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Primary Ocular Irritation Potential of Nitrosoguanidine in Male Rabbits

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and
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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

September 1989

Toxicology Series: 166

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

Primary Ocular Irritation Potential of Nitrosoguanidine in Male Rabbits (Toxicology Series 166)--Hiatt and Korte

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William C. Cole 11 Sept 89

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

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The potential for nitrosoguanidine to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. Slight conjunctival vasodilation (indicative of mild inflammation) was the predominant response observed. Slight iridial hyperemia was noted in one animal and one animal had a small fluorescein-staining area in the cornea. The results indicate that nitrosoguanidine is not a primary ocular irritant under conditions of this study. (Keywords: Explosives; propellants;)

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ABSTRACT

The potential for nitrosoguanidine to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. Slight conjunctival vasodilation (indicative of mild inflammation) was the predominant response observed. Slight iridial hyperemia was noted in one animal and one animal had a small fluorescein-staining area in the cornea. The results indicate that nitrosoguanidine is not a primary ocular irritant under conditions of this study.

Key Words: Nitrosoguanidine, Ocular Irritation, Mammalian Toxicology, Rabbit, Munition, Propellant, Nitroguanidine

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 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: 85010

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

PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

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: Nitrosoguanidine

INCLUSIVE STUDY DATES: 10 October 1985 - 15 November 1985

OBJECTIVE:

The objective of this study was to determine the primary ocular irritation potential of nitrosoguanidine in male New Zealand White rabbits.

ACKNOWLEDGMENTS

MAJ Earl W. Morgan, DVM, SP4 John R.G. Ryabik, BS, and SP4 Paul B. Simboli, BS, provided technical assistance. SSG James D. Justus, SP4 James J. Fischer, SP4 Scott L. Schwebe, Richard D. Spieler, Obie B. Goodrich, and Diane Arevalo provided care for the animals. Colleen S. Kamiyama, Ann Wilkinson, and Julie Peacock provided administrative and clerical support during the performance of this study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS INVCLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85010 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. 5 Sep 89

DON W. KORTE, JR., PhD / DATE
LTC, MSC
Study Director

Gerald F.S. Hiatt

GERALD F.S. HIATT, PhD / DATE
DAC
Principal Investigator

Conrad Wheeler 5 Sep 89

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

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

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 6 September 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85Ø1Ø

1. This is to certify that the protocol for LAIR GLP Study 85Ø1Ø was reviewed on 1Ø May 1985.

2. The institute report entitled "Primary Ocular Irritation Potential of Nitrosoguanidine in Male Rabbits," Toxicology Series 166, was audited on 21 August 1989.

Carolyn M. Lewis

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

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

Primary Ocular Irritation Potential of Nitrosoguanidine in Male Rabbits– Hiatt and Korte

INTRODUCTION

Nitrosoguanidine is a potential anaerobic degradation product of nitroguanidine (1), a primary component of US Army triple-base propellants, which 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 (2). 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.

Objective of Study

The objective of this study was to determine the primary ocular irritation potential of nitrosoguanidine in male New Zealand White rabbits.

MATERIALS

Test Substance

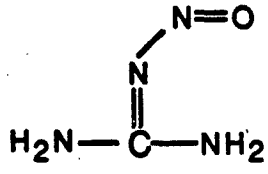
Chemical Name: Nitrosoguanidine

Chemical Abstracts Registry No.: 674-81-7

LAIR Code Number: TP48

Physical State: Yellow powder

Molecular Structure:



Molecular Formula: CH_4N_4O

Other test substance information is presented in Appendix A.

Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA) were identified individually with ear tattoos numbered 85F250 - 85F255. Animal weights on dosing day ranged from 2.7 to 3.1 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dumptanks. The diet consisted of approximately 150 g/day 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°C to 22.2°C and relative humidity ranged from 49% to 71%, except for occasional humidity spikes as high as 80% (room washing). The photoperiod was 12 hours of light per day.

METHODS

Conduct of this study was in accordance with the LAIR Standard Operating Procedure OP-STX-33, "Primary Eye Irritation Study", and guidelines promulgated by the EPA for ocular irritation testing (3,4).

Group Assignment/Acclimation

Study rabbits were assigned to two dose groups of 3 males each. These animals were quarantined in the Division of Animal Care and Services for 14 days and acclimated for 5-19 days in the GLP Suite before dosing. While in quarantine the animals were treated once with Canex® and mineral oil for ear mites. During these periods they were observed daily for signs of illness.

Dosage Levels and Administration

One-tenth milliliter (0.06 g) of nitrosoguanidine was administered once to one 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 instilled. Upper and lower lids were then held gently together for one second to prevent loss of material.

Compound Preparation

Nitrosoguanidine is a powder and was administered neat (without any physical modification).

Test Procedures

On 28 Oct 85, both eyes of each Group 1 animal were examined, for any preexisting abnormalities, by the procedure detailed under the "Ocular Examination/Grading" subheading. For each animal, the eye with the nearest normal appearance was designated for treatment, the other eye serving as an untreated control. On 29 Oct 85, a dose of 0.1 ml nitrosoguanidine was placed in the designated eye of each rabbit in this group. Group 2 rabbits underwent the same examination on 11 Nov 85 and the same treatment procedure on 12 Nov 85.

Ocular Examination/Grading

Initially, each eye was observed unaided in a darkened room with focal illumination (penlight). Structures examined included the lids and surrounding fur, the conjuntiva (semilunar, palpebral, and bulbar), the cornea, and the iris. Grading of the cornea, iris, and conjunctiva was performed according to Table 1 (5). During each observation, the eyes were also examined with a slit lamp. Special attention was given to integrity of the corneal surface, thickness of the corneal stroma, clarity of anterior chamber fluid, iridial morphology, clarity of the lens, and lenticular surface morphology (6). Additionally, any areas appearing grossly abnormal were examined under high magnification. All observations, including normal appearance, were detailed on the grading sheet. Following this, fluorescein dye (Fluor-I-Strips, Ayerst Laboratories, Inc., New York, NY) was introduced into the eye, which was then observed under ultraviolet light. Any corneal areas reacting with the dye (a sign of discontinuity of the corneal epithelium) were described with respect to area and intensity of fluorescence. Examination and grading of ocular reactions were performed in this fashion at 1, 4, 24, 48, and 72 hours after dosing. Fluorescein staining was omitted from the 1- and 4-hour observations. Due to an almost total lack of reaction during the 72 hours after dosing, the study was terminated in accordance with the protocol, and the animals were submitted for necropsy. No scoring or observations were performed at 7, 14, and 21 days.

Duration of Study

Appendix C is a complete historical listing of study events.

Changes/Deviations

Slit lamp examination was added to the standard observation procedures. The slit lamp enables one to detect subtle reactions not grossly observable and to evaluate more thoroughly those abnormalities which are grossly observable. Color photographic documentation was not performed due to lack of significant response to the test compound.

TABLE 1: Grades for Ocular Lesions*

CORNEA	
Opacity: degree of density (area of greatest density taken for reading)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1†
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through opacity	4
IRIS	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumiridial hyperemia or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive)	1†
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
CONJUNCTIVA	
Redness: (refers to palpebral and bulbar conjunctiva, excluding cornea and iris)	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	2†
Diffuse, beefy red	3
Chemosis: (lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal including nictitating membranes	1
Obvious swelling with partial eversion of lids	2†
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4

* Adapted from Table 6 in Draize *et al.* (4).

† Indicates minimum level for a positive response.

With these exceptions, this study was completed in accordance with the appropriate protocol and addenda. It is believed that none of these changes/deviations had a negative effect on the performance of the study or the validity of the results.

Storage of Raw Data and Final Report

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

RESULTS

Tabulation of the Draize-type ocular grading results is presented in Appendix D and a summary of the ocular observations in Appendix E.

Cornea

Nitrosoguanidine produced no grossly observable effects in the cornea. All treated eyes were assigned zero scores for both opacity and area involvement at all observations after dosing.

Slit lamp examination revealed a slight thickening in the cornea in rabbit 85F251 at 4 hours after dosing and after fluorescein staining one small (approximately 0.2 mm) area at 24 and 48 hours after dosing in rabbit 85F255. All other slit lamp observations revealed corneas of normal thickness, indicating lack of edema, and smooth surfaces, indicating epithelial integrity.

Iris/Anterior Chamber

One rabbit (85F254) had slight iridial hyperemia at 1 hour after dosing. Iridial scores were consistently zero at all other observation times. No iridial abnormalities were detected by slit lamp examination of the treated eyes. Circumiridial vessels and surface morphology were normal at all times after dosing. Close examination of anterior chamber fluid revealed no evidence of the presence of protein or cells (signs of iridial inflammation).

Lens

The lens was not scored under the Draize-type grading system because of the difficulty in making unaided observations. At all times after dosing, the lens appeared normal during slit lamp examination. No changes were observed in clarity or surface morphology.

Conjunctiva

In this study, nitrosoguanidine produced only one grossly observable response—slight conjunctival redness. At 1 hour after dosing, all treated eyes exhibited slight vasodilatation in the bulbar (sclera) or semilunar (nictitating membrane) conjunctiva. Conjunctival redness scores of 1 were assigned to the treated eyes and slit lamp examination confirmed the presence of dilated vessels within the outer layers of the sclera and the nictitating membrane. This slight conjunctival redness had cleared in 3 rabbits by 4 hours after dosing. It persisted through 24 hours after dosing in 3 rabbits (85F253, 85F254, and 85F255).

Control Eyes

At no time during the study did the untreated eyes exhibit any change from their normal condition on the day of dosing. A mucous-like area on the cornea that stained with fluorescein was observed in one of the control eyes (85F250) at the preliminary examination 24 hours before dosing. This resolved by the day of dosing and no further abnormalities were observed during the study.

Pathology Report

No lesions were observed at necropsy. The pathologist's report is presented in Appendix F.

DISCUSSION

The primary goal of ocular toxicity testing is to determine the potential for ocular damage resulting from accidental contact of the test compound with

the eye. For this purpose, the Draize-type irritation test, used in the present study, is especially well-suited. An important feature of this test is that the route and type of exposure (ocular instillation followed by a forced blink) closely mimics potential human exposures.

Consumer Product Safety Commission Guidelines, which the EPA recommends for ocular irritation testing, state that an animal has 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 with partial eversion of the lids; or a diffuse crimson-red coloration in the conjunctiva with individual vessels not easily discernible (3).

Guidelines for classification of chemicals as ocular irritants or nonirritants have been published and form the basis for evaluation in the present study (7). These Interagency Regulatory Liaison Group (IRLG) guidelines state: "[a] test result is considered positive if four or more animals exhibit a positive reaction. If only one animal exhibits a positive reaction, the test result is regarded as negative."

In this study, nitrosoguanidine produced no positive reactions, as defined by the IRLG. Slight conjunctival redness, indicating mild inflammation, was the predominant response. Slight iridial hyperemia was noted in one animal and one animal had a small fluorescein-staining area in the cornea. These reactions, although scorable, did not achieve sufficient severity to warrant consideration as a "positive response." Due to this lack of positive response, nitrosoguanidine is classified as a nonirritant by the results of the present study.

CONCLUSION

Nitrosoguanidine exhibited minimal potential to produce ocular irritation under conditions of this study.

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2. 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.

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4. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Primary eye irritation. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

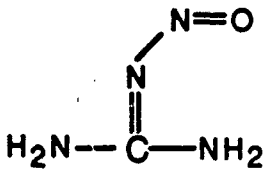
5. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82:377-390.

6. MacDonald TO, Baldwin HA, Beasley CH. Slit lamp examination of experimental animal eyes I. Techniques of illumination and the normal animal eye. J Soc Cosmet Chem 1973; 24:163-180.

7. Interagency Regulatory Liaison Group. Recommended guidelines for acute eye irritation testing. January 1985.

Appendix A: CHEMICAL DATA

Chemical Name: Nitrosoguanidine
Chemical Abstracts Service Registry No.: 674-81-7
Lot Number: WCC-2-002
LAIR Code: TP48
Chemical Structure:



Molecular Formula: CH_4N_4O
Molecular Weight: 88
Physical State: Yellow powder
Analytical Data:

HPLC: Nitrosoguanidine was analyzed using conditions similar to those employed by Burrows *et al.*[1] Conditions were as follows: column, Brownlee RP-18 (4.6 mm x 25 cm); mobile phase, water; flowrate, 0.8 ml/min. The effluent was monitored at 255 nm. The retention times for nitrosoguanidine and nitroguanidine were 4.4 and 6 min, respectively. The HPLC data demonstrated that the nitrosoguanidine contained approximately 2.5% nitroguanidine.[2]

IR (KBr): 3378, 3096, 1690, 1649, 1508, 1341, 1266,1134, 1088, 1035, 690, 668 cm^{-1}.[3]

Solubility:

A saturated solution of nitrosoguanidine in water was prepared at room temperature. A 1:500 dilution of this solution produced an absorbance of 0.533 units. Using an extinction coefficient of 13,305 L/moles·cm, the concentration of nitrosoguanidine in the original saturated solution was calculated to be 1.76 mg/ml.[4]

Source: Alan Rosencrance
US Army Biomedical Research
and Development Laboratory
Fort Detrick, Maryland

---

[1]Burrows EP, Brueggeman EE, Hoke SH. Chromatographic trace analysis of guanidine, substituted guanidines and striazines in water. Chromatog 1984;16:494-8.

[2]Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 37. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]*Ibid.* p 30.

[4]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: 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: 2.7 - 3.1 kg

Identification procedures:

Ear tattoo numbers 85F250 - 35F255.

Pretest conditioning:

1. Quarantine from 10 Oct - 24 Oct 1985
2. Animal eyes were examined 24 hours before dosing using slit lamp, fluorescein dye, and ultraviolet light.

Justification:

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

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
10 Oct 85	Animals arrived at LAIR. Animals were examined for illness and placed under a two-week quarantine.
10-24 Oct 85	Animals were checked daily by quarantine personnel.
11 Oct 85	Animals were weighed.
18 Oct 85	Animals were weighed and given one application of Canex®/mineral oil.
24 Oct 85	Rabbits were certified healthy by a staff veterinarian and moved from quarantine to the GLP Suite. Rabbits were separated into test groups and weighed.
28 Oct 85	Animals were checked for preexisting ocular injury (Group 1).
29 Oct 85	Group 1 rabbits were dosed and weighed. Eyes were scored 1 and 4 hours after exposure.
30 Oct 85	Eyes were scored 24 hours after exposure (Group 1).
31 Oct 85	Eyes were scored 48 hours after exposure (Group 1).
1 Nov 85	Eyes were scored 72 hours after exposure. Study was terminated (Group 1).
5 Nov 85	Group 1 animals were weighed and submitted to necropsy.
11 Nov 85	Animals were checked for preexisting ocular injury (Group 2).
12 Nov 85	Group 1 animals were weighed and submitted to necropsy. Group 2 rabbits were dosed and weighed. Eyes were scored 1 and 4 hours after exposure.
13 Nov 85	Eyes were scored 24 hours after exposure (Group 2).
14 Nov 85	Eyes were scored 48 hours after exposure (Group 2).
15 Nov 85	Eyes were scored 72 hours after exposure (Group 2). Study (Group 2) was terminated and animals were weighed and submitted for necropsy.

Appendix D: TABULATED OCULAR DATA

CORNEAL OPACITY
(score by animal)

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F250	0	0	0	0	0	0
85F251	0	0	0	0	0	0
85F252	0	0	0	0	0	0
85F253	0	0	0	0	0	0
85F254	0	0	0	0	0	0
85F255	0	0	0	0	0	0

IRIS
(score by animal)

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F250	0	0	0	0	0	0
85F251	0	0	0	0	0	0
85F252	0	0	0	0	0	0
85F253	0	0	0	0	0	0
85F254	0	1	0	0	0	0
85F255	0	0	0	0	0	0

Appendix D (cont.): TABULATED OCULAR DATA

CONJUNCTIVA (CHEMOSIS)
(score by animal)

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F250	0	0	0	0	0	0
85F251	0	0	0	0	0	0
85F252	0	0	0	0	0	0
85F253	0	0	1	0	0	0
85F254	0	0	0	0	0	0
85F255	0	0	0	0	0	0

CONJUNCTIVA (REDNESS)
(score by animal)

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F250	0	1	0	0	0	0
85F251	0	1	0	0	0	0
85F252	0	1	0	0	0	0
85F253	0	1	1	1	0	0
85F254	0	1	1	1	0	0
85F255	0	1	1	1	0	0

Appendix E: SUMMARY OF OCULAR OBSERVATIONS

One Hour After Dosing

Slight hyperemia was present in all 6 test rabbits and was confined to the lower bulbar and palpebral conjunctiva and the nictitating membrane. This vasodilatation was visible with the unaided eye. Slight hyperemia was present in the iris of one rabbit (85F254). All other structures appeared normal.

Four Hours After Dosing

Slight hyperemia was present in the conjunctiva of 3 of 6 rabbits. Slight corneal thickening was observed in one rabbit (85F251). All other structures appeared normal.

Twenty-four Hours After Dosing:

Slight hyperemia persisted in 3 of 6 rabbits. A small spot (0.2 mm) was noticed in one rabbit (85F255) after fluorescein staining. All other structures appeared normal.

Forty-eight Hours After Dosing

The small spot in rabbit 85F255 was still present. All other structures in each treated eye appeared normal.

Seventy-two Hours After Dosing

All structures examined by slit lamp appeared normal and no fluorescein staining was present.

Appendix F: PATHOLOGY REPORT

Study: GLP #85010, Toxicology Group.

Test: Primary Ocular Irritation Test.

Investigator: Dr. Hiatt.

Test Substance: Nitroseguanidine (CAS #674-81-7).

Species: Rabbit, NZW.

Age: Approximately 4 months old, 6 males.

History: See LAIR SOP-OP-STX-33. All animals were killed by exsanguination following sodium pentobarbital anesthesia.

Gross Findings:

LAIR ACC#	ANIMAL ID#	DIAGNOSIS
38439	85F250	Not remarkable (NR)
38440	85F251	NR
38441	85F252	NR
38466	85F253	NR
38467	85F254	NR
38468	85F255	NR

G. TRACY MAKOVEC, DVM
MAJ, VC
Comparative Pathology Branch

12 February 1986

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