PRIMARY EYE IRRITATION OF GUANIDINE NITRATE IN MALE RABBITS (U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA E W MORGAN ET AL. JAN 86 LAIR-212

UNCLASSIFIED
PRIMARY EYE IRRITATION OF GUANIDINE NITRATE IN MALE RABBITS

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JOY W. BAUSERMAN, MEd
and
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TOXICOLOGY BRANCH
DIVISION OF COMPARATIVE MEDICINE
AND TOXICOLOGY

JANUARY 1986

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129
Primary Eye Irritation of Guanidine Nitrate in Male Rabbits
(Toxicology Series 86)—Morgan et al

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

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)

[Signature and date]

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The primary eye irritation potential of guanidine nitrate was determined in male New Zealand White rabbits by using a modified Draize method. The compound produced a positive test response as it caused mild-to-moderate irritation. Signs of irritation were erythema and chemosis of the conjunctiva, iritis, and corneal lesions. All 6 animals tested showed one or more of these lesions. Potential corrosive properties of guanidine nitrate were indicated by observation of pannus (1 of 6) and corneal erosions (2 of 6) that persisted through termination at day 21.
ABSTRACT

The primary eye irritation potential of guanidine nitrate was determined in male New Zealand White rabbits by using a modified Draize method. The compound produced a positive test response as it caused mild-to-moderate irritation. Signs of irritation were erythema and chemosis of the conjunctiva, iritis, and corneal lesions. All 6 animals tested showed one or more of these lesions. Potential corrosive properties of guanidine nitrate were indicated by observation of pannus (1 of 6) and corneal erosions (2 of 6) that persisted through termination at day 21.

Key words: Primary Eye Irritation, Toxicology, Guanidine Nitrate, Mammalian Toxicology, Nitroguanidine, Rabbit
PREFACE

TYPE REPORT: Primary Eye Irritation GLP Study Report

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

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT: 3E162720A835
Nitrocellulose-Nitroguanidine Projects,
WU 180, APC TLO9

GLP STUDY NUMBER: 84021

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

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

CO-PRINCIPAL INVESTIGATOR: Joy W. Bauserman, MEd

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: Guanidine Nitrate

INCLUSIVE STUDY DATES: 2 Jul - 31 Jul 84

OBJECTIVE: The objective of this study was to determine the primary
eye irritation potential of guanidine nitrate in male New Zealand White rabbits.
ACKNOWLEDGMENT

SP4 Paul D. Mauk, BS, SP4 Steven K. Sano, BA, Gerald F. S. Hiatt, PhD, and Yvonne C. Johnson, BS assisted in the research; Richard D. Spieler, Richard Katona, Charlotte Speckman, Roosevelt Cunningham, and Edward M. Sands provided animal care; and Lynda Araiza and JoAnn Nishimoto prepared the typescripts.
SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84021 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., PhD / DATE
MAJ, MSC
Study Director

EARL W. MORGAN, DVM / DATE
CPT, VC
Principal Investigator

JOY W. BAUSERMAN, MD / DATE
Co-Principal Investigator

CONRAD WHEELER, PhD / DATE
DAC
Analytical Chemist
MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

2. The report and raw data for this study were audited on 10 October 1985.

3. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 1 October 1984 report to Management and the Study Director.

GARY L. DUTCHER
SSG, USA
Quality Assurance Unit
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Nitroguanidine, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Bioengineering Research and Development Laboratory (USAMBRDL), as part of its mission to evaluate the environmental and health hazards of military-unique pollutants 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 USAMBRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. Guanidine nitrate is an intermediate and an anticipated by-product of nitroguanidine production.

Objective of Study

The objective of this study was to determine the primary eye irritation potential of guanidine nitrate in male New Zealand White rabbits.

MATERIALS

Test Substance

Chemical name: Guanidine Nitrate

Chemical Abstract Service Registry No.: 506-93-4

Molecular structure:

\[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{C} = \text{NH}_2 \end{array}
\begin{array}{c}
\text{H}_2\text{N} \text{NO}_3^-
\end{array}
\]

Molecular formula: C\text{H}_4\text{N}_2\text{HNO}_3

Other test substance information is presented in Appendix A.
Vehicle

None

Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA) were identified individually with ear tattoos numbered 84F269, 84F270, 84F272, 84F274, 84F275 and 84F276. The animal weights on dosing day ranged from 3.9 to 4.5 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed (no bedding), battery-type cages with automatically flushing dump tanks. The diet consisted of approximately 150 g of Certified Purina Chow Diet 5322 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 17.8 to 22.2°C with a relative humidity range of 56 to 70%, except for an 8-hour period when the steamline was being repaired during which the relative humidity rose to 76%. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with guidelines promulgated by the EPA for ocular irritation testing (2) and LAIR SOP-OP-STX-3 (3).

Group Assignment/Acclimation

Study rabbits were divided into two dose groups of 3 males each. The animals were acclimated for 8 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

One-tenth milliliter (0.092 g) of guanidine nitrate was administered once to the right eye of each rabbit by gently pulling the lower lid away from the conjunctival cul-de-sac to form a cup into which the compound was dropped. The upper and lower lids were then held together for one second to prevent loss of material. The first group, (A), was dosed on 3 July, and the second group, (B), was dosed on 10 July.
Compound Preparation

Since the dose of guanidine nitrate administered was expressed in milliliters, it was necessary to equate milliliters to grams because the guanidine nitrate was administered in crystalline powder form. By using a pipette, it was determined that 0.1 ml of guanidine nitrate tightly packed weighed 0.0920 grams.

Test Procedures

On 2 July, both eyes of Group A animals were examined, unaided and with fluorescein dye, for any corneal, iridial, or conjunctival abnormalities. On 3 July, 0.092 g of guanidine nitrate was placed in the right eye of each rabbit. The left eye was untreated and served as the control. Group B rabbits underwent the same procedures on 9 and 10 July, respectively.

Observations

The grading and scoring for ocular reactions were performed according to Table 1 (3). Observations were made daily from 2 July to 31 July. Scoring and grading of ocular reactions were performed at 1 and 4 hours, 1, 2, 3, 7, 14, and 21 days for both groups A and B. Fluorescein dye was used for scoring and grading at 24 hours, 7, 14 and 21 days.
## TABLE 1

### GRADES FOR OCULAR LESIONS (2)

#### CORNEA

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No ulceration or opacity.</td>
</tr>
<tr>
<td>1*</td>
<td>Scattered or diffuse areas of opacity (other than slight dulling of normal luster) details of iris clearly visible.</td>
</tr>
<tr>
<td>2</td>
<td>Easily discernible translucent areas, details of iris slightly obscured.</td>
</tr>
<tr>
<td>3</td>
<td>Nacreous areas, no details of iris visible, size of pupil barely discernible.</td>
</tr>
<tr>
<td>4</td>
<td>Opaque cornea, iris not discernible through opacity.</td>
</tr>
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</table>

#### IRIS

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Markedly deepened rugae, congestion, swelling, moderate circumrirdial hyperemia or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive).</td>
</tr>
<tr>
<td>1*</td>
<td>No reaction to light, hemorrhage, gross destruction (any or all of these).</td>
</tr>
</tbody>
</table>

#### CONJUNCTIVA

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>Blood vessels normal.</td>
</tr>
<tr>
<td>1</td>
<td>Some blood vessels definitely hyperemic (injected).</td>
</tr>
<tr>
<td>2*</td>
<td>Diffuse, crimson color, individual vessels not easily discernible.</td>
</tr>
<tr>
<td>3</td>
<td>Diffuse beefy red.</td>
</tr>
<tr>
<td>4</td>
<td>Chemosis: lids and/or nictitating membranes</td>
</tr>
<tr>
<td>0</td>
<td>No swelling.</td>
</tr>
<tr>
<td>1</td>
<td>Any swelling above normal (including nictitating membranes).</td>
</tr>
<tr>
<td>2*</td>
<td>Obvious swelling with partial eversion of lids.</td>
</tr>
<tr>
<td>3</td>
<td>Swelling with lids about half-closed.</td>
</tr>
<tr>
<td>4</td>
<td>Swelling with lids more than half-closed.</td>
</tr>
</tbody>
</table>

*Indicates minimum level for a positive response.
Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

Animals for this study were transferred from Non-GLP Study 74021, in which they were untreated breeders in a teratology study. They were quarantined for 8 days instead of 14 days because they had been in the room since 5 April 84 and needed only to be adapted to the certified chow.

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

Results from scoring the ocular irritation in each rabbit are tabulated in Appendix D.

Slight corneal opacity (score 1) was observed in five rabbits. This was first observed at 4 hours, reached a maximum incidence at 24 hours, and had cleared in 3 of the rabbits by 72 hours. The opacity in Rabbit 84F272 persisted through day 14 but cleared by day 21. In Rabbit 84F275 the opacity not only continued through day 21 when the study was terminated but also increased in severity as a result of the developing pannus.

In four rabbits, slight (score 1) iritis (vascular injection) was observed. This was noted as early as the 1-hour observation and cleared by day 14.

In evaluating the conjunctiva for both redness and chemosis, all rabbits showed a positive reaction at some point after dosing. All rabbits exhibited slight conjunctival redness (score 1). This condition was present in 4 of 6 at the 1-hour observation. The redness in all animals cleared by the 14-day observation except for rabbits 84F272 and 84F276 in which redness had cleared by day 21. Rabbit 84F275 showed moderate redness (score 2) at the 4-hour and 7-day observation periods. Conjunctival chemosis (score 1) was observed in all rabbits by the 24-hour observation. The swelling cleared by day 14 except one reading of slight chemosis at day 21 (84F275). In earlier observations, chemosis had been an edematous swelling, however, in Rabbit 84F275 at day 21, both palpebra lids were about twice normal thickness and leathery. Rabbit 84F270 showed moderate (score 2) conjunctival chemosis at the 48-hour and 72-hour observations.
In addition to the ocular reactions, four rabbits developed corneal erosions. In two of the animals the erosions cleared by the 7-day observation, but in two rabbits the erosions persisted through day 21. In the animals where the erosions remained, the areas of the cornea covered by the ulcerations were 5 mm in Rabbit 84F275 and 2 mm in Rabbit 84F272. Also, a large area of the nictitating membrane became devitalized and was sloughed in three rabbits, 84F270, 84F272, and 84F275.

Animal 84F275 developed the most severe reaction to the test compound. One-hour after exposure, vascularization of the iris was observed. After 24 hours this rabbit showed a slight reaction (score 1) in all areas of the eye. These conditions persisted through day 7. By the 14-day observation, all conditions had cleared except the corneal opacity and erosion. However, pannus (vascularization of the cornea) was observed from day 14 through termination of the study on day 21. The pannus began as a slight vascularization in the left ventral portion of the cornea and spread until it covered the lower half of the eye. Other ocular reactions observed were slight-to-marked tearing and free-floating exudate.

The control eye remained normal throughout the study.

DISCUSSION

The EPA recommends that investigators follow the Consumer Product Safety Commission Guidelines on ocular irritation testing (3). These guidelines state that an animal is considered to have exhibited a positive reaction if the test substance produces one or more of the following signs: ulceration of the cornea (other than a fine stippling); opacity of the cornea (other than a slight dulling of the normal luster); inflammation of the iris (other than a slight deepening of the rugae or a slight hyperemia of the circumcorneal blood vessels); an obvious swelling in the conjunctiva (excluding the cornea and iris) with partial eversion of the lids; or a diffuse crimson-red coloration in the conjunctiva with individual vessels not easily discernible. The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. The EPA also classifies irritation in terms of duration of response. A test compound which produces a response which is reversible in 21 days is classified as irritative, while a test compound that produces an irreversible response (present at 21 days) is classified as corrosive. This system requires that classification be dependent on the most severe responder.

Thus, according to EPA guidelines, since four animals exhibited corneal opacity/ulceration, the primary ocular irritation test for guanidine nitrate was positive in male New Zealand White rabbits. Furthermore, due to the persistence of the corneal erosions in 2 rabbits (84F272 and 84F275) through the termination of the study at day 21 the compound should also be considered a corrosive agent.
The severe erosions may be caused in part by the physical state of the compound, and in part because guanidine nitrate was not irrigated from the eye. If the eye had been irrigated with large volumes of water, the compound would have been washed from the eye. Thus, prompt and thorough irrigation of the eye may ameliorate, if not completely eliminate, any clinical symptomatology.

CONCLUSION

Guanidine nitrate is a mild irritant with corrosive properties. It produced a positive test response.
REFERENCES


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Appendices
CHEMICAL DATA

Chemical name: Guanidine Nitrate

LAIR Code: TP030

Chemical Abstracts Service Registry No.: 506-93-4

Chemical structure:

\[
\begin{align*}
& \text{H}_2\text{N} \\
C &= \text{NH}_2 \\
& \text{H}_2\text{N} \\
\text{NO}_3
\end{align*}
\]

Molecular formula: \( \text{CH}_6\text{N}_4\text{O}_3 \)

Molecular weight: 122.1

pH*: 4.8 (saturated solution of GuN in MCT80 dosing vehicle)
4.3 (MCT80 dosing vehicle alone)

Physical state: White granular crystalline powder

Melting point: 214°C†

Source: Chemical Dynamics Corporation
Hadley Road, P.O. Box 395
South Plainfield, NJ

Lot No.: 123820

Purity: Infrared spectrophotometry was performed on 1 Mar 84 and the spectrum obtained† was identical to the Sadtler spectrum§ for guanidine nitrate. The grade of material obtained for this study is referred to as the Ultralog Grade by the manufacturer. The label on the bulk container states that the purity is at least 99.99%.


Appendix A
ANIMAL DATA

Species: Oryctolagus cuniculus

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male

Age: Young Adults

Animals in each group: 3 males

Condition of animals at start of study: Normal

Body weight range at dosing: 3.9 - 4.5 kg

Identification procedures: Ear tattoo; tag numbers 84F269, 84F270, 84F272, 84F274, 84F275, 84F276.

Pretest conditioning:

1. Quarantine from 25 June - 2 July 1984

2. Animal eyes were examined 24 hours before dosing using fluorescein dye and ultraviolet light.

Justification: Laboratory rabbits are a proven sensitive animal model for ocular testing.

Appendix B
<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Jul 84</td>
<td>Animals received from Non-GLP Study 74021 and were checked for pre-existing ocular injury.</td>
</tr>
<tr>
<td>3 Jul 84</td>
<td>Rabbits were weighed and group A animals numbered 84F269, 270, and 272 dosed. One and 4-hour post-exposure scoring were conducted.</td>
</tr>
<tr>
<td>4-31 Jul 84</td>
<td>All rabbits were checked daily.</td>
</tr>
<tr>
<td>4 Jul 84</td>
<td>Group A 24-hour post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>5 Jul 84</td>
<td>Group A 48-hour post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>6 Jul 84</td>
<td>Group A 72-hour post-exposure scoring was conducted. All animals were weighed.</td>
</tr>
<tr>
<td>9 Jul 84</td>
<td>Group B animals numbered 84F274-84F276, were checked for pre-existing ocular injury.</td>
</tr>
<tr>
<td>10 Jul 84</td>
<td>Group A 7-day post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>10 Jul 84</td>
<td>Rabbits were weighed and group B dosed. One and 4-hour post-exposure scoring were conducted.</td>
</tr>
<tr>
<td>11 Jul 84</td>
<td>Group B 24-hour post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>12 Jul 84</td>
<td>Group B 48-hour post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>13 Jul 84</td>
<td>Group A animals were observed for the tenth day and weighed.</td>
</tr>
<tr>
<td>13 Jul 84</td>
<td>Group B 72-hour post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>17 Jul 84</td>
<td>All animals were weighed and group A 14-day post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>Date</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>17 Jul 84</td>
<td>Group B 7-day post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>24 Jul 84</td>
<td>Group A animals were weighed and 21-day post-exposure scoring was conducted. The animals were sacrificed.</td>
</tr>
<tr>
<td>24 Jul 84</td>
<td>Group A animals were weighed and 14-day post-exposure scoring was conducted.</td>
</tr>
<tr>
<td>31 Jul 84</td>
<td>Group B animals were weighed and 21-day post-exposure scoring was conducted. The animals were sacrificed and the study terminated.</td>
</tr>
</tbody>
</table>
TABULAR SCORING DATA
ON
ACUTE EYE IRRITATION SUMMARY FORMS

Table 1. Conjunctiva Chemosis Scores............................19
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Appendix D-1
TABLE 1

Summary of Conjunctival Chemosis

Score by Animal

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Base Line</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
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<tbody>
<tr>
<td>84F269</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>84F270</td>
<td>0</td>
<td>0</td>
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<td>2</td>
<td>2</td>
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<td>0</td>
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<tr>
<td>84F275</td>
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<td>84F276</td>
<td>0</td>
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TABLE 2

Summary of Conjunctival Redness

Score by Animal

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Base Line</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>84F269</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>84F270</td>
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<td>0</td>
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<td>1</td>
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<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>84F272</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>84F274</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>0</td>
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</tr>
<tr>
<td>84F275</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>84F276</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 3

Summary of Iris Score by Animal

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Base Line</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>84F269</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>84F270</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>84F272</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>84F274</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>84F275</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>84F276</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Appendix D-4
TABLE 4
Summary of Cornea Opacity
Score by Animal

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Base Line</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>84F269</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>84F270</td>
<td>0</td>
<td>0</td>
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<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>84F272</td>
<td>0</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td>0</td>
<td>1</td>
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</tr>
<tr>
<td>84F274</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>84F275</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>84F276</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>1 Hr</th>
<th>4 Hr</th>
<th>24 Hr</th>
<th>48 Hr</th>
<th>72 Hr</th>
<th>7 D</th>
<th>14 D</th>
<th>21 D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tearing - slight</td>
<td>269,270</td>
<td>269,270</td>
<td></td>
<td>269,270</td>
<td>270</td>
<td>274</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- moderate</td>
<td>272,274</td>
<td>272,274</td>
<td></td>
<td>272</td>
<td></td>
<td>270</td>
<td>272</td>
<td>275</td>
</tr>
<tr>
<td>- marked</td>
<td>275,276</td>
<td>275,276</td>
<td></td>
<td>270</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Devitalization/Fluorescein staining of nictitating membrane/conjunctiva</td>
<td></td>
<td>269,272, 274,275, 276</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Fibrinonecrotic cast on conjunctival surface</td>
<td></td>
<td></td>
<td>270,272</td>
<td>270,272</td>
<td>270,272</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corneal Erosion</td>
<td></td>
<td></td>
<td></td>
<td>270,272, 274,275</td>
<td>270,272, 275, 275</td>
<td>270,272, 272,275</td>
<td>272,275</td>
<td>272</td>
</tr>
<tr>
<td>Pannus</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
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