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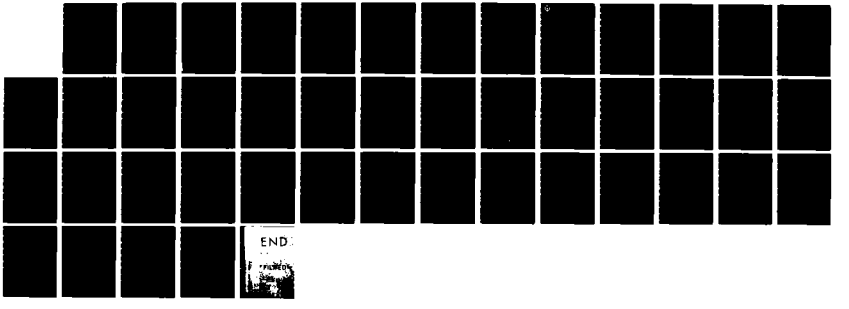

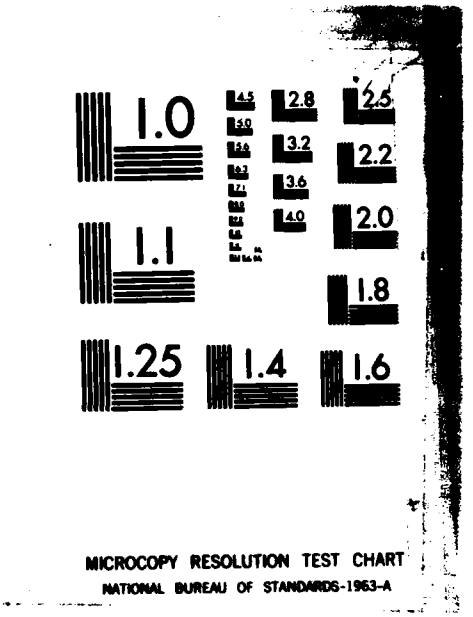
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MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

*INSTITUTE REPORT NO. 160*

# ACUTE DERMAL TOXICITY OF 1,2,3,4-Tetrahydro-6-Methyl-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6) IN RABBITS

*JUSTO RODRIGUEZ, BS, SP4*
*MARTHA A. HANES, DVM, CPT VC*
*and*
*GLEN E. MARRS, DVM, MS, MAJ VC*

**TOXICOLOGY GROUP,**
**DIVISION OF RESEARCH SUPPORT**



*SEPTEMBER 1983*

**Toxicology Series 47**

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

Acute Dermal Toxicity of 1,2,3,4-Tetrahydro-6-Methyl-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6) in Rabbits (Toxicology Series 47)--Rodriguez, Hanes and Marrs

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|---|
| 1. REPORT NUMBER<br>LAIR Institute Report No. 160 | 2. GOVT ACCESSION NO.<br>AD-A133134 | 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE *(and Subtitle)*<br>Acute Dermal Toxicity of 1,2,3,4-Tetrahydro-6-Methyl-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6) in Rabbits | | 5. TYPE OF REPORT & PERIOD COVERED<br>Final<br>21 Jul - 26 Aug 82<br>6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s)<br>Justo Rodriguez, BS, SP4<br>Martha A. Hanes, DVM, CPT VC<br>Glen E. Marrs, DVM, MS, MAJ VC | | 8. CONTRACT OR GRANT NUMBER(s) |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>Toxicology Group, Division of Research Support<br>Letterman Army Institute of Research<br>Presidio of San Francisco, CA 94129 | | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS<br>Project 3M161770A871 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS<br>US Army Medical Research and Development Command<br>Fort Detrick<br>Frederick, MD 21701 | | 12. REPORT DATE<br>September 1983<br>13. NUMBER OF PAGES<br>42 |
| 14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)* | | 15. SECURITY CLASS. *(of this report)*<br>UNCLASSIFIED<br>15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |

16. DISTRIBUTION STATEMENT *(of this Report)*

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18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Acute Dermal Toxicity, Insect Repellents, Toxicology, 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline, CHR6

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The acute dermal toxicity potential of the candidate insect repellent CHR6* was determined in rabbits using abraded skin sites and plastic covering over the exposed area for 24 hours. No animals died or showed clinical signs of toxicity. The treated animals showed a slight irritation on the back, primarily along the abraded areas. This compound should be exposed to further toxicological testing for human use potential as an insect repellent.

*Code Name for 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl)Quinoline

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

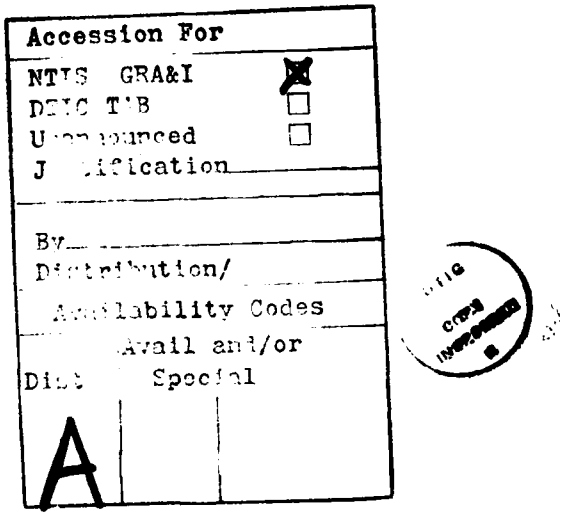
SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

## ABSTRACT

The acute dermal toxicity potential of the candidate insect repellent CHR6* was determined in rabbits using abraded skin sites and plastic covering over the exposed area for 24 hours. No animals died or showed clinical signs of toxicity. The treated animals showed a slight irritation on the back, primarily along the abraded areas. This compound should be exposed to further toxicological testing for human use potential as an insect repellent.


*Code Name for 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline


KEY WORDS: Acute Dermal Toxicity, Insect Repellents, Toxicology, 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline, CHR6

## PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

PROJECT/WORK UNIT/APC: Prevention of Military Disease Hazards
3M16770A871, WU: 201, APC: TL01

GLP STUDY NUMBER: 82020

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Martha A. Hanes, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP4 Justo Rodriguez, BS

PATHOLOGIST: MAJ Glen Marrs, DVM, MS, VC, Diplomate of American College of Veterinary Pathologists

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

TEST SUBSTANCE: 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)

INCLUSIVE STUDY DATES: 21 Jul - 26 Aug 82

OBJECTIVE: The purpose of this study was to determine the acute dermal toxicity potential of CHR6 in rabbits.

## ACKNOWLEDGMENTS

The authors wish to thank Leonard J. Sauers, SP5, MS; Thomas P. Kellner, SP4, BA; Lawrence Mullen, SP4, BS; Evelyn Zimmerman, SP4; and Carolyn M. Lewis, MS; for their assistance in conducting this study.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 82020 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Medical Laboratory Studies, outlined by the Food and Drug Administration.

JOHN T. FRUIN / DATE
COL, VC
Study Director

JUSTO RODRIGUEZ / DATE
SP4, USA
Co-Principal Investigator

GLEN E. MARRS / DATE 27 Jul 83
MAJ, VC
Pathologist

MARTHA A. HANES/ DATE 27 Jul 83
CPT, VC
Principal Investigator

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

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

30 Jun 83

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82020 the following inspections were made:

11 Aug 82
12 Aug 82
19 Aug 82

The report and raw data for this study were audited on 23 Jun 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct 82 report to management and the Study Director.

NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

TABLE OF CONTENTS

Table of Contents (continued)

Acute Dermal Toxicity of 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6) in Rabbits--Rodriguez et al

The goal of the insect repellent program is to develop better insect repellents for the protection of soldiers from insects and insect-borne diseases in the field. In the last several years the Division of Cutaneous Hazards, Letterman Army Institute of Research (LAIR), has tested a large number of chemical compounds, submitted by SRI International, the U.S. Department of Agriculture (USDA), and private industry, against a variety of mosquitoes, sand flies, fleas, bugs, ticks, and mites in animals and in vitro test systems. Several of these materials have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of, or in conjunction with, the current troop-issue insect repellent, 71.25% N,N-diethyl-m-toluamide (m-DEET) in ethanol. The Division of Cutaneous Hazards, LAIR, has also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent than the current troop-issue repellent in tests on animals.

## Toxicity Testing Repellent Program

It is now planned to test the best of the new compounds and formulations on human volunteers to confirm the results that have been obtained in the in vitro and animal tests and to evaluate their performance under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to insure that it is safe for application to the skin. The toxicity tests required for registration of a new insect repellent are prescribed by the Environmental Protection Agency (EPA). The basic animal toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and USAMRDC Human Use Committees. An acute dermal toxicity ($LD_{50}$) test of 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline (CHR6) is one of the animal toxicity tests requested by the Division of Cutaneous Hazards so that the chemical could be considered for human testing. If adverse toxicity data are obtained with the animal tests, the chemical will be eliminated from consideration, and the prospective tests on human volunteers will not be carried out. The toxicity testing program thereby serves as both a safety assessment and secondary screen in the repellent development scheme.

## Description of Test

Methods of testing compounds for their potential irritancy or toxicity have become standardized over the years by the cooperative efforts of EPA, FDA, the U.S. Consumer Product Safety Commission and numerous subcommittees, and Armed Forces Research departments (1-3).

A test for acute dermal toxicity evaluates the potential for systemic toxic effects of chemicals expected to come in contact with the skin. This is done by determining the median lethal dose ($LD_{50}$) of a single dermal exposure to the animal.

Dermal toxicity is one of the three categories of toxicity defined by route of exposure in the Federal Hazardous Substances Act (FHSA). The adult albino rabbit is the preferred species, for such reasons as size, ease of handling and restraint, and because its skin is the most permeable of all model species. The rabbit appears to be very sensitive to dermal insult. The animal's dorsal and lateral sections are close-clipped so that no less than 10% of the body surface area is available for application of material (4).

The maximum quantity of liquid test substance applied is 2 ml/kg. The test dose must remain in contact with the skin throughout the 24-hour exposure period. For liquids, this is assured by application of the dose inside an impermeable cuff made of plastic film. The cuff or sleeve is constructed so that the reinforced ends fit snugly around the trunk of the animal. The ends are tucked to permit the central portion to "balloon" and to furnish a reservoir for the dose. Such devices occlude the skin and thereby enhance penetration and potential toxicity of the test material. For this reason, routine use of occlusive dressing is not recommended unless anticipated human exposure warrants it. For materials of anticipated low toxicity, an initial range-finding dose of 2 g/kg of body weight (or approximately 2 ml/kg of body weight for a compound of unknown specific gravity) applied to five or more animals of each sex with abraded skin is sufficient to demonstrate a lack of appreciable dermal toxicity. At the end of the exposure periods, any residual material is gently removed with a gauze compress and the exposed area is examined daily for signs of systemic toxicity and localized dermal reaction. After the 14-day observation period, animals are sacrificed, a gross necropsy performed, and two sections of the exposed skin are processed for histopathology (5).

## Objective of Study

The objective of this study is to determine the acute dermal toxicity potential of CHR6 in rabbits.

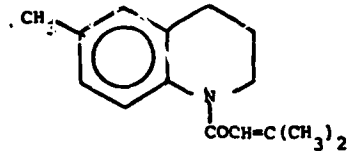
METHODS

Test Substance

Chemical name: 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)

CAS: Unknown

Molecular structure:




Empirical formula: $C_{15}H_{19}NO$

Other test substance information appears in Appendix A.

Compound Preparation

CHR6 was stored at 4 C and an undiluted aliquot was used for testing.

Animal Data

The animal data appear in Appendix B.

Environmental Conditions

The environmental conditions are listed in Appendix C.

Dosing

Dosing Levels: The test was conducted as a limit test (1) where 6 males and 6 females were assigned in two groups. Group 1 was the dose group (dose level of 2 ml of the test compound (CHR6)/kg body weight). Group 2 was a "nothing applied" control group. Six rather than 5 animals were used to assure compliance of the study in the event that one animal of either sex would need to be eliminated from the study due to non-compound related health problems.

Dose Volume: According to weight; actual range 4.2 - 5.6 ml of CHR6.

Duration of Exposure: 24 hours.

Method and Frequency of Administration: The application sites were abraded by using an instrument designed for this experiment. It has four small metal points mounted onto a flat piece of metal that is attached to a handle (1). The instrument was drawn across the area to be exposed so that only the stratum corneum was disrupted. The lines were approximately 2 cm apart over the entire exposed surface along the long axis of the body. Animals were wrapped with roll-type Kling® gauze (Johnson and Johnson, New Brunswick, NJ 08903, Lot 3608C228) and test material was administered by using a syringe of the appropriate dose volume. The gauze was then covered with plastic wrap from GSA bag (#NSN8105-00-655-8285) and taped on the ends and seam with Conform® adhesive tape (Kendall Hospital Products, Boston, MA 02110, Code No. 7233). Clinical signs were recorded within six hours of administration of the test substance. The bandages were removed after 24 hours. Excess material was wiped from the area.

## Observations

Animals were weighed six times during the study test period. Observations were recorded once a day between the hours of 1700 - 1900. At the end of the 2-week period, animals were sacrificed with sodium pentobarbital and necropsies were performed. Skin was taken from two sites on either side of the midline extending the length of exposed area and examined histopathologically. On the day prior to sacrifice the animals were bled by ear vein for a complete blood count (CBC) for hematology training.

## Duration of Study

Appendix D is a listing of historical study events.

## Changes in the Original Procedures or Protocol During the Study

1. Animals were received from quarantine on 9 August 1982, rather than 3 August 1982 as originally scheduled.

2. Animals were not weighed as per protocol during quarantine. Weights were taken on the day animals were received from quarantine.

3. Ear labeling was performed with indelible ink using number system specified in SOP OP-ARG-1.

# RESULTS

## Clinical Observations

During the course of the study, observations were split into two major categories - those that applied to the general systemic health of the animal and those which were related to skin exposure.

Systemic: No clinical signs were interpreted as significant signs of toxicity. Of the animals exposed to CHR6, 2 of 12 appeared excited and 2 of 12 appeared irritable. In the control animals, male and female, 3 of 12 appeared excited and 2 of 12 appeared irritable. No animals were eliminated from the study due to non-compound related health problems.

Dermal: The most notable signs related to dermal toxicity were erythema, edema, scaling, skin thickening, skin cracking, and scab and scar formation.

Summaries by symptom and group were created from the data. To elucidate the most severe reaction that could be expected, a table was created, for each clinical sign, that portrayed the maximum changes (intensity) and the maximum area involved (Tables 1a - 7a, Appendix E). Location, approximate percentage of area, and intensity were "graded" according to a code at the bottom of the summary sheets. To elucidate the most probable reaction of a group of animals exposed to the chemical, an additional table ("b") was created to demonstrate the most frequent reaction for each clinical sign. Figures 1 and 2 (Appendix F) were prepared for each sex to show the evolution of the irritation and healing processes.

Erythema, redness of the skin, was seen from the day after dosing and approximately 4 days subsequently along the abrasions of the back in 12 of 12 animals exposed to CHR6. The maximum severe intensity was moderate erythema; the maximum and most frequent area involved was greater than (>) 50% seen in 10 of 12 exposed animals (Tables 1a,1b).

Edema was associated with the first two days of redness and was seen along the abrasions on the back. Only 1 of 12 animals exposed to CHR6 developed edema (Tables 2a, 2b).

Scaling along the abrasions at the back, flanks, and sides was observed in 11 of 12 animals exposed to CHR6 (Tables 3a, 3b). One of the 12 animals had a defined (D) scaling (Table 3a). The most frequent intensity was slight (9 of 12 animals, Table 3b).

Skin thickening appeared in 7 of 12 animals exposed to CHR6, the thickening was scored as slight to very slight $\leq$ 5% of the area exposed. The most severe intensity was a moderate skin thickening which was exhibited in 2 of 12 exposed animals. The most severe and frequent locations were along the abrasions of the back (Tables 4a, 4b). Skin thickening was seen for approximately 4 days in female rabbits (Figures 1 and 2).

Skin cracking was found in 2 of 12 animals exposed to the test compound (CHR6). In both animals, the most severe and frequent intensity skin cracking was scored as "slight." The greatest area was $\leq$ 50% along the abrasions in 1 of 12 animals (Tables 5a, 5b).

Scar formation occurred in 4 of 12 animals for a period of approximately 3 days. A scar of slight intensity was apparent in 1 of 12 exposed animals. The most severe area covered by the scar along the back was >50% (1 of 12 treated animals). The most frequent intensity was a very slight scar formation, $\leq$5% of the back area, on 4 of 12 animals (Tables 6a, 6b).

Scab formation was seen for a period of approximately 2 days in 8 of 12 animals exposed to CHR6 (Tables 7a, 7b). The most severe intensity, seen in only one animal, was a moderate scab; the maximum area involved was $\leq$ 10% on the back and flank areas. A slight or very slight scab formation was observed on approximately 4 of 12 animals; the most frequent location was on the back.

No dermal signs were recorded as significant in the control animals.

Treatment of Animal Disease and Injury

The rabbits for Study 82020 were treated prophylactically for coccidiosis with sulfaquinoline during the quarantine for 17 days rather than two weeks, as stated in the protocol.

Gross Pathological Observations

Appendix G contains gross pathological observations.

DISCUSSION

The acute dermal toxicity test revealed that CHR6 was not toxic at 2 ml/kg when applied to approximately 10% of the body surface of rabbits during a 24-hour exposure period.

The dermal toxicity of the compound was made on the basis of three major catergories: lethality, clinical observations (systemic and dermal), and pathologic findings.

No animals died during the period of the experiment; therefore, exposure to CHR6 at the levels tested did not demonstrate lethality. During clinical observations, the greatest percent of animals exposed showed erythema, scaling, and skin thickening. Those particular results demonstrate that the chemical caused skin reactivity although the severity was mostly in the slight to very slight categories.

The pathology report indicated that CHR6 caused or intensified inflammatory response in the skin of the exposed rabbits.

## CONCLUSION

During a 24-hour exposure period and 14-day observation period, rabbits exposed to CHR6 demonstrated minimal dermal irritation on clipped and abraded skin sites and no toxicity.

## RECOMMENDATION

On the basis of acute dermal toxicity studies and primary dermal irritation studies, CHR6 should be considered for continued toxicology testing for potential human use as an insect repellent.

REFERENCES

1. Environmental Protection Agency. Good Laboratory Practices proposed regulations (40 CFR 770,771,772) and preamble as published in the Federal Register, 22 Aug 78, 9 May 79, 26 Jul and 18 Apr 80, (45 FR26373).

2. Food and Drug Administration. Good Laboratory Pracatices regulations (21 CFR58) and preamble, published in the Federal Register, 22 Dec 78 (45 FR59986-60025).

3. Interagency Regulatory Liaison Group Testing Standards and Guidelines Work Group. Recommended guideline for acute dermal toxicity test. 1981.

4. Association of Food and Drug Officials of U.S. Appraisal of the safety of chemicals in foods, drugs and cosmetics (4th printing). 1959.

5. Committee for the Revision of NAS Publication 1138. Committee on Toxicology, National Research Council. Principles and procedures for evaluating the toxicity of household substances. Prepared for the Consumer Product Safety Commission. Washington, DC: National Academy of Sciences, 1977.

Page



APPENDICES

## DATA SHEET FOR COMPOUNDS

HAM

SB NR. WR2502021A

SUBMITTER: Starks Associates, Inc.
1280 Niagara St.
Buffalo, New York 14213

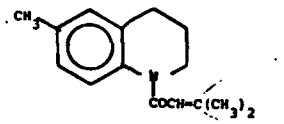
SUBMITTER KEY NR.: 0205

| DATE SHIPPED | DAY | MO | YR | DATE RECEIVED | DAY | MO | YR | DATE ACKNOWLEDGED | DAY | MO | YR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | 5 | 82 | | 27 | 5 | 82 | | | | |

NAME OF COMPOUND: 1,2,3,4-Tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline

STRUCTURE

2.5




BK22960

MM
AP

Ship 1200g to LAIR with all data sheets & spectra
attn: Maj. Eisenberg
Pls Ship on priority 1

| MOL FORMULA | MOL WT |
|---|---|
| $C_{15}H_{19}NO$ | 229.171 |

APPEARANCE: viscous yellow oil

QUANTITY: 1268 g

CODE NR.: JF16-29-1

NOTEBOOK REF.: JF16-29-1

PREPARED BY: S. L. Mucha

TEST SYSTEM:

B.P. | M.P. | REFRACT. INDEX

I.R.: See attached spectrum

U.V.: See attached spectrum

ANALYSES

| ELEMENT | CALCULATED | FOUND | WE FOUND |
|---|---|---|---|
| C | 78.57 | 78.43 | |
| H | 8.35 | 8.30 | |
| N | 6.11 | 6.24 | |
| S | | | |

CHROMATOGRAPHY: *Homogeneous (JTB precoated TLC plates, glass support, 5 cm x 10 cm, 0.25 mm silica gel F-254; detection - ultraviolet light.)

LITERATURE
1. The compound is unknown to the chemical literature.

STABILITY (Check Where Applicable)

| | STABLE | UNSTABLE | | STABLE | UNSTABLE |
|---|---|---|---|---|---|
| ACID | 56 | | OTHER | | |
| BASE | 57 | | | 60 | |
| HEAT | 58 | | | 61 | |
| LIGHT | 59 | | | 62 | |

SOLUBILITY (Check Where Applicable)

| | V. SOL | MOD. SOL | SL SOL | | V. SOL | MOD. SOL | SL SOL |
|---|---|---|---|---|---|---|---|
| WATER | 64 | | | CHLOROFORM | 70 | | |
| ACID | 65 | | | ETHER | 71 | | |
| BASE | 66 | | | PET. ETHER | 72 | | |
| METHANOL | 67 | | | BENZENE | 73 | | |
| ETHANOL | 68 | | | | 74 | | |
| ACETONE | 69 | | | | 75 | | |

HYGROSCOPIC: YES ☐ NO ☒ 63

EQUATIONS INDICATING SYNTHETIC ROUTE

PRIORITY 1

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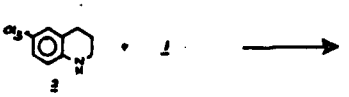
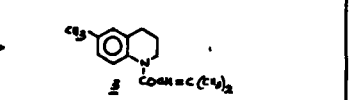
b.  (2) + (1) ⟶  (3)

REMARKS: * 1. Ether; Rf=0.80
2. Ethyl acetate; Rf=0.77
3. Methylene chloride; Rf=0.44; faint spot Rf=0.65.

N.M.R. - see attached spectrum.

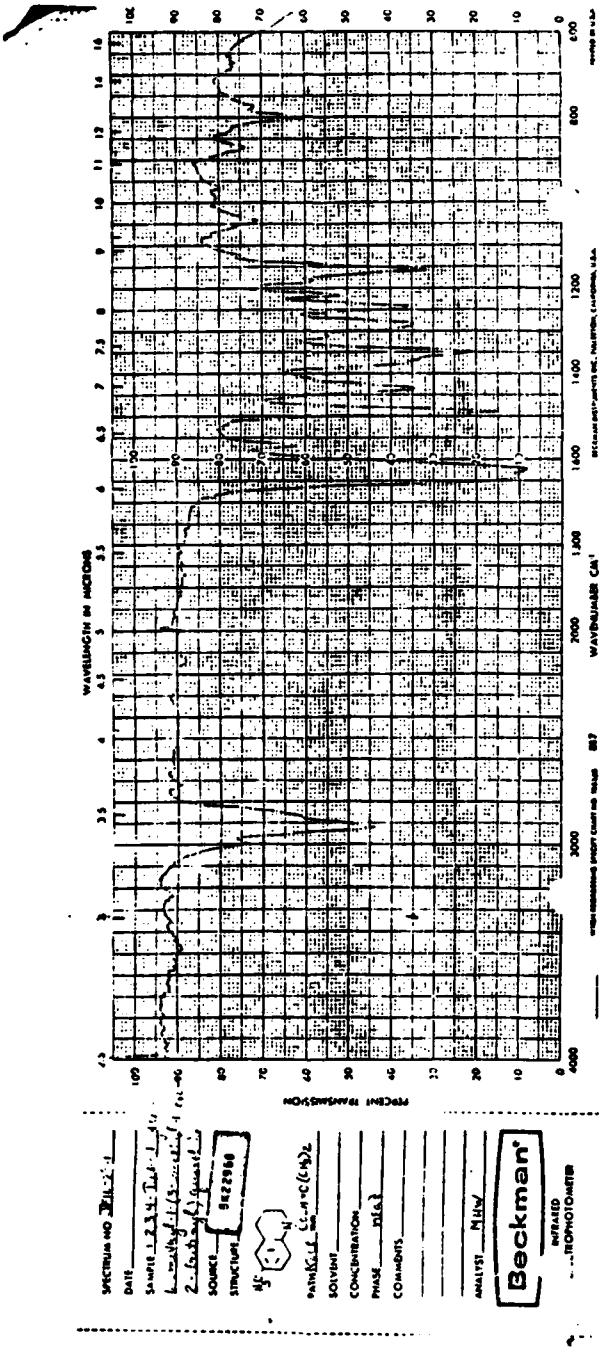
(L)

| TO COMMERCIALLY DESCRIBE? | SYNTHESIZED UNDER GOV'T SUPPORT? | GIFT? | PURCHASED? |
|---|---|---|---|
| ☐ | ☒ | ☐ | ☐ |

WRAMC FORM 108 1 APR 1964 SUPERSEDES WRAMC FORM 108, 10 AUG 61, WHICH IS OBSOLETE.



APPENDIX A

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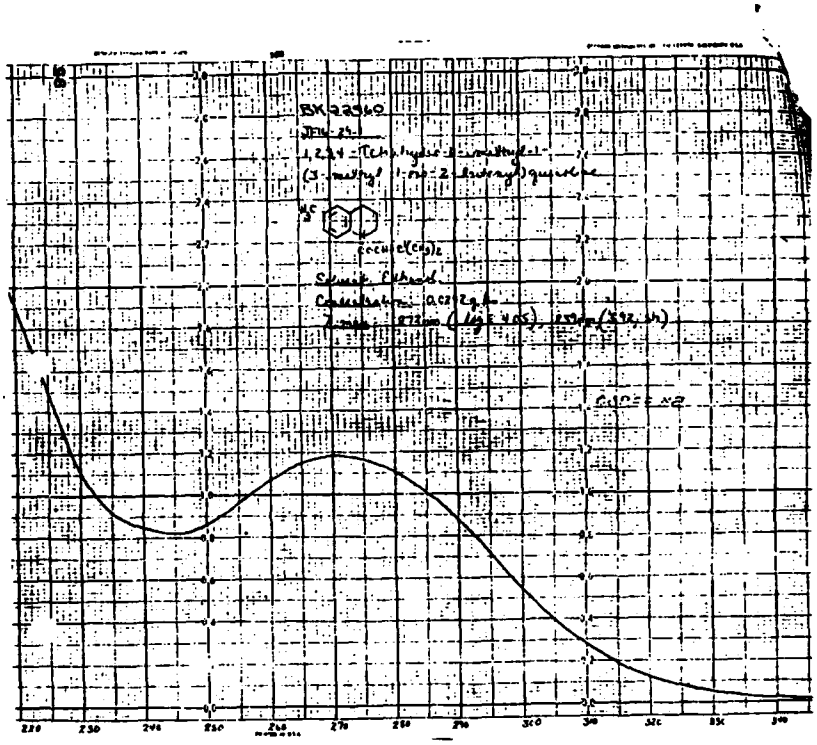
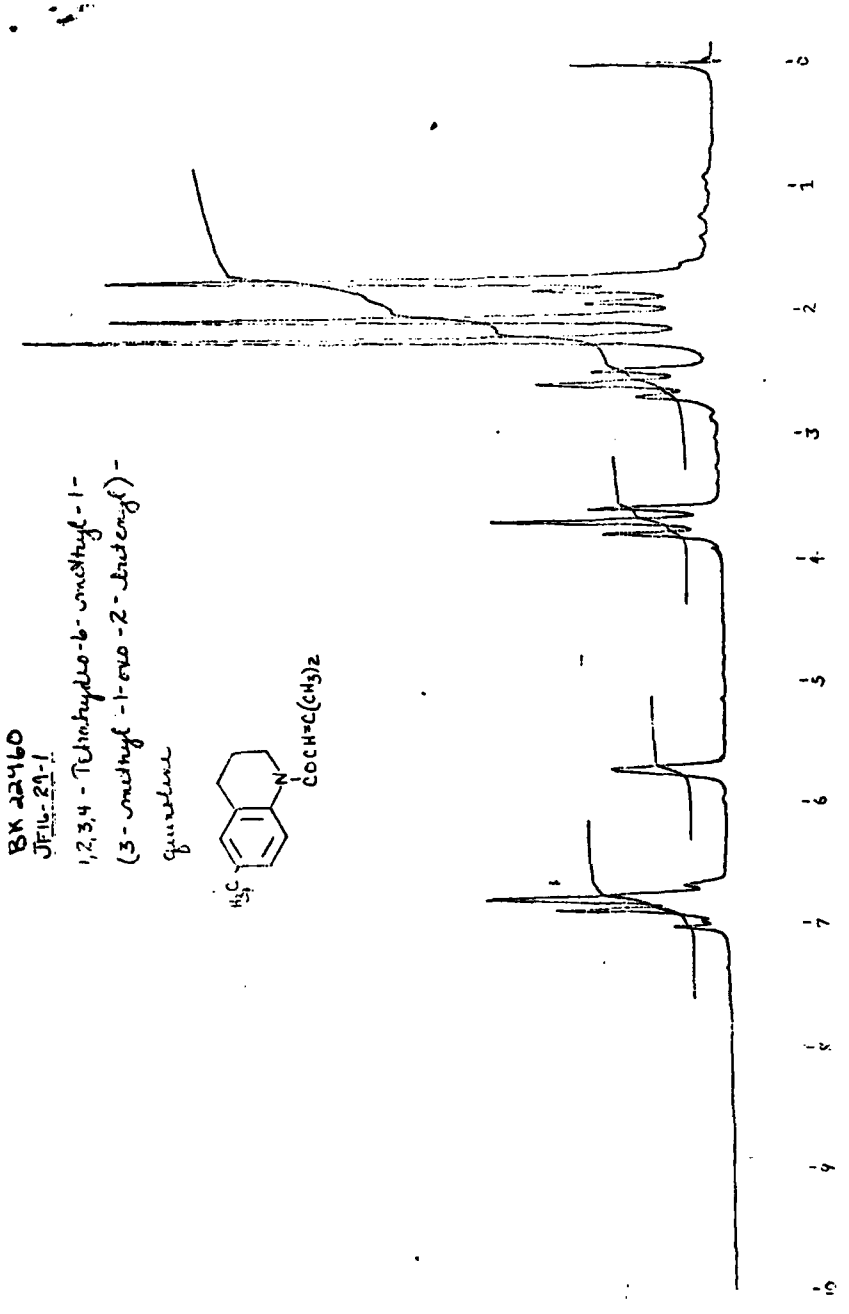


APPENDIX A (cont.)




APPENDIX A (Concluded)

# ANIMAL DATA

Species: Rabbit

Strain: New Zealand White

Rationale for selection: The New Zealand White Rabbit is a proven mammalian model for acute dermal studies because of its size, ease of handling, restraint and skin permeability.

Source: Elkhorn Rabbitry
565 Starr Way
Watsonville, CA 95076

Pretest Conditioning:

a. Quarantine for 17 days

b. Animals clipped the day before dosing

Restraint: Manual restraint during dosing. Animals left their bandages alone over the 24-hour period. Some bandages slipped in the flank area; this did not affect the systemic toxicity of the chemical in question.

Sex: Male and female

Age: Young adult

Method of Randomization: Random Numbers Table

Animals in each group: 2 groups of 12 animals. 6 males and 6 females in each group. Group 1 test animals, Group 2 wrapped control animals.

Condition of animals at start of study: Normal

Mean weight ($\pm$ 1 standard deviation) at dosing:

2440 ($\pm$ 215 g ) for test animals
2390 ($\pm$ 224 g) for control animals

Mean weight ($\pm$ 1 standard deviation) at sacrifice:

2475 ($\pm$ 198 g) for test animals
2412 ($\pm$ 154 g) for control animals

Identification procedures: Ear labeled with laboratory indelible ink, using number system specified in SOP-OP-ARG-1.

## ENVIRONMENTAL CONDITIONS

Caging: Number/cage = 1; type used = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralston Purina Rabbit Chow 5322.

Water: Central line to cage battery.

Temperature: 71 $\pm$ 2 F (21 + 1 C)

Relative humidity: 47 $\pm$ 2%

Photoperiod: 0600 - 2000 hour/day (light 14 hours).

APPENDIX C

## HISTORICAL LISTING OF STUDY EVENTS

| Date | Day | Event |
|---|---|---|
| 22 Jul 82 | A0-A18 | Animals arrived at LAIR. They were examined for illness, and treated for coccidiosis. The animals were held in acclimation status for 17 days until all of them were free of signs of the diseases. |
| 9 Aug 82 | A19-A21 | Rabbits were received from quarantine, weighed, randomized, separated into test groups and close clipped along the back and side areas IAW SOP-OP-STX-30. |
| 12 Aug 82 | 0 | Rabbits were weighed and dosed IAW SOP-OP-STX-30. |
| 12-15 Aug 82 | 0-3 | 1/2, 24, 48, and 72 hour scores were recorded. |
| 13-26 Aug 82 | 1-14 | Observations recorded at 0900 hours. |
| 13 Aug 82 | 1 | Bandaging materials were removed and rabbits were observed at 0900 hours, approximately 24 hours after dosing. |
| 9,12,16,19, 13,26 Aug 82 | A19,0,4, 7,11,14 | Rabbits were weighed. |
| 25 Aug 82 | 13 | Rabbits were bled by ear vein method for hematology training. |
| 26 Aug 82 | 14 | Rabbits were not fed. They were close clipped (as necessary),euthanized; exposed tissue, obtained at necropsy was for histopathological study. |

APPENDIX D

# LIST OF TABLES



APPENDIX E

GLP Study 82020
Dermal Summary Sheet

ERYTHEMA

(Table 1a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX (%) | ALL LOCATIONS | |
|---|---|---|---|---|---|---|---|---|
| CHR6 | M | 6/6 | 1/6 | M | 5/6 | 51 | 6/6 | B |
| CHR6 | F | 6/6 | 1/6 | M | 5/6 | 51 | 6/6 | B |
| Control | F/M | 0/12 | _____ | | _____ | | _____ | |

ERYTHEMA

(Table 1b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY FREQ. | AREA INVOLVED NO. | AREA INVOLVED FREQ. (%) | LOCATION | |
|---|---|---|---|---|---|---|---|---|
| CHR6 | M | 6/6 | 6/6 | SL | 5/6 | 10-51 | 6/6 | B |
| CHR6 | F | 6/6 | 4/6 | SL | 5/6 | 51 | 6/6 | B |
| Control | F/M | 0/12 | _____ | | _____ | | _____ | |

| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| SL = | Slight | 10 = | ≤ 10% | B = | Back |
| M = | Moderate | 25 = | ≤ 25% | C = | Thorax |
| D = | Defined | 49 = | ≤ 50% | K = | Flank |
| S = | Severe | 51 = | ≥ 50% | S = | Lateral |
| | | | | O = | Abrasions |
| | | | | T = | Teat |
| | | | | U = | Umbilicus |

GLP Study 82020
Dermal Summary Sheet

EDEMA

(Table 2a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX | ALL LOCATIONS |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 0/6 | ——— | | ——— | | ——— |
| CHR6 | F | 1/6 | 1/6 | SL | 1/6 | 51 | 1/6 B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— |

EDEMA

(Table 2b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY FREQ. | AREA INVOLVED NO. | AREA INVOLVED FREQ. | LOCATION |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 0/6 | ——— | | ——— | | ——— |
| CHR6 | F | 1/6 | 1/6 | SL | 1/6 | 51 | 1/6 B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— |

| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| SL = | Slight | 10 = | ≤ 10% | B = | Back |
| M = | Moderate | 25 = | ≤ 25% | C = | Thorax |
| D = | Defined | 49 = | ≤ 50% | K = | Flank |
| S = | Severe | 51 = | ≥ 50% | S = | Lateral |
| | | | | O = | Abrasions |
| | | | | T = | Teat |
| | | | | U = | Umbilicus |

GLP Study 82020
Dermal Summary Sheet

SCALING

(Table 3a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX | ALL LOCATIONS |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 6/6 | 1/6 | D | 3/6 | 51 | 6/6B;3/6 K; 3/6 S; 1/6 O |
| CHR6 | F | 5/6 | 2/6 | M | 1/6 | 51 | 4/6B;1/6 K; 2/6 S |
| Control | F/M | 0/12 | ——— | | ——— | | ——— |

SCALING

(Table 3b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY FREQ. | AREA INVOLVED NO. | AREA INVOLVED FREQ. | LOCATION |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 6/6 | 5/6 | SL-V | 5/6 | 10 | 6/6 B |
| CHR6 | F | 5/6 | 4/6 | SL | 4/6 | 5 | 4/6 B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— |

| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| SL = | Slight | 10 = | ≤ 10% | B = | Back |
| M = | Moderate | 25 = | ≤ 25% | C = | Thorax |
| D = | Defined | 49 = | ≤ 50% | K = | Flank |
| S = | Severe | 51 = | ≥ 50% | S = | Lateral |
| | | | | O = | Abrasions |
| | | | | T = | Teat |
| | | | | U = | Umbilicus |

GLP Study 82020
Dermal Summary Sheet

SKIN THICKENING

(Table 4a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX | ALL LOCATIONS | |
|---|---|---|---|---|---|---|---|---|
| CHR6 | M | 3/6 | 1/6 | SL | 1/6 | 25 | 3/6 | B |
| CHR6 | F | 4/6 | 2/6 | M | 1/6 | 51 | 4/6 | B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— | |

SKIN THICKENING

(Table 4b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY FREQ. | AREA INVOLVED NO. | AREA INVOLVED FREQ. | LOCATION | |
|---|---|---|---|---|---|---|---|---|
| CHR6 | M | 3/6 | 2/6 | V | 3/6 | 5 | 3/6 | B |
| CHR6 | F | 4/6 | 3/6 | V | 2/6 | 5-10 | 4/6 | B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— | |

| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| SL = | Slight | 10 = | ≤ 10% | B = | Back |
| M = | Moderate | 25 = | ≤ 25% | C = | Thorax |
| D = | Defined | 49 = | ≤ 50% | K = | Flank |
| S = | Severe | 51 = | ≥ 50% | S = | Lateral |
| | | | | O = | Abrasions |
| | | | | T = | Teat |
| | | | | U = | Umbilicus |

GLP Study 82020
Dermal Summary Sheet

SKIN CRACKING

(Table 5a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX | ALL LOCATIONS |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 0/6 | _____ | | _____ | | _____ |
| CHR6 | F | 2/6 | 2/6 | SL | 1/6 | 49 | 2/6 B |
| Control | F/M | 0/12 | _____ | | _____ | | _____ |

SKIN CRACKING

(Table 5b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY FREQ. | AREA INVOLVED NO. | AREA INVOLVED FREQ. | LOCATION |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 0/6 | _____ | | _____ | | _____ |
| CHR6 | F | 2/6 | 2/6 | SL | 1/6 | 5-10-49 | 2/6 B |
| Control | F/M | 0/12 | _____ | | _____ | | _____ |

| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| | | | | B = | Back |
| SL = | Slight | 10 = | ≤ 10% | C = | Thorax |
| | | | | K = | Flank |
| M = | Moderate | 25 = | ≤ 25% | S = | Lateral |
| | | | | O = | Abrasions |
| D = | Defined | 49 = | ≤ 50% | T = | Teat |
| | | | | U = | Umbilicus |
| S = | Severe | 51 = | ≥ 50% | | |

GLP Study 82020
Dermal Summary Sheet

SCAR FORMATION

(Table 6a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX | ALL LOCATIONS | |
|---|---|---|---|---|---|---|---|---|
| CHR6 | M | 3/6 | 1/6 | SL | 3/6 | 5 | 3/6 | B |
| CHR6 | F | 1/6 | 1/6 | V | 1/6 | 5-51 | 1/6 | B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— | |

SCAR FORMATION

(Table 6b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY FREQ. | AREA INVOLVED NO. | AREA INVOLVED FREQ. | LOCATION | |
|---|---|---|---|---|---|---|---|---|
| CHR6 | M | 3/6 | 3/6 | V | 3/6 | 5 | 3/6 | B |
| CHR6 | F | 1/6 | 1/6 | V | 1/6 | 5-51 | 1/6 | B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— | |

| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| | | | | B = | Back |
| SL = | Slight | 10 = | ≤ 10% | C = | Thorax |
| | | | | K = | Flank |
| M = | Moderate | 25 = | ≤ 25% | S = | Lateral |
| | | | | O = | Abrasions |
| D = | Defined | 49 = | ≤ 50% | T = | Teat |
| | | | | U = | Umbilicus |
| S = | Severe | 51 = | > 50% | | |

APPENDIX E (Cont.)

GLP Study 82020
Dermal Summary Sheet

SCAB FORMATION

(Table 7a)

Summary for Most Severe Intensity, Maximum Area, and All Locations

| GROUP | SEX | TOTAL | INTENSITY NO. | INTENSITY MAX | AREA INVOLVED NO. | AREA INVOLVED MAX | ALL LOCATIONS |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 4/6 | 2/6 | SL | 1/6 | 10 | 3/6 B; 1/6 K |
| CHR6 | F | 4/6 | 1/6 | M | 4/6 | 5 | 3/6 B; 1/6 K |
| Control | F/M | 0/12 | ——— | | ——— | | ——— |

SCAB FORMATION

(Table 7b)

Summary for Most Frequent Intensity, Area, and Location

| GROUP | SEX | TOTAL | INTENSITY NO | INTENSITY NO. | AREA INVOLVED FREQ.EQ. | | LOCATION |
|---|---|---|---|---|---|---|---|
| CHR6 | M | 4/6 | 2/6 | V-SL | 3/6 | 5 | 3/6 B |
| CHR6 | F | 4/6 | 2/6 | V | 4/6 | 5 | 3/6 B |
| Control | F/M | 0/12 | ——— | | ——— | | ——— |

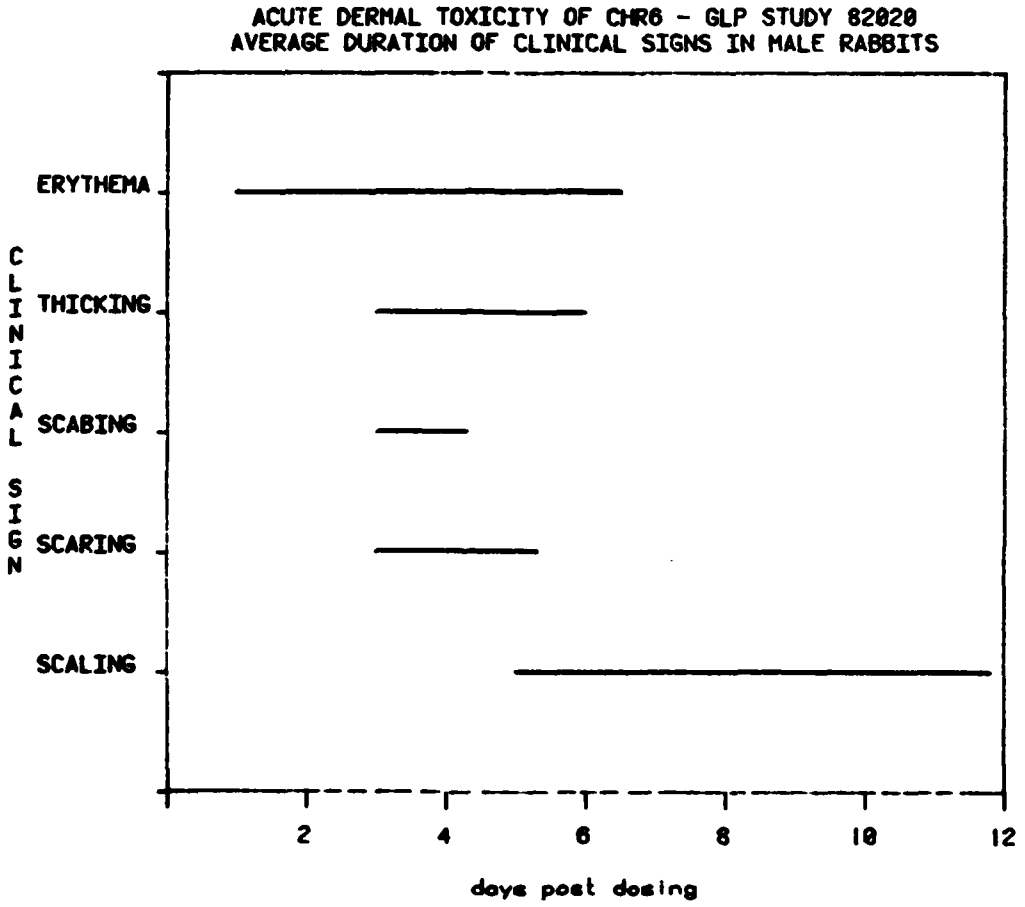
| Severity | | Exposed Area | | Location | |
|---|---|---|---|---|---|
| Symbol | Explanation | Symbol | Explanation | Symbol | Explanation |
| V = | Very Slight | 5 = | ≤ 5% | A = | Abdomen |
| SL = | Slight | 10 = | ≤ 10% | B = | Back |
| M = | Moderate | 25 = | ≤ 25% | C = | Thorax |
| D = | Deined | 49 = | ≤ 50% | K = | Flank |
| S = | Severe | 51 = | ≥ 50% | S = | Lateral |
| | | | | O = | Abrasions |
| | | | | T = | Teat |
| | | | | U = | Umbilicus |

APPENDIX E (Concluded)

## LIST OF FIGURES

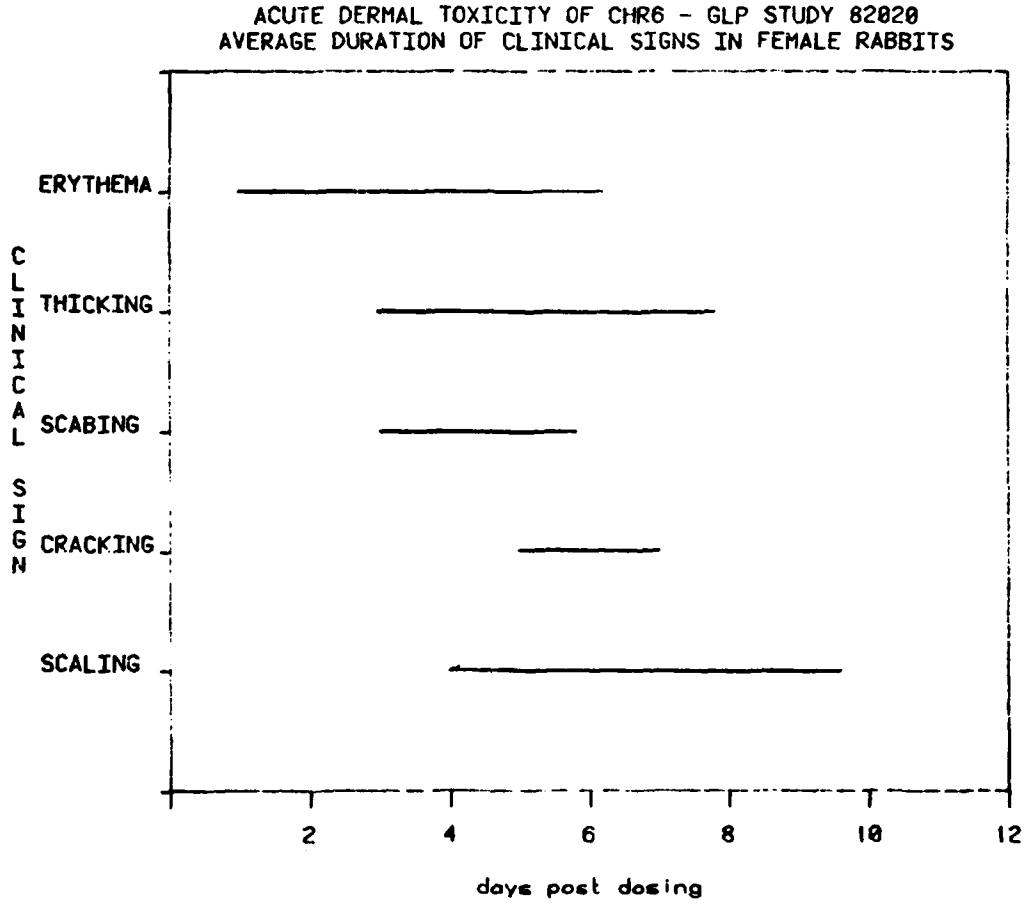
Page

FIGURE 1




APPENDIX F (Cont.)

FIGURE 2




APPENDIX F (Concluded)

PATHOLOGY REPORT

GLP Study 82020

Acute Dermal Toxicity Study of CHR6

History: The purpose of this study was to determine the acute dermal toxicity of 1,2,3,4 - Tetrahydro-6-methyl-1-(3-methyl-oxo-2-butenyl) quinoline (CHR6) in male and female New Zealand White Rabbits. Two ml/kg of tested material was applied to the clipped and abraded skin of the rabbits in group 1 for 24 hours. The skin of the control rabbits in group 2 was clipped and abraded.

After a 14 day observation period, the rabbits were submitted for necropsy. They were killed by exsanguination from severed axillary vessels while under anesthesia produced by intravenous injection of pentobarbitol. Complete gross necropsies were performed and two specimens of skin from each exposed area were fixed in neutral buffered formalin, embedded in paraffin, sectioned at approximately 6 micrometers, and stained with hematoxylin and eosin for microscopic examination.

Gross necropsy findings: No gross lesions were observed in any of the controls or rabbits exposed to the tested compound.

Microscopic findings: Three types of microscopic lesions were observed in the rabbit skin from the clipped and abraded sites. The most common type lesion was a minimal to mild, focal, multifocal, or diffuse infiltration of macrophages, lymphocytes and plasma cells (collectively referred to as mononuclear inflammatory cells) in the upper 15% of the dermis, immediately beneath the epidermis. The second most common lesion was a minimal to mild, focal or multifocal epidermal hyperplasia. The epidermis in these foci was 2 to 3 times the thickness of the more normal epidermis. The third lesion was a minimal to mild, focal or diffuse proliferation of fibroblasts and increased amounts of collagen (referred to as fibrosis). The fibrosis was restricted to the superficial dermis. Microscopic findings in each skin section examined are tabulated in Table I. Table II is a summary of the incidence of skin lesions by sex and experimental group.

Mononuclear inflammatory cell infiltration was present in the superficial dermis of 6/6* male and 5/6 female rabbits exposed to CHR6 and 2/6 male and 1/6 female control rabbits. Epidermal hyperplasia was present in 3/6 male and 5/6 female rabbits exposed to CHR6 and 1/6 male and 2/6 female control rabbits. Dermal

*Number of rabbits affected/Number of rabbits in treatment group

fibrosis was present in 4/6 male and 3/6 female rabbits exposed to CHR6 and 0/6 male and 0/6 female control rabbits. The mononuclear inflammatory cell infiltration and epidermal hyperplasia, though present in both exposed and controls, occurred in greater incidence and/or relative severity in the exposed rabbits. The CHR6 either caused or intensified these two lesions in the abraded skin of the exposed rabbits. The dermal fibrosis, present only in exposed rabbits, was either caused by exposure to CHR6 or was due to the intensified inflammatory response that resulted from the CHR6 exposure.

In summary, application of CHR6 to close clipped abraded skin of rabbits for 24 hours causes or intensifies an inflammatory response in skin that can be detected 14 days after application.

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS
Diplomate, A.C.V.P.
MAJ, VC
Pathology Services Group
Division of Research Support

16 February 1983

GLP Study 82-020

Acute Dermal Toxicity of CHR6

Group I Male Rabbits Exposed to 2 ml CHR6/kg Body Weight

| Animal# | Pathology Accession# | Microscopic Findings |
|---|---|---|
| 82F00116 | 32693-1 | Epidermal hyperplasia, focal, mild<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal |
| | 32693-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal |
| 82F00121 | 32697-1 | Mononuclear infiltrate cell infiltrate, diffuse, minimal |
| | 32697-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal |
| 82F00123 | 32699-1 | Epidermal hyperplasia, focal, minimal<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, focal, minimal |
| | 32699-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, focal, minimal |
| 82F00125 | 32700-1 | Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, focal, minimal |
| | 32700-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, focal, minimal |
| 82F00127 | 32702-1 | Mononuclear inflammatory cell infiltrate, diffuse, minimal |
| | 32702-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, diffuse, mild |
| 82F00129 | 32704-1 | Epidermal hyperplasia, focal, minimal<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, focal, minimal |
| | 32704-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal |

Group II - Male Rabbits - Control

| | | |
|---|---|---|
| 82F00117 | 32694-1 | Essentially normal skin |
| | 32694-2 | Mononuclear inflammatory cell infiltrate, multifocal, minimal |
| 82F00118 | 32695-1 | Essentially normal skin |
| | 32695-2 | Essentially normal skin |
| 82F00120 | 32696-1 | Essentially normal skin |
| | 32696-2 | Essentially normal skin |
| 82F00122 | 32698-1 | Essentially normal skin |
| | 32698-2 | Mononuclear inflammatory cell infiltrate, multifocal, minimal |
| 82F00126 | 32701-1 | Epidermal hyperplasia, focal, minimal |
| | 32701-2 | Essentially normal skin |
| 82F00128 | 32703-1 | Essentially normal skin |
| | 32703-2 | Essentially normal skin |

GLP Study 82-020

Acute Dermal Toxicity of CHR6

Group I Female Rabbits Exposed to 2 ml CHR6/kg Body Weight

| Animal ID# | Pathology Accession# | Microscopic Findings |
|---|---|---|
| 82F00102 | 32681-1 | Epidermal hyperplasia, focal, minimal |
| | 32681-2 | Essentially normal skin |
| 82F00108 | 32685-1 | Epidermal hyperplasia, multifocal, minimal<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, diffuse, minimal |
| | 32685-2 | Mononuclear inflammatory cell infiltrate, diffuse, mild<br>Fibrosis, diffuse, mild |
| 82F00110 | 32687-1 | Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, diffuse, minimal |
| | 32687-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal |
| 82F00111 | 32688-1 | Epidermal hyperplasia, multifocal, minimal<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, diffuse, minimal |
| | 32688-2 | Mononuclear inflammatory cell infiltrate, diffuse, minimal<br>Fibrosis, diffuse, minimal |
| 82F00113 | 32690-1 | Epidermal hyperplasia, multifocal, minimal<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal |
| | 32690-2 | Epidermal hyperplasia, focal, minimal |
| 82F00115 | 32692-1 | Epidermal hyperplasia, focal, minimal |
| | 32692-2 | Epidermal hyperplasia, focal, minimal<br>Mononuclear inflammatory cell infiltrate, diffuse, minimal |

Group II Female Rabbits - Control

| | | |
|---|---|---|
| 82F00104 | 32682-1 | Essentially normal skin |
| | 32682-2 | Essentially normal skin |
| 82F00106 | 32683-1 | Essentially normal skin |
| | 32683-2 | Essentially normal skin |
| 82F00107 | 32684-1 | Mononuclear inflammatory cell infiltrate, focal, minimal |
| | 32684-2 | Essentially normal skin |
| 82F00109 | 32686-1 | Epidermal hyperplasia, focal, minimal |
| | 32686-2 | Essentially normal skin |
| 82F00112 | 32689-1 | Epidermal hyperplasia, focal, minimal |
| | 32689-2 | Epidermal hyperplasia, focal, minimal |
| 82F00114 | 32691-1 | Essentially normal skin |
| | 32691-2 | Essentially normal skin |

APPENDIX G (Cont.)

GLP Study 82-020

Acute Dermal Toxicity of CHR6

Table II

Incidence of Microscopic Skin Lesions by Sex and Experimental Group

| Group# | Sex | Dosage | Normal Skin | Infiltration | Fibrosis | Hyperplasia |
|---|---|---|---|---|---|---|
| I | Male | 2 ml/kg | 0/6 | 6/6 | 4/6 | 3/6 |
| II | Male | Control | 3/6 | 2/6 | 0/6 | 1/6 |
| I | Female | 2 ml/kg | 0/6 | 5/6 | 3/6 | 5/6 |
| II | Female | Control | 3/6 | 1/6 | 0/6 | 2/6 |

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