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Acute Oral Toxicity of Nitroguanidine in Mice

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Acute oral toxicity of nitroguanidine in mice (Toxicology Series 116)--Hiatt et al.

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20. ABSTRACT (continued)

crystalline material in the urine. In a previous study, chemical analyses of a whitish crystalline material isolated from the urinary bladder of a rat administered nitroguanidine orally indicated that the crystalline material was nitroguanidine.

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ABSTRACT

The acute oral toxicity of nitroguanidine was determined in male and female Institute of Cancer Research (ICR) mice using the oral gavage method. In males, tests at or above the LIMIT value of 5000 mg/kg produced less than 50% mortality. In females, the median lethal dose was 4345 mg/kg. Clinical signs produced by nitroguanidine were consistent with general malaise, effects on the gastrointestinal (GI) tract and urogenital system, and stimulation of the central nervous system (CNS). A frequent observation was the presence of a whitish crystalline material in the urine. In a previous study, chemical analyses of a whitish crystalline material isolated from the urinary bladder of a rat administered nitroguanidine orally indicated that the crystalline material was nitroguanidine. ←

Key Words: Acute Oral Toxicity, Nitroguanidine, Mouse, RA III, Munitions

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PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

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Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

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

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

GLP STUDY NUMBER: 84009

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

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DATA MANAGER: Yvonne C. Johnson, 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, and an aliquot of the
test compound will be retained
in the LAIR Archives.

TEST SUBSTANCE: Nitroguanidine

INCLUSIVE STUDY DATES: 26 September - 7 November 1984

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

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SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that the GLP Study 84009 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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30 March 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for Study 84009

1. I hereby certify that LAIR GLP Protocol 84009 was reviewed by Quality Assurance on 24 February 1984.
2. The report entitled "Acute Oral Toxicity of Nitroguanidine in Mice," Tox Series 116, and the raw data were reviewed on 24 November 1986.

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

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Acute Oral Toxicity of Nitroguanidine in Mice--
Hiatt 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 Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. *cont'd pg 2*

Objective of Study

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

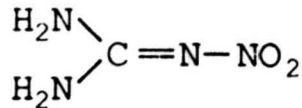
MATERIALS

Test Substance

Chemical name: Nitroguanidine

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

Molecular structure:



Molecular formula: CH₄N₄O₂

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

Vehicle

Nitroguanidine was prepared as a suspension in 0.2% methylcellulose and 0.4% Tween® 80 in sterile water. Methylcellulose (4000 grade), Lot 82F-0634, with assigned expiration date of April 1994, was obtained from Sigma Chemical Co (St Louis, MO). Tween® 80 (polyoxyethylene (20) sorbitan monooleate), Lot 713137, with assigned expiration date of December 1986, was obtained from Fisher Scientific Products (Fairlawn, NJ). Sterile water for injection (Lot 49-420-DM-03, expiration date 1 February 1985) was obtained from Abbott Laboratories (Chicago, IL).

Animal Data

Seventy-nine male and 80 female ICR mice were received from Harlan-Sprague Dawley Inc. (Indianapolis, IN) for GLP studies 84009 and 84014. These mice were identified individually with ear tags numbered 84C00347 to 84C00357 and 84C00369 to 84C00436 (inclusive) for the males and 84C00358 to 84C00368 and 84C00437 to 84C00505 (inclusive) for the females. Two males and two females were submitted for quality control necropsy evaluation at receipt. One male and two females were selected at random for this purpose; the other male selected for evaluation exhibited a scrotal bulge on receipt. On receipt, the animals' weights ranged from 19 to 33 g. Additional animal data appear in Appendix B.

Husbandry

Mice assigned to this study 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, Certified Purina Rodent Chow Diet 5002 (Ralston Purina Company, Checkerboard Square, St Louis, MO), was provided *ad libitum*; water was provided by continuous drip from a central line. Temperature in the animal room was maintained in the range of 18.3°C to 26.1°C, and relative humidity in the range of 34% to 68%. The photoperiod was 12 hours of light per day.

METHODS

This study was performed in accordance with LAIR Standard Operating Procedure OP-STX-36, "Acute Oral Toxicity Study" (2) and EPA guidelines (3).

Group Assignment/Acclimation

Mice assigned to the test phases of the study were randomized into dose groups comprised of 10 males or 10 females each. Allocation was accomplished using the Beckman TOXSYS® Animal Allocation Program, a computer-based, stratified, weight-biased method.

The test phase mice were acclimated for 20 days in the animal room before dosing. During this period, they were observed daily for signs of illness.

Dosage Levels

A pilot study, performed with a dose above the "LIMIT" value of 5000 mg/kg (3), was conducted. Results for the males were equivocal, and the LIMIT test was repeated during the test phase. The test phase for the males consisted of a treated group, which received a dose (5620 mg/kg) that was in excess of the LIMIT test dose, and a vehicle control group. For the females, preliminary results suggested an approximate lethal dose slightly lower than the LIMIT value of 5000 mg/kg. Dose levels for the test phase in the females were set accordingly (Table 1).

TABLE 1
NITROGUANIDINE DOSAGES
(Female Mice)

Group	Dosage Level mg/kg
1F	6310
2F	5010
3F	3980
4F	Vehicle
5F	2820

Preparation of Compound

At the concentrations necessary to achieve the dosage levels set for this study, nitroguanidine is insoluble in water or saline. Dosing was therefore performed with a highly concentrated suspension of nitroguanidine in a vehicle consisting of 0.2% methylcellulose and 0.4% Tween® 80 in sterile water. This suspension was prepared immediately before dosing.

Chemical Analysis of Dosing Solution

Nitroguanidine was stable in the vehicle for at least 14 days after preparation (Appendix A). This was sufficient since dosing was begun as soon as the suspension was prepared and completed within 2 hours. Tests for homogeneity of the test compound in the suspension indicated that the concentrations for the top, middle, and bottom of the suspensions varied less than 1.5% (Appendix A).

Test Procedures

The volume of the dosing suspension that each animal received was based upon its assigned dosage level, its body weight, and the nitroguanidine concentration of the suspension. To keep the volume administered at any one time below 10 ml/kg, all nitroguanidine groups received split dosings (2 separate gavages), administered within 90 minutes of each other. Half of the vehicle control animals also received split dosings while the other half received a single gavage. For any single gavage, the volume administered ranged from 0.28 to 0.40 ml in the males and from 0.14 to 0.32 ml in the females.

Dosing was performed using the oral gavage method without sedating or anesthetizing the animal. Sterile, disposable 1 ml syringes fitted with 20-gauge, 1-1/2-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. Males in the LIMIT test group and females in groups 1F, 2F, and 3F were dosed between 1145 and 1319 hours on 17 October 1984. Males in the vehicle control group and females in groups 4F and 5F were dosed between 0950 and 1111 hours on 24 October 1984.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical

examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 2 and 4 hours after dosing 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 weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for necropsy. Those that survived the 14-day study period were submitted for necropsy immediately after sacrifice by Necropsy Suite personnel.

Statistical Analysis

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

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

This study was performed in accordance with GLP protocol 84009 and amendments with the following exceptions:

- (1) Pilot studies indicated that the median lethal dose in males would exceed 5000 mg/kg; consequently, only a single LIMIT dose in excess of 5000 mg/kg was evaluated in the males.
- (2) Vehicle control groups were increased in size from 5 to 10 animals.
- (3) Animals were not weighed on the days of dosing; weights for calculating doses were obtained the previous day. Since multiple gavages were required for dosing, inclusion of the weighing step on the day of dosing would unduly prolong the dosing procedure. Subsequent weighings were scheduled to occur at weekly intervals.

- (4) One afternoon "walk-through" observation (28 Oct 84, day 18 of the study) was not performed. All animals received an especially thorough inspection the next morning, and all were found to be healthy.
- (5) Due to a failure to wind the hygrothermograph recorder adequately, the environmental data for 3-5 Nov 84 were not recorded. However, the environmental conditions as recorded by the hygrothermograph were constant before and after the unrecorded period. Also the daily log for the room as recorded by the animal caretakers reflected that the environmental conditions while the caretakers were in the room on the affected days did not differ from that recorded by the hygrothermograph during the study.

None of these changes/deviations is believed to have had an adverse effect on the study.

Raw Data and Storage of Final Report

A copy of the final report, study protocol and amendments, raw data, retired SOPs, analytical, stability and purity data of the test compound and an aliquot of the test compound will be retained in the Letterman Army Institute of Research (LAIR) Archives.

RESULTS

Mortality

Mortality in the male LIMIT group (5620 mg/kg) was 40%; 4 of the 10 animals died. Two of these deaths occurred within 24 hours of dosing, one within 48 hours, and one 11 days after dosing.

Of the 40 females administered doses ranging from 2820 to 6310 mg/kg, 20 died during the observation period. Eleven (55%) of the deaths occurred during the initial 24 hours after dosing and 8 more died between 24 and 50 hours. Thus, 19 of the 20 (95%) deaths occurred within 50 hours of dosing. The remaining death occurred within 75 hours after dosing.

Table 2 lists the compound-related deaths by group and by percent mortality. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2
Compound-Related Deaths by Group

Dose Level mg/kg		Compound-Related Deaths/ Number in Group	% Mortality
GROUP			
MALE			
2M	5620	4/10	40.0
4M	Vehicle	0/10	0.0
FEMALE			
1F	6310	9/10	90.0
2F	5010	6/10	60.0
3F	3980	3/10	30.0
5F	2820	2/10	20.0
4F	Vehicle	0/9*	0.0

*Reduced number in this group was due to one misdosed animal which was excluded from statistical analysis and removed from study.

Lethal Dose Calculations

Lethal dose values for the females were calculated by probit analysis, and the equation for the probit regression line was: $Y = -16.4 + 5.89 \log X$, where X is the dose and Y the corresponding probit value. Misdosed animals were excluded from statistical analysis and eliminated from the study. Figure 1 graphically presents the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

FIGURE 1

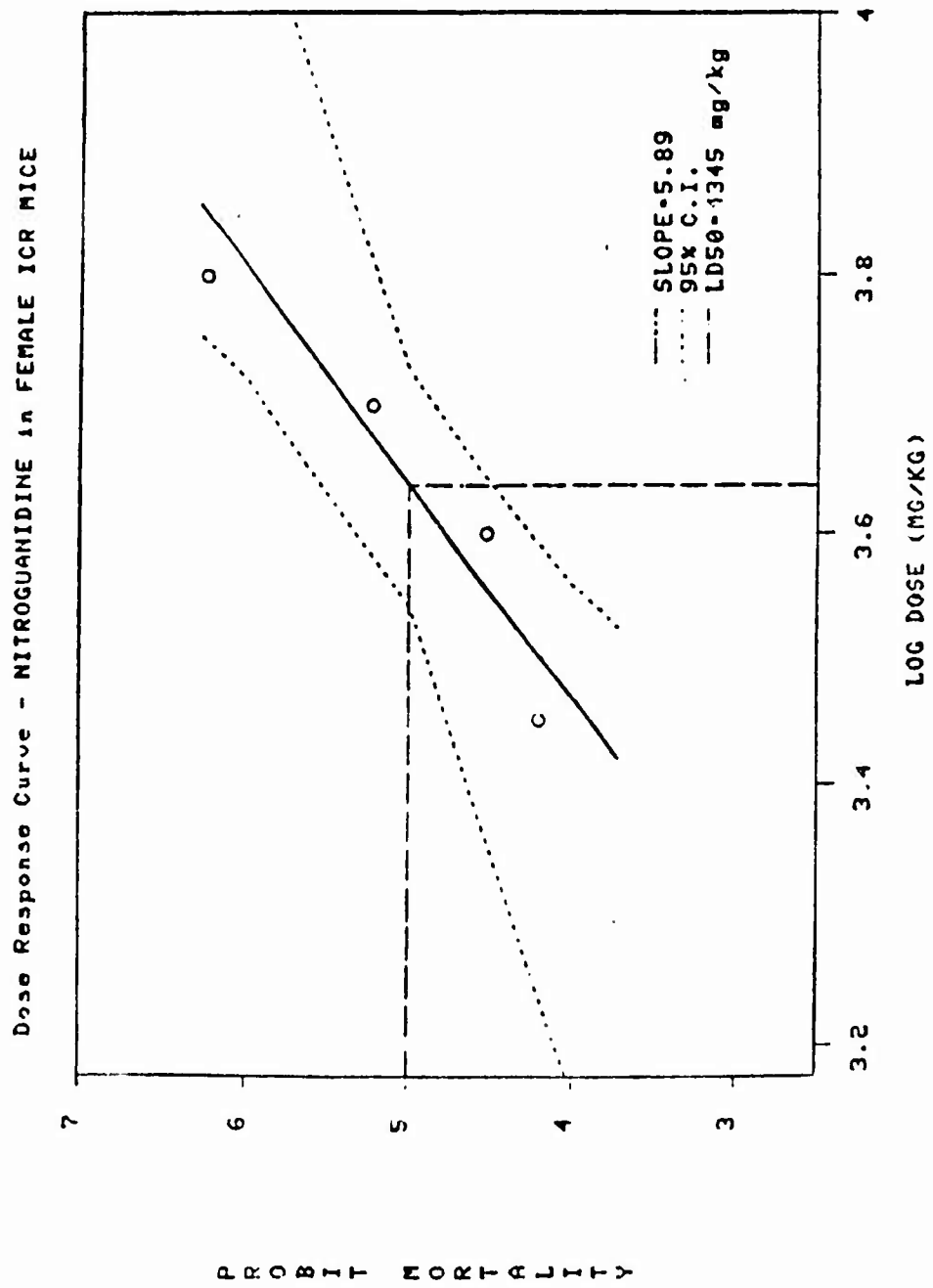


TABLE 3
Calculated Lethal Doses (LD) of Nitroguanidine

Effect Level	Dose (mg/kg)	95% Confidence Limits (mg/kg)
FEMALES		
LD10	2632	(1108; 3341)
LD50	4345	(3483; 5335)
LD90	7171	(5693; 16,378)

Because less than 50% mortality was produced by a dose (5620 mg/kg) in excess of the LIMIT test dose, dose-response data were not obtained for the males.

Clinical Signs

Nitroguanidine produced clinical signs primarily referable to the gastrointestinal (GI) tract and the central nervous system (CNS). However, since the males responded with less than 50% mortality to a dose above the LIMIT value, they also exhibited a reduced number of signs associated with nitroguanidine toxicity. Inactivity was observed in 7 of 10 (70%) males, and hunched posture was noted in 8 of 10 (80%). In addition, rough hair coat was observed in 3 of the 10 (30%) males.

The females exhibited a wider variety of clinical signs than the males. Hunched posture and inactivity were the two most prevalent signs, eventually being displayed by 58% (inactivity) to 68% (hunched posture) of the females dosed with nitroguanidine. Both signs were present in a dose-related pattern as 80-90% of the high dose animals were affected and the signs were more prevalent in animals eventually dying. Inactivity was noted in only 6 of 20 (30%) survivors but was present in 18 of the 20 (90%) females that died. Of 20 survivors, only 12 (60%) exhibited hunched posture, while 18 of the 20 (90%) animals that eventually died displayed this sign.

Seizures were observed in almost half (19 of 40) of the treated females, especially in those animals eventually

dying. The incidence of seizures ranged from 20% at the lowest dose to 90% at the highest dose and from 10% in survivors to 85% in eventual fatalities. Seizures were first observed within 3 hours of dosing and were not present more than 30 hours after dosing. These were not agonal seizures that could be attributed to CNS hypoxia. The overwhelming majority of these seizures were stimulated by handling, especially by the tail, and were of limited duration. Upon being picked up, the animal would arch its neck and back, extend all four legs, become rigid, and display a fine tremor. At this time, the eyes appeared glassy while the face and whiskers quivered. After a few seconds, the body would relax and the animal would begin to exhibit purposeful movements. In a few cases, animals were also observed in their cages lying on their sides performing paddling movements with all four legs. Following the seizure, the animal appeared normal as before. Seizures were not observed in any animals before dosing nor in the vehicle control animals at any time. Seizures were not observed nor stimulated by handling in any of the males dosed with 5620 mg/kg nitroguanidine.

In general, clinical signs developed rapidly and were of short duration. Most (47 of 50) of the animals treated with nitroguanidine exhibited at least one clinical sign at the first observation after dosing. Except for those animals succumbing, the overwhelming majority returned to normal between 24 and 48 hours after receiving nitroguanidine. Only occasional mild signs were observed thereafter, except in one male (84C00397) which developed bilateral ocular opacities that persisted until study termination.

A white, crystalline material was observed in the urine of many of the animals dosed with nitroguanidine. The white, crystalline material was not present in an obvious dose-response relationship, although it was noted twice as often in females surviving as opposed to those dying after treatment.

With one exception, all vehicle control animals (male and female) were judged normal at all observations after dosing. The one exception was a female (85C00494) that escaped from her cage 5 days after dosing (29 Oct 85). She was found within 15 minutes of being reported missing. At this time, and for 2 days following, her muzzle was slightly swollen and inflamed -- probably the result of an accident either during or following her escape from the cage.

Clinical signs are summarized by group in Table 4 for male mice. Clinical signs of the female mice are summarized

TABLE 4

Incidence Summary for Clinical Observations in Male Mice
Dosed With Nitroguanidine

Clinical Signs	Group	Limit	Vehicle
	Dose (mg/kg)	5620	Control
	(n=)	10	10
Hunched Posture		8	0
Inactivity		7	0
Rough Coat		3	0
White Crystalline Material in Urine		4	0
Yellow Perianal Staining		3	0

by group in Table 5. Weight gains of survivors were not significantly affected by dosing. Table 6 presents mean body weights by group. Appendix E details individual body weights.

Necropsy Observations

All study animals, whether dying acutely or surviving the entire 14-day observation period, were examined grossly at necropsy. Few gross lesions were observed and none of these appeared related to administration of nitroguanidine.

Two of the four males succumbing to nitroguanidine exhibited urinary bladder distention; penile paraphimosis was observed in one of these two. The other two males dying acutely and five of the six survivors were not remarkable at necropsy. One of the surviving males, which had developed ocular opacities, exhibited cataract formation in the one lens examined microscopically.

At necropsy, none of the 20 females dying acutely after administration of nitroguanidine (at doses 2820 to 6310 mg/kg)

TABLE 5

Incidence Summary for Clinical Observations in Female Mice
Dosed With Nitroguanidine

Clinical Signs	Group	5F	3F	2F	1F	4F
	Dose (mg/kg)	2820	3980	5010	6310	Vehicle
	(n=)	10	10	10	10	9
Hunched Posture		6	7	5	9	0
Inactivity		4	5	6	8	0
Seizures		2	4	4	9	0
White Crystalline Material in Urine		9	3	3	7	0
Irritability		1	3	0	1	0
Hyperactivity		0	3	0	0	0

exhibited any gross lesions attributable to nitroguanidine. One female survivor (3980 mg/kg) presented at study termination with focal lesions in the right salivary gland and the liver. The salivary gland was not remarkable upon microscopic examination. Foci of coagulative necrosis in the liver were observed during microscopic examination. These areas did not respond to special bacterial stains and may have been due to mouse hepatitis virus. Microscopic examination of the eyes from one control female (vehicle only) revealed loss or reduction of retinal rod and cone layers. All of the other females surviving until study termination were not remarkable at necropsy.

The complete pathology report appears in Appendix F.

TABLE 6
Mean Body Weights In Grams + S.D. (N)

Dose (mg/kg)	Receipt	Dosing	Mid-Observation Period	Terminal Sacrifice
MALES				
Limit	28 ± 2 (10)	34 ± 2 (10)	34 ± 3 (7)	36 ± 3 (6)
Vehicle	27 ± 2 (10)	35 ± 2 (10)	35 ± 2 (10)	35 ± 2 (10)
FEMALES				
2820	21 ± 2 (10)	28 ± 3 (10)	28 ± 3 (8)	29 ± 3 (8)
3980	22 ± 2 (10)	25 ± 2 (10)	28 ± 2 (7)	28 ± 2 (7)
5010	21 ± 1 (10)	26 ± 2 (10)	26 ± 2 (4)	27 ± 2 (4)
6310	22 ± 2 (10)	27 ± 3 (10)	25 ± 1 (1)	26 ± 1 (1)
Vehicle	21 ± 2 (10)	28 ± 3 (10)	28 ± 2 (9)	28 ± 3 (9)

DISCUSSION

Mortality

Nitroguanidine exhibited very low toxicity in the present study. A dose in excess of the LIMIT dose produced less than 50% mortality in male mice and the MLD in females was relatively close to this LIMIT dose. Clinical signs of toxicity produced by nitroguanidine were associated with effects on either the GI tract or the CNS. Very little gross pathology was observed in the animals dosed with nitroguanidine, none of which explained the observed signs.

In the males, oral administration of nitroguanidine produced only 40% mortality at 5620 mg/kg. Because this dose, which was in excess of the 5000 mg/kg "LIMIT" value, produced less than 50% mortality no further testing was deemed necessary (3).

Nitroguanidine was sufficiently toxic in females that a dose-response relationship could be established. Thus, the MLD (95% confidence limits) of nitroguanidine in female mice was calculated to be 4345 mg/kg (3483, 5335). Although nitroguanidine was more potent in female mice, the mechanism of its toxic action appeared to be similar to its action in

male mice as most of the deaths in both sexes occurred during the 48 hours following administration of nitroguanidine.

These results are consistent with two previous reports on the acute oral toxicity of nitroguanidine in rodents. A study of the toxicity of a series of thioureas in domestic Norway rats reported that the LD₅₀ of nitroguanidine was in excess of 500 mg/kg (5). A US Army report on nitroguanidine (6) cites a study performed at Hazelton Laboratories in which a single oral dose of 4640 mg/kg nitroguanidine produced no irreversible toxic effects in male albino rats.

On the basis of the toxicity classification scheme of Hodge and Sterner (7, Appendix G), these results place nitroguanidine in the "practically nontoxic" to "slightly toxic" range. For comparative purposes, a compound of similar acute oral toxicity is sodium chloride, with a MLD (in rats) of approximately 4000 mg/kg (8, Appendix G).

Clinical Signs

Most of the clinical signs observed in response to nitroguanidine were referable to the gastrointestinal tract, the urogenital system or the central nervous system. Maintenance of hunched posture was the single most common clinical sign in both males and females; the second most common was inactivity. These two signs often occur together since animals feeling poorly often sit hunched over in one corner of the cage. Both of these signs are characteristic of general malaise and may be related to GI upset/nausea. Rough hair coat (a related sign) was also present in 3 of the 10 males dosed with nitroguanidine. At necropsy none of the animals succumbing to nitroguanidine, nor the survivors, exhibited any pathology of the GI tract.

Three of the 10 dosed males also exhibited a yellow stain in the perianal region, which appeared to be dried urine. Two of these three were the males succumbing to nitroguanidine within 24 hours of dosing. Perianal staining was noted in these two at both recorded inspections on the day of dosing. In the third male, perianal staining appeared 72 hours after dosing and was noted for three consecutive days; this male survived until study termination.

Two males, the one that died within 48 hours and the one that died after 11 days, were observed at necropsy to have distention of the urinary bladder. Penile paraphimosis was also present in one of these males. Following dosing, a white crystalline material was observed in the urine of many of the animals, male and female, receiving nitroguanidine.

This material is presumed to be nitroguanidine because in a related study, rats dosed with nitroguanidine excreted a similar white crystalline material which was identified by high pressure liquid chromatography as nitroguanidine (9, Appendix H). It is conceivable that larger aggregates of these nitroguanidine crystals occluded the urethra and may, therefore, have contributed to the development of the gross pathology in these two males.

A white urinary precipitate was also observed in 15 of the 20 female survivors but only 7 of the 20 females that died. Such a differential may indicate that many of the deaths occurred before the animals starting excreting nitroguanidine. Alternatively, there may have been a selective survival advantage in the ability to excrete large amounts of the nitroguanidine rapidly. There was no evidence at necropsy of impaired renal function in the animals dying acutely.

The most common CNS sign observed after administration of nitroguanidine was seizure activity, especially in response to handling. Other CNS signs included hyperactivity and an exaggerated startle response. All of these signs are consistent with increased excitability or stimulation of the CNS.

Nitroguanidine induced seizures in a dose-related manner in female rats. These seizures were of two types. Those seizures induced by handling were of a tonic, extensor type, while those that occurred spontaneously were of a clonic nature. Females were more susceptible to seizure induction than males. No seizures were observed in males treated with 5620 mg/kg of nitroguanidine, a dose which produced 40% mortality, while females administered doses of nitroguanidine (3980 and 5010 mg/kg) which bracketed (30 and 60% mortality, respectively) the 40% mortality observed in males exhibited a 40% incidence of seizures.

The dose-response nature of the handling-induced seizures implicates nitroguanidine as the causative agent. Despite much handling and close observation, no seizures were observed in either the vehicle control mice or the treated mice before dosing. The highest incidence of seizures occurred in the high-dose female group, the lowest incidence occurred in the low-dose female group.

Many of the clinical signs produced by nitroguanidine in the present study are consistent with CNS stimulation. The dose-related increase in handling-induced, spontaneous seizure activity in females is the most dramatic evidence. Hyperactivity and exaggerated startle reflex behavior, both

occurring in the females albeit at much lower incidences, can also result from CNS stimulation. An exact mechanism for nitroguanidine in the development of these CNS signs cannot be inferred from this study. At necropsy, no pathology was observed that could explain the CNS-related symptoms. However, the possibility that nitroguanidine may produce CNS stimulation should be considered.

One of the males dosed with nitroguanidine developed large bilateral ocular opacities within 48 hours of dosing. Other clinical signs were minimal in this animal. These lesions were present at study termination as intraocular opacities. Upon microscopic examination of one involved lens, the condition was diagnosed as cataract formation. However, since this was an isolated occurrence, it is unlikely that the cataract was formed in response to administration of nitroguanidine.

CONCLUSIONS

The MLD values for nitroguanidine in ICR mice were 4345 mg/kg for the females and in excess of 5620 mg/kg for the males. This places nitroguanidine in the "practically nontoxic" to "slightly toxic" classification. Target organ systems for nitroguanidine were the gastro-intestinal tract, the urogenital system, and the central nervous system.

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Appendix A: CHEMICAL DATA

Chemical Name: Nitroguanidine (NGu)

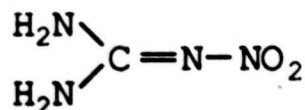
Other Listed Names: Guanidine, Nitro; alpha-Nitroguanidine;
beta-Nitroguanidine

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

Lot Number: SOW83H001-004

LAIR Code: TP36

Chemical Structure:



Molecular Formula: CH₄N₄O₂

Molecular Weight: 104.1

Physical State: White powder

Melting Point: 232° C¹

pH of a Saturated Aqueous Solution: 5.7

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

Appendix A (cont.): CHEMICAL DATA

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:	269.0		
% Target concentration:	94.1		

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

Appendix A (cont.): CHEMICAL DATA

mg/ml) was determined by the analysis of three aliquots removed from the suspension.⁷ The results were as follows:

<u>Sample #</u>	<u>Concentration of Nitroguanidine (mg/ml)</u>
1	277.1
2	276.6
3	277.8
<hr/>	
Mean value:	277.2
% target concentration:	97.0

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

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

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

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

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

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

⁷Ibid, p 34.

Appendix A (cont.): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC (FORM 213-R, 10 AUG 77)		EXEMPT. PAGE 7-2a AR 335-15	PAGE 1 OF 1
TO: Commander US Army Ammunition Munitions and Chemical Command Actg: DASMPC-AD Rock Island, ILL. 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>Min.</u> <u>Max.</u>	<u>Analysis</u>	
Purity, %	99.0	99.6	
Ash Content, %		0.30	
pH Value	4.5	7.0	
Acidity (as H ₂ SO ₄), %		0.06	
Total Volatiles, %		0.25	
Sulfates (as NaSO ₄), %		0.20	
Impurities, H ₂ O Insoluble, %		0.20	
Particle Size, Microns		3.0 *	
Particle Size, Std. Dev.		± 0.5	
		4.0 ****	
		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 285. 25 pounds per drum per RAD 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.	
TESTING CONDUCTED BY Hercules Aerospace Division		<i>13 Sept 83</i> <i>A. K. Enrich</i>	
THE ABOVE DESCRIBED MATERIAL HAS BEEN ACCEPTED		FOR THE COMMANDER	
<i>14 Sept 83</i> <i>Quality Assurance Specialist</i>		<i>[Signature]</i>	

Appendix B: ANIMAL DATA

Species: *Mus musculus*

Strain: ICR

Source: Harlan Sprague Dawley, Inc.
Indianapolis, IN

Sex: Male and female

Date of birth: 27 July 1984

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

Animals/group: 10 males and 10 females/group

Condition of animals at start of study: Normal

Body weight range at dosing: 21-40 g

Identification procedures: Ear tagging procedure (SOP-OP-
ARG), tag numbers between 84C00347
to 84C00368 and 84C00369 to
84C00505 inclusive.

Pretest conditioning: Quarantine/acclimation 26 September -
17 October 1984

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

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
26 Sep 84	Animals arrived at LAIR. They were sexed, observed for illness, ear tagged, weighed, and caged.
26 Sep-9 Oct 84	Animals were observed daily.
30 Sep 84	Ventilation fan in animal room was shut down for maintenance (1130 to 1445 hrs).
3 Oct 84	Animals were weighed.
10 Oct 84	Food was removed at approximately 0600 hours. Animals were weighed and dosed at 1000 hours. Observations were conducted 2 and 4 hours after dosing. Food was reintroduced 2 to 4 hours after dosing. Eighteen successful dosings, 3 misdosings (pilot study).
11 Oct-16 Oct 84	Animals were observed for clinical signs in AM and PM (pilot study).
11 Oct 84	Eight compound-related deaths in pilot animals.
12 Oct 84	Four compound-related deaths in pilot animals.
16 Oct 84	Animals were weighed. Survivors from pilot study were sacrificed.
17 Oct 84	Food was removed at approximately 0600 hours. Animals were dosed at 1000 hours. Observations were conducted 2 and 4 hours after dosing. Food was reintroduced after initial observations. Groups 1M, 1F, 2M, 2F, 3M, and 3F dosed with 59 successful dosings and 1 misdose. No compound-related deaths (Phase I).
18-30 Oct 84	Animals were observed daily for clinical signs in AM and PM (Phase I).
18 Oct 84	Nineteen compound-related deaths.

Appendix C(cont.): HISTORICAL LISTING OF STUDY EVENTS

19 Oct 84 Eight compound-related deaths.

20 Oct 84 One compound-related death.

23 Oct 84 Animals were weighed.

24 Oct 84 Food was removed at approximately 0600 hours. Animals were dosed at 1000 hours. Observations were conducted 2 and 4 hours after dosing. Food was reintroduced after initial observations. Groups 4M, 4F and 5F dosed with 29 successful dosings and 1 misdose (Phase II).

25 Oct 84 Two compound-related deaths.

25 Oct-6 Nov 84 Animals were observed daily for clinical signs in AM and PM (Phase II).

28 Oct 84 One compound-related death.

30 Oct, 6 Nov 84 Animals were weighed.

31 Oct 84 Food was removed at approximately 0600 hours. Animals were observed for clinical signs at 0730 hours. Animals were delivered to the Necropsy Suite for sacrifice and gross necropsy (27 animals, Phase I).

7 Nov 84 Food was removed at approximately 0600 hours. Animals were observed for clinical signs at 0730 hours. Animals were delivered to the Necropsy Suite for sacrifice and gross necropsy (11 animals, Phase II). Unassigned animals were transferred to other protocols.

Appendix D

Cumulative Mortality Data (Deaths/Group)
(10 Animals/Group)

Dose (mg/kg)	Time After Dosing														
	Hours				Days										
	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MALES															
Limit	0	2	3	3	3	3	3	3	3	3	3	4	4	4	4
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FEMALES															
2820	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3980	0	0	2	2	3	3	3	3	3	3	3	3	3	3	3
5010	0	3	6	6	6	6	6	6	6	6	6	6	6	6	6
6310	0	6	8	9	9	9	9	9	9	9	9	9	9	9	9
Control*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	13	21	22	23							24			24

*9 animals per group

Appendix E

Individual Body Weights in Grams

5620 mg/kg
MALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00369	27	32	32	Dead
84C00379	26	32	31	34
84C00382	28	36	36	36
84C00384	29	36	38	39
84C00389	26	33	Dead	
84C00392	33	36	35	38
84C00394	29	36	Dead	
84C00397	27	35	35	35
84C00405	27	31	32	32
84C00423	26	30	Dead	

Mean	28	34	34	36
Standard Deviation	2	2	3	3

Appendix E (cont.)

Individual Body Weights in Grams

Vehicle

MALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00370	30	37	38	37
84C00372	25	34	35	34
84C00373	26	33	33	34
84C00376	24	32	32	34
84C00381	28	36	34	36
84C00383	27	35	34	34
84C00408	24	33	33	34
84C00414	29	40	40	40
84C00422	28	35	35	35
84C00428	27	35	34	34
Mean	27	35	34	36
Standard Deviation	2	2	3	3

Appendix E (cont.)

Individual Body Weights in Grams

2820 mg/kg

FEMALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00444	20	27	28	29
84C00447	21	27	27	28
84C00449	21	29	Dead	
84C00452	21	28	28	29
84C00466	19	26	26	26
84C00473	24	30	Dead	
84C00478	24	33	33	34
84C00485	19	28	27	28
84C00492	21	30	30	30
84C00503	19	26	24	26

Mean	21	28	28	29
Standard Deviation	2	2	3	3

Appendix E (cont.)

Individual Body Weights in Grams

3980 mg/kg

1 MALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00438	21	28	30	31
84C00439	22	26	26	27
84C00441	19	21	Dead	
84C00442	25	25	Dead	
84C00443	23	26	29	29
84C00448	20	24	Dead	
84C00469	24	29	29	30
84C00481	20	26	28	27
84C00486	24	24	26	26
84C00490	20	23	25	25
Mean	22	25	28	28
Standard Deviation	2	2	2	2

Appendix E (cont.)

Individual Body Weights in Grams

5010 mg/kg

FEMALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00440	22	24	Dead	
84C00445	20	25	Dead	
84C00446	22	28	Dead	
84C00450	20	23	Dead	
84C00462	19	23	24	25
84C00463	21	26	26	29
84C00467	24	29	Dead	
84C00480	21	27	26	26
84C00491	21	25	28	28
84C00499	20	25	Dead	

Mean	21	26	26	27
Standard Deviation	1	2	2	2

Appendix E (cont.)

Individual Body Weights in Grams

6310 mg/kg

FEMALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00454	20	25	25	26
84C00456	25	30	Dead	
84C00461	21	29	Dead	
84C00472	20	24	Dead	
84C00482	24	28	Dead	
84C00488	21	26	Dead	
84C00495	20	24	Dead	
84C00497	22	27	Dead	
84C00504	21	23	Dead	
84C00505	22	30	Dead	
Mean	22	27	25	26
Standard Deviation	2	3		

Appendix E (cont.)

Individual Body Weights in Grams

Vehicle

FEMALES

Animal	Receipt	Dosing	Mid-Observation Period	Study Termination
84C00437	26	32	32	34
84C00451	21	26	27	28
84C00470	20	27	27	27
84C00474	22	31	31	32
84C00475	19	24	24	25
84C00476	22	29	Dead	
84C00477	19	29	29	28
84C00487	21	25	26	27
84C00494	19	28	27	26
84C00496	20	27	29	26
Mean	21	28	28	28
Standard Deviation	2	3	2	3

Appendix F: PATHOLOGY REPORT

Pathology Report
 GLP Study #4000
 Oral Lethal Dose (LD50)

ID#: GLP Study #4000

Substance: Nitroguanidine.

Species: Mouse Strain: ICR.

History: See IAR 502-02-87X-16. Animals that did not die were killed with sodium pentobarbital anesthesia and axillary examination.

Gross Findings:

Dose Group 1 - female (6310 mg/kg)

<u>Path. No.</u>	<u>Animal No.</u>	<u>Gross Findings</u>
36022	04 00 0451	Live - Not Remarkable (NR)
36022	04 00 0456	Dead - NR (wet tissue saved)
36000	01 00 0461	Dead - NR (wet tissue saved)
36011	04 00 0472	Dead - NR (wet tissue saved)
36011	01 00 0480	Dead - NR (wet tissue saved)
36004	01 00 0488	Dead - NR (wet tissue saved)
36024	04 00 0495	Dead - NR (wet tissue saved)
36005	04 00 0497	Dead - NR (wet tissue saved)
36012	04 00 0504	Dead - NR (wet tissue saved)
36026	04 00 0505	Dead - NR (wet tissue saved)

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Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84909

Dose group 2 - female (5010 mg/kg)

<u>Path. Acc. No.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36020	94 C0 0440	Dead - NR
36006	94 C0 0445	Dead - NR
36007	84 C0 0446	Dead - NR
36008	84 C0 0450	Dead - NR
36113	94 C0 0462	Live - NR
36134	84 C0 0463	Live - NR
36023	94 C0 0467	Dead - NR
36136	94 C0 0480	Live - NR
36140	84 C0 0491	Live - NR
36016	84 C0 0499	Dead - NR

Dose group 3 - female (3980mg/kg)

<u>Path. Acc. No.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36129	94 C0 0438	Live - NR (eyes processed for control)
36130	84 C0 0439	Live - Right salivary gland had 3, 2mm, red foci. Liver - There were approximately 25, 1 mm or smaller diameter, discrete white foci.
36027	84 C0 0441	Dead - NR

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Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84009

36015	04 CO 0442	Dead - NR
36131	04 CO 0443	Live - NR
36021	04 CO 0448	Dead - NR
36135	04 CO 0469	Live - NR
36137	04 CO 0481	Live - NR
36138	04 CO 0486	Live - NR
36139	04 CO 0490	Live - NR

Dose group 4-female (Vehicle Control)

<u>Path. Acc. No.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36234	04 CO 0437	Live - NR (wet tissue saved)
36237	04 CO 0451	Live - NR
36240	04 CO 0470	Live - NR
36241	04 CO 0474	Live - NR
36242	04 CO 0475	Live - NR
36243	04 CO 0477	Live - NR
36246	04 CO 0487	Live - NR
36248	04 CO 0494	Live - NR
36249	04 CO 0496	Live - NR

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Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 94009

Dose group 3 - female (2825 mg/d)

<u>Path. Acc. No.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36235	94 C0 0444	Live - NR
36236	94 C0 0447	Live - NR
36077	94 C0 0449	Dead - NR
36238	94 C0 0452	Live - NR
36239	94 C0 0466	Live - NR
36078	94 C0 0473	Dead - NR
36244	94 C0 0478	Live - NR
36245	94 C0 0485	Live - NR
36247	94 C0 0492	Live - NR
36250	94 C0 0503	Live - NR

Dose group 4 - male (Vehicle control)

<u>Path. Acc. No.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36224	94 C0 0370	Live - NR (wet tissue saved)
36225	94 C0 0372	Live - NR
36226	94 C0 0373	Live - NR
36227	94 C0 0376	Live - NR
36228	94 C0 0391	Live - NR

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Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 34000

36229	84 C0 0393	Live - NR
36230	84 C0 0400	Live - NR
36231	84 C0 0414	Live - NR
36232	84 C0 0422	Live - NR
36233	84 C0 0428	Live - NR

Dose group 2- male (5620 mg/kg)

<u>Path. Acc. No.</u>	<u>Animal ID No.</u>	<u>Gross Findings</u>
36106	84 C0 0369	Dead - Urinary bladder distended Postmortem autolysis; moderate, severe.
36112	84 C0 0379	Live - NR
36113	84 C0 0382	Live - NR
36114	84 C0 0384	Live - NR
36019	84 C0 0389	Dead - Penis - paraphimosis; Urinary- bladder distended
36115	84 C0 0392	Live - NR
36000	84 C0 0394	Dead - NR
36117 intraocular	84 C0 0397	Live - Eyes, bilateral white areas,
36120	84 C0 0405	Live - NR
36003	84 C0 0423	Dead - NR

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Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 94009

Microscopic findings:

Animal ID#: 84C00439 Pathology Accession #: 36130.

Slide 4: Salivary gland - Not remarkable. The pale foci observed grossly may not have microscopic morphologic correlates.

Slide 9: Liver - The liver contain several, small (approximately 0.25 mm diameter) foci of coagulation necrosis.

Dx: Hepatitis, necrotizing, acute, multifocal, mild, liver.

Comment: The cause of the hepatitis is unknown. Special stains were negative for bacteria. The hepatitis may have been caused by mouse hepatitis virus.

Animal ID#: 84C00397 Pathology Accession #: 36117.

Slide 19A: Eye - There is Morgagnian globule formation in the lens. The lens epithelium is vacuolated.

Dx: Cataract, eye.

Slide 19B: Eye - NR. Lens not present.

Animal ID#: 84C00438 Pathology Accession #: 36120.

Slide 19A: Eye - The lens is only partially present, and the globe is artifactually distorted. One of the granular layers and the rod and cone layers are reduced or missing.

Slide 19B: Eye - One granular layer is absent, and the rod and cone layers are reduced or missing.

Dx: Retinal Atrophy or Hypoplasia or Abiotrophy, eye.

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Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84009

Table I - Males

Group No. Dose	2 (5000/mg/kg)	4 (Vehicle Control)
Animals/group	10	10
Deaths	4	0
% Deaths	40	0
Animals with gross lesions	3	0
Gross lesions Urinary bladder Distention	2	0
Penis Paraphimosis	1	0
Eyes Cataracts	1	0

Table II - Females

Group No. Dose	1 (6310/mg/kg)	2 (5000/mg/kg)	3 (3000/mg/kg)	4 (Vehicle Control)	5 (2800/mg/kg)
Animals/group	10	10	10	0	10
Deaths	0	6	1	0	2
% Deaths	0%	60%	11%	0%	20%
Animals with gross lesions	0	0	1	0	1
Gross lesions Salivary gland	0	0	0	0	0
Liver Necrosis, multifocal	0	0	1	0	0

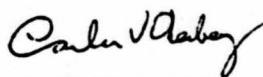
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Appendix F (cont.): PATHOLOGY REPORT

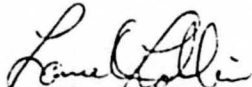
Pathology Report
GLP Study 84009

Summary: The gross lesions were not related to the test compound (urinary bladder distension, paraphimosis, cataracts, liver necrosis). A dose effect was clearly present in the females percent deaths. A range of doses was not done in the males.

There were no gross lesions to explain the central nervous system and gastrointestinal signs observed.



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Pathology Services Group



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Chief, Pathology Services Group

Appendix G

Toxicity Classification Based on Lethal Dose (7)

Commonly Used Term	LD50 Single Oral Dose for Rats (gm/kg)	LD50 Skin for Rabbits (gm/kg)	Probable Lethal Dose for Man
Extremely toxic	0.001 or less	0.005 or less	Taste (1 grain)
Highly toxic	0.001 to 0.05	0.005 to 0.043	1 tsp (4cc)
Moderately toxic	0.05 to 0.05	0.044 to 0.340	1 oz (30 gm)
Slightly toxic	0.5 to 5.0	0.35 to 2.81	1 pint (250gm)
Practically nontoxic	5.0 to 15.0	2.82 to 22.6	1 quart
Relatively harmless	>15.0	>22.6	>1 quart

Approximate Acute LD50s of a Selected Variety of Chemical Agents (8)

Agent	LD50 (mg/kg)
Ethyl alcohol	10,000
Sodium chloride	4,000
Ferrous sulfate	1,500
Morphine sulfate	900
Phenobarbital sodium	150
DDT	100
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
d-Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.1
Dioxin (TCDD)	0.001
Botulinus toxin	0.00001

Appendix H**URINARY NITROGUANIDINE IDENTIFICATION**

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 μ m column (100 x 2.1 mm). Dr. Bert Ho of the LAIR Division of Toxicology performed the analysis.*

The retention time of the bladder crystals (1.13 min; flow rate 0.3 ml/min; solvent system 5% MeOH in H₂O) was identical to that of nitroguanidine standards. The UV spectrum (obtained via a diode-array detector) of the bladder crystals was virtually identical to that of nitroguanidine standards (λ max = 264 nm).

*Ho, B. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-03-009, p 2. Letterman Army Institute of Research, Presidio of San Francisco, CA.

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