.00	1 0.60
HEART	# EX 5	# SEV 5	# SEV 5	# SEV 5
KIDNEY	# EX 5	# SEV 5	# SEV 5	# SEV 5
TESTES	# EX 5	# SEV 5	# SEV 5	# SEV 5

Severity Calculated by No. of Tissues Scored

(3) - 20 mg/kg/day

(1) - 0 mg/kg/day

(4) - 100 mg/kg/day

(2) - 4 mg/kg/day

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

SEVERITY SUMMARY

STUDY ID : TRL168

STUDY NUMBER: TRL168

FATE: ALL

SEX: FEMALE

GROUP:	1F (1)	2F (2)	3F (3)	4F (4)
NUMBER OF ANIMALS:	5	5	5	5
	# EX	# SEV	# SEV	# SEV
BRAIN	5	5	5	5
LIVER	# EX 5	5	5	5
Inflammation, chronic	0 0.00	1 0.20	0 0.00	1 0.20
Granulopoiesis	0 0.00	0 0.00	0 0.00	1 0.20
SPLEEN	# EX 5	5	5	5
Necrosis, lymphocyte	0 0.00	0 0.00	0 0.00	3 1.20
Erythropoiesis	3 0.60	4 1.20	3 0.60	5 1.40
Granulopoiesis	0 0.00	0 0.00	0 0.00	3 1.00
HEART	# EX 5	5	5	5
KIDNEY	# EX 5	5	5	5
OVARY	# EX 5	5	5	5

Severity Calculated by No. of Tissues Scored

(3) - 20 mg/kg/day

(1) - 0 mg/kg/day

(4) - 100 mg/kg/day

(2) - 4 mg/kg/day

DRAFT

SECTION IV
TABULATED ANIMAL DATA

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

TABULATED ANIMAL DATA

STUDY ID : TRL168

FATE: ALL

STUDY NUMBER: TRL168

GROUP: 1M: 0 mg/kg/day

SEX: MALE

ANIMAL ID:	0251	0252	0253	0254	0255
BRAIN	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN	N	N	N	N	N
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
TESTES	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

TABULATED ANIMAL DATA

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 2M: 4 mg/kg/day
SEX: MALE

ANIMAL ID:	0261	0262	0263	0264	0265
BRAIN	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN	N	N	N	N	N
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
TESTES	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

TABULATED ANIMAL DATA

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 3M: 20 mg/kg/day
SEX: MALE

ANIMAL ID:	0271	0272	0273	0274	0275
BRAIN	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN	N	N	N	N	N
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
TESTES	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

TABULATED ANIMAL DATA

STUDY ID : TRL168

STUDY NUMBER: TRL168

FATE: ALL

GROUP: 4M: 100 mg/kg/day

SEX: MALE

ANIMAL ID:	0281	0282	0283	0284	0285
BRAIN	N	N	N	N	N
LIVER	N		N	N	N
Fatty change	-	1	-	-	-
SPLEEN					
Necrosis, lymphocyte	2	2	2	2	2
Depletion, lymphocyte	-	3	-	-	-
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
TESTES	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

 TABULATED ANIMAL DATA

 STUDY ID : TRL168
 FATE: ALL

 STUDY NUMBER: TRL168
 GROUP: 1F: 0 mg/kg/day
 SEX: FEMALE

ANIMAL ID:	0256	0257	0258	0259	0260
BRAIN	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN		N	N		
Erythropoiesis	1	-	-	1	1
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
OVARY	N	N	N	N	N

 See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

 TABULATED ANIMAL DATA

STUDY ID : TRL168
 FATE: ALL

STUDY NUMBER: TRL168
 GROUP: 2F: 4 mg/kg/day
 SEX: FEMALE

ANIMAL ID:	0266	0267	0268	0269	0270
BRAIN	N	N	N	N	N
LIVER	N	N	N	N	
Inflammation, chronic	-	-	-	-	1
SPLEEN					N
Erythropoiesis	2	1	2	1	-
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
OVARY	N	N	N	N	N

 See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

 TABULATED ANIMAL DATA

STUDY ID : TRL168

STUDY NUMBER: TRL168

FATE: ALL

GROUP: 3F: 20 mg/kg/day

SEX: FEMALE

ANIMAL ID:	0276	0277	0278	0279	0280
BRAIN	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN	N	N			
Erythropoiesis	-	-	1	1	1
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
OVARY	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
 STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
 TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

TABULATED ANIMAL DATA

STUDY ID : TRL168

FATE: ALL

STUDY NUMBER: TRL168

GROUP: 4F: 100 mg/kg/day

SEX: FEMALE

ANIMAL ID:	0286	0287	0288	0289	0290
BRAIN	N	N	N	N	N
LIVER	N			N	N
Inflammation, chronic	-	1	-	-	-
Granulopoiesis	-	-	1	-	-
SPLEEN					
Necrosis, lymphocyte	3	1	-	2	-
Erythropoiesis	1	1	2	1	2
Granulopoiesis	-	-	2	2	1
HEART	N	N	N	N	N
KIDNEY	N	N	N	N	N
OVARY	N	N	N	N	N

See Reports Code Table for Symbol Definitions

DRAFT

SECTION V

CORRELATION OF GROSS AND MICROSCOPIC (MICRO) FINDINGS

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 1M: 0 mg/kg/day
SEX: MALE

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168

FATE: ALL

STUDY NUMBER: TRL168

GROUP: 2M: 4 mg/kg/day

SEX: MALE

No Gross Observations for any animal in this group

DRAFT

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 3M: 20 mg/kg/day
SEX: MALE

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 4M: 100 mg/kg/day
SEX: MALE

Animal ID: 0281
Animal Fate: Moribund sacrifice

Reference to Necropsy Record:
SPLEEN - SMALL, 9 MM X 3 MM

Related Histopathology:
SPLEEN - Necrosis, lymphocyte

Animal ID: 0282
Animal Fate: Found dead

Reference to Necropsy Record:
SPLEEN - SMALL, 11 MM X 2 MM

Related Histopathology:
SPLEEN - Necrosis, lymphocyte; SPLEEN - Depletion,
lymphocyte

Animal ID: 0283
Animal Fate: Moribund sacrifice

Reference to Necropsy Record:
SPLEEN - SMALL, 11 MM X 2 MM

Related Histopathology:
SPLEEN - Necrosis, lymphocyte

Animal ID: 0284
Animal Fate: Moribund sacrifice

Reference to Necropsy Record:
SPLEEN - SMALL

Related Histopathology:
SPLEEN - Necrosis, lymphocyte

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 1F: 0 mg/kg/day
SEX: FEMALE

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 2F: 4 mg/kg/day
SEX: FEMALE

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168
FATE: ALL

STUDY NUMBER: TRL168
GROUP: 3F: 20 mg/kg/day
SEX: FEMALE

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 168

DRAFT

CORRELATION OF GROSS & MICRO

STUDY ID : TRL168

FATE: ALL

STUDY NUMBER: TRL168

GROUP: 4F: 100 mg/kg/day

SEX: FEMALE

No Gross Observations for any animal in this group

DRAFT

APPENDIX 9
Protocol and Protocol Amendments

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

1.0 PURPOSE OF THE STUDY:

The purpose of this study is to determine the oral toxicity of halofantrine hydrochloride in B6C3F1 mice following four weeks of daily administration by gavage. The result of this study will be used to select dose levels for a 13 week oral toxicity study. This study will be conducted in accordance with the specifications of the Sponsor as described in Task Order UIC-11. The protocol for this study was approved by the UIC Animal Care Committee (Appendix 1).

2.0 SPONSOR:

- 2.1 Name: U.S. Army Medical Materiel
Development Activity
- 2.2 Address: Fort Detrick
Frederick, MD 21702-5009
- 2.3 Representative: George J. Schieferstein, Ph.D.

3.0 TESTING FACILITY:

- 3.1 Name: Toxicology Research Laboratory (TRL)
- 3.2 Address: University of Illinois at Chicago (UIC)
Department of Pharmacology
1940 W. Taylor St.
Chicago, IL 60612-7353
- 3.3 Study Director: Barry S. Levine, D.Sc., D.A.B.T.

4.0 DATES:

- 4.1 Proposed Initiation of Dosing: 09/28/94
- 4.2 Proposed Necropsy Date: 10/26/94
- 4.3 Proposed Study Completion
Date (Draft Study Report): 12/30/94

5.0 TEST ARTICLE

5.1 Name or Code No: Halofantrine HCl (WR171669)

5.2 TRL Chemical No: 1950614

5.3 Physical Description: White powder

5.4 Stability and Handling of Test Article:

5.4.1 Storage Conditions to Maintain Stability:

5.4.1.1 Temperature: Room temperature

5.4.1.2 Humidity: Ambient conditions at room temperature

5.4.1.3 Light: No requirements

5.4.1.4 Special Requirements: None

5.4.2 Special Handling Procedures: Standard safety precautions including gloves, eye protection, mask, and lab coats.

5.4.3 Log of Test Article: The amount, date, identity of person(s) removing aliquots and the purpose for which each aliquot of the test article was removed from the batch will be documented. At termination of the study, all unused test article will be returned to the Sponsor if requested.

6.0 PERSONNEL:

Study Director	Barry S. Levine, D.Sc., D.A.B.T.
Toxicologist	Clyde W. Wheeler, Ph.D.
Pathologist	Robert L. Morrissey, D.V.M., Ph.D.; D.A.C.V.P.
Clinical Veterinarian	James E. Artwohl, D.V.M., M.S., D.A.C.L.A.M.
Clinical Pathology	Maria Lang, A.H.T., C.V.T.
Lab Supervisor	Soudabeh Soura, B.S.
Lead Technician	To be documented in the raw data

7.0 TEST SYSTEM:

7.1 Species: Mouse

7.2 Strain: B6C3F1 (Virus Antibody Free)

7.3 Number and Sex: 20 Males and 20 Females

- 7.4 Age of Animals: Approximately 6-7 weeks old at dosing initiation.
- 7.5 Weight of Animals: Approximately 22 - 26 g (males) and approximately 18 - 22 g (females) at dosing initiation.
- 7.6 Source of Animals: Charles River Breeding Laboratories. The specific breeding facility will be documented in the raw data.
- 7.7 Justification for Selection of Test System: This study is being conducted to select dose levels for a 13 week toxicity study, prior to the conduct of a carcinogenicity study in mice. The mouse is a standard and accepted rodent species for carcinogenicity studies, and is specified by the Sponsor.
- 7.8 Procedure for Unique Identification of Test System: Upon arrival, each animal will be given a study-unique quarantine/pretest number. During the animal selection process, each animal will be assigned an animal number unique to it within the population making up the study. This number will appear as an ear tag and will also appear on a cage card visible on the front of each cage. The cage card will additionally contain the study number, test article identification, treatment group number and dose level. Cage cards will be color-coded as a function of treatment group. Raw data records and specimens will also be identified by the unique test animal number.
- 7.9 Housing: The animals will be housed in an AAALAC-accredited facility. Animals will be singly housed in polycarbonate cages with Anderson bed-o-cob bedding (Heinhold, Kankakee, IL) in a temperature (65-78°F) and humidity (approx. 30-70%) controlled room with a 14 hour light/10 hour dark cycle. The cage size will be adequate to house mice at the upper weight range as described in the *Guide for the Care and Use of Laboratory Animals*, DHEW (NIH) No. 86.23. All animals will be routinely transferred to clean cages once weekly.
- 7.10 Quarantine Procedure: Animals will be quarantined for approximately one week. During that time, the animals will be observed daily for signs of illness or death, and all unusual observations will be reported to the Study Director, Toxicologist or Clinical Veterinarian. Animals will be examined during quarantine and approved for use by the Clinical Veterinarian prior to being placed on test. Any sickly animals will be eliminated prior to the test animal selection process. If a selected animal appears sickly prior to initiation of treatment, it will be replaced by a healthy animal prior to initiation of treatment under the direction of the Study Director or Toxicologist. Quarantine release will be documented by the veterinarian prior to study initiation.
- 7.11 Food: Certified Rodent Chow No. 5002 (PMI Feeds, Inc. St. Louis, MO) will be provided *ad libitum* from arrival until termination.

- 7.12 Water: Tap water from an automatic watering system in which the room distribution lines are flushed daily will be provided *ad libitum* from arrival until termination. The water is not treated with additional chlorine or HCl.
- 7.13 There are no known contaminants in the feed or water which are expected to influence the study. A copy of the feed certification will be kept with the study records. The results of bimonthly comprehensive chemical analyses of Chicago water are documented in files maintained by Quality Assurance.
- 7.14 It is not known if the animals will experience pain or distress during the study. Analgesic or anesthetic agents will confound the ability to determine the toxic potential of the test article, and therefore will not be used. If an animal is in severe pain or distress, following consultation with the veterinary staff, it will be euthanized in accordance with standard operating procedures.

8.0 EXPERIMENTAL DESIGN:

8.1 Treatment Groups:

Treatment Group	Dose Level (mg/kg/day)	Number of Males	Number of Females
1	0	5	5
2	4	5	5
3	20	5	5
4	100	5	5

Dose levels are selected on the basis of subchronic toxicity data in rats following discussions with the Sponsor.

If toxicity is not observed after two weeks of treatment, the mid dose may be escalated above the high dose at that time. Additional dose level escalation may occur in order to elicit frank toxicity, and will be documented in the raw data.

- 8.2 Frequency and Route of Administration of the Test Article: The test article will be administered by gavage once daily for at least 4 weeks. Control animals will receive the test article vehicle. All animals will receive vehicle by gavage for at least 4 days during Week -1 to acclimate them to the procedure. All animals will be dosed up to and including the day prior to their scheduled necropsy. Dosing volume will be 10 ml/kg, adjusted on the basis of each animal's most recent body weight. The actual volume (ml) administered will be documented in the raw data.
- 8.3 Justification of Route(s): Oral treatment is the intended clinical route of administration and is specified by the Sponsor.

8.4 Procedure to Control Bias during the Assignment of Animals to Treatment Groups: During the quarantine/pretest period, the animals will be randomized by sex into the four groups shown in Section 8.1 using a computer-generated randomization procedure on the basis of body weight.

8.5 Test Article Vehicle: 0.5% Aqueous methylcellulose

8.6 Test Article Dosage Form Preparation and Analyses: The test and control materials will be assumed to be 100% pure for dosing calculations. Formulations will be prepared at least weekly and will be administered daily by gavage, at 10 ml/kg/day, 7 days a week. Since no adjustment for purity will be made, suspensions will be prepared on the basis of weight of the salt, halofantrine hydrochloride. The methylcellulose vehicle will be prepared at least weekly by placing the required amount of deionized water in a beaker and then adding the required amount of methylcellulose which will be weighed on an analytical balance (0.5 g of methylcellulose per 100 ml of deionized water). The same lot no. of methylcellulose will be used for the 4 and 13 week studies. The mixture will be stirred until homogeneous and then refrigerated.

A stock test article dosing suspension, which will also be used for high dose animals, will be prepared by triturating the appropriate amount of halofantrine hydrochloride with approximately one-third to one-half of the required 0.5% methylcellulose vehicle in a mortar. The mix will be transferred to a graduated cylinder, the mortar will be rinsed with vehicle and added to the graduated cylinder, and the final volume will be brought to mark with vehicle. The entire mixture will then be thoroughly mixed. The mid and low dose level suspensions will be prepared by diluting an appropriate volume of the high dose formulation with additional vehicle. All suspensions will be stored at 2 - 8°C. Approximately 10 ml reserve samples from each weekly dosing suspensions will be frozen and retained for possible analysis.

8.7 Type and Frequency of Observations, Tests, Analyses and Measurements:

8.7.1 Mortality Check: All animals will be observed for moribundity/mortality immediately prior to dosing in the morning and in the afternoon.

8.7.2 Clinical Signs: All animals will be observed for clinical signs of toxicity approximately 1 - 2 hours after dosing.

8.7.3 Clinical Observations: All animals will be subjected to a physical examination including examination of eyes and all orifices, once weekly starting in Week -1.

8.7.4 Body Weight: Body weights of all animals will be recorded weekly starting in Week -1 and at scheduled necropsy.

8.7.5 Food Consumption: Food consumption for all animals will be measured weekly starting in Week -1.

8.7.6 Clinical Pathology: Hematology and clinical chemistry parameters will be measured for all animals at necropsy. The non-fasted animals will be anesthetized by carbon dioxide inhalation (CO₂/O₂:80%/20%), and approximately 0.5 - 0.75 ml of blood will be collected from the orbital sinus to measure the following parameters. The samples will be processed in the same random order as collected.

Hematology

*Erythrocyte count and morphology	Mean corpuscular volume(MCV)
Hematocrit	Mean corpuscular hemoglobin (MCH)
Hemoglobin	Mean corpuscular hemoglobin concentration (MCHC)
Leukocyte count, total and differential	Platelet count
	Reticulocyte count

*Includes nucleated RBCs.

Clinical Chemistry

The clinical chemistry tests will be prioritized as shown on the basis of the sample volume obtained.

- | | |
|------------------------------------|-------------------|
| (1) Alanine aminotransferase (ALT) | (4) Glucose |
| (2) Alkaline phosphatase | (5) BUN |
| (3) Cholesterol | (6) Triglycerides |

8.7.8 Pathology: All animals which die on test or are sacrificed if moribund will be necropsied on that day. Surviving animals will be sacrificed and necropsied following 4 weeks of treatment. Euthanasia will be accomplished by carbon dioxide asphyxiation (CO₂/O₂:80%/20%), and an extensive necropsy will be performed under the direction and supervision of the pathologist. Terminal body weights will be collected prior to routine sacrifice. The necropsy procedure will be a thorough and systematic examination and dissection of the animal viscera and carcass, and collection and fixation of the following tissues/organs in 10% neutral buffered formalin. The ear with its attached identification tag will be saved with the wet tissues.

Adrenal glands	Ovaries
*Brain	Pancreas
Cecum	Pituitary
Colon	Prostate
Duodenum	Salivary gland (submaxillary)
Epididymides	Sciatic nerve
Esophagus	Skeletal muscle
Eyes with harderian glands	Skin (abdominal) with Mammary gland
Femur with marrow	Spinal cord (thoracic)
Gall bladder	*Spleen
Gross lesions	Stomach
*Heart	*Testes
Ileum	Thymus
Jejunum	Thyroid gland/Parathyroids
*Kidneys	Tongue
*Liver	Trachea
*Lungs/Bronchi	Ureter
Lymph node (mesenteric)	Urinary bladder
	Uterus
	Vagina

*Weighed at scheduled necropsy. Paired organs will be weighed as a unit.

The following tissues will be examined microscopically in all animals in all groups.

Brain (fore-, mid-, hind-)	Liver
Gross lesions	Ovaries
Heart	Spleen
Kidneys	Testes

- 8.7.9 Statistical Analyses: For each sex, Analysis of Variance tests will be conducted on body weight, weight gains, food consumption, hematology, clinical chemistry and organ weight data. Organ weight analysis will consider weights relative to brain weight. If a significant F ratio is obtained ($p \leq 0.05$), Dunnett's t test will be used for pair-wise comparisons to the control group.

Quantitative data will be tabulated and presented in the report. In addition to the written report, summary data tables of parameters and variability will be transmitted to the Sponsor on magnetic media (computer diskette) in "ASCII" form.

9.0 RECORDS TO BE MAINTAINED:

All data generated during the conduct study, except those that are generated as direct computer input, shall be recorded directly, promptly, and accurately in ink in bound books with prenumbered pages or on worksheets that shall be bound during or at the conclusion of the nonclinical laboratory study. All appropriate computer and machine output shall be bound during or at the conclusion of the study. All data entries shall be dated on the day of entry and signed or initialed by the person entering the data. Any changes in entries for whatever reason (e.g., to correct an error or transposition) shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or identified at the time of data input. In computer driven collection systems, the operator responsible for direct input shall be identified at the time of data input. Any changes in computer entries for whatever reason (e.g., to correct an error or transposition) shall be made in such manner so as not to obscure the original entry, if possible, shall indicate the reason for such change, and shall be dated and the responsible individual shall be identified.

All recorded data shall be reviewed, signed, and dated by a knowledgeable person, other than the person making the entry, to assure adherence to procedures and to verify observations.

Upon completion of the study and submission of the final report, all raw data, documentation, specimens, test article reserves and other materials necessary to reconstruct the study will be stored in the TRL archives maintained by Quality Assurance, unless specified by the Sponsor.

All changes or revisions, and reasons therefore, to this protocol once it is approved shall be documented, signed by the Study Director and Sponsor, dated and maintained with the protocol.

10.0 REGULATORY REQUIREMENTS:

This study will be performed within the spirit of the UIC/TRL Quality Assurance Program designed to conform with FDA Good Laboratory Practice Regulations and EPA Good Laboratory Practice Standards.

Will this study be submitted to a regulatory agency? Yes

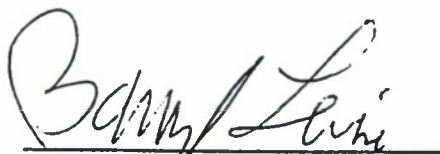
If so, to which agency(ies)? U.S. Food and Drug Administration

Does the Sponsor request that remaining test article be returned? Possibly; direction will be provided by the Sponsor.

Does the Sponsor request that samples of the test article/carrier mixture(s) be sent to the Sponsor? No

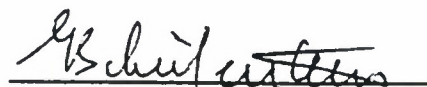
11.0 PROTOCOL APPROVAL:

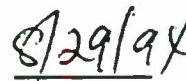
STUDY DIRECTOR:


Barry S. Levine, D.Sc., D.A.B.T.


Date

SPONSOR APPROVAL:


George Schieferstein, Ph.D.
Contracting Officer's
Representative (COR)


Date

COMMENTS FROM THE COR:

Office of the Vice Chancellor for Research (M/C 672)
310 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227
(312) 996-4995

APPENDIX 1

August 23, 1994

Barry S. Levine
Pharmacology
312 BGRC, M/C 868

Dear Dr. Levine:

The modifications requested in your correspondence of August 19, 1994 pertaining to your approved protocol ACC: #93-031-15: "Four Week Oral (Gavage) Dose Range-Finding Study of Halofantrine Hydrochloride in Mice" have been reviewed in accordance with the Animal Care and Use Policies of the University of Illinois at Chicago. You will be pleased to know that the modifications were approved on August 23, 1994 and consequently the records of Animal Care Committee will be revised to reflect these changes.

Thank you for complying with the Animal Care Policies and Procedures of UIC.

Sincerely yours,


Josephine B. Miller, Ph.D.
Chair, Animal Care Committee

JBM:st
xc: BRL

PROTOCOL AMENDMENT

Study No.: 168

Title: Four Week Oral (Gavage) Dose Range-Finding
Study of Halofantrine Hydrochloride in Mice

1. Page 1 Section 4.0

Add the study dates as follows:

4.1	<u>Proposed Initiation of Dosing:</u>	09/28/94
4.2	<u>Proposed Necropsy Dates:</u>	10/26/94
4.3	<u>Proposed Study Completion Date (Draft Study Report):</u>	12/30/94

Reason: The study dates have been finalized.

2. Page 2 Section 5.1

Include the Walter Reed identification number, "WR171669".

Reason: Clarification of the test article used in the study.

3. Page 2 Section 5.3

Add the physical description of the test article: "White powder".

Reason: Physical description of test article was provided by the Sponsor.

4. Page 2 Section 5.4

Add the following storage conditions for maintenance of stability:

5.4.1.1	<u>Temperature:</u>	Room temperature
5.4.1.2	<u>Humidity:</u>	Ambient conditions at room temperature.
5.4.1.3	<u>Light:</u>	No requirements
5.4.1.4	<u>Special Requirements:</u>	None

Reason: Storage conditions were provided by the Sponsor.

PROTOCOL AMENDMENT

Study No.: 168

Title: Four Week Oral (Gavage) Dose Range-Finding
Study of Halofantrine Hydrochloride in Mice

5. Page 2 Section 6.0

Change the Pathologist from "Michael J. Tomlinson, D.V.M., Ph.D., D.A.C.V.P." to
"Robert L. Morrissey, D.V.M., Ph.D., D.A.C.V.P.".

Reason: Dr. Tomlinson has resigned from Pathology Associates Inc. (our pathology
subcontractor).

6. Page 4 Section 8.2

Add the following sentence "All animals will receive vehicle by gavage for at least 4
days during Week -1 to acclimate them to the procedure."

Reason: Clarification of the protocol.

7. Page 7 Section 8.7.8

Change the histopathology requirements as follows by replacing the last two paragraphs
with the following clarification.

The following tissues will be examined microscopically in all animals in all groups.

Brain (fore-, mid-, hind-)	Liver
Gross lesions	Ovaries
Heart	Spleen
Kidneys	Testes

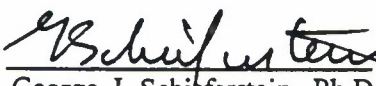
Approvals:

STUDY DIRECTOR:


Barry S. Levine, D.Sc. D.A.B.T.

10/3/94
Date

SPONSOR APPROVAL:


George J. Schieferstein, Ph.D.
Contracting Officer's
Representative (COR)

10/18/94.
Date

DRAFT

APPENDIX 10
Study Deviations

FOUR WEEK ORAL (GAVAGE) DOSE RANGE-FINDING
STUDY OF HALOFANTRINE HYDROCHLORIDE IN MICE

Study Deviations*

DRAFT

<u>Deviation Type</u>	<u>Specific Deviation</u>	<u>Effect on Study</u>
Protocol	On one occasion, the relative humidity deviated outside the specified range by -5% in the animal room.	None

*The detailed "Deviation Reports" are contained in the raw data which are archive at the University of Illinois at Chicago, Department of Pharmacology, Chicago, Illinois.

The above deviation did not affect the integrity of the study.

Barry S. Levine, D.Sc., D.A.B.T.

Date