Institute Report No. 419

Acute Dermal Toxicity of Guanidine Hydrochloride in Rabbits

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

December 1989 Toxicology Series: 98

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

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

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William C Corby 29 December 89

for Donald G. Corby (date)
COL, MC
Commanding

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The acute dermal toxicity of guanidine hydrochloride was evaluated in five male and five female New Zealand White rabbits. Guanidine hydrochloride (2 g/kg) was applied topically to the clipped dorsal skin surface for 24 hours. No compound-related deaths or clinical signs were observed; however, guanidine hydrochloride did produce dermal irritation, necrosis, and eschar formation under conditions of the study.

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ABSTRACT

The acute dermal toxicity of guanidine hydrochloride was evaluated in five male and five female New Zealand White rabbits. Guanidine hydrochloride (2 g/kg) was applied topically to the clipped dorsal skin surface for 24 hours. No compound-related deaths or clinical signs were observed; however, guanidine hydrochloride did produce dermal irritation, necrosis, and eschar formation under conditions of the study.

KEY WORDS: Acute Dermal Toxicity, Guanidine hydrochloride, Rabbit, Mammalian Toxicology, Nitroguanidine

PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY:

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

SPONSOR:

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

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 84006

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

PRINCIPAL INVESTIGATOR: Gerald F. S. Hiatt, PhD

CO-PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SP5

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

REPORT AND DATA MANAGEMENT:

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

TEST SUBSTANCE: Guanidine hydrochloride

INCLUSIVE STUDY DATES: 21 June 1984 - 1 August 1984

OBJECTIVE:

The objective of this study was to evaluate the acute dermal toxicity of guanidine hydrochloride in male and female New Zealand White rabbits.

ACKNOWLEDGMENTS

SP5 Thomas P. Kellner, BA, SP4 Paul D. Mauk, BS, and Joy Bauserman, MED, assisted in conducting this research; Richard A. Spieler, Richard Katona, Roosevelt Cunningham, and Charlotte Speckman provided care for the animals; and Callie B. Crosby, MA, Lynda Araiza, Brenda Goce, and Colleen S. Kamiyama provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84006 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. 23 AUG 85
DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

19 Aug 85
LANCE O. LOLLINI, DVM / DATE
LTC, VC
Pathologist

Gerald F.S. Hiatt 19 Aug 85
GERALD F.S. HIATT, PhD / DATE
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Steven Kano 19 AUG 85
STEVEN K. SANO, BA / DATE
SP4 USA
Co-Principal Investigator

DEPARTMENT OF THE ARMY

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

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ATTENTION OF:

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27 December 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84006

1. This is to certify that in relation to LAIR GLP Study 84006 the following inspections were made:

15 February 1984	-	Protocol Review
15 July 1984	-	Necropsy

2. The institute report entitled "Acute Dermal Toxicity of Guanidine Hydrochloride in Rabbits," Toxicology Series 98, was audited on 23 March 1987.

Carolyn M. Lewis

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

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INTRODUCTION

Guanidine nitrate is an intermediate product in the synthesis of nitroguanidine. Nitroguanidine, which is 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. Guanidine hydrochloride was also evaluated to define more completely the contribution of the guanidine base to the toxicity of guanidine nitrate.

Objective of Study

The objective of this study was to determine the acute dermal toxicity of guanidine hydrochloride in male and female New Zealand White rabbits.

MATERIALS

Test Substance

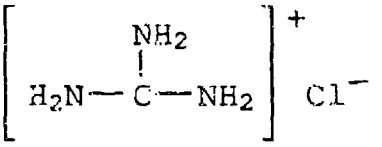
Chemical Name: Guanidine hydrochloride

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

LAIR Code Number: TP28

Physical State: White powder

Chemical Structure:



Molecular Formula: $CH_5N_3 \cdot HCl$

Other test substance information is presented in Appendix A.

Vehicle

To enhance its penetration into the skin, guanidine hydrochloride was moistened with 0.9% saline at the time of application. Saline was obtained from Travenol Laboratories, Inc. (Deerfield, IL). The lot number was 3C865A4 and the expiration date was December 1984.

Animal Data

Five male and five female young New Zealand White rabbits (Elkhorn Rabbitry, Watsonville, CA) from a shipment that arrived at LAIR on 21 June 1984 were assigned to the study. The 10 rabbits were identified individually by ear tattoos. The animal weights ranged from 2.5 to 3.4 kg on receipt and from 2.8 to 3.7 kg at dosing. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. Water was provided *ad libitum* by continuous drip from a central line. The diet consisted of approximately 150 g per day of Purina Certified Rabbit Chow® No. 5322 (Ralston Purina Company, St. Louis, MO). The animal room temperature was maintained at 17° to 24°C and the relative humidity was maintained at 46% to 74%, except for spikes to 94% during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was performed in accordance with LAIR Standard Operating Procedure OP-STX-30, "Acute Dermal Toxicity Study" (2) and Environmental Protection Agency guidelines (3).

Acclimation/Group Assignment

Study rabbits were quarantined for two weeks before being certified healthy by a staff veterinarian. During quarantine the rabbits were treated with Canex®/mineral oil (Pitman-Moore, Inc., Washington Crossing, NJ) for ear mite protection.

Randomization for group assignment was unnecessary as there was only one dose level for each sex.

Dose Levels

A "limit test" was conducted in which 5 male and 5 female rabbits were assigned to a test group receiving 2.0 g/kg of guanidine hydrochloride applied topically to the dorsum (skin over back). According to body weight, 5.7 to 7.4 g of guanidine hydrochloride was applied to each rabbit.

Compound Preparation

The calculated amount of guanidine hydrochloride was mixed with 0.9% saline to form a paste. This paste was prepared immediately before applying it to the animal.

Chemical Analysis of Guanidine Hydrochloride

Previous testing had indicated that guanidine hydrochloride was stable in an aqueous vehicle for a period exceeding the time needed to prepare and apply the paste to the rabbits' backs (Appendix A).

Test Procedures

The application sites on the dorsal and lateral sections of the animals (surface area approximately 240 cm^2) were close-clipped with electric clippers (Oster® Model A5, Size 40 blade, Sunbeam Corp, Milwaukee, WI) 24 hours before applying the test compound. The animals were weighed, and the quantity of compound required to provide the 2.0 g/kg limit dose was measured. This quantity of the test compound was evenly distributed over the surface of a 8 x 8 in. piece of gauze dressing (Curity Cover Sponges, Kendall Co. Hospital Products, Boston, MA) which was then taped to the animal's back with hypoallergenic tape (Durapore® Surgical Tape, 3M Corp, St. Paul, MN). The trunk of the animal was then wrapped with Vetrap® bandaging tape (Animal Care Products, 3M Corp, St. Paul, MN) to hold the compound in place and prevent the animal from ingesting the compound. The Vetrap® was anchored in place cranially and caudally by strips of Conform® elastic tape (Kendall Co. Hospital Products, Boston, MA). The patch and wrappings were left in place for 24 hours. Animals were not restrained except during the wrapping procedure. When the wrappings and patch were removed, the exposed area was gently wiped with a piece of saline-moistened gauze to remove any remaining test compound.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (1) animals were observed undisturbed in their cages, (2) animals were removed from their cages and given a physical examination, and (3) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Observations were recorded daily for the remainder of the two-week test period. A second "walk-through" observation was performed each day, with only significant observations recorded. The exposed area was examined for signs of dermal reaction 0.5, 24, 48, and 72 hours after patch removal. Animal weights were recorded seven times during the study test period.

During evaluation of the exposure site, area and intensity of each dermal reaction were graded. Grading was performed according to a scale which included five categories to describe area and a 4-point scale for severity. Area categories were 0 - 5%, > 5 - 10%, > 10 - 25%, > 25 - 50% and > 50%; severity was defined as slight, mild, moderate, and severe.

Necropsy

All study animals were submitted for necropsy. Those that survived the 14-day study period were necropsied immediately after being given an overdose of sodium pentobarbital followed by exsanguination from severed axillary vessels. Skin was taken from the exposed area and examined microscopically.

Duration of Study

The study period was 14 days and was preceded by a 25-day quarantine/acclimation period. Historical study events are listed in Appendix C.

Changes/Deviations from Protocol

The hygrothermograph pen recording temperature used in the study malfunctioned twice failing to record temperature during the periods 14-15 July and 21-22 July. The exhaust fans were shut off for 2-4 hours on 12 July and there was a steam outage for 8-10 hours on 14 July with a resulting fluctuation in the temperature and humidity readings during those days. Animals were fed ~150 g/day of their feed instead of 110 g/day since experience indicated that 110 g/day was not sufficient to maintain growth. None of these changes appeared to have any effect on the study.

Raw Data and Final Report Storage

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

RESULTS

Twenty-four hour dermal exposure to guanidine hydrochloride at a limit dose of 2.0 g/kg produced no mortality in the 10 rabbits evaluated in the study. During the course of the study, observations were split into two major categories: systemic (general health of the animal) and dermal.

Systemic: There were no clinical signs (other than the dermal signs described below) that were interpreted as signs of toxicity attributable to the test compound. One male rabbit exhibited a necrotic toe, a result of self-mutilation secondary to a condition diagnosed as infectious pododermatitis. Individual body weights are presented in Appendix D.

Dermal: Skin irritation signs are presented in Appendix E. Erythema was observed in all rabbits 1/2 hour after patch removal. By 24 hours after patch removal the erythema had disappeared in five animals. Slight erythema in association with necrosis and ulceration was observed in one rabbit (84F444) after 72 hours. Loss of hair from the patch site or alopecia was observed in 9 of 10 animals. Typically, the hair covering more than 50% of the exposure site was removed by the gentle washing procedure at the time of patch removal. Initial signs of regrowth were evident within 24 hours after patch removal in all affected animals. Bruising was observed in 3 females (84F431, 84F447, 84F449). The bruising resolved between 48 and 72 hours after patch removal. Burns and slight edema were observed in two males (84F441, 84F444) at 1/2 hour after patch removal The edema resolved by 24 hours after patch removal while the burns were still present at 72 hours after patch removal. Necrosis was present in one male (84F444) 1/2 hours after patch removal. This lesion was observed throughout the 14-day observation period during which time it was characterized by eschar formation.

Pathology: There were no gross findings in these rabbits at necropsy, following the 2-week observation period, that could be attributed to dermal exposure to guanidine hydrochloride other than the one male (84F444) which presented with a 3.5 cm scab. This lesion upon microscopic examination was characterized as epidermal ulceration covered by a fibrinocellular exudate. A copy of the Pathology Report appears in Appendix F.

DISCUSSION

Acute dermal toxicity testing is designed to evaluate both systemic toxicity due to percutaneous absorption of the test material and local toxicity from its contact with the skin. From these observations it can be determined whether absorption of the test material across the skin is sufficient to produce systemic effects or lethality. In the present study, guanidine hydrochloride produced moderate to severe dermal reactions with no evidence of systemic effects.

All of the animals exposed to a limit dose of 2.0 g/kg guanidine hydrochloride survived to the end of the test. None of these test animals exhibited any clinical signs suggestive of a systemic action by guanidine hydrochloride. Typical clinical signs observable after oral administration of guanidine hydrochloride include retching, diarrhea, tremors/twitching, disorientation, and exaggerated response to handling (4). Therefore, it is concluded that dermal exposure to guanidine hydrochloride, at 2.0 g/kg, either does not result in sufficient percutaneous absorption to produce systemic toxicity or is not a systemic toxin at doses tested in the rabbit. The dermal median lethal dose of guanidine hydrochloride, as indicated by this study, is above the limit value of 2.0 g/kg.

Local dermal toxicity was observed at the site of exposure. Slight to moderate erythema was present in all 10 animals after the removal of test compound wrappings. Erythema is a relative nonspecific reaction to a dermal insult; however, the degree and persistence of erythema in these rabbits may suggest a more specific response to the test compound. Alopecia was the second most frequent observation reported and was present in 9 of 10 animals. There was evidence of regrowth in all affected animals by 24 hours after patch removal, indicating that guanidine hydrochloride did not irreversibly damage the mechanism responsible for hair production. Ulceration was present in two males (burns, necrosis). In both cases the lesions were focal and involved less than 25% of the exposed area. This pattern suggests either: (a) the test compound became highly concentrated in these regions by shifting under the patch or (b) this effect was manifested on unusually

susceptible skin. These ulcerative lesions were present at 72 hours after patch removal in both animals, and were present at necropsy in one male. Similar ulcerative lesions were produced by guanidine hydrochloride in an acute dermal irritation study in which necrotic lesions with eschar formation were primarily responsible for ranking guanidine hydrochloride as a severe dermal irritant (5).

CONCLUSION

A limit dose of 2.0 g/kg guanidine hydrochloride was not lethal to rabbits nor did it produce significant systemic effects; however, it did produce dermal irritation and eschar formation following dermal exposure for 24 hours.

REFERENCES

1. Kenyon KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1982, DTIC No. ADA 125591.

2. Acute dermal toxicity study. LAIR Standard Operating Procedure OP-STX-30, Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.

3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, dermal toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

4. Morgan EW, Sano SK, Korte DW. Acute oral toxicity (LD_{50}) of guanidine hydrochloride in male and female rats. Toxicology Series 77. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1985; Institute Report No. 204.

5. Morgan EW, Mullen L, Korte DW. Primary dermal irritation potential of guanidine hydrochloride in rabbits. Toxicology Series 91. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1986; Institute Report No. 213.

Appendix A: CHEMICAL DATA

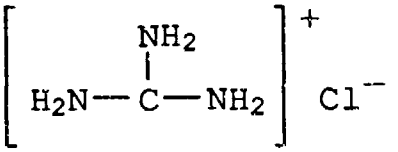
Chemical Name: Guanidine hydrochloride

Alternate Name(s): Aminomethanamidine hydrochloride,
Carbamamidine hydrochloride,
Carbamidine hydrochloride,
Aminoformamidine hydrochloride,
Iminourea hydrochloride

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

LAIR Code: TP28

Chemical Structure:



Molecular Formula: $CH_5N_3 \cdot HCl$

Molecular Weight: 95.5

Physical State: White powder

Melting Point: 182°-184°C (184°-185°C)[1]

Analytical Data:

Water content 0.1% by Karl Fischer analysis.[1] The material is at least 98% pure and chromatographs as one spot by thin layer chromatography.[2] Elemental analysis: Calculated for $CH_5N_3 \cdot HC–Cl$, 37.1; Found: Cl, 36.6.[2] An IR spectrum was obtained upon receipt of the compound. IR (KBr): 3400, 2750, 1650, 1535, 1050 (broad) cm^{-1}. A comparison of this spectrum to the Sadtler standard spectrum confirmed the identity of the material.[3]

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

[2] Sigma Chemical Company, St. Louis, MO. Becky Goodloe, PhD, personal communication, 5 March 1985.

[3] Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #8676.

Appendix A (cont.): CHEMICAL DATA

Source: Sigma Chemical Company
St. Louis, MO

Lot Number: 103F-5623

Stability in Aqueous Vehicle:

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

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

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

---

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

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and female

Age: Young adult

Animals in each group: 5 males and 5 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.8 - 3.7 kg

Identification procedures: Ear tattoo.

Pretest conditioning:

1. Quarantine/Acclimation from 21 June - 17 July 1984
2. Animals were close-clipped and examined 24 hours before dosing

Justification:

The laboratory rabbit is a proven mammalian model for dermal toxicity studies because of its size, ease of restraint, and skin permeability.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

DATE	EVENT
21 Jun 84	Animals arrived at LAIR. They were weighed, tattooed, observed for illness, and held for a two-week quarantine period.
21 Jun - 17 Jul 84	Animals were observed daily.
28 Jun, 5,12,17,24 Jul 84	Animals were weighed.
5 Jul 84	Animals completed quarantine.
17 Jul 84	Animals were close-clipped.
18 Jul 84	Animals were dosed and observed for clinical signs.
19-22 Jul 84 -	Animals were observed daily for dermal signs.
19 Jul- 1 Aug 84	Animals were observed daily for clinical signs.
1 Aug 84	Feed was removed during the morning observation. Animals were weighed and submitted to necropsy.

Appendix D: BODY WEIGHT DATA*

Animal Number	Day 01	07	014	026	7	14
			Females			
84F431	2851	2608	2680	3174	3190	3174
84F446	3296	3343	3073	3558	3545	3508
84F447	3363	3316	2904	3414	3487	3655
84F449	3438	3602	3346	3722	4009	3740
84F450	2727	2889	2695	3243	3311	3218
Mean	3135	3152	2940	3422	3508	3459
Standard Deviation	323	397	279	225	313	254
			Males			
84F436	2782	2802	2790	3060	3088	2969
84F441	2598	2776	2621	3234	3349	3405
84F443	2558	2357	2410	2848	2935	2947
84F444	2494	2579	2510	3007	2913	2924
84F445	2761	2711	2792	3184	3290	3473
Mean	2639	2645	2625	3067	3115	3144
Standard Deviation	127	183	169	153	200	271

* Weights are given in grams.

Appendix E: INDIVIDUAL DERMAL SIGNS

Animal Number	Dermal Signs	Duration of Dermal Signs(Days)§	Severity*	Area†
		Females		
84F431	Erythema	1	B	5
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5
	Bruising	1-4	D	2
84F446	Erythema	1-3	B	5
84F447	Erythema	1	A	5
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5
	Bruising	1-2	A	1
84F449	Erythema	1-2	A	5
	Alopecia	1	D	4
	Hair Growth	2-4	N/A	4
	Bruising	1	A	1
84F450	Erythema	1	A	5
	Alopecia	1	D	3
	Hair Growth	2-4	N/A	3

§ Day 1 represents 30 min. after patch removal, Day 2 - 24 hours later, Day 3 - 48 hours later, and Day 4 - 72 hours later.

* Severity Scores

A = Slight
B = Mild
C = Moderate
D = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation. This value is determined by visual approximation.

1 = 5%
2 = > 5 to 10%
3 = >10 to 25%
4 = >25 to 50%
5 = >50%

Appendix E (cont.): INDIVIDUAL DERMAL SIGNS

Animal Number	Dermal Signs	Duration of Dermal Signs(Days)§	Severity*	Area†
		Males		
84F436	Erythema	1	A	5
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5
84F441	Erythema	1-2	B	5
	Edema	1	A	2
	Burn	1-4	A	1
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5
84F443	Erythema	1	A	2
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5
84F444	Erythema	1-4	B	5
	Edema	1	A	3
	Burn	1-4	B	1
	Necrosis	1-4	D	3
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5
84F445	Erythema	1	A	5
	Alopecia	1	D	5
	Hair Growth	2-4	N/A	5

§ Day 1 represents 30 min. after patch removal, Day 2 - 24 hours later, Day 3 - 48 hours later, and Day 4 - 72 hours later.

* Severity Scores

A = Slight
B = Mild
C = Moderate
D = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation. This value is determined by visual approximation.

1 = 5%
2 = > 5 to 10%
3 = >10 to 25%
4 = >25 to 50%
5 = >50%

Appendix F: PATHOLOGY REPORT

PATHOLOGY REPORT

GLP Study 84006

Acute Dermal Toxicity (Limited Test) of Guanidine.HCl (CH_5N_3HCl) In Male and Female New Zealand White Rabbits

Purpose: This study was done to determine the acute dermal toxicity of Guanadine.HCl (CAS #22661-876). A test dose of 2 g/kg was applied to clipped but unabraded skin of the rabbit. Animals that died were necropsied within 16 hours after death. The remaining animls were killed by exsanguination while under pentobarbital anesthesia after a 14-day observation period. Complete gross necropsies were performed and two specimens of skin from each exposed area were processed for histologic evaluation. Five male and five female rabbits were treated. Two of each sex (84F00327, 84F00332 males, and 84F00334, 84F00326 females) died within 24 hours. The rest survived until the end of the test.

Gross Necropsy Findings:

84F00334 - 35646 - The skin of the application site was erythematous and had foci of mild subcutaneous hemorrhage and edema.

84F00326 - 35643 - The skin of the application site was erythematous and had foci of mild subcutaneous hemorrhage and edema.

84F00325 - 35668 - The skin had a 5 mm in diameter scab in the application site. Purulent otitis media was present in the left ear.

84F00327 - 35644 - The skin of the application site was erythematous and had foci of mild hemorrhage and edema.

84F00332 - 35645 - The skin of the application site was erythematous and had foci of mild hemorrhage and edema. The fundus of the stomach had multiple pinpoint black foci on the mucosal surface.

84F00331 - 35671 - The skin of the application site had a 2 mm in diameter dermal scar and two 2 mm in diameter scabs.

Appendix F (cont.): PATHOLOGY REPORT

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Microscopic Findings:

84F00326 - 35643 - Slides 1 and 2, Skin, 4 sections (two per slide) - Lesions varied from section to section; tissues from slide 1 were maximally involved and those of slide 2 were minimally involved. There were multiple foci of hemorrhage and edema in the dermis. Microthrombi were present in some of the small blood vessels and some small vessels had necrotic walls which contained from a few to several heterophils. Epithelial cells of adjacent adenexae were often dissociated and occasionally necrotic. The underlying sketetal muscle was fragmented and had small foci of coagulation necrosis along with heterophilic infiltrates between muscle fibers.

84F00327 - 35644 - Slides 1 and 2 - Skin - The lesions were similar to but less severe than those in 35643. There was less muscle necrosis, absence of muscular heterophilic infiltrate, and vascular wall necrosis was not present. Microthrombi were present in some vessels.

84F00332 - 35645 - Slides 1 and 2 - Skin - There were several foci of mild to moderate hemorrhage in the dermis.

84F00334 - 35646 - Slides 1 and 2 - Skin - There were a few small foci of dermal hemorrhage. Some small vessels had necrotic walls, others had a few vacuolated cells and very low numbers of heterophils in their walls. A few muscle fibers were degenerating and had foci of necrosis.

84F00323 - 35666 - Slides 1 and 2 - Skin - Not remarkable (NR).

84F00324 35667 - Slides 1 and 2 - Skin - Three sections were unremarkable. One section had multiple foci of minimal hemorrhage in the dermis and degenerative changes in a few muscle fibers.

84F00325 - 35668 - Slides 1 and 2 - Skin - Three sections were unremarkable. One section had an ulcer of the epidermis with superficial bacterial colonization of the surface exudate. The subadjacent dermis was necrotic and a heterophilic infiltrate was present in that region. Mixed inflammatory infiltrates were present in the deeper dermis.

84F00328 - 35669 - Slides 1 and 2 - Skin - NR.

84F00330 - 35670 - Slides 1 and 2 - Skin - NR.

Appendix F (cont.): PATHOLOGY REPORT

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84F00331 -- 35671 - Slides 1 and 2 - Skin - Two sections were unremarkable. One of the other sections had a focal scar in the dermis with acanthotic, hyperkeratotic epithelium covering the scar. A fibrinocellular exudate was present over the epithelial surface covering the scar. The remaining section had ulceration of the epithelial surface and acanthotic epithelium bordering the ulcer. Numerous mixed leukocytes were present beneath the ulcer and in adjacent tissue. Adjacent adenexae were undergoing lytic changes.

Summary: This study is being repeated; therefore, further evaluation of this phase will not be done at present.

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