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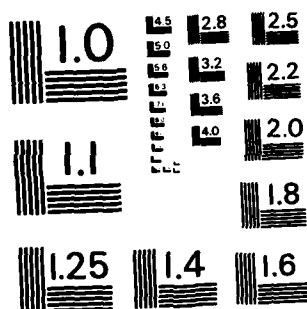
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INSTITUTE REPORT NO. 151

ACUTE DERMAL TOXICITY POTENTIAL OF (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) IN RABBITS

LAWRENCE MULLEN, BS, SP4
MARTHA A. HANES, DVM, CPT VC
and
PAUL MELLICK, DVM, PhD, LTC VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

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AUG 25 1983
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JUNE 1983

Toxicology Series 46

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

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Acute Dermal Toxicity Potential of (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5) in Rabbits (Toxicology Series 46)--Mullen, Hanes and Mellick

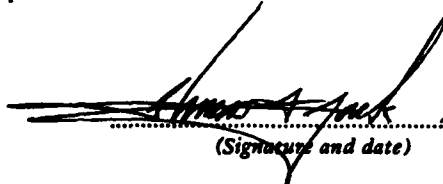
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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute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


..... 13 June 83
(Signature and date)

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4. TITLE (and Subtitle) Acute Dermal Toxicity Potential of (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-1-Butenyl) Quinoline (CHR5) in Rabbits	5. TYPE OF REPORT & PERIOD COVERED Final 6 May 1982 - 2 March 1983	
7. AUTHOR(s) Lawrence Mullen, BS, SP4 Martha A. Hanes, DVD, CPT VC Paul Mellick, DVM, PhD, LTC VC	6. PERFORMING ORG. REPORT NUMBER	
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18. SUPPLEMENTARY NOTES (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-Butenyl) Quinoline, Acute Dermal Toxicity, Insect Repellent, Toxicology		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The acute dermal toxicity potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR5) was determined in rabbits using abraded skin sites and plastic covering over the exposed areas for 24 hours. No animals died or showed clinical signs of toxicity. This compound should be exposed to further toxicological testing for human use potential.		

ABSTRACT

The acute dermal toxicity potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR5) was determined in rabbits by using abraded skin sites and plastic covering over the exposed areas for 24 hours. No animals died or showed clinical signs of toxicity. This compound should be exposed to further toxicological testing for human use potential.

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

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 94192

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

GLP STUDY NUMBER: 82013

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

PATHOLOGIST: LTC Paul Mellick, DVM, PhD, VC, Diplomate of American
College of 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: (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-
Butenyl) Quinoline. (CHR5)

INCLUSIVE STUDY DATES: 6 May 1982 - 2 March 1983

OBJECTIVE: The purpose of this study was to determine the acute
dermal toxicity potential of CHR5 in rabbits.

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ACKNOWLEDGEMENTS

The authors wish to thank SSG Lance White; SP5 Joseph Alletto, BS; SP5 Florence McKinley, BS; SP5 Marlin McKinley, BS; SP4 Thomas Kellner, BA; SP4 Justo Rodriguez, BS; SP4 Evelyn Zimmerman; Carolyn Lewis, MS; Thomas Hironaga; Lucille Cote; and John Dacey for their assistance in performing the research. The authors also wish to thank William Riefenrath, PhD, for providing the chemical and Louis Rutledge, MS, for the background information.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 82013 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 Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

John T. Fruin 5 Dec 82
JOHN T. FRUIN / DATE
COL, VC
Study Director

Martha A. Hanes 14 Dec 82
MARTHA A. HANES / DATE
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Principal Investigator

Paul W. Mellick 6 Jan 83
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DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

3 March 1983

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82013, the following inspections were made:

2 Jun 82
8 Jun 82
16 Jun 82

The report and raw data for this study were audited on 2 Mar 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 1982 report to management and the Study Director.

JOHN C. JOHNSON
CPT (P), MSC
Quality Assurance Officer

TABLE OF CONTENTS

	Page
Abstract.	i
Preface	iii
Acknowledgments	iv
Signatures of Principal Scientists.	v
Report of Quality Assurance Unit.	vi
Table of Contents	vii
BODY OF REPORT	
INTRODUCTION.	1
Toxicity Testing.	1
Description of Test	2
Objective of Study.	3
METHODS	
Chemical Analysis.	3
Test Substance	3
Compound Preparation	3
Animal Data.	4
Environmental Conditions	4
Dosing	4
Observations	4
Duration of Study.	5
Changes to Original Procedures	5
RESULTS	
Clinical Observations.	5
Treatment of Animal Disease and Injury	6
Gross Pathological Observations.	6
DISCUSSION	7
CONCLUSION	7
RECOMMENDATION	7
REFERENCES	8

Table of Contents (continued)

	Page
APPENDICES	
Appendix A Chemical Analysis Data.	11
Appendix B Animal Data	17
Appendix C Environmental Conditions.	19
Appendix D Historical Listing of Study Events.	21
Appendix E Figures 1 and 2	23
Appendix F Pathology Report.	27
OFFICIAL DISTRIBUTION LIST	32

ACUTE DERMAL TOXICITY POTENTIAL OF (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) IN RABBITS--Mullen et al

The goal of the insect repellent program is to develop insect repellents for the protection of soldiers from insect 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 (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR5) 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 factor 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 species under test.

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 has been the preferred species for such reasons as size, ease of handling and restraint, and because its skin is the most permeable of all species studied. The rabbit appears to be very sensitive to dermal insult. The animal's dorsal and lateral sections were close clipped so that no less than 10% of the body surface area was available for application of material (4). The abdominal section was not clipped.

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 ends are reinforced and 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, the exposed area examined at least 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 processed for histopathology (5).

Objective of Study

The objective of this study is to determine the acute dermal toxicity potential of CHR5 in rabbits.

METHODS

Chemical Analysis

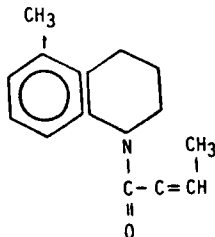
Our information on the chemical analysis of CHR5 was obtained from Starks Associates (Appendix A).

Test Substance

Chemical name: (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5)

CAS: Unknown

Molecular structure:



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

Other test substance information appears in Appendix A.

Compound Preparation

CHR5 was supplied by Starks Associates, Inc. through Mr. L. Rutledge, of Cutaneous Hazards Division, Letterman Army Institute of Research, and used in the form provided. No vehicle was used. CHR5 was kept in vials in a water bath maintained at a temperature of 39-42 C. The compound maintains a liquid state at this temperature and becomes light yellow in color.

Animal Data

Animal data appear in Appendix B.

Environmental Conditions

Environmental conditions are described in Appendix C.

Dosing

Dosing Levels: The test was conducted as a limit test (SOP-OP-STX-30) wherein 5 males and 5 females are assigned to the test chemical CHR5. A "nothing applied" control group of 5 males and 5 females was tested. The dose level was 2 ml/kg for CHR5. According to "the state of the art," if a test is conducted at this dose level and no compound related mortality occurs, then a full study using 3 dose levels is not necessary (1). For a standard test, 10 animals per dose group would have been used -- one half of these animals would have the exposed area abraded and the other half would remain intact. (3).

Dose Volume: According to weight; range 4.5 to 5.3 ml of test compound.

Duration of Exposure: 24 h.

Method and Frequency of Administration: The application sites in the abraded-skin group were abraded by use of an abrading tool designed for this experiment (6). It has four small metal points mounted onto a flat piece of metal that is attached to a handle. It was drawn across the area to be exposed so that only the stratum corneum was disrupted. The lines were approximately 2 cm apart along the axis of the backbone over the entire exposed surface. Test material was administered with a needle-less syringe at the appropriate dose volume. The dorsal and abdominal areas were then covered with plastic wrap (5 mm polyethylene) derived from GSA bags (#NSN8105-00-655-8285) and taped on the ends and seam with Conform[®] adhesive tape (Kendall Hospital Products, Boston, MA 02110, Code No. 7233). The animals were observed and clinical signs recorded within six hours of administration of the test substance. The bandage was removed after 24 hours.

Observations

Animals were weighed seven times over the study test period. Observations were recorded once a day. At the end of the 2-week period, animals were anesthetized with sodium pentobarbital, sacrificed by exsanguination from severed axillary vessels and necropsied. Skin was taken from an abraded and non-abraded area and examined microscopically.

Duration of Study

Historical study events are listed in Appendix D.

Changes in the Original Procedures or Protocol During the Study

The shipping documents provided by Starks Associates, Inc. incorrectly identified the test compound. Consequently, the original protocol stated that CHR6 would be tested; CHR5 was actually tested. CHR5 was not diluted with carboxymethyl cellulose, and no vehicle was tested.

The rabbits were reclipped at dosing to assure adequate area exposed.

Blood samples from the rabbits were taken before necropsy, for the purpose of obtaining base line data for comparison in future studies. This had no effect on the study and are not reported as part of the study. However, data are retained in the LAIR Archives.

The dose level was delivered at 2 ml/kg rather than 2 g/kg due to the physical properties of the substance.

RESULTS

Clinical Observations

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

Systemic: No clinical systemic signs were noted that were interpreted as signs of toxicity attributable to the test compound. Two of the five control group females developed diarrhea. One of five females in the CHR5 dose group appeared irritable. One of five control group males demonstrated clipper burns. Animal number 82F00066 in the CHR5 group developed diarrhea and lost weight for a period of eight days. However, the animal was gaining weight by the end of the study.

Dermal: The most notable signs related to dermal toxicity were erythema, scabbing, scaling, skin cracking, scratching, and skin thickening. Figures 1 and 2 (Appendix E) show the average duration of clinical signs in male and female rabbits, respectively.

Erythema, redness of the skin, was seen in three of five CHR5 females and three of five CHR5 males. When data were taken, location, area and intensity were graded according to a code seen at the top of the summary sheets. Slight erythema occurred on the back, involving a maximum area $\leq 10\%$. Rabbits exhibited erythema for an average of three days.

Scab formation was noted in all five CHR5 males and in four of five CHR5 females. The scabbing occurred along the back and right flank, involving a maximum area $\leq 10\%$. The maximum intensity was moderate. The average duration of scabbing was approximately 4 days.

Scaling, dermal lichenification, was also quite prevalent, occurring in four of five CHR5 females and two of five CHR5 males. Scaling was noted along the back and lateral areas; the maximum area was noted to be $\leq 10\%$ and the maximum intensity was noted as slight. The average duration was noted as approximately 4 days.

Skin cracking occurred in four of five CHR5 females and four of five CHR5 males. Skin cracking was noted on the back and along the abrasions. The maximum area with skin cracking was $\leq 10\%$. The maximum intensity was slight. Skin cracking lasted an average of approximately three days.

Skin thickening occurred in two of five CHR5 animals of each sex. Skin thickening was observed on the back; the maximum area involved was $\leq 10\%$ and the maximum intensity was slight. The average duration was approximately two days.

Slight necrosis was noted on less than 5% of the exposed area in one female and persisted for a period of five days, beginning 10 days after exposure. One CHR5 female and one CHR5 male each had slight edema on the back and involved a maximum area of $\leq 5\%$.

Three of five males had scratches. The scratches had a maximum intensity of moderate and occurred in a maximum of $\leq 25\%$ of the exposed area. Scratches were noted along the abrasions and the back. One male had a scratch on the right flank area. Two males developed the scratches during the last four days of the study.

Treatment of Animal Disease and Injury

Rabbits were placed on therapeutic levels of sulfaquinoline (SQ) in the drinking water for coccidiosis prophylaxis during quarantine. They did not receive SQ after they were placed in the GLP suite.

Gross Pathological Observations

Report of gross pathological observations appears in Appendix F.

DISCUSSION

The acute dermal toxicity test revealed that CHR5 did not cause clinical signs of systemic toxicity at 2 ml/kg when applied to approximately 10% of the body surface of rabbits. CHR5 caused slight inflammatory response in skin samples examined microscopically 14 days after exposure. The acute dermal toxicity test evaluates the potential for systemic toxic effects of a given substance. Rabbits are an appropriate animal model for this test as their unique sensitivity presents the "worst possible" conditions of chemical exposure.

CONCLUSION

CHR5 causes slight dermal irritation and inflammation to the clipped skin of rabbits exposed for 24-hour periods and observed for two weeks.

RECOMMENDATION

CHR5 should undergo further toxicity testing for eventual human use screening, dependent on efficacy findings.

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 Practices regulations (21 CFR58) and preamble, published in the Federal Register, 22 Dec 78 (45 FR59986-60025).
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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.
6. Haley TJ, Hunziker J. Instrument for producing standardized skin abrasion. J Pharm Sci 1974;63:106.

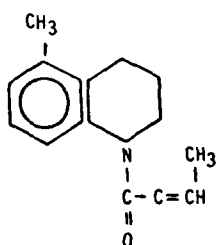
	Page
Appendix A, Chemical Analysis Data.	11
Appendix B, Animal Data	17
Appendix C, Environmental Conditions.	19
Appendix D, Historical Listing of Study Events.	21
Appendix E, Figures 1 and 2	23
Appendix F, Pathology Report.	27

APPENDICES

CHEMICAL DATA

Chemical Name: (E)-1,2,3,4-Tetrahydro-6-Methyl-1(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5)

Chemical structure:



Empirical formula: C₁₅H₁₉NO

Molecular weight: 229

pH: Non Aqueous

Physical state: White crystalline solid

Melting point: 37-37.5 C

Compound density: Unknown

Compound refractory index: Unknown

Compound stability: Unknown but presumed to be stable at room temperature.

Purity: Unknown

Contaminants: Unknown

Manufacturer Lot No: 0205

APPENDIX A

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Mullen--12

Manufacturer: Starks Associates, Inc.
1280 Niagara Street
Buffalo, New York 14213

Published Toxicity Data: The compound is unknown to the chemical literature.

Other information: Unknown

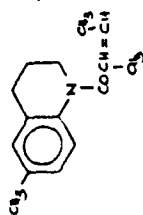
APPENDIX A (Cont.)

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WR250018AA		Starks Associates, Inc. 1280 Niagara Street Buffalo, New York 14213						0205																																											
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STRUCTURE																																																			
ST E 2.5						BK16177																																													
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APPEARANCE		white crystalline solid				ELEMENT		CALCULATED		FOUND																																									
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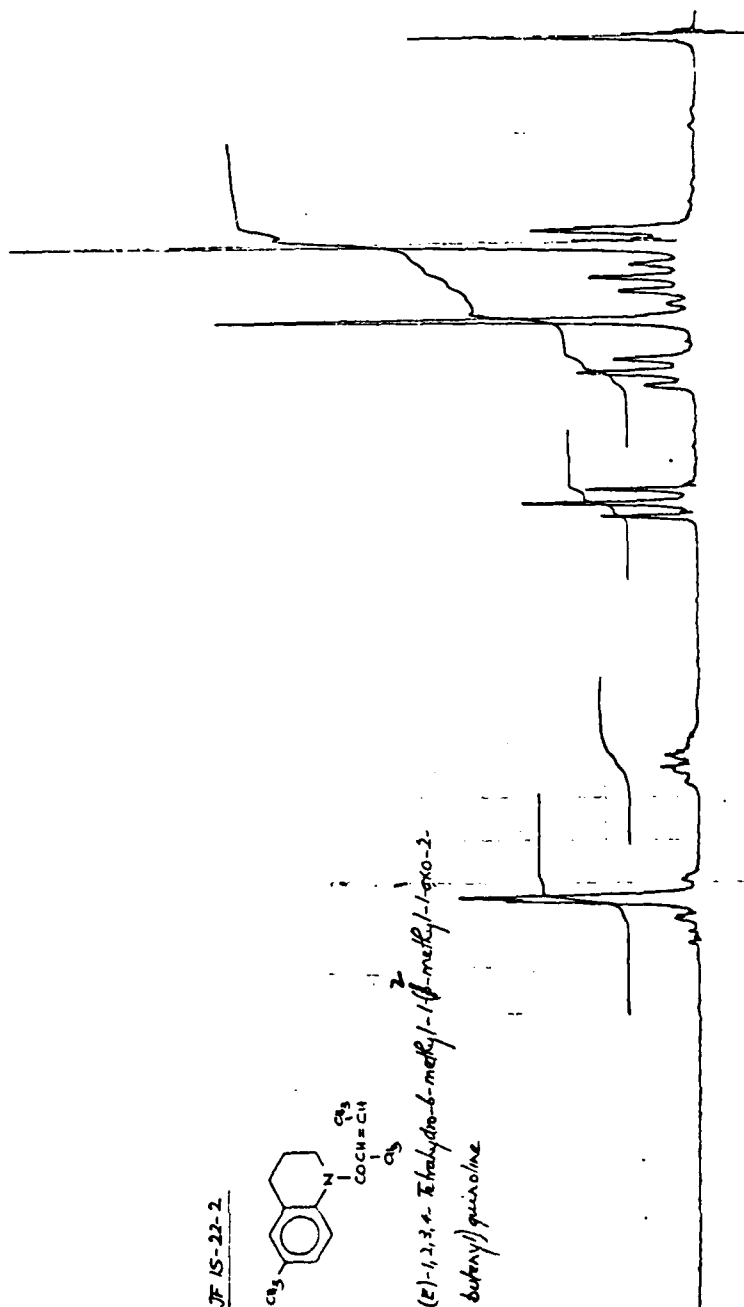
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(E)-1,2,3,4-tetrahydro-6-methyl-1H-pyrido[1,2-a]pyrimidin-2-one
butenyl quinoxaline

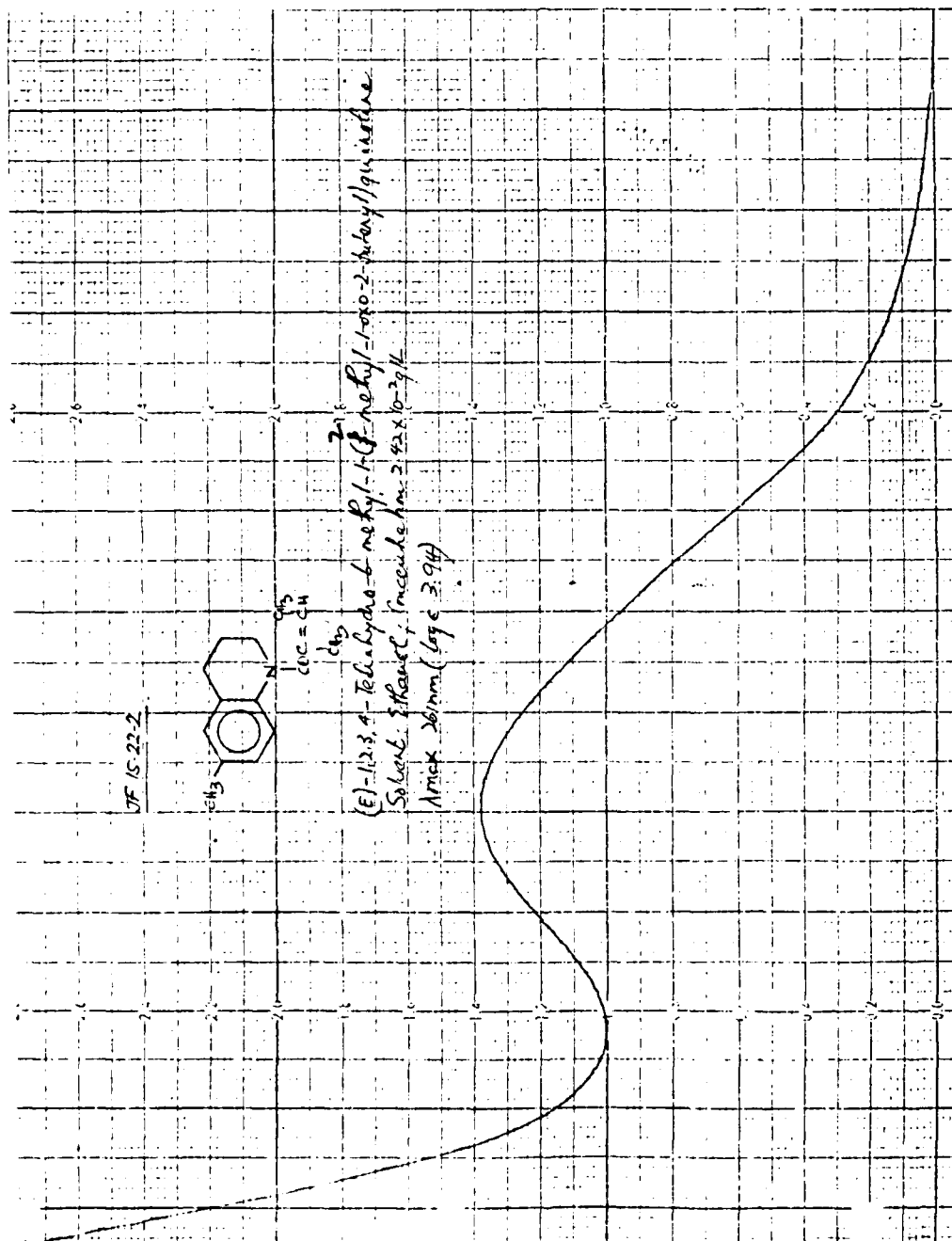


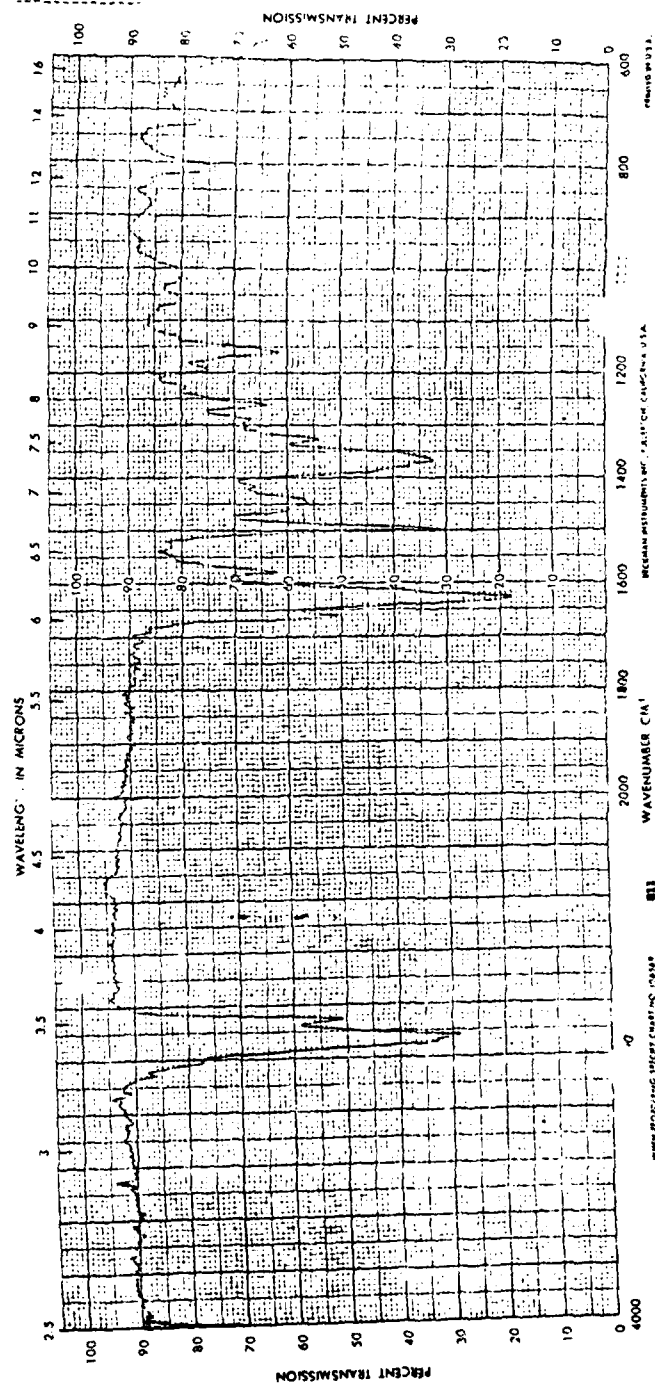
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CDCl₃

PPM	INTEGRATION	SWEEP TIME
10.0	10/10	(10/9.6)
8.0	0.5	SWEEP 10.000
6.0		END OF SWEEP





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. Arrival at LAIR 6 May 82 quarantine time 20 days.
- b. Animals clipped the day before dosing.
- c. Animals given sulfaquinoline (SQ) during quarantine, at a standard dosage of 3.2 ml SQ per 8 oz. water bottle ad lib for seven days.

Restraint: Manual restraint during application. Animals left their bandages alone over the 24-hour period.

Sex: Male and female.

Age: Young adult

Method of Randomization: Manually by Random Numbers Table

Animals in Each Group: 5 males and 5 females per test chemical; 5 males and 5 females in wrapped control.

Condition of Animals at Start of Study: Normal

Mean Weight (+ 1 standard deviation) at Dosing:

2494.8 (+ 118) g for test animals
2422.0 (+ 157) g for control

Mullen--18

Mean Weight ($\pm$ 1 standard deviation) at Sacrifice:

2517.4 ($\pm$ 127) g for test animals

2461.1 ($\pm$ 151) g for control

Identification Procedures: Ear Tattooed IAW SOP OP-ARG-1

APPENDIX B (cont.)

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: 75 ± 5 F (24 ± 3 C)

Relative humidity: $45 \pm 5\%$

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

HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
6 May 82	Male and female rabbits arrived at LAIR. They were checked for illness and quarantined in Room RS1409.
26 May 82	10 males and 10 females were removed from quarantine, separated into test groups and prepared for study conduction. Hair was clipped from the back and sides.
2 Jun 82	Rabbits were dosed according to SOP-OP-STX-30. The clipped areas were abraded and test substance applied. Rabbits were observed frequently after dosing. Clinical signs were recorded once after dosing.
3 Jun 82	Bandaging materials were removed. Animals were observed.
4-16 Jun 82	Clinical observations were recorded once a day.
15 Jun 82	Rabbits were bled from the ear vein.
16 Jun 82	Animals were not fed; euthanasia and necropsies were performed, and several sites selected for histopathologic observation.

APPENDIX D

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	Page
Figure 1. GLP Study No. 82013 - Acute Dermal Toxicity of CHR5 Average Duration of Clinical Signs in Male.....	24
Figure 2. GLP Study No. 82013 - Acute Dermal Toxicity of CHR5 Average Duration of Clinical Signs in Female.....	25

APPENDIX E

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Figure 1

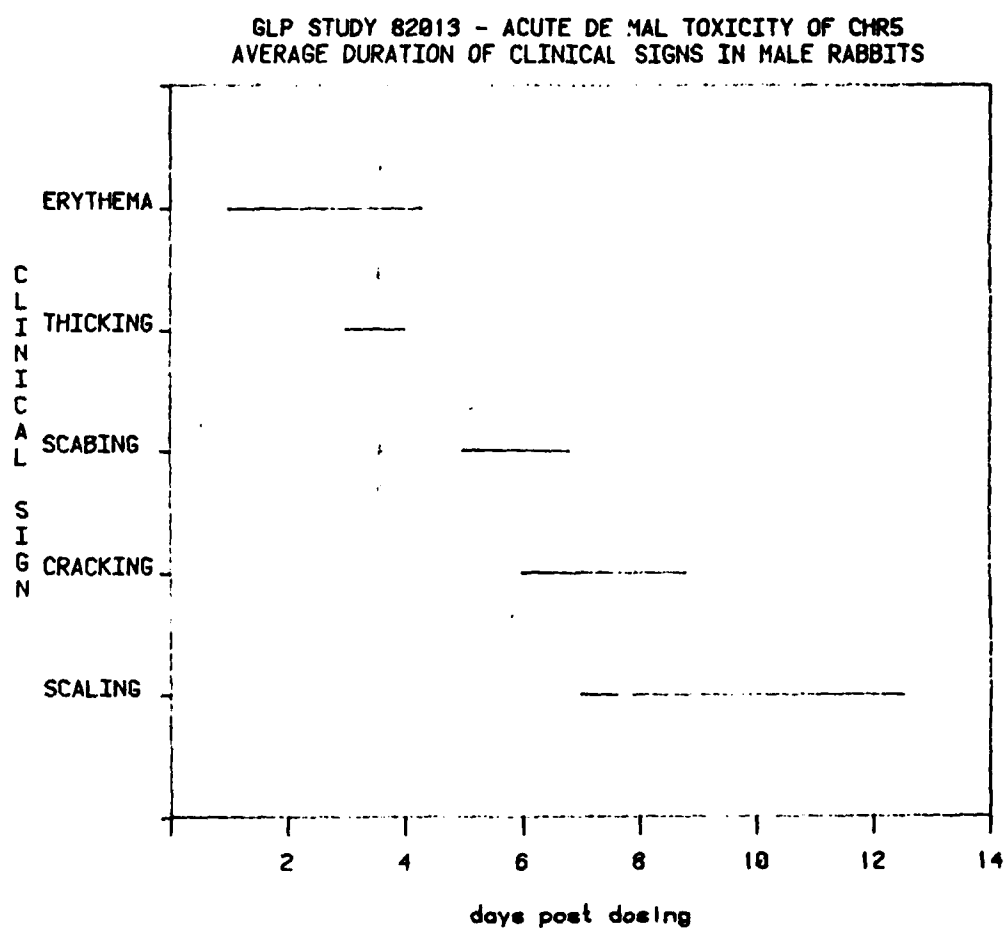
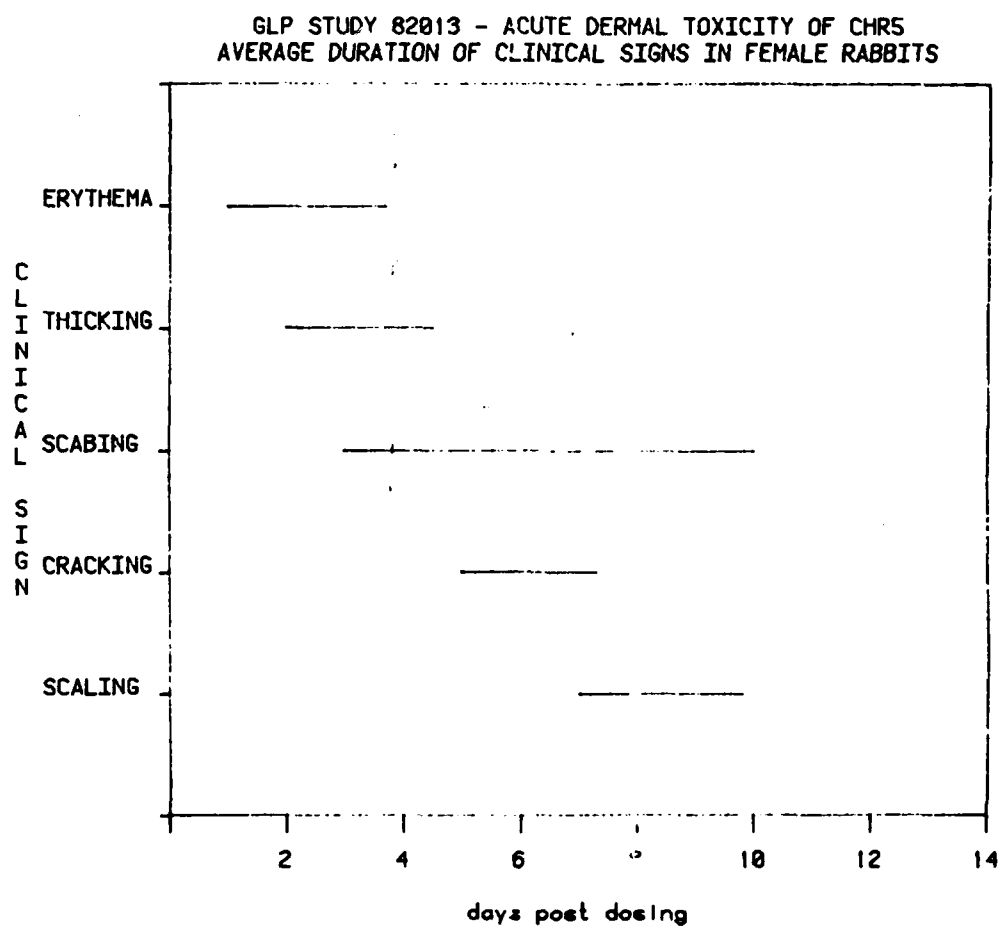


Figure 2



PATHOLOGY REPORT

GLP Study 82013

Acute Dermal Toxicity Study of CHR5

History: The purpose of this study was to determine the acute dermal toxicity of (E) - 1,2,3,4 - Tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR5) in male and female New Zealand White Rabbits.

After a 14 day observation period, animals were submitted for necropsy. They were killed by exsanguination from severed axillary vessels while under anesthesia produced by intravenous injection of pentobarbital. 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. Microscopic examination was done by a pathologist who did not know which sections were from control rabbits and which were from rabbits exposed to the tested compound.

Gross necropsy findings: There were no gross lesions detected in the skin of any of the control or exposed rabbits. One of five male rabbits in the control group (82F67) had numerous abscesses ranging from 1 - 10 mm in diameter diffusely scattered throughout the right caudal lobe of the lung. The liver of this animal contained multiple white linear fibrous tracts. These measured approximately 1- 2 mm in diameter. The pulmonary lesions were typical of bacterial infection and the liver lesions were compatible with those caused by Eimeria stiedae, a protozoan parasite that frequently infects intrahepatic bile ducts of rabbits. No other gross lesions were observed in rabbits used in this study.

Microscopic findings: Two types of microscopic lesions were observed in the skin from exposed sites in rabbits. The most common type was either diffuse or multifocal infiltration of macrophages, lymphocytes, and plasma cells (collectively referred to as mononuclear inflammatory cells) in the superficial dermis. These cells, when present, were always located immediately beneath the epidermis. The other lesion was mild diffuse proliferation of fibroblasts and increased amounts of collagen (referred to as fibrosis). This lesion, when present, was also located in the superficial dermis and was accompanied by infiltration of mononuclear inflammatory cells. Both lesions were very mild. No lesion was recognized in the epidermis of any skin section examined. 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.

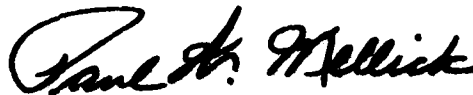
APPENDIX F

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Five of five female and 4/5* male rabbits exposed to CHR5 had mononuclear inflammatory cell infiltration in the superficial dermis at the site of application. In addition, fibrosis was present in the superficial dermis of 2/5 exposed females. Fibrosis was not present in any of the male rabbits. No microscopic lesions were observed in any of the male or female rabbits in the control group.

In summary, application of CHR5 to the close clipped abraded skin of rabbits for 24 hours causes a mild inflammatory response in the skin that can be detected 14 days after application. There were no gross lesions in other organs or tissues in rabbits attributable to the single cutaneous application of CHR5.

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



PAUL W. MELLICK, DVM, PhD
Diplomate, ACVP
LTC, VC
Chief, Pathology Services Group
Division of Research Support

6 October 1982

GLP Study 82-013
Acute Dermal Toxicity of CHR5

TABLE I

Group I Female Rabbits Exposed to 2 ml CHR5/kg Body Weight

Animal ID#	Pathology Accession#	Microscopic findings
82F47	32321-1	Mononuclear inflammatory cell infiltration, minimal Fibrosis, diffuse, minimal
82F49	32323-1 32323-2	Mononuclear inflammatory cell infiltration, minimal Mononuclear inflammatory cell infiltration, minimal
82F50	32324-1 32324-2	Mononuclear inflammatory cell infiltration, mild Mononuclear inflammatory cell infiltration, minimal
82F51	32325-1 32325-2	Mononuclear inflammatory cell infiltration, mild Fibrosis, diffuse, minimal Mononuclear inflammatory cell infiltration, minimal
82F52	32326-1 32326-2	Mononuclear inflammatory cell infiltration, minimal Mononuclear inflammatory cell infiltration, minimal

Group II Female Rabbits - Control

82F44	32319-1 32319-2	Essentially normal skin Essentially normal skin
82F46	32320-1 32320-2	Essentially normal skin Essentially normal skin
82F48	32322-1 32322-2	Essentially normal skin Essentially normal skin
82F54	32327-1 32327-2	Essentially normal skin Essentially normal skin
82F55	32328-1 32328-2	Essentially normal skin Essentially normal skin

TABLE I (continued)

Group I Male Rabbits Exposed to 2 ml CHR5/kg Body Weight

Animal ID#	Pathology Accession#	Microscopic Findings
82F56	32329-1	Essentially normal skin
	32329-2	Essentially normal skin
82F60	32332-1	Mononuclear inflammatory cell infiltration, minimal
	32332-2	Mononuclear inflammatory cell infiltration, minimal
82F61	32333-1	Mononuclear inflammatory cell infiltration, minimal
	32333-2	Mononuclear inflammatory cell infiltration, minimal
82F63	32334-1	Mononuclear inflammatory cell infiltration, minimal
	32334-2	Mononuclear inflammatory cell infiltration, minimal
82F66	32337-1	Essentially normal skin
	32337-2	Mononuclear inflammatory cell infiltration, minimal

Group II Male Rabbits - Control

82F58	32330-1	Essentially normal skin
	32330-2	Essentially normal skin
82F59	32331-1	Essentially normal skin
	32331-2	Essentially normal skin
82F64	32335-1	Essentially normal skin
	32335-2	Essentially normal skin
82F65	32336-1	Essentially normal skin
	32336-2	Essentially normal skin
82F67	32338-1	Essentially normal skin
	32338-2	Essentially normal skin

GLP Study 82-013

Acute Dermal Toxicity of CHR5

TABLE II

Incidence of Microscopic Skin Lesions by Sex and Experimental Group

Group#	Sex	Treatment	Normal Skin	Mononuclear Infiltration	Fibrosis
I	Female	2 ml CHR5/kg	0/5	5/5	2/5
I	Male	2 ml CHR5/kg	1/5	4/5	0/5
II	Female	Control	5/5	0/5	0/5
II	Male	Control	5/5	0/5	0/5

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

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