ARMSTRONG LABORATORY

AL/OE-TR-1994-0101
WRAIR/TR-1994-0008

REPRODUCTIVE TOXICITY SCREEN OF LIQUID PROPELLANT XM46 ADMINISTERED IN THE DRINKING WATER OF SPRAGUE-DAWLEY RATS

E. R. Kinkead
R. E. Wolfe
S. A. Salins
C. D. Flemming
H. F. Leahy

MANTECH ENVIRONMENTAL TECHNOLOGY, INC.
P. O. BOX 31009
DAYTON, OH 45437

D. J. Caldwell
C. R. Miller

US ARMY MEDICAL RESEARCH DETACHMENT
WALTER REED ARMY INSTITUTE OF RESEARCH

G. B. Marit

OCCUPATIONAL AND ENVIRONMENTAL HEALTH DIRECTORATE
TOXICOLOGY DIVISION, ARMSTRONG LABORATORY
WRIGHT-PATTERSON AFB, OH 45433-7400

June 1994

FINAL REPORT FOR THE PERIOD JUNE THROUGH OCTOBER 1993

AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

NOTICES

DISCLAIMER

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1994-0101

WRAIR/TR-1994-0008

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Terry A. Childress

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1994	Final - June - October 1993

4. TITLE AND SUBTITLE
Reproductive Toxicity Screen of Liquid Propellant XM46 Administered in the Drinking Water of Sprague-Dawley Rats

5. FUNDING NUMBERS
Contract F33615-90-C-0532
PE 62202F
PR 6302
TA 630200
WU 63020002

6. AUTHOR(S)
E.R. Kinkead, R.E. Wolfe, S.A. Salins, C.D. Flemming, H.F. Leahy, D.J. Caldwell, C.R. Miller, and G.B. Marit

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER
TR-4300-94-06

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB OH 45433-7400

10. SPONSORING;MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1994-0101
WRAIR/TR-1994-0008

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Liquid propellant XM46 is being considered as a replacement for solid propellants, both as part of a regenerative injection gun system as a working fluid in an electrothermal gun system. The XM46 formulation contains hydroxylammonium nitrate, triethanolammonium nitrate, and water. Male and female Sprague-Dawley rats received XM46 drinking water containing 2.0, 1.0, 0.2, or 0.0 g XM46/L throughout a 90-day study. Mating occurred following 14 days of treatment. One half the male rats per group were necropsied after 28 days of treatment, the remaining males and all dams were necropsied following 90 days. No mortality occurred in any of the parental animals during the study. The study did not produce any adverse effects on reproduction or litter parameters. Hemolytic anemia and methemoglobinemia were common findings in both sexes of rats. Splenomegaly was found in both sexes; in male rats, as early as 28 days. Exposure via drinking water containing XM46 for 90 days did not result in any decrease in reproductive performance in male or female rats, but it did result in clinical signs of hemolytic anemia at doses as low as 17 mg/kg/day.

14. SUBJECT TERMS

Drinking Water	Hemolytic Anemia	Liquid Propellant
Methemoglobinemia	Nitrate	Reproduction
Sprague-Dawley Rat	XM46	

15. NUMBER OF PAGES
36

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

THIS PAGE INTENTIONALLY LEFT BLANK

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, OH. This document serves as a final report on the reproductive toxicity screen of liquid propellant XM46 administered in the drinking water of Sprague-Dawley rats. The research described in this report began in June 1993 and was completed in October 1993 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. A03). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was sponsored by the U.S. Army under the direction of LTC Daniel J. Caldwell, USAMRD/WRAIR, USA.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

dL	Deciliter
EMH	Extramedullary hematopoiesis
fL	Femtoliter
g	Gram
h	Hour
HAN	Hydroxylammonium nitrate
HCT	Hematocrit
HGB	Hemoglobin
in.	Inch
kg	Kilogram
L	Liter
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
mmol	Millimole
mV	Millivolt
N	Number
p	Probability
pg	Picogram
ppm	Parts per million
RBC	Red blood cells
RO	Reverse osmosis
s	seconds
SEM	Standard error of the mean
TEAN	Triethanolammonium nitrate
XM46	Liquid propellant 1846

SECTION I

INTRODUCTION

Liquid propellant 1846 (XM46) is a developmental propellant for the next generation Advanced Field Artillery System. It will be used as part of the regenerative injection gun system instead of powder charges for the 155-mm cannon and as a working fluid in an electrothermal gun system. The components of XM46 (hydroxylammonium nitrate [HAN] and triethanolammonium nitrate [TEAN]) are both strongly reducing and oxidizing agents and can react with many organic and inorganic materials (Jet Propulsion Laboratory, 1989). Impurities are generated by reaction of XM46 with components of the gun systems (e.g. metals), manufacturing intermediates, or materials introduced by improper handling after production. Decomposition leads to the formation of nitric acid and ammonium nitrate, with subsequent destabilization of the propellant mixture (Klein et al., 1991; Hansen et al., 1990).

Individuals working with XM46 have reported burning sensations and lesions within 24 h following exposure. Sensitization has been reported in one worker who handled HAN (Parmer et al., 1991). XM46 is a moderate to severe skin irritant and may cause a burning sensation immediately upon contact. If the material is not flushed, erythema will occur on contaminated areas within several hours, followed by severe dermatitis within 24 h (Weller et al., 1989).

When applied to guinea pig skin, XM46 proved to be a strong skin sensitizer. The sensitization response to XM46 may be associated with hydroxylamine, a potent skin sensitizer (Gross, 1985) and to triethylamine, also reported to be a skin sensitizer (Grosselin et al., 1984). Single- and repeat-dose dermal studies on animals have shown that XM46 and HAN penetrate the skin to produce systemic effects. A 21-day repeated dermal application of XM46 and HAN to rabbit skin produced erythrocyte destruction, Heinz body formation, anemia, spleen enlargement, and dermal necrosis (Asaki et al., 1982). Rinsing the skin within 4 h of exposure effectively reduces the severity of the skin lesions (Witt et al., 1992).

XM46 is also a strong eye irritant, producing iritis, chemosis, and corneal opacity that lasts for up to 1 week. Washing the eye 30 s after application eliminated the corneal opacity and reduced the duration and severity of the remaining effects. Washing at 10 min postapplication further reduced the symptoms, but signs of ocular irritation were still present (Justus and Korte, 1988).

Inhalation of XM46 vapor is not a hazard because the vapor decomposes almost entirely to water. Inhalation of HAN aerosol, however, may produce respiratory irritation, blood dyscrasia, and elevated methemoglobin (Snodgrass et al., 1985). Extensive genotoxicity studies have been negative (Jagannath, 1979; Balter et al., 1982; Bakke, 1990; Rudd and Lee, 1990; Blachman, 1990) and XM46 is classified as a nonmutagen.

The purpose of this study was to evaluate the potential of XM46 to produce alterations in paternal and/or maternal fertility, maternal gestation and lactation, and growth and development of offspring of Sprague-Dawley rats. The XM46 was administered to rats via drinking water.

SECTION II
MATERIALS AND METHODS

Test Compound

The XM46 formulation is a mixture of HAN (61%), TEAN (19%), and water (20%). The compound is a eutectic salt and does not exist as an aqueous solution. The XM46 formulation is acidic and has a density of 1.42 g/mL at 20 °C.

Analysis of Drinking Water Solutions

Nitrate ion concentration and pH were tested from fresh and representative stored samples of drinking water to ensure stability of diluted liquid propellant XM46. A Model 25 pH/ion meter (Fisher Scientific, Pittsburgh, PA) using a pH probe (Fisher Cat. # 13-620-285) with accompanying temperature probe (Fisher Cat. # 13-620-16) was used for pH analysis, and an Orion Model 93-07 nitrate ion probe (Orion Instruments, Boston, MA) with a companion reference electrode (Fisher, Double Junction Electrode, ISE Cat. # 13620-47) was used for nitrate analysis.

The reference buffers were pH 4.00 and 10.00 from Aldrich (Aldrich Chemical Co, Milwaukee, WI) and pH 7.00 from Fisher. The nitrate ion standards were 100 and 1000 ppm obtained from Orion; the 1 and 10 ppm standards were made by dilution of the 100 ppm standard with laboratory supplied reverse osmosis (RO) water.

The 2.0 g/L dilution of XM46 was performed while monitoring the pH. The results of this test demonstrated that the initial pH approximated that expected from the 0.1% nitric acid added to stabilize XM46, and this occurred immediately on the addition of the XM46 to the water. Little change was observed when maintained for short-term storage, and after 2 months, the change in the highest concentration solution was from a pH of 4.3 to 3.1 (Appendix A).

The nitrate ion probe measures only nitrate ion activity. Both HAN and TEAN, at the dilutions used in this study, ionized immediately and almost completely, with the molar concentration of the solutions being in the range expected from the combined concentrations. Nitrate ion activity remained constant even during long-term storage (Appendix B). Nitrate standards were run with each batch of test samples. The slopes of all curves were between -56 and -60 mV for one log difference in concentration and were linear across the range of samples and standards.

Preparation of Drinking Water Solutions

The drinking water dosing solutions were routinely prepared every 5 to 7 days, or if consumption was greatly increased (i.e., during gestation), on an as needed basis. Solutions were prepared by adding a specific volume of XM46 (calculated using density of 1.42 g/mL at 20 °C) to a known volume of water obtained from the animal drinking water system. The animal drinking water was supplied via commercial water conditioning system (Osmotics Incorporated, Minnetonka, MN). This recirculating system consists of an activated carbon filter, softener, and RO system. The water pH normally ranged between 6.5 and 7.0.

Group Assignments and Dose Levels

Group	Number of Animals: Males	Number of Animals: Females	Dose Level of XM46 (mg/L drinking water)	Target Dose Level of XM46 (mg/kg body wt/day)[a]
Control	12	12	0.0	0.0
Low	12	12	200.0	12.0
Middle	12	12	1000.0	60.0
High	12	12	2000.0	120.0

[a] Assumed daily water consumption of 30 mL per 500 g rat.

Test Animals

Fifty male and 50 female Sprague-Dawley derived outbred albino rats [Crl:CD^R(sd)BR], known as Charles River CD rats, were purchased from Charles River Breeding Laboratories, Raleigh, NC. The rats were 14 weeks of age upon arrival and 16 weeks of age at initiation of the treatment period. All rats were identified by tail tattoo and were subjected to a 2-week acclimatization period. XM46-treated water and control drinking water from an RO system and feed (Purina Formulab #5002, St. Louis, MO) were available *ad libitum*. Rodent drinking water was supplied via glass bottles equipped with stainless steel sipper tubes and neoprene stoppers. Bottles, stoppers, and sipper tubes were changed weekly. Animal room temperatures were maintained at 21 to 25 °C, and the light/dark cycle was set at 12-h intervals. Parental rats were single housed (except during the mating period) in clear plastic cages with hardwood-chip bedding (Bettachip, Northeastern Products Corp., Warrensburg, NY). During the mating period, the animals were housed in clear plastic cages with stainless steel wire bottoms. Rats were assigned to groups of 12 per sex by means of a computer-generated randomization, stratified by body weight such that the mean body weights of all groups were homogeneous by statistical analysis at study initiation.

Rats were observed twice daily for signs of toxic stress. Male rat body weights were measured weekly. Female body weights were measured in the same manner until confirmation of mating. During gestation, females were weighed on Gestation Days 0, 4, 7, 14, and 20. Dams producing litters were weighed on Days 0, 4, 7, 14, and 21 postpartum, and weekly thereafter.

Water consumption was determined during the prebreeding period for both male and female rats. Water consumption of individual dams was measured for Gestation Days 0-7, 7-14, and 14-20 and for Postpartum Days 0-7 and 7-14. Male water consumption was calculated weekly through study termination. Water consumption was not measured during the mating period when more than one rat was in a cage, nor during Days 14-21 postpartum when pups were beginning to drink from the water bottles. Water bottles were cleaned on a weekly basis, at which time, all leftover water was discarded. Water consumption was measured every 2 to 3 days; after postpartum, it was measured daily in the female rats. The live and dead pups were counted and sexed on Postpartum/Lactation Day 0. All pups were counted and sexed, and live pups were weighed on 1, 4, 7, 14, and 21 days after birth. Standardization of litter sizes, four per sex when possible, occurred on Day 4. Pups were examined for external abnormalities. Pup water consumption was not measured.

General Study Design

Six male rats per group were dosed from 14 days prior to mating and throughout the mating period for a total of 28 days. The remaining six male rats per group were dosed through the 90-day study. Female rats also began treatment 14 days prior to mating and continued through mating, gestation, postpartum (21 days), and for 3 to 4 weeks postweaning, for a total of 90 days. Pups were maintained on treated water through 3 to 4 weeks postweaning.

One male and one female, within their respective dose groups, were cohabited on Day 14. The pairs remained cohabited for up to 14 days until either a copulation plug was present or sperm were present in the vaginal wash. The day a copulation plug was present or sperm were found in the vaginal wash was defined as Day 0 of gestation.

Blood samples were taken via the vena cava from fasted parental animals at necropsy. Methemoglobin assays, measured within 1 h of blood collection, were performed using a Model IL282 CO-oximeter (Instrumentation Laboratory, Lexington, MA).

An Opto-Varimex open-field behavioral evaluation test (Columbus Instruments, Columbus, OH) was performed on the parental rats. The test was performed on male rats prior to dosing, postmating, and prior to sacrifice and on dams prior to dosing, during the postpartum period, and again prior to sacrifice.

Four test chambers were used in the Opto-Varimex test, each having an observation area of approximately 17×17.5 in., with sensors at 1-in. intervals (15 per side). Exposure sessions consisted of a single 5-min interval, conducted with the room lights turned off. Following each run, the chambers were sprayed with a disinfectant/deodorant and wiped clean.

One-half of the male rats (six per group) were necropsied following 28 treatment days; those remaining were necropsied following 90 days of treatment. The testes and epididymides were weighed. Sperm count and motility were evaluated at sacrifice. Sperm were removed from the right cauda epididymis and were analyzed microscopically using a videomicrography system (Cell

Soft Automated Semen Analyzer, Cryo Resources, Ltd., Montgomery, NY) (Toth et al., 1992). Bouin's fixative was used to fix the testes and epididymides.

Female rats were necropsied following 90 treatment days. The spleen, liver, and kidneys were removed from representative animals of both sexes and fixed in 10% buffered formalin solution. After routine processing, the tissues were embedded in paraffin and stained with hematoxylin and eosin for histopathologic examination. Pups were examined for gross lesions at necropsy.

Statistical Analysis

Maternal body weights, pup weights, organ weights, organ weight ratios, serum chemistry, hematology, and XM46 dose calculations were analyzed for statistical significance using a one-factorial analysis of variance with Bonferroni multiple comparisons (Rosner, 1990). A one-factorial repeated measures analysis of variance with Bonferroni multiple comparisons was used for water consumption and paternal body weights (Barcikowski, 1983). Mating indices and histopathologic results were analyzed by a chi-square test of proportions applied to the incidence data (Rosner, 1990). Tissue lesion severity data were analyzed using the Kruskal-Wallis analysis of variance (Rosner, 1990).

Sperm analysis and Opto-Varimex data were analyzed using one-way analysis of variance, employing Dunnett's technique (Sokal and Rohlf, 1981) for multiple comparisons between controls and treatments when significant differences ($p < 0.05$) occurred. Parametric analysis techniques were preferred, but a Kruskal-Wallis rank-based analysis of variance (Sokal and Rohlf, 1981) was used when transformation techniques failed to present a normal distribution.

SECTION III
RESULTS

General Toxicity

No mortality occurred in parental rats during the study. No treatment-related differences were noted in mean body weights of treated rats when compared to their respective control group. Water consumption (Figures 1 and 2) decreased significantly in both sexes of treated rats when treatment began and continued to be significantly less than controls throughout the 90-day study. For the duration of the study, male rats consumed approximately 30, 30, and 37 mL/day resulting in a mean dose of 136, 67, and 17 mg XM46/kg/day for the high-, mid-, and low-dose groups, respectively (Figure 3). Female rats consumed approximately 18, 22, and 25 mL/day during the premating and postweaning periods resulting in a mean dose of 140, 80, and 20 mg XM46/kg/day for the high-, mid-, and low-dose groups, respectively (Figure 4). During gestation, the dose increased to 230, 120, and 30 mg XM46/kg/day for the high-, mid-, and low-dose groups, respectively, and during lactation, the dose was 375, 220, and 50 mg XM46/kg/day for each group, respectively. Because of the wide variation in water consumption of the female rats, a median value was used to determine the overall dose of 155, 107, and 25 mg XM46/kg/day for the respective dose groups.

No clinical signs of motor skills loss were noted during the study. This was confirmed by the Opto-Varimex tests during the course of the study, where no differences in locomotor skills were found in treated or control animals.

One half of the male rats (six/group) were necropsied following the mating period (28 days treatment). Treatment-related splenomegaly (splenic enlargement) was evident. Relative spleen weights were increased by 400, 290, and 140% at the high-, mid-, and low-dose levels, respectively (Table 1). Following 90 days of treatment, the remaining male rats were sacrificed. Relative spleen weights measured at sacrifice were increased by 550, 290, and 130% at the high-, mid-, and low-dose levels, respectively. No differences were noted at either sacrifice period in absolute or relative weights of liver, kidneys, testes, epididymides, brain, or thymus.

For male rats sacrificed following mating and those sacrificed following 90-days treatment, measurements of sperm concentration, motility, and morphology of treated rats did not differ significantly from those of the controls (Table 2).

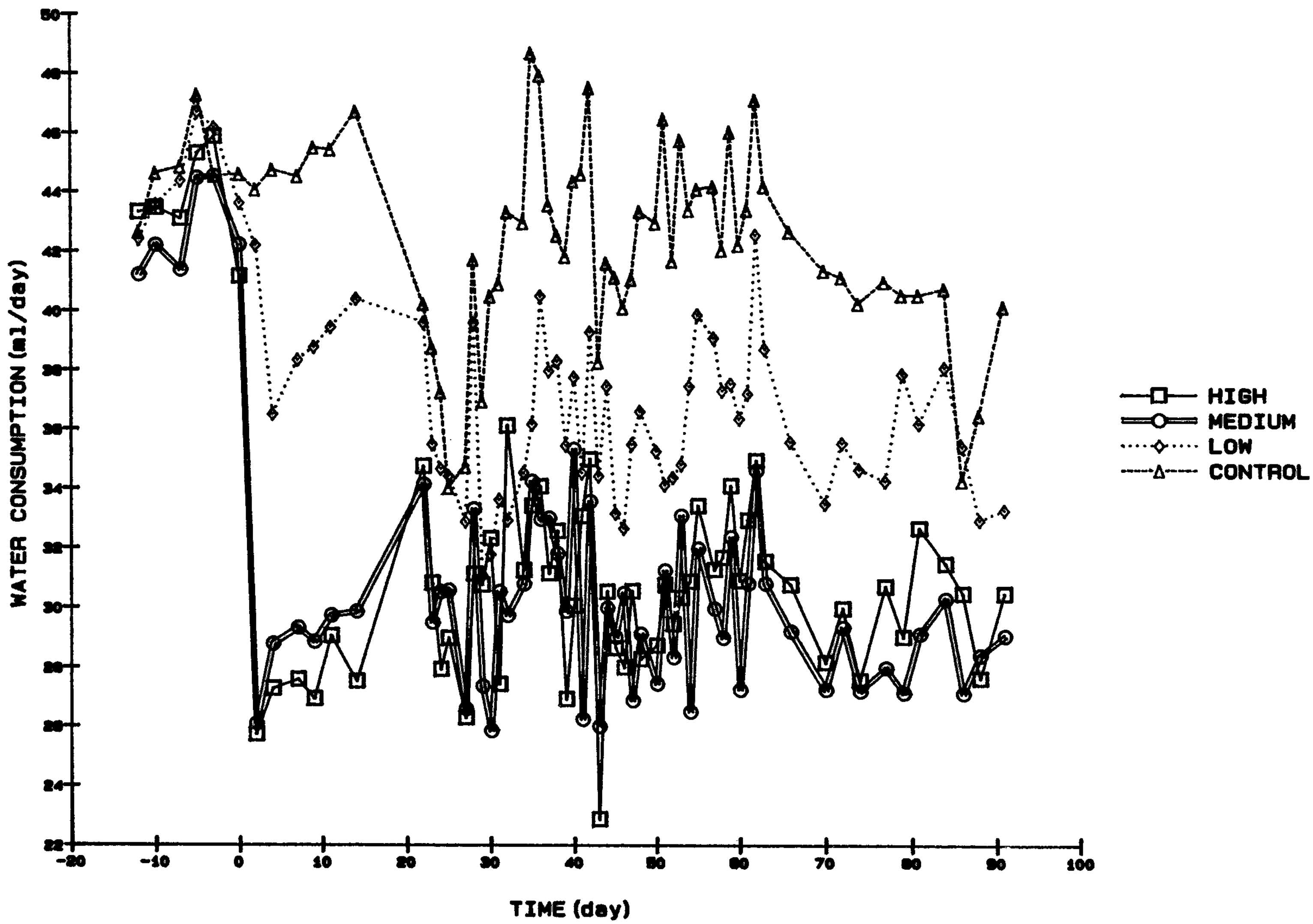

Figure 1. Mean Water Consumption of Male Rats During 90 Days of Treatment with XM46.

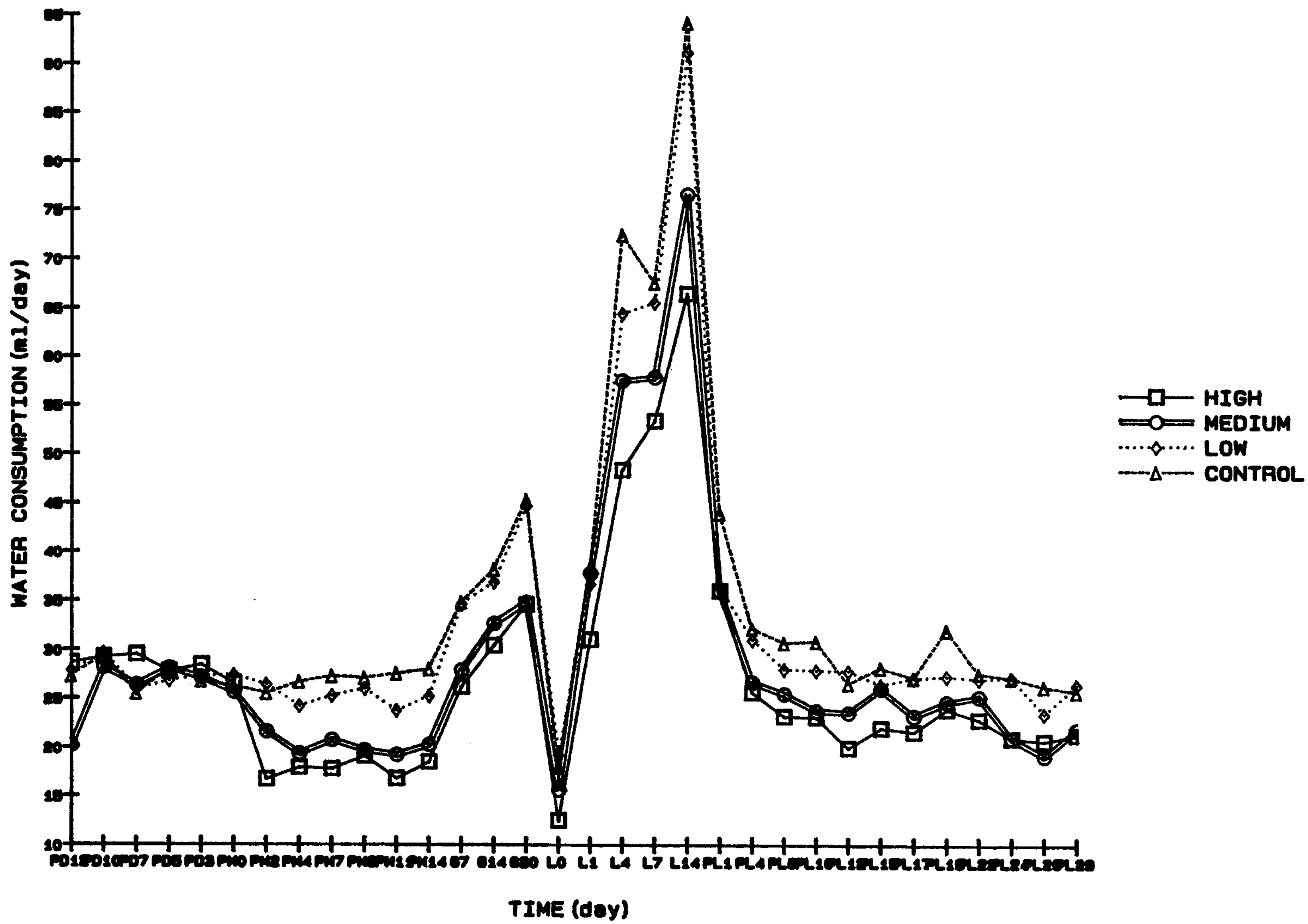

Figure 2. Mean Water Consumption of Female Rats During 90 Days of Treatment with XM46.

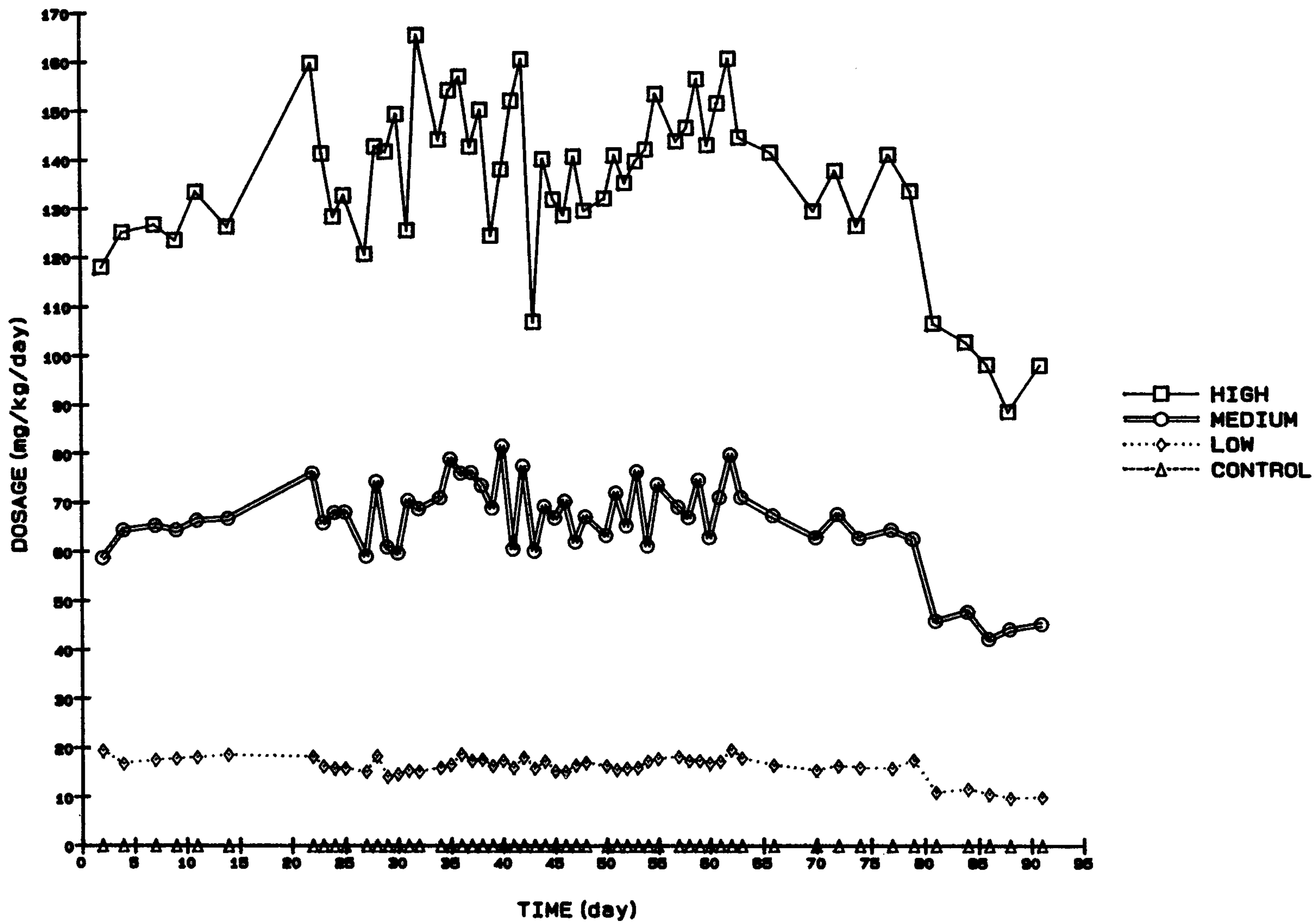

Figure 3. Mean Dose of Male Rats During 90 Days of Treatment with XM46.

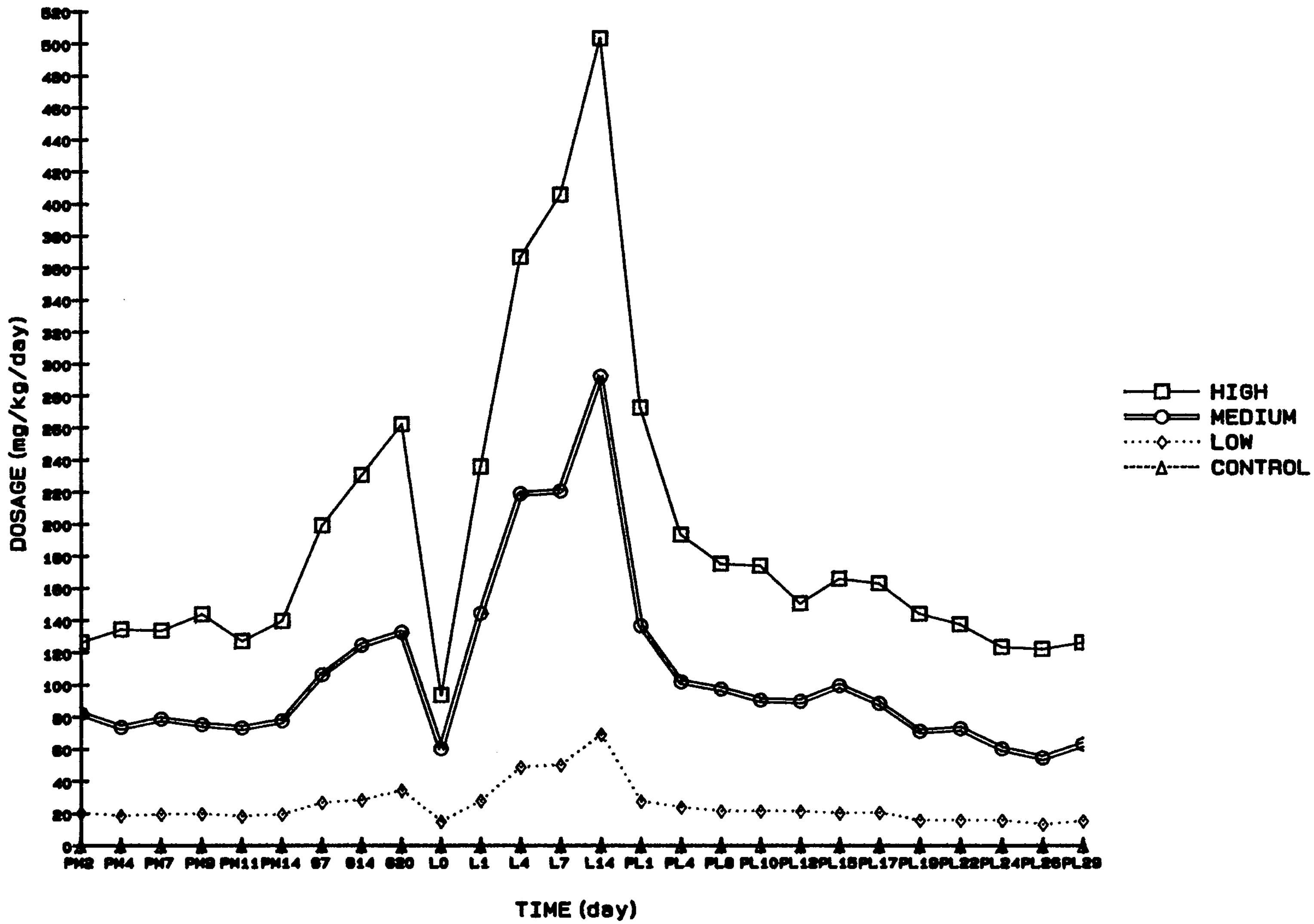

Figure 4. Mean Dose of Female Rats During 90 Days of Treatment with XM46.

TABLE 1. ABSOLUTE WEIGHT (g) AND ORGAN/BODY WEIGHT RATIOS (%) OF RATS TREATED WITH XM46[a]

Organ	Control	Low	Medium	High
		MALES[b]		
Spleen	0.90 ± 0.03	1.29 ± 0.07[c]	2.67 ± 0.19[d]	3.48 ± 0.10[d]
Ratio	0.17 ± <0.01	0.24 ± 0.01[d]	0.49 ± 0.04[d]	0.68 ± 0.02[d]
		MALES[e]		
Spleen	1.01 ± 0.07	1.34 ± 0.15	2.93 ± 0.11[d]	5.60 ± 0.81[d]
Ratio	0.15 ± 0.01	0.20 ± 0.01[c]	0.46 ± 0.02[d]	0.91 ± 0.14[d]
		FEMALES[f]		
Kidneys	2.38 ± 0.05	2.39 ± 0.04	2.57 ± 0.04[c]	2.57 ± 0.05[c]
Ratio	0.72 ± 0.01	0.71 ± 0.02	0.76 ± 0.02	0.79 ± 0.01[d]
Spleen	0.62 ± 0.02	0.81 ± 0.02[c]	2.39 ± 0.17[d]	3.96 ± 0.27[d]
Ratio	0.19 ± <0.01	0.24 ± <0.01[c]	0.71 ± 0.05[d]	1.22 ± 0.08[d]

[a] Mean ± SEM.
[b] 28 Days treatment, N=6.
[c] Statistically different from control at $p < 0.05$.
[d] Statistically different fromcontrol at $p < 0.01$.
[e] 90 Days treatment, N=6.
[f] 90 Days treatment, N=12.

TABLE 2. SPERM EVALUATIONS FROM RATS ADMINISTERED XM46 IN DRINKING WATER

Parameter	Control (N=5)	Low (N=5)	Mid (N=5)	High (N=5)
			28 Days of Treatment	
Mean (± SEM) Number Motile Cells Analyzed	312 ± 22	330 ± 42	275 ± 46	176 ± 57
Concentration Mobile (million/mL)	0.99	1.05	0.87	0.51
Mean (± SEM) Number of Cells Traveling in a Circular Pattern	68 ± 7	81 ± 9	59 ± 10	55 ± 13
Percent Cells Traveling in a Circular Pattern	13	17	12	17
Percent in Circular Pattern Compared to Total Cells	22	25	22	25
			90 Days of Treatment	
Mean (± SEM) Number Motile Cells Analyzed	343 ± 22	232 ± 42	283 ± 42	343 ± 51
Concentration Motile (million/mL)	1	0.68	0.85	1.08
Mean (± SEM) Number of Cells Traveling in a Circular Pattern	72 ± 11	45 ± 7	52 ± 16	72 ± 16
Percent Cells Traveling in a Circular Pattern	15	14	11	15
Percent in Circular Pattern Compared to Total Cells	21	20	17	20

Splenomegaly was also evident in the female rats sacrificed after 90 days of treatment (Table 1). Relative spleen weights were increased by 645, 375, and 128% in the high-, mid-, and low-dose groups, respectively. Relative kidney weights of the high-dose female rats were also significantly increased over control values. No treatment-related differences were noted in absolute or relative weights of liver, brain, or thymus.

A treatment-related decrease in red blood cells, hemoglobin, and mean corpuscular hemoglobin concentration (MCHC) occurred in both sexes of rats at the conclusion of the study. These same parameters also showed a significant decrease in male rats following 28 days of treatment. Also, a treatment-related increase occurred in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values (Tables 3 through 5). After 28 days of treatment, there were no abnormalities noted in the selected clinical chemistry parameters measured (Table 6), though there were differences between treated and control male and female rats following 90 days of treatment (Tables 7 and 8).

TABLE 3. BLOOD HEMATOLOGY VALUES[a] OF MALE RATS FOLLOWING 28 DAYS OF TREATMENT WITH XM46

Parameter[b]	Control	Low	Medium	High
WBC (10^3)	11.58 ± 0.61	12.38 ± 0.83	13.92 ± 1.18	13.23 ± 1.11
RBC (10^6)	8.00 ± 0.13	6.97 ± 0.13[c]	5.76 ± 0.30[c]	5.10 ± 0.16[c]
HGB (g/dL)	14.27 ± 0.26	12.95 ± 0.15[c]	11.82 ± 0.29[c]	11.42 ± 0.29[c]
HCT (%)	45.78 ± 0.59	43.43 ± 0.54	43.50 ± 2.59	41.13 ± 0.49
MCV (fL)	56.97 ± 0.79	62.40 ± 1.00	70.38 ± 2.83[c]	80.92 ± 2.28[c]
MCH (pg)	17.77 ± 0.16	18.60 ± 0.24	20.73 ± 0.60[c]	22.43 ± 0.46[c]
MCHC (g/dL)	31.15 ± 0.33	29.82 ± 0.24[d]	29.50 ± 0.45[c]	27.75 ± 0.43[c]
Platelets (10^3)	924 ± 35	940 ± 29	1015 ± 84	898 ± 43
Neutrophils (%)	14 ± 3	14 ± 1	19 ± 3	19 ± 1
Lymphocytes (%)	81 ± 3	81 ± 1	75 ± 3	75 ± 1
Monocytes (%)	1.4 ± 0.1	1.5 ± 0.2	2.1 ± 0.1[c]	2.8 ± 0.4[c]
Eosinophils (%)	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.1
Basophils (%)	0.6 ± 0.1	0.7 ± <0.1	0.70 ± 0.1	0.68 ± 0.06

[a] Mean ± SEM, N=6.
[b] WBC=white blood cell, RBC=red blood cell, HGB=hemoglobin, HCT=hematocrit, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, and MCHC=mean corpuscular hemoglobin concentration.
[c] Significantly different from control at $p<0.01$.
[d] Significantly different from control at $p<0.05$.

TABLE 4. BLOOD HEMATOLOGY VALUES[a] OF MALE RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46

Parameter[b]	Control	Low	Medium	High
WBC (10^3)	14.59 ± 1.04	15.34 ± 1.14	16.13 ± 1.11	16.12 ± 1.00
RBC (10^6)	8.53 ± 0.11	7.65 ± 0.10[d]	6.22 ± 0.09[c]	5.12 ± 0.18[c]
HGB (g/dL)	14.37 ± 0.28	13.37 ± 0.30	12.30 ± 0.22[c]	11.15 ± 0.15[c]
HCT (%)	45.25 ± 0.67	43.27 ± 0.74	40.28 ± 0.55	39.97 ± 1.06
MCV (fL)	53.13 ± 0.61	56.58 ± 0.93	64.85 ± 1.21[c]	78.5 ± 2.96[c]
MCH (pg)	16.85 ± 0.27	17.5 ± 0.34	19.80 ± 0.28[c]	21.88 ± 0.75[c]
MCHC (g/dL)	31.7 ± 0.25	30.9 ± 0.29	30.53 ± 0.39	27.92 ± 0.55[c]
Platelets (10^3)	738 ± 44	845 ± 37	841 ± 31	706 ± 41
Neutrophils (%)	13 ± 2	15 ± 2	14 ± 2	17 ± 1
Lymphocytes (%)	80 ± 2	79 ± 2	80 ± 2	76 ± 1
Monocytes (%)	1.8 ± 0.1	2 ± 0.2	1.8 ± 0.9	2.8 ± 0.8
Eosinophils (%)	1.2 ± 0.1	1 ± 0.1	0.8 ± 0.1	0.6 ± <0.1
Basophils (%)	0.8 ± 0.1	0.8 ± 0.1	0.80 ± 0.1	0.8 ± <0.1

[a] Mean ± SEM, N=6.
[b] WBC=white blood cell, RBC=red blood cell, HGB=hemoglobin, HCT=hematocrit, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, and MCHC=mean corpuscular hemoglobin concentration.
[c] Significantly different from control at $p<0.01$.
[d] Significantly different from control at $p<0.05$.

TABLE 5. BLOOD HEMATOLOGY VALUES[a] OF FEMALE RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46

Parameter[b]	Control	Low	Medium	High
WBC (10^3)	8.35 ± 0.69	8.13 ± 0.60	11.35 ± 0.76	12.70 ± 1.14[c]
RBC (10^6)	8.31 ± 0.09	7.30 ± 0.11[c]	5.66 ± 0.14[c]	4.52 ± 0.16[c]
HGB (g/dL)	15.30 ± 0.15	13.68 ± 0.25[c]	12.36 ± 0.24[c]	10.50 ± 0.27[c]
HCT (%)	47.18 ± 0.49	43.35 ± 0.78[c]	40.73 ± 0.59[c]	38.66 ± 0.73[c]
MCV (fL)	56.84 ± 0.56	59.41 ± 0.58	72.32 ± 1.24[c]	86.36 ± 2.09[c]
MCH (pg)	18.43 ± 0.18	18.75 ± 0.20	21.91 ± 0.24[c]	23.36 ± 0.31[c]
MCHC (g/dL)	32.43 ± 0.13	31.57 ± 0.08	30.32 ± 0.22[c]	27.14 ± 0.43[c]
Platelets (10^3)	797 ± 33	864 ± 25	801 ± 26	806 ± 32
Neutrophils (%)	12 ± 1	11 ± 1	11 ± 1	12 ± 1
Lymphocytes (%)	82 ± 2	84 ± 1	84 ± 1	82 ± 1
Monocytes (%)	2.0 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.1
Eosinophils (%)	1.4 ± 0.2	0.8 ± 0.1[d]	0.7 ± 0.1[c]	0.5 ± 0.1[c]
Basophils (%)	0.7 ± 0.1	0.5 ± <0.1	0.70 ± 0.1	0.7 ± <0.1

[a] Mean ± SEM, N=12.
[b] WBC=white blood cell, RBC=red blood cell, HGB=hemoglobin, HCT=hematocrit, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, and MCHC=mean corpuscular hemoglobin concentration.
[c] Significantly different from control at $p<0.01$.
[d] Significantly different from control at $p<0.05$.

TABLE 6. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR MALE RATS FOLLOWING 28 DAYS OF TREATMENT WITH XM46

Parameter	Control	Low	Medium	High
BUN (mg/dL)	12.92 ± 0.59	11.77 ± 0.82	12.43 ± 0.69	13.52 ± 1.31
Creatinine (mg/dL)	0.49 ± 0.03	0.51 ± 0.05	0.54 ± 0.03	0.54 ± 0.02
Calcium (mg/dL)	1.07 ± 0.03	1.17 ± 0.03	1.12 ± 0.03	1.18 ± 0.04
Total Protein (g/dL)	6.85 ± 0.10	6.40 ± 0.08	6.42 ± 0.08	6.50 ± 0.11
AST (IU/L)	106.33 ± 6.05	105.67 ± 5.00	137.67 ± 19.67	150.67 ± 9.17
ALT (IU/L)	60.67 ± 6.15	49.83 ± 2.55	59.00 ± 6.81	63.50 ± 4.85
Alkaline phosphatase (IU/L)	173.83 ± 20.85	162.83 ± 13.44	126.83 ± 13.43	169.67 ± 44.26
Albumin (g/dL)	3.50 ± 0.10	3.45 ± 0.10	3.42 ± 0.08	3.50 ± 0.11
ALB/GLOB (%)	1.05 ± 0.04	1.17 ± 0.03	1.12 ± 0.03	1.17 ± 0.03

[a]Mean ± SEM, N=6.

TABLE 7. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR MALE RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46

Parameter	Control	Low	Medium	High
BUN (mg/dL)	13.82 ± 0.94	14.4 ± 0.63	12.97 ± 0.76	14.78 ± 0.63
Creatinine (mg/dL)	0.57 ± 0.04	0.58 ± 0.02	0.6 ± 0.04	0.67 ± 0.03
Chloride (mmol/L)	100.20 ± 0.39	99.88 ± 0.36	100.32 ± 0.74	100.38 ± 0.72
Calcium (mg/dL)	6.30 ± 2.24	6.40 ± 2.28	6.34 ± 2.24	6.38 ± 2.20
Total Protein (g/dL)	5.95 ± 0.11	6.20 ± 0.05	5.82 ± 0.14	5.58 ± 0.15
Phosphorus (mg/dL)	9.08 ± 0.23	9.32 ± 0.29	9.73 ± 0.47	11.53 ± 0.86[b,c]
AST (IU/L)	175.83 ± 14.82	189.00 ± 18.87	193.83 ± 15.17	202.50 ± 15.90
ALT (IU/L)	54.50 ± 3.59	59.83 ± 9.33	51.50 ± 2.87	60.33 ± 6.87
Alkaline phosphatase (IU/L)	99.00 ± 10.10	121.67 ± 12.59	144.83 ± 19.65	93.17 ± 11.51
Albumin (g/dL)	3.33 ± 0.08	3.50 ± 0.04	3.33 ± 0.12	3.28 ± 0.10
ALB/GLOB (%)	1.25 ± 0.03	1.28 ± 0.03	1.33 ± 0.06	1.42 ± 0.04
Glucose (mg/dL)	180.00 ± 6.05	182.17 ± 15.15	164.17 ± 12.22	130.67 ± 10.75[b,e]
Sodium (mmol/L)	144.17 ± 0.65	143.5 ± 0.62	144.33 ± 0.49	144.00 ± 0.26
Triglycerides (mg/dL)	103.17 ± 19.9	176.67 ± 41.39	86.67 ± 11.78	50.83 ± 5.12
Magnesium (mg/dL)	2.66 ± 0.10	2.85 ± 0.20	3.15 ± 0.22	4.02 ± 0.22[d,e,f]
Potassium (mmol/L)	6.05 ± 0.48	5.61 ± 0.25	5.51 ± 0.18	6.17 ± 0.31
GGT (IU/L)	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
Cholesterol (mg/dL)	57.17 ± 5.92	56.17 ± 3.75	46.17 ± 1.17	46.50 ± 1.50
Total Bilirubin (mg/dL)	0.13 ± 0.02	0.15 ± 0.03	0.20 ± 0.03	0.33 ± 0.02[d,e,g]
LDH (IU/L)	518.83 ± 99.64	588.00 ± 138.43	461.00 ± 82.70	643.00 ± 161.00
CO_2 (mmol/L)	34.00 ± 0.28	33.10 ± 0.25	32.33 ± 0.49[d]	32.27 ± 0.22[d]
Uric Acid (mg/dL)	1.13 ± 0.23	1.37 ± 0.12	1.73 ± 0.19	2.57 ± 0.41[b]
Creatine Kinase (IU/L)	122.00 ± 16.76	157.00 ± 22.4	111.83 ± 17.55	124.33 ± 21.39

[a] Mean ± SEM, N=6
[b] Significantly different from control at $p<0.05$.
[c] Significantly different from low at $p<0.05$.
[d] Significantly different from control at $p<0.01$.
[e] Significantly different from low at $p<0.01$.
[f] Significantly different from medium at $p<0.05$.
[g] Significantly different from medium at $p<0.01$.

TABLE 8. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR FEMALE RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46

Parameter	Control	Low	Medium	High
BUN (mg/dL)	12.00 ± 0.99	15.44 ± 0.63	16.27 ± 1.63	15.29 ± 1.68
Creatinine (mg/dL)	0.55 ± 0.04	0.59 ± 0.03	0.61 ± 0.03	0.69 ± 0.06
Chloride (mmol/L)	102.48 ± 0.70	102.52 ± 0.54	101.78 ± 0.70	102.07 ± 0.57
Calcium (mg/dL)	11.34 ± 0.09	11.60 ± 0.08	11.64 ± 0.12	11.53 ± 0.13
Phosphorus (mg/dL)	7.40 ± 0.42	8.03 ± 0.50	8.91 ± 0.44	10.01 ± 0.65[c]
Total Protein (g/dL)	6.85 ± 0.09	7.09 ± 0.10	6.69 ± 0.14	6.58 ± 0.14
AST (IU/L)	136.50 ± 18.12	133.92 ± 9.61	140.42 ± 12.50	153.00 ± 20.65
ALT (IU/L)	58.33 ± 6.07	50.33 ± 5.75	49.92 ± 2.84	49.83 ± 5.12
Alkaline phosphatase (IU/L)	96.25 ± 11.98	70.67 ± 6.14	85.25 ± 12.06	71.33 ± 5.59
Albumin (g/dL)	4.12 ± 0.09	4.33 ± 0.15	4.30 ± 0.14	4.28 ± 0.16
ALB/GLOB (%)	1.52 ± 0.06	1.59 ± 0.09	1.82 ± 0.08	1.88 ± 0.10[b]
Glucose (mg/dL)	172.92 ± 6.12	158.58 ± 4.88	163.92 ± 8.46	139.83 ± 7.97[b]
Sodium (mmol/L)	145.00 ± 0.51	143.75 ± 0.41[b]	143.25 ± 0.39[b]	143.25 ± 0.39[b]
Triglycerides (mg/dL)	84.93 ± 12.13	70.92 ± 7.86	82.67 ± 9.34	72.17 ± 7.64
Magnesium (mg/dL)	3.31 ± 0.10	3.46 ± 0.08	3.85 ± 0.16[b]	4.49 ± 0.16[c,d,e]
Potassium (mmol/L)	5.74 ± 0.27	5.68 ± 0.19	6.12 ± 0.23	6.54 ± 0.23
GGT (IU/L)	8.00 ± 0.00	8.00 ± 0.00	8.08 ± 0.08	8.00 ± 0.00
Cholesterol (mg/dL)	61.92 ± 3.55	64.33 ± 3.42	51.33 ± 3.44	49.00 ± 1.92[b,d]
Total Bilirubin (mg/dL)	0.27 ± 0.03	0.22 ± 0.02	0.37 ± 0.04	0.58 ± 0.05[c,d,e]
LDH (IU/L)	523.67 ± 75.93	412.50 ± 43.36	443.58 ± 44.67	556.58 ± 50.57
CO_2 (mmol/L)	33.53 ± 0.35	33.41 ± 0.33	32.91 ± 0.44	32.70 ± 0.59
Uric Acid (mg/dL)	1.58 ± 0.12	1.70 ± 0.13	2.80 ± 0.21[c,d]	4.06 ± 0.19[c,d,e]
Creatine Kinase (IU/L)	77.50 ± 9.37	75.08 ± 6.05	79.17 ± 6.28	100.08 ± 9.86

[a]Mean ± SEM, N=12
[b]Significantly different from control at $p<0.05$.
[c]Significantly different from control at $p<0.01$
[d]Significantly different from low at $p<0.01$.
[e]Significantly different from medium at $p<0.01$.

Mean phosphorus, magnesium, total bilirubin, and uric acid values were significantly increased in both male and female high-dose groups. A decrease was noted in glucose in both sexes, whereas decreases in sodium and cholesterol values were noted in the female high-dose group only.

Methemoglobin concentrations measured at the conclusion of the study were higher in both male and female rats (Table 9). Among males, levels were increased by approximately 475, 425, and 200% over control values in the high-, mid-, and low-dose treatment groups, respectively. One half of the female rats (six/group) received untreated (no XM46) water for 24 h prior to sacrifice, whereas the remaining rats continued on treated water until sacrifice. Female rats receiving water that did not contain XM46 for 24 h had methemoglobin values approximately 275, 280, and 125% above control values in the high-, mid-, and low-dose treatment groups, respectively, whereas those receiving XM46-treated water until sacrifice were increased approximately 580, 450, and 175% over controls.

TABLE 9. METHEMOGLOBIN VALUES (%)[a] OF RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46

	Control	Low	Medium	High
Males	1.13 ± <0.1	2.22 ± <0.01[b]	4.93 ± <0.1[b]	5.38 ± 0.1[b]
Females	1.13 ± <0.1	2.00 ± <0.1[b]	5.23 ± <0.1[b]	6.60 ± 0.5[b]
Females[c]	1.27 ± <0.1	1.70 ± <0.1[b]	3.55 ± 0.1[b]	3.51 ± 0.2[b]

[a] Mean ± SEM, N=6.
[b] Significantly different from control at p<0.01.
[c] Animals on untreated water 24 h prior to sacrifice.

At necropsy, all rats utilized in this study were in good general condition. Splenomegaly was consistently observed in the mid- and high-dose groups of both sexes and sporadically in the low-dose male group. Discoloration/pigmentation of the kidneys ranging from dark brown to purple mottling was frequently observed in all groups including controls. Gross examination of the pups revealed lesions in only two animals, both from control dams.

Histopathology

Observed histopathologic lesions of statistical, clinical, and pathologic significance were limited to the liver, spleen, kidney, and bone marrow (Tables 10 through 12). Hemosiderosis was observed in livers at increased incidence and/or severity in the mid- and high-dose female rats after 90 days of treatment. Hepatic extramedullary hematopoiesis (EMH) was increased in the mid- and high-dose groups of both sexes. This was also observed in male rats sacrificed after 28 days of of treatment with XM46.

Hemosiderosis of the spleen was increased in all female treated groups (in severity, not incidence) and in the mid- and high-dose male groups. The incidence of splenomegaly was significantly increased in the mid- and high-dose groups of both sexes. Renal tubular pigmentation was increased in the mid- and high-dose female and high-dose male groups after 90 days of treatment. Hypercellular bone marrow (myeloid hyperplasia) also showed a significant increase in incidence in the mid- and high-dose groups of both sexes. Splenomegaly and bone marrow myeloid hyperplasia were observed in the male rats sacrificed after 28 days of treatment (Table 10).

TABLE 10. INCIDENCE SUMMARY OF SELECTED MICROSCOPIC LESIONS OF MALE RATS FOLLOWING 28 DAYS OF TREATMENT WITH XM46[a]

Organ/Lesion	Control	Low	Medium	High
Liver (N)	6	6	6	6
Hemosiderosis	0	0	0	0
(severity)	0.0	0.0	0.0	0.0
Extremedullary hematopoiesis	0	0	83[b]	100[b]
(severity)	0.0	0.0	0.8[b]	1.0[b]
Spleen (N)	6	6	6	6
Hemosiderosis	0	0	100[b]	100[b]
(severity)	0.0	0.0	1.7[b]	2.0[b]
Splenomegaly	0	33	100[b]	100[b]
(severity)	0.0	0.3	1.3[b]	2.3[b]
Kidney (N)	6	6	6	6
Tubular pigmentation	0	0	0	0
(severity)	0.0	0.0	0.0	0.0
Bone Marrow, femur (N)	6	6	6	6
Myeloid hyperplasia	0	17	100[b]	100[b]

[a]Mean grades of severity based on 0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked, and 5=severe.
[b]Statistically different from control at $p<0.01$.

TABLE 11. INCIDENCE SUMMARY OF SELECTED MICROSCOPIC LESIONS OF MALE RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46[a]

Organ/Lesion	Control	Low	Medium	High
Liver (N)	6	6	6	6
Hemosiderosis	0	0	50	100[b]
(severity)	0.0	0.0	0.5[b]	1.7[b]
Extremedullary hematopoiesis	0	17	83[c]	100[b]
(severity)	0.0	0.2	0.8[b]	1.2[b]
Spleen (N)	6	6	6	6
Hemosiderosis	67	100	100[b]	100[b]
(severity)	0.8	2.3[b]	2.7[b]	2.8[b]
Splenomegaly	0	33	100[b]	100[b]
(severity)	0.0	0.3	1.3[b]	2.7[b]
Kidney (N)	6	6	6	6
Tubular pigmentation	0	0	50	100
(severity)	0.0	0.0	0.8[b]	1.3[b]
Bone Marrow, femur (N)	6	6	6	6
Myeloid hyperplasia	0	0	50	100[b]

[a] Mean grades of severity based on 0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked, and 5=severe.
[b] Statistically different from control at $p<0.01$.
[c] Statistically different from control at $p<0.05$.

TABLE 12. INCIDENCE SUMMARY OF SELECTED MICROSCOPIC LESIONS OF FEMALE RATS FOLLOWING 90 DAYS OF TREATMENT WITH XM46[a]

Organ/Lesion	Control	Low	Medium	High
Liver (N)	12	12	12	12
Hemosiderosis	0	0	67[b]	92[b]
(severity)	0.0	0.0	0.8[b]	1.2[b]
Extremedullary hematopoiesis	0	25	83[b]	100[b]
(severity)	0.0	0.3	0.8[b]	1.1[b]
Spleen (N)	12	12	12	12
Hemosiderosis	92	100	100	100
(severity)	1.3	2.6[b]	2.2[b]	2.6[b]
Splenomegaly	0	0	100[b]	100[b]
(severity)	0.0	0.0	1.3[b]	2.8[b]
Kidney (N)	12	12	12	12
Tubular pigmentation	0	0	83[b]	100[b]
(severity)	0.0	0.0	1.1[b]	2.6[b]
Bone Marrow, femur (N)	12	12	12	12
Myeloid hyperplasia	0	8	100[b]	100[b]

[a] Mean grades of severity based on 0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked, and 5=severe.
[b] Statistically different from control at $p<0.01$.

Reproductive Indices

The treatment showed no adverse effects on mating because 100% of the animals mated (Table 13). The fertility index was 90% in groups given the control and high-dose treatment, but was 100% in the mid- and low-dose groups. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-day lactation phase, mean pup weights showed no statistically significant difference between treated and control groups.

TABLE 13. LITTER DATA FOR RATS TREATED WITH XM46

	Dose Level			
	Control	**Low**	**Medium**	**High**
No. of Mated Pairs	12	12	12	12
No. of Copulated Pairs	12	12	12	12
No. of Dams with Pups Born	11	12	12	11
No. of Dams with Pups Alive	11	12	12	11
Gestation Index (%)[a]	91.7	100.0	100.0	91.7
Live Birth Index (%)[b]	100.0	97.6	96.4	99.3
4-Day Survival Index (%)	98.8	95.7	98.1	95.1
7-Day Survival Index (%)	100.0	100.0	100.0	98.8
14-Day Survival Index (%)	100.0	98.9	100.0	100.0
21-Day Survival Index (%)	100.0	100.0	100.0	100.0
Lactation Index (%)[c]	100.0	98.9	100.0	98.8

[a] $\frac{\text{Number of females with live litters}}{\text{Number of females pregnant}}$

[b] $\frac{\text{Number of live pups at birth}}{\text{Total number of pups born}}$

[c] $\frac{\text{Number of pups surviving 21 days}}{\text{Number of pups surviving 4 days}}$

SECTION IV
DISCUSSION

Administering XM46 in the drinking water of male and female Sprague-Dawley rats produced no adverse effects on reproductive performance, litter, or pup parameters. The clinical pathology and pathologic findings in the treated rats are characteristic of nitrate poisoning (Valli, 1993) and are similar to findings in rabbits following dermal exposure to XM46 (Asaki, 1982). Significant lesions and abnormalities noted in the clinical pathology data are attributed to the effects if XM46 on erythrocytes. Exposure to high doses of nitrates can result in hemolytic anemia and icterus (Valli, 1993), though icterus was not observed in this study. Nitrates that are converted to high levels of nitrites can produce methemoglobinemia (Menzer, 1991).

Gross examination failed to reveal the brown discoloration of blood characteristic of methemoglobinemia (Valli, 1993); however, chemical analysis did reveal its presence. Icterus was not evident at necropsy and although total bilirubin tended to increase in a dose-related manner, the values were within normal reference ranges. Anemia was noted, using hemoglobin concentration and red blood cell count decreases as the basis of comparison. Females showed statistically significant dose-related decreases in hematocrit, but the values were within the normal reference range. Therefore, what is observed is a compensated hemolytic anemia (Duncan and Prasse, 1987).

When blood and tissues were collected for examination, the bone marrow had likely compensated for the hemolytic anemia. The hemolysis is interpreted as occurring extravascularly based on the gross and histologic evidence of hypersplenism and the decreased MCHC. The increased MCH and MCV and decreased MCHC indicate the presence of large numbers of circulating reticulocytes (Duncan and Prasse, 1987). Because of their larger size and the exposure period allowing for a continued maximal bone marrow response, marked reticulocytosis could elevate the hematocrit into the low normal range. Unfortunately, automated hematology analyzers do not count reticulocytes or nucleated red blood cells, and blood smears were not taken on these animals.

The increased white blood cell count (leukocytosis; primarily in the females) is attributed to an absolute neutrophilia, which is common in hemolytic conditions and may be in response to erythrocyte breakdown products or stress. The eosinopenia observed in the females is most likely induced by stress. The monocytosis observed in the males may also be due to stress, but frequently accompanies the need for phagocytic removal of abnormal red cells seen in hemolytic anemia (Duncan and Prasse, 1987). Increases in total serum bilirubin are indicative of the hemolytic breakdown. The increase in mean phosphorus and magnesium levels is also related to lyses of erythrocytes. The cause of the increase in uric acid levels is unknown. An increase in glucose levels is of questionable relevance in rats (Meeks, 1989; Loeb, 1989). Slight increases in the total bilirubin concentration with increasing dose is consistent with an extravascular hemolytic anemia (Duncan and Prasse, 1987). The consistent and at times statistically significant dose-related elevations of magnesium and phosphorus may be the result of hemolysis, although the ion in the highest concentrations in red blood cells (potassium) is only slightly elevated (Riley and Cornelius, 1989). This may be an adaptive response because potassium concentration is regulated by a sensitive homeostatic mechanism.

Gross and histological examination supports the clinical pathologic interpretations of a compensated extravascular hemolytic anemia. Two key anatomical features for this diagnosis are splenomegaly due to EMH and/or increased phagocytosis of damaged erythrocytes by splenic macrophages with resulting hemosiderosis, and a hypercellular erythroid bone marrow. These lesions are present in this study and follow a dose-response relationship that is statistically significant. Related hepatic lesions include hemosiderosis and EMH (erythroid). Another related and statistically significant change is pigmentation (hemosiderosis) of renal tubular epithelial cells.

SECTION V

REFERENCES

Asaki, A.E., L.W. Metker, J.G. Harvey, and E.A. Haight. 1982. Health hazard evaluation of liquid monopropellants, Phase 3, Range findings studies on the effects of hydroxylammonium nitrate in animals, Special Study No. 75-51-0132-84. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD.

Bakke, J. 1990. Evaluation of the potential of LP1846 liquid gun propellant to induce unscheduled DNA synthesis in the *in vitro* hepatocyte DNA repair assay. Final Report, SRI Project LSC-7662-2, SRI Study No. 7662-A01-89. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Balter, N., R. Davis, G. Cortes, M. Rosario, and B. Rivera. 1982. Determination of the acute oral LD_{50} and dominant lethal effects of HAN in mice. Omni Research, Inc., Baltimore, MD, Contract No. 82-MD-441, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD.

Barcikowski, R.S., ed. 1983. *Computer Packages and Research Design.* Lanham, MD: University Press of America.

Blachman, D. 1990. An assessment of the clastogenic potential of LP1846 utilizing the mammalian cell cytogenetics assay with Chinese hamster ovary (CHO) cells. Final Report, SRI Project No. LSC-7662-4, SRI Study No. 7662-C01-89, Project No. 89M4505. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Duncan, J.R. and K.W. Prasse. 1987. *Veterinary Laboratory Medicine: Clinical Pathology.* Iowa State University Press, Ames, IA, pp. 1-27, 236-238.

Gross, P. 1985. Biological activity of hydroxylamine: A review. *CRC Crit. Rev. Toxicol.* **14**(1):87-115.

Grosselin, R.E, R.P. Smith, and H.C. Hodge. 1984. *Clinical Toxicology of Commercial Products*. 5th Edition.

Hansen, R., E. Backoff, H.J. DeGrieff, and N. Klein. 1990. Processes for assessing the thermal stability of HAN-based liquid propellants, Final Report. U.S. Army Ballistics Research Laboratory Contractor Report 635. U.S. Army Ballistics Research Laboratory, Aberdeen Proving Ground, MD 21005.

Jagannath, D.R. 1979. Mutagenic evaluation of HAN 13.2 Batch #R149/151 in the Ames *Salmonella*/microsome plate test. Final Report, Litton Bionetics, Inc., Kensington, MD. LBI Project No. 20988, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD.

Jet Propulsion Laboratory. 1989. Safe liquid propellant technology. Report on liquid propellant logistics systems study. Phase II. JPL Report D-6876. U.S. Army Armament Research, Development and Engineering Center.

Justus, J.D. and D.W. Korte. 1988. Ocular irritation evaluation with eye wash regimen of liquid propellant 1846. Institute Report No. 299, Letterman Army Institute of Research, Presidio of San Francisco, CA.

Klein, N., C.S. Leveritt, and P.G. Baer. 1991. The effects of composition variation and nitric acid on the stability and reactivity of the HAN-based liquid propellants. U.S. Army Ballistics Research Laboratory Technical Report 3179. U.S. Army Ballistics Research Laboratory, Aberdeen Proving Ground, MD.

Loeb, W.F. 1989. The primate. In: *The Clinical Chemistry of Laboratory Animals*. W.F. Loeb and F.W. Quimby, eds. Pergamon Press, New York, NY. pp. 56-60.

Meeks, R.G. 1989. The rat. In: *The Clinical Chemistry of Laboratory Animals*. W.F. Loeb and F.W. Quimby, eds. Pergamon Press, New York, NY. pp. 19-25.

Menzer, R.E. 1991. Water and soil pollutants. In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*, Fourth Edition. M.O. Amdur, J. Doull, and C.D. Klaassen, eds. Pergamon Press, New York, NY. pp. 893-894.

Parmer, D.L., W.G. Palmer, D.A. Smart, and R.A. Finch. 1991. Developmental medical research on liquid gun propellants. In: *Conference on Chemical Risk Assessment in the DOD: Science, Policy, and Practice*. H.J. Clewell III ed., ACGIH, Cincinnati, OH. pp. 95-104.

Riley, J.H. and L.M. Cornelius. 1989. Electrolytes, blood gases, and acid base balance. In: *The Clinical Chemistry of Laboratory Animals*. W.F. Loeb and F.W. Quimby, eds. Pergamon Press, New York, NY. pp. 360-372.

Rosner, B. 1990. *Fundamentals of Biostatistics*. PWS-Kent Publishing Co., Boston, MA.

Rudd, C.J. and P.S. Lee. 1990. CHO/HGPRT gene mutation assay of LP1846 liquid gun propellant. Final Report, SRI Project No. LSC-7662-3, SRI Study No. 7662-B01-89. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*, 2nd Ed. W.W. Freeman and Company, New York, NY.

Snodgrass, H.L., A.E. Asaki, J.G. Harvey, and L.W. Metker. 1985. Health hazard evaluation of liquid monopropellants, Phase 4, Subchronic inhalation of hydroxylammonium nitrate. Special Study No. 75-51-1-0132-85, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD.

Toth, G.P., S.R. Wang, H. McCarthy, D.R. Tocco, and M.K. Smith. 1992. Effects of three male reproductive toxicants on rat cauda epididymal sperm motion. *Reproductive Toxicology*, **6**:507-515.

Valli, V.E.O. 1993. The hematopoietic system. In: *Pathology of Domestic Animals*, Vol. 3. K.V.F. Jubb, P.C. Kennedy, and N. Palmer, eds. Academic Press, New York, NY. pp. 208-209.

Weller, R.E., J.E. Morris, B.J. McClanahan, and R.F. Jostes. 1989. Evaluate the dermal toxicity of nitrate compounds and liquid gun propellants. Final Report, Project Number 87PP7806, U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Witt, W.M., R.M. Parker, J.D. Thurman, and P.A. Gosnell. 1992. LP1846 liquid gun propellant dermal toxicity study in male miniature Hanford swine. Final Report, Project No. 3E162720A835, U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.