Institute Report No. 264

Acute Oral Toxicity of Nitroguanidine in Male and Female Rats

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

March 1988

Toxicology Series: 104

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

Acute oral toxicity of nitroguanidine in male and female rats (Toxicology Series 104)--Brown et al.

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.

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

for Richard A. Cushman 30 MAR 88

Edwin S. Beatrice (date)
COL, MC
Commanding

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The acute oral toxicity of nitroguanidine was determined in male and female Sprague-Dawley rats by using the oral gavage single-dose limit test method. Test results indicated that the median lethal dose was greater than 5000 mg/kg body weight in both male and female rats. The predominant clinical signs associated with nitroguanidine administration were urinary excretion of a whitish precipitate (nitroguanidine) in the first 24 hours followed by excretion of a reddish urine for up to a week. Nitroguanidine also affected the gastrointestinal tract as it produced diarrhea with perianal staining and irritation of the mucosa of the stomach and small intestine. Excessive secretion from the harderian gland was also observed as a red nasal discharge and staining around the nose and mouth. These results place nitroguanidine in the practically nontoxic category based on the toxicity classification system of Hodge and Sterner.

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ABSTRACT

The acute oral toxicity of nitroguanidine was determined in male and female Sprague-Dawley rats by using the oral gavage single-dose limit test method. Test results indicated that the median lethal dose was greater than 5000 mg/kg body weight in both male and female rats. The predominant clinical signs associated with nitroguanidine administration were urinary excretion of a whitish precipitate (nitroguanidine) in the first 24 hours followed by excretion of a reddish urine for up to a week. Nitroguanidine also affected the gastrointestinal tract as it produced diarrhea with perianal staining and irritation of the mucosa of the stomach and small intestine. Excessive secretion from the harderian gland was also observed as a red nasal discharge and staining around the nose and mouth. These results place nitroguanidine in the practically nontoxic category based on the toxicity classification system of Hodge and Sterner.

Key Words: Nitroguanidine, Mammalian Toxicology, Rat, Munitions, Propellants, Acute Toxicity, Explosives.

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

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US Army Medical Research and Development Command
Letterman Army Institute of Research
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SPONSOR:

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

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

GLP STUDY NUMBER: 84008

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: MAJ Larry D. Brown, DVM, MPVM, VC
Diplomate, American Board of Toxicology

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

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

DATA MANAGERS: Carolyn M. Lewis, MS, Yvonne C. Le Tellier, BS

REPORT AND DATA MANAGEMENT:

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

TEST SUBSTANCE: Nitroguanidine ($CH_4N_4O_2$)

INCLUSIVE STUDY DATES: 9 August - 18 September 1984

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

ACKNOWLEDGMENTS

SP5 Paul D. Mauk, BS, SGT Steven K. Sano, BA, Carolyn M. Lewis, MS, and Yvonne C. Le Tellier, BS, provided research assistance; Richard A. Spieler, Richard Katona, Charlotte Speckman, and Roosevelt Cunningham provided animal and facility management; and Callie B. Crosby, JoAnn Nishimoto, Lynda Araiza, Colleen S. Kamiyama, Brenda V. Goce, Dianna Johnson, and Mara W. Joshua provided secretarial assistance. Eleanor M. Baker proofread the manuscript. CPT Earl W. Morgan, VC, served as the LAIR Project Director for the acute toxicity studies on nitroguanidine.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84008 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr. 29 MAR 88
DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

Larry D. Brown 22 Mar 1988
LARRY D. BROWN, DVM / DATE
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Principal Investigator

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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 23 March 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for GLP Study 84008

1. I hereby certify that in relation to LAIR GLP Study 84008, Tox Series 104, the following inspections were made:

24 February 1984	-	Protocol Review
10 August 1984	-	Necropsy

2. The report and raw data were audited on 30 December 1986.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Acute Oral Toxicity of Nitroguanidine in Male and Female Rats--Brown *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 compounds 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 Study

The objective of this study was to determine the acute oral toxicity of nitroguanidine in male and female albino Sprague-Dawley rats.

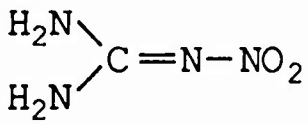
MATERIALS

Test Substance

Chemical name: Nitroguanidine (NGu)

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

Chemical structure:



Molecular formula: $CH_4N_4O_2$

Other information about the test substance is presented in Appendix A.

Vehicle

For oral dosing, nitroguanidine was prepared as a suspension in 0.2% methylcellulose, 0.4% Tween® 80 in double distilled water. Methylcellulose (4000 grade; viscosity of 2% solution in water = 4000 centipoises at 25°C; Lot 82F-0634, expiration date Apr 1994) was obtained from Sigma Chemical Co. (St Louis, MO). Tween® 80 (polyoxyethylene (20) sorbitan monooleate; Lot 713137, expiration date Dec 1986) was obtained from Fisher Scientific Products (Fairlawn, NJ). Double distilled water was obtained from the Chemistry Branch, LAIR.

Animal Data

Fourteen male and 14 female Sprague-Dawley rats from Bantin & Kingman, Inc., Fremont, CA, were used for this study. They were identified individually with ear tags. Two males and 2 females were selected for quality-control necropsy evaluation at receipt. Ten of the animals were used for a preliminary range-finding, approximate lethal dose (ALD) determination. Fourteen animals were used for a GLP limit test as defined by the EPA (2). The animal weights on receipt (9 Aug 84) ranged from 115 to 172 g with females the heavier of the two sexes. Additional animal data appear in Appendix B.

Husbandry

Rats were caged individually in stainless-steel wire-mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow Diet 5002 (Ralston Purina Company, St Louis, MO); water was provided by continuous drip from a central line. The temperature of the animal room was maintained in a range from 22.2°C to 25.5°C; the relative humidity ranged from 38% to 52% with temporary spikes to 60% during room washing. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Rats were randomized into quality-control, ALD, and limit test groups. The Beckman TOXSYS Animal Allocation Program was used in conjunction with a Beckman TOXSYS Data Collection Terminal. The limit test animals were acclimated for 14 days before dosing. During this period, they were

observed daily for signs of illness. At the end of this period males were approximately 90 g heavier than females.

Dosage Levels

The results of a literature search and the range-finding (ALD) study suggested that the median lethal dose (MLD) value was greater than 5000 mg/kg. Based on these data, a limit test was conducted using a dose of 5500 mg/kg.

Compound Preparation

Dosing suspensions were prepared by mixing nitroguanidine in an appropriate volume of methylcellulose/Tween® 80 vehicle before dosing the animals. At the concentrations required to achieve limit test dosage levels, nitroguanidine is insoluble in water or saline. Dosing was performed with a highly concentrated suspension prepared immediately prior to dosing.

Chemical Analysis of Dosing Solution

Nitroguanidine was stable in an aqueous suspension for 24 hours after preparation (Appendix A). This was deemed sufficient as the dosing suspensions were prepared fresh and dosing was completed within three hours of preparation. Tests for homogeneity of the test compound in the suspension indicated a variation in concentration of the top, middle, and bottom layers of less than 0.5% (Appendix A).

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3). Animals were fasted (food only) for approximately 16 hours prior to dosing.

Dosing was performed using the oral gavage method without animal sedation or anesthesia. Sterile, disposable 3 ml syringes (Becton, Dickenson & Co., Rutherford, NJ) fitted with 16-gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. All limit test animals were dosed between 0929 and 1210 hours on 5 September 1984.

Due to the viscosity of the nitroguanidine suspension (286 mg/ml was the highest concentration successfully administered in the ALD study), the maximum volume (10 mg/kg body weight) routinely administered to the rat as a single oral gavage (4), and the requirement to administer a limit dose, it was necessary to split the nitroguanidine limit dose

into three injections given at hourly intervals. The concentration of nitroguanidine in the dosing suspension for all three injections was 270 mg/ml.

The total volume administered in the three doses ranged from 5.93 to 6.48 ml in males and 4.20 to 4.79 ml in females.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 2, 3, and 4 hours after dosing (split dose given over 2-hour period) was concluded and once daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily, and only significant observations were recorded. Body weights were recorded twice weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those that survived the 13-day study period were necropsied immediately after sacrifice by barbiturate overdose.

Statistical Analysis

Statistical analyses were not conducted for this limit test because there was only one dose level. In accordance with appropriate guidelines (3) a limit test may be performed using a minimum of 5 animals of each sex at 5000 mg/kg. Group body weight means $\pm$ 1 standard error were calculated.

Duration of Study

Appendix C is a complete listing of historical events. The study was performed over a 41-day period, from 9 August 1984 to 18 September 1984.

Changes/Deviations From Original Protocol

All phases of this study were accomplished according to the protocol and applicable amendments with three exceptions: (a) only one test compound group of 7 males and 7 females was used; (b) historical cage control and vehicle control data

were used to conserve animals; and (c) post-dosing observations on the day of dosing were conducted approximately 2,3, and 4 hours after dosing was concluded, and (d) the post-dosing observation period was 13, not 14, days in duration because of an error in preparing the addendum schedule. None of these changes were thought to have an effect on the outcome of the study.

Storage of Raw Data and Final Report

A copy of the final report, study protocols and amendments, raw data, retired SOPs, analytical, stability and purity data of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Fourteen animals were dosed for the limit test. One misdosed female was removed from the study. Four (2 male and 2 female) of the 13 remaining limit test animals died. One female death (84D01200) occurred approximately 48 hours after dosing. The remaining three animals were found dead on the mornings of the fifth (84D01131, 84D01205) and sixth (84D01132) day. Mortality in the male group was 2 of 7 (28.5%) and in the female group 2 of 6 (33.3%). Appendix D is a tabular presentation of cumulative mortality.

Clinical Observations

The most frequently observed clinical signs were urinary system symptoms (13 of 13 animals dosed), a dark, reddish staining around eyes, nose and/or mouth (10 of 13), and gastrointestinal (GI) tract symptoms (6 of 13). On the day of dosing, the main clinical sign noted was the presence of a white precipitate on the tip of the penis or around the female urethral orifice. The white urinary precipitate was collected and analyzed. It was identified as nitroguanidine by HPLC (Appendix A). Seven of 7 males and 4 of 6 females exhibited this sign. The white precipitate was observed 2 to 4 hours after dosing was concluded and was usually followed by red urine/red perianal staining within the first 24 hours after dosing. Light microscopic examination of the red urine revealed numerous red blood cells. The red urine persisted for a week in some animals. A dark, reddish staining around the eyes, nose, and/or mouth was also observed beginning on the day of dosing and generally resolving within a week. The most common GI sign was diarrhea as indicated by fecal

staining of the perianal region. One animal presented with whitish fecal material which could have been contamination from the urine. Other clinical signs observed included irritation, hypotonia, anorexia, rough coat, and cyanosis.

Clinical signs are summarized in Tables 1 (males) and 2 (females). Individual animal histories are presented in Appendix E. Weight gains of survivors were not significantly affected by dosing. Table 3 presents the mean body weights for the male and female groups. Appendix F contains individual body weight tables.

Gross Pathological Observations

Lesions were found in the digestive and urinary systems. The nine animals that survived until terminal sacrifice had no recognizable gross lesions. The mucosa of the stomachs of 3 (84D01205, 84D01131, 84D01132) of the 4 animals which died after dosing contained multiple pinpoint sites of petechial hemorrhage. One animal (84D01200) had petechiae in the jejunum and red and pale areas in the glandular mucosa of the stomach. Three (84D01205, (84D01131, 84D01132) of the 4 animals that died had red contents in the small intestine and one (84D01132) had a bladder calculus. Appendix G contains the report of the veterinary pathologist.

DISCUSSION

Nitroguanidine exhibited low toxicity in this acute oral toxicity study. The MLD value is greater than the limit dose of 5000 mg/kg in both sexes as a dose of 5500 mg/kg produced less than 50% mortality in both male and female rats. Based on the toxicity classification scheme of Hodge and Sterner these results place nitroguanidine in the practically nontoxic range (5).

Clinical sign data indicated that nitroguanidine had a primary effect on the urinary system. Urinary tract symptoms included almost immediate excretion of nitroguanidine in the urine. Whitish-colored nitroguanidine crystals formed in the urine and the crystals accumulated at the urethral opening. The whitish urine was followed by a reddish urine (hematuria), which persisted for a week in some animals. On necropsy no gross lesions were noted in the urinary system except for one bladder calculus. Microscopic examination of the kidneys of animals surviving to the end of the observation period revealed no compound-related changes. Most animals also exhibited a dark red nasal and/or oral staining. The red nasal and/or oral discharge was attributed

TABLE 1

Incidence Summary for Clinical Observations in Male Rats Administered Nitroguanidine (5000 mg/kg)

	Animal (84D01___)							
Clinical Signs	131*	132*	134	135	136	140	155	Totals
Urinary precip. white crystall.	x	x	x	x	x	x	x	7/7
Red perianal stains/red urine	x	x			x			3/7
Stain, nasal and/or oral	x		x	x	x	x		5/7
Gastrointestinal>				x	x	x	x	4/7
Irritable		x			x	x		3/7
Rough coat		x	x					2/7
Hypotonia		x			x			2/7
Anorexia		x			x			2/7

*Animal died during observation period.

>Includes whitish feces and staining of perianal region.

TABLE 2

Incidence Summary for Clinical Observations in Female Rats Administered Nitroguanidine (5000 mg/kg)

	Animal (84D01___)						
Clinical Signs	196	200*	205*	206	208	212	Totals
Urinary precip. white crystall.	x	x	x	x			4/6
Red perianal stains/red urine	x	x	x	x	x	x	6/6
Stain, nasal and/or oral	x	x	x	x	x	x	6/6
Gastrointestinal>	x				x		2/6
Irritable		x	x			x	3/6
Rough coat stained coat	x						1/6
Cyanosis		x					1/6
Inactive		x					1/6

*Animal died during observation period.

>Includes diarrhea and staining of perianal region.

TABLE 3

Mean Body Weights In Grams of Rats Administered Nitroguanidine (5000 mg/kg)

Group	At Receipt	Dosing Day (Fasted)	Mid-Observation Period - Day 7	Terminal Sacrifice (Fasted)
Male	142.0 ± 1.9 (7)*	304.6 ± 3.7 (7)	333.0 ± 5.2 (5)	329.8 ± 3.9 (5)
Female	166.3 ± 1.2 (6)Δ	214.8 ± 4.2 (6)	230.3 ± 1.7 (4)	225.8 ± 7.0 (4)

* Values represent mean ± SE (number of animals).

Δ Female group contained one less animal than did the male group because one animal (84D01207) was removed from study due to misdosing.

to secretion of porphyrin from the harderian gland which is often observed in rats subjected to stress or disease (6). Nitroguanidine also produced GI tract signs. Fecal staining of the perianal region is associated with diarrhea which may indicate irritation to the lining of the GI tract. Necropsy of animals that died as a result of the test compound indicated that nitroguanidine produced slight irritation in the stomach and small intestine. Erythema and petechial hemorrhage of the gastric, jejunal, and duodenal mucosa were observed.

The results from this study are consistent with two previous reports on the acute oral toxicity of nitroguanidine. Dieke *et al* reported in 1947 that the MLD of nitroguanidine in the Norway rat was in excess of 5000 mg/kg (7). Kenyon (1) reviewed an extramural report in which a single oral dose (4640 mg/kg) of nitroguanidine produced no mortality or irreversible toxic effects in the male albino rat.

CONCLUSIONS

The MLD values for nitroguanidine were in excess of 5000 mg/kg in both male and female Sprague-Dawley rats, which classifies nitroguanidine as practically nontoxic. The predominate clinical signs associated with nitroguanidine administration were urinary excretion of nitroguanidine in the first 24 hours followed by hematuria lasting up to a week.

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Appendix A: CHEMICAL DATA

Chemical Name: Nitroguanidine (NGu)

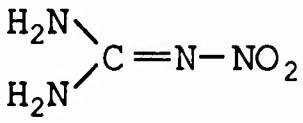
Other Listed Names: Guanidine, Nitro; alpha-Nitroguanidine; beta-Nitroguanidine

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

Lot Number: SOW83H001-004

LAIR Code: TP36

Chemical Structure:



Molecular Formula: $CH_4N_4O_2$

Molecular Weight: 104.1

Physical State: White powder

Melting Point: 232° C[1]

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^{-1}.[2] The spectrum was identical to the Sadtler spectrum for nitroguanidine.[3]

Stability:

An aqueous solution of NGu (48.1 µmolar) 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.[4]

The stability of nitroguanidine suspended in the dosing vehicle was also examined.[5] A suspension of nitroguanidine (50 mg/ml) was prepared and six samples removed. Three of the samples were diluted and analyzed (UV spectroscopy, 264 nm) immediately while the remaining three were analyzed 24 hours later. The results are presented below in terms of mg of nitroguanidine per gram of dosing suspension.

Sample Number	Time of Analysis 0 hour	24 hours
1	56.4	56.3
2	56.2	55.9
3	56.2	55.7
Average:	56.3	56.0

The average concentration at 24 hours was 99.5% of the initial concentration.

Homogeneity of Nitroguanidine Suspensions:

A solution of methylcellulose (0.2%) and Tween®-80 (0.4%) in sterile water was added to 10 g of nitroguanidine to produce a volume of 35 ml (i.e., 285.7 mg nitroguanidine per ml of dosing vehicle). After homogenization, three samples were taken from the top, middle, and bottom layers of the suspension for analysis by UV spectroscopy.[6]

	Concentration of Nitroguanidine (mg/ml) in each level of the suspension		
Sample #	Top	Middle	Bottom
1	266.5	270.7	275.2
2	269.0	271.2	264.3
3	261.7	270.3	274.6
Average for each level:	265.7	270.7	271.4
Average of all levels:		269.3	
% Target concentraton:		94.3	

A comparison of the overall average to the average for each level shows that no deviation exceeds 1.5%, thus demonstrating that homogeneous suspensions of nitroguanidine can be prepared.

Analysis of Dosing Suspension:

The concentration of nitroguanidine in the dosing suspension prepared on 5 Sep 84 (target concentration 285.7 mg/ml) was determined by the analysis of three aliquots

removed from the suspension.[7] The results were as follows:

Sample #	Concentration of Nitroguanidine (mg/ml)
1	277.1
2	276.7
3	277.8
Mean value:	277.2
% target concentration:	97.0

[1]Fedoroff BT, Sheffield OE. Encyclopedia of explosives and related items. Vol 6. Dover, New Jersey: Picatinny Arsenal, 1975: G154.

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

[3]Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #21421.

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

[5]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-12-022, pp 14-17. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[6]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 30. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[7]*Ibid*, p 34-35.

Appendix A (cont.): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC
(CRSAR-P-702-109)

DTIC CONTROL SYMBOL: EXEMPT-Para 7-7e AR 335-15 | PAGE: 1 OF 1

TO:	FROM:	DATE
Commander US Army Armament Munitions and Chemical Command Attn: DRSMC-QAD Rock Island, ILL. 61201	Sunflower Army Ammunition Plant DeSoto, Kansas 66018	September 13, 1983 MATERIAL: Nitroguanidine Type II, Class 2 *

MANUFACTURER	CONTRACT NO.
Hercules Aerospace Division, Hercules Incorporated	DAAA-09-77-C-4016, CLIN 0270

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
SOW83H001-004		1	7,000 lbs.

PLACE MANUFACTURED	SPECIFICATION AND AMENDMENT/DRAWING NO.
Sunflower Army Ammunition Plant, DP Facility	MIL-N-494A w/Int. Amend 6 (AR) dated 25 March 1981 *

SECTION B - DESCRIPTION OF MATERIAL

Property	Requirement Min.	Requirement Max.	Analysis
Purity, %	99.0		99.6
Ash Content, %		0.30	0.03
pH Value	4.5	7.0	7.55 **
Acidity (as H_2SO_4), %		0.06	ND ***
Total Volatiles, %		0.25	0.03
Sulfates (as $NaSO_4$), %		0.20	0.01
Impurities, H_2O Insoluble, %		0.20	0.01
Particle Size, Microns		3.0 *	4.0 ****
Particle Size, Std. Dev.		± 0.5	0.168

* 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

REMARKS

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 285. 25 pounds per drum per HAD letter dated Aguust 1, 1983, to COR.

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY	THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.
Hercules Aerospace Division	13 Sept 83 A. W. Erdlich
TESTING CONDUCTED BY: Hercules Aerospace Division	DATE — A. W. ERDLICH, SIGNATURE

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

FOR THE COMMANDER

14 Sep 83 — Quality Assurance Specialist — M. A. Ford, SIGNATURE

DATE — TITLE

ARRCOM FORM 213-R, 18 AUG 77

REPLACES ARRCOM FORM 213-R, 15 JUL 75 (STEMP) WHICH MAY BE USED.

Appendix A (cont.): CHEMICAL DATA

ANALYSIS OF URINARY CRYSTALS

Rats dosed orally with nitroguanidine in GLP Study 84008 excreted urine that contained a white crystalline substance. Inspection of the dosed animals at necropsy revealed a crystalline substance in the bladder of one animal. High pressure liquid chromatographic (HPLC) analysis of these crystals provided evidence that the substance in the bladder and urine was nitroguanidine.

The crystalline substance obtained from the bladder was dissolved in water, filtered, and analyzed by HPLC; the instrument used was a Hewlett-Packard 1090 Liquid Chromatograph equipped with a Hypersil ODS 5 µm column (100 x 2.1 mm). Dr. Bert Ho of the LAIR Division of Toxicology performed the analysis.*

The retention time of the bladder compound (1.13 min; flow rate 0.3 ml/min; solvent system 5% MeOH in H_2O) was identical to that of nitroguanidine standards. The UV spectrum (obtained via a diode-array detector) of the bladder compound was virtually identical to that of nitroguanidine standards (lambda max = 264 nm).

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*Ho, B. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-03-009, p2. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: Rattus norvegicus

Strain: Sprague-Dawley

Source: Bantin & Kingman, Inc.
Fremont, CA 94538

Sex: Male and female

Date of birth: Male: 2 July 1984
Female: 25 June 1984

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

Condition of animals at start of study: Normal

Body weight range at dosing: 206-318 g

Identification procedures: Ear tagging procedure (SOP OP-ARG-1). Tag numbers [84D01131, 132, 134, 135, 136, 140, 155, 196, 200, 205, 206, 207, 208, and 212] were used for Limit Test animals.

Pretest conditioning: Quarantine/acclimation 10-24 Aug 84.

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

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
9 Aug 84	Received 14 male and 14 female Sprague-Dawley rats. Rats were checked for physical condition, sexed, weighed, ear-tagged, and individually caged.
10 Aug 84	Four rats (2 male and 2 female) were submitted for necropsy quality control.
10 Aug-4 Sep 84	Animals were observed daily.
13 Aug 84	Animals weighed and randomized into dose groups.
14 Aug 84	Ten ALD animals were weighed, dosed, and observed.
24 Aug 84	Limit Test animals were cleared from quarantine.
4 Sep 84	Food was removed from the Limit Test animals at 1600 hours.
5 Sep 84	Fourteen Limit Test animals were weighed, dosed, and observed at 1, 2, and 4 hours after dosing was concluded. One misdose was removed from the study.
6-18 Sep 84	Animals observed daily for clinical signs in AM and PM.
7 Sep 84	One compound-related death occurred.
10 Sep 84	Two compound-related deaths occurred.
11 Sep 84	One compound-related death occurred.
5,7,12, 14,18 Sep 84	Animals were weighed.
18 Sep 84	Nine surviving animals were weighed, sacrificed, and necropsied. The in-life phase of the study was terminated.

Appendix D

Survival and Cumulative Mortality Data
Nitroguanidine (5000 mg/kg) Limit Test

Animal Number	Time After Dosing (Days) 1	2	3	4	5	6	7	8	9	10	11	12	13
	MALES												
84D01131	0*	0	0	0	D								
132	0	0	0	0	0	D							
134	0	0	0	0	0	0	0	0	0	0	0	0	0
135	0	0	0	0	0	0	0	0	0	0	0	0	0
136	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0	0	0	0	0	0	0
155	0	0	0	0	0	0	0	0	0	0	0	0	0
	FEMALES												
196	0	0	0	0	0	0	0	0	0	0	0	0	0
200	0	D											
205	0	0	0	0	D								
206	0	0	0	0	0	0	0	0	0	0	0	0	0
207	MD[Δ]												
208	0	0	0	0	0	0	0	0	0	0	0	0	0
212	0	0	0	0	0	0	0	0	0	0	0	0	0
Cumulative Mortality	0	1	1	1	3	4	4	4	4	4	4	4	4

* O = Alive; D = Died

Δ MD = Misdosed, not included in cumulative mortality total

STUDY: 00000004
COMPOUND: NITROGUANIDINE
START DATE: 9/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N402)

ANIMAL HISTORY

ANIMAL ID: 44061131 SPECIES: D SEX: MALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OPERATOR	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
9/24/84	9:45:24	0000537	266.0	* NO OBSERVATIONS RECORDED.
9/31/84	9:09:04	0076478	317.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:04:54	0084799	344.0	QUARANTINE COMPLETED / NORMAL
9/05/84	9:17:52	0084799	312.0	DOSED
9/05/84	12:19:10	0081799		URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA
9/05/84	15:33:44	0010186		URINARY PRECIPITATE WHITE FROM TIP PENI /URETHRA MODERATE
9/05/84	15:04:15	0010186		NORMAL
9/06/84	9:53:56	0010186		NORMAL
9/07/84	7:57:06	0010186	254.0	* NO OBSERVATIONS RECORDED.
9/07/84	9:59:14	0010186		NORMAL
9/08/84	9:23:14	0053842		STAIN DARK NOSE MODERATE,STAIN RED PERIANAL MODERATE
9/09/84	7:10:22	0084799		STAIN DARK NOSE MODERATE,STAIN RED PERIANAL SLIGHT,STAIN DARK EYE MARKED
9/10/84	9:57:04	0084799		DEATH

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 8/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE ($CH_4N_4O_2$)

ANIMAL HISTORY

ANIMAL ID: 84001132 SPECIES: D SEX: MALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OPERATOR	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
8/24/84	8:46:30	0000337	259.0	* NO OBSERVATIONS RECORDED.
8/31/84	9:09:46	0078478	303.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:06:36	0084799	321.0	QUARANTINE COMPLETED / NORMAL
9/05/84	8:25:50	0084799	294.0	DOSED
9/05/84	12:26:50	0010186		NORMAL
9/05/84	13:35:30	0010186		URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA MODERATE
9/05/84	15:04:46	0010186		URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA MODERATE
9/06/84	6:54:30	0010136		IRRITABLE SLIGHT,URINE RED SLIGHT
9/07/84	7:55:10	0010186	261.0	* NO OBSERVATIONS RECORDED.
9/07/84	9:59:32	0010186		URINE RED SLIGHT
9/08/84	8:24:22	0054832		URINE RED SLIGHT
9/09/84	7:15:02	0054832		URINE RED SLIGHT
9/10/84	9:13:06	0084799		HYPOTONIA,ROUGH COAT SLIGHT,ANOREXIA
9/11/84	6:37:02	0010186		DEATH

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N4O2)

ANIMAL HISTORY

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 9/15/84

ANIMAL ID: [illegible] SPECIES: R SEX: MALE GROUP: 5000 MG/KG

DATE	TIME	OBSERVATIONS	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
9/24/84	9:47:34	00005437	285.0	* NO OBSERVATIONS RECORDED.
9/21/84	9:11:10	00784474	314.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:00:00	00843709	332.0	QUARANTINE COMPLETED / NORMAL
9/05/84	-2:59:14	00843709	345.0	DOSED
9/05/84	12:29:46	00101146		STAIN DARK NOSE SLIGHT
9/05/84	14:37:34	00101146		STAIN DARK NOSE SLIGHT,SCAB RIGHT EAR SLIGHT, URINARY PRECIPITATE WHITE FOCI TIP PENIS/URETHRA MODERATE
9/05/84	15:06:04	00101146		STAIN DARK NOSE SLIGHT,SCAB RIGHT EAR SLIGHT
9/06/84	7:55:24	00101146		STAIN DARK NOSE SLIGHT,SCAB RIGHT EAR SLIGHT
9/07/84	7:59:00	00101146	325.0	* NO OBSERVATIONS RECORDED.
9/07/84	11:00:22	00101146		STAIN DARK NOSE SLIGHT
9/08/84	-2:40:00	00549452		STAIN DARK NOSE SLIGHT
9/09/84	7:14:54	00549452		STAIN DARK NOSE SLIGHT
9/10/84	6:14:04	00001557		NORMAL
9/11/84	8:58:55	00101146		NORMAL
9/12/84	6:44:40	00101146	347.0	ROUGH COAT SLIGHT
9/13/84	7:04:42	00101146		NORMAL
9/14/84	10:04:00	00000547	355.0	* NO OBSERVATIONS RECORDED.
9/16/84	10:49:22	00101146		NORMAL
9/17/84	10:22:54	00101146		NORMAL
9/18/84	9:59:54	00849791	342.0	NORMAL / EUTHANIZED

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N4O2)

ANIMAL HISTORY

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 4/13/84.

ANIMAL ID: [illegible] SPECIES: R SEX: MALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	DEGREES	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
4/23/84	[illegible]	0000799	255.0	QUARANTINE COMPLETED / DOSED
4/31/84	9:12:10	0074478	307.0	* NO OBSERVATIONS RECORDED.
5/05/84	8:41:04	0041799	402.0	DOSED
5/05/84	12:40:54	0010146		SCAB RIGHT EAR SLIGHT,ORDINARY PRECIPITATE WHITE FROM TIP REDUCED
5/05/84	13:40:04	0010146		SCAB RIGHT EAR SLIGHT
5/05/84	15:00:44	0010146		SCAB RIGHT EAR SLIGHT,STAIN ORANGE NOSE SLIGHT
5/06/84	8:57:24	0010146		SCAB RIGHT EAR SLIGHT,STAIN FECES TAIL SLIGHT
5/07/84	7:54:36	0010146	272.0	* NO OBSERVATIONS RECORDED.
5/07/84	10:01:02	0010146		SCAB RIGHT EAR SLIGHT
5/08/84	8:41:04	0058642		SCAB RIGHT EAR SLIGHT,STAIN DARK NOSE SLIGHT
5/09/84	7:17:52	0084799		SCAB RIGHT EAR SLIGHT,STAIN DARK NOSE SLIGHT
5/11/84	8:55:04	0010146		SCAB RIGHT EAR SLIGHT
5/12/84	8:49:40	0010146	423.0	SCAB RIGHT EAR SLIGHT
5/13/84	7:06:56	0010146		SCAB RIGHT EAR SLIGHT
5/14/84	10:42:08	0000337	526.0	* NO OBSERVATIONS RECORDED.
5/16/84	16:49:52	0010146		SCAB RIGHT EAR SLIGHT
5/17/84	10:23:22	0010146		NORMAL
5/19/84	7:00:32	0084799	424.0	NORMAL / EUTHANIZED

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 8/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH40402)

ANIMAL HISTORY

ANIMAL ID: 84001156 SPECIES: D SEX: MALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OPERATOR	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
8/24/84	8:50:04	0000337	275.0	* NO OBSERVATIONS RECORDED.
8/31/84	9:12:45	0078478	330.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:11:36	0081799	351.0	QUARANTINE COMPLETED / NORMAL
9/05/84	6:43:54	0084799	318.0	DOSED
9/05/84	12:26:36	0010186		STAIN DARK NOSE SLIGHT, URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA SLIGHT
9/05/84	15:41:26	0010186		STAIN DARK NOSE SLIGHT, URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA SLIGHT
9/05/84	15:07:54	0010186		STAIN DARK NOSE MODERATE, URINE WHITE MODERATE
9/06/84	6:58:46	0010186		STAIN DARK NOSE MODERATE, URINE RED MODERATE, IRRITABLE SLIGHT
9/07/84	8:00:15	0010186	283.0	* NO OBSERVATIONS RECORDED.
9/07/84	10:01:53	0010186		IRRITABLE SLIGHT, STAIN DARK NOSE MARKED, STAIN YELLOW PERIANAL SLIGHT
9/09/84	7:19:52	0054332		STAIN YELLOW PERIANAL SLIGHT
9/10/84	9:18:35	0000337		HYPOTONIA, ANOREXIA
9/11/84	6:55:40	0010186		NORMAL
9/12/84	6:50:24	0010186	328.0	IRRITABLE SLIGHT
9/13/84	7:07:36	0010186		IRRITABLE SLIGHT
9/14/84	10:44:32	0000337	334.0	* NO OBSERVATIONS RECORDED.
9/14/84	10:49:44	0010186		IRRITABLE SLIGHT
9/17/84	10:23:44	0010186		IRRITABLE SLIGHT
9/18/84	7:01:32	0084799	334.0	NORMAL / EUTHANIZED

STUDY: 004N003
COMPOUND: NITROGUANIDINE
START DATE: 9/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N402)

ANIMAL HISTORY

ANIMAL ID: 0000140 SPECIES: R SEX: MALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OBSERVATION	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
9/29/84	8:55:22	0000337	254.0	* NO OBSERVATIONS RECORDED.
9/31/84	9:15:08	0073478	305.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:12:35	0084799	316.0	QUARANTINE COMPLETED / NORMAL
9/05/84	8:47:00	0084799	291.0	DOSED
9/05/84	12:41:46	0010186		URINARY PRECIPITATE WHITE F204 TIP PENIS/URETHRA SLIGHT
9/05/84	13:45:12	0010186		URINE WHITE SLIGHT
9/05/84	13:50:16	0010186		IRRITABLE MODERATE
9/05/84	16:09:06	0010186		STAIN DARK NOSE SLIGHT
9/06/84	7:03:54	0010186		STAIN DARK NOSE MODERATE
9/07/84	8:40:24	0010186	240.0	* NO OBSERVATIONS RECORDED.
9/07/84	10:21:12	0010186		STAIN DARK TAIL SLIGHT
9/08/84	8:34:32	0054832		STAIN DARK TAIL SLIGHT
9/09/84	7:22:36	0054832		STAIN DARK TAIL SLIGHT
9/10/84	9:20:22	0000337		NORMAL
9/12/84	5:51:06	0010186	319.0	NORMAL
9/13/84	7:04:16	0010186		NORMAL
9/14/84	10:45:08	0000337	318.0	* NO OBSERVATIONS RECORDED.
9/14/84	10:49:56	0010186		NORMAL
9/17/84	10:24:50	0010186		NORMAL
9/18/84	7:05:20	0084799	315.0	NORMAL / EUTHANIZED

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N4O2)

ANIMAL HISTORY

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 9/14/84

ANIMAL ID: [illegible] SPECIES: R SEX: MALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	CUMULATIVE HOURS	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
9/20/84	[illegible]	0.0	275.0	QUARANTINE COMPLETED / NORMAL
9/21/84	[illegible]	24.0	325.0	* NO OBSERVATIONS RECORDED.
9/22/84	[illegible]	48.0	311.0	DOSED
9/23/84	[illegible]	[illegible]		NORMAL
9/24/84	[illegible]	[illegible]		FECES WHITE MODERATE,URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA SLIGHT
9/25/84	[illegible]	[illegible]		URINE WHITE MODERATE
9/26/84	[illegible]	[illegible]		NORMAL
9/27/84	[illegible]	[illegible]	282.0	* NO OBSERVATIONS RECORDED.
9/28/84	[illegible]	[illegible]		NORMAL
9/29/84	[illegible]	[illegible]		NORMAL
9/30/84	[illegible]	[illegible]		NORMAL
10/1/84	[illegible]	[illegible]		NORMAL
10/2/84	[illegible]	[illegible]	333.0	NORMAL
10/3/84	[illegible]	[illegible]		NORMAL
10/4/84	[illegible]	[illegible]	357.0	* NO OBSERVATIONS RECORDED.
10/5/84	[illegible]	[illegible]	384.0	* NO OBSERVATIONS RECORDED.
10/6/84	[illegible]	[illegible]		NORMAL
10/7/84	[illegible]	[illegible]		NORMAL
10/8/84	[illegible]	[illegible]	397.0	NORMAL / EUTHANIZED

STUDY: 0047004
COMPOUND: NITROGUANIDINE
START DATE: 8/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N402)

ANIMAL HISTORY

ANIMAL ID: 84001196 SPECIES: D SEX: FEMALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OBSERVER	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
8/24/84	9:55:22	0000347	231.0	* NO OBSERVATIONS RECORDED.
8/31/84	9:03:10	0073078	245.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:16:56	0084799	258.0	QUARANTINE COMPLETED / NORMAL
9/05/84	8:42:19	0084799	245.0	DOSED
9/05/84	12:46:34	0084799		SCAB RIGHT EAR SLIGHT,STAIN URINE PERIANAL MODERATE, URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA SLIGHT
9/05/84	13:51:06	0084799		SCAB RIGHT EAR SLIGHT,STAIN FECES PERIANAL MARKED
9/05/84	15:12:39	0010186		SCAB RIGHT EAR SLIGHT,STAIN RED PERIANAL MARKED
9/06/84	7:10:16	0010196		SCAB RIGHT EAR SLIGHT,STAIN RED PERIANAL MARKED,STAIN DARK NOSE SLIGHT, STAIN DARK MOUTH SLIGHT,STAIN BROWN TAIL MARKED,URINE RED MODERATE
9/07/84	8:01:24	0010186	217.0	* NO OBSERVATIONS RECORDED.
9/07/84	10:04:06	0010186		SCAB RIGHT EAR SLIGHT,STAIN BROWN TAIL SLIGHT,STAIN DARK NOSE SLIGHT,STAIN DARK PERIANAL SLIGHT
9/08/84	8:37:36	0084799		SCAB RIGHT EAR SLIGHT,STAIN BROWN TAIL SLIGHT,STAIN DARK NOSE SLIGHT,STAIN DARK PERIANAL SLIGHT
9/09/84	7:24:50	0084799		SCAB RIGHT EAR SLIGHT,STAIN BROWN TAIL SLIGHT,STAIN DARK NOSE SLIGHT,STAIN DARK PERIANAL SLIGHT
9/10/84	9:22:12	0000347		SCAB RIGHT EAR SLIGHT,STAIN BROWN TAIL MODERATE,STAIN BROWN PERIANAL MODERATE
9/11/84	8:59:16	0010186		SCAB RIGHT EAR SLIGHT,STAIN BROWN TAIL SLIGHT
9/12/84	8:52:33	0010186	233.0	SCAB RIGHT EAR SLIGHT,STAIN BROWN TAIL SLIGHT,ROUGH COAT SLIGHT
9/13/84	7:04:58	0010186		SCAB RIGHT EAR MODERATE,STAIN BROWN TAIL SLIGHT
9/14/84	9:29:58	0000347	256.0	* NO OBSERVATIONS RECORDED.
9/15/84	10:50:42	0010186		SCAB RIGHT EAR MODERATE,STAIN BROWN TAIL SLIGHT
9/17/84	10:25:26	0010186		SCAB RIGHT EAR MODERATE,STAIN BROWN TAIL SLIGHT
9/18/84	7:05:43	0084799	242.0	SCAB RIGHT EAR MODERATE,STAIN ORANGE TAIL SLIGHT,EUTHANIZED

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N402)

ANIMAL HISTORY

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 4/13/84

ANIMAL ID: [illegible] SPECIES: R SEX: FEMALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OBSERVATION	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
4/24/84	[illegible]	00000367	214.0	* NO OBSERVATIONS RECORDED.
4/25/84	[illegible]	00000479	226.0	* NO OBSERVATIONS RECORDED.
4/26/84	[illegible]	00000599	235.0	QUARANTINE COMPLETED / NORMAL
4/27/84	[illegible]	00000719	242.0	DOSED
4/27/84	[illegible]	00010146		SCAR RIGHT EAR SLIGHT
4/27/84	[illegible]	00000239		SCAR RIGHT EAR SLIGHT,ORDINARY PRECIPITATE SMILE FROM TIP PENIS/UNDETERM SLIGHT
4/27/84	[illegible]	00010146		IRRITABLE SLIGHT,STAIN DARK NOSE SLIGHT
4/27/84	[illegible]	00010146		STAIN DARK NOSE SLIGHT,STAIN RED PERIANAL SLIGHT,STAIN RED TAIL SLIGHT, URINE RED MODERATE
4/28/84	7:13:24	00010146		STAIN DARK NOSE SLIGHT,STAIN RED TAIL SLIGHT,URINE RED SLIGHT,STAIN RED/U MOUTH SLIGHT,SCAB RIGHT EAR SLIGHT
4/28/84	[illegible]	00010146	182.0	* NO OBSERVATIONS RECORDED.
4/29/84	[illegible]	00010146		MOVEMENT,INACTIVE RAPID,CYANOSIS SLIGHT,SCAB RIGHT EAR SLIGHT,URINE RED MARKED
4/29/84	[illegible]	00000749		DEATH

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

STUDY: 0084004
COMPOUND: NITROGUANIDINE
START DATE: /84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE ($CH_4N_4O_2$)

ANIMAL HISTORY

ANIMAL ID: 84001205 SPECIES: 0 SEX: FEMALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OPERATOR	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
8/24/84	9:41:40	0060537	210.0	* NO OBSERVATIONS RECORDED.
8/31/84	9:45:16	0075478	225.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:19:00	0084799	229.0	QUARANTINE COMPLETED / NORMAL
9/05/84	8:47:44	0084799	210.0	DOSED
9/05/84	12:49:55	0084799		SCAB RIGHT EAR SLIGHT,URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA SLIGHT
9/05/84	13:54:54	0084799		SCAB RIGHT EAR SLIGHT,STAIN DARK NOSE SLIGHT,IRRITABLE SLIGHT, URINARY PRECIPITATE WHITE FROM TIP PENIS/URETHRA SLIGHT
9/05/84	15:15:16	0010186		SCAB RIGHT EAR SLIGHT,IRRITABLE SLIGHT,STAIN DARK NOSE MODERATE
9/06/84	7:15:14	0084799		SCAB RIGHT EAR SLIGHT,STAIN DARK NOSE SLIGHT,STAIN RED TAIL MARKED,URINE RED MODERATE,STAIN DARK FRONT LEG MODERATE,STAIN DARK HIND LEG MODERATE
9/07/84	8:29:56	0010186	193.0	* NO OBSERVATIONS RECORDED.
9/07/84	10:19:54	0010186		SCAB RIGHT EAR SLIGHT,STAIN RED TAIL MARKED,URINE RED MODERATE,STAIN RED HIND LEG MODERATE,STAIN RED ABDOMEN MODERATE
9/08/84	8:59:00	0054432		SCAB RIGHT EAR SLIGHT,STAIN RED TAIL MARKED,URINE RED MODERATE,STAIN RED HIND LEG MODERATE,STAIN RED ABDOMEN MODERATE
9/09/84	7:26:20	0054332		SCAB RIGHT EAR SLIGHT,STAIN RED TAIL MARKED,URINE RED MODERATE,STAIN RED HIND LEG MODERATE,STAIN RED ABDOMEN MODERATE
9/10/84	7:44:13	0084799		DEATH

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

STUDY: NCS4004
COMPOUND: NITROGUANIDINE
START DATE: 8/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N4O2)

ANIMAL HISTORY

ANIMAL ID: 84001245 SPECIES: 0 SEX: FEMALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OPERATOR	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
8/28/84	9:42:02	0000437	207.0	* NO OBSERVATIONS RECORDED.
8/31/84	9:46:56	0074479	231.0	* NO OBSERVATIONS RECORDED.
9/04/84	9:15:44	0084799	241.0	QUARANTINE COMPLETED / NORMAL
9/05/84	8:50:25	0084799	213.0	DOSED
9/05/84	12:51:12	0010186		URINE WHITE SLIGHT
9/05/84	11:00:20	0010186		STAIN DARK NOSE MODERATE,URINARY PRECIPITATE WHITE FROM URETHRA SLIGHT
9/05/84	15:16:00	0010186		STAIN DARK NOSE MODERATE,URINE RED MODERATE
9/06/84	7:15:24	0010186		STAIN DARK NOSE MODERATE
9/07/84	8:05:55	0010186	193.0	* NO OBSERVATIONS RECORDED.
9/07/84	10:04:30	0010186		STAIN DARK NOSE SLIGHT
9/08/84	8:41:54	0054842		STAIN DARK NOSE SLIGHT
9/09/84	7:30:00	0054842		STAIN DARK NOSE SLIGHT
9/10/84	9:23:25	0000437		NORMAL
9/11/84	6:59:44	0010186		NORMAL
9/12/84	6:55:44	0010186	235.0	NORMAL
9/13/84	7:11:04	0010186		NORMAL
9/14/84	4:40:15	0000337	235.0	* NO OBSERVATIONS RECORDED.
9/14/84	10:50:55	0010186		NORMAL
9/17/84	10:25:45	0010186		NORMAL
9/18/84	7:36:14	0084799	227.0	NORMAL / EUTHANIZED

STUDY: [illegible]
COMPOUND: NITROGUANIDINE
START DATE: 4/13/84

ORAL LETHAL DOSE (LD50) TEST IN RATS OF
NITROGUANIDINE (CH4N4O2)

ANIMAL HISTORY

ANIMAL ID: [illegible] SPECIES: R SEX: FEMALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OBSERVATION	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
4/20/84	9:44:[illegible]	0000537	216.0	* NO OBSERVATIONS RECORDED.
4/21/84	9:45:17	0075878	242.0	* NO OBSERVATIONS RECORDED.
4/22/84	9:21:12	0084799	243.0	QUARANTINE COMPLETED / NORMAL
4/25/84	9:55:[illegible]6	0084799	213.0	DOSED
4/25/84	12:55:59	0010186		NORMAL
4/25/84	14:31:54	0010186		URINE RED MARKED,STAIN BROWN NOSE SLIGHT
4/25/84	15:16:22	0010186		URINE RED MARKED,STAIN BROWN NOSE SLIGHT
4/26/84	7:19:32	0010186		URINE RED MODERATE,STAIN BROWN NOSE SLIGHT,SCAB RIGHT EAR SLIGHT,STAIN DARK MOUTH SLIGHT
4/27/84	9:06:12	0010186	211.0	* NO OBSERVATIONS RECORDED.
4/27/84	14:09:22	0010186		SCAB RIGHT EAR SLIGHT,STAIN DARK MOUTH SLIGHT
4/28/84	8:32:43	0054832		SCAB RIGHT EAR SLIGHT,STAIN DARK MOUTH SLIGHT,STAIN YELLOW PERIANAL MODERATE STAIN DARK TAIL SLIGHT,STAIN DARK NOSE SLIGHT
4/29/84	7:4[illegible]:52	0084799		SCAB RIGHT EAR SLIGHT,STAIN DARK MOUTH SLIGHT,STAIN YELLOW PERIANAL MODERATE STAIN DARK TAIL SLIGHT,STAIN DARK NOSE SLIGHT
4/30/84	9:27:20	0000537		NORMAL
5/1/84	7:[illegible]:[illegible]	0010186		SCAB RIGHT EAR MODERATE,STAIN BROWN TAIL SLIGHT
5/2/84	8:55:55	0010186	229.0	SCAB RIGHT EAR MODERATE
5/3/84	7:11:15	0010186		SCAB RIGHT EAR MODERATE
5/4/84	9:41:54	0000537	235.0	* NO OBSERVATIONS RECORDED.
5/4/84	13:51:14	0010186		DIARRHEA SLIGHT,SCAB RIGHT EAR SLIGHT
5/7/84	10:25:54	0010186		SCAB RIGHT EAR SLIGHT
5/14/84	7:07:00	0084799	226.0	SCAB RIGHT EAR SLIGHT,EUTHANIZED

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

STUDY: 00000000 ORAL LETHAL DOSE (LD50) TEST IN RATS OF
COMPOUND: NITROGUANIDINE NITROGUANIDINE (CH4N402)
START DATE: 4/13/84

ANIMAL HISTORY

ANIMAL ID: 40001212 SPECIES: 0 SEX: FEMALE GROUP: 5000 MG/KG

OBSERVATIONS

DATE	TIME	OBTAIN	WEIGHT (GRAMS)	CLINICAL OBSERVATIONS
4/24/84	9:44:48	0000337	214.0	* NO OBSERVATIONS RECORDED.
4/31/84	9:44:34	0084799	227.0	QUARANTINE COMPLETED / NORMAL
4/25/84	8:57:30	0084799	206.0	DOSED
4/25/84	13:06:42	0010186		SCAB RIGHT EAR SLIGHT,STAIN RED NOSE SLIGHT,URINE RED SLIGHT
4/25/84	15:17:12	0010186		SCAB RIGHT EAR SLIGHT,STAIN RED NOSE MODERATE,URINE RED MODERATE
4/26/84	8:26:34	0010186	194.0	* NO OBSERVATIONS RECORDED.
4/26/84	10:18:52	0010186		SCAB RIGHT EAR SLIGHT,STAIN BROWN MOUTH SLIGHT
4/26/84	8:46:04	0084799		SCAB RIGHT EAR SLIGHT,STAIN BROWN MOUTH SLIGHT,URINE RED PERIANAL SLIGHT
4/26/84	7:52:14	0084799		SCAB RIGHT EAR SLIGHT,STAIN BROWN MOUTH SLIGHT,URINE RED PERIANAL SLIGHT, IRRITABLE MARKED
4/10/84	9:27:56	0000337		NORMAL
4/11/84	7:00:54	0010186		NORMAL
4/12/84	6:34:26	0010186	226.0	NORMAL
4/13/84	7:11:42	0010186		NORMAL
4/14/84	9:44:14	0000337	237.0	* NO OBSERVATIONS RECORDED.
4/14/84	10:52:14	0010186		NORMAL
4/17/84	10:26:14	0010186		NORMAL
4/14/84	7:07:52	0084799	208.0	NORMAL / EUTHANIZED

Appendix F

Individual Body Weights (in grams) of Rats Dosed with 5000 mg/kg Nitroguanidine

Males

Animal Number	At Receipt	Dosing*	Day 7	Termination* Day 13
84D01131	141	312	Dead	N/A
84D01132	147	294	Dead	N/A
84D01134	148	304	347	334
84D01135	136	302	323	329
84D01136	143	318	328	334
84D01140	144	291	319	315
84D01155	135	311	343	337
Mean	142.0	304.6	332.2	329.8
Stan. Dev.	5.0	9.8	11.6	8.8
Stan. Error	1.9	3.7	5.2	3.9

Females

Animal Number	At Receipt	Dosing*	Day 7	Termination* Day 13
84D01196	164	235	233	242
84D01200	167	212	Dead	N/A
84D01205	171	210	Dead	N/A
84D01206	165	213	233	227
84D01208	168	213	229	226
84D01212	163	206	226	208
Mean	166.3	214.8	230.3	225.8
Stan. Dev.	2.9	10.2	3.4	13.9
Stan. Error	1.2	4.2	1.7	7.0

* animals fasted overnight

Appendix G: PATHOLOGY REPORT

LAIR Pathology Report
GLP Study 84008
Acute Oral Toxicity Limit Study in Rats
of Nitroguanidine ($CH_4N_4O_2$)
(CAS No., 556-88-7), Dose 5000 mg/kg

History: Rats were tested in accordance with LAIR SOP-OP-STX-36. Some rats had red tinged urine after dosing; therefore, limited tissues were examined microscopically. Rat 84D01207 was removed from the study because of misdosing.

Gross Findings

Path #	Animal #	Sex	Dead	Findings
35920	84D01207	F	Ø	Removed from study/misdosed.
35921	84D01200	F	+	Perineal hair stained red; Jejunem multifocal petechiae; stomach red and pale areas glandular mucosa.
35922	84D01205	F	+	Posterior hair red stained, Duodenum contained red material, Stomach multiple 1 mm red foci glandular mucosa.
35923	84D01131	M	+	Abdomen hair red stained; Stomach pinpoint red foci glandular mucosa; Small intestine contained red contents.
35925	84D01132	M	+	Stomach pinpoint red foci glandular mucosa; Duodenum contents red; Kidney pinpoint white foci capsule; Bladder contained a calculus.
35928	84D01134	M	0	NR.
35929	84D01135	M	0	NR.
35930	84D01136	M	0	NR.
35931	84D01140	M	0	NR.
35932	84D01155	M	0	NR.
35933	84D01196	F	0	NR.
35934	84D01206	F	0	NR.
35935	84D01208	F	0	NR.
35936	84D01212	F	0	NR.

Appendix G (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84008

Histologic Findings

35928: Kidneys - NR.

35929: Kidneys - NR.

35980: Kidneys - NR.

35931: Kidneys - NR.

35932: Kidneys - NR.

35933: Kidneys - multifocal tubular mineral, minimal.

35934: Kidneys - multifocal tubular mineral, minimal.

35935: Kidneys - multifocal tubular mineral, minimal.

35936: Kidneys - multifocal tubular mineral, minimal.

Comment: Two males and two females died, the remaining animals lived to the end of the test. No compound related changes were present in the tissues examined. The renal mineral is considered to be a normal background finding in these rats. The females are slightly more susceptable to renal tubular mineralization, most likely because of sexual dimorphism.

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

Distribution List

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Fort Detrick, Frederick, MD 21701-5010

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

US Army Medical Research and Development Command (2)
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Fort Detrick, Frederick, MD 21701-5012

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USAEHA Regional Division, South
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