

AD-A211 981

Institute Report No. 344

Acute Oral Toxicity of
Trimethylolethane Trinitrate (TMETN)
in Sprague-Dawley Rats

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DIVISION OF TOXICOLOGY

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July 1989

Toxicology Series: 134

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Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in Sprague-Dawley Rats
(Toxicology Series 134)--LeTellier *et al.*

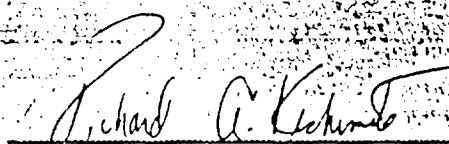
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Richard A. Kishimoto
COL, MSC
Acting Commander

28 July 1981
(date)

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 344		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology	6b. OFFICE SYMBOL (If applicable) SGPN-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory	
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 62720	PROJECT NO. A835
		TASK NO. AB	WORK UNIT ACCESSION NO. DA303913
11. TITLE (Include Security Classification) (U) Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in Sprague-Dawley Rats			
12. PERSONAL AUTHOR(S) YC LeTellier, SK Sano, CR Wheeler, and DW Korte, Jr.			
13a. TYPE OF REPORT Institute	13b. TIME COVERED FROM 11 OCT 84 TO NOV 84	14. DATE OF REPORT (Year, Month, Day) July 1989	15. PAGE COUNT 67
16. SUPPLEMENTARY NOTES Toxicology Series No. 134 <i>Keywords: munitions; nitroglycerine substitute;</i>			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
		Acute Oral Toxicity, TMETN, Trimethylolethane Trinitrate, Rat, Mammalian Toxicology, Propellants (RT)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The acute oral toxicity of trimethylolethane trinitrate (TMETN) was determined in male and female Sprague-Dawley rats by using the oral gavage single-dose method. The median lethal dose (MLD) for male rats was 1587.3 ± 191.5 mg/kg and for female rats was 1027.4 ± 63.7 mg/kg. Clinical signs observed following TMETN administration were inactivity, tremors, irritability, clonic convulsions, depression of grasping and righting reflexes, changes in the startle reflex, disorientation, hunched posture, and rough coat. Most animals exhibited signs within 2 hours of dosing and had either died or returned to normal by 72 hours after dosing. The extent of the neurotoxic component of this clinical signs profile suggests that TMETN possesses pharmacological properties in addition to those routinely associated with nitrate esters. According to the classification scheme of Hodge and Steiner, these results indicate that TMETN is a slightly toxic compound.			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL RICHARD A. KISHIMOTO, COL, MSC		22b. TELEPHONE (Include Area Code) (415) 561-3600	22c. OFFICE SYMBOL SGRD-ULZ

ABSTRACT

The acute oral toxicity of trimethylolethane trinitrate (TMETN) was determined in male and female Sprague-Dawley rats by using the oral gavage single-dose method. The median lethal dose (MLD) for male rats was 1587.3 ± 191.5 mg/kg and for female rats was 1027.4 ± 63.7 mg/kg. Clinical signs observed following TMETN administration were inactivity, tremors, irritability, clonic convulsions, depression of grasping and righting reflexes, increases in the startle reflex, disorientation, hunched posture, and rough coat. Most animals exhibited signs within 2 hours of dosing and had either died or returned to normal by 72 hours after dosing. The extent of the neurotoxic component of this clinical signs profile suggests that TMETN possesses pharmacological properties in addition to those routinely associated with nitrate esters. According to the classification scheme of Hodge and Sterner, these results indicate that TMETN is a slightly toxic compound.

KEY WORDS: Acute Oral Toxicity, Trimethylolethane Trinitrate, TMETN, Mammalian Toxicology, Rat, Propellants



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PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A535/180/TLB0

GLP STUDY NUMBER: 84013

STUDY DIRECTOR: LTC Don W. Korte, Jr., PhD, MSC
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CO-PRINCIPAL INVESTIGATOR: Steven K. Sanc, BA, SGT

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Diplomate, American College of Veterinary Pathologists

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT: A copy of the final report,
study protocol, SOPs, raw data,
analytical, stability, and
purity data of the test
compound, tissues, and an
aliquot of the test compound
will be retained in the LAIR
Archives.

TEST SUBSTANCE: Trimethylolethane trinitrate (TMETN)

INCLUSIVE STUDY DATES: 11 October - 8 November 1984

OBJECTIVE: The objective of this study was to determine the
acute oral toxicity of trimethylolethane trinitrate
(TMETN) in male and female Sprague-Dawley rats.

ACKNOWLEDGMENTS

MAJ Earl W. Morgan, VC, LTC Larry D. Brown, VC, Charlotte L. Speckman, and SP4 James J. Fischer, provided research assistance; SP4 James J. Fischer, SP4 Scott L. Schwebe, and Charlotte L. Speckman provided animal care; and Colleen S. Kamiyama, Julie Peacock, and Dorothy Davis provided secretarial assistance.

**SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS
INVOLVED IN THE STUDY**

We, the undersigned, declare that GLP Study 84013 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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REPLY TO
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24 July 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84013

1. This is to certify that the protocol for LAIR GLP Study 84013 was reviewed on 29 February 1984.
2. The institute report entitled "Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in Sprague-Dawley Rats," Toxicology Series 134, was audited on 26 June 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

TABLE OF CONTENTS

Abstract.....	i
Preface.....	iii
Acknowledgments.....	iv
Signatures of Principal Scientists.....	v
Report of Quality Assurance Unit.....	vi
Table of Contents.....	vii
 INTRODUCTION.....	 1
Objective of Study.....	1
 MATERIALS.....	 1
Test Substance.....	1
Vehicle.....	2
Animal Lata.....	2
Husbandry.....	2
 METHODS.....	 2
Group Assignment/Acclimation.....	2
Dose Levels.....	3
Compound Preparation.....	3
Chemical Analysis of TMETN and Dosing Suspensions.....	3
Test Procedures.....	4
Observations.....	4
Necropsy.....	4
Statistical Analysis.....	5
Duration of Study.....	5
Changes/Deviations.....	5
Storage of Raw Data and Final Report.....	5
 RESULTS.....	 5
Mortality.....	5
Lethal Dose Calculations.....	6
Clinical Observations.....	9
Gross Pathological Observations.....	10
 DISCUSSION.....	 10
 CONCLUSION.....	 13
 REFERENCES.....	 14

TABLE OF CONTENTS (cont.)

APPENDICES.....	15
Appendix A. Chemical Data.....	16
Appendix B. Animal Data.....	23
Appendix C. Historical Listing of Study Events.....	24
Appendix D. Cumulative Mortality Data.....	25
Appendix E. Individual Animal Histories.....	26
Appendix F. Individual Body Weights.....	50
Appendix G. Pathology Report.....	61
OFFICIAL DISTRIBUTION LIST.....	67

Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in Sprague-Dawley Rats--LeTellier et al.

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABDRL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABDRL has tasked the Division of Toxicology, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute oral toxicity of trimethylolethane trinitrate (TMETN) in male and female Sprague-Dawley rats.

MATERIALS

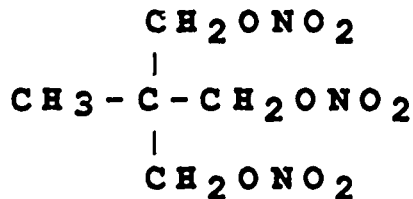
Test Substance

Chemical Name: Trimethylolethane trinitrate (TMETN)

Chemical Abstract Service Registry No.: 3032-55-1

LAIR Code No.: TA35

Chemical Structure:



Molecular Formula: C₅H₉N₃O₉

Source: Naval Ordnance Station
Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for TMETN was corn oil (Mazola, Best Foods, CPC International, Englewood Cliffs, NJ).

Animal Data

Sprague-Dawley rats (Bantin-Kingman Inc., Fremont, CA) used in this study were identified individually with ear tags. Two males and 2 females were selected randomly for quality control necropsy evaluation at receipt. The animal weights on receipt (11 Oct 84) ranged from 125 to 169 g. Additional animal data are given in Appendix B.

Husbandry

Rats were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, St Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 22.2°C to 26.1°C with a relative humidity range of 34 to 50 percent. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study rats were randomized into five dose groups of 10 males and 10 females each and vehicle control groups of five males and five females each. Allocation was accomplished using a computer-based stratified, weight-biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. The animals were acclimated for 12 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of an approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was between 1000 and 2000 mg/kg for the males, and between 500 and 1500 mg/kg for the females. Based on these data, the test doses were selected (Table 1).

TABLE 1: TMETN Doses

Group*	Dosage Level mg/kg	
	<u>Males</u>	<u>Females</u>
4	1000	775
5	1670	1290
6	2780	2150
7	1290	600
8	3600	1000
9 vehicle	corn oil (10 ml/kg)	corn oil (10 ml/kg)

Compound Preparation

TMETN used in this study was received as a 10% solution in ethanol. Neat TMETN was obtained by rotoevaporation removal of the ethanol. All dosing suspensions were prepared by mixing measured volumes of neat TMETN in an appropriate volume of corn oil immediately before dosing the animals.

Chemical Analyses of TMETN and Dosing Suspensions

Periodic analysis of the ethanol solutions and neat TMETN by HPLC analysis has shown no evidence of decomposition for up to 9 weeks. Since the neat TMETN contained no impurities as determined in an NMR analysis and TMETN

chromatographed as a single peak by HPLC analysis, it was considered to be at least 98% pure. TMETN was shown to be stable in the corn oil vehicle for at least 30 days based on storage time prior to analyses of dosing emulsions (Appendix A). Tests to verify concentration and to prove that homogenous emulsions of the test compound in the vehicle could be prepared were conducted (Appendix A).

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3). The volume of dosing solution each animal received was based upon the desired dose level, and the compound concentration in solution. The dose level was increased by varying the concentration of each solution. Volumes ranged from 1.61 to 2.46 ml in the males and 1.46 to 1.94 ml in females. The vehicle control group was given 10.0 ml/kg of corn oil. Dosing was performed using the oral gavage method without animal sedation or anesthesia. Sterile disposable 1-ml Monoject® syringes (Sherwood Medical, St. Louis, MO) fitted with 16-gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. Male and female animals from groups 4, 5, and 6 were dosed between 1029 and 1234 hours on 23 October 1984. After analysis of the preliminary data, male and female rats from groups 7, 8, and 9 were dosed between 0811 and 0915 hours on 25 October 1984.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 1, 2, and 4 hours after dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those that survived the 14-day study period were submitted for necropsy immediately after receiving a barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD₁₀, LD₅₀, and LD₉₀ were derived by probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with no deviations.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

All deaths (51) occurred within 24 hours of dosing. Twenty-four of the 46 males exposed to TMETN died within the first 8 hours after dosing. An additional three male animals were found dead the next morning. Twenty-three of the 47 females exposed to TMETN died within the first 24 hours after dosing. Twenty of these female deaths occurred within the first 8 hours after dosing and the remaining three by 24 hours. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2: Compound-Related Deaths by Group

<u>Group</u>	<u>Dose Level</u> (mg/kg)	<u>Deaths/</u> <u>Group</u>	<u>Percent</u> <u>Mortality</u>
Males			
4	1000	1/8	12.5
7	1290	4/9	44.4
5	1670	6/10	60.0
6	2780	7/10	70.0
8	3600	9/9	100.0
9	Vehicle	0/4	0
Females			
7	600	0/10	0
4	775	1/8	12.5
8	1000	5/10	50.0
5	1290	8/10	80.0
6	2150	9/9	100.0
9	Vehicle	0/5	0

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equation for the probit regression line was: $Y = -8.06 + 4.08 \log X$ for males and $Y = -24.29 + 9.73 \log X$ for females, where X is the dose and Y the corresponding probit value. Animals removed from the study were not included in the calculations. Figures 1 and 2 graphically present the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

Figure 1
TMETN Dose Response Curve for Male Rats

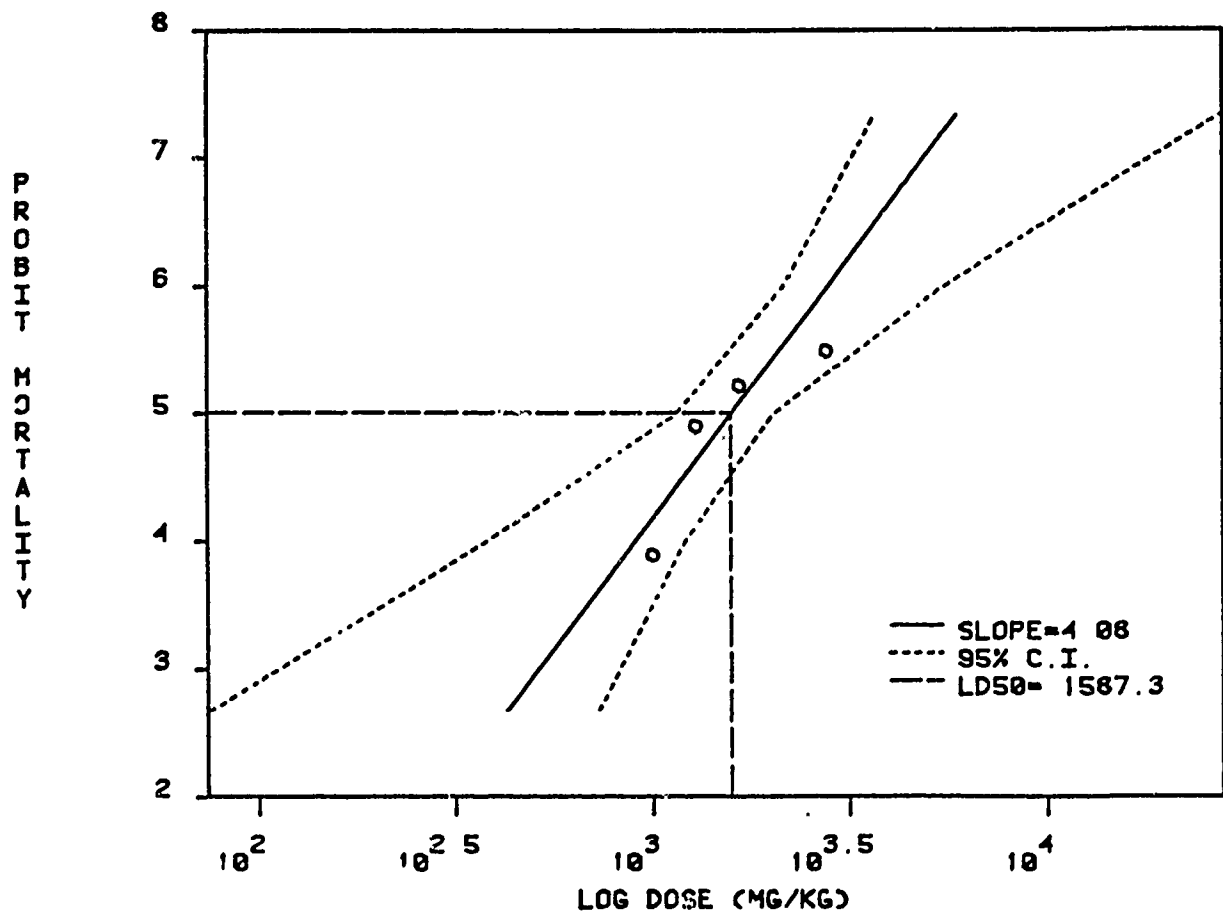


Figure 2

TMETN Dose Response Curve for Female Rats

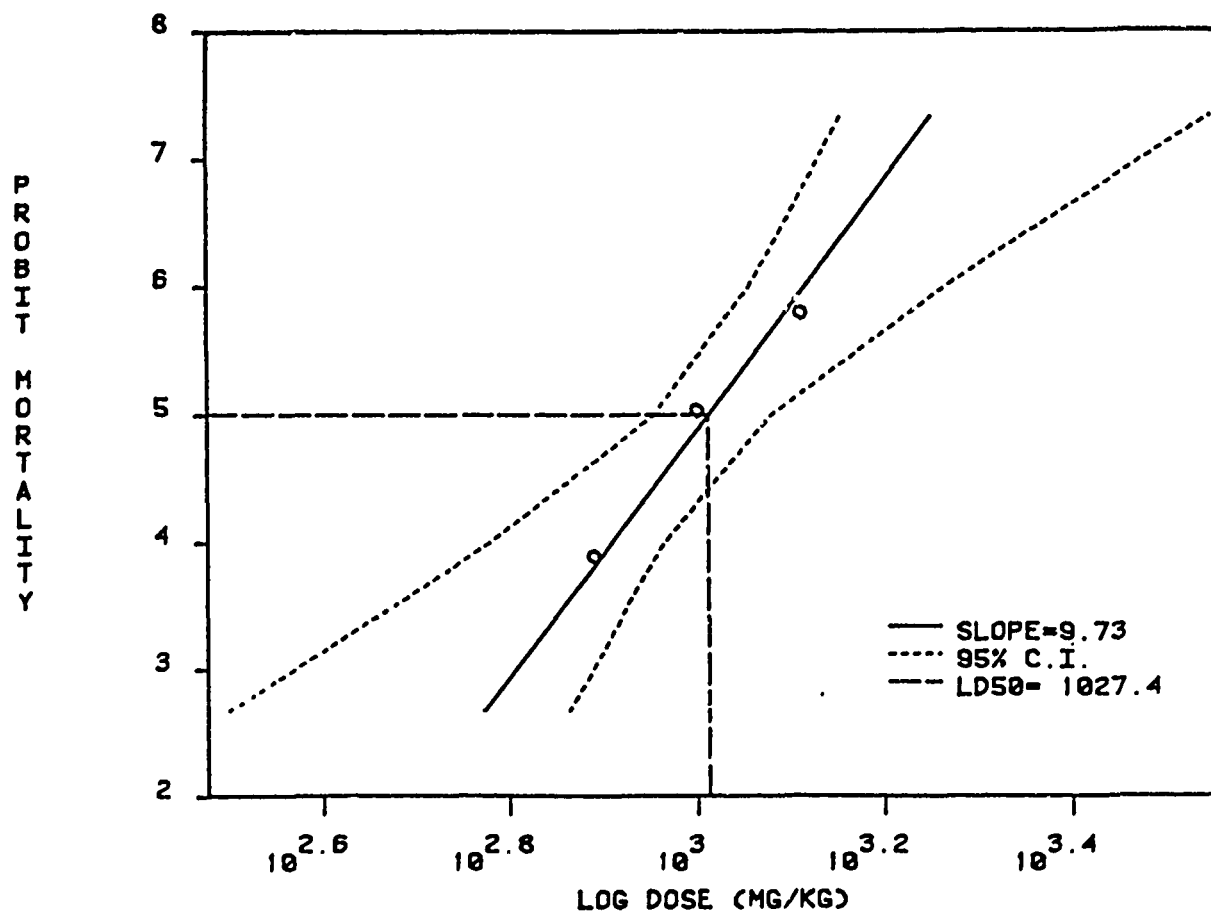


TABLE 3: Calculated Lethal Doses (LD) of TMETN in Sprague-Dawley Rats

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
Males		
LD10	770.2 $\pm$ 196.9	(271.1, 1086.9)
LD50	1587.3 $\pm$ 191.5	(1150.2, 2035.4)
LD90	3271.5 $\pm$ 701.8	(2428.8, 7657.8)
Females		
LD10	758.5 $\pm$ 74.4	(526.1, 873.9)
LD50	1027.4 $\pm$ 63.7	(898.0, 1198.0)
LD90	1391.6 $\pm$ 151.0	(1194.5, 2106.9)

* Calculated dose $\pm$ standard error.

Clinical Observations

The most frequently observed categories of clinical signs in animals administered TMETN were behavioral disturbances (77 of 93 animals dosed) and hunched posture (74 of 93), and changes in reflex activity (56 of 93). Behavioral signs included irritability, inactivity, disorientation, hyperactivity, tremors, and twitching. Changes in reflex activity include depressed grasping and righting reflexes and changes in the startle reflex. Although clinical signs were observed at each dose level, there was no clear dose-response relationship for severity or duration of the symptoms.

Twenty-three female and 27 male rats died during the study. All vehicle control animals survived until study termination at 14 days. TMETN toxicity was acute as all deaths were observed within the first 24 hours after dosing. The majority of clinical signs were observed within the first 48 hours after dosing. Table 4 contains a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not affected by dosing. Table 5 presents the mean body weights by groups. Appendix F contains individual weight tables.

Gross Pathological Observations

All study animals, whether dying acutely from TMETN or sacrificed at the end of the 14-day observation period, were examined grossly at necropsy. Few gross lesions were observed and none of these appeared clearly related to TMETN administration. Several animals that died had red staining of the nose and muzzle that was considered to be an incidental finding. Other incidental findings included dilated renal pelvis in five male animals and focal alopecia in one female. This female was removed from the study on day 7. The veterinary pathologist's report appears in Appendix G.

DISCUSSION

Female Sprague-Dawley rats were more sensitive than males to TMETN toxicity. The calculated MLD for TMETN was 1587.3 ± 191.5 mg/kg in male Sprague-Dawley rats and 1027.4 ± 63.7 mg/kg in female Sprague-Dawley rats. These MLD values place TMETN in the "slightly toxic" range by the system of Hodge and Sterner (5).

TMETN appeared to have a neurotoxic effect as indicated by the clinical signs data. These signs included increased startle reflex, inactivity, twitching, clonic convulsions, depressed grasping and righting reflexes, and hyperactivity. Other signs frequently reported such as rough coat, hunched posture, diarrhea, irritability, tremors, disorientation, and various stains were also observed in the vehicle control animals and thus are attributed to administration of the corn oil.

The increased respiratory rate observed in 35 of 93 (37.6%) animals could be associated with the neurotoxicity observed following TMETN administration or it could be secondary to methemoglobin formation. However, there was little evidence of methemoglobin formation since cyanosis was observed in only two females and the pathologist did not report the presence of chocolate-colored or darkened blood in any animals at necropsy. Anderson and Mehl (6) have reported a significant neurologic component to the lethality of a nitrate ester (TEGDN) compared with lethality due to methemoglobin formation alone as reported for EGDN or PGDN.

**TABLE 4: Incidence Summary for Clinical Observations
in Rats Administered TMETN**

MALES	Group (n=)	9	4	7	5	6	8
		<u>Vehicle</u>	<u>1000</u>	<u>1290</u>	<u>1670</u>	<u>2780</u>	<u>3600</u>
		4	8	9	10	10	9
Respiratory ^a		0	7	0	7	7	2
Behaviorial ^b		1	7	7	9	9	5
Convulsions ^c		0	1	1	1	2	0
Skin/Hair ^d		4	7	7	8	6	5
Gastrointestinal ^e		0	4	2	1	0	1
Stains ^f		1	5	6	8	6	4
Ocular ^g		0	1	0	1	0	0
Hunched posture		0	7	7	9	8	5
Reflex ^h		0	6	5	5	6	2
Prostrate/Moribund		0	0	0	0	0	1
Deaths before signs		0	1	2	1	0	4
Deaths		0	1	4	6	7	9
FEMALES							
	Group (n=)	9	7	4	8	5	6
		<u>Vehicle</u>	<u>600</u>	<u>775</u>	<u>1000</u>	<u>1290</u>	<u>2150</u>
		5	10	8	10	10	9
Respiratory ^a		0	1	5	2	5	1
Behaviorial ^b		2	10	8	8	8	4
Convulsions ^c		0	0	0	0	0	2
Skin/Hair ^d		5	9	7	4	8	3
Gastrointestinal ^e		1	3	2	0	0	2
Stains ^f		2	9	5	8	6	2
Ocular ^g		0	0	0	1	0	0
Hunched posture		4	10	8	9	8	3
Reflex ^h		0	9	8	5	7	3
Prostrate/Moribund		0	0	0	0	0	1
Deaths before signs		0	0	0	1	2	5
Deaths		0	0	1	5	8	9

^a Includes changes in rate or depth.

^b Includes disorientation, irritability, inactivity, hyperactivity, tremors, and twitching.

^c Includes clonic convulsions.

^d Includes cyanosis and rough coat.

^e Includes diarrhea and increased salivation.

^f Includes urine and feces on tail and perineum and reddish stains on mouth, eyes, and nose.

^g Includes chromodacryorrhea and squinting.

^h Includes changes in grasping, righting, and startle reflexes.

TABLE 5: Mean Body Weights in Grams $\pm$ S.E (N)

<u>Dose Groups</u> (mg/kg)	<u>At</u> <u>Receipt</u>	<u>Dosing</u> <u>Day</u>	<u>Midtrial</u> <u>Day</u>	<u>Termination</u> <u>Day*</u>
MALES				
1000	140.1 ± 2.6 (10)	206.0 ± 3.4 (10)	280.9 ± 5.0 (7)	280.6 ± 6.1 (7)
1290	141.0 ± 2.4 (10)	228.6 ± 13.8 (10)	285.6 ± 9.4 (5)	296.8 ± 12.6 (5)
1670	143.9 ± 3.1 (10)	208.9 ± 3.3 (10)	298.5 ± 6.4 (4)	289.0 ± 6.1 (4)
2780	139.9 ± 2.4 (10)	203.1 ± 5.4 (10)	278.3 ± 8.4 (3)	281.7 ± 6.4 (3)
3600	136.9 ± 8.6 (10)	225.0 ± 2.9 (10)	N/A	N/A
Vehicle	140.8 ± 4.0 (5)	229.8 ± 3.6 (5)	299.5 ± 5.9 (4)	313.5 ± 8.4 (4)
FEMALES				
600	145.2 ± 2.5 (10)	177.9 ± 4.3 (10)	197.7 ± 4.7 (10)	213.8 ± 3.4 (10)
775	143.4 ± 1.9 (10)	172.6 ± 3.1 (10)	197.4 ± 4.8 (7)	200.7 ± 5.8 (7)
1000	145.2 ± 2.4 (10)	181.4 ± 5.3 (10)	202.8 ± 8.0 (5)	199.6 ± 8.3 (5)
1290	143.1 ± 2.1 (10)	169.2 ± 1.8 (10)	199.0 ± 5.0 (2)	209.5 ± 2.5 (2)
2150	143.7 ± 1.9 (10)	172.3 ± 2.6 (10)	N/A	N/A
Vehicle	148.2 ± 2.7 (5)	178.0 ± 4.4 (5)	187.6 ± 5.4 (5)	205.8 ± 6.8 (5)

* Weight after overnight fast.

- The data suggest that the lethality following acute administration of TMETN is due to its neurotoxicity rather than its ability to cause methemoglobinemia.

CONCLUSION

TMETN is a nitrate ester with neurotoxic effects. Females were more sensitive to TMETN than were males. Calculated MLD values were 1587.3 ± 191.5 mg/kg in male and 1027.4 ± 63.7 mg/kg in female Sprague-Dawley rats. TMETN is thus classified as a "slightly toxic" compound.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC NO. ADA 127846.
2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, oral toxicity. In: Health effects test guidelines. Washington, DC; Environmental Protection Agency, August 1982; EPA 560/6-82-001.
3. Acute oral toxicity study (ALD and LD 50). LAIR Standard Operating Procedure OP-STX-36, Presidio of San Francisco, California: Letterman Army Institute of Research, 15 June 1984.
4. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.
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6. Andersen ME, Mehl, RG. A comparison of the toxicology of triethylene glycol dinitrate and propylene glycol dinitrate. Amer Ind Hyg Assoc J 1973; 34:526-532.

Appendix A. Chemical Data.....	16
Appendix B. Animal Data.....	23
Appendix C. Historical Listing of Study Events.....	24
Appendix D. Cumulative Mortality Data.....	25
Appendix E. Individual Animal Histories.....	26
Appendix F. Individual Body Weights.....	50
Appendix G. Pathology Report.....	61

Appendix A: CHEMICAL DATA

Chemical Name: 1,3-Propanediol, 2-methyl-2
[(nitroxymethyl)-dinitrate (ester)]

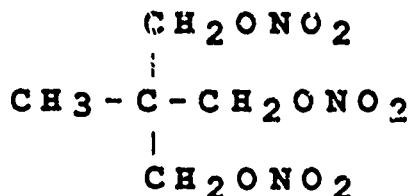
Other Names: 1,3-Propanediol-2-(hydroxymethyl)-2-methyl-,
trinitrate; 1,1,1-trimethylolethane trinitrate
(TMETN), metriol trinitrate (MTN);
nitropentaglycerin

Lot Number: 53-84A

Chemical Abstracts Service Registry No.: 3032-55-1

LAIR Code No.: TA35

Structur 1 Formula:



Molecular Formula: $\text{C}_5\text{H}_9\text{N}_3\text{O}_9$

Molecular Weight: 255.15

Physical State: Light brown oil

Melting Point: -3° 1,2

Compound Density: 1.47 g/cm 1,2

Source: Naval Ordnance Station, Indian Head, MD, 20640

¹ Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p 17.

² Lindner V. Properties of explosive aliphatic nitrate esters. Table 5. In: Grayson M., exec. ed. Kirk-Othmer Encyclopedia of Chemical Technology. Volume 9. 3rd ed. New York: John Wiley and Sons, Inc., 1980:573.

Appendix A (cont.): CHEMICAL DATA

Analytical Data: Ultra-violet (UV) spectra were obtained using a Hitachi 110-A Spectrophotometer (Hitachi Instruments, Inc., Mountain View, CA), infra-red spectra (IR) were obtained with a Perkin-Elmer Model 457 Infra-red Spectrophotometer (Perkin-Elmer, Norwalk, CT) and nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT-80 NMR (Varian, Palo Alto, CA) using tetramethylsilane as an internal standard. Chromatographic analysis was performed using a 1090B HPLC with diode array detector (Hewlett-Packard, Santa Clara, CA) and a Brownlee RP-18 Spheri-5 Column, 4.6 x 250 mm (Brownlee Labs, Inc., Santa Clara, CA). The following conditions were employed for the HPLC assay: solvent system, 70% methanol, 30% water; flow rate, 0.9 ml/min; detector wavelength, 215 nm; oven temperature, 50°C.

UV Spectrum: For UV analysis TMETN was dissolved in acetonitrile. UV absorbance begins at approximately 240 nm and increases with decreasing wavelength.³ No absorption peak was observed. IR (KBr windows): 2900, 1645 (asymmetric stretch of NO group), 1470, 1375, 1280 (symmetric stretch of NO₂ group), 990, 860, and 755 cm.⁴ ¹H NMR (CDCl₃, 80 MHz): δ 1.22 (s, 3H, CH₃), 4.44 (s, 6H, -CH₂-).⁵ TMETN subjected to HPLC analysis eluted as two peaks with retention times of 5.5-5.6 and 12.5 min.⁶ Based on integration of peak areas, the first peak represented 98% of the sample. The second peak was not identified. No decomposition of TMETN was detected by HPLC after storage of TMETN (neat or in ethanol) for a period of nine weeks.⁷

³ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 51. Letterman Army Institute of Research, Presidio of San Francisco, CA.

⁴ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 67. Letterman Army Institute of Research, Presidio of San Francisco, CA.

⁵ *Ibid.*, p 68.

⁶ Wheeler, CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 72-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

⁷ Wheeler, CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p 34. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

ANALYSIS OF TMETN DOSING FORMULATIONS

INTRODUCTION

Emulsions of trimethylolethane trinitrate (TMETN) in corn oil were prepared by shaking or stirring mixtures of the two components. The emulsions were subsequently used for dosing animals in the GLP studies 84013 (acute oral toxicity in rats) and 84014 (acute oral toxicity in mice). After dosing, the remainder of the emulsion was stored at 4°C for analysis. Determination of the TMETN concentration was accomplished by reverse-phase high pressure liquid chromatography.

MATERIALS

Chromatographic analysis was performed using a Hewlett-Packard 1090 high pressure liquid chromatography (HPLC) system with diode array detector (Hewlett-Packard, Palo Alto, CA). Separations were obtained on a Brownlee RP-18 column (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA). HPLC grade acetonitrile and water were obtained from the J. T. Baker Chemical Co., Phillipsburg, NJ.

METHODS

Analysis of TMETN solutions was accomplished under the following HPLC conditions: solvent 70% acetonitrile-30% water; solvent flow, 0.9 ml/min; injections volume, 10 µL; detector wavelength, 205 nm.⁸ The HPLC mobile phase was used to prepare standards as well as to extract the TMETN/corn oil mixtures. Standards were prepared by weighing TMETN on aluminium foil (0.5 mm squares) using a microbalance. The weigh boats containing TMETN were added to volumetric flasks. The flasks were filled to volume with the HPLC solvent and the contents were mixed well by shaking. The concentration of the standards ranged from 52 to 494 or 511 µg/ml and a set of 10 standards covering this range was analyzed both before and after each set of samples (diluted dosing emulsions).

⁸ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p. 69-74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

To extract the dosing preparations the TMETN/corn oil mixtures were removed from the refrigerator and warmed to room temperature. After rapidly stirring each sample for a minimum of five minutes, an aliquot of approximately one ml was removed and transferred to a tared volumetric flask. The weight of each aliquot transferred was recorded and the flask filled to volume. A second dilution was required prior to analysis by HPLC.

To determine if the emulsions of TMETN in corn oil prepared for dosing were homogenous, a series of emulsions were prepared with TMETN concentrations spanning the range of concentrations employed in the dosing preparations. Four emulsions containing 50, 200, 400 and 800 mg of TMETN per ml were prepared in 20 ml scintillation vials. After stirring with a magnetic stir bar for at least 5 minutes, aliquots from the top, middle, and bottom of the emulsions were removed and transferred to tared 25 ml volumetric flasks. The exact weight of the aliquot was recorded and the flask filled to volume. One ml of this solution was transferred to a second volumetric flask for a further dilution prior to HPLC analysis.

RESULTS

Under the conditions of the analysis, TMETN eluted with a retention time of 5.3 min. A plot of TMETN concentration versus peak area was linear within the range of concentrations (5.2 to 511 µg/ml) employed as standards. Consecutive analyses ($n = 10$) were performed with standards containing 52, 257, and 494 µg TMETN/ml. The coefficient of variation for each set of peak area values was 0.37%, 0.16%, and 0.6%, respectively.⁸ Standards were analyzed both before and after the analysis of samples prepared from dosing emulsions. The differences in peak areas between corresponding standards run before and after was less than 1%. In addition, the difference among standards analyzed several days apart was also less than 1%.

Extraction of the dosing emulsions with 70% acetonitrile-30% water resulted in quantitative recovery of TMETN with no peaks in the chromatogram from corn oil. The results for the determination of homogeneity are presented in Table 1. The deviation of individual values from the mean of each set of three samples (top, middle, bottom) did not exceed 0.8% for any emulsion prepared.

⁸ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p. 69-74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

DISCUSSION

The linearity of the calibration plot and the precision of the assay within and between days indicate the assay is a valid method to quantitate TMETN. The data in Table 1 demonstrate that the dispersion of TMETN in corn oil provides a homogenous emulsion over a range of 50 to 800 mg/ml. Since the dosing preparations were prepared in an identical manner they were, by implication, homogenous.

The data from the analysis of the dosing emulsions is presented in Table 2. The concentration of TMETN determined by analysis was very close to the target value for almost every suspension prepared. In only one case (study 84014, 159.0 mg TMETN/ml) did the actual concentration come close to a 10% deviation from the target concentration.

CALCULATIONS

A series of standards were analyzed before and after the samples (diluted dosing emulsions) for each study. The two peak area values for each standard solution were averaged and linear least squares regression performed on the concentration versus peak area data. This provided the equation for the best fitting line in the form of Equation 1 in which:

$$\text{Equation 1} \quad y \text{ (peak area)} = mx + b$$

m is the slope, x is the concentration ($\mu\text{g/ml}$) and b is the intercept. The concentration of TMETN in the final dilution was calculated by substituting for the y the peak area obtained from HPLC analysis and solving for x.

The total amount of TMETN (mg) in the sample analyzed was calculated as shown in Equation 2:

$$\text{Equation 2} \quad \text{Total TMETN (mg)} = \frac{X \times (\text{dilution factor})}{10^3 \mu\text{g/mg}} = Y$$

The volume corresponding to the weight of TMETN calculated above was determined by dividing by the density of TMETN (Equation 3):

$$\text{Equation 3.} \quad \text{Total TMETN (ml)} = \frac{\text{Total TMETN (mg)}}{1470 \text{ mg/ml}} = \frac{Y}{1470} = Z$$

Appendix A (cont.): CHEMICAL DATA

The contribution by corn oil to the volume of the original aliquot of emulsion removed for analysis was calculated as follows (Equation 4):

$$\text{Volume of corn oil} = \frac{\text{weight of aliquot removed for analysis} - \text{weight of TMETN}}{\text{Density of corn oil}} = \frac{\text{wgt (mg) of aliquot} - Y}{918 \text{ mg/ml}} = V$$

The concentration of TMETN could be determined as follows (Equation 5):

$$\text{Conc. of TMETN (mg/ml)} = \frac{\text{mg TMETN total volume of aliquot removed for analysis}}{\text{ml TMETN + corn oil}} = \frac{Y}{Z + V}$$

TABLE 1

Assessment of homogeneity for TMETN/corn oil emulsions. Aliquots of approximately one ml were withdrawn from the top (T), middle (M), and bottom (B) of emulsions prepared to represent the range of TMETN concentrations [TMETN] employed in dosing. Equation for line obtained by linear least squares regression: $Y = 0.0653X + 0.0380$ ($R = 0.9996$).⁹

Target [TMETN] (mg/ml)	Site of Sampling	[TMETN] Determined by Analysis (mg/ml)	Mean [TMETN] (mg/ml)	Deviation from Mean (%)
50	T	49.9	49.9	0.0
	M	49.8		0.2
	B	49.9		0.0
200	T	200.4	200.4	0.0
	M	200.0		0.2
	B	200.7		0.2
400	T	404.3	403.0	0.1
	M	404.9		0.5
	B	399.8		0.8
800	T	797.1	799.4	0.3
	M	803.2		0.5
	B	798.0		0.2

⁹ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 10-14, 26-35. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

TABLE 2

Concentration of TMETN [TMETN] in dosing formulations prepared for GLP studies 84013 and 84014.

Study #	Target [TMETN] (mg/ml)	Date Prepared	Date Analyzed	[TMETN] determined by analysis (mg/ml)	% target [TMETN]
84013 ¹⁰	400.0	19 Oct 84	21 Feb 85	405.3	101.3
	800.0	19 Oct 84	21 Feb 85	795.3	99.4
	77.5	23 Oct 84	21 Feb 85	80.7	104.1
	100.0	23 Oct 84	21 Feb 85	96.5	96.5
	129.0	23 Oct 84	21 Feb 85	128.0	99.2
	167.0	23 Oct 84	21 Feb 85	166.6	99.8
	215.0	23 Oct 84	21 Feb 85	220.9	102.7
	278.0	23 Oct 84	21 Feb 85	273.6	98.4
	60.0	25 Oct 84	21 Feb 85	59.6	99.3
	100.0	25 Oct 84	21 Feb 85	100.8	100.8
	129.0	25 Oct 84	21 Feb 85	128.1	99.3
	360.0	25 Oct 84	21 Feb 85	373.5	103.8
84014 ¹¹	100.0	9 Nov 84	25 Feb 85	97.2	97.2
	63.1	13 Nov 84	25 Feb 85	64.9	102.8
	79.4	13 Nov 84	25 Feb 85	79.4	100.0
	100.0	13 Nov 84	25 Feb 85	96.2	96.2
	126.0	13 Nov 84	25 Feb 85	130.5	103.6
	159.0	13 Nov 84	25 Feb 85	174.2	109.6
	39.8	23 Jan 85	25 Feb 85	38.4	96.5
	50.1	23 Jan 85	25 Feb 85	49.6	99.0
	79.4	23 Jan 85	25 Feb 85	78.9	99.4
	100.0	23 Jan 85	25 Feb 85	98.8	98.8
	56.2	24 Jan 85	25 Feb 85	55.5	98.8

¹⁰ Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 6-14, 26-35. Letterman Army Institute of Research, Presidio of San Francisco, CA. The equation obtained for the standard curve was $y = 0.0653X + 0.0380$ ($R = 0.9996$).

¹¹ Ibid. p. 15-17, 26-35. The equation obtained for the standard curve was $y = 0.0639X + 0.2973$ ($r = 0.9998$).

Appendix B: ANIMAL DATA

Species: *Rattus norvegicus*

Strain: Albino, Sprague-Dawley

Source: Bantin & Kingman, Inc.
Fremont, CA

Sex: Male and female

Date of birth: 03 September 1984 (males),
27 August 1984 (females)

Method of randomization: Weight bias, stratified animal
allocation using the Toxsys
Software Package (Standard
Procedures for Assigning Animals to
Treatment Groups, SOP OP-ISG-24).

Animals in each group: 10 males and 10 females
5 each for control group

Condition of animals at start of study: Normal

Body weight range at dosing: 146 - 246 g

Identification procedures: Ear tag

Pretest conditioning: Quarantine/acclimation 11-22 Oct 1984.

Justification: The laboratory rat has proven to be a
sensitive and reliable animal model for
acute toxicity studies.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
11 Oct 84	Rats were received, sexed, observed for illness, ear-tagged, weighed, and individually caged.
12 Oct 84	Two male and two female rats were submitted for necropsy quality control.
12-22 Oct 84	Animals were observed daily.
15 Oct 84	Animals were weighed and randomized into dose groups.
17,19 Oct 84	ALD animals were weighed, dosed, and observed.
22 Oct 84	Food was removed from groups 4, 5, and 6 by 2200 hours.
23 Oct 84	Groups 4, 5, and 6 were weighed dosed, and observed at 1, 2, and 4 hours after dosing.
24 Oct-8 Nov 84	All animals were observed daily in a.m. and p.m.
24 Oct 84	Food was removed from groups 7, 8, and 9 by 2200 hours.
25 Oct 84	Groups 7, 8, and 9 were weighed dosed, and observed at 1, 2, and 4 hours after dosing.
31 Oct 84	All animals were weighed.
5 Nov 84	Food was removed from groups 4, 5, and 6 by 2200 hours.
6 Nov 84	All surviving animals of groups 4, 5, and 6 were weighed, observed, and submitted for necropsy.
7 Nov 84	Food was removed from groups 7, 8, and 9 by 2200 hours.
8 Nov 84	All surviving animals of groups 7, 8, and 9 were weighed, observed, and submitted for necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing									
		Hours			Days						
		2	4	8	1	2	3	4	5	6	7-14
MALES											
1000	8	1	1	1	1	1	1	1	1	1	1
1290	9	2	3	4	4	4	4	4	4	4	4
1670	10	1	2	5	6	6	6	6	6	6	6
2780	10	1	5	6	7	7	7	7	7	7	7
3600	9	4	5	8	9	9	9	9	9	9	9
Vehicle	4	0	0	0	0	0	0	0	0	0	0
FEMALES											
600	10	0	0	0	0	0	0	0	0	0	0
775	8	0	0	1	1	1	1	1	1	1	1
1000	10	1	3	5	5	5	5	5	5	5	5
1290	10	1	3	5	8	8	8	8	8	8	8
2150	9	6	9	9	9	9	9	9	9	9	9
Vehicle	5	0	0	0	0	0	0	0	0	0	0
TOTAL		17	31	44	51	51	51	51	51	51	51

Appendix E: INDIVIDUAL ANIMAL HISTORIES

MALE: 1000 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01275	Misdosed	N/A	N/A
84D01276	Stain, Perianal, Brown	Oct. 23,24	Marked
	Inactive	Oct. 23	Moderate
	Hunched Posture	Oct. 23-25,29	
	Irritable	Nov. 4	Marked
	Incr. Respiration Depth	Oct. 23	Slight
	Incr. Salivation	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23,24	Marked
	Rough Coat	Oct. 23	Moderate
	Incr. Startle Reflex	Oct.23-26,30,31	Moderate
		Oct. 23	Slight
84D01277	Death	Oct. 23	2.0 h
84D01292	Misdosed	N/A	N/A
84D01294	Tremors	Oct. 23	Slight
	Rough Coat	Oct. 23-26,29	Moderate
	Incr. Respiration Rate	Oct. 23,24	Moderate
	Hunched Posture	Oct. 23-25	Moderate
	Incr. Startle Reflex	Oct. 24	Slight
84D01303	Stain, Perianal, Brown	Oct. 23	Slight
	Stain, Mouth, Red	Oct. 23,24	Marked
	Stain, Perianal, Yellow	Oct. 24	Marked
	Stain, Red, Both Eyes	Oct. 24	Slight
	Incr. Respiration Rate	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Twitching	Oct. 23	Slight
	Hunched Posture	Oct. 23-25	Moderate
	Chromodacryorrhea	Oct. 23	Moderate
	Rough Coat	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1000 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01305	Irritable	Oct. 23,26	Slight
	Hunched Posture	Oct. 23,24	Moderate
	Incr. Respiration Rate	Oct. 23	Moderate
	Inactive	Oct. 23	Slight
	Tremors	Oct. 23	Slight
	Rough Coat	Oct. 23,24,26	Slight
	Incr. Startle Reflex	Oct. 24	Slight
84D01315	Hunched Posture	Oct. 23-25	Marked
	Inactive	Oct. 23	Marked
	Incr. Startle Reflex	Oct. 23,24	Slight
	Clonic Convulsion	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23,24	Slight
	Incr. Respiration Depth	Oct. 23	Slight
	Tremors	Oct. 23-25	Moderate
	Diarrhea	Oct. 23	Marked
	Rough Coat	Oct. 23-26	Marked
	Stain, Perianal, Yellow	Oct. 24	Moderate
84D01318	Incr. Salivation	Oct. 23	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Incr. Respiration Depth	Oct. 23	Slight
	Tremors	Oct. 23,24	Moderate
	Hunched Posture	Oct. 23-26	Marked
	Inactive	Oct. 23	Marked
	Rough Coat	Oct. 23-26	Marked
	Stain, Perianal, Yellow	Oct. 23	Moderate
84D01321	Tremors	Oct. 23,24	Slight
	Hunched Posture	Oct. 23-25	Moderate
	Inactive	Oct. 23	Moderate
	Stain, Nose, Red	Oct. 23,30-Nov.4	Slight
	Stain, Perianal, Brown	Oct. 23,24	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23	Moderate
	Depr. Grasping Reflex	Oct. 23,24	Moderate
	Scab, Right Ear	Oct. 25	Slight
	Irritable	Nov. 1	Slight
	Diarrhea	Nov. 6	Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1290 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01267	Misdosed	N/A	N/A
84D01274	Death	Oct. 25	2.0 h
84D01278	Hunched posture	Oct. 25	Moderate
	Inactive	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Stain, Mouth, Red	Oct. 25	Slight
	Stain, Perianal, Brown	Oct. 25, 26	Slight
	Depr. Grasping Reflex	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Slight
	Incr. Salivation	Oct. 25	Moderate
	Rough Coat	Oct. 25, 26	Slight
84D01279	Hunched Posture	Oct. 25	Moderate
	Tremors	Oct. 25	Moderate
	Stain, Nose, Red	Oct. 25, 26	Slight
	Depr. Grasping Reflex	Oct. 25	Moderate
	Inactive	Oct. 25	Marked
	Rough Coat	Oct. 25, 26	
		Nov. 7	Slight
84D01280	Hunched Posture	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Inactive	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
	Death	Oct. 25	3.3 h
84D01291	Hunched Posture	Oct. 25	Moderate
	Irritable	Oct. 25	Moderate
	Tremors	Oct. 25	Marked
	Incr. Startle Reflex	Oct. 25	Marked
	Rough Coat	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25, 26	Slight
	Depr. Grasping Reflex	Oct. 25	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1290 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01308	Hunched Posture	Oct. 25	Slight
	Inactive	Oct. 25	Moderate
	Rough Coat	Oct. 25, 26	Slight
	Stain, Nose, Red	Oct. 25	Moderate
	Tremors	Oct. 25	Slight
84D01326	Hunched Posture	Oct. 25	Marked
	Stain, Nose, Red	Oct. 25	Moderate
	Rough Coat	Oct. 25	Moderate
	Inactive	Oct. 25	Marked
	Incr. Startle Reflex	Oct. 25	Slight
	Irritable	Oct. 25	Slight
	Death	Oct. 25	7.7 h
84D01327	Death	Oct. 25	1.7 h
84D01331	Hunched Posture	Oct. 25	Moderate
	Rough Coat	Oct. 25, 26	Slight
	Incr. Startle Reflex	Oct. 25	Moderate
	Diarrhea	Oct. 25	Moderate
	Stain, Nose, Red	Oct. 25, Nov. 8	Moderate
	Stain, Perianal, Brown	Oct. 26	Slight
	Irritable	Oct. 25	Moderate
	Tremors	Oct. 25	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1670 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01266	Incr. Respiration Rate	Oct. 23	Moderate
	Hunched Posture	Oct. 23-25	Slight
	Stain, Perianal, Brown	Oct. 23	Slight
	Rough Coat	Oct. 23, 24	Slight
	Tremors	Oct. 23	Slight
	Stain, Mouth, Red	Oct. 24	Moderate
84D01271	Tremors	Oct. 23	Moderate
	Inactive	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Marked
	Hunched Posture	Oct. 23-25, 29	Marked
	Stain, Perianal, Brown	Oct. 23	Moderate
	Irritable	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23-26, 29	Moderate
	Diarrhea	Oct. 23	Moderate
	Stain, Nose, Red	Oct. 24	Slight
	Stain, Mouth, Red	Oct. 24	Slight
	Stain, Perianal, Yellow	Oct. 24, 25	Moderate
84D01288	Depr. Grasping Reflex	Oct. 26	Slight
	Hunched Posture	Oct. 23, 24	Moderate
	Stain, Nose, Red	Oct. 23	Slight
	Stain, Tail, Brown	Oct. 30, 31	Slight
	Stain, Perianal, Yellow	Oct. 24	Marked
	Stain, Perianal, Brown	Oct. 23	Moderate
	Depr. Grasping Reflex	Oct. 23	Slight
	Twitching	Oct. 23	Slight
	Inactive	Oct. 23	Moderate
	Irritable	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Nov. 1-5	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1670 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01290	Stain, Mouth, Red	Oct. 23	Moderate
	Hunched Posture	Oct. 23	Slight
	Disoriented	Oct. 23	Slight
	Inactive	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23	Slight
	Rough Coat	Oct. 23	Moderate
	Tremors	Oct. 23	Slight
	Death	Oct. 23	4.7 h
84D01293	Incr. Respiration Rate	Oct. 23	Moderate
	Hunched Posture	Oct. 23	Marked
	Tremors	Oct. 23	Marked
	Rough Coat	Oct. 23	Slight
	Squinting	Oct. 23	Slight
	Clonic Convulsion	Oct. 23	Slight
	Death	Oct. 24	21.3 h
84D01311	Hunched Posture	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23	Slight
	Stain, Nose, Red	Oct. 23	Slight
	Inactive	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Rough Coat	Oct. 23	Slight
	Irritable	Oct. 23	Slight
	Death	Oct. 23	5.1 h
84D01316	Hunched Posture	Oct. 23	Slight
	Twitching	Oct. 23	Slight
	Incr. Startle Reflex	Oct. 23	Slight
	Stain, Perianal, Brown	Oct. 23	Moderate
	Death	Oct. 23	3.8 h
84D01317	Death	Oct. 23	1.2 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1670 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01322	Hunched Posture	Oct. 23	Moderate
	Rough Coat	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Slight
	Stain, Nose, Red	Oct. 23	Slight
	Death	Oct. 23	5.2 h
84D01324	Tremors	Oct. 23, 24	Moderate
	Incr. Respiration Rate	Oct. 23	Marked
	Hunched Posture	Oct. 23-25	Marked
	Incr. Startle Reflex	Oct. 23	Marked
	Inactive	Oct. 23	Marked
	Stain, Mouth, Red	Oct. 23	Slight
	Rough Coat	Oct. 23-25	Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2780 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01263	Tremors	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23	Slight
	Rough Coat	Oct. 23	Slight
	Stain, Nose, Red	Oct. 23	Slight
	Death	Oct. 23	4.3 h
84D01265	Tremors	Oct. 23	Marked
	Inactive	Oct. 23	Moderate
	Twitching	Oct. 23	Slight
	Incr. Startle Reflex	Oct. 23	Marked
	Hunched Posture	Oct. 23-25	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Depr. Grasping Reflex	Oct. 23	Slight
	Rough Coat	Oct. 23-26, 29- Nov. 5	Marked
	Stain, Perianal, Yellow	Oct. 23-26	Marked
	Stain, Mouth, Red	Oct. 24	Marked
84D01270	Hunched Posture	Oct. 23-25	Moderate
	Incr. Respiration Rate	Oct. 23, 24	Moderate
	Stain, Nose, Red	Oct. 23, 24	Moderate
	Incr. Startle Reflex	Oct. 23, 24	Slight
	Rough Coat	Oct. 24	Slight
84D01283	Hunched Posture	Oct. 23	Moderate
	Incr. Respiration Depth	Oct. 23	Moderate
	Inactive	Oct. 23	Moderate
	Depr. Grasping Reflex	Oct. 23	Moderate
	Depr. Righting Reflex	Oct. 23	Slight
	Death	Oct. 23	2.0 h
84D01284	Clonic Convulsion	Oct. 23	Slight
	Inactive	Oct. 23	Moderate
	Hunched Posture	Oct. 23	Slight
	Depr. Grasping Reflex	Oct. 23	Slight
	Incr. Startle Reflex	Oct. 23	Slight
	Death	Oct. 23	2.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2780 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01310	Tremors	Oct. 23	Moderate
	Twitching	Oct. 23	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Hunched Posture	Oct. 23	Marked
	Stain, Perianal, Brown	Oct. 23	Moderate
	Rough Coat	Oct. 23	Marked
	Inactive	Oct. 23	Moderate
	Clonic Convulsion	Oct. 23	Marked
	Death	Oct. 24	20.5 h
84D01319	Twitching	Oct. 23	Slight
	Hunched Posture	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23	Slight
	Death	Oct. 23	3.1 h
84D01320	Twitching	Oct. 23	Slight
	Stain, Nose, Red	Oct. 23	Slight
	Death	Oct. 23	2.2 h
84D01328	Hunched Posture	Oct. 23	Marked
	Inactive	Oct. 23	Moderate
	Rough Coat	Oct. 23	Moderate
	Tremors	Oct. 23	Slight
	Stain, Nose, Red	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23	Slight
	Incr. Startle Reflex	Oct. 23	Moderate
	Death	Oct. 23	3.9 h
84D01329	Tremors	Oct. 23, 24	Moderate
	Inactive	Oct. 23	Slight
	Hunched Posture	Oct. 23-26	Moderate
	Depr. Grasping Reflex	Oct. 23, 24	Moderate
	Rough Coat	Oct. 24-26	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3600 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01269	Death	Oct. 25	1.5 h
84D01273	Hunched Posture	Oct. 25	Moderate
	Inactive	Oct. 25	Marked
	Tremors	Oct. 25	Moderate
	Incr. Respiration Rate	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
	Stain, Perianal, Brown	Oct. 25	Slight
	Stain, Mouth, Red	Oct. 25	Slight
	Irritable	Oct. 25	Marked
	Death	Oct. 23	5.7 h
84D01281	Hunched Posture	Oct. 25	Marked
	Inactive	Oct. 25	Marked
	Tremors	Oct. 25	Marked
	Incr. Respiration Rate	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
	Stain, Mouth, Red	Oct. 25	Moderate
	Stain, Perianal, Brown	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25	Moderate
	Incr. Startle Reflex	Oct. 25	Moderate
	Death	Oct. 26	23.7 h
84D01289	Death	Oct. 25	1.7 h
84D01298	Inactive	Oct. 25	Marked
	Hunched Posture	Oct. 25	Moderate
	Tremors	Oct. 25	Moderate
	Rough Coat	Oct. 25	Slight
	Irritable	Oct. 25	Slight
	Stain, Mouth, Red	Oct. 25	Slight
	Diarrhea	Oct. 25	Moderate
	Moribund	Oct. 25	
	Death	Oct. 25	6.7 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3600 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01299	Hunched Posture	Oct. 25	Marked
	Inactive	Oct. 25	Marked
	Tremors	Oct. 25	Slight
	Irritable	Oct. 25	Moderate
	Rough Coat	Oct. 25	Moderate
	Stain, Mouth, Red	Oct. 25	Moderate
	Incr. Startle Reflex	Oct. 25	Slight
	Death	Oct. 25	5.8 h
84D01304	Hunched Posture	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
	Death	Oct. 25	3.5 h
84D01307	Death	Oct. 25	1.8 h
84D01309	Death	Oct. 25	1.8 h
84D01312	Misdosed	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01296	Irritable Rough Coat	Oct. 25 Oct. 29	Moderate Slight
84D01301	Rough Coat	Oct. 25	Slight
84D01302	Rough Coat	Oct. 25, 26	Slight
84D01325	Rough Coat Stain, Nose, Red	Oct. 25 Nov. 8	Slight Slight
84D00532	Misdosed	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 600 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01342	Hunched Posture	Oct. 25	Moderate
	Stain, Nose, Red	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Rough Coat	Oct. 25, 26	Slight
	Depr. Grasping Reflex	Oct. 26	Slight
84D01360	Hunched Posture	Oct. 25	Slight
	Stain, Mouth, Red	Oct. 25	Moderate
	Stain, Perianal, Brown	Oct. 25, 26	Moderate
	Diarrhea	Oct. 25	Moderate
	Irritable	Oct. 25	Moderate
	Incr. Startle Reflex	Oct. 25	Slight
	Rough Coat	Oct. 26	Slight
84D01368	Hunched Posture	Oct. 25	Marked
	Inactive	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
84D01370	Hunched Posture	Oct. 25	Moderate
	Incr. Startle Reflex	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25	Slight
	Tremors	Oct. 25	Slight
84D01376	Tremors	Oct. 25	Marked
	Hunched Posture	Oct. 25, 29	Moderate
	Inactive	Oct. 25	Moderate
	Rough Coat	Oct. 25, 26	Moderate
	Diarrhea	Oct. 25	Slight
	Stain, Nose, Red	Oct. 25	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 600 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01379	Hunched Posture	Oct. 25	Moderate
	Inactive	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25, 26	Slight
	Rough Coat	Oct. 25	Slight
	Stain, Perianal, Brown	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 26	Slight
	Stain, Nose, Red	Oct. 25	Moderate
	Tremors	Oct. 25	Slight
	Diarrhea	Oct. 25	Slight
	Incr. Respiration Rate	Oct. 25	Slight
84D01392	Tremors	Oct. 25	Slight
	Hunched Posture	Oct. 25	Moderate
	Stain, Nose, Red	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25	Slight
84D01397	Stain, Nose, Red	Oct. 25, 26	Moderate
	Hunched Posture	Oct. 25	Moderate
	Tremors	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25	Moderate
	Rough Coat	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Slight
84D01399	Hunched Posture	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Rough Coat	Oct. 25, 26	Slight
	Incr. Startle Reflex	Oct. 25	Slight
84D01400	Incr. Startle Reflex	Oct. 25	Slight
	Hunched Posture	Oct. 25	Slight
	Tremors	Oct. 25	Slight
	Stain, Perianal, Brown	Oct. 25	Slight
	Irritable	Oct. 25	Moderate
	Stain, Perianal, Yellow	Oct. 25	Moderate
	Cyanosis	Oct. 25	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 775 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01333	Hunched Posture	Oct. 23-25	Moderate
	Disoriented	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23,24	Moderate
	Depr. Grasping Reflex	Oct. 23,24,26	Moderate
	Stain, Perianal, Yellow	Oct. 23,24	Moderate
84D01337	Inactive	Oct. 23	Marked
	Hunched Posture	Oct. 23-25	Marked
	Tremors	Oct. 23,24	Moderate
	Stain, Perianal, Yellow	Oct. 23,24	Marked
	Stain, Mouth, Red	Oct. 25	Slight
	Rough Coat	Oct. 23-25	Slight
	Depr. Grasping Reflex	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23,24	Marked
	Incr. Respiratio. Rate	Oct. 23	Moderate
84D01345	Removed from Study (Ulceration on Head)	N/A	N/A
84D01348	Hunched Posture	Oct. 23	Moderate
	Inactive	Oct. 23	Moderate
	Disoriented	Oct. 23	Slight
	Rough Coat	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Moderate
	Tremors	Oct. 23	Slight
	Diarrhea	Oct. 23	Slight
	Stain, Perianal, Yellow	Oct. 24	Moderate
	Depr. Grasping Reflex	Oct. 24	Moderate
84D01354	Hunched Posture	Oct. 23,25	Marked
	Inactive	Oct. 23	Moderate
	Tremors	Oct. 23,24	Marked
	Rough Coat	Oct. 23-26	Marked
	Incr. Startle Reflex	Oct. 23,24	Marked
	Depr. Grasping Reflex	Oct. 24	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 775 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01361	Hunched Posture	Oct. 23	Marked
	Inactive	Oct. 23	Marked
	Tremors	Oct. 23	Moderate
	Diarrhea	Oct. 23	Moderate
	Depr. Grasping Reflex	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23	Slight
	Death	Oct. 23	4.5 h
84D01362	Hunched Posture	Oct. 23-25	Moderate
	Tremors	Oct. 23,24	Moderate
	Rough Coat	Oct. 23-26	Moderate
	Stain, Perianal, Yellow	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Marked
84D01369	Hunched Posture	Oct. 23-25	Marked
	Tremors	Oct. 23,24	Moderate
	Rough Coat	Oct. 23-25	Moderate
	Depr. Grasping Reflex	Oct. 23,24	Moderate
	Disoriented	Oct. 23	Slight
	Incr. Startle Reflex	Oct. 23,24	Moderate
	Hyperactive	Oct. 30	Slight
84D01375	Hunched Posture	Oct. 23-25	Marked
	Inactive	Oct. 23,25	Slight
	Tremors	Oct. 23,24	Moderate
	Depr. Grasping Reflex	Oct. 23,24	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23,24,26	Marked
	Stain, Perianal, Yellow	Oct. 24	Moderate
84D01398	Misdosed	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1000 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01332	Death	Oct. 25	1.8 h
84D01338	Hunched Posture	Oct. 25	Moderate
	Tremors	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Slight
	Stain, Nose, Red	Oct. 25	Slight
	Death	Oct. 25	3.5 h
84D01339	Tremors	Oct. 25	Moderate
	Inactive	Oct. 25	Moderate
	Hunched Posture	Oct. 25	Moderate
	Stain, Nose, Red	Oct. 25	Slight
	Stain, Perianal, Yellow	Oct. 25, 26	Slight
84D01341	Tremors	Oct. 25	Marked
	Hunched Posture	Oct. 25	Moderate
	Incr. Startle Reflex	Oct. 25	Slight
	Stain, Nose, Red	Oct. 25	Slight
84D01349	Hunched Posture	Oct. 25	Marked
	Inactive	Oct. 25	Moderate
	Tremors	Oct. 25	Marked
	Incr. Startle Reflex	Oct. 25	Moderate
	Rough Coat	Oct. 25	Slight
84D01355	Tremors	Oct. 25	Slight
	Hunched Posture	Oct. 25, 29	Moderate
	Stain, Mouth, Red	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
84D01365	Hunched Posture	Oct. 25	Slight
	Stain, Nose, Red	Oct. 25	Slight
	Death	Oct. 25	3.6 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1000 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01377	Hunched Posture	Oct. 25,26	Moderate
	Tremors	Oct. 25	Moderate
	Stain, Nose, Red	Oct. 25	Slight
	Disoriented	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Slight
	Cyanosis	Oct. 25	Moderate
	Incr. Respiration Depth	Oct. 25	Moderate
84D01387	Hunched Posture	Oct. 25	Moderate
	Tremors	Oct. 25	Slight
	Stain, Mouth, Red	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
	Death	Oct. 25	6.2 h
84D01388	Hunched Posture	Oct. 25	Marked
	Inactive	Oct. 25	Marked
	Tremors	Oct. 25	Marked
	Stain, Perianal, Yellow	Oct. 25	Moderate
	Irritable	Oct. 25	Slight
	Incr. Startle Reflex	Oct. 25	Moderate
	Chromodacryorrhea	Oct. 25	Moderate
	Incr. Respiration Rate	Oct. 25	Moderate
	Death	Oct. 25	6.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1290 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01351	Hunched Posture	Oct. 23	Moderate
	Inactive	Oct. 23	Slight
	Tremors	Oct. 23	Slight
	Depr. Grasping Reflex	Oct. 23	Slight
	Rough Coat	Oct. 23	Slight
	Death	Oct. 23	3.0 h
84D01357	Tremors	Oct. 23	Moderate
	Hunched Posture	Oct. 23	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23	Slight
	Depr. Grasping Reflex	Oct. 23	Moderate
	Inactive	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Slight
	Death	Oct. 23	5.3 h
84D01359	Death	Oct. 23	2.3 h
84D01367	Death	Oct. 23	2.0 h
84D01372	Hunched Posture	Oct. 23, 24	Moderate
	Tremors	Oct. 23, 24	Moderate
	Stain, Mouth, Red	Oct. 23	Slight
	Rough Coat	Oct. 23-26	Moderate
	Incr. Respiration Rate	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23-26	Slight
	Depr. Grasping Reflex	Oct. 23, 24	Slight
84D01374	Hunched Posture	Oct. 23-25	Marked
	Inactive	Oct. 23	Marked
	Disoriented	Oct. 23	Slight
	Depr. Grasping Reflex	Oct. 23, 24	Moderate
	Rough Coat	Oct. 23-25	Moderate
	Stain, Mouth, Red	Oct. 23, 24	Marked
	Tremors	Oct. 23, 24	Moderate
	Incr. Startle Reflex	Oct. 23, 25	Moderate
	Irritable	Oct. 23, 25	Moderate
	Stain, Perianal, Yellow	Oct. 24, 25	Marked
	Hyperactive	Oct. 25	Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1290 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01378	Hunched Posture	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Stain, Perianal, Yellow	Oct. 23	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23	Moderate
	Stain, Nose, Red	Oct. 23	Slight
	Death	Oct. 23	4.9 h
84D01384	Disoriented	Oct. 23	Marked
	Hunched Posture	Oct. 23	Marked
	Inactive	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Stain, Mouth, Red	Oct. 23	Marked
	Depr. Grasping Reflex	Oct. 23	Slight
	Rough Coat	Oct. 23	Moderate
	Incr. Respiration Rate	Oct. 23	Slight
	Incr. Startle Reflex	Oct. 23	Moderate
	Stain, Perianal, Brown	Oct. 23	Slight
	Death	Oct. 24	20.8 h
84D01386	Hunched Posture	Oct. 23	Marked
	Tremors	Oct. 23	Marked
	Inactive	Oct. 23	Marked
	Disoriented	Oct. 23	Marked
	Depr. Grasping Reflex	Oct. 23	Marked
	Rough Coat	Oct. 23	Marked
	Stain, Mouth, Red	Oct. 23	Marked
	Incr. Startle Reflex	Oct. 23	Marked
	Stain, Perianal, Yellow	Oct. 23	Slight
	Irritable	Oct. 23	Marked
	Death	Oct. 24	20.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1290 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01396	Hunched Posture	Oct. 23	Marked
	Inactive	Oct. 23	Moderate
	Twitching	Oct. 23	Marked
	Depr. Grasping Reflex	Oct. 23	Slight
	Incr. Respiration Rate	Oct. 23	Moderate
	Incr. Respiration Depth	Oct. 23	Moderate
	Incr. Startle Reflex	Oct. 23	Marked
	Rough Coat	Oct. 23	Marked
	Tremors	Oct. 23	Marked
	Irritable	Oct. 23	Marked
	Stain, Perianal, Yellow	Oct. 23	Marked
	Death	Oct. 24	20.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2150 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01334	Death	Oct. 23	1.2 h
84D01336	Hunched Posture	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Inactive	Oct. 23	Moderate
	Diarrhea	Oct. 23	Slight
	Rough Coat	Oct. 23	Slight
	Stain, Perianal, Yellow	Oct. 23	Slight
	Clonic Convulsion	Oct. 23	Marked
	Incr. Startle Reflex	Oct. 23	Marked
	Death	Oct. 23	3.6 h
84D01340	Misdosed	N/A	N/A
84D01343	Death	Oct. 23	1.3 h
84D01346	Death	Oct. 23	1.3 h
84D01366	Hunched Posture	Oct. 23	Marked
	Inactive	Oct. 23	Moderate
	Disoriented	Oct. 23	Moderate
	Tremors	Oct. 23	Moderate
	Depr. Grasping Reflex	Oct. 23	Moderate
	Stain, Mouth, Red	Oct. 23	Marked
	Rough Coat	Oct. 23	Marked
	Death	Oct. 23	3.4 h
84D01371	Death	Oct. 23	1.6 h
84D01373	Prostrate	Oct. 23	
	Tremors	Oct. 23	Marked
	Incr. Salivation	Oct. 23	Moderate
	Death	Oct. 23	1.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2150 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01389	Hunched Posture	Oct. 23	Marked
	Tremors	Oct. 23	Marked
	Depr. Grasping Reflex	Oct. 23	Marked
	Incr. Respiration Rate	Oct. 23	Moderate
	Rough Coat	Oct. 23	Marked
	Clonic Convulsion	Oct. 23	Marked
	Inactive	Oct. 23	Marked
	Death	Oct. 23	3.7 h
84D01401	Death	Oct. 23	1.3 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D01353	Hunched Posture	Oct. 25	Slight
	Rough Coat	Oct. 25	Slight
84D01363	Hunched Posture	Oct. 25	Slight
	Irritable	Oct. 25, 26	Marked
	Rough Coat	Oct. 26	Slight
84D01385	Hunched Posture	Oct. 25	Moderate
	Stain, Perianal, Yellow	Oct. 25	Slight
	Rough Coat	Oct. 25	Moderate
84D01391	Hunched Posture	Oct. 25	Moderate
	Tremors	Oct. 25	Moderate
	Disoriented	Oct. 25	Slight
	Diarrhea	Oct. 25	Moderate
	Rough Coat	Oct. 25	Slight
84D01393	Rough Coat	Oct. 25, 26	Slight
	Stain, Perianal, Yellow	Oct. 25	Moderate

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS

MALES: 1000 mg/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01275	136	184	Misdosed	—
84D01276	155	210	261	262
84D01277	129	209	Dead	—
84D01292	133	200	Misdosed	—
84D01294	145	223	298	310
84D01303	144	212	288	272
84D01305	136	204	287	286
84D01315	131	195	265	266
84D01318	148	213	285	284
84D01321	144	210	282	284

Mean	140.1	206.0	280.9	280.6
Standard Deviation	8.36	10.85	13.21	16.07
Standard Error of the Mean	2.64	3.43	4.99	6.07

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALES: 1290 mg/kg

Animal Number	Receipt	D ing	Day 8	Termination Day 14
84D01267	133	219	Misdosed	—
84D01274	150	243	Dead	—
84D01278	149	238	289	314
84D01279	142	226	284	305
84D01280	151	246	Dead	—
84D01291	130	228	300	305
84D01308	143	234	304	313
84D01326	141	218	Dead	—
84D01327	138	237	Dead	—
84D01331	133	197	251	247

Mean	141.0	228.6	285.6	296.8
Standard Deviation	7.51	14.56	20.96	28.16
Standard Error of the Mean	2.38	13.8	9.37	12.60

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**MALES: 1670 mg/kg**

Animal Number	Receipt	Dosing	Day 8	Termination
				Day 14
84D01266	146	221	298	293
84D01271	144	206	279	283
84D01288	145	219	301	304
84D01290	141	207	Dead	—
84D01293	141	207	Dead	—
84D01311	138	199	Dead	—
84D01316	132	188	Dead	—
84D01317	139	220	Dead	—
84D01322	169	215	Dead	—
84D01324	144	207	276	276

Mean	143.9	208.9	288.5	289.0
Standard Deviation	9.73	10.34	12.82	12.19
Standard Error of the Mean	3.08	3.27	6.41	6.10

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALES: 2780 mg/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01263	143	216	Dead	---
84D01265	147	221	295	290
84D01270	136	203	272	286
84D01283	144	202	Dead	---
84D01284	154	221	Dead	---
84D01310	141	209	Dead	---
84D01319	132	203	Dead	---
84D01320	131	192	Dead	---
84D01328	141	202	Dead	---
84D01329	130	162	268	269

Mean	139.9	203.1	278.3	281.7
Standard Deviation	7.69	17.18	14.57	11.15
Standard Error of the Mean	2.43	5.43	8.41	6.44

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**MALES: 3600 mg/kg**

Animal Number	Receipt	Dosing	Day 8	Termination
				Day 14
84D01269	143	227	Dead	—
84D01273	146	233	Dead	—
84D01281	137	219	Dead	—
84D01289	127	211	Dead	—
84D01298	125	224	Dead	—
84D01299	149	230	Dead	—
84D01304	129	236	Dead	—
84D01307	131	220	Dead	—
84D01309	145	237	Dead	—
84D01312	137	213	Misdosed	—

Mean	136.9	225.0	—	—
Standard Deviation	8.62	9.19	—	—
Standard Error of the Mean	2.73	2.91	—	—

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALES: 600 mg/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01342	142	181	214	207
84D01360	148	186	191	209
84D01368	144	182	165	197
84D01370	144	146	197	210
84D01376	151	176	210	225
84D01379	162	165	203	214
84D01392	137	176	183	201
84D01397	135	192	208	225
84D01399	140	185	201	222
84D01400	149	190	205	228

Mean	145.2	177.9	197.7	213.8
Standard Deviation	7.81	13.66	14.70	10.80
Standard Error of the Mean	2.47	4.32	4.65	3.41

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALES: 775 mg/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01333	142	171	213	198
84D01337	141	165	212	180
84D01345	145	184	Deleted	—
84D01348	137	161	193	185
84D01354	137	164	176	198
84D01361	145	157	Dead	—
84D01362	144	184	199	215
84D01369	141	179	195	222
84D01375	158	184	194	207
84D01398	144	177	Misdosed	—

Mean	143.4	172.6	197.4	200.7
Standard Deviation	5.91	10.34	12.61	15.21
Standard Error of the Mean	1.87	3.27	4.77	5.75

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALES: 1000 mg/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01332	141	213	Dead	—
84D01338	140	162	Dead	—
84D01339	130	166	201	187
84D01341	141	155	189	186
84D01349	149	194	232	231
84D01355	152	188	204	200
84D01365	143	183	Dead	—
84D01377	153	187	188	194
84D01387	151	184	Dead	—
84D01388	152	182	Dead	—

Mean	145.2	181.4	202.8	199.6
Standard Deviation	7.45	16.84	17.80	18.45
Standard Error of the Mean	2.36	5.33	7.96	8.25

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALES: 1290 mc/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01351	138	168	Dead	—
84D01357	147	166	Dead	—
84D01359	150	173	Dead	—
84D01367	142	164	Dead	—
84D01372	147	176	194	207
84D01374	143	180	204	212
84D01378	130	162	Dead	—
84D01384	153	170	Dead	—
84D01386	139	167	Dead	—
84D01396	142	166	Dead	—

Mean	143.1	169.2	199.0	209.5
Standard Deviation	6.61	5.61	7.07	3.54
Standard Error of the Mean	2.09	1.78	5.00	2.50

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALES: 2150 mg/kg

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
84D01334	133	163	Dead	—
84D01336	139	161	Dead	—
84D01340	143	171	Misdosed	—
84D01343	152	180	Dead	—
84D01346	143	181	Dead	—
84D01366	140	173	Dead	—
84D01371	147	187	Dead	—
84D01373	150	169	Dead	—
84D01389	150	172	Dead	—
84D01401	140	166	Dead	—

Mean	143.7	172.3	—	—
Standard Deviation	6.00	8.29	—	—
Standard Error of the Mean	1.90	2.62	—	—

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Vehicle Controls

Animal Number	Receipt	Dosing	Day 8	Termination Day 14
Males				
84D01296	138	231	302	314
84D01301	128	220	292	302
84D01302	151	237	315	337
84D01325	140	223	289	301
84D01330	147	238	Misdosed	—

Mean	140.8	229.8	299.5	313.5
Standard Deviation	8.87	8.11	11.73	16.74
Standard Error of the Mean	3.97	3.62	5.87	8.37
Females				
84D01353	139	176	185	204
84D01363	152	163	168	184
84D01385	153	186	195	218
84D01391	152	188	191	201
84D01393	145	177	199	222

Mean	148.2	178.0	187.6	205.8
Standard Deviation	6.06	9.92	12.12	15.11
Standard Error of the Mean	2.71	4.44	5.42	6.76

Appendix G: PATHOLOGY REPORT

GLP Study 84013

Substance: 1,3 - Propanediol, 2-(hydroxymethyl)- 2-methyl -, trinitrate (TMETN).

Species: Rat Strain: Sprague-Dawley.

History: See LAIR SOP-OP-STX-36. Animals that did not die were killed under sodium pentobarbital anesthesia by axillary exsanguination.

Gross Findings:

MALES/1000 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36041	84 D0 1277	Dead - red stains, eyes, muzzle
36042	84 D0 1292	Dead - hydrothorax, red stains, eyes, muzzle
36050	84 D0 1275	Dead - hydrothorax and hydropericardium, red stains, eyes, muzzle
36205	84 D0 1276	Live - Not Remarkable (NR)
36207	84 D0 1294	Live - NR
36208	84 D0 1303	Live - NR
36209	84 D0 1305	Live - NR
36210	84 D0 1315	Live - NR
36212	84 D0 1321	Live - Thymus - multifocal hemorrhage, 1-2 mm
36211	84 D0 1318	Live - NR

MALES/1290 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36080	84 D0 1274	Dead - NR
36082	84 D0 1327	Dead - NR
36083	84 D0 1280	Dead - NR
36096	84 D0 1326	Dead - 1 cm focal scrotal dermatitis
36255	84 D0 1278	Live - NR
36256	84 D0 1279	Live - NR
36257	84 D0 1291	Live - NR
36261	84 D0 1308	Live - NR
36264	84 D0 1331	Live - NR

Appendix G (cont.): PATHOLOGY REPORT

MALES/1670 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36043	84 D0 1317	Dead - red stains, eyes, muzzle
36058	84 D0 1316	Dead - red stains, muzzle
36062	84 D0 1290	Dead - NR
36063	84 D0 1311	Dead - NR
36064	84 D0 1322	Dead - NR
36071	84 D0 1293	Dead - NR
36202	84 D0 1266	NR
36204	84 D0 1271	NR
36206	84 D0 1288	NR
36213	84 D0 1324	Live - NR

MALES/2780 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36051	84 D0 1283	Dead - NR
36054	84 D0 1320	Dead - NR
36057	84 D0 1284	Dead - dilated right renal pelvis
36059	84 D0 1319	Dead - NR
36061	84 D0 1263	Dead - NR
36065	84 D0 1328	Dead - red stains, eyes, muzzle
36072	84 D0 1310	Dead - eye, 3 mm opaque area on cornea
36201	84 D0 1265	Live - NR
36203	84 D0 1270	Live - thymus multifocal hemorrhage, lungs red exudate, red mottled surface
36214	84 D0 1329	Live - dilated right renal pelvis, thymus multifocal hemorrhage, lungs white exudate

Appendix G (cont.): PATHOLOGY REPORT

MALES/3600 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36079	84 D0 1269	Dead - NR
36081	84 D0 1289	Dead - dilated right renal pelvis
36084	84 D0 1304	Dead - NR
36085	84 D0 1307	Dead - NR
36086	84 D0 1309	Dead - NR
36092	84 D0 1273	Dead - NR
36093	84 D0 1281	Dead - 1 cm focal scrotal dermatitis
36094	84 D0 1298	Dead - NR
36095	84 D0 1299	Dead - NR

MALES/control

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36258	84 D0 1296	Live - dilated right renal pelvis.
36259	84 D0 1301	Live - NR
36260	84 D0 1302	Live - NR
36262	84 D0 1325	Live - dilated right renal pelvis

FEMALES/600 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36267	84 D0 1342	Live - NR
36271	84 D0 1360	Live - NR
36273	84 D0 1368	Live - NR
36274	84 D0 1370	Live - NR
36275	84 D0 1376	Live - NR
36277	84 D0 1379	Live - NR
36280	84 D0 1392	Live - NR
36282	84 D0 1397	Live - NR
36283	84 D0 1399	Live - NR
36284	84 D0 1400	Live - NR

Appendix G (cont.): PATHOLOGY REPORT

FEMALES/775 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36060	84 D0 1361	Dead - NR
36110	84 D0 1345	Live - Focal alopecia, head
36215	84 D0 1333	Live - NR
36216	84 D0 1337	Live - NR
36217	84 D0 1348	Live - NR
36218	84 D0 1354	Live - NR
36219	84 D0 1362	Live - NR
36220	84 D0 1369	Live - NR
36223	84 D0 1375	Live - NR

FEMALES/1000 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36088	84 D0 1332	Dead - NR
36089	84 D0 1338	Dead - NR
36090	84 D0 1365	Dead - NR
36097	84 D0 1387	Dead - NR
36098	84 D0 1388	Dead - NR
36265	84 D0 1339	Live - NR
36266	84 D0 1341	Live - NR
36268	84 D0 1349	Live - NR
36270	84 D0 1355	Live - NR
36276	84 D0 1377	Live - NR

FEMALES/1290 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36048	84 D0 1359	Dead - NR
36052	84 D0 1367	Dead - NR
36055	84 D0 1351	Dead - red stains, eyes, muzzle
36067	84 D0 1357	Dead - NR
36069	84 D0 1378	Dead - NR
36073	84 D0 1384	Dead - NR
36074	84 D0 1386	Dead - urinary bladder 8 mm x 4 mm white crystalline ovoid
36075	84 D0 1396	Dead - NR
36221	84 D0 1372	Live - NR
36222	84 D0 1374	Live - NR

Appendix G (cont.): PATHOLOGY REPORT

FEMALES/2150 mg/kg

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36044	84 D0 1334	Dead - NR
36045	84 D0 1340	Dead - hydrothorax, red stains, eyes, muzzle
36046	84 D0 1343	Dead - NR
36047	84 D0 1346	Dead - NR
36049	84 D0 1401	Dead - NR
36053	84 D0 1371	Dead - red stains, eyes, muzzle
36056	84 D0 1373	Dead - NR
36066	84 D0 1336	Dead - NR
36068	84 D0 1366	Dead - NR
36070	84 D0 1389	Dead - NR

FEMALES/control

<u>Path. Acc.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36269	84 D0 1353	Live - NR
36272	84 D0 1363	Live - NR
36278	84 D0 1385	Live - NR
36279	84 D0 1391	Live - NR
36281	84 D0 1393	Live - NR

Comments:

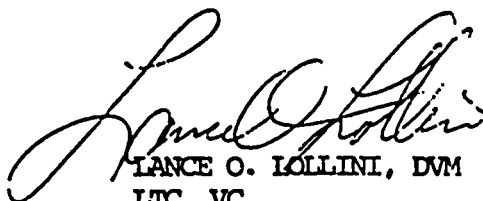
1. Dose-related toxicity of the compound was clearly present and the difference noted between the male and females was possibly due to sexual dimorphism.
2. Most deaths occurred within 7 hours of dosing (48/54). A few died during the first night and were recorded as dead the following morning.
3. Compound-related lesions were not observed.
4. Several animals that died had red staining of the hair and skin around the eyes and muzzle that was considered to be an incidental finding. Animals that died during the night had diffuse post mortem autolysis of all organs to include opaque corneas and white precipitate in the urinary bladder.

Appendix G (cont.): PATHOLOGY REPORT

Other incidental findings included dilated renal pelves, scrotal dermatitis, and focal alopecia. Iatrogenic lesions include hydropericardium as a result of esophageal puncture during dosing, and thymus hemorrhage and pulmonary edema resulting from restraint during the I.P. injection of pentobarbital.



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