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Institute Report No. 272

**Fourteen-Day Subchronic Oral Toxicity  
Study of Nitroguanidine in Rats**

AD-A196 666

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June 1988

Toxicology Series: 146

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Fourteen-day subchronic oral toxicity study of nitroguanidine in rats (Toxicology Series 146)--Morgan et al.

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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## SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION			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. 272			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Branch Division of Toxicology		6b. OFFICE SYMBOL (if applicable) SGRD-UL-TO-M	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, MD 21701-5012			10. SOURCE OF FUNDING NUMBERS		
		PROGRAM ELEMENT NO 627204	PROJECT NO 835	TASK NO AB	WORK UNIT ACCESSION NO. DA 303913
11. TITLE (Include Security Classification) Fourteen-day subchronic oral toxicity study of nitroguanidine in rats					
12. PERSONAL AUTHOR(S) Earl W. Morgan, Larry D. Brown, Carolyn M. Lewis, Robert R. Dahlgren, and Don W. Korte Jr.					
13a. TYPE OF REPORT Institute		13b. TIME COVERED FROM 3/20/85 TO 4/19/85	14. DATE OF REPORT (Year, Month, Day) June 1988		15. PAGE COUNT 78
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	subchronic oral toxicity, nitroguanidine, toxicology, rat		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The 14-day subchronic oral toxicity of nitroguanidine was evaluated in male and female rats. Nitroguanidine was administered in the diet at dose levels of 0, 100, 316, and 1000 mg/kg/day for 14 days. The addition of nitroguanidine to the diet did not have an effect on food consumption, but there was a significant dose-response increase in water consumption. Clinical signs attributable to the test compound were not observed during the study. At necropsy, blood samples were taken for hematological and serum clinical analyses. Serum potassium and calcium values were decreased in the treated dose groups. Microscopic examination of tissues from the control and 1000-mg/kg/day dose group animals revealed no lesions attributable to the administration of nitroguanidine. These findings indicate that nitroguanidine is nontoxic in rats when administered at doses as high as 1000 mg/kg/day for 14 days. The findings of serum electrolyte decreases coupled with increased water consumption suggest that nitroguanidine, which is excreted unchanged in the rat's urine, may be acting as an osmotic diuretic.					
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 Edwin S. Beatrice, COL MC			22b. TELEPHONE (Include Area Code) (415) 561-3600		22c. OFFICE SYMBOL SGRD-ULZ

**ABSTRACT**

The 14-day subchronic oral toxicity of nitroguanidine was evaluated in male and female rats. Nitroguanidine was administered in the diet at dose levels of 0, 100, 316, and 1000 mg/kg/day for 14 days. The addition of nitroguanidine to the diet did not have an effect on food consumption, but there was a significant dose-response increase in water consumption. Clinical signs attributable to the test compound were not observed during the study. At necropsy, blood samples were taken for hematological and serum clinical analyses. Serum potassium and calcium values were decreased in the treated dose groups. Microscopic examination of tissues from the control and 1000-mg/kg/day dose group animals revealed no lesions attributable to the administration of nitroguanidine. These findings indicate that nitroguanidine is nontoxic in rats when administered at doses as high as 1000 mg/kg/day for 14 days. The findings of serum electrolyte decreases coupled with increased water consumption suggest that nitroguanidine, which is excreted unchanged in the rat's urine, may be acting as an osmotic diuretic.

Key Words: Subchronic Oral Toxicity; Nitroguanidine; Toxicology; Rat; *response (biology); physiological effects; toxic tolerances; triple base propellants; ammunition; hazardous wastes; (KT)*

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Justification	
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## PREFACE

TYPE REPORT: 14-Day Subchronic 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, Maryland 21701-5010  
Project Officer: Gunda Reddy, PhD

WORK UNIT/APC: 180 Environmental Health Effects of Army  
Materials/TL09

GLP STUDY NUMBER: 84040

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

TEST SUBSTANCE: Nitroguanidine

INCLUSIVE STUDY DATES: 20 March - 19 April 1985

OBJECTIVE: The objective of this study was to determine the  
14-day subchronic toxicity of nitroguanidine in  
male and female Sprague-Dawley rats.

## ACKNOWLEDGMENTS

SSG James D. Justus, BS, SP4 John R.G. Ryabik, BS, SP4 James J. Fischer, SP4 Scott L. Schwebe, Richard A. Spieler, and Charlotte L. Speckman provided research assistance and animal care; Conrad R. Wheeler, PhD, and SP4 Paul B. Simboli, BS, provided chemical preparation and analysis; MAJ Carlin V. Okerberg, DVM, ACVP, MAJ George T. Makovec, DVM, ACVP, SGT Thomas W. Johnson, BA, and SGT Lisa E. Carson provided necropsy and pathology support; SSG Clinton H. Beckett, SGT Samuel S. Liu, BS, and SP4 Gary E. Mattison provided hematology support; SP4 Julius Harmon and Mary E. Lyons provided clinical chemistry support; Lucille M. Cote, ASCP, and Tom Hironaga provided histology preparation support; and Colleen S. Kamiyama provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS  
INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84040 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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SGRD-ULZ-QA (77-1n)

8 June 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for GLP Study 84040

1. I hereby certify that in relation to GLP Study 84040, the following inspection was :

17 April 1985 - Weighing and Observations

2. The report entitled "Fourteen-Day Subchronic Oral Toxicity Study of Nitroguanidine in Rats," Toxicology Series 146, and the raw data for this study were audited on 17 May 1988.

*Walter G. Bell*

WALTER G. BELL  
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Quality Assurance Auditor



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# Fourteen-Day Subchronic Oral Toxicity Study of Nitroguanidine in Rats -- Morgan et al

## INTRODUCTION

Nitroguanidine, a primary component of US Army triple-base propellants, is now 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 Toxicology Branch, 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.

### Objective of the Study

The objective of this study was to determine the 14-day subchronic toxicity of nitroguanidine in male and female Sprague-Dawley rats.

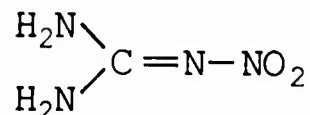
## MATERIALS

### Test Substance

Chemical name: Nitroguanidine

Chemical Abstract Service Registry No.: 556-88-7

Structural formula:



Molecular formula: CH<sub>4</sub>N<sub>4</sub>O<sub>2</sub>

Other test substance information is presented in Appendix A.

### Vehicle for Test Substance

The test compound was mixed into the feed (see Husbandry).

### Animals

Forty-five male and 45 female albino Sprague-Dawley rats (Bantin-Kingman Breeding Laboratories, Fremont, CA) were used in this study. Ear tags were used to identify each animal individually. Tag numbers from 85D00300 to 85D00389 were used, without exclusions. Five males and five females were used for necropsy quality controls or baseline control animals to ensure the animals were healthy and within normal limits for all measurements at receipt and after quarantine. The rats' weights on receipt (21 March 1985) ranged from 137 to 185 g. Additional animal data are presented in Appendix B.

### Husbandry

The animals assigned to this study were housed individually in clear, polycarbonate shoe boxes in drawer rack cages. Cellubed®, a cellulose fiber, was used as bedding. The shoe boxes and bedding were changed twice weekly. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow 5002 Meal Form (Ralston Purina, St Louis, MO). Water was provided by 16 ounce water bottles with stoppers and sipper tubes. Both feed and water consumption were measured weekly.

The temperature range maintained throughout this study was 23.9°-26.7°C with a relative humidity of 29%-43%. The photoperiod was 12 hours of light daily.

## **METHODS**

This study was performed in accordance with LAIR Standard Operating Procedure OP-STX-52 "14-Day Subchronic Oral Toxicity Testing in Rodents" (2) and EPA guidelines (3).

### Group Assignment/Acclimation

The animals were acclimated for 14 or 16 days (males and females, respectively) from receipt to the onset of dosing. During the acclimation period, the animals were observed for signs of illness daily. Food and water consumption was measured during the second week of quarantine.

Ten animals per sex were assigned to each of four dose groups. Allocation was accomplished using a computer-based, stratified, weight-biased randomization method (LAIR SOP-OP-STX-78).

#### Dose Levels

Dose levels were selected on the basis of the results of an acute toxicity study (4) and a 14-day pilot study. The acute oral median lethal dose exceeded a LIMIT dose of 5000 mg/kg. Thus, the upper dose level used in the pilot study was a LIMIT dose of 1000 mg/kg (3). At this dose level no deaths nor obvious toxicity were observed. Using a logarithmic progression table the following dose levels were selected: 0 mg/kg/day, 100 mg/kg/day, 316 mg/kg/day, and 1000 mg/kg/day.

#### Compound and Diet Preparation

The nitroguanidine was received as a dry white powder, 99.6% pure. All diet preparations were done in accordance with LAIR SOP OP-STX-16 (5). A premix consisting of 50 mg nitroguanidine/kg of the Rodent Chow was prepared. Since the compound tends to clump, it was further ground in a jar mill (Norton Inc, Akron, OH) using porcelain grinding pellets for two hours to break up the clumps. The nitroguanidine was then mixed into the meal in a series of 2-, 4-, and 6-fold dilutions. Each dilution was mixed for 15 minutes in the jar mill. The dilutions were then sieved through a 10-mesh screen to ensure the grinding was complete and to remove the grinding pellets.

On the day of the diet change, after the new diet concentrations had been calculated, the appropriate amounts of premix and meal were blended together using a Model A200D mixer (Hobart Inc, Troy, OH) for at least 15 minutes. Nitroguanidine was mixed into the feed at a level that, based on the feed consumption of the previous week and the animals's weight, would provide the desired dose (mg/kg) on a daily basis. All diet mixes were within 6.5% of target concentration and were adequately homogeneous. Additional mixing data and analyses are presented in Appendix C.

#### Test Procedures

Feed consumption and water consumption were measured on a weekly basis. Individual feed jars were used. They were weighed at the beginning and at the end of each week. The feed was sifted using a 10-mesh sieve to remove bedding and feces prior to the final weighing. If there were signs of

spillage in the bedding, the bedding was also sifted and the feed obtained was returned to the jar prior to weighing. Records for water bottles with obvious spillage were flagged and the weights were omitted. Recordkeeping was initiated during the final week of quarantine and provided the baseline consumption data to calculate the first week's diet mixture.

Early on the day of diet change, the animals were weighed, observed, and their water bottles and feeders were weighed. These data were collected on a Beckman TOXSYS® data collection terminal. The Beckman Diet Computation Subsystem was used for the calculations. After the new diet was mixed, the feeders and water bottles were filled, weighed, and returned to the cages.

Observations were performed twice daily throughout the two-week test period. During the morning observations, the animals were observed undisturbed in their cages, outside of their cages, and after return to their cages. All findings were recorded. A second "walk through" observation was performed in the afternoon and only significant observations were recorded. Body weights were recorded weekly and on the day of sacrifice. Appendix D contains a listing of the historical events.

All animals were subjected to a complete necropsy under sodium pentobarbital anesthesia. Blood was collected from the right ventricle for hematology and clinical chemistry measurements. A listing of the measurements and SOPs is provided in Appendix E. A listing of the tissues examined microscopically is provided in Appendix F. Animals were terminated by exsanguination while under anesthesia.

#### Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with the following exceptions: 1) Recorded observations were inadvertently omitted on 5 and 15 Apr 85; 2) daily observation records were lost on 8 Apr 85 on 5 animals due to computer malfunction; and 3) due to an oversight the necropsy quality control animals were not submitted until day 5 of the quarantine period. None of these changes had any effect on the results of the study.

#### Statistics

The animal weights, the results from hematology, and the blood chemistry results were analyzed statistically with packaged programs available on BMDP software (6). The

equality of the variances of the groups was tested using the Levene's Test. If the variances were equal, the vehicle control group and the dose groups were compared by the standard one-way analysis of variance (ANOVA). Otherwise, the Welch one-way ANOVA, which is not based on the assumption that the variances are equal, was performed. If the F-statistic was significant in either case, the Dunnett's test was performed to determine whether or not the vehicle control group was significantly different from any of the dose groups. Total bilirubin values are nonparametric data and were analyzed using the Kruskal-Wallis one-way ANOVA.

#### 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**

#### Mortalities

No deaths occurred during the study.

#### Feed and Water Consumption

Feed consumption increased slightly in the males and decreased slightly in the females during the first week while both males and females had increases in their feed consumption during the second week. None of these differences were significant. Table 1 presents the average daily doses of nitroguanidine achieved. Water consumption by both sexes increased in a dose-related manner during both weeks. For the 316 and 1000 mg/kg/day dose groups this increased water consumption was significant. Table 2 presents daily feed and water consumption data. Appendix G contains individual feed and water data.

#### Clinical Signs

No clinical signs attributable to nitroguanidine administration were observed. Five rats (85D00318, 85D00329, 85D00358, 85D00361, 85D00369) exhibited increased startle reflex. One rat (85D00347) was irritable. In all but one rat (85D00318) these signs were only observed once. Five rats (85D00346-49, 85D00352) exhibited dehydration due to failure to insert sipper tubes adequately into the cages. One animal (85D00316) became anorectic and emaciated as a result of water deprivation from a faulty sipper tube. These signs were seen in the control, 100-, and 1000-mg/kg/day dose

groups. Fifty-two rats exhibited swelling, bleeding, and/or scabs on the ear that was tagged. Finally, a small mass near the base of the tail was observed on one rat (85D00360).

TABLE 1:  
Daily Consumption of Nitroguanidine

Group	Week	Males mg/kg/day	Females mg/kg/day
Controls (n=10)	1	0 ± 0*	0 ± 0
	2	0 ± 0	0 ± 0
100 mg/kg (n=10)	1	95 ± 1	89 ± 2
	2	109 ± 1	111 ± 3
316 mg/kg (n=10)	1	314 ± 9	297 ± 3
	2	346 ± 8	346 ± 8
1000 mg/kg (n=10)	1	993 ± 18	933 ± 41
	2	1077 ± 21	1108 ± 38

\*Mean ± Standard Error

This mass had resolved by the second week of observation. Clinical signs data are presented in Appendix H.

#### Animal Weights

The mean body weights for each group are given in Table 3. For the females the body weights for the control and 1000-mg/kg/day dose group were significantly different (at the 95% confidence level) when compared by ANOVA and the Dunnett's Test. Although the animals were randomized using a weight-biased stratified method at the end of the first week of quarantine, their weights were significantly different by the end of quarantine (second week). This weight difference persisted throughout the study. Since the difference first occurred during quarantine before the introduction of the test compound, this weight difference appeared to be due to individual growth rates of the animals and not to the test compound. The rate of gain for the 100- and 1000-mg/kg/day dose groups compared to the control group was significantly lower during the second week of quarantine, due at least in



part to one animal in each group that lost weight. Water bottle problems contributed to these weight losses. Body weight data are presented in Appendix I.

TABLE 2:

Effect of Nitroguanidine on Food and Water Consumption\*

Dose (mg/kg/day)	Food (g/day)			Water (g/day)		
	Base- line	Week 1	Week 2	Base- line	Week 1	Week 2
FEMALES						
0	19.3 ±1.2	17.2 ±0.4	18.5 ±0.7	26.5 ±1.2	28.7 ±0.9	25.0 ±1.2
100	17.9 ±1.0	16.3 ±0.4	18.4 ±0.6	25.5 ±1.3	28.5 ±0.7	26.5 ±0.8
316	16.8 ±0.6	16.2 ±0.4	17.6 <sup>†</sup> ±0.7	27.7 ±0.9	33.4« ±0.7	31.5« ±1.2
1000	15.9« ±0.8	15.1 ±0.7	16.7 ±0.7	23.9 ±1.3	35.4« ±1.3	31.2« ±1.1
MALES						
0	18.0 ±1.4	21.7 ±0.7	23.6 ±0.7	28.9 ±2.5	37.7 ±1.8	36.9 ±2.0
100	21.3« ±0.6	21.7 ±0.5	24.3 ±0.4	33.3 ±1.0	39.0 ±1.5	40.0 ±1.3
316	21.4« ±0.5	22.8 ±0.5	25.5 ±0.6	35.1« ±0.7	45.6« ±1.3	43.3« ±1.4
1000	21.3« ±0.7	22.4 ±0.5	24.7 ±0.6	32.8 ±1.1	47.3« ±1.4	42.4 ±1.9

\* Mean ± Standard Error, 10 animals/group

† 9 animals/group.

« Significantly different than the control group ( $p \leq 0.05$ ) by the Dunnett's test.

TABLE 3:  
Effect of Nitroguanidine on Body Weights (g) of Rats

	<u>Study Day</u>				
	Q00	Q5	0	7	14†
FEMALES					
Controls (n=10)	170 ± 2*	199 ± 3	230 ± 4	244 ± 4	246 ± 5
100 mg/kg (n=10)	173 ± 3	200 ± 4	220 ± 4	234 ± 4	236 ± 4
316 mg/kg (n=10)	175 ± 2	203 ± 3	232 ± 5	240 ± 6	237 ± 6
1000 mg/kg (n=10)	174 ± 3	200 ± 3	216 ± 3«	222 ± 5«	223 ± 5«
MALES					
Controls (n=10)	169 ± 2	218 ± 4	263 ± 11	305 ± 9	317 ± 8
100 mg/kg (n=10)	168 ± 3	221 ± 4	273 ± 4	311 ± 6	325 ± 5
316 mg/kg (n=10)	168 ± 3	217 ± 6	277 ± 6	318 ± 5	332 ± 6
1000 mg/kg (n=10)	172 ± 1	221 ± 3	279 ± 3	314 ± 2	327 ± 3

◇ Q = quarantine period

† Fasted overnight

\* Mean ± Standard Error

«Significantly different than the control group ( $p \leq 0.05$ ) by Dunnett's test.

### Organ Weights and Ratios

Organ weight, organ-to-body weight ratio, and organ-to-brain weight ratios were compared for liver, spleen, adrenal, kidneys, heart, testes/ovaries, and brain weights. For the males, the 316-mg/kg/day dose group's heart weight and heart-to-brain weight ratio were significantly higher by both one-way ANOVA and by the Dunnett's test. The heart-to-body weight ratio data were significantly different by one-way ANOVA but were not significantly different by the Dunnett's test. For the females, heart weight (1000-mg/kg/day dose group significant) and heart-to-brain weight ratio (316- and 1000-mg/kg/day dose groups significant) both exhibited a decreasing trend. The brain-to-body weight ratio exhibited an increasing trend with the 1000-mg/kg/day dose group being significantly elevated. For the 100-mg/kg/day dose group, the ovaries-to-body weight ratio was significantly elevated. The ovaries-to-brain weight ratio was also higher by one-way ANOVA but the difference was not significant by the Dunnett's test. Group mean organ weights and the comparative ratios are presented in Tables 4 through 6. Individual organ weight data are presented in Appendix J.

### Clinical Chemistry

The effect of nitroguanidine on the level of several serum electrolytes (Table 7), various serum biochemistry measurements (Tables 8 and 9), and the activity of several serum enzymes (Table 10) was examined. For the females, comparing the control and treatment groups by ANOVA indicated significant differences in the levels of aspartate aminotransferase, potassium, magnesium, and uric acid. However, when the Dunnett's test was performed, there were no significant differences between the control and treatment groups. For the males, comparing the control and treatment groups by ANOVA and Dunnett's test indicated significant differences were present in potassium and calcium levels. For calcium, the group means were lower for all dose groups. However, only the 100-mg/kg/day dose group was significantly lower. For potassium, there was no apparent trend with only the high-dose group being significantly lower. Individual clinical chemistry values are presented in Appendices K, L, and M.

### Hematology

The effect of nitroguanidine on various hematological measurements was examined. These data are summarized in Tables 11 (females) and 12 (males). For the males, no significant differences in any of the hematological

TABLE 4: Organ Weights

	Control (n=10)	100 mg/kg (n=10)	316 mg/kg (n=10)	1000 mg/kg (n=10)
FEMALES				
Liver (g)	6.85* ±0.19	7.07 ±0.33	6.93 ±0.31	6.45 ±0.26
Kidneys (g)	1.80 ±0.06	1.78 ±0.06	1.76 ±0.05	1.68 ±0.05
Heart (mg)	914 ±33	872 ±23	847 ±36	801« ±34
Ovaries (g)	161 ±11	195 ±15	163 ±8	171 ±11
Brain (g)	1.77 ±0.04	1.75 ±0.05	1.86 ±0.03	1.83 ±0.03
Spleen (mg)	543 ±27	549 ±19	547 ±33	535 ±40
Adrenals (mg)	108 ±10	112 ±9	101 ±8	102 ±8
MALES				
Liver (g)	9.40* ±0.36	9.73 ±0.25	10.16 ±0.25	9.26 ±0.18
Kidneys (g)	2.53 ±0.08	2.70 ±0.11	2.77 ±0.07	2.55 ±0.07
Heart (g)	1.12 ±0.04	1.19 ±0.04	1.29« ±0.04	
Testes (g)	2.82 ±0.05	2.91 ±0.05	2.81 ±0.05	2.71 ±0.12
Brain (g)	1.95 ±0.02	1.90 ±0.05	1.89 ±0.03	1.88 ±0.05
Spleen (mg)	726 ±62	786 ±56	826 ±26	695 ±55
Adrenals (mg)	86 ±14	99 ±18	103 ±14	111 ±27

\*Mean ± Standard Error

«Significantly different from the control ( $p \leq 0.05$ ) by Dunnett's test.

TABLE 5: Organ-to-Body Weight Ratios

	Control (n=10)	100 mg/kg (n=10)	316 mg/kg (n=10)	1000 mg/kg (n=10)
FEMALES				
Liver (%)	2.80* ±0.05	2.99 ±0.10	2.92 ±0.09	2.89 ±0.06
Kidneys (%)	0.736 ±0.017	0.755 ±0.016	0.743 ±0.008	0.755 ±0.017
Heart (%)	0.374 ±0.012	0.370 ±0.008	0.356 ±0.009	0.359 ±0.009
Ovaries (%)	0.066 ±0.004	0.083< ±0.007	0.069 ±0.003	0.076 ±0.003
Brain (%)	0.723 ±0.018	0.742 ±0.021	0.787 ±0.018	0.826« ±0.020
Spleen (%)	0.221 ±0.008	0.233 ±0.007	0.229 ±0.010	0.239 ±0.015
Adrenals (%)	0.044 ±0.003	0.048 ±0.004	0.042 ±0.003	0.046 ±0.003
MALES				
Liver (%)	2.96 ±0.08	2.99 ±0.05	3.06 ±0.05	2.83 ±0.04
Kidneys (%)	0.798 ±0.019	0.828 ±0.027	0.834 ±0.018	0.780 ±0.022
Heart (%)	0.354 ±0.011	0.366 ±0.011	0.391 ±0.015	0.340 ±0.011
Testes (%)	0.892 ±0.025	0.896 ±0.021	0.851 ±0.026	0.858 ±0.033
Brain (%)	0.616 ±0.017	0.585 ±0.017	0.571 ±0.017	0.575 ±0.015
Spleen (%)	0.228 ±0.018	0.241 ±0.016	0.250 ±0.009	0.213 ±0.017
Adrenals (%)	0.027 ±0.004	0.030 ±0.006	0.031 ±0.004	0.034 ±0.008

\*Mean ± Standard Error

«Significantly different from the control (p ≤ 0.05) by Dunnett's test.

TABLE 6: Organ-to-Brain Weight Ratios

	Control (n=10)	100 mg/kg (n=10)	316 mg/kg (n=10)	1000 mg/kg (n=10)
FEMALES				
Liver (%)	388* ±15	407 ±20	373 ±15	353 ±16
Kidneys (%)	102 ±4	102 ±4	95 ±2	92 ±3
Heart (%)	51.7 ±2.0	50.0 ±1.1	45.5« ±1.6	43.8« ±1.9
Ovaries (%)	9.11 ±0.66	11.26 ±0.94	8.75 ±0.34	9.28 ±0.49
Spleen (%)	30.7 ±1.5	31.4 ±0.9	29.4 ±1.6	29.3 ±2.4
Adrenals (%)	6.13 ±0.61	6.45 ±0.56	5.43 ±0.42	5.56 ±0.37
MALES				
Liver (%)	483 ±18	515 ±16	540 ±15	497 ±17
Kidneys (%)	130 ±5	143 ±6	147 ±5	136 ±4
Heart (%)	57.7 ±2.2	63.1 ±3.0	68.6« ±1.9	59.2 ±1.4
Testes (%)	145 ±3	154 ±4	149 ±3	150 ±6
Spleen (%)	37.3 ±3.1	41.1 ±2.6	43.8 ±1.4	36.7 ±2.5
Adrenals (%)	4.38 ±0.72	5.16 ±0.96	5.45 ±0.79	6.16 ±1.75

\*Mean ± Standard Error

«Significantly different from the control ( $p \leq 0.05$ ) by Dunnett's test.

TABLE 7: Serum Electrolyte Levels

	Control (n=10)	100 mg/kg (n=10)	316 mg/kg (n=10)	1000 mg/kg (n=10)
FEMALES				
Sodium (mEq/dl)	165.6* ±2.4	166.7 ±1.9	166.9† ±1.1	163.1 ±2.4
Potassium (mEq/dl)	6.74 ±0.21	6.71 ±0.22	7.21† ±0.35	6.14 ±0.15
Chloride (mEq/dl)	122.2 ±2.5	122.5 ±1.6	122.6† ±1.4	118.8† ±0.9
Calcium (mg/dl)	10.78 ±0.16	10.96 ±0.12	10.92† ±0.19	10.58 ±0.11
Phosphorus (mg/dl)	10.4 ±0.5	10.7 ±0.4	10.6† ±0.3	9.4† ±0.4
Magnesium (mg/dl)	3.17 ±0.12	3.32 ±0.09	3.41† ±0.10	2.96 ±0.10
MALES				
Sodium (mEq/dl)	182.1† ±5.7	182.1 ±3.4	178.1 ±4.0	169.0 ±5.5
Potassium (mEq/dl)	8.02† ±0.52	7.48 ±0.21	7.71 ±0.28	6.57« ±0.27
Chloride (mEq/dl)	112.7 ±1.3	109.7 ±1.4	110.2 ±2.0	113.8 ±1.8
Calcium (mg/dl)	11.99 ±0.21	11.23« ±0.08	11.61 ±0.20	11.51 ±0.19
Phosphorus (mg/dl)	14.3 ±0.4	13.4 ±0.3	13.6 ±0.4	13.1 ±0.4
Magnesium (mg/dl)	3.27 ±0.16	3.08 ±0.15	2.94 ±0.09	2.96 ±0.10

\*Mean ± Standard Error

†n=9

«Significantly different from the control ( $p \leq 0.05$ ) by Dunnett's test.

TABLE 8: Serum Biochemistry Measurements for Female Rats

	Controls (n=10)	100mg/kg (n=10)	316mg/kg (n=9)	1000mg/kg (n=10)
Triglycerides (mg/dl)	63.7* ±4.4	65.6 ±5.4	58.8 ±5.3	56.9† ±3.9
Cholesterol (mg/dl)	82 ±4	87 ±3	80 ±3	77† ±4
Glucose (mg/dl)	168 ±13	174 ±12	170 ±13	181 ±12
Creatinine (mg/dl)	0.69 ±0.04	0.71 ±0.03	0.70 ±0.03	0.68† ±0.03
Blood Urea Nitrogen (mg/dl)	17.41 ±0.95	18.36 ±0.57	18.47 ±0.98	18.24 ±1.04
Uric Acid (mg/dl)	2.5 ±0.2	2.7 ±0.2	3.3 ±0.3	2.4 ±0.2
Albumin (g/dl)	2.92◊ ±0.05	3.16 ±0.05	3.07 ±0.10	3.13† ±0.05
Globulin (g/dl)	2.79◊ ±0.08	2.75 ±0.05	2.69 ±0.07	2.62† ±0.04
Total Protein (g/dl)	5.77 ±0.10	5.91 ±0.07	5.71 ±0.18	5.74 ±0.07
Total Bilirubin< (mg/dl)	0.70 ±0.06	0.60 ±0.06	0.70 ±0.06	0.60 ±0.03
Serum Iron (µg/dl)	293 ±22	328 ±27	332 ±31	323 ±24

\*Mean ± Standard Error

†n=9

◊n=8

&lt;Median ± Standard Error



TABLE 9: Serum Biochemistry Measurements for Male Rats

	Controls (n=10)	100mg/kg (n=10)	316mg/kg (n=10)	1000mg/kg (n=10)
Triglycerides (mg/dl)	81.6* ±6.0	72.7 ±4.0	83.7 ±5.5	107.3 ±13.3
Cholesterol (mg/dl)	70 ±3	63 ±2	64 ±4	68 ±3
Glucose (mg/dl)	165 ±13	158 ±10	182 ±12	162 ±10
Creatinine (mg/dl)	0.55 ±0.02	0.65 ±0.08	0.59 ±0.02	0.56 ±0.02
Blood Urea Nitrogen (mg/dl)	16.42 ±0.38	18.35 ±1.54	17.21 ±0.86	17.02 ±0.85
Uric Acid (mg/dl)	2.1 ±0.3	1.9 ±0.1	2.2 ±0.2	1.9 ±0.2
Albumin (g/dl)	2.80 ±0.09	2.72 ±0.08	2.80 ±0.09	2.63 ±0.06
Globulin (g/dl)	2.96 ±0.06	2.80 ±0.06	2.88 ±0.07	2.81 ±0.12
Total Protein (g/dl)	5.77 ±0.11	5.52 ±0.10	5.67 ±0.10	5.44 ±0.11
Total Bilirubin< (mg/dl)	0.72 ±0.09	0.64 ±0.06	0.64 ±0.09	0.59 ±0.04
Serum Iron (µg/dl)	144† ±15	165 ±17	164† ±13	175 ±15

\*Mean ± Standard Error

&lt;Median ± Standard Error

†n=9

TABLE 10: Serum Enzyme Activity

	Control (n=10)	100 mg/kg (n=10)	316 mg/kg (n=10)	1000 mg/kg (n=10)
FEMALES				
Aspartate Amino- transferase (I.U.)	87.58* ±7.39	80.38 ±3.78	67.12† ±3.66	87.40 ±10.62
Alanine Amino- transferase (I.U.)	29.42 ±0.98	29.73 ±1.16	27.54† ±1.16	28.99 ±1.41
Lactate Dehydro- genase (I.U.)	569.64 ±55.27	454.53† ±57.68	400.59† ±58.33	566.98 ±81.63
Creatine Phospho- kinase (I.U.)	320.95 ±66.65	287.85 ±15.70	210.77† ±21.87	262.58† ±29.51
Alkaline Phospho- kinase (I.U.)	64.12 ±3.88	74.87 ±5.34	59.99† ±5.31	66.78 ±5.36
MALES				
Aspartate Amino- transferase (I.U.)	84.76 ±4.86	77.09 ±3.09	80.35 ±5.93	79.53 ±5.18
Alanine Amino- transferase (I.U.)	34.62 ±2.04	30.39 ±0.72	33.30 ±1.74	32.32 ±1.02
Lactate Dehydro- genase (I.U.)	595.68 ±65.78	565.45 ±49.71	455.20 ±73.92	593.80 ±77.92
Creatine Phospho- kinase (I.U.)	244.72 ±20.13	213.36 ±14.76	234.42 ±26.70	219.30 ±26.27
Alkaline Phospho- kinase (I.U.)	129.70 ±8.05	120.38 ±9.48	141.37 ±9.33	135.07 ±9.52

\*Mean ± Standard Error  
†n=9

TABLE 11: Hematology Values in Female Rats

	Control (n=10)	100 mg/kg (n=9)	316 mg/kg (n=10)	1000 mg/kg (n=9)
Erythrocytes (x 10 <sup>6</sup> /μl)	7.12* ±0.29	7.49 ±0.13	7.63 ±0.16	7.55 ±0.17
Hemoglobin (g/dl)	15.6 ±0.2	15.3 ±0.1	15.6 ±0.2	14.9 ±0.3
Hematocrit (%)	43.5 ±0.5	42.1 ±0.5	43.0 ±1.0	41.4 ±0.9
Mean Cell Volume (μ <sup>3</sup> )	56.9 ±0.6	55.2« ±0.4	55.5 ±0.5	54.7« ±0.3
Mean Corpuscular Hemoglobin (pg)	20.6 ±0.2	20.2 ±0.3	20.4 ±0.2	19.9 ±0.2
Mean Corpuscular Hemoglobin Conc (%)	35.4 ±0.2	35.8 ±0.5	36.0 ±0.4	35.4 ±0.2
Platelets (x 10 <sup>6</sup> /μl)	1.236 ±0.085	1.231 ±0.035	1.319 ±0.092	1.155 ±0.085
Leukocytes (total) (x 10 <sup>3</sup> /μl)	6.01 ±0.35	4.88 ±0.52	6.49 ±0.67	5.34 ±0.41
Neutrophils (x 10 <sup>3</sup> /μl)	0.92† ±0.11	0.54« ±0.08	0.76 ±0.10	0.60 ±0.12
Lymphocytes (x 10 <sup>3</sup> /μl)	5.07† ±0.29	4.24 ±0.49	5.51 ±0.55	4.59 ±0.41
Eosinophils (x 10 <sup>3</sup> /μl)	0.11† ±0.01	0.03« ±0.01	0.10 ±0.02	0.08 ±0.01
Monocytes (x 10 <sup>3</sup> /μl)	0.12† ±0.01	0.06« ±0.02	0.13 ±0.02	0.08 ±0.01

\*Mean ± Standard Error

«Significantly different from the control group (p ≤ 0.05) by the Dunnett's test.

†n=9

TABLE 12: Hematology Values for Male Rats

	Control (n=10)	100 mg/kg (n=10)	316 mg/kg (n=9)	1000 mg/kg (n=10)
Erythrocytes (x 10 <sup>6</sup> /μl)	7.34* ±0.14	7.19 ±0.18	7.39 ±0.11	7.43 ±0.10
Hemoglobin (g/dl)	15.3 ±0.3	15.3 ±0.2	15.2 ±0.3	15.3 ±0.2
Hematocrit (%)	43.9 ±0.8	44.1 ±0.6	44.1 ±0.9	44.6 ±0.5
Mean Cell Volume (μ <sup>3</sup> )	59.5 ±0.6	59.4 ±0.6	59.3 ±0.5	59.0 ±0.7
Mean Corpuscular Hemoglobin (pg)	20.9 ±0.2	20.9 ±0.2	20.7 ±0.2	20.4 ±0.2
Mean Corpuscular Hemoglobin Conc (%)	34.3 ±0.3	34.2 ±0.2	34.1 ±0.3	33.8 ±0.2
Platelets (x 10 <sup>6</sup> /μl)	1.277 ±0.074	1.246 ±0.029	1.297 ±0.061	1.287 ±0.043
Leukocytes (total) (x 10 <sup>3</sup> /μl)	6.72 ±0.49	6.65 ±0.46	6.68 ±0.42	7.26 ±0.44
Neutrophils (x 10 <sup>3</sup> /μl)	0.52 ±0.05	0.75 ±0.08	0.70 ±0.08	0.81 ±0.10
Lymphocytes (x 10 <sup>3</sup> /μl)	6.03 ±0.48	5.71 ±0.47	5.81 ±0.40	6.23 ±0.44
Eosinophils (x 10 <sup>3</sup> /μl)	0.05 ±0.02	0.07 ±0.02	0.05 ±0.02	0.09 ±0.02
Monocytes (x 10 <sup>3</sup> /μl)	0.11 ±0.02	0.11 ±0.03	0.12 ±0.02	0.11 ±0.02

\*Mean ± Standard Error

measurements were found. For the females, mean cell volume was decreased in the 100- and 1000-mg/kg/day dose groups. Analysis of variance indicated significant differences in the hemoglobin values, but there were no significant differences between control and treatment groups when the Dunnett's test was applied. Total numbers of eosinophils, neutrophils, and monocytes were significantly lower in the 100-mg/kg/day dose group than in the control group. The values of the 316- and 1000-mg/kg/day dose groups for neutrophils and the 1000-mg/kg/day dose groups for eosinophils and monocytes were also lower, although not significantly. Individual hematology values are presented in Appendix N.

#### Pathology

Two males, one in the 100-mg/kg/day and one in the 316-mg/kg/day dose groups, had mild-to-moderately dilated renal pelves. One male in the 1000-mg/kg/day dose group exhibited testicular atrophy. No gross abnormalities were found in any of the females. There were no microscopic lesions that could be attributed to the test compound. The veterinary pathologist's report is presented in Appendix O.

#### **DISCUSSION**

No clinical signs of toxicity attributable to nitroguanidine administration were observed during the 14-day study period. In addition, there were no mortalities nor lesions noted at necropsy or on microscopic examination that could be attributed to nitroguanidine administration. The mean body weight of the female 1000 mg/kg/day dose group was significantly lower from the second week of quarantine to termination of the study because of faulty placement of sipper tubes during the quarantine period. Water deprivation and the resultant decrease in food consumption and depletion of stored fat could account for the weight loss observed and the small but significant increases in brain-to-body weight ratios observed in the female animals. The increase in ovary-to-body weight ratio in the 100-mg/kg/day dose group was heavily influenced by two females. They were probably experiencing an early estrus. No consistent treatment-related changes in organ weights or organ ratios were observed in the male dose groups.

The lack of toxicity observed in this study is consistent with the results of a previously reported single-dose oral toxicity study (4). Metabolism studies (7) have indicated that nitroguanidine is rapidly absorbed following oral administration and excreted in the urine over a dose

range from 20 mg/kg to 200 mg/kg. Absorption and excretion were not measured at doses equivalent to the 1000-mg/kg/day dose administered in this study. However, the lack of toxicity observed in this study suggests that nitroguanidine might also be rapidly absorbed following oral administration and excreted in the urine at dose levels up to 1000-mg/kg/day.

Serum calcium levels in the 100-mg/kg/day male group and potassium in the 1000-mg/kg/day male group were significantly lower than in the male control group. While the levels of serum calcium and potassium in the other dose groups were lower, no overall trend was apparent. Although not significant by the Dunnett's test, the serum potassium and magnesium levels in the 1000-mg/kg/day female group were also lower.

Nitroguanidine may be acting as an osmotic diuretic in this study. Urea, a chemically related compound, has been used as an osmotic diuretic (8). Since nitroguanidine is considerably less soluble in water than guanidine or urea (9), the excretion of nitroguanidine in the urine would require considerably more urinary volume than would be required to excrete a similar quantity of guanidine or urea. The dose-related increases in water consumption following nitroguanidine administration observed in this study are consistent with an increased urinary volume requirement for excretion of nitroguanidine. The serum electrolyte decreases also observed in the study could then be an indirect or secondary response to the nitroguanidine-induced diuresis.

#### CONCLUSION

Nitroguanidine fed at dose levels from 100-mg/kg/day to 1000-mg/kg/day in the diet for 14 days was nontoxic to Sprague-Dawley rats.

## REFERENCES

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### Appendix A: CHEMICAL DATA

Chemical Name: Nitroguanidine (NGu)

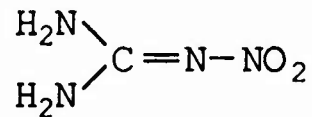
Other Listed Names: Guanidine, Nitro; alpha-Nitroguanidine;  
beta-Nitroguanidine

Chemical Abstracts Service Registry No.: 556-88-7

Lot Number: SOW83H001-004

LAIR Code: TP36

Chemical Structure:



Molecular Formula: CH<sub>4</sub>N<sub>4</sub>O<sub>2</sub>

Molecular Weight: 104.1

Physical State: White powder

Melting Point: 232°C<sup>1</sup>

Purity: 99.6% (Data Sheet Attached)

Names of Contaminants and Percentages: (Data Sheet Attached)

Source: Hercules Aerospace Division  
Sunflower Ammunition Plant  
DeSoto, Kansas

Analytical Data:

An infrared spectrum was obtained upon receipt of the compound; major absorption peaks were observed at 3330 (broad), 1660, 1630, 1525, 1400, 1300, 1050, and 780 cm<sup>-1</sup>.<sup>2</sup> The spectrum was identical to the Sadtler spectrum for nitroguanidine.<sup>3</sup>

<sup>1</sup>Fedoroff BT, Sheffield OE. Encyclopedia of explosives and related items. Vol 6. Dover, NJ: Picatinny Arsenal, 1975: G154.

<sup>2</sup>Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 39. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>3</sup>Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #21421.

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

**Stability:**

An aqueous solution of NGu (48.1  $\mu$ molar) was prepared and the absorption at 264 nm determined to be 0.689 AUFS. Three weeks later the same solution was reexamined spectroscopically and the absorption at 264 nm found to be 0.689 AUFS. A full spectrum scan revealed the characteristic pattern of absorption in the UV range with peak maxima at 215 and 264 nm. These data indicate that NGu is stable in aqueous solution for at least three weeks.<sup>4</sup>

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<sup>4</sup>Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, pp 22 and 36. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC (CRSAR-P-102-109)			USE CONTROL SYMBOL EXEMPT-Para 7-2e AR 335-15	PAGE 1 of 1
TO: Commander US Army Ammunition Munitions and Chemical Command Attn: DRSMC-OAD Rock Island, IL 61201		FROM: Sunflower Army Ammunition Plant DeSoto, Kansas 66018		DATE September 13, 1983
MANUFACTURER Hercules Aerospace Division, Hercules Incorporated		CONTRACT NO. DAAA-09-77-C-4016, CLIN 0270		
SECTION A - DESCRIPTION OF LOTS				
FROM NUMBER SOW83H001-004	THRU NUMBER	TOTAL NO. LOTS 1	TOTAL NET AMOUNT ACCEPTED 7,000 lbs.	
PLACE MANUFACTURED Sunflower Army Ammunition Plant, DP Facility		SPECIFICATION AND AMENDMENT/DRAWING NO. MIL-N-494A w/Int. Amend 6 (AR) dated 25 March 1981 *		
SECTION B - DESCRIPTION OF MATERIAL				
<u>Property</u>	<u>Requirement</u>		<u>Analysis</u>	
	<u>Min.</u>	<u>Max.</u>		
Purity, %	99.0		99.6	
Ash Content, %		0.30	0.03	
pH Value	4.5	7.0	7.55 **	
Acidity (as H <sub>2</sub> SO <sub>4</sub> ), %		0.06	ND ***	
Total Volatiles, %		0.25	0.03	
Sulfates (as NaSO <sub>4</sub> ), %		0.20	0.01	
Impurities, H <sub>2</sub> O Insoluble, %		0.20	0.01	
Particle Size, Microns		3.0 *	4.0 ****	
Particle Size, Std. Dev.		± 0.5	0.168	
<p>* As amended by Contract Scope of Work                  ** Approved by Waiver No. NQ83-1 dated Sept. 2, 1983                  *** ND = None Detected                  **** Approved by Waiver No. NQ83-2 dated Sept. 9, 1983</p>				
REMARKS				
<p>1.) Manufactured under SOW ES 1A-3-8423, Nitroguanidine Particle Size, dated 1 Feb. 83.                  2.) Packaging: Level B - fiber drums to Spec. DOT 21C60. Drums numbered 3 thru 243 and 247 thru 265. 25 pounds per drum per IAD letter dated August 1, 1983, to COR.</p>				
SECTION C - CERTIFICATION				
SAMPLING CONDUCTED BY Hercules Aerospace Division		THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.  <i>13 Sept 83</i> <i>M. A. ...</i> DATE SIGNATURE		
TESTING CONDUCTED BY Hercules Aerospace Division				
THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED		FOR THE COMMANDER		
<i>14 Sep 83</i> DATE		<i>M. A. ...</i> SIGNATURE		
Quality Assurance Specialist				

**Appendix B: ANIMAL DATA**

Species: Rattus norvegicus

Strain: Sprague-Dawley

Source: Bantin Kingman  
Fremont, CA

Sex: Male and female.

Date of birth: Male: 4 February 1985  
Female: 28 January 1985

Method of randomization: Weight bias, stratified animal  
allocation (RANDOM Computer Program,  
SOP OP-ISG-21)

Animals in each group: 10 male and female animals, 3 each  
for baseline controls

Condition of animals at start of study: Normal

Body weight range at start of dosing: 173-304 g

Identification procedures: Ear tagging procedure (SOP OP-  
ARG-1).

Pretest conditioning: Quarantine/acclimation from 20 March to  
2 April 1985

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

**Appendix C: ANALYSIS OF FEED MIXTURES****INTRODUCTION**

Feed mixtures containing nitroguanidine (NGu) were prepared for GLP Study #84040 to provide dose levels of nitroguanidine ranging from 100 to 1000 mg NGu/kg body weight/day. Separate diets were prepared for male and female rats due to differences in food consumption and body weights. New diets were prepared for each week of the two-week study to account for changes in food consumption and body weights due to growth. The target concentration of NGu in the feed mixtures ranged from 1.23 to 14.74 mg NGu/g diet. The samples of the feed mixtures were analyzed to determine concentration, homogeneity, and stability of NGu in the mixtures. The method of analysis was an HPLC method in which methylnitroguanidine (MNGu) was used as an internal standard.

**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).

The nitroguanidine was obtained from the Sunflower Army Ammunition Plant, Desoto, KS (Lot No. SOW84K010-A-001). The methylnitroguanidine was synthesized previously according to the method of McKay (1) using 1-methyl-3-nitro-1-nitrosoguanidine, 97% (MNNG, Lot No. 8228CK) and methylamine (40 wt% in water, Lot No. 0719AL) from the Aldrich Chemical Company, St Louis, MO. Certified Rodent Chow #5002 (Lot Nos. AUG1684BBMEAL and FEB288512MEAL) was obtained from Ralston Purina, St. Louis, MO. HPLC grade methanol (Lot No. 440127) was obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. The water used for the HPLC solvent was distilled and the trace organic compounds removed using Organicpure<sup>®</sup> oxidizer (Sybron/Barnstead, Boston, MA).

**METHODS**

Stock solutions of NGu (1 mg/ml water) and MNGu (1 mg/ml water) were prepared as the first step in making standards for calibration. The standards were prepared by adding varying amounts of the stock solutions and water (Table 1).

## Appendix C (cont): ANALYSIS OF FEED MIXTURES

TABLE 1

Tube #	Target Conc. NGu (mg/ml)	Target Conc. MNGu(mg/ml)	Mls of NGu Stock Soln.	Mls of MNGu Stock Soln.	Mls of Water
1	0.01	0.04	0.25	1.00	23.75
2	0.02	0.04	0.50	1.00	23.50
3	0.03	0.04	0.75	1.00	23.25
4	0.04	0.04	1.00	1.00	23.00
5	0.05	0.04	1.25	1.00	22.75
6	0.06	0.04	1.50	1.00	22.50
7	0.08	0.04	2.00	1.00	22.00

The standards were analyzed at the beginning and end of each run. Samples from the feed mixtures and premix were prepared by adding varying amounts of water and the MNGu stock solution (1 mg/ml) as described in Table 2.

TABLE 2

Dose Level (mg/kg/day)	Gm of Diet Analyzed	Mls of MNGu Soln Added	Mls of Water Added	Total Volume (Dilution Factor)
100	1.00	1	24	25
316	1.00	4	96	100
1000	1.00	10	240	250
Premix (50 mg/g)	0.25	10	240	250

The samples were stirred for an hour and then centrifuged at 3000g for 10 minutes. The supernatant from each tube was filtered through a Pasteur pipette with a tightly packed glass wool plug. The filtrate was then passed through a millipore filter (0.2  $\mu$ m) using a syringe with a Swinney adapter. The filtrate from the ultrafiltration was subsequently analyzed using HPLC.

### Appendix C (cont): ANALYSIS OF FEED MIXTURES

To determine the homogeneity of the feed mixtures, samples were removed from the top, middle, and bottom of the first batch of premix and from the feed mixtures for each dose level. Samples for testing homogeneity were also removed from the last set of feed mixtures prepared. The samples were prepared for analysis as described above.

The stability of the test compound in the feed was determined by analyzing the feed mixtures from the first week approximately one week, three weeks, and three months after their preparation. The stability of the standard solutions was determined by comparing the NGu/MNGu ratios obtained from freshly prepared solutions with standards prepared approximately three months before. The standard solutions were held at approximately 4°C in screw-cap test tubes with parafilm around the edge of the cap to prevent evaporation.

The analysis of NGu feed mixtures was accomplished under the following HPLC conditions: solvent, 10% methanol-90% water; solvent flow, 0.7 ml/min; injection volume, 10 ul; detector wavelength, 265 nm. The NGu was analyzed using methylnitroguanidine (MNGu) as an internal standard.

#### Calculations

The ratio of NGu to MNGu was calculated for all the standards and samples. The two peak area values for each standard from the beginning and the end of the run were averaged. Least squares linear regression analysis of the standard concentrations versus the peak area ratios was performed to obtain the equation of the best fitting line in the form of Equation 1

$$y = mx + b \quad (1)$$

where y is the peak area ratio, m is the slope, x is the concentration (mg/ml) and b is the intercept. The concentration of each extract was calculated by substituting for y the peak area obtained from HPLC analysis and solving for x. To calculate the concentration in the diet in terms of mg of NGu per g diet, the concentration of the extract was multiplied by the dilution factor and divided by the weight of the diet sample extracted (Equation 2).

$$\text{Conc. in diet} = \frac{\text{Conc. of NGu in extract} \times \text{Dilution factor}}{\text{Grams of diet extracted}} \quad (2)$$

## Appendix C (cont): ANALYSIS OF FEED MIXTURES

## RESULTS

Under the conditions of the analysis NGu eluted with a retention time of approximately 5.05 minutes and MNGu eluted with a retention time of approximately 6.40 minutes. The plots of the NGu concentration versus peak area ratio were linear within the range of concentrations analyzed. The correlation coefficients for each of these runs were greater than 0.9995. The equation of the line obtained by the regression analysis for each run is as follows:

$$\begin{array}{ll} 10 \text{ Apr } 85 & y = 0.00570 + 28.07180 x \\ 17 \text{ Apr } 85 & y = -0.00889 + 29.04639 x \\ 23 \text{ Apr } 85 & y = -0.00844 + 28.84959 x \\ 19 \text{ Jul } 85 & y = -0.00713 + 29.00340 x \end{array}$$

The results from the analysis of the diet mixtures are presented in Table 3.

TABLE 3

Target Concentration (mg/g)	Date Prepared	Date Analyzed	Concentration Determined by Analysis (mg/g)	% of Target Concentration
1.28	3 Apr 85	10 Apr 85	1.32	103.1
1.23	5 Apr 85	10 Apr 85	1.24	100.8
1.43	10 Apr 95	19 Jul 85	1.50	104.9
1.43	12 Apr 85	17 Apr 85	1.40	97.9
4.09	3 Apr 85	10 Apr 85	4.21	102.9
4.35	5 Apr 85	10 Apr 85	4.49	103.2
4.40	10 Apr 85	17 Apr 85	4.31	98.0
4.69	12 Apr 85	17 Apr 85	4.60	98.1
13.13	3 Apr 85	23 Apr 85	13.56	103.3
13.60	5 Apr 85	10 Apr 85	14.49	106.5
13.98	0 Apr 85	17 Apr 85	14.56	104.1
14.74	12 Apr 85	17 Apr 85	14.74	100.0



## Appendix C (cont): ANALYSIS OF FEED MIXTURES

Table 4 contains the results for the determination of homogeneity in the diets and premix.

TABLE 4

Target Concentration of NGu (mg/g)	Site of Sampling	Concentration Determined by Analysis (mg/g)	Mean Concentration (mg/g)	Deviation from Mean (%)
1.28	Top	1.31	1.32	0.8
	Middle	1.26		4.5
	Bottom	1.38		4.8
1.43	Top	1.43	1.40	2.1
	Middle	1.39		0.7
	Bottom	1.38		1.4
4.09	Top	4.20	4.21	0.2
	Middle	4.15		1.4
	Bottom	4.27		1.4
4.69	Top	4.59	4.60	0.2
	Middle	4.60		0.0
	Bottom	4.62		0.4
13.13	Top	13.55	13.56	0.1
	Middle	13.54		0.1
	Bottom	13.61		0.4
14.74	Top	14.68	14.74	0.4
	Middle	15.12		2.6
	Bottom	14.43		2.1
50.00 (Premix)	Top	50.30	50.41	0.2
	Side	50.40		0.0
	Middle	50.14		0.5
	Bottom	50.79		0.7

**Appendix C (cont): ANALYSIS OF FEED MIXTURES**

Results from the stability determinations of the test compound in the feed mixtures are shown in Table 5.

TABLE 5

Target Concentration (mg/ml)	Observed Concentration (mg/ml)		
	10 Apr 85	23 Apr 85	19 Jul 85
1.23	1.24	1.24	1.25
4.35	4.49	4.40	4.48
13.60	14.49	14.17	14.31

The stability determinations of the standard solutions are shown in Table 6 by comparison of the NGu/MNGu ratios.

TABLE 6

Date of Preparation	<u>Standard Solutions (mg/ml)</u>						
	0.01	0.02	0.03	0.04	0.05	0.06	0.08
16 Apr 85	0.3004	0.5813	0.8639	1.1559	1.4404	1.6976	2.3461
18 Jul 85	0.2894	0.5752	0.8644	1.1554	1.4300	1.7148	2.3319

## DISCUSSION

The concentration of NGu in the diet mixtures as determined by analysis was within 6.5% of the target concentrations. According to the EPA and NIH criteria for homogeneity (1), the data demonstrate that the dispersion of NGu in the feed provides a homogenous mixture over the range of concentrations tested. Nitroguanidine was stable in the feed mixtures for at least three months.

## REFERENCES

1. EPA, GLP Standards, Final Rule (40 CFR part 160) as published in the Federal Register, 29 Nov 1983, Vol. 48, no. 230 pp 53955-53959

## Appendix D: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Events</u>
20 Mar 85	Animals arrived at LAIR. They were sexed, observed for illness, and caged in the GLP Suite.
21 Mar 85	The rats were ear tagged and weighed.
21 Mar - 2 Apr 85	Animals were checked daily.
25 Mar 85	Two males and 2 females were submitted to the LAIR Pathology Group for quality control necropsy examination.
22, 29 Mar 85	All animals were weighed, males and then females respectively. Food and water consumption monitoring was initiated. Feeders and water bottles were weighed.
3 Apr 85	Animals were removed from quarantine, and dietary concentration was calculated for males based upon food consumption. Males were weighed and started on diet containing test compound. Three males were submitted for baseline hematology and serology.
4-19 Apr 85	Observations were conducted twice daily throughout the study period.
5 Apr 85	Dietary concentration was calculated for females based upon food consumption. Females were weighed and started on diet containing test compound. Three females were submitted for baseline hematology and serology.
10 Apr 85	Males were observed, weighed, and water bottles and feeders weighed. Diet requirements were recalculated and new diet mixes prepared. Feeders were changed to new mix.

Appendix D (cont): HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Events</u>
12 Apr 85	Females were observed, weighed, and water bottles and feeders weighed. Diet requirements were recalculated and new diet mixes prepared. Feeders were changed to new mix.
16 Apr 85	Food was removed from males at 1600 hours.
17 Apr 85	Observed and weighed males. Submitted them for necropsy. Blood and tissue samples were taken for the measurements specified.
18 Apr 85	Food was removed from females at 1600 hours.
19 Apr 85	Observed, weighed, and submitted females for necropsy. Blood and tissue samples were taken for the measurements specified.

**Appendix E: HEMATOLOGY/CLINICAL CHEMISTRY INDICES**

The following are LAIR GLP SOPs for the Hematology measurements performed during the study:

1. Complete Blood Count - OP-PSG-40 (WBC, RBC, Hb, HCT, MCV, MCH, and MCHC).
2. Platelets - OP-PSG-39
3. WBC Differential - OP-PSG-26 (neutrophils, lymphocytes, eosinophils, and monocytes)

Counts for the neutrophils, lymphocytes, eosinophils, and monocytes are obtained by multiplying the WBC by the appropriate percentage obtained from the differential count.

The following are LAIR GLP SOPs for the Clinical Chemistry measurements performed during the study:

1. Calcium - OP-ACH-17
2. Sodium and Potassium - OP-ACH-19
3. Chloride - OP-ACH-20
4. Magnesium - OP-ACH-50
5. Phosphorus - OP-ACH-18
6. Glucose - OP-ACH-7
7. Cholesterol - OP-ACH-11
8. Triglycerides - OP-ACH-9
9. Creatinine - OP-ACH-15
10. Blood Urea Nitrogen - OP-ACH-16
11. Uric Acid - OP-ACH-14
12. Albumin - OP-ACH-12
13. Total Protein - OP-ACH-13
14. Total Bilirubin - OP-ACH-8
15. Serum Iron - OP-ACH-22
16. Aspartate Amino-Transferase - OP-ACH-4
17. Alanine Amino-Transferase - OP-ACH-3
18. Lactate Dehydrogenase - OP-ACH-5
19. Creatine Phosphokinase - OP-ACH-6
20. Alkaline Phosphatase - OP-ACH-10

Globulin values were calculated by subtracting the albumin values from the total protein values.

**Appendix F: HISTOPATHOLOGY TISSUES**

The following is a list of all tissues submitted for light microscopic examination following necropsy:

Cerebrum	Pancreas
Cerebellum	Cecum
Trachea	Colon
Thyroid	Rectum
Parathyroid	Stomach
Esophagus	Skeletal Muscle
Salivary Gland	Sciatic Nerve
Harderian Gland	Tongue
Exorbital Gland	Skin
Heart	Mammary Gland
Aorta	Nasal Region
Lung	Sternum
Thymus	Femur
Spleen	Vertebrae
Mesenteric Lymph Node	Spinal Cord
Liver	Adrenals
Kidney	Pituitary
Urinary Bladder	Eye(s)
Duodenum	Middle Ear
Jejunum	Auditory Sebaceous Gland
Ileum	
MALE	FEMALE
Accessory Sex Glands	Uterus
Epididymis	Ovaries
Testes	

## Appendix G: INDIVIDUAL FEED AND WATER DATA

## FEMALES

Dose mg/kg per day	Animal ID (85D00---)	Feed (g/wk)			Water (g/wk)		
		Base- line	Week 1	Week 2	Base- line	Week 1	Week 2
0	345	105	115	94	147	181	142
	355	142	127	123	178	182	182
	357	117	127	118	155	183	174
	358	125	124	116	190	207	194
	362	163	127	128	196	219	206
	365	114	114	96	178	179	160
	367	130	131	108	215	210	204
	369	117	100	101	198	210	204
	379	189	113	102	162	187	168
	381	149	123	125	233	229	127
100	348	117	115	111	174	191	185
	351	153	102	99	171	178	156
	353	117	116	119	178	195	217
	361	140	124	134	176	204	171
	364	161	116	107	215	213	203
	368	135	114	105	197	202	176
	370	122	129	122	188	226	189
	375	85	111	99	110	206	204
	385	115	110	104	165	173	171
	388	108	107	104	211	204	193
316	354	114	123	109	194	242	207
	356	100	97	96	186	238	234
	363	120	116	113	201	243	229
	376	121	119	118	192	227	213
	382	118	109	95	232	258	259
	383	122	117	101	168	215	247
	384	130	125	---	217	251	---
	386	142	119	127	180	226	217
	387	105	97	91	178	210	173
	389	106	110	100	190	231	205
1000	346	123	92	89	178	221	196
	347	99	87	85	139	221	229
	352	121	93	110	213	257	267
	359	109	113	105	154	242	197
	360	94	84	81	145	203	183
	366	130	127	106	182	253	230
	371	96	100	91	162	255	208
	373	87	129	125	122	302	226
	377	139	116	108	200	250	216
	378	116	114	105	177	273	236

## Appendix G (cont): INDIVIDUAL FEED AND WATER DATA

## MALES

Dose mg/kg per day	Animal ID (85D00---)	Feed (g/wk)			Water (g/wk)		
		Base- line	Week 1	Week 2	Base- line	Week 1	Week 2
0	308	140	155	140	246	240	299
	309	143	168	157	190	308	278
	313	125	144	141	237	330	228
	316	48	172	151	56	298	284
	322	158	159	152	215	224	210
	327	138	174	156	238	276	237
	332	138	149	135	220	258	251
	333	118	131	119	203	239	338
	337	137	147	139	238	265	266
	341	116	126	124	181	202	190
100	300	143	140	138	214	258	255
	301	138	162	152	265	343	336
	306	156	159	146	238	293	287
	312	132	142	142	242	263	280
	324	168	169	155	261	292	276
	325	164	162	149	220	224	235
	326	151	150	159	211	243	278
	329	156	154	142	254	296	311
	331	131	138	132	221	259	280
	336	155	146	144	227	261	259
316	302	152	156	143	270	319	307
	304	143	152	147	237	300	274
	310	129	152	150	249	317	358
	314	146	149	141	240	303	290
	319	143	153	151	238	310	297
	323	160	180	166	243	323	282
	328	138	155	144	224	285	258
	335	164	173	161	271	351	343
	343	157	156	151	239	302	309
	344	168	171	176	248	381	315
1000	305	153	157	144	232	338	292
	307	149	152	145	237	351	346
	311	138	142	144	206	293	257
	318	178	180	174	277	366	359
	320	148	163	151	221	362	342
	330	143	153	141	251	335	319
	334	152	169	161	242	358	237
	338	156	158	134	225	325	287
	340	121	146	140	195	279	247
	342	151	151	147	212	303	284



**Appendix H: Clinical Signs**

Coding for Clinical Signs

- - Normal
- \* - Observation not performed/record lost due to computer malfunction
- A - Emaciated/Anorectic
- D - Dehydration
- E - Ear scab/swelling and/or bleeding
- I - Irritable
- M - Small mass at base of tail
- S - Increased startle reflex



Appendix H (cont): Clinical Signs

Group	Animal ID	Days of Study														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group 2 100 mg/kg	85D00300	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
	85D00301	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
	85D00306	-	-	*	-	-	-	-	-	E	E	E	E	E	E	E
	85D00312	-	-	*	-	-	-	-	-	-	-	-	-	-	E	E
	85D00324	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
	85D00325	-	-	*	-	-	-	-	-	-	-	-	-	-	E	E
	85D00376	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
	85D00329	-	-	*	-	S	E	E	E	E	E	E	E	E	E	E
Males	85D00331	-	-	*	-	-	-	-	E	E	E	E	E	E	E	E
	85D00336	-	-	*	-	-	-	-	E	E	E	E	E	E	E	E
	85D00348	*	D <sup>1</sup>	-	E	E	E	E	E	E	E	E	E	E	E	E
	85D00351	*	D <sup>1</sup>	-	-	-	-	-	E	E	E	E	E	E	E	E
	85D00353	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00361	*	S	-	-	-	-	-	-	-	-	-	-	E	E	E
	85D00364	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00368	*	-	-	-	-	-	-	-	E	E	E	E	E	E	E
Group 2 100 mg/kg	85D00379	*	-	-	E	E	E	E	E	E	E	E	E	E	E	E
	85D00375	*	-	-	-	-	-	-	E	E	E	E	E	E	E	E
	85D00385	*	-	-	-	-	-	-	-	-	-	-	-	E	E	E
	85D00348	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00348	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<sup>1</sup>Not given water bottle.



Appendix H (cont): Clinical Signs

Group	Animal ID	Days of Study														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group 4 1000 mg/kg	85D00305	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00307	-	-	*	-	-	E	E	E	E	E	E	E	E	E	E
	85D00311	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
	85D00318	-	-	*	S	S	-	-	-	-	-	-	-	-	E	E
	85D00320	-	-	*	-	-	E	E	E	E	E	E	E	E	E	E
	85D00330	-	-	*	-	-	-	-	-	-	-	-	-	-	E	E
	85D00334	-	-	*	-	-	E	E	E	E	E	E	E	E	E	E
	85D00338	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Group 4 1000 mg/kg	85D00340	-	-	*	-	-	-	-	-	-	-	-	-	-	E	E
	85D00342	-	-	*	-	-	-	-	-	-	-	-	-	-	E	E
	85D00346	*	DI	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00347	*	ID	-	-	-	E	E	E	E	E	E	E	E	E	E
	85D00352	*	DI	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00359	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85D00360	*	M	M	M	M	M	M	M	M	M	M	M	M	M	M
	85D00366	*	-	-	-	-	-	E	E	E	E	E	E	E	E	E
Females	85D00371	*	-	-	-	-	E	E	E	E	E	E	E	E	E	E
	85D00373	*	-	-	-	-	E	E	E	E	E	E	E	E	E	E
	85D00377	*	-	-	-	-	E	E	E	E	E	E	E	E	E	E
	85D00378	*	-	-	-	-	E	E	E	E	E	E	E	E	E	E

<sup>1</sup>Not given water bottle.

## Appendix I: INDIVIDUAL BODY WEIGHT (g) /FEMALES

<u>Dose</u>	<u>Animal ID</u>	<u>QDay0</u>	<u>QDay5</u>	<u>Day0</u>	<u>Day7</u>	<u>Day14</u>
0 mg/kg/day	84D00345	176	206	230	248	239
	84D00355	166	210	243	263	266
	84D00357	179	211	238	261	259
	84D00358	173	204	238	250	251
	84D00362	166	181	229	252	259
	84D00365	166	193	225	233	234
	84D00367	169	207	236	247	246
	84D00369	168	191	212	227	231
	84D00379	159	185	207	222	220
84D00381	177	200	239	238	250	
100 mg/kg/day	84D00348	180	208	227	240	240
	84D00351	170	191	221	229	224
	84D00353	179	193	211	230	233
	84D00361	172	198	225	246	253
	84D00364	160	182	207	222	220
	84D00368	167	202	232	245	247
	84D00370	183	218	239	253	258
	84D00375	177	205	192	220	225
	84D00385	179	212	231	234	236
84D00388	161	189	211	224	221	
316 mg/kg/day	84D00354	176	207	232	255	250
	84D00356	164	184	203	202	207
	84D00363	176	204	233	238	252
	84D00376	188	217	251	262	267
	84D00382	182	208	230	237	233
	84D00383	171	203	231	247	246
	84D00384	185	212	251	264	219
	84D00386	171	207	243	245	257
	84D00387	171	193	218	214	210
84D00389	170	198	224	235	231	
1000 mg/kg/day	84D00346	172	195	219	219	211
	84D00347	166	186	196	200	198
	84D00352	178	204	219	214	212
	84D00359	165	190	222	233	234
	84D00360	177	200	215	206	210
	84D00366	165	192	215	215	222
	84D00371	186	209	218	216	215
	84D00373	184	215	212	224	233
	84D00377	182	216	234	251	254
84D00378	164	191	214	243	241	

## Appendix I (cont.): INDIVIDUAL BODY WEIGHT (g)/MALES

<u>Dose</u>	<u>Animal ID</u>	<u>QDay0</u>	<u>QDay5</u>	<u>Day0</u>	<u>Day7</u>	<u>Day14</u>
0 mg/kg/day	84D00308	172	222	278	321	327
	84D00309	180	239	304	349	362
	84D00313	172	226	274	308	321
	84D00316	169	226	173	256	290
	84D00322	169	225	283	331	313
	84D00327	171	218	271	313	333
	84D00332	167	212	273	307	327
	84D00333	157	197	249	277	287
	84D00337	168	215	270	309	326
	84D00341	167	205	252	277	288
100 mg/kg/day	84D00300	166	216	263	299	313
	84D00301	175	225	279	315	324
	84D00306	159	213	272	314	321
	84D00312	157	206	256	295	311
	84D00324	170	237	293	341	354
	84D00325	178	226	280	286	331
	84D00326	164	219	273	342	348
	84D00329	179	238	283	322	332
	84D00331	159	207	254	290	309
	84D00336	176	227	275	303	308
316 mg/kg/day	84D00302	170	225	283	319	325
	84D00304	159	209	260	302	320
	84D00310	164	214	272	301	332
	84D00314	179	229	277	316	332
	84D00319	170	221	275	317	331
	84D00323	168	171	242	301	296
	84D00328	169	216	272	311	334
	84D00335	145	225	291	334	348
	84D00343	176	231	289	321	329
	84D00344	182	226	310	355	373
1000 mg/kg/day	84D00305	170	226	287	316	321
	84D00307	172	221	280	316	328
	84D00311	172	223	278	310	323
	84D00318	167	229	290	324	342
	84D00320	175	231	283	317	328
	84D00330	169	216	282	313	325
	84D00334	176	225	284	320	339
	84D00338	174	225	281	320	323
	84D00340	167	217	272	304	318
	84D00342	177	193	256	298	319

## Appendix J: INDIVIDUAL ORGAN WEIGHTS/FEMALES

Dose /day	Animal (85D00-)	Liver g	Spleen g	Adrenals g	Kidneys g	Heart g	Ovaries g	Brain g
0 mg/kg	345	6.055	0.488	0.069	1.630	0.934	0.119	1.792
	355	6.983	0.634	0.139	1.930	0.936	0.194	1.887
	357	7.439	0.623	0.166	1.962	0.850	0.192	1.586
	358	7.166	0.575	0.125	2.011	1.049	0.195	1.914
	362	7.721	0.651	0.109	1.916	0.925	0.180	1.806
	365	6.515	0.446	0.090	1.799	0.799	0.146	1.832
	367	7.290	0.525	0.126	1.937	1.121	0.207	1.722
	369	5.925	0.507	0.090	1.516	0.830	0.127	1.870
	379	6.371	0.392	0.083	1.714	0.831	0.108	1.507
	381	6.990	0.593	0.081	1.581	0.861	0.144	1.813
100 mg/kg	348	6.786	0.452	0.086	1.733	0.888	0.145	1.701
	351	6.167	0.570	0.070	1.583	0.917	0.148	1.878
	353	7.501	0.505	0.105	1.825	0.817	0.170	1.484
	361	9.403	0.584	0.110	2.056	0.940	0.240	1.845
	364	6.679	0.476	0.154	1.733	0.835	0.202	1.642
	368	6.983	0.622	0.121	1.726	0.899	0.208	1.684
	370	8.086	0.619	0.116	2.170	0.974	0.196	1.943
	375	5.895	0.608	0.157	1.739	0.902	0.262	1.875
	385	6.786	0.545	0.082	1.623	0.810	0.126	1.880
	388	6.439	0.506	0.115	1.638	0.734	0.254	1.552
316 mg/kg	354	7.150	0.460	0.127	1.795	0.826	0.166	1.795
	356	5.970	0.399	0.112	1.489	0.722	0.143	1.804
	363	7.425	0.645	0.113	1.828	0.882	0.173	1.958
	376	7.588	0.702	0.114	1.960	0.994	0.166	1.933
	382	6.147	0.512	0.054	1.710	0.805	0.127	1.780
	383	7.526	0.626	0.120	1.855	0.968	0.220	2.025
	384	5.241	0.412	0.069	1.647	0.725	0.151	1.895
	386	8.248	0.654	0.127	1.879	0.933	0.183	1.841
	387	6.165	0.528	0.085	1.600	0.678	0.144	1.722
	389	7.831	0.532	0.090	1.858	0.940	0.159	1.838
1000 mg/kg	346	5.883	0.445	0.064	1.364	0.744	0.153	1.746
	347	5.580	0.486	0.071	1.665	0.684	0.122	1.753
	352	5.570	0.433	0.094	1.601	0.800	0.135	1.785
	359	7.140	0.434	0.090	1.810	0.937	0.186	1.780
	360	5.561	0.505	0.110	1.532	0.610	0.164	1.895
	366	6.549	0.492	0.120	1.836	0.772	0.160	1.879
	371	5.986	0.459	0.118	1.574	0.818	0.182	1.907
	373	7.432	0.826	0.121	1.724	0.878	0.174	1.696
	377	7.253	0.662	0.146	1.873	0.960	0.255	2.065
	378	7.525	0.610	0.090	1.811	0.808	0.177	1.809



## Appendix J (cont.): INDIVIDUAL ORGAN WEIGHTS/MALES

<u>Dose</u> /day	<u>Animal</u> (85D00-)	<u>Liver</u> g	<u>Spleen</u> g	<u>Adrenals</u> g	<u>Kidneys</u> g	<u>Heart</u> g	<u>Testes</u> g	<u>Brain</u> g
0 mg/kg	308	9.814	0.625	0.056	2.929	1.225	3.032	1.939
	309	10.530	0.799	0.054	2.818	1.140	2.867	1.899
	313	10.313	0.737	0.106	2.766	1.054	2.987	1.950
	316	8.145	0.818	0.128	2.433	1.102	2.818	1.843
	322	10.375	0.817	0.095	2.658	1.179	2.574	1.971
	327	10.850	1.171	0.181	2.645	1.376	2.873	2.002
	332	8.491	0.661	0.066	2.413	1.087	2.615	1.942
	333	7.803	0.493	0.031	2.185	0.861	2.808	1.925
	337	9.375	0.505	0.048	2.300	1.183	2.714	1.915
	341	8.350	0.630	0.090	2.170	1.022	2.884	2.072
	100 mg/kg	300	8.754	0.673	0.089	2.320	1.075	2.940
301		9.818	0.716	0.105	2.789	1.276	2.890	1.902
306		9.155	0.365	0.010	2.488	1.261	2.733	1.579
312		10.289	0.901	0.144	2.854	1.273	2.921	2.102
324		10.945	0.879	0.112	2.833	1.267	2.745	2.018
325		10.375	0.923	0.190	3.348	1.305	3.198	1.918
326		10.543	0.944	0.149	3.025	1.331	2.995	1.781
329		9.696	0.913	0.012	2.580	1.176	2.953	1.997
331		8.881	0.702	0.113	2.460	0.960	2.623	1.774
336		8.877	0.839	0.063	2.263	0.980	3.086	1.967
316 mg/kg		302	9.795	0.839	0.080	3.037	1.423	2.854
	304	9.411	1.003	0.075	2.473	1.354	2.681	1.900
	310	9.939	0.775	0.028	2.657	1.085	2.680	1.794
	314	9.700	0.810	0.062	2.964	1.174	2.959	1.827
	319	9.941	0.780	0.116	2.557	1.566	2.860	1.955
	323	10.153	0.842	0.138	2.647	1.284	3.059	2.080
	328	9.656	0.721	0.186	2.811	1.293	2.675	1.781
	335	10.890	0.892	0.142	2.941	1.246	2.992	1.801
	343	10.038	0.747	0.099	2.579	1.215	2.710	1.876
	344	12.112	0.851	0.102	3.011	1.300	2.653	1.925
	1000 mg/kg	305	9.284	0.355	0.336	2.155	0.837	2.544
307		8.755	0.666	0.090	2.361	1.132	3.008	1.860
311		9.436	0.642	0.084	2.577	1.208	2.909	1.799
318		10.218	0.894	0.115	2.696	1.270	3.283	2.045
320		9.090	0.833	0.117	2.502	1.062	2.870	1.846
330		9.241	0.784	0.130	2.661	1.202	2.695	2.111
334		9.455	0.641	0.050	2.581	1.080	2.895	1.745
338		9.842	0.563	0.077	2.980	1.150	2.023	1.868
340		8.183	0.622	0.056	2.350	1.133	2.603	1.985
342		9.101	0.950	0.052	2.623	1.037	3.215	1.939

## Appendix K: SERUM ELECTROLYTE LEVELS/FEMALES

<u>Dose</u> /day	<u>Animal</u> (85D00-)	<u>Sodium</u> mg/dl	<u>Potassium</u> mEq/L	<u>Chloride</u> mEq/L	<u>Calcium</u> mg/dl	<u>Phosphorus</u> mg/dl	<u>Magnesium</u> mg/dl
0 mg/kg	345	163	6.80	118	10.51	8.83	3.16
	355	178	7.30	133	11.50	12.30	3.68
	357	173	7.70	131	11.34	12.29	3.53
	358	162	6.20	115	10.85	10.12	3.30
	362	154	6.40	115	10.05	9.62	2.92
	365	168	6.60	128	10.83	10.52	2.92
	367	174	7.20	128	11.25	12.35	3.75
	369	158	5.80	117	10.41	8.74	2.85
	379	165	7.50	126	11.02	10.30	2.63
	381	161	5.90	111	10.03	8.88	2.97
	100 mg/kg	348	170	6.90	121	11.22	8.65
351		171	7.00	118	10.59	10.95	3.32
353		172	6.90	128	11.04	12.17	3.59
361		165	5.70	121	10.84	10.82	3.54
364		169	7.40	126	11.54	11.95	3.42
368		171	7.20	128	11.15	12.20	3.26
370		152	5.30	112	10.15	8.88	2.63
375		163	6.50	124	10.96	10.02	3.09
385		166	7.00	122	10.89	10.21	3.54
388		168	7.20	125	11.27	11.36	3.48
316 mg/kg		354	169	8.10	129	11.32	11.45
	356	172	6.30	117	11.42	11.87	3.57
	363	166	8.50	125	11.16	11.21	3.73
	376*						
	382	167	7.00	123	10.68	9.99	3.29
	383	168	8.20	124	10.93	10.68	3.54
	384	167	5.30	126	9.75	9.03	3.00
	386	160	7.80	115	10.30	11.07	3.44
	387	166	6.40	122	11.43	9.20	2.87
	389	167	7.30	122	11.31	11.29	3.53
	1000 mg/kg	346	160	5.80	121	10.39	7.38
347		161	6.10	119	10.02	7.88	2.78
352		182	7.10	---	10.63	----	2.93
359		163	6.30	115	10.80	10.09	3.14
360		165	6.10	119	10.48	10.12	3.48
366		166	5.90	124	11.08	11.37	2.47
371		160	6.70	116	10.29	10.14	3.14
373		159	5.80	119	10.58	9.48	2.60
377		154	5.50	116	10.33	9.05	2.73
378		161	6.10	120	11.16	8.97	3.08

\*In sufficient sample collected.

**Appendix K (cont): SERUM ELECTROLYTE LEVELS/MALES**

<u>Dose</u> /day	<u>Animal</u> (85D00-)	<u>Sodium</u> mg/dl	<u>Potassium</u> mEq/L	<u>Chloride</u> mEq/L	<u>Calcium</u> mg/dl	<u>Phosphorus</u> mg/dl	<u>Magnesium</u> mg/dl
0 mg/kg	308	195	10.68	114	12.72	15.61	3.46
	309	161	6.32	118	12.27	14.58	3.19
	313	180	7.75	111	11.52	14.75	2.72
	316	194	8.07	114	12.15	14.15	3.26
	322	155	5.93	118	13.15	15.74	2.99
	327	175	7.00	105	11.12	12.45	2.90
	332	179	7.77	109	11.07	13.31	3.15
	333	192	9.56	110	12.13	15.58	4.53
	337	208	9.14	116	11.92	13.50	3.28
	341	---	----	112	11.88	13.75	3.20
100 mg/kg	300	181	7.26	111	11.20	12.28	2.81
	301	192	6.98	114	11.21	12.21	2.28
	306	161	6.63	117	11.77	13.45	2.98
	312	176	7.15	108	11.06	13.90	3.01
	324	176	7.00	101	10.90	13.34	2.93
	325	183	8.45	108	11.02	14.12	3.20
	326	178	7.65	106	11.05	12.49	2.86
	329	186	7.12	110	11.30	13.30	3.56
	331	187	8.70	111	11.54	15.01	4.08
	336	201	7.91	111	11.27	13.52	3.11
316 mg/kg	302	185	8.86	113	11.48	13.24	2.54
	304	182	8.19	115	11.26	12.42	2.92
	310	149	5.89	117	12.42	14.65	2.86
	314	181	7.31	112	11.37	12.91	2.75
	319	191	8.40	114	11.78	15.16	3.36
	323	169	6.81	100	11.21	12.06	2.96
	328	177	8.12	107	10.99	12.76	3.18
	335	171	7.19	99	10.80	12.28	2.76
	343	187	8.37	109	12.65	15.50	3.39
	344	189	7.95	116	12.12	14.83	2.70
1000 mg/kg	305	155	5.73	115	11.17	11.86	2.46
	307	200	7.86	123	11.77	12.84	2.80
	311	151	6.60	120	12.72	11.65	3.15
	318	193	7.12	115	11.93	15.75	3.45
	320	154	5.74	111	11.81	13.58	2.99
	330	175	6.71	104	10.60	11.75	2.77
	334	159	6.67	112	11.62	15.19	3.42
	338	161	5.59	115	11.57	12.62	2.87
	340	161	5.85	116	10.98	13.35	2.62
	342	181	7.83	107	10.96	12.36	3.10

## Appendix L: SERUM BIOCHEMISTRIES/FEMALES

Dose	Animal (85D00-)	Trigly- cerides mg/dl	Choles- terol mg/dl	Glucose mg/dl	Creati- nine mg/dl	Blood Urea Nitrogen mg/dl	Uric Acid mg/dl
0 mg/kg/day	345	74.40	86.27	116.38	0.74	18.11	2.01
	355	66.95	83.22	216.47	0.84	21.31	3.43
	357	59.42	73.50	174.82	0.77	18.40	2.42
	358	82.54	107.70	168.76	0.66	14.97	2.72
	362	40.39	82.23	182.71	0.64	15.42	1.80
	365	64.24	77.51	152.30	0.58	21.48	2.23
	367	74.55	93.68	169.51	0.87	18.85	3.38
	369	53.50	64.75	106.31	0.52	18.73	2.34
	379	75.90	90.19	242.94	0.66	12.41	2.38
	381	44.78	62.68	154.32	0.63	14.40	1.96
100 mg/kg/day	348	57.35	84.83	133.52	0.77	21.06	2.40
	351	75.04	69.26	130.78	0.77	19.66	2.67
	353	81.11	85.48	185.05	0.83	17.20	2.91
	361	58.87	84.19	152.41	0.74	20.22	2.42
	364	53.78	84.68	212.19	0.62	17.61	3.21
	368	106.35	103.30	145.65	0.74	19.34	2.48
	370	57.04	77.26	202.71	0.50	14.99	1.39
	375	59.49	87.22	161.69	0.75	16.97	3.24
	385	51.68	85.22	250.94	0.72	17.75	3.74
	388	55.25	104.44	161.13	0.59	18.75	2.69
316 mg/kg/day	354	85.99	93.43	174.40	0.78	21.89	3.43
	356	81.57	78.07	138.29	0.89	21.87	2.56
	363	61.41	67.64	149.17	0.72	21.17	4.05
	376*						
	382	40.71	78.94	158.83	0.64	17.89	2.67
	383	51.37	84.61	215.93	0.67	20.38	5.02
	384	51.09	61.45	101.29	0.72	17.23	1.41
	386	46.47	78.61	166.76	0.59	14.98	3.94
	387	46.41	90.47	202.22	0.62	14.30	2.93
	389	64.63	82.60	221.90	0.70	16.53	3.32
1000 mg/kg/day	346	74.97	89.19	171.34	0.87	18.87	2.79
	347	58.89	84.66	166.54	0.66	15.41	1.78
	352	-----	-----	231.18	-----	15.44	2.73
	359	66.75	70.54	156.48	0.71	25.81	2.18
	360	48.27	62.43	137.46	0.69	19.83	2.61
	366	58.15	83.25	176.46	0.66	17.58	1.49
	371	69.38	65.69	179.81	0.50	19.27	3.41
	373	48.68	68.70	262.15	0.59	13.80	2.13
	377	42.54	75.65	181.39	0.74	18.16	2.67
	378	44.78	93.59	147.87	0.67	18.22	2.36

\*Insufficient sample collected.

## Appendix L (cont): SERUM BIOCHEMISTRIES/FEMALES (cont)

<u>Dose</u>	<u>Animal</u> (85D00-)	<u>Albumin</u> g/dl	<u>Globulin</u> g/dl	<u>Total Protein</u> g/dl	<u>Total Bilirubin</u> mg/dl	<u>Serum Iron</u> µg/dl
0 mg/kg/day	345	3.12	2.67	5.79	1.00	329
	355	----	----	5.65	0.60	292
	357	----	----	6.33	0.50	316
	358	3.07	3.26	6.33	0.70	243
	362	2.80	3.00	5.80	0.50	292
	365	2.79	2.58	5.37	0.80	259
	367	3.02	2.74	5.76	0.70	423
	369	3.02	2.65	5.67	0.80	149
	379	2.71	2.75	5.46	0.80	332
	381	2.86	2.65	5.51	0.60	298
100 mg/kg/day	348	3.21	2.85	6.06	0.50	228
	351	3.00	2.44	5.44	0.70	319
	353	3.41	2.76	6.17	0.70	447
	361	3.08	2.73	5.81	0.50	240
	364	3.09	2.88	5.97	0.50	---
	368	3.13	2.94	6.07	0.60	393
	370	3.32	2.52	5.84	0.50	252
	375	3.01	2.76	5.77	0.60	322
	385	3.41	2.78	6.19	0.80	429
	388	2.94	2.84	5.78	0.60	319
316 mg/kg/day	354	2.94	2.74	5.68	0.60	405
	356	3.42	2.68	6.10	0.60	240
	363	3.29	2.88	6.17	0.50	222
	376*					
	382	3.19	2.91	6.10	0.80	353
	383	2.94	2.75	5.69	0.70	365
	384	2.41	2.09	4.50	0.70	505
	386	2.95	2.33	5.28	0.60	246
	387	3.22	2.63	5.85	0.70	273
	389	3.25	2.77	6.02	0.80	380
1000 mg/kg/day	346	3.04	2.76	5.80	0.60	295
	347	2.87	2.54	5.41	0.60	435
	352	----	----	5.62	1.20	---
	359	3.45	2.71	6.16	0.60	371
	360	3.14	2.69	5.83	0.70	295
	366	3.23	2.68	5.91	0.50	383
	371	3.07	2.45	5.52	0.80	314
	373	3.15	2.45	5.60	0.70	219
	377	3.06	2.64	5.70	0.60	362
	378	3.13	2.70	5.83	0.60	234

\*Insufficient sample collected.

## Appendix L (cont): SERUM BIOCHEMISTRIES/MALES

<u>Dose</u>	<u>Animal</u> (85D00-)	<u>Trigly- cerides</u> mg/dl	<u>Choles- terol</u> mg/dl	<u>Glucose</u> mg/dl	<u>Creati- nine</u> mg/dl	<u>Blood Urea Nitrogen</u> mg/dl	<u>Uric Acid</u> mg/dl
0 mg/kg/day	308	77.26	77.48	200.51	0.57	16.09	2.43
	309	61.09	74.10	122.73	0.61	17.11	1.30
	313	67.00	76.34	163.36	0.52	16.07	1.31
	316	91.35	77.74	79.22	0.51	15.82	2.11
	322	96.94	75.62	185.43	0.50	17.59	1.50
	327	92.34	84.34	158.29	0.45	14.97	1.77
	332	49.63	52.03	157.11	0.49	14.26	1.48
	333	71.30	58.20	177.17	0.68	17.43	3.80
	337	106.33	63.99	170.95	0.56	18.11	2.38
	341	102.43	63.95	236.85	0.57	16.71	2.78
100 mg/kg/day	300	61.84	63.72	127.51	0.74	20.70	1.90
	301	65.91	56.99	159.57	0.64	16.63	1.09
	306	85.40	53.69	157.14	0.61	15.56	2.05
	312	95.69	69.33	109.04	0.52	16.27	2.18
	324	62.18	69.99	161.31	0.53	17.36	1.45
	325	72.32	62.37	160.13	0.52	18.16	2.31
	326	53.83	62.57	170.47	0.48	18.43	1.48
	329	76.14	73.88	203.13	0.51	13.63	1.87
	331	71.16	66.02	126.89	1.34	31.12	1.97
	336	82.83	49.91	207.20	0.57	15.63	2.22
316 mg/kg/day	302	71.59	67.69	151.42	0.58	16.42	2.49
	304	90.16	78.62	125.61	0.63	18.85	2.14
	310	84.12	64.31	181.53	0.59	14.00	1.32
	314	112.93	84.50	125.74	0.60	22.56	1.43
	319	64.63	68.41	199.68	0.66	19.35	2.24
	323	75.61	50.42	213.09	0.49	15.43	2.69
	328	76.28	65.44	176.87	0.45	17.12	2.73
	335	61.05	52.28	219.08	0.61	13.87	1.69
	343	109.08	55.17	213.92	0.65	15.55	2.96
	344	91.47	53.37	214.69	0.65	18.96	2.31
1000 mg/kg/day	305	101.38	65.17	165.96	0.60	17.41	1.09
	307	68.37	66.00	150.73	0.69	21.44	1.67
	311	122.67	70.72	203.50	0.56	20.24	3.11
	318	131.29	70.97	145.16	0.70	18.11	1.79
	320	67.88	51.61	175.64	0.58	15.09	2.05
	330	103.00	61.30	117.60	0.48	13.70	1.80
	334	97.85	85.12	196.51	0.61	14.37	1.83
	338	210.91	77.19	197.86	0.42	14.28	2.22
	340	90.36	68.68	150.06	0.47	19.12	1.31
	342	78.81	59.50	118.69	0.50	16.44	1.98

## Appendix L (cont): SERUM BIOCHEMISTRIES/MALES (cont)

<u>Dose</u>	<u>Animal</u> (85D00-)	<u>Albumin</u> g/dl	<u>Globulin</u> g/dl	<u>Total Protein</u> g/dl	<u>Total Bilirubin</u> mg/dl	<u>Serum Iron</u> µg/dl
0 mg/kg/day	308	2.87	3.06	5.93	0.65	255
	309	2.97	3.10	6.07	0.46	167
	313	2.50	2.95	5.45	0.85	109
	316	2.50	2.92	5.42	0.87	106
	322	2.77	2.67	5.44	0.76	136
	327	2.56	2.96	5.52	0.85	149
	332	3.18	2.71	5.89	0.76	133
	333	2.91	2.84	5.75	0.69	121
	337	3.29	3.21	6.50	0.55	122
	341	2.49	3.20	5.69	0.37	---
100 mg/kg/day	300	3.15	2.66	5.81	0.80	286
	301	2.74	2.74	5.48	0.52	160
	306	2.57	3.13	5.70	0.84	194
	312	2.64	2.59	5.23	0.65	112
	324	2.47	3.01	5.48	0.62	158
	325	2.48	2.71	5.19	0.58	182
	326	2.52	2.57	5.09	0.47	146
	329	2.99	3.05	6.04	0.74	121
	331	3.03	2.71	5.74	0.66	185
	336	2.62	2.81	5.43	0.54	103
316 mg/kg/day	302	2.50	3.10	5.60	0.77	152
	304	2.38	3.22	5.60	0.97	155
	310	3.19	2.82	6.01	0.76	216
	314	2.63	2.73	5.36	0.43	126
	319	2.62	2.78	5.40	0.74	182
	323	2.94	2.68	5.62	0.53	216
	328	2.62	2.69	5.31	0.69	191
	335	3.19	3.10	6.29	0.43	109
	343	3.04	2.63	5.67	0.46	126
	344	2.85	3.02	5.87	0.60	---
1000 mg/kg/day	305	2.17	3.11	5.28	0.95	233
	307	2.66	3.64	6.30	0.66	176
	311	2.73	2.76	5.49	0.64	200
	318	2.60	2.77	5.37	0.62	146
	320	2.73	2.64	5.37	0.57	261
	330	2.79	2.89	5.68	0.50	176
	334	2.70	2.85	5.55	0.51	109
	338	2.68	2.50	5.18	0.37	190
	340	2.58	2.67	5.25	0.63	122
	342	2.67	2.30	4.97	0.46	139

## Appendix M: ENZYME ACTIVITIES/FEMALES

<u>Dose</u> (mg/kg/day)	<u>Animal</u> (85D00-)	<u>AST*</u> (IU)	<u>ALT*</u> (IU)	<u>LDH*</u> (IU)	<u>CPK*</u> (IU)	<u>AP*</u> (IU)
0	345	95.39	32.8	646.68	286.24	66.23
	355	74.66	30.5	570.72	240.84	55.88
	357	94.33	28.3	470.63	305.16	56.22
	358	83.42	28.7	915.93	317.64	56.50
	362	57.78	24.8	379.33	172.08	62.13
	365	132.31	34.1	680.16	896.21	63.56
	367	70.63	29.5	324.12	199.83	95.56
	369	119.04	32.7	712.31	326.16	55.90
	379	81.19	26.4	502.70	298.06	71.82
	381	67.03	26.4	493.84	167.30	57.34
100	348	79.98	35.0	397.90	205.24	68.63
	351	90.33	26.7	763.23	303.08	78.52
	353	68.83	33.3	432.36	351.72	111.16
	361	66.51	26.8	303.79	246.57	51.39
	364	96.88	28.7	676.79	290.00	74.25
	368	96.36	29.6	-----	367.09	88.70
	370	64.17	26.7	532.45	266.09	61.94
	375	73.33	35.9	236.26	247.34	61.73
	385	83.16	28.6	403.67	318.42	83.77
	388	84.28	26.0	344.30	282.93	68.65
316	354	71.17	28.6	605.82	206.16	94.48
	356	74.32	30.3	546.31	321.55	61.85
	363	60.92	28.1	315.32	266.09	46.24
	376†					
	382	72.32	21.6	547.30	195.26	69.90
	383	61.34	29.5	131.25	175.84	42.03
	384	78.96	31.8	442.92	130.69	51.97
	386	70.15	29.2	412.11	227.54	54.86
	387	42.28	22.3	130.34	116.94	51.09
	389	72.59	26.5	473.93	256.84	67.47
1000	346	89.15	34.3	839.20	253.32	60.65
	347	79.38	29.4	745.30	222.09	60.37
	352	134.19	28.7	649.22	409.29	67.11
	359	74.14	24.5	697.26	279.87	59.13
	360	69.00	26.1	394.59	211.58	55.24
	366	81.09	28.8	811.13	401.28	60.67
	371	158.94	38.8	780.39	-----	101.35
	373	77.74	26.8	360.06	240.41	70.18
	377	50.12	28.0	102.34	191.07	89.72
	378	60.22	24.5	290.35	154.29	43.35

\* AST=Aspartate Amino-transferase; ALT=Alanine Amino-transferase; LDH=Lactate Dehydrogenase; CPK=Creatine Phosphokinase; AP=Alkaline Phosphatase  
†Insufficient sample collected.



## Appendix M (cont): ENZYME ACTIVITIES/MALES

<u>Dose</u> (mg/kg/day)	<u>Animal</u> (85D00-)	<u>AST*</u> (IU)	<u>ALT*</u> (IU)	<u>LDH*</u> (IU)	<u>CPK*</u> (IU)	<u>AP*</u> (IU)	
0	308	86.52	31.5	263.13	227.68	174.22	
	309	91.33	48.3	864.94	261.69	140.69	
	313	88.80	37.2	742.34	174.82	116.07	
	316	118.21	35.2	710.83	369.34	106.19	
	322	81.31	33.3	527.11	226.14	123.25	
	327	82.47	29.5	701.26	246.18	104.18	
	332	77.81	31.3	587.81	283.25	136.26	
	333	65.56	37.1	323.97	210.59	156.79	
	337	92.18	38.7	818.80	299.95	145.36	
	341	63.40	24.1	416.61	147.60	94.00	
	100	300	71.24	29.9	343.11	205.53	101.51
		301	64.97	28.6	408.52	193.36	172.65
306		94.08	31.6	672.71	245.06	148.73	
312		65.66	34.1	397.62	146.65	94.36	
324		86.00	32.7	774.91	261.27	88.24	
325		73.70	32.2	585.00	258.88	133.21	
326		68.27	28.6	478.51	172.57	95.14	
329		82.89	28.7	680.45	205.70	129.18	
331		80.40	30.7	540.90	161.25	148.77	
336		83.68	26.8	772.80	283.35	92.03	
316		302	60.86	28.0	315.39	127.35	86.76
		304	75.93	41.0	331.71	136.35	151.58
	310	85.85	41.2	799.46	240.98	117.23	
	314	113.75	33.4	742.76	323.83	137.18	
	319	78.70	32.2	591.12	224.97	144.14	
	323	78.63	37.5	734.46	249.49	177.52	
	328	60.94	31.8	272.42	130.51	135.48	
	335	111.36	32.0	314.55	381.69	183.94	
	343	66.70	23.4	232.89	278.99	117.84	
	344	70.74	32.5	217.20	250.08	162.01	
	1000	305	100.38	29.2	761.68	344.90	119.28
		307	76.70	36.4	410.77	230.25	140.15
311		69.93	29.6	640.28	186.15	136.18	
318		95.31	38.3	872.40	301.96	199.42	
320		58.14	30.3	220.23	115.42	97.89	
330		85.30	33.6	693.40	201.00	139.00	
334		94.06	29.5	910.66	319.23	139.19	
338		93.96	30.7	735.87	237.92	103.14	
340		63.70	34.7	425.26	138.11	112.86	
342		57.85	30.9	267.49	118.06	163.57	

\* AST=Aspartate Amino-transferase; ALT=Alanine Amino-transferase; LDH=Lactate Dehydrogenase; CPK=Creatine Phosphokinase; AP=Alkaline Phosphatase

## Appendix N: HEMATOLOGY DATA/FEMALES

Dose	Animal ID	RBC	HGB	HCT	MCV	MCH	MCHC
		Count x10 <sup>6</sup> /μl					
0 mg/kg/day	85D00345	6.15	16.2	45.8	56	19.9	34.8
	85D00355	7.11	15.4	42.7	60	21.7	35.5
	85D00357	7.61	15.5	42.4	55	20.6	36.1
	85D00358	7.34	15.5	43.4	59	21.3	35.3
	85D00362	7.24	14.5	41.3	57	20.2	34.7
	85D00365	5.11	16.2	44.6	55	20.1	35.8
	85D00367	7.16	15.1	42.7	59	21.2	34.9
	85D00369	8.43	16.7	45.4	54	19.9	36.2
	85D00379	7.35	15.3	42.3	57	20.8	35.6
	85D00381	7.71	15.9	44.2	57	20.6	35.5
100 mg/kg/day	85D00348	7.79	15.4	42.9	55	19.9	35.4
	85D00351	6.82	15.4	43.2	55	19.8	35.2
	85D00353	7.63	15.0	42.4	55	19.8	34.9
	85D00361*						
	85D00364	7.50	14.7	41.8	55	19.7	34.8
	85D00368	7.83	15.6	43.8	56	20.1	35.1
	85D00370	7.39	14.8	41.4	56	20.1	35.2
	85D00375	7.85	15.6	43.0	55	20.1	35.9
	85D00385	7.74	15.3	41.2	53	19.9	36.6
	85D00388	6.89	15.6	39.2	57	22.7	39.2
316 mg/kg/day	85D00354	7.59	15.2	41.7	55	20.1	35.8
	85D00356	7.66	15.5	42.8	56	20.3	35.7
	85D00363	8.54	17.2	48.9	57	20.3	34.6
	85D00376	7.82	15.4	43.1	55	19.8	35.3
	85D00382	7.78	15.7	44.0	56	20.4	35.4
	85D00383	6.91	14.9	37.9	55	21.7	38.8
	85D00384	7.25	14.9	39.4	54	20.6	37.2
	85D00386	7.47	15.6	43.2	58	20.9	35.5
	85D00387	7.02	15.4	42.5	53	19.3	35.8
	85D00389	8.22	16.7	46.3	56	20.4	35.6
1000 mg/kg/day	85D00346	8.25	15.8	44.5	54	19.3	35.1
	85D00347	7.29	13.9	38.9	53	19.2	35.2
	85D00352	6.81	13.5	37.3	54	19.9	35.5
	85D00359	7.54	15.1	41.7	55	20.1	35.6
	85D00360	7.90	15.4	44.0	55	19.6	34.6
	85D00366	6.86	13.8	38.3	56	20.2	35.4
	85D00371	8.03	15.4	43.4	54	19.4	35.0
	85D00373*						
	85D00377	7.81	15.6	43.0	55	20.1	35.8
	85D00378	7.46	15.5	41.9	56	20.9	36.5

\*Sample clotted.

## Appendix N (cont): HEMATOLOGY DATA/FEMALES (cont)

Dose	Animal ID	Plate- lets x10 <sup>6</sup> /μl	WBC Count x10 <sup>3</sup> /μl	White Blood Cell Differential			
				Neutro- phils %	Lympho- cytes %	Eosino- phils %	Mono- cytes %
0 mg/kg/day	85D00345	1748	7.1	12	84	2	2
	85D00355	1172	4.9	15	81	2	2
	85D00357	856	4.1	--	--	-	-
	85D00358	1254	5.3	20	77	1	2
	85D00362	1346	7.1	24	72	2	2
	85D00365	1206	5.3	15	82	1	2
	85D00367	1238	6.6	8	88	2	2
	85D00369	1540	7.0	12	83	2	3
	85D00379	880	7.1	12	86	1	1
	85D00381	1122	5.6	16	79	3	2
100 mg/kg/day	85D00348	1410	5.8	18	79	2	1
	85D00351	1164	4.0	18	80	0	2
	85D00353	1266	4.5	12	87	1	0
	85D00361*						
	85D00364	1224	2.9	11	87	1	1
	85D00368	1378	8.2	7	91	0	2
	85D00370	1106	5.6	8	90	0	2
	85D00375	1234	3.3	7	89	2	2
	85D00385	1152	5.0	10	88	1	1
	85D00388	1146	4.6	10	90	0	0
316 mg/kg/day	85D00354	1486	5.5	12	85	1	2
	85D00356	1552	3.7	18	82	2	2
	85D00363	1270	6.9	11	87	0	2
	85D00376	1278	9.5	14	82	2	2
	85D00382	1182	5.8	14	82	2	2
	85D00383	1014	5.1	9	88	2	1
	85D00384	922	8.9	7	89	2	2
	85D00386	1276	5.8	12	85	2	1
	85D00387	1950	4.3	9	89	0	2
	85D00389	1258	9.4	13	82	2	3
1000 mg/kg/day	85D00346	1162	4.1	10	86	2	2
	85D00347	1248	5.2	11	86	2	1
	85D00352	522	3.7	11	87	1	1
	85D00359	1270	4.9	9	89	1	1
	85D00360	1382	5.0	28	69	1	2
	85D00366	1098	6.4	5	91	2	2
	85D00371	1178	6.2	16	81	2	1
	85D00373*						
	85D00377	1160	7.7	6	91	1	2
	85D00378	1372	4.9	8	90	1	1

\*Sample clotted.

## Appendix N (cont): HEMATOLOGY DATA/MALES

<u>Dose</u>	<u>Animal ID</u>	<u>RBC</u> <u>Count</u> x10 <sup>6</sup> /μl	<u>HGB</u> g/dl	<u>HCT</u> %	<u>MCV</u> μ <sup>3</sup>	<u>MCH</u> pg	<u>MCHC</u> %
0 mg/kg/day	85D00308	7.79	16.0	45.9	59	20.6	34.4
	85D00309	7.10	14.8	42.1	59	20.9	34.6
	85D00313	6.80	14.2	41.2	60	21.0	34.0
	85D00316	6.85	14.8	42.9	62	21.5	33.9
	85D00322	7.23	14.4	41.0	56	20.0	34.7
	85D00327	7.19	14.8	44.8	62	20.6	32.5
	85D00332	7.42	14.6	42.2	57	19.8	34.2
	85D00333	8.28	17.1	49.0	59	20.8	34.5
	85D00337	7.58	15.8	45.5	60	21.0	34.3
85D00341	7.21	16.1	44.2	61	22.4	35.9	
100 mg/kg/day	85D00300	7.55	15.7	45.1	60	20.9	34.2
	85D00301	7.53	15.4	43.9	58	20.6	34.6
	85D00306	7.59	15.2	43.8	57	20.1	34.3
	85D00312	5.89	14.0	41.8	60	20.4	32.9
	85D00324	7.42	15.2	43.9	59	20.6	34.2
	85D00325	7.25	14.8	42.8	59	20.5	34.1
	85D00326	7.00	15.5	42.4	60	22.2	35.9
	85D00329	7.77	16.5	48.4	62	21.3	33.6
	85D00331	6.69	15.5	44.0	57	20.3	34.7
85D00336	7.20	15.5	45.0	62	21.7	34.0	
316 mg/kg/day	85D00302	7.28	15.1	42.4	58	20.8	35.1
	85D00304	6.98	14.7	41.4	59	21.2	35.1
	85D00310	7.37	16.0	45.7	62	21.8	34.4
	85D00314	6.90	13.8	39.7	57	20.1	34.2
	85D00319	7.43	15.3	44.5	60	20.6	33.9
	85D00323	7.55	15.7	44.9	59	20.9	34.4
	85D00328	7.28	14.3	42.5	58	19.8	33.1
	85D00335*						
	85D00343	7.89	16.0	47.8	60	20.4	33.0
85D00344	7.82	16.2	47.7	61	20.3	33.5	
1000 mg/kg/day	85D00305	7.07	15.3	44.9	63	21.7	33.5
	85D00307	7.07	14.5	41.7	59	20.7	34.4
	85D00311	7.21	14.6	42.9	59	20.4	33.6
	85D00318	7.34	15.9	46.1	63	21.7	34.0
	85D00320	7.72	15.1	45.2	58	19.7	33.0
	85D00330	7.78	15.6	44.6	57	20.2	34.6
	85D00334	7.16	15.9	46.4	57	19.6	33.9
	85D00338	7.72	15.0	44.4	57	19.6	33.3
	85D00340	7.95	15.8	46.4	58	20.0	33.5
85D00342	7.24	14.9	43.0	59	20.6	34.1	

\*Insufficient sample collected.

## Appendix N (cont): HEMATOLOGY DATA/MALES (cont)

Dose	Animal ID	Plate- lets x10 <sup>6</sup> /μl	WBC Count x10 <sup>3</sup> /μl	White Blood Cell Differential			
				Neutro- phils %	Lympho- cytes %	Eosino- phils %	Mono- cytes %
0 mg/kg/day	85D00308	1234	6.1	7	91	0	2
	85D00309	1290	7.6	7	88	2	3
	85D00313	1320	6.5	8	89	1	2
	85D00316	1782	4.3	17	80	1	2
	85D00322	1044	6.1	9	87	2	2
	85D00327	1044	9.5	9	89	0	2
	85D00332	1110	6.8	7	91	0	2
	85D00333	1442	8.6	5	94	1	0
	85D00337	1434	6.7	4	96	0	0
	85D00341	1066	5.0	9	87	1	2
100 mg/kg	85D00300	1328	7.9	10	86	2	2
	85D00301	1048	6.8	9	88	1	2
	85D00306	1260	5.5	14	83	1	2
	85D00312	1334	4.4	25	71	1	3
	85D00324	1320	9.4	6	89	2	3
	85D00325	1292	5.3	14	84	2	0
	85D00326	1290	7.4	17	81	0	2
	85D00329	1240	6.5	6	94	0	0
	85D00331	1188	7.3	7	92	1	0
	85D00336	1156	6.0	13	84	1	2
316 mg/kg/day	85D00302	1398	6.2	10	86	1	3
	85D00304	990	7.6	9	89	0	2
	85D00310	1684	4.1	10	88	0	2
	85D00314	1280	5.4	16	81	2	1
	85D00319	1298	7.0	6	89	2	3
	85D00323	1252	7.2	12	85	1	2
	85D00328	1308	6.9	14	83	1	2
	85D00335*						
	85D00340	1186	7.7	6	93	0	1
	85D00344	1278	8.0	13	87	0	0
1000 mg/kg/day	85D00305	1088	5.8	10	87	0	3
	85D00307	1482	8.8	11	86	2	1
	85D00311	1354	5.6	21	76	1	2
	85D00318	1396	8.9	7	89	2	2
	85D00320	1172	7.4	12	84	2	2
	85D00330	1188	8.7	4	95	0	1
	85D00334	1456	7.2	15	84	0	1
	85D00338	1152	6.5	6	86	2	2
	85D00340	1344	8.3	15	83	1	1
	85D00342	1236	5.4	14	84	2	0

\*Insufficient sample collected.

## Appendix O: PATHOLOGY REPORT

### Pathology Report

#### Fourteen-Day Subchronic Toxicity Study of Nitroguanidine in Male and Female Albino Sprague-Dawley Rats

Study 84040

#### 1. Introduction

The objective of this study was to determine the subchronic effects of nitroguanidine given orally for 14 days in male and female Sprague-Dawley rats. The rats were divided randomly into three dose groups and a control group. The dosage levels were as follows:

Group 1 -	0 mg/kg/day
2 -	100 mg/kg/day
3 -	316 mg/kg/day
4 -	1000 mg/kg/day

Following anesthesia with sodium pentobarbital, administered by intraperitoneal injection, blood was collected from the right ventricle of each rat and submitted for hematologic examination [red blood cell count (RBC), hemoglobin, concentration (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), WBC differential and blood morphology]. Additional blood was submitted to Analytical Chemistry Services Group, Division of Research Support, for chemical analysis. All rats were killed by exsanguination and complete gross necropsy examinations were performed. Tissue specimens from all major organs and systems were fixed in 10% neutral buffered formalin for subsequent microscopic examination. Tissues were embedded in paraffin, sectioned at approximately 6 microns thickness and stained with hematoxylin and eosin. All tissues itemized in SOP-OP-PSG-13 were examined microscopically in controls and the 1000 mg/kg dosage level. In the 100 mg/kg and 316 mg/kg dosage levels only gross examination was done.

#### 2. Results, Interpretation, and Discussion

The gross and microscopic findings are itemized in Incidence Tables 1 and 2. Appendix 1 lists the abnormal microscopic findings by individual animal.

a. Table 1 tabulates the gross lesions observed in all of the rats utilized in the study.

b. Table 2 tabulates the tissue inventory and the abnormal microscopic findings in the tissues of each rat in the control and high dose groups.

c. Clinical pathology:

The results of complete blood counts and serum chemistry were submitted to the investigator for evaluation.

Appendix O (cont): PATHOLOGY REPORT

Study 84040

d. Gross necropsy:

There were no deaths in any of the dosage groups that were attributable to the toxic effects of the test article.

e. Microscopic findings and conclusions:

(1) There were no microscopic lesions which were caused by the test article.

(2) Lymphoid infiltration in the peribronchiolar and peribronchial areas of the lung and in the periportal tract of the liver is a common microscopic observation in both control and test article groups. These lesions are considered as part of the normal background in rats.

(3) Animal #37426, female control - Sections of liver contained a single area of clear cells. The hepatic cells in these areas contain excessive lipid and have reduced oxidative enzymatic activity. It has been suggested that these foci are preneoplastic (Toxicologic Pathology, 1982; Symposium: Preneoplastic Changes in the Rat Liver).

  
ROBERT R. DAMLGREN, DVM  
LTC, VC  
I.H.A., Pathology Services Group

Appendix O (cont): PATHOLOGY REPORT

TABLE 1

Gross Observations - Males

	Group 1 - Control				Group 2 - 100 mg/kg			
95000	3	3	3	3	3	3	3	3
	0	1	2	3	0	0	1	2
	6	3	6	7	1	0	1	6
	-	-	-	-	-	-	-	-
Normal	+	+	+	+	+	+	+	+
Kidney, Pelvis Dilated, mild								+

	Group 3 - 316 mg/kg				Group 4 - 1000 mg/kg			
95000	3	3	3	3	3	3	3	3
	0	1	1	2	0	3	1	2
	2	4	0	3	0	5	3	4
	-	-	-	-	-	-	-	-
Normal	+	+	+	+	+	+	+	+
Kidney, Pelvis Dilated, Moderate Testicle Atrophy								+

There were no gross abnormalities found in females in any of the 4 groups.





Appendix O (cont): PATHOLOGY REPORT

Female Controls (Cont'd)

85000	3	3	3	3	3	3	3	3	3
	4	5	5	5	6	6	6	6	7
	5	5	7	8	2	5	7	9	9
	1								
Cecum	N	N	N	N	N	N	NW	N	N
Rectum	N	N	N	N	N	N	NW	N	N
Colon	N	N	N	N	N	N	NW	N	N
Stomach	N	N	N	N	N	N	NW	N	N
Skeletal Muscle	N	N	N	N	N	N	N	N	N
Sciatic Nerve	N	N	N	N	N	N	N	N	N
Tongue	N	N	N	N	N	N	N	N	N
Skin	N	N	N	N	N	N	N	N	N
Mammary Gland	N	N	N	N	N	N	N	N	N
Nasal	N	N	N	N	N	N	N	N	N
Sternum	N	N	N	N	N	N	N	N	N
Femur	N	N	N	N	N	N	N	N	N
Vertebrae	N	N	N	N	N	N	N	N	N
Spinal Cord	N	N	N	N	N	N	N	N	N
Adrenals	N	N	N	N	N	N	N	A	N
Pituitary	N	N	N	N	N	N	N	N	N
Eye	N	N	N	N	N	N	N	N	N
Middle Ear	N	N	N	N	N	N	N	N	N
Auriliary Sebaceous Gland	N	N	N	N	N	N	N	N	N

N = Normal; NW = Not present in wet tissue; (1) = One eye available; A = Abnormal



## Appendix O (cont): PATHOLOGY REPORT

## Female Dose Group 1000 mg/kg (Cont'd)

	RSP#									
	3	3	3	3	3	3	3	3	3	3
	4	4	5	5	6	6	7	7	7	7
	6	7	2	9	0	6	1	3	7	8
	-	-	-	-	-	-	-	-	-	-
Pancreas	N	N	A	N	N	N	N	N	N	N
Cecum	N	N	N	N	N	N	N	A	N	N
Colon	N	A	N	N	N	N	N	N	N	N
Pectun	N	N	N	N	N	N	N	N	N	N
Stomach	N	N	N	N	N	N	N	N	N	N
Skeletal Muscle	N	N	N	N	N	N	N	N	N	N
Sciatic Nerve	N	N	N	N	N	N	N	N	N	N
Tongue	N	N	N	N	N	N	N	N	N	N
Skin	N	N	N	N	N	N	N	N	N	N
Mammary Gland	N	N	N	N	N	N	N	N	N	N
Nasal	N	N	N	N	N	N	N	N	N	N
Sternum	N	N	N	N	N	N	N	N	N	N
Femur	N	N	N	N	N	N	N	N	N	N
Vertebrae	N	N	N	N	N	N	N	N	N	N
Spinal Cord	N	N	N	N	N	N	N	N	N	N
Adrenals	N	N	N	N	N	N	N	N	N	N
Pituitary	N	N	N	N	N	N	N	N	N	N
Eye(s)	N	N	N	N	N	N	N	N	N	N
Middle Ear	N	N	N	N	N	N	N	N	N	N
Auditory Sebaceous Gland	N	N	N	N	N	N	N	N	N	N

N = Normal; NP = Not present on slide; NW = Not present in wet tissue;  
 (1) = One eye available; A = Abnormal





## Appendix O (cont): PATHOLOGY REPORT

Table 2: Male Controls (Cont'd)

	85000									
	3	3	3	3	3	3	3	3	3	3
	0	0	1	1	2	2	3	3	3	4
	8	9	3	6	2	7	2	3	7	1
	-	-	-	-	-	-	-	-	-	-
Eye(s)	N	N	N	N	N	N	N	N	N	N
Middle Ear	N	N	N	N	N	N	N	N	N	N
Auditory Sebaceous Gland	N	N	N	N	N	N	N	N	N	N

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N = Normal; NP = Not present on slide; NI = Not present in wet tissue;  
 (1) = One eye available; A = Abnormal

Appendix O (cont): PATHOLOGY REPORT

TABLE 2  
Males 1000 mg/kg

95D00	3	3	3	3	3	3	3	3	3	3
	0	0	1	1	2	3	3	3	4	4
	5	7	1	9	9	0	4	9	0	2
	-	-	-	-	-	-	-	-	-	-
Cerebrum	N	N	N	N	N	N	N	N	N	N
Cerebellum	N	N	N	N	N	N	N	N	N	N
Trachea	N	N	N	N	N	N	N	N	N	N
Thyroid	N	N	N	N	N	N	N	N	N	N
Parathyroid	N	N	N	N	N	N	N	N	N	NP
Esophagus	N	N	N	N	N	N	N	N	N	N
Salivary Gland	N	N	N	N	N	N	N	N	N	N
Harderian Gland	N	N	N	N	A	N	N	N	N	N
Exorbital Gland	N	N	N	N	N	N	N	N	N	N
Heart	N	N	N	N	N	N	N	A	N	N
Aorta	N	N	N	N	N(1)	N	N	N	N	N
Lung	N	A	A	N	N	N	N	N	N	N
Thymus	N	N	N	N	N	N	N	N	N	N
Spleen	N	N	N	N	N	N	N	N	N	N
Mesenteric Lymph Node	N	N	N	N	A	N	N	N	N	N
Liver	N	N	N	A	N	N	N	N	N	N
Kidney	N	A	N	A	A	N	N	A	N	N
Urinary Bladder	N	N	N	N	N	N	N	N	N	N
Accessory Sex Glands	N	N	N	N	N	N	N	N	N	N
Epididymis	N	N	N	N	N	N	N	N	N	N

(1) Aorta on Slide #7





Appendix O (cont): PATHOLOGY REPORT

Table 2: Males 1000 mg/kg (Cont'd)

85000	3	3	3	3	3	3	3	3	3	3
	0	0	1	1	2	3	3	3	4	4
	5	7	1	8	0	0	4	8	0	2
	-	-	-	-	-	-	-	-	-	-
Middle Ear	N	N	N	N	N	N	N	N	N	N
Auditory Sebaceous Gland	N	N	N	N	N	N	N	N	N	N

N = Normal; NP = Not present on slide; NI = Not present in wet tissue;  
 (1) = One eye available; A = Abnormal

## Appendix O (cont): PATHOLOGY REPORT

## APPENDIX 1

## Microscopic Findings

Group 1 - Control - Males

85D00308

Harderian Gland - Lymphoid infiltration, mild, focal.  
Lung - Peribronchiolar and peribronchial lymphoid hyperplasia, mild, multifocal.

85D00309

Harderian Gland - Lymphoid infiltration - mild, multifocal.  
Exorbital Gland - Lymphoid infiltration - mild, multifocal.  
Lung - Peribronchiolar and peribronchial lymphoid hyperplasia, mild, multifocal.

85D00313

Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.

85D00316

Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.

85D00322

Harderian Gland - Lymphoid infiltration, mild, multifocal.  
Exorbital Gland - Lymphoid infiltration, mild, multifocal.  
Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.  
Mesenteric Lymph Node - Lymphoid hyperplasia, moderate, diffuse.

85D00327

Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.  
Heart - Lymphoid infiltration, myocardium, mild, focal.

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**Appendix O (cont): PATHOLOGY REPORT**

Appendix 1, Study #04040 (Cont'd)

- 85D00332
- Lung - Peribronchial and peribronchiolar lymphoid hyperplasia, mild, multifocal.
- 85D00337
- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.
- 85D00341
- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.
- Group 4 - 1000 mg/kg - Males
- 85D00307
- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.  
Nasal Region - Lymphoid hyperplasia, mild, focal.  
Kidney - Lymphoid infiltration, interstitial, mild, focal.
- 85D00311
- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.
- 85D00318
- Liver - Periportal lymphoid infiltration, mild, multifocal.  
Kidney - Hydronephrosis, severe, diffuse, unilateral.  
Kidney - Interstitial nephritis, chronic, moderate, focal, unilateral.
- 85D00320
- Kidney - Lymphoid infiltration, moderate, focal, interstitium, unilateral.  
Harderian Gland - Lymphoid infiltration, mild, multifocal.
- 85D00338
- Heart - Lymphoid infiltration, mild, focal.  
Kidney - Hydronephrosis, moderate, diffuse, bilateral.  
Testes - Atrophy, severe, unilateral.

## Appendix O (cont): PATHOLOGY REPORT

## Appendix 1, Study #84040 (Cont'd)

Group 1 - Control Females

85D00345

- Lung - Peribronchiolar lymphoid hyperplasia, mild, multifocal.  
 Liver - Periportal lymphoid infiltration, mild, multifocal.

85D00355

- Lung - Peribronchiolar lymphoid hyperplasia, mild, multifocal.  
 Kidney - Renal pelvis, lymphoid infiltration, mild, diffuse, unilateral.

85D00357

- Lung - Peribronchiolar and bronchial lymphoid hyperplasia, mild, multifocal.

85D00358

- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.

85D00362

- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.

85D00365 (37426)

- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.  
 Liver - Clear cell focus (nodule).

85D00367

- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.

Appendix O (cont): PATHOLOGY REPORT

Appendix 1, Study #84040 (Cont'd)

85D00369

- Heart - Mild, multifocal lymphoid infiltration, left ventricle.
- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.
- Liver - Periportal lymphoid infiltration, mild, multifocal.
- Adrenal - Post-cortical hyperplasia, mild, unilateral.

85D0370

- Trachea - Submucosal lymphoid infiltration, mild, multifocal.
- Lung - Peribronchial and perivascular lymphoid hyperplasia, moderate, multifocal.

Group 4 - 1000 mg/kg Females

85D00347

- Mesenteric Lymph Node - Lymphoid hyperplasia, moderate, diffuse.
- Colon - Lymphoid infiltration, mild, multifocal, submucosal.

85D00352

- Kidney - Tubular mineralization, deep medulla, mild, focal.
- Pancreas - Periarthritis, mild, focal.

85D00359

- Lung - Peribronchiolar and bronchial lymphoid hyperplasia, mild, multifocal.
- Kidney - Tubular mineralization, deep medulla, mild, multifocal.

85D00366

- Lung - Peribronchial lymphoid hyperplasia, mild, multifocal.
- Kidney - Tubular mineralization, corticomedullary junction, mild multifocal.

Appendix O (cont): PATHOLOGY REPORT

Appendix 1, Study #84040 (Cont'd)

85D00371

Harderian Gland - Lymphoid infiltration, mild, focal.  
Lung - Peribronchial lymphoid hyperplasia, mild,  
multifocal.

85D00373

Uterus - Dilatation of lumen, moderate, diffuse.  
Cecum - Submucosal hemorrhage, mild, multifocal.

95D00377

Lung - Peribronchial lymphoid hyperplasia, mild,  
multifocal.

85D00377

Lung - Peribronchiolar and peribronchial lymphoid  
hyperplasia, mild, multifocal.

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