

DTIC FILE COPY

2



AD-A217 618

Institute Report No. 418

Acute Oral Toxicity of Guanidine Hydrochloride in Mice

Earl W. Morgan, DVM, MAJ, VC  
Gerald F. S. Hiatt, PhD  
and  
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY

DTIC  
ELECTE  
JAN 30 1990  
S E D

December 1989

Toxicology Series: 87

DISTRIBUTION STATEMENT A  
Approved for public release;  
Distribution Unlimited

LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

50 01 30 034

Acute Oral Toxicity of Guanidine Hydrochloride in Mice (Toxicology Series 87)--  
Morgan *et al.*

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)

*William C. Cole*      *29 December '89*  
for \_\_\_\_\_ (date)  
Donald G. Corby  
COL, MC  
Commanding

UNCLASSIFIED

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 APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.			
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 418			
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 418		5. MONITORING ORGANIZATION REPORT NUMBER(S)			
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology		6b. OFFICE SYMBOL (If applicable) SGRD-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 Guanidine Hydrochloride in Mice					
12. PERSONAL AUTHOR(S) EW Morgan, GFS Hiatt, and DW Korte, Jr.					
13a. TYPE OF REPORT Institute		13b. TIME COVERED FROM 2FEB84 TO 7FEB84	14. DATE OF REPORT (Year, Month, Day) December 1989		15. PAGE COUNT 50
16. SUPPLEMENTARY NOTATION Toxicology Series No. 87					
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)			
FIELD	GROUP	SUB-GROUP	Acute Oral Toxicity; Guanidine hydrochloride; Mouse; Mammalian Toxicology; Nitroguanidine		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The acute oral toxicity of guanidine hydrochloride was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose was $570.8 \pm 39.6$ mg(base)/kg for male and $621.4 \pm 83.4$ mg(base)/kg for female mice. Clinical signs included behavioral and respiratory changes. Behavioral signs observed were tremors, irritability, inactivity, disorientation, hyperactivity, jumping, and twitching. Respiratory changes included increases in rate and depth, and gasping. Lethality was observed only during the first 4.5 hours after dosing and clinical signs were observed primarily during the first 24 hours after dosing. These results place guanidine hydrochloride in the slightly toxic category.					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL DONALD G. CORBY, COL, MC		22b. TELEPHONE (Include Area Code) (415) 561-3600		22c. OFFICE SYMBOL SGRD-ULE	

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

**ABSTRACT**

The acute oral toxicity of guanidine hydrochloride was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose was  $570.8 \pm 39.6$  mg(base)/kg for male and  $621.4 \pm 83.4$  mg(base)/kg for female mice. Clinical signs included behavioral and respiratory changes. Behavioral signs observed were tremors, irritability, inactivity, disorientation, hyperactivity, jumping, and twitching. Respiratory changes included increases in rate and depth, and gasping. Lethality was observed only during the first 4.5 hours after dosing and clinical signs were observed primarily during the first 24 hours after dosing. These results place guanidine hydrochloride in the slightly toxic category.

KEY WORDS: Acute Oral Toxicity, Guanidine hydrochloride, Mouse, Mammalian Toxicology, Nitroguanidine



<b>Accession For</b>	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

## 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

WORK UNIT/APC: 180, Environmental Health Effects of Army  
Materials/TLB0

GLP STUDY NUMBER: 83020

STUDY DIRECTOR: LTC Don W. Korte, Jr., PhD, MSC  
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: MAJ Earl W. Morgan, DVM, VC  
Diplomate, American College of  
Veterinary Preventive Medicine,  
American Board of Toxicology

CO-PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

PATHOLOGIST: LTC Lance O. Lollini, DVM, VC, Diplomate,  
American College of Veterinary Pathologists

DATA MANAGER: Carolyn M. Lewis, MS

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

TEST SUBSTANCE: Guanidine hydrochloride

INCLUSIVE STUDY DATES: 2 - 27 February 1984

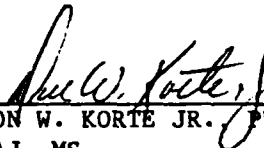
OBJECTIVE: The objective of this study was to determine the  
acute oral toxicity of guanidine hydrochloride in  
male and female ICR mice.


### **ACKNOWLEDGMENTS**

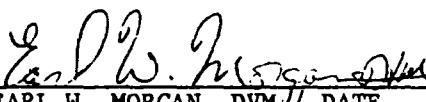
SP5 Thomas P. Kellner, BA, SP5 Justo Rodriguez, BS, Carolyn M. Lewis, MS, and SP4 Steven Sano provided research assistance; Edward Sands and Susan Hernandez provided animal care; and Callie Crosby, MA, and Brenda V. Goce provided secretarial assistance.

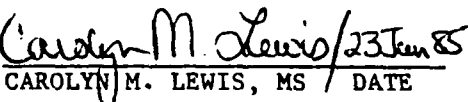
SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS  
INVOLVED IN THE STUDY

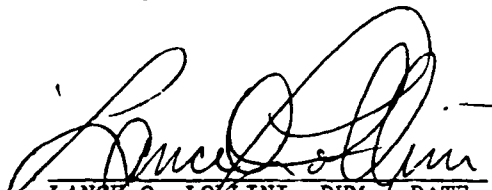
We, the undersigned, declare that GLP Study 83020 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

  
Don W. Korte Jr. / 15 July 85  
DON W. KORTE JR., PhD / DATE  
MAJ, MS  
Study Director

  
Gerald F.S. Hiatt / 19 June 85  
GERALD F.S. HIATT, PhD / DATE  
DAC  
Co-Principal Investigator

  
Earl W. Morgan / 23 May 85  
EARL W. MORGAN, DVM / DATE  
CPT, VC  
Principal Investigator

  
Carolyn M. Lewis / 23 June 85  
CAROLYN M. LEWIS, MS / DATE  
DAC  
Data Manager

  
Lance O. Lollini /  
LANCE O. LOLLINI, DVM / DATE  
LTC, VC  
Pathologist

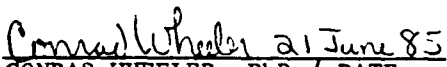
  
Conrad Wheeler / 21 June 85  
CONRAD WHEELER, PhD / DATE  
DAC  
Analytical Chemist

TABLE OF CONTENTS

	Page
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 Data.....	2
Husbandry.....	2
METHODS.....	3
Group Assignment/Acclimation.....	3
Dosage Levels.....	3
Compound Preparation.....	3
Chemical Analysis of Dosing Solution.....	4
Test Procedures.....	4
Observations.....	4
Necropsy.....	5
Statistical Analysis.....	5
Duration of Study.....	5
Changes/Deviations.....	5
Raw Data and Final Report Storage.....	5
RESULTS.....	5
Mortality.....	5
Lethal Dose Calculation .....	6
Clinical Observations.....	7
Gross Pathology Observations.....	10

TABLE OF CONTENTS (cont.)

DISCUSSION.....10

CONCLUSION.....13

REFERENCES.....14

APPENDICES.....15

    Appendix A. Chemical Data.....16

    Appendix B. Animal Data.....18

    Appendix C. Historical Listing of Study Events.....19

    Appendix D. Cumulative Mortality Data.....20

    Appendix E. Individual Animal Histories.....21

    Appendix F. Individual Body Weights.....36

    Appendix G. Pathology Report.....48

OFFICIAL DISTRIBUTION LIST.....50

**Acute Oral Toxicity of Guanidine Hydrochloride in Mice--Morgan et al.**

**INTRODUCTION**

Guanidine nitrate is an intermediate product in the synthesis of nitroguanidine. Nitroguanidine is a primary component of US Army triple-base propellants and is now being produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. Guanidine hydrochloride was also evaluated to define more completely the contribution of the guanidine base to the toxicity of guanidine nitrate.

Objective of Study

The objective of this study was to determine the acute oral toxicity of guanidine hydrochloride in male and female Institute of Cancer Research (ICR) mice.

**MATERIALS**

Test Substance

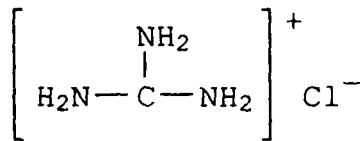
Chemical Name: Guanidine hydrochloride

Chemical Abstract Service Registry No.: 050-01-1

LAIR Code Number: TP28

Physical State: White powder

Molecular Structure:



Molecular Formula:  $\text{CH}_5\text{N}_3 \cdot \text{HCl}$

Other test substance information is presented in Appendix A.

#### Vehicle

The vehicle for guanidine hydrochloride was sterile water for injection (Cutter Laboratory, Inc., Emeryville, CA). The expiration date was April 1984.

#### Animal Data

Seventy-seven male and 76 female ICR mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used for this study. They were identified individually with tags numbered 84C00045 - 84C00197 (inclusive) placed in the dorsal skin of the neck. Two males and 2 females were selected at random for quality control necropsy evaluation at receipt. Twenty-nine of the animals were used in an Approximate Lethal Dose (ALD) determination. The animal weights on receipt (2 Feb 84) ranged from 23 to 32 g. Additional animal data appear in Appendix B.

#### Husbandry

Mice were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow Diet 5002 (Ralston Purina Company, St. Louis, MO); water was provided by lixit valves on a central line. The animal room temperature was constantly monitored and maintained in a range from 21.1°C to 25.5°C with a relative humidity range of 44% to 72% with occasional spikes to 80% due to room cleaning. The photoperiod was 12 hours of light per day.

**METHODS**Group Assignment/Acclimation

Study mice were randomized into five dose groups of 10 males and 10 females each and vehicle and cage control groups of 5 males and 5 females each. Allocation was accomplished by 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 10 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

The results of the ALD determination suggested that the median lethal dose (MLD) was between 400 and 500 mg(base)/kg for both male and female mice. Based on these data, test doses were selected (Table 1).

**TABLE 1: Group Dose Schedule**

Group	Dose mg (base) /kg
1	794
2	316
3	398
4	501
5	631
6 (vehicle)	0.2 ml sterile water for injection
7 (cage control)	N/A

Compound Preparation

Since the dose of guanidine hydrochloride administered was expressed in terms of the base equivalent, it was necessary to correct for the weight of the hydrochloride

moiety. On a molecular weight basis, the hydrochloride salt is 1.63 times the weight of the base equivalent and this factor was used to calculate the quantity of guanidine hydrochloride required to achieve the required dose levels. Since guanidine hydrochloride is freely soluble in water, all dosing solutions were prepared by mixing the guanidine hydrochloride in an appropriate volume of sterile water for injection immediately before dosing the animals.

#### Chemical Analysis of Dosing Solution

Guanidine hydrochloride was stable in the vehicle for four hours (Appendix A). This was sufficient since dosing was begun as soon as the solutions were prepared and completed within 2 hours. Tests for homogeneity of the test compound in the vehicle were not applicable as the test compound was in solution.

#### Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP OP-STX-36 (3). The volume of dosing solution given each animal was based upon the desired dose level and the compound concentration in suspension. The dose level was increased by varying the concentration of each suspension, and animals received calculated volumes based upon weight. Volumes ranged from 0.18 to 0.24 ml in the male and 0.16 to 0.21 ml in female mice. The vehicle control group was given 0.2 ml of the vehicle. Dosing was performed by oral gavage without animal sedation or anesthesia. Sterile, disposable syringes (Becton, Dickenson & Co., Rutherford, NJ) fitted with 20-gauge, 1-1/2-inch, ball-tipped, stainless steel Perfektum® oral gavage animal tubes (Popper & Sons, Inc., New Hyde Park, NY) were used for dosing. All animals were dosed between 0916 and 1050 hours on 13 February 1984 except for Group 1 mice (794 mg(base)/kg) which were dosed between 0916 and 0935 hours on 14 February 1984 after analysis of the data from 13 February.

#### Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: 1) animals were observed undisturbed in their cages, 2) animals were removed from their cages and given a physical examination, and 3) animals were observed after being returned to their cages. On the day of dosing, the mice 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 periodically during the course of the study.

#### Necropsy

Animals that died during the observation period were submitted for necropsy. Those that survived the 14-day study period were also submitted for necropsy after termination by barbiturate overdose.

#### Statistical Analysis

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

#### Duration of Study

Appendix C is a historical listing of study events.

#### Changes/Deviations

The study was performed as described in the protocol and applicable amendments with two exceptions. Acclimatization of the animals to the dosing procedure was omitted because previous studies indicated that acclimatization alerted the animals to the process and made them wary and difficult to handle. This change had no effect on the study. Group 2 males were inadvertently misdosed. Due to an error in mixing the dosing solution, they actually received 495 mg(base)/kg.

#### Raw Data and Final Report Storage

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 (42) occurred within 4 hours 24 minutes of dosing. Table 2 lists the compound-related deaths by group. Appendix D is a tabular presentation of cumulative mortality.

**TABLE 2: Compound-Related Deaths by Group**

Group	Dose (mg/kg)	Compound-Related Death/ Number in Group	Percent Mortality
<b>MALE</b>			
3	398	1/10	10
2	495*	4/10	40
4	501	4/10	40
5	631	6/10	60
1	794	8/10	80
6	Vehicle	0/5	0
7	Cage	0/5	0
<b>FEMALE</b>			
2	316	1/10	10
3	398	3/10	30
4	501	4/10	40
5	631	4/10	40
1	794	7/10	70
6	Vehicle	0/5	0
7	Cage	0/5	0

\* These animals should have received 316 mg(base)/kg, but were misdosed due to an error in mixing the dosing solution. They actually received 495 mg(base)/kg.

#### Lethal Dose Calculation

Lethal dose values were calculated by probit analysis. The equation for the probit regression line was:  $Y = -12.79 + 6.45 \log X$  for males and  $Y = -5.41 + 3.73 \log X$  for females, where X is the dose and Y the corresponding probit value. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

**TABLE 3: Calculated Lethal Doses (LD) of Guanidine Hydrochloride in ICR Mice**

Effect Level	Calculated Dose* (mg(base)/kg)	95% Confidence Limits (mg(base)/kg)
<b>MALES</b>		
LD10	361.3 ± 53.1	(181.8, 437.9)
LD50	570.8 ± 39.6	(491.2, 694.7)
LD90	901.8 ± 152.3	(726.0, 2015.8)
<b>FEMALES</b>		
LD10	281.5 ± 73.0	(48.3, 386.7)
LD50	621.4 ± 83.4	(492.1, 1183.9)
LD90	1371.7 ± 522.6	(877.9, 20689.0)

\*Lethal Dose ± standard error.

#### Clinical Observations

The most frequently observed categories of clinical signs in animals administered guanidine hydrochloride were respiratory disturbances (68 of 100 animals dosed), behavioral disturbances (60 of 100). Respiratory signs included changes in rate or depth, and gasping. Behavioral signs included irritability, inactivity, disorientation, hyperactivity, hypotonia, jumping, tremors, and twitching. Although clinical signs were observed at each dose level, there was no clear dose-response relationship for severity or duration of the symptoms.

The term "disorientation" describes a behavior pattern in which the animal appeared dazed and confused in response to external stimuli. "Disoriented" animals were observed sitting in their cages with vacant stares and exhibiting little or no response to noise, movement of their cages, or handling. Their movements were hesitant and appeared random except upon perceiving a threat which elicited grossly exaggerated escape movements. The dehydration observed in 4 mice was attributed to a malfunctioning water delivery system in their cages.

Figure 1  
Dose Response Curve for Guanidine Hydrochloride in Male Mice

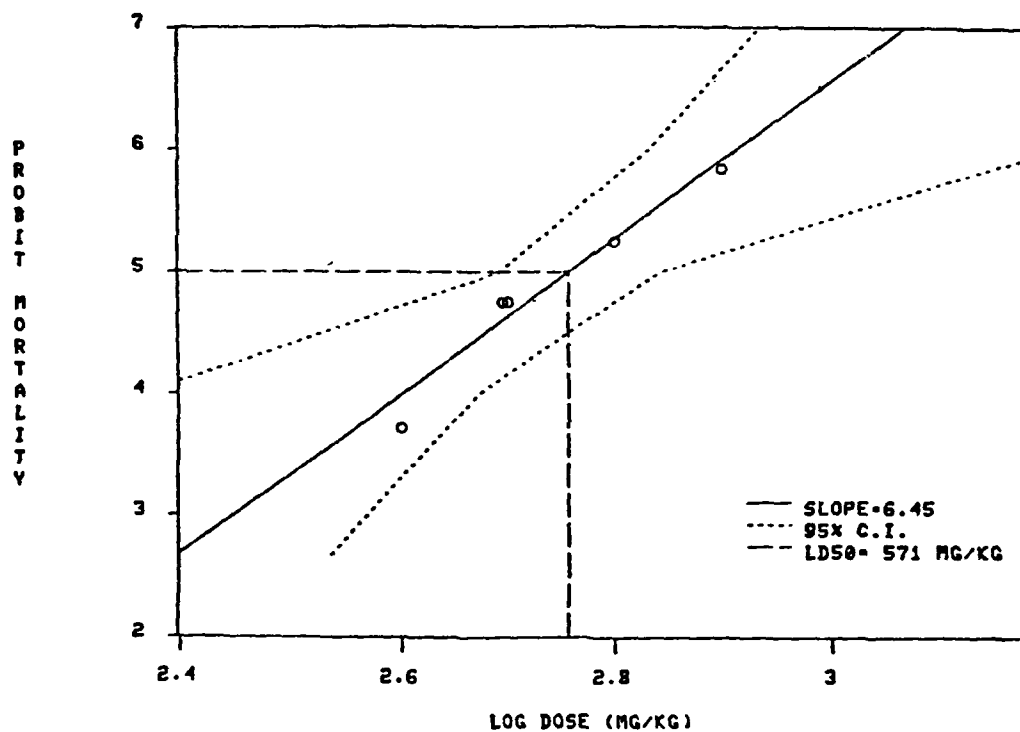
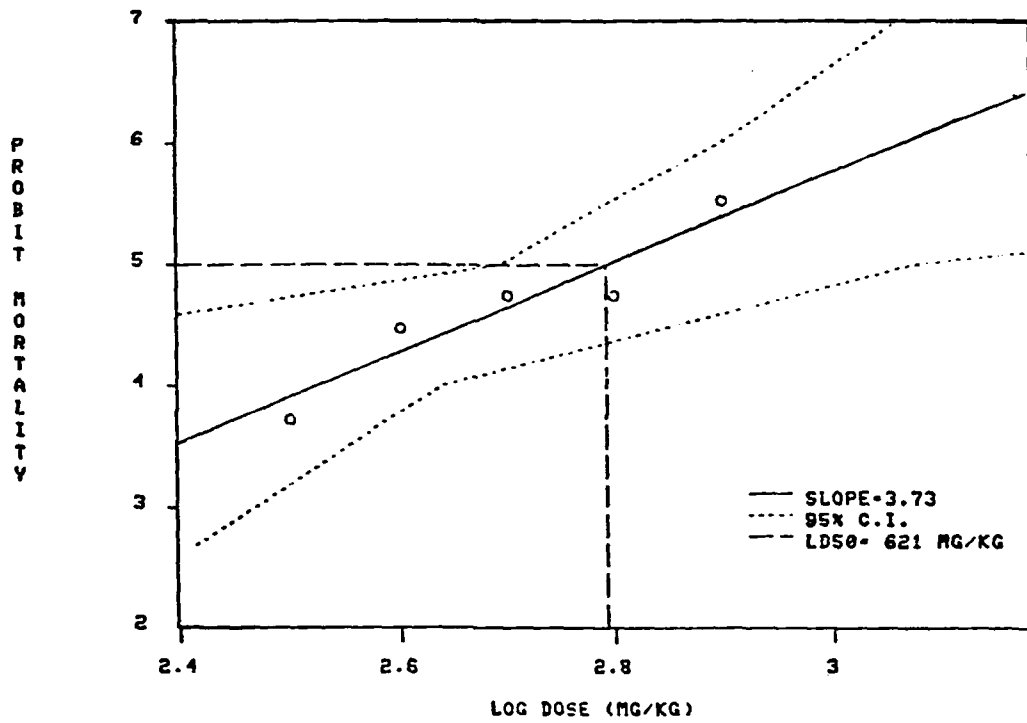


Figure 2  
Dose Response Curve for Guanidine Hydrochloride in  
Female Mice



Twenty-three male mice died during the study. Of the 27 surviving male mice, only 8 were normal within 48 hours of dosing. Nineteen female mice died during the study. Of the 31 surviving females, 18 were normal within 48 hours of dosing. All vehicle control animals survived until study termination at 14 days. 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 Pathology Observations

Pulmonary edema was observed in 6 of 20 animals dosed at 794 mg/kg. No other lesions were observed at necropsy. The veterinary pathologist's report appears at Appendix G.

#### **DISCUSSION**

The calculated MLD for guanidine hydrochloride was  $570.8 \pm 39.6$  mg(base)/kg in male and  $621.4 \pm 83.4$  mg(base)/kg in female ICR mice. These MLD values are within the slightly toxic range (5).

Orally administered guanidine hydrochloride was rapidly absorbed in mice as indicated by the onset of toxic signs including death within one hour of dosing. The toxicity observed, other than death, was predominately respiratory and behavioral signs. Respiratory signs included increased rate and depth, and gasping. Behavioral signs included disorientation, inactivity, tremors, twitching, hyperactivity, irritability, hypotonia, and jumping. Guanidine hydrochloride augments the release of acetylcholine from nerve endings (6) and a similar effect in the respiratory center and central nervous system may be responsible for the clinical signs observed in this study. The fact that guanidine hydrochloride is a small molecule, highly soluble in water and therefore readily permeable into the central nervous system is consistent with the rapid onset of toxicity following its oral administration.

**TABLE 4: Incidence Summary for Clinical Observations in Mice Administered Guanidine Hydrochloride**

Category of Clinical Signs	Group Dose (mg/kg)	1 Control <sup>a</sup>	2 316 <sup>b</sup>	3 398	4 501	5 631	6 794
<b>MALES</b>	(N=)	(10)	(10)	(10)	(10)	(10)	(10)
Respiratory <sup>c</sup>		2	5	8	4	4	7
Behavioral <sup>d</sup>		4	6	4	7	6	7
Convulsions <sup>e</sup>		-	-	-	1	-	1
Gastrointestinal <sup>f</sup>		-	2	-	1	-	2
Rough Coat		2	1	4	5	1	4
Squinting		-	-	1	1	-	-
Hunched Posture		-	2	2	1	1	2
Reflex <sup>g</sup>		-	1	2	-	-	3
Prostrate/Moribund		-	-	-	-	-	2
Miscellaneous <sup>h</sup>		2	3	2	2	3	-
Death		-	4	1	4	6	8
Normal		4	-	1	-	-	-
<b>FEMALES</b>	(N=)	(10)	(10)	(10)	(10)	(10)	(10)
Respiratory <sup>c</sup>		2	9	9	7	7	8
Behavioral <sup>d</sup>		5	5	4	6	8	7
Convulsions <sup>e</sup>		-	-	-	-	-	1
Gastrointestinal <sup>f</sup>		-	1	-	1	-	1
Rough Coat		-	-	-	1	-	-
Squinting		-	-	-	-	-	-
Hunched Posture		1	1	1	2	2	2
Reflex <sup>g</sup>		1	1	2	2	2	-
Prostrate/Moribund		-	-	-	-	-	1
Miscellaneous <sup>h</sup>		-	3	1	2	-	-
Death		-	1	3	4	4	7
Normal		5	1	-	-	-	-

<sup>a</sup> Includes vehicle and cage controls.

<sup>b</sup> Males actually received 495 mg/kg.

<sup>c</sup> Includes changes in rate or depth, and gasping.

<sup>d</sup> Includes disorientation, irritability, inactivity, hyperactivity, hypotonia, jumping, tremors, and twitching.

<sup>e</sup> Includes clonic and/or tonic convulsions.

<sup>f</sup> Includes retching, dehydration, diarrhea and increased salivation.

<sup>g</sup> Includes depressed grasping and/or righting reflexes, and changes in the startle reflex.

<sup>h</sup> Includes material and stains on perineum/flank and granulation/scabs at tag site.

**TABLE 5: Mean Body Weights in Grams  $\pm$  S.E  $\dagger$** 

Dose (mg/kg)	At Receipt	Dosing Day	Day 8	Day 14*
<b>MALES</b>				
Vehicle	28.4 $\pm 0.5$ (5)	31.4 $\pm 0.4$ (5)	33.8 $\pm 0.9$ (5)	29.0 $\pm 0.6$ (5)
398	27.5 $\pm 0.7$ (10)	31.4 $\pm 0.6$ (10)	33.9 $\pm 1.0$ (9)	28.8 $\pm 0.6$ (9)
495	26.8 $\pm 0.4$ (10)	30.6 $\pm 0.8$ (10)	31.7 $\pm 1.7$ (6)	27.7 $\pm 1.0$ (6)
501	28.2 $\pm 0.5$ (10)	31.0 $\pm 0.3$ (10)	33.0 $\pm 1.7$ (6)	28.8 $\pm 0.5$ (6)
631	27.9 $\pm 0.5$ (10)	31.2 $\pm 0.7$ (10)	33.8 $\pm 1.0$ (4)	29.3 $\pm 0.5$ (4)
794	27.1 $\pm 0.7$ (10)	31.7 $\pm 0.9$ (10)	31.0 $\pm 2.0$ (2)	28.0 $\pm 2.0$ (2)
Cage (not dosed)	27.2 $\pm 0.8$ (5)	32.6 $\pm 1.0$ (5)	34.4 $\pm 0.6$ (5)	29.4 $\pm 0.6$ (5)
<b>FEMALES</b>				
Vehicle	27.8 $\pm 0.6$ (5)	26.8 $\pm 1.4$ (5)	28.4 $\pm 0.8$ (5)	23.4 $\pm 1.2$ (5)
316	28.1 $\pm 0.5$ (10)	27.2 $\pm 0.7$ (10)	28.1 $\pm 1.0$ (9)	24.3 $\pm 0.6$ (9)
398	26.7 $\pm 0.4$ (10)	26.0 $\pm 0.4$ (10)	27.7 $\pm 0.8$ (7)	23.3 $\pm 0.6$ (7)
501	28.1 $\pm 0.5$ (10)	27.0 $\pm 0.4$ (10)	27.3 $\pm 1.1$ (6)	24.5 $\pm 0.4$ (6)
631	28.1 $\pm 0.4$ (10)	27.8 $\pm 0.7$ (10)	29.0 $\pm 0.3$ (6)	24.5 $\pm 0.5$ (6)
794	27.3 $\pm 0.4$ (10)	28.2 $\pm 0.6$ (10)	29.3 $\pm 1.5$ (3)	24.0 $\pm 1.5$ (3)
Cage (not dosed)	28.4 $\pm 0.2$ (5)	28.8 $\pm 0.6$ (5)	29.8 $\pm 1.0$ (5)	24.8 $\pm 0.7$ (5)

$\dagger$  Number in parenthesis = number of animals.

\* Weight after an overnight fast before necropsy.

The toxic signs were similar to those reported for guanidine hydrochloride in rats (7) although relative incidence was not consistent. For example, although approximately the same percentage of rats and mice exhibited behavioral effects, 50% more mice than rats responded with respiratory stimulation. Also, the gastrointestinal tract was not involved as significantly in the mouse study as in the rat study. At necropsy, guanidine hydrochloride had produced red glandular gastric mucosa in 20 of 36 rats that died during the study. In the mouse study, similar lesions were not observed. This may be a reflection of the shorter time between dosing and death in the mice versus the rats. While all deaths of the mice occurred within 4.5 hours of dosing, all deaths of the rats had not occurred until 48 hours after dosing. Although the relative lack of a gastrointestinal effect in the mouse may be due to species differences with the rat, it may simply be due to the fact that death occurred so rapidly in the mouse that there was not sufficient time for recognizable lesions to develop.

#### CONCLUSION

Guanidine hydrochloride is a slightly toxic compound that produces respiratory and behavioral signs of toxicity. Calculated median lethal dose values were  $570.8 \pm 39.6$  mg(base)/kg in male and  $621.4 \pm 83.4$  mg(base)/kg in female ICR mice.

### REFERENCES

1. Kenyon KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1982. DTIC No. ADA 125591.
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 1982; EPA 560/6-82-001.
3. Acute oral toxicity study (ALD and LD50). LAIR Standard Operating Procedure OP-STX-36, Letterman Army Institute of Research, Presidio of San Francisco, CA. 22 December 1982.
4. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.
5. Hodge HC, Sterner JH. Tabulation of toxicity classes. Amer Ind Hyg Assoc Q 1943; 10:93-96.
6. Morgan EW, Sano SK, Korte, DW Jr. Acute oral toxicity (LD50) of guanidine hydrochloride in rats. Toxicology Series 77. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1985; Institute Report No. 204.
7. Otsuka M, Endo M. The effect of guanidine on neuromuscular transmission. J Pharmacol Exp Ther 1960; 128:273-82.

	Page
Appendix A. Chemical Data .....	16
Appendix B. Animal Data .....	18
Appendix C. Historical Listing of Study Events .....	19
Appendix D. Cumulative Mortality Data .....	20
Appendix E. Individual Animal Histories .....	21
Appendix F. Individual Body Weights .....	36
Appendix G. Pathology Report .....	48

**Appendix A: CHEMICAL DATA**

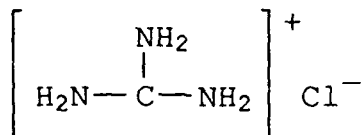
Chemical Name: Guanidine hydrochloride

Alternate Name(s): Aminomethanamide hydrochloride,  
Carbamamide hydrochloride,  
Carbamidine hydrochloride,  
Aminoformamide hydrochloride,  
Iminourea hydrochloride

Chemical Abstracts Service Registry No.: 050-01-1

LAIR Code: TP28

Chemical Structure:



Molecular Formula:  $\text{CH}_5\text{N}_3 \cdot \text{HCl}$

Molecular Weight: 95.5

Physical State: White powder

Melting Point:  $182^\circ\text{-}184^\circ\text{C}$  ( $184^\circ\text{-}185^\circ\text{C}$ )<sup>1</sup>

Analytical Data:

Water content 0.1% by Karl Fischer analysis.<sup>1</sup> The material is at least 98% pure and chromatographs as one spot by thin layer chromatography.<sup>2</sup> Elemental analysis: Calculated for  $\text{CH}_5\text{N}_3 \cdot \text{HCl}$ , 37.1; Found: Cl, 36.6.<sup>2</sup> An IR spectrum was obtained upon receipt of the compound. IR (KBr): 3400, 2750, 1650, 1535, 1050 (broad)  $\text{cm}^{-1}$ . A comparison of this spectrum to the Sadtler standard spectrum confirmed the identity of the material.<sup>3</sup>

<sup>1</sup> Zygmunt R. Analytical data sheet for guanidine hydrochloride, lot number 103F-5623. Sigma Chemical Co., St. Louis, MO. 16 Feb 84.

<sup>2</sup> Sigma Chemical Company, St. Louis, MO. Becky Goodloe, PhD, personal communication, 5 March 1985.

<sup>3</sup> Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #8676.

**Appendix A (cont.): CHEMICAL DATA**

Source: Sigma Chemical Company  
St. Louis, MO

Lot Number: 103F-5623

Stability in Aqueous Vehicle:

A preliminary study was conducted to determine the stability of guanidine hydrochloride in sterile water for injection. A solution of guanidine hydrochloride (18.825 µg/ml water) was assayed after preparation and 4 hours later by using the Voges-Proskauer Method.<sup>4</sup> This method is specific for unsubstituted and monosubstituted guanidines and yields a colored derivative which is monitored spectrophotometrically. Three samples were analyzed for each time point and the results were as follows:

Absorbance Value (1st Assay)	Absorbance Value (2nd Assay)
2.190	2.053
2.165	2.190
<u>2.160</u>	<u>2.191</u>
X = 2.172	X = 2.145

The values for the two assays are within 1.5% of each other, which is within the error for repeated sampling using this assay. This indicates that the compound is stable in aqueous solution for at least 4 hours.

<sup>4</sup> Micklus MJ, Stein IM. The colorimetric determination of mono- and disubstituted guanidines. Anal Biochem 1973; 54:545-553.

**Appendix B: ANIMAL DATA**

Species: *Mus musculus*

Strain: ICR

Source: Harlan Sprague-Dawley, Inc.  
P.O. Box 29176  
Indianapolis, IN 46229

Sex: Male and female

Date of birth: 30 December 1983

Method of randomization: TOXSYS Animal Allocation Program  
(SOP OP-ISG-24)

Animals in each group: 10 male and female animals,  
Controls contained 5/sex.

Condition of animals at start of study: Normal

Body weight range at dosing: 23-36 g

Identification procedures: Cervical tag, tag numbers  
84C00045 to 84C00197, inclusive.

Pretest conditioning: Quarantine/acclimation 2-12 Feb 84

Justification: The laboratory mouse has proven to be a  
sensitive and reliable system for lethal dose  
determination.

**Appendix C: HISTORICAL LISTING OF STUDY EVENTS**

<u>Date</u>	<u>Event</u>
2 Feb 84	Received 77 male and 76 female ICR mice. Mice were checked for physical condition, sexed, weighed, cervical skin tagged, and individually caged.
3-12 Feb 84	Animals were observed daily during quarantine/acclimation period.
3 Feb 84	Four mice (2 male and 2 female) were submitted for necropsy quality control.
6,9,12 Feb 84	Animals were weighed and randomized (6 Feb) into dose groups.
7,8 Feb 84	ALD animals were weighed, dosed, and observed.
13 Feb 84	Group 2-6 animals were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
14 Feb 84	Group 1 animals were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
14-26 Feb 84	Animals were observed daily in a.m. and p.m.
17,21,24 Feb 84	Animals were weighed.
26 Feb 84	Food was removed at 1600 hours.
27 Feb 84	All surviving animals were weighed and submitted for necropsy.

## Appendix D: CUMULATIVE MORTALITY DATA\*

Dose <sup>∞</sup>	Animals Dosed	Time after Dosing									
		Hours			Days						
		1	2	4	1	2	3	4	5	6	7-14
MALES											
0	10	0	0	0	0	0	0	0	0	0	0
398	10	0	0	1	1	1	1	1	1	1	1
495†	10	0	3	4	4	4	4	4	4	4	4
501	10	0	3	4	4	4	4	4	4	4	4
631	10	2	4	5	6	6	6	6	6	6	6
794	10	1	3	7	8	8	8	8	8	8	8
FEMALES											
0	10	0	0	0	0	0	0	0	0	0	0
316	10	0	0	1	1	1	1	1	1	1	1
398	10	0	1	3	3	3	3	3	3	3	3
501	10	1	2	4	4	4	4	4	4	4	4
631	10	0	0	4	4	4	4	4	4	4	4
794	10	1	2	7	7	7	7	7	7	7	7
Totals		5	18	40	42	42	42	42	42	42	42

\* Values are deaths/dose group.

<sup>∞</sup> Guanidine•HCl doses were administered as mg(base)/kg.

† Animals were misdosed--should have received 316 mg(base)/kg.

**Appendix E: INDIVIDUAL ANIMAL HISTORIES**

MALE: 398 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00048	Incr. Respiration Rate	Feb 13	Slight
84C00054	Rough Coat	Feb 13,21	Slight
84C00072	Normal	N/A	N/A
84C00084	Incr. Respiration Rate	Feb 13,15	Slight
	Stain, Yellow, Perianal	Feb 15	Slight
	Rough Coat	Feb 22	Slight
84C00087	Incr. Startle Reflex	Feb 13	Marked
	Irritable	Feb 13,14,16	Marked
	Incr. Respiration Rate	Feb 13	Slight
	Incr. Respiration Depth	Feb 13	Slight
	Twitching	Feb 13	Slight
	Inactive	Feb 17	Slight
84C00098	Material, Yellow, Perianal	Feb 13,24	Slight
	Material, Brown, Perianal	Feb 13-15,21	Moderate
	Rough Coat	Feb 13,14	Slight
	Tremors	Feb 13	Slight
	Incr. Respiration Rate	Feb 13-15	Slight
	Hunched Posture	Feb 13	Slight
84C00100	Incr. Respiration Rate	Feb 15	Slight
84C00102	Incr. Respiration Rate	Feb 13	Slight
	Tremors	Feb 13	Moderate
	Hunched Posture	Feb 13	Slight
84C00110	Incr. Respiration Rate	Feb 13	Moderate
	Incr. Respiration Depth	Feb 13	Slight
	Hunched Posture	Feb 18-20	Slight
84C00118	Incr. Respiration Rate	Feb 13	Slight
	Incr. Respiration Depth	Feb 13	Slight
	Squinting	Feb 13	Slight
	Inactive	Feb 13	Marked
	Twitching	Feb 13	Slight
	Incr. Startle Reflex	Feb 13	Moderate
	Hypotonia	Feb 13	Slight
	Death	Feb 13	2.9 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 495 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00046	Granulation, Ear Tag	Feb 17-20	Present
	Pus, Ear Tag	Feb 17-20	Present
	Inactive	Feb 13,27	Slight
84C00047	Irritable	Feb 13	Slight
	Dehydrated	Feb 21	Marked
84C00057	Irritable	Feb 13	Slight
	Incr. Respiration Rate	Feb 13	Slight
	Material, Brown, Perianal	Feb 13	Slight
	Hunched Posture	Feb 13	Slight
84C00076	Hunched Posture	Feb 13	Slight
	Rough Coat	Feb 13	Slight
	Retching	Feb 13	Slight
	Incr. Respiration Rate	Feb 13	Slight
	Tremors	Feb 13	Marked
	Incr. Salivation	Feb 13	Marked
	Death	Feb 13	1.8 h
84C00088	Incr. Respiration Rate	Feb 13,15	Moderate
	Inactive	Feb 13	Slight
	Incr. Startle Reflex	Feb 13	Slight
	Twitching	Feb 13	Slight
	Disoriented	Feb 13	Moderate
	Tremors	Feb 13	Slight
84C00090	Death	Feb 13	1.5 h
84C00095	Death	Feb 13	1.5 h
84C00101	Inactive	Feb 13	Slight
	Twitching	Feb 13	Slight
	Tremors	Feb 13	Slight
	Incr. Respiration Rate	Feb 13	Slight
84C00107	Death	Feb 13	2.2 h
84C00112	Material, Brown, Perianal	Feb 13	Slight
	Incr. Respiration Rate	Feb 13	Slight
	Stain, Yellow, Perianal	Feb 13	Slight

## Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 501 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00058	Irritable	Feb 13,15	Moderate
84C00064	Irritable Rough Coat	Feb 13-16 Feb 13	Slight Slight
84C00068	Death	Feb 13	1.3 h
84C00083	Squinting Irritable Hyperactive Stain, Yellow, Perianal Rough Coat Inc. Respiration Rate	Feb 13 Feb 13 Feb 13,15 Feb 15-17,23 Feb 14 Feb 21,22	Slight Moderate Slight Slight Slight Slight
84C00086	Death	Feb 13	1.4 h
84C00089	Rough Coat Irritable Incr. Respiration Rate Inactive	Feb 13 Feb 13 Feb 13 Feb 15	Slight Moderate Slight Slight
84C00111	Death	Feb 13	1.3 h
84C00114	Twitching Incr. Respiration Depth Rough Coat Hypotonia Inactive Death	Feb 13 Feb 13 Feb 13 Feb 13 Feb 13 Feb 13	Marked Moderate Moderate Moderate Marked 3.3 h
84C00120	Irritable Rough Coat Stain, Yellow, Flank Dehydrated	Feb 13 Feb 13 Feb 15 Feb 21	Slight Slight Slight Marked
84C00121	Inactive Hunched Posture Incr. Respiration Depth Hypotonia Disoriented Clonic Convulsion Incr. Respiration Rate	Feb 13 Feb 13 Feb 13 Feb 13 Feb 13 Feb 13 Feb 14	Marked Moderate Marked Moderate Marked Moderate Slight

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 631 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00053	Death	Feb 13	1.1 h
84C00060	Hyperactive	Feb 13	Moderate
84C00062	Death	Feb 13	1.1 h
84C00063	Incr. Respiration Rate	Feb 13	Marked
	Incr. Respiration Depth	Feb 13	Marked
	Inactive	Feb 13	Marked
	Death	Feb 13	2.9 h
84C00078	Incr. Respiration Rate	Feb 13	Moderate
	Material, Yellow, Perianal	Feb 13	Slight
	Disoriented	Feb 13	Moderate
	Jumping	Feb 13	Moderate
	Twitching	Feb 13	Marked
	Hunched Posture	Feb 13	Moderate
	Hypotonia	Feb 13	Moderate
	Death	Feb 13	4.2 h
84C00091	Death	Feb 13	0.9 h
84C00093	Incr. Respiration Rate	Feb 13, 14, 22	Moderate
	Tremors	Feb 13	Slight
	Hyperactive	Feb 14-17	Slight
84C00097	Irritable	Feb 13	Moderate
	Tremors	Feb 13	Slight
	Stain, Yellow, Perianal	Feb 13, 15	Slight
84C00099	Death	Feb 13	1.0 h
84C00108	Material, Brown, Perianal	Feb 13	Slight
	Tremors	Feb 13	Slight
	Stain, Yellow, Perianal	Feb 13, 15, 17, 23	Slight
	Incr. Respiration Rate	Feb 14	Slight
	Rough Coat	Feb 15-17	Slight

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 794 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00049	Prostrate	Feb 14	Present
	Clonic Convulsion	Feb 14	Marked
	Jumping	Feb 14	Marked
	Retching	Feb 14	Moderate
	Gasping	Feb 14	Slight
	Incr. Respiration Depth	Feb 14	Slight
	Rough Coat	Feb 14	Moderate
	Inactive	Feb 14	Marked
	Death	Feb 14	3.8 h
84C00055	Irritable	Feb 14	Slight
	Hyperactive	Feb 14-16	Slight
	Rough Coat	Feb 14,15	Slight
	Incr. Respiration Rate	Feb 14-16	Slight
	Tremors	Feb 14,15	Slight
84C00059	Inactive	Feb 14	Slight
	Incr. Respiration Rate	Feb 14-17	Slight
	Incr. Startle Reflex	Feb 15	Slight
84C00061	Incr. Respiration Rate	Feb 14	Marked
	Hunched Posture	Feb 14	Slight
	Disoriented	Feb 14	Moderate
	Twitching	Feb 14	Moderate
	Prostrate	Feb 14	Present
	Tremors	Feb 14	Marked
	Death	Feb 14	4.4 h
84C00081	Incr. Respiration Rate	Feb 14	Slight
	Incr. Respiration Depth	Feb 14	Slight
	Hyperactive	Feb 14	Slight
	Rough Coat	Feb 14	Slight
	Disoriented	Feb 14	Slight
	Retching	Feb 14	Slight
	Death	Feb 14	2.5 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 794 mg/kg Guanidine•HCl (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00094	Death	Feb 14	1.1 h
84C00105	Death	Feb 14	0.4 h
84C00106	Incr. Respiration Rate	Feb 14	Slight
	Rough Coat	Feb 14	Slight
	Incr. Startle Reflex	Feb 14	Slight
	Depr. Grasping Reflex	Feb 14	Moderate
	Disoriented	Feb 14	Moderate
	Death	Feb 14	3.4 h
84C00109	Death	Feb 14	1.1 h
84C00115	Incr. Respiration Rate	Feb 14	Slight
	Hunched Posture	Feb 14	Slight
	Incr. Startle Reflex	Feb 14	Slight
	Depr. Grasping Reflex	Feb 14	Slight
	Twitching	Feb 14	Slight
	Hyperactive	Feb 14	Moderate
	Death	Feb 15	3.6 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: Control

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
<b>Vehicle</b>			
84C00051	Inactive	Feb 13	Marked
	Rough Coat	Feb 13	Moderate
84C00052	Inactive	Feb 13	Slight
84C00074	Inactive	Feb 13	Slight
84C00103	Normal	N/A	N/A
84C00119	Rough Coat	Feb 13	Slight
	Irritable	Feb 15, 21	Slight
	Stain, Yellow, Perianal	Feb 15, 16, 22-24	Moderate
	Incr. Respiration Rate	Feb 16, 17	Slight
<b>Cage</b>			
84C00077	Normal	N/A	N/A
84C00080	Granulation, Tag Site	Feb 18	N/A
	Scab, Neck	Feb 19, 20	N/A
84C00085	Normal	N/A	N/A
84C00092	Incr. Respiration Rate	Feb 22	Slight
84C00116	Normal	N/A	N/A

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 316 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00123	Incr. Respiration Rate	Feb 13	Slight
	Incr. Respiration Depth	Feb 13	Slight
	Hunched Posture	Feb 13	Slight
	Death	Feb 13	2.6 h
84C00139	Inactive	Feb 13	Slight
	Incr. Respiration Rate	Feb 13	Slight
	Material, Yellow, Perianal	Feb 14	Slight
	Dehydrated	Feb 21,22	Marked
84C00141	Incr. Respiration Rate	Feb 13	Slight
	Tremors	Feb 13	Slight
	Disoriented	Feb 13	Slight
84C00143	Incr. Respiration Depth	Feb 13	Marked
84C00148	Incr. Respiration Rate	Feb 13	Slight
84C00152	Incr. Respiration Rate	Feb 13	Slight
	Irritable	Feb 13	Slight
	Stain, Brown, Perianal	Feb 13	Slight
84C00154	Irritable	Feb 13,15,24,25	Moderate
	Incr. Respiration Rate	Feb 13,14	Slight
	Hyperactive	Feb 14	Slight
84C00176	Incr. Respiration Rate	Feb 13,15	Slight
84C00188	Incr. Respiration Rate	Feb 13	Slight
	Incr. Startle Reflex	Feb 13	Slight
	Tremors	Feb 13	Slight
	Stain, Brown, Perianal	Feb 13	Slight
84C00191	Normal	N/A	N/A

## Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 398 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00131	Incr. Respiration Rate	Feb 13,14	Slight
84C00137	Incr. Respiration Rate	Feb 13	Slight
84C00157	Incr. Respiration Rate	Feb 13	Slight
84C00158	Incr. Respiration Rate	Feb 13	Slight
84C00166	Incr. Respiration Rate	Feb 13	Slight
84C00171	Incr. Respiration Rate	Feb 13	Slight
	Incr. Startle Reflex	Feb 13	Moderate
	Twitching	Feb 13	Marked
	Hyperactive	Feb 14-17	Slight
84C00181	Death	Feb 13	1.6 h
84C00184	Incr. Respiration Rate	Feb 13	Moderate
	Incr. Startle Reflex	Feb 13	Slight
	Stain, Brown, Perianal	Feb 13	Slight
	Irritable	Feb 13	Slight
84C00195	Inactive	Feb 13	Moderate
	Incr. Respiration Rate	Feb 13	Slight
	Hunched Posture	Feb 13	Slight
	Hypotonia	Feb 13	Slight
	Death	Feb 13	2.9 h
84C00196	Incr. Respiration Depth	Feb 13	Moderate
	Disoriented	Feb 13	Slight
	Twitching	Feb 13	Marked
	Death	Feb 13	2.2 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 501 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00132	Inactive	Feb 13	Marked
	Incr. Respiration Rate	Feb 13	Marked
	Incr. Respiration Depth	Feb 13	Slight
	Hypotonia	Feb 13	Moderate
	Tremors	Feb 13	Slight
	Hyperactive	Feb 16	Slight
84C00134	Incr. Respiration Rate	Feb 13	Slight
84C00136	Incr. Respiration Rate	Feb 13, 14	Marked
	Hunched Posture	Feb 13	Slight
	Irritable	Feb 13	Slight
	Stain, Brown, Perianal	Feb 13	Slight
	Dehydrated	Feb 21	Marked
84C00140	Death	Feb 13	1.2 h
84C00153	Death	Feb 13	2.3 h
84C00167	Hunched Posture	Feb 13	Slight
	Material, Yellow, Perianal	Feb 13	Slight
	Inactive	Feb 13	Moderate
	Incr. Respiration Depth	Feb 13	Moderate
	Death	Feb 13	3.1 h
84C00169	Incr. Respiration Rate	Feb 13	Slight
	Rough Coat	Feb 13	Slight
	Incr. Startle Reflex	Feb 13	Slight
	Jumping	Feb 13	Slight
84C00173	Incr. Respiration Rate	Feb 13	Moderate
	Disoriented	Feb 13	Slight
84C00175	Death	Feb 13	1.0 h
84C00189	Incr. Respiration Rate	Feb 13, 14	Slight
	Incr. Startle Reflex	Feb 13	Moderate
	Twitching	Feb 13	Slight
	Irritable	Feb 13, 14	Marked
	Tremors	Feb 13	Slight
	Hyperactive	Feb 15-17, 24, 25	Slight

## Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 631 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00128	Incr. Respiration Rate	Feb 13	Slight
	Tremors	Feb 13	Slight
	Twitching	Feb 13	Marked
	Incr. Respiration Depth	Feb 13	Moderate
	Disoriented	Feb 13	Moderate
	Hypotonia	Feb 13	Moderate
	Death	Feb 13	4.1 h
84C00130	Incr. Respiration Rate	Feb 13	Slight
	Incr. Respiration Depth	Feb 13	Slight
	Tremors	Feb 13	Slight
	Inactive	Feb 13	Slight
	Death	Feb 13	3.5 h
84C00142	Hunched Posture	Feb 13	Slight
	Incr. Respiration Rate	Feb 13	Slight
	Irritable	Feb 13	Slight
84C00156	Incr. Respiration Rate	Feb 13	Slight
84C00160	Irritable	Feb 16	Slight
	Hyperactive	Feb 17	Slight
84C00161	Incr. Respiration Rate	Feb 13, 14	Moderate
	Hyperactive	Feb 13	Marked
	Irritable	Feb 13, 14	Slight
84C00164	Incr. Respiration Rate	Feb 13, 16, 17	Moderate
	Incr. Startle Reflex	Feb 13	Slight
	Hyperactive	Feb 13	Moderate

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 631 mg/kg Guanidine•HCl (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00183	Death	Feb 13	2.1 h
84C00186	Twitching	Feb 13	Moderate
	Incr. Startle Reflex	Feb 13	Slight
	Tremors	Feb 13	Slight
	Irritable	Feb 13	Slight
	Disoriented	Feb 13,16	Slight
	Hyperactive	Feb 15-17	Slight
84C00194	Incr. Respiration Depth	Feb 13	Marked
	Inactive	Feb 13	Marked
	Hunched Posture	Feb 13	Slight
	Hypotonia	Feb 13	Moderate
	Death	Feb 13	2.7 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 794 mg/kg Guanidine•HCl

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00127	Hyperactive	Feb 14-17	Slight
	Incr. Respiration Rate	Feb 14,15	Marked
	Hunched Posture	Feb 14	Slight
	Twitching	Feb 14	Moderate
84C00129	Death	Feb 14	0.2 h
84C00145	Hyperactive	Feb 14	Slight
	Gasping	Feb 14	Slight
	Death	Feb 14	2.5 h
84C00147	Irritable	Feb 14	Slight
	Incr. Respiration Rate	Feb 14-17	Marked
	Hyperactive	Feb 16	Slight
84C00159	Incr. Respiration Rate	Feb 14	Slight
	Death	Feb 14	2.5 h
84C00168	Death	Feb 14	1.1 h
84C00170	Incr. Respiration Rate	Feb 14,15	Moderate
	Incr. Respiration Depth	Feb 14	Slight
	Disoriented	Feb 14,15	Moderate
	Hunched Posture	Feb 14	Slight
	Inactive	Feb 14	Moderate
	Irritable	Feb 15,16	Slight
84C00174	Incr. Respiration Rate	Feb 14	Slight
	Twitching	Feb 14	Slight
	Hypotonia	Feb 14	Slight
	Death	Feb 14	2.4 h
84C00182	Disoriented	Feb 14	Moderate
	Retching	Feb 14	Moderate
	Incr. Respiration Depth	Feb 14	Moderate
	Inactive	Feb 14	Moderate
	Irritable	Feb 14	Slight
	Death	Feb 14	3.5 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 794 mg/kg Guanidine•HCl (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00193	Irritable	Feb 14	Slight
	Incr. Respiration Rate	Feb 14	Moderate
	Clonic Convulsions	Feb 14	Marked
	Tonic Convulsions	Feb 14	Marked
	Prostrate	Feb 14	Present
	Death	Feb 14	3.5 h

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: Control

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
<b>Vehicle</b>			
84C00122	Incr. Startle Reflex	Feb 13	Slight
	Hunched Posture	Feb 13	Slight
	Irritable	Feb 14	Slight
84C00149	Normal	N/A	N/A
84C00162	Hyperactive	Feb 13	Slight
84C00163	Normal	N/A	N/A
84C00187	Incr. Respiration Rate	Feb 13	Slight
	Irritable	Feb 14	Slight
<b>Cage</b>			
84C00125	Normal	N/A	N/A
84C00133	Normal	N/A	N/A
84C00135	Normal	N/A	N/A
84C00179	Incr. Respiration Rate	Feb 13	Moderate
	Irritable	Feb 13,16	Slight
	Hyperactive	Feb 14,15	Slight
84C00192	Irritable	Feb 14	Slight

**Appendix F: INDIVIDUAL BODY WEIGHTS†**

MALES: 398 mg/kg Guanidine•HCl

Animal Number	Receipt	Dosing	Day 4	Day 8	Day 10	Day 14*
84C00048	28	32	33	34	35	29
84C00054	30	33	35	36	36	28
84C00072	28	30	29	32	33	27
84C00084	32	33	32	34	38	30
84C00087	26	28	31	27	31	26
84C00098	24	32	35	35	36	29
84C00100	25	30	31	34	33	28
84C00102	27	33	36	36	37	31
84C00110	28	33	36	37	38	31
84C00118	27	30	Dead			
Mean	27.5	31.4	33.1	33.9	35.2	28.8
Standard Deviation	2.3	1.8	2.5	3.0	2.4	1.7
Standard Error	0.7	0.6	0.8	1.0	0.8	0.6

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

MALES: 495 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 10</u>	<u>Day 14*</u>
84C00046	27	30	29	31	32	27
84C00047	26	30	31	24	33	27
84C00057	26	28	31	32	32	25
84C00076	28	29	Dead			
84C00088	27	29	30	32	33	26
84C00090	27	34	Dead			
84C00095	25	33	Dead			
84C00101	26	32	33	36	36	30
84C00107	27	27	Dead			
84C00112	29	34	35	35	37	31
Mean	26.8	30.6	31.5	31.7	33.8	27.7
Standard Deviation	1.4	2.5	2.2	4.2	2.1	2.3
Standard Error	0.4	0.8	0.9	1.7	0.9	1.0

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

MALES: 501 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 10</u>	<u>Day 14*</u>
84C00058	26	31	34	36	36	29
84C00064	30	32	35	36	36	30
84C00068	29	30	Dead			
84C00083	30	31	32	33	33	28
84C00086	30	31	Dead			
84C00089	27	33	36	34	38	30
84C00111	26	29	Dead			
84C00114	28	31	Dead			
84C00120	27	31	32	25	33	27
84C00121	29	31	32	34	34	29
Mean	28.2	31.0	33.5	33.0	35.0	28.8
Standard Deviation	1.6	1.1	1.8	4.1	2.0	1.2
Standard Error	0.5	0.3	0.7	1.7	0.8	0.5

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

MALES: 631 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 11</u>	<u>Day 14*</u>
84C00053	28	27	Dead			
84C00060	29	32	32	36	36	30
84C00062	26	30	Dead			
84C00063	29	32	Dead			
84C00078	29	34	Dead			
84C00091	30	31	Dead			
84C00093	27	31	32	34	34	29
84C00097	27	30	32	34	34	28
84C00099	29	34	Dead			
84C00108	25	31	34	31	35	30
Mean	27.9	31.2	32.5	33.8	34.8	29.3
Standard Deviation	1.6	2.0	1.0	2.1	1.0	1.0
Standard Error	0.5	0.7	0.5	1.0	0.5	0.5

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

MALES: 794 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 11</u>	<u>Day 14*</u>
84C00049	27	32	Dead			
84C00055	27	31	26	33	35	30
84C00059	23	27	29	29	32	26
84C00061	25	30	Dead			
84C00081	28	28	Dead			
84C00094	26	31	Dead			
84C00105	28	35	Dead			
84C00106	30	36	Dead			
84C00109	27	33	Dead			
84C00115	30	34	Dead			
Mean	27.1	31.7	27.5	31.0	33.5	28.0
Standard Deviation	2.1	2.9	2.1	2.8	2.1	2.8
Standard Error	0.7	0.9	1.5	2.0	1.5	2.0

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

FEMALES: 316 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 10</u>	<u>Day 14*</u>
84C00123	30	28	Dead			
84C00139	27	25	28	22	30	24
84C00141	28	31	31	32	32	26
84C00143	29	29	30	30	29	26
84C00148	28	25	28	28	30	26
84C00152	26	24	26	26	27	22
84C00154	26	26	28	27	27	22
84C00176	30	29	30	29	30	25
84C00188	30	29	28	31	30	25
84C00191	27	26	27	28	27	23
Mean	28.1	27.2	28.4	28.1	29.1	24.3
Standard Deviation	1.6	2.3	1.6	3.0	1.8	1.7
Standard Error	0.5	0.7	0.5	1.0	0.6	0.6

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

FEMALES: 398 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 11</u>	<u>Day 14*</u>
84C00131	26	26	27	26	27	24
84C00137	25	27	30	29	28	24
84C00157	29	26	26	27	26	22
84C00158	26	24	26	27	26	22
84C00166	25	25	25	26	26	22
84C00171	27	25	26	28	27	23
84C00181	28	26	Dead			
84C00184	26	28	29	31	30	26
84C00195	28	26	Dead			
84C00196	27	27	Dead			
Mean	26.7	26.0	27.0	27.7	27.1	23.3
Standard Deviation	1.3	1.2	1.8	1.8	1.5	1.5
Standard Error	0.4	0.4	0.7	0.8	0.6	0.6

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

FEMALES: 501 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 11</u>	<u>Day 14*</u>
84C00132	27	27	27	28	28	23
84C00134	29	26	27	29	28	24
84C00136	30	28	28	22	30	24
84C00140	28	26	Dead			
84C00153	29	29	Dead			
84C00167	30	28	Dead			
84C00169	28	26	28	29	30	25
84C00173	26	27	28	27	30	25
84C00175	26	25	Dead			
84C00189	28	28	29	29	31	26
Mean	28.1	27.0	27.8	27.3	29.5	24.5
Standard Deviation	1.5	1.3	0.8	2.7	1.2	1.1
Standard Error	0.5	0.4	0.3	1.1	0.5	0.4

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

FEMALES: 631 mg/kg Guanidine•HCl

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 11</u>	<u>Day 14*</u>
84C00128	30	30	Dead			
84C00130	28	32	Dead			
84C00142	27	27	28	28	29	24
84C00156	27	27	27	29	29	24
84C00160	27	26	28	29	29	24
84C00161	29	30	31	29	32	27
84C00164	28	28	29	30	30	24
84C00183	30	25	Dead			
84C00186	28	27	28	29	29	24
84C00194	27	26	Dead			
Mean	28.1	27.8	28.5	29.0	29.7	24.5
Standard Deviation	1.2	2.2	1.4	0.6	1.2	1.2
Standard Error	0.4	0.7	0.6	0.3	0.5	0.5

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

## Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†

FEMALES: 794 mg/kg Guanidine•HCl

Animal Number	Receipt	Dosing	Day 4	Day 8	Day 11	Day 14*
84C00127	26	27	27	29	28	23
84C00129	27	29	Dead			
84C00145	27	29	Dead			
84C00147	26	26	27	76	26	22
84C00159	26	27	Dead			
84C00168	27	27	Dead			
84C00170	30	31	31	32	31	27
84C00174	27	27	Dead			
84C00182	28	28	Dead			
84C00193	29	31	Dead			
Mean	27.3	28.2	28.3	29.3	28.3	24.0
Standard Deviation	1.3	1.8	2.3	2.5	2.5	2.7
Standard Error	0.4	0.6	1.3	1.5	1.5	1.5

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

## Vehicle Control

Animal Number	Receipt	Dosing	Day 4	Day 8	Day 11	Day 14*
<b>MALES</b>						
84C00051	28	32	32	33	34	28
84C00052	27	30	31	32	34	28
84C00074	29	32	33	32	34	28
84C00103	30	31	33	36	36	31
84C00119	28	32	31	36	36	30
Mean	28.4	31.4	32.0	33.8	34.8	29.0
Standard Deviation	1.1	0.9	1.0	2.1	1.1	1.4
Standard Error	0.5	0.4	0.5	0.9	0.5	0.6
<b>FEMALES</b>						
84C00122	29	29	30	30	30	25
84C00149	26	24	25	26	26	21
84C00162	27	23	26	27	27	21
84C00163	28	28	29	30	29	23
84C00187	29	30	30	29	33	27
Mean	27.8	26.8	28.0	28.4	29.0	23.4
Standard Deviation	1.3	3.1	2.4	1.8	2.7	2.6
Standard Error	0.6	1.4	1.1	0.8	1.2	1.2

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS†**

## Cage Control

<u>Animal Number</u>	<u>Receipt</u>	<u>Dosing</u>	<u>Day 4</u>	<u>Day 8</u>	<u>Day 11</u>	<u>Day 14*</u>
<b>MALES</b>						
84C00077	27	31	32	33	34	28
84C00080	26	33	34	33	35	28
84C00085	25	32	29	35	36	31
84C00092	29	34	34	35	35	30
84C00116	29	33	34	36	36	30
Mean	27.2	32.6	32.6	34.4	35.2	29.4
Standard Deviation	1.8	1.1	2.2	1.3	0.8	1.3
Standard Error	0.8	0.5	1.0	0.6	0.4	0.6
<b>FEMALES</b>						
84C00125	29	30	30	32	31	27
84C00133	29	30	31	32	32	25
84C00135	28	27	27	27	28	23
84C00179	28	28	28	29	30	25
84C00192	28	29	29	29	28	24
Mean	28.4	28.8	29.0	29.8	29.8	24.8
Standard Deviation	0.6	1.3	1.6	2.2	1.8	1.5
Standard Error	0.2	0.6	0.7	1.0	0.8	0.7

† Weight is given in grams.

\* Animals were fasted overnight before necropsy.

Appendix G: PATHOLOGY REPORT

PATHOLOGY REPORT

GLP Study 83-020

Oral Lethal Dose (LD<sub>50</sub>) Test in Mice of  
Guanidine HCL(CH<sub>5</sub>N<sub>3</sub>HCL) (CAS No. 050-01-1)

History: 60 male and 60 female ICR strain young adult mice were divided into seven groups per sex. Ten each were placed in the 5 dose groups, 5 each in the vehicle control groups and five each in the cage control groups. Dosage was done by oral gavage. Sterile water was used as the vehicle for the test compound which was dosed as follows:

Group #	Dose mg/kg	No. Mice/Sex
1	794	10 male, 10 female
2	316*	10 male, 10 female
3	398	10 male, 10 female
4	501	10 male, 10 female
5	631	10 male, 10 female
6	0.2 ml H <sub>2</sub> O veh. cntr.	5 male, 5 female
7	Cage control	5 male, 5 female

Forty two mice died within one to four hours after dosing and were necropsied on the same day. The remaining mice survived until completion of the study 14 days after dosing. The mice were anesthetized with pentobarbital, intra-peritoneal injection, and killed by exsanguination from severed axillary vessels.

Gross necropsy findings: One high dose male mouse and five high dose female mice had dark red lungs which did not collapse and foamy fluid freely exuded from the trachea (compatible with pulmonary edema). None of the remaining mice had gross lesions.

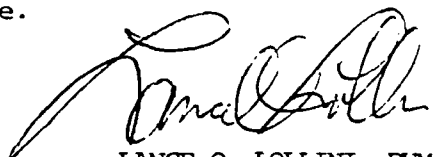
\*Received 495 mg (base)/kg due to formulation error.

## Appendix G (cont.): PATHOLOGY REPORT

Table: Summary of Deaths and Lesions by Group

<u>Group #</u>	<u>Died</u>	<u>Euthanized</u>	<u>Gross Lesions</u>	<u>Number of Animals</u>
1 male	8	2	1 pulmonary edema	10
1 female	7	3	5 pulmonary edema	10
2 male	4	6	none	10
2 female	1	9	none	10
3 male	1	9	none	10
3 female	3	7	none	10
4 male	4	6	none	10
4 female	4	6	none	10
5 male	6	4	none	10
5 female	4	6	none	10
6 male	0	5	none	5
6 female	0	5	none	5
7 male	0	5	none	5
7 female	0	5	none	5

Summary: The pulmonary edema seen in 6 of 20 high dose mice was probably test compound related. No other gross lesions were seen in test mice. No lesions were seen in the vehicle control or cage control mice.



LANCE O. LOLLINI, DVM, ACVP  
LTC, VC  
Chief, Pathology Services Group

8 May 1985

## Distribution List

Commander  
US Army Biomedical Research and  
Development Laboratory (12)  
ATTN: SGRD-UBZ-C  
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center  
(DTIC) (2)  
ATTN: DTIC-DLA  
Cameron Station  
Alexandria, VA 22304-6145

US Army Medical Research and  
Development Command (2)  
ATTN: SGRD-RMI-S  
Fort Detrick, Frederick, MD 21701-5012

Commandant  
Academy of Health Sciences, US Army  
ATTN: AHS-CDM  
Fort Sam Houston, TX 78234

Chief  
USAEHA Regional Division, West  
Fitzsimmons AMC  
Aurora, CO 80045

Chief  
USAEHA Regional Division, North  
Fort George G. Meade, MD 20755

Chief  
USAEHA Regional Division, South  
Bldg. 180  
Fort McPherson, GA 30330

Commander  
USA Health Services Command  
ATTN: HSPA-P  
Fort Sam Houston, TX 78234

Commander US Army Materiel  
Command  
ATTN: AMSCG  
5001 Eisenhower Avenue  
Alexandria, VA 22333

Commander  
US Army Environmental Hygiene  
Agency  
ATTN: Librarian, HSDH-AD-L  
Aberdeen Proving Ground, MD 21010

Dean  
School of Medicine  
Uniformed Services University of the  
Health Sciences  
4301 Jones Bridge Road  
Bethesda, MD 20014

Commander  
US Army Materiel Command  
ATTN: AMCEN-A  
5001 Eisenhower Avenue  
Alexandria, VA 22333

HQDA  
ATTN: DASG-PSP-E  
Falls Church, VA 22041-3258

HQDA  
ATTN: DAEN-RDM  
20 Massachusetts, NW  
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous  
Material Agency  
ATTN: DRXTH/ES  
Aberdeen Proving Ground, MD 21010

Commandant  
Academy of Health Sciences  
United States Army  
ATTN: Chief, Environmental  
Quality Branch  
Preventive Medicine Division  
(HSHA-IPM)  
Fort Sam Houston, TX 78234