AD NUMBER

ADB112150

LIMITATION CHANGES

TO:

Approved for public release; distribution is unlimited.

FROM:

Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; OCT 1986. Other requests shall be referred to U.S. Army Medical Research and Development Command, Attn: SGRD-RMI-S, Fort Detrick, Frederick, MD 21701-5012.

AUTHORITY

USAMRDC notice dtd 1 Nov 1989

L (2)

AD

REPORT NUMBER
2100414504-008 ✓

TITLE
Controlled-Release Personal Use
Arthropod Repellent Formulation - Phase II

TYPE OF REPORT
Final Technical Report

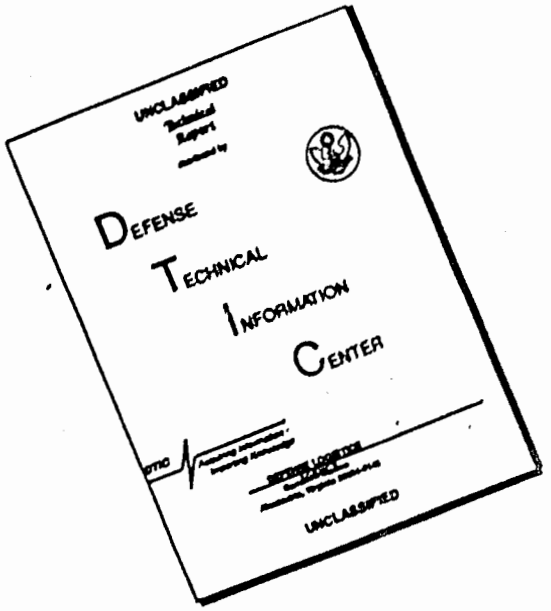
AUTHOR
Neil A. Randen, Ph.D.

DATE
Typed September 15, 1986;
Period of January 21, 1986 through September 20, 1986

Prepared Under Contract Number
DAMD17-85-C-5017 for U.S. Army
Medical Research Acquisition Activity
Fort Detrick, Frederick, Maryland 21701-5014

3M Company
Personal Care Products
St. Paul, Minnesota 55144-1000

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

AD

REPORT NUMBER
2100414504-008

TITLE
Controlled-Release Personal Use
Arthropod Repellent Formulation - Phase II

TYPE OF REPORT
Final Technical Report

AUTHOR
Neil A. Randen, Ph.D.

DATE
Typed September 15, 1986;
Period of January 21, 1986 through September 20, 1986

Prepared Under Contract Number
DAMD17-85-C-5017 for U.S. Army
Medical Research Acquisition Activity
Fort Detrick, Frederick, Maryland 21701-5014

3M Company
Personal Care Products
St. Paul, Minnesota 55144-1000

AD-B112150L

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp. Date: Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION
Unclassified

1b. RESTRICTIVE MARKINGS

2a. SECURITY CLASSIFICATION AUTHORITY

3. DISTRIBUTION/AVAILABILITY OF REPORT
Limited to U.S. Government Agencies only; proprietary information, October 1986

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
3M Company

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)
Personal Care Products
St. Paul, Minnesota 55144-1000

7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command

8b. OFFICE SYMBOL (If applicable)
SGRD-RMI-S

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
DAMD17-85-C-5017

8c. ADDRESS (City, State, and ZIP Code)
Fort Detrick, Frederick, Maryland 21701-5012

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
64758A	3S464758D849	EA	024

11. TITLE (Include Security Classification)
(U) Controlled-Release Personal Use Arthropod Repellent Formulation

12. PERSONAL AUTHOR(S)
Neil A. Randen, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final - Phase II	FROM 10/3/85 TO 9/20/86	1986 September 15	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	06		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Judy Pawlus	301-663-7325	SGRD-RMI-S

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

0.0 ABSTRACT

An improved controlled-release arthropod repellent formulation for topical application to a person's exposed skin areas that provides extended protection against biting arthropods, which is safe and agreeable to use, which is more compatible with other current and projected military materials and systems than the Army's current 75% N,N-diethyl-m-toluamide (DEET) in alcohol formulation and which complies with the registration requirements of the Environmental Protection Agency (EPA) has been developed. The Phase I formulation containing 30% DEET and an acrylate polymer served as the starting point for the refinement and development in Phase II. The final Phase II submission contained 35% DEET and the acrylate polymer. This formulation provided 95% repellency against Aedes aegypti mosquitoes for 14-15 hours, 10-11 hours and 14-15 hours when evaluated in the constant high humidity, the variable high humidity and the basic hot climatic conditions using a modified standard mosquito repellency test method (ASTM:E951-83). In field evaluations in Louisiana against Aedes sollicitans and Anopheles quadrimaculatus mosquitoes, the formulation provided complete protection times of 10.7 ± 2.6 hours and 12.3 ± 1.8 hours, respectively. The climatic conditions for both tests were the variable high humidity condition. The test method employed was a modified ASTM:E951-83 in which the products were applied according to label directions. In addition the formulation was shown to be acceptable to 88% of men and women of military age and was shown to be less toxic to animals and humans than the 75% DEET formulation. The formulation is packaged in individual 2 ounce, olive drab, high density polyethylene tubes with a noiseless spouted cap. The tubes are labeled per the EPA Registration Standard and Guidance Package. An EPA Registration Data Package for the EPA has been prepared as well as a User-Training Package.

1.0 INTRODUCTION

Personal Care Products (PCP)/3M received a Phase II contract from the Department of the Army to refine and correct any product deficiencies revealed during Government testing of PCP's Phase I formulation[1]. During Phase I "...a controlled-release arthropod repellent formulation for topical application to a soldier's exposed skin areas which will provide extended protection against biting arthropods, be safe and agreeable to use, be compatible with other current and projected military materials and systems and comply with registration requirements of the Environmental Protection Agency"[2] was developed. This formulation was an oil-in-water emulsion which contained 30.00 percent N,N-diethyl-m-toluamide (DEET) and 5.00 percent of a 3M proprietary acrylate polymer.

---

1 - Modification Number P60006, Contract Number DAMD17-85-C-5017, p.2, 1986.

2 - Contract Number DAMD17-85-C-5017, p.4, 1984.

FOREWORD

Citations of commercial organizations and tradenames in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in the report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of The Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, Revised 1978).

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CRF46.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

This data shall not be disclosed outside the government and shall not be duplicated, used, or disclosed in whole or in part for any purpose other than that provided in the contract. This restriction does not limit the government's right to use information contained in the data if it is obtained from another source without restriction.

TABLE OF CONTENTS

0.0 ABSTRACT

1.0 INTRODUCTION

2.0 DISCUSSION

2.1 Phase II Final Formulation

2.1.1 Composition
2.1.2 Toxicology Testing
2.1.3 Mosquito Repellency Tests

2.1.3.1 Final Laboratory Repellency Test in Three Basic Climatic Conditions
2.1.3.2 Field Mosquito Repellency Tests

2.1.3.2.1 Aedes Species
2.1.3.2.2 Anophoies Species
2.1.3.2.3 Estimated 95% Repellency Values

2.1.4 Troop Acceptance Study
2.1.5 Compatibility Tests

2.1.5.1 Fabric Materials
2.1.5.2 Solid Plastic Materials
2.1.5.3 Compatibility of Repellents with Plastic Materials
2.1.5.4 Repellent Compatibility with Rubber Materials and Leather
2.1.5.5 Repellent Compatibility with Camouflage Paint

2.1.6 Odor Comparison Study
2.1.7 Package Design
2.1.8 Registration Data Package
2.1.9 Label
2.1.10 In-Vitro Penetration/Evaporation Test
2.1.11 User Training Package Development
2.1.12 Shelf Life Studies
2.1.13 Deliverables

2.2 Formulation Refinement and Development

2.2.1 Formulation Containing 40% DEET
2.2.2 First 35% DEET Formulation Design Experiment
2.2.3 Second 35% DEET Formulation Design Experiment

2.2.3.1 Aesthetic and DEET Substantivity Studies
2.2.3.2 Comparison to Phase I Submission
2.2.3.3 Mosquito Repellency Test

2.2.4 Third 35% DEET Formulation Design Experiment
2.2.5 Fourth 35% DEET Formulation Design Experiment
2.2.6 Final Seven 35% DEET Formulations

2.2.6.1 Aesthetic Evaluation
2.2.6.2 Mosquito Repellency Test
2.2.6.3 Final Phase II Arthropod Repellent Selection

2.2.7 Final Formulation Aesthetic Improvement

2.3 Toxicology Testing

2.4 Package Design

2.5 Label

2.6 Shelf Life Studies

2.6.1 Phase I Submission
2.6.2 Phase II Submission

3.0 SUMMARY

4.0 CONCLUSION

5.0 LIST OF APPENDICES

5.1 Appendix A - Figures

5.1.1 Figure I - 40% DEET Formula
5.1.2 Figure II - 35% DEET Oil-in-Water Formulation - Design I
5.1.3 Figure III - Mosquito Repellent Aesthetic Evaluation
5.1.4 Figure IV - 35% DEET Design I Response Factors
5.1.5 Figure V - Preferred Formulation From 35% DEET Design I
5.1.6 Figure VI - 35% DEET Oil-in-Water Formulation - Design II
5.1.7 Figure VII - 35% Design II Dependent Variables
5.1.8 Figure VIII - Preferred Formulation From 35% DEET Design II
5.1.9 Figure IX - 35% DEET Oil-in-Water Formulation - Design III
5.1.10 Figure X - 35% DEET Oil-in-Water Formulation - Design IV
5.1.11 Figure XI - Mosquito Repellent Formulations
5.1.12 Figure XII - Percent Mosquito Repellency Versus Time
5.1.13 Figure XIII - Phase II Second Repellency Test Formulations
5.1.14 Figure XIV - Phase II Second Mosquito Repellency Test Data
5.1.15 Figure XV - Final Seven Formulations Aesthetic Evaluation

5.1.16 Figure XVI - Phase I Aging Data
5.1.17 Figure XVII - Formulations For Third Mosquito Repellency Test
5.1.18 Figure XVIII - Third Phase II Mosquito Repellency Test
5.1.19 Figure XIX - Final Mosquito Repellency Tests
5.1.20 Labels

5.1.20.1 Figure XXa - Prototype Label (Front)
5.1.20.2 Figure XXb - Prototype Label (Back)

5.1.21 Figure XXI - Improved Back Label
5.1.22 Figure XXII - Field Repellency Test Against Aedes sollicitans Mosquitoes
5.1.23 Figure XXIII - Field Mosquito Repellency Test
5.1.24 Figure XXIV - Laboratory Mosquito Repellency Test
5.1.25 Figure XXV - Percent Repellency For Hypothetical Mosquito Repellency Data
5.1.26 Figure XXVI - Field Repellency Test Against Anopholes quadramaculatus Mosquitoes

5.1.27.1 Figure XXVIIa - Front Label
5.1.27.2 Figure XXVIIb - Back Label

5.1.28 Figure XXVIII - In-Vitro Evaporation of 3M Formulation - Study 213-26-31
5.1.29 Figure XXIX - In-Vitro Penetration and Evaporation of the 3M Formulation
5.1.30 Figure XXX - Package Aging Data
5.1.31 Figure XXXI - In-Vitro Evaporation Study 213-33
5.1.32 Figure XXXII - In-Vitro Penetration and Evaporation of the 3M Formulation

5.2 Appendix B - Mosquito Repellency Test

5.2.1 Final Laboratory Mosquito Repellency Test
5.2.2 Field Mosquito Repellency Tests

5.3 Appendix C - Troop Acceptance Studies/Odor Comparison Study

5.4 Appendix D - Toxicology Testing

5.4.1 Toxicology Summary
5.4.2 Acute Oral
5.4.3 Acute Dermal
5.4.4 Primary Skin Irritation
5.4.5 Eye Irritation
5.4.6 Repeated Insult Human Patch Test

5.5 Appendix E - Compatibility Testing

5.5.1 Table I - Compatibility Test - Fabrics
5.5.2 Table II - Repellent Compatibility with Painted Surfaces - 24-Hours
5.5.3 Repellent Compatibility with Plastic Materials - 24 Hours
5.5.4 Repellent Compatibility with Rubber Materials and Leather - 24 Hours
5.5.5 Repellent Compatibility with Chemical Protective Suit

5.6 Appendix F - Package Design

5.7 Appendix G - Label Copy

5.8 Appendix H - User Training Package Development

6.0 ANNEX

This formulation provided protection times with 95 percent mosquito repellency for 16 hours, 12 hours and 16 hours when evaluated under the basic hot, the variable high humidity and the constant high humidity Basic Climatic Conditions using a modified ASTM:E951-83 mosquito repellency test method. The formulation was shown to be acceptable to 95 percent of men and women of military age and was less toxic than the Army's current arthropod repellent (75% DEET in alcohol). Also it was shown to be less damaging to current and projected military materials and systems than the current Army issue repellent.

At the end of Phase I, the Department of the Army evaluated all of the repellent submissions from the six contract companies, with respect to mosquito repellency and cosmetic acceptability. In a letter dated January 8, 1986, to the author, Colonel John F. Reinert, Product Manager for Arthropod Repellents, stated that the "3M formulation needs improvement in its cosmetic properties". The data provided also indicated that the repellency attributes should be improved.

Increased mosquito repellency for the formulation could be obtained by increasing the percent DEET, by increasing the amount of acrylate polymer, or by increasing the molecular weight of the polymer. Since the last two would have a negative impact on formulation aesthetics, the former was chosen as the approach to take. The cosmetic acceptability of the formulation had already been improved by PCP during the interim between Phase I and Phase II contracts. Additional improvements could still be achieved by optimizing the levels of the various ingredients in the formulation with the use of statistical design experiments.

2.0 DISCUSSION

Personal Care Products' final Phase I arthropod repellent formulation served as the starting point for the Phase II refinement and development program. Herein statistical design experiments were used for the optimization process. The raw materials used in the formulation were the independent variables. The dependent variables were one or more of the following: formulation resistance, viscosity, aesthetic properties, six(6)-hour DEET substantivity and mosquito repellency. Using this approach, the effect that each raw material had on each dependent variable was determined and then adjustments were made in the formula. This process was repeated a number of times until the best formulation was obtained. Once identified, the following evaluation studies were run on this formulation: final laboratory mosquito repellency tests in the three basic climatic conditions, field mosquito repellency tests against Aedes and Anopholes species of mosquitoes, troop acceptance study, toxicology tests, compatibility tests and odor comparison tests.

The discussion which follows will present the final Phase II arthropod repellent formulation first. This will be followed by the various evaluation studies dealing specifically with this formulation and the other requirements defined in the contract. The second and subsequent sections will present the general work flow plan used to develop the final formulation. This will include the studies used to select the final formulation.

2.1 Phase II Final Formulation

Personal Care Products' final Phase II arthropod repellent formulation is a thick, white lotion which contains 35% N,N-diethyl-m-toluamide as the sole repellent. The formulation is packaged in a 2-ounce, olive drab, high density, polyethylene tube with a push-up spout cap. This will deliver 20-22 applications to an area the size of an average forearm.

2.1.1 Composition

The specific composition of the final formulation as well as the function of each ingredient is as follows:

FINAL REPELLENT FORMULATION (35% DEET)

INGREDIENTS (CTFA NAMES)		
Fumed Silica	Thickener	2.75
Polyethylene Glycol	Humectant	.98
Glycereth-7	Humectant	2.26
Magnesium Aluminum Silicate	Thickener	.70
Hydroxyethyl Cellulose	Thickener	.50
PEG-82 Glyceryl Monotallowate	Emulsifier	1.03
Glyceryl Monostearate	Emulsifier	4.06
3M Polymer (85:7.5:7.5 Mole Ratio Iso-Octyl Acrylate:Stearyl Methacrylate:Acrylic Acid)	Polymer	5.83
DEET	Repellent	35.00
Propylene Glycol Dicaprylate/ Dicaprate	Emollient Oil	3.22
PPG-15 Stearyl Ether	Emollient Oil	.43
Cetyl-Stearyl Alcohol	Thickener/Stabilizer	.86
Cetyl Palmitate	Waxy Emollient	.65
PEG-200 Glyceryl Monotallowate	Emulsifier	.65
Diazolidinyl:Urea:Methyl Paraben: Propyl Paraben: Propylene Glycol	Preservative	.24
Deionized Water		q.s. to 100

2.1.2 Toxicology Testing

The toxicology testing of PCP/3M's final formulation (T-3896) and 75% DEET/alcohol formulation (T-3755) have been summarized by Dr. F. D. Griffith, Manager, Toxicology Services, Medical Department/3M. One will see below that the PCP/3M formulation is less toxic than the 75% DEET/alcohol formulation. One can also see that the PCP/3M formulation meets all the criteria set forth by the Environmental Protection Agency. The complete summary of testing procedures and the data are in Appendix D.

Results - "Eye Irritation - T-3755 - Mild to moderate irritation in both the washed and unwashed eyes. Pain response in one of six animals in the unwashed group but none in the washed group. Conjunctival blanching and corneal epithelial peeling in all unwashed and one washed animal. Petite hemorrhage in some animals in the washed eyes. One unwashed eye has neo-vascularization at 7 days. Signs persisted at 7 days but not at 14 days. Washing alleviated but did not prevent serious damage.

T-3896 - Mild to moderate irritation in both washed and unwashed eyes. No pain response. Conjunctival blanching in all eyes. Corneal epithelial peeling in unwashed eyes and in two of three washed eyes. Five of six unwashed eyes had all zero scores at 7 days and one had all zero scores at 14 days. Two of three washed eyes had all zero scores at 7 days but one had approximately 15% corneal epithelial peeling at 21 days.

In a repeat of the wash procedure, two eyes were all zero scores at 7 days and one was all zero scores at 14 days.

Primary Dermal Irritation - T-3755 - No irritation reported. T-3896 - Minimal erythema in three animals at 24 hours and two animals at 48 hours. Minimal edema in one animal at 24 and 48 hours.

Acute Oral Toxicity - T-3755 - Three males and all females died within one day following dosing. The rat acute oral LD50 is "less" than 5 g/kg body weight. T-3896 - Red stained face on study Days 1 and 2. No other signs. The rat oral LD50 is "greater" than 5 g/kg body weight.

Acute Dermal Toxicity - T-3755 - All appeared clinically normal. Irritation consisted of slight to severe erythema and edema, slight to marked atonia, desquamation, conaceousness and fissuring. The rabbit acute dermal LD50 is greater than 2 g/kg body weight. T-3896 - One female had signs of diarrhea on Days 4, 5 and 7. There was

slight to severe erythema, silght to moderate edema, desquamation, fissuring and some subcutaneous hemorrhaging. The acute dermal LD50 in rabbits is greater than 2 g/kg body weight.

Repeated Insult Human Patch Test - T-3755 and T-3896 - Mild, transient irritation with no indication of sensitization."

2.1.3 Mosquito Repellency Tests

The Phase II contract requires that the final Phase II submission be tested for laboratory and field mosquito repellency using the application directions on the product label.

2.1.3.1 The laboratory mosquito repellency testing was conducted at Hazleton Laboratories America in Madison, Wisconsin, using a modified ASTM:E951-83 procedure. Specifically, fifteen fresh, 5-15 day old, female Aedes aegypti mosquitoes, acclimated to the room conditions for at least an hour prior to exposure were used to assess the mosquito repellency attributes of PCP's final formulation and the Army's current 75% DEET in alcohol formulation.

The application rate for the 3M formulation was 2 mg of lotion per square centimeter as suggested by the Army's RFP (DAMD17-84-R-0056, page 8). For the Army's 75% DEET/alcohol repellent, the directions on the container which read "shake about 12 drops into one hand, rub hands together and apply thoroughly in a thin layer to all areas of exposed skin..." were interpreted as 6 drops per arm, and this amount was applied. The exact weight applied in each situation was recorded. The treated sites were exposed to fresh mosquitoes every two hours starting at time zero (time of application) and continued through 16 hours or until the percent repellency of a particular formulation fell below 95% for two consecutive exposure times. The formulations were evaluated in three basic climatic conditions: A) constant high humidity, 75°F, 100-95-100% relative humidity (R.H.); B) variable high humidity, 78-95-78°F, 100-74-100% R.H.; and, C) basic hot, 86-110-86°F, 44-14-14% R.H.

A summary of the data is shown in Figure XIX.

The 95% repellency break points for the PCP formulation and the 75% DEET/alcohol formulation occurred at 14-15 hours versus 10-11 hours, respectively, in the constant high humidity climatic condition, at 10-11 hours versus 2-3 hours in the variable high humidity condition, and at 14-15 hours versus 10-11 hours in the basic hot condition. The PCP formulation always lasted longer than the 75% DEET/alcohol formulation.

The percent repellency for the PCP formulation at 12 hours in the variable high humidity climate was 93%, which is below the 95% requirement. At 14 hours it was still 90%. In two previous tests conducted in the same climatic condition, repellency values of 99% (see Figure XIV) and 98% (see Figure XVIII) at "16" hours were obtained for this same formulation. In addition the Phase II formulation has been shown to be superior to PCP's Phase I submission (see Figure XII).

2.1.3.2 Field Mosquito Repellency Testing

A modified ASTM:E939-83 protocol submitted by Hazleton Laboratories America was sent to Col. Reinert on June 6, 1986, for his comments and suggestions and to determine if we would have to make any revisions in the testing procedure. Basically, a pair-comparison test was proposed to be run between the 3M candidate formulation and 75% DEET in alcohol. The products would be applied following the "use directions" on the label. Mosquito avidity would be run during the course of the test to determine biting pressure. We were required to evaluate the repellent formulation against two species of mosquitoes - Aedes and Anopholes, which meant two separate tests.

The field test was conducted in the Jefferson Davis Parish Mosquito Abatement District No. 1, near Jennings, Louisiana, from July 16 through 22, 1986. The conditions at the times of the test were the variable high humidity basic climatic condition. Temperatures during the day reached 95-100°F with relative humidities in the 70%s. During morning and evenings, temperatures of 80° and humidities of 90-100% were prevalent.

2.1.3.2.1 Field Study Using Aedes Mosquitoes

The first test was run against Aedes soiiicitans mosquitoes in a swampy pasture. The formulations were applied in a random order to the arms and to the iegs. The sites were exposed for 10 minutes each hour starting at time 0. An untreated site was also run each hour to determine mosquito biting pressure. Failure of a site via the ASTM:E939-83 was defined as two bites in a 10 minute test period, or one bite 30 minutes apart by the same species. Via this procedure, the 75% DEET in alcohol had a complete protection time of 5.5 $\pm$ 1.8 hours. The 3M candidate formulation had a complete protection time of 10.7 $\pm$ 2.6 hours. These protection times are statistically different at the 99.5% confidence interval via the students' t test for two sample averages with unknown. The data for the six replications are shown in Figure XXii. Figure XXiii is a graphic representation. The results seen in this field study are very similar to the variable high humidity results in the Laboratory Mosquito Repellency test (see Figure XXiV).

2.1.3.2.2 Field Study Using Anopholes Mosquitoes

The second field study was against the Anopholes mosquito species. The test area was next to a soon-to-be drained rice field, a wooded area, and a pasture. The testing protocol was as before. Again, the products were applied per the use directions on the packages. The Anopholes species of mosquito had a peak activity time, determined the day before around 8:00 P.M. Therefore, the respective repellents were applied 10 (3M) and 6 (75% DEET) hours prior to this peak time. The

conditions through the day were the variable high humidity. Exposure times were started around 17:00 hours and continued until everyone failed. An untreated control was run each hour to determine biting pressure. However, very few bites were received by the untreated site because of the way the control was run. The subject would walk to the site, sit down, and immediately expose their untreated arms and legs, and of course, weren't bitten. The Anopholes mosquitoes are very wary and non-aggressive. The test participants had to remain in a test area for a short length of time before they would be bitten. They seemed to get most of their bites 5 to 10 minutes after they've been there as opposed to the first 5 minutes.

The complete protection times determined against Anopholes quadrimaculatus mosquitoes for the 75% DEET/alcohol formulation and the 3M candidate formulation were 7.7 $\pm$ 1.8 hours and 12.4 $\pm$ 1.9 hours respectively (see Figure XXVI). These were statistically different at the 99.5 percent confidence interval via the students' t test for two sample averages with σ unknown.

2.1.3.2.3 Estimated 95% Repellency Values

The contract states that the "contractor" shall use ASTM standard E939-83..." (with appropriate modifications) to comply with the greater than 95% protection level requirement"..., for our final Phase II submission.

The protocol which PCP/3M used for its field tests inadvertently did not include determining 95% repellency times. Instead, the complete protection times of the formulations were determined using direct comparison testing methods. The

biting pressure which is required to determine 95% repellency times was obtained for the Aedes solilicitans but not for the Anophoies mosquito species for the reasons mentioned earlier. While the actual avidity on an untreated site was not determined for the later, the Anophoies mosquitoes were biting as evidenced by the "confirmed species" bites received on the treated sites throughout the test. The complete protection times which were determined for this mosquito species are very realistic and representative for the climatic conditions and biting pressures at the time the test was conducted. These protection times do represent the relative effectiveness of the two repellent formulations at that particular site, conditions, etc.

A person should be able to compare the complete protection time data for the Aedes mosquito species to another set of data which was run to determine 95% repellency. The complete protection times (for the second data set) could be calculated using the two bite failure criteria and compared to the first taking into account the biting pressure for each test. Since the control was run improperly for the Anophoies mosquitoes, a similar comparison would be more difficult, if not impossible, to do.

In an attempt to see if any meaningful 95% repellency values could be salvaged from the data, 3M biostatisticians were contacted. After reviewing the data shown in Figures XXII and XXVI, they stated that: 1) there were too many missing data points; 2) the missing data points were not missing in a random fashion; 3) the missing data points were not random or independent from each other, and; 4) therefore

estimated values for these missing data points could not be obtained. This means that 95% repellency values could not be obtained either. On the other hand, the statistician could find nothing wrong with representing the missing data with hypothetical data, as long as the assumptions and methods of generation were stated up front. For the Aedes mosquito species a hypothetical 95% repellency value could be determined since the avidity had been run during the field study.

In the field study a test site was closed to further mosquito exposure after it had failed via the two or more bite criteria. If one assumed that the site would have received twice as many bites at the next exposure, if the site had not been closed and then twice as many the next, etc., one could generate hyupothetical bites for the missing data points and calculate a hypothetical percent repellency. Similiarly, one could have assumed that the site would have received 3 times as many bites each time the site had been exposed, and so on. This hypothetical data appears in Figure XXV.

For twice as many bites for each succeeding exposure time, the 75% DEET/alcohol formulation would have had 96.0% repellency at 5 hours, 96.2% at 6 hours and 84.3% at 7 hours. The 3M formulation would have had 99.9% at 9 hours, 98.9% at 10 hours, 97.1% at 11 hours and 86.5% at 12 hours. For the threefold increase each time, the 75% DEET formulation would have had 96.0% repellency at 5 hours and 94.3% at 6 hours. The 3M formulation would have had 98.4% at 10 hours and 93.8% at 11 hours.

Another approach to generate the missing data points would be to use the regression equation which would

define the loss of repellency for the two formulations in the "laboratory testing" against "Aedes aegypti mosquitoes" in the same climatic condition. Using the percent repellency determined thusly, and the mosquito biting pressure, one could calculate a hypothetical number of bites for each subsequent missing data point. The regression equation determined from the 95% repellency data of the Final Laboratory Mosquito Repellency Test for the appropriate climatic conditions for the Phase II submission is $y=102 - 3.35x$ where y = percent repellency and x = time in hours past the point of 100% repellency. For the 75% DEET/alcohol formulation, the regression equation is $y = 96 - 9.0 x$. The data determined in this manner are shown in Figure XXXIII and are marked with *. One can see that the Phase II submission has 97.4% repellency at 11 hours, 93.7% at 12 hours, 93.0% at 13 hours and 81.3% at 14 hours. The 75% DEET formulation has 96.0% repellency at 5 hours, 91.5% at 6 hours and 84.3% at 7 hours. The results of this data are very similar to the hypothetical situations proposed above.

In summary, against Aedes sollicitans mosquitoes, the Phase II submission had a complete protection time of 10.7 ± 2.6 hours and a hypothetical 96.1% repellency at 11 hours (average of the three hypothetical bases). The 75% DEET/alcohol formulation had a complete protection time of 5.5 ± 1.8 hours and a hypothetical 96% repellency at 5 hours. For the Anopholes quadrimaculatus mosquitoes, only a complete protection time could be calculated. These were 12.4 ± 1.9 hours and 7.7 ± 1.8 hours for the two repellents, respectively.

2.1.4 Troop Acceptance Studies

The consumer acceptance study was conducted by 3M Corporate Marketing Research in Dallas, Texas, during the first part of July. The testing was conducted outside with 200 respondents (10% female and 30% non-caucasian) in 82-100°F temperatures with humidities ranging from 9-78%.

"To test absolute acceptability, respondents were asked whether or not they would be likely to use each formulation if they were involved in an outdoor activity, given that no other insect repellent was available. immediately after application, 94.5% of respondents (189) stated that they would be at least somewhat likely to use the 3M formulation. After being outdoors for 10 minutes, 88% (176) stated that they would be at least somewhat likely to use the 3M formulation. Immediately after application, 96.5% (193) stated that they would be at least somewhat likely to use the military standard formulation (75% DEET/alcohol). After being outdoors for 10 minutes, 91% (182) stated that they would be at least somewhat likely to use the military standard formulation. These results are projectable to the general population of military age personnel of similar demographics with an accuracy of $\pm$ 6% at the 90% confidence level.

In comparative testing, which was conducted after being outdoors, the respondents were asked which of the two repellents they would prefer to use if they were involved in an outdoor activity. Of these, 46.5% (93) preferred the 3M formulation and 53% (106) preferred the military standard product. A 12% difference would be statistically significant at the 90% confidence level (14% at 95% confidence), therefore no difference in preference between the products can be confirmed.

The 3M formulation far exceeds the 75% user acceptability requirement of the Army contract. There is no statistically significant difference between the acceptability of the 3M insect repellent formulation and that of the military standard insect repellent." (Peter A. Schamel, Corporate Marketing Research/3M to Craig A. Sterling, Personal Care Products/3M; August 1, 1986 - Rough Draft - Arthropod Repellent Project, User Acceptability Testing, Phase II Resuits, CMR Project #1570).

2.1.5 Compatibility Testing

The Phase II submission was compared to the 75% DEET/alcohol formulation to see what effect each had on various materials typically found in an Army environment.

2.1.5.1 Compatibility of Repellents With Fabric Materials

Tensile strength and percent elongations were determined on natural and/or synthetic fabrics. The fabrics were cut into 1 x 6 cm strips. The center square centimeter area was treated with the two repellents at two application rates - 5 g/m^2 and total saturation (immersion) respectively. The strips were aged at room temperature or 71°C (160°F) and then evaluated at 1, 6 and 24 hours. An Instron was used to take the measurements at a crosshead speed of 10 inches/minute, a chart speed of 10 inches/minute and a gauge length of 1 inch.

As in Phase I, the vinyl material was disintegrated by the 75% DEET/alcohol formulation and to a slightly lesser degree by the Phase II submission. The data shown in Table I for this material is probably due to the fabric backing. The tensile strengths and percent elongations for the rest of the fabrics are also shown in the Table.

As a person can see the percent elongation data indicates that fabric materials treated with the Phase II submission did not appear to be affected as much as those treated with the 75% DEET/alcohol formulation. The Kevlar fabric broke outside of the treated area in all of the tests which were conducted for both formulations.

2.1.5.2 Compatibility of Repellents with Plastic and Painted Materials

Various types of paint were tested with the Phase II submission and 75% DEET/alcohol to compare the pitting tendency of the formulations (ASTM 6-46). The samples were applied at two application rates - 5 g/m^2 and total saturation (immersion) and aged at two temperatures - room temperature and 71°C (160°F) for 24 hours. Both formulations caused deterioration of the painted surfaces, especially when saturated. At the lower application levels, the Phase II submission was less harsh on the surfaces than the 75% DEET formulation (see Table II).

2.1.5.3 Compatibility of Repellents with Plastic Materials

The Shore Hardness was determined on a number of plastic substrates before and after treatment with the two repellent formulations (see Table III). The formulations were applied at two levels and aged at two temperatures. Overall it appears that the Phase II submission affects the plastics slightly less than the 75% DEET/alcohol formulation.

2.1.5.4 Repellent Compatibility with Rubber Materials and Leather

The rubbers shown in Table IV were treated with the two formulations at two levels of application and aged at room temperature and 71°C for 24 hours. At the lower application level, there appeared to be very little affect. At the higher level, the Phase II submission caused the natural and neoprene rubber to soften. The formulation literally stuck to the rubber.

2.1.5.5 Repellent Compatibility with Camouflage Paint

The compatibility of the two repellent formulations was determined using camouflage face paint, compact-type container in two ways. First, the two repellent formulations - Phase II submission and 75% DEET/alcohol were applied at the use level as dictated on the label. Then the camouflage face paints were applied and observations made. The second way consisted of applying the face paints first, and then the repellents.

Applying the repellents first and then camouflage paints netted the following: The white and green paints covered the treated arms very well; the brown and the green paints covered the arm treated with 6 drops of 75% DEET/alcohol better than the arm treated with our Phase II submission. When the camouflage paints were applied first, both repellents smeared the paints severely. Bottom line is that it is better to apply the repellents first, and then the paints.

2.1.6 Odor Comparison Study

The odor comparison study was conducted in Dallas at the same time as the troop acceptance study. At a distance of 5 feet, 11.5% of the 200 respondents stated they could detect the odor of the 3M formulation and 10% stated they could detect the odor of the 75% DEET/alcohol formulation. The odor detectability of both formulations was essentially the same.

2.1.7 Package Design

The package for PCP/3M's Phase II arthropod repellent is an olive drab, 2 ounce, high density polyethylene tube with a cap with a flip-up spout.

Color: Olive Drab
Material: High Density Polyethylene
Size: 1-1/2" x 3-1/2" Tube
Neck Finish: 22/400
Orifice: 0.500
Decorating: Plain
Interior Lacquer: None
External Coat: #1004 Barrier Coat
Cap: Olive Drab Polytop Dispenser, Polyethylene 22/400

2.1.8 Registration Data Package

An Environmental Protection Agency (EPA) Registration Data Package was compiled for PCP/3M's insect repellent lotion. It contained the following:

Application for Pesticide Registration
Confidential Statement of Formula
Formulator's Exemption Statement
Chemistry Information Matrix (and Data)
Acute Toxicology Data
Draft Label

This was sent in to the Army as an annex to the technical data package.

2.1.9 Label

The final label for PCP/3M's arthropod repellent formulation which is in compliance with Sections III through VII of the EPA Registration Standard and Section V of the Guidance Package follows. Please note that the percent N,N-diethyl-m-toluamide is different that that which appears in PCP/3M's Phase II products. The labels for the tubes were prepared before the EPA Registration

Data Package. During the completion of the latter it was learned that the minimum amount of DEET which could appear in the product during manufacturing had to be the label amount.

Front Label

YYYY-YY-YYY-YYYY
INSECT REPELLENT LOTION (CREAM)
TYPE (XXX)
Federal Specification XXXXXXX
Contents: 2 Fluid Ounces

Repels biting flies, chiggers, deer flies, mosquitoes, fleas and stable flies. Also repels terrestrial leeches in tropical areas where pest occurs.

Provides 95% or greater protection against mosquitoes for 12 or more hours under normal use conditions.

ACTIVE INGREDIENTS: N,N-Diethyl-m-toluamide 31.58%
Other isomers 1.58% inert ingredients 66.75%.

FOR EXTERNAL USE ONLY
Keep out of reach of children.

Caution - Avoid contact with eyes and lips. In case of eye contact, flush with plenty of water. Do not apply to excessively sunburned or damaged skin.

Contract No. DAMD17-85-C-5017

Back Label

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Squeeze into one hand a 2.5 ml strip of repellent, equal in length and width to the diagram on the side of the tube. Rub hands together and apply thoroughly in a thin layer to both forearms. Use additional lotion for upper arms. Repeat for other exposed areas. **To apply to face** squeeze lotion into palm of hand and spread on face and neck. **Avoid Contact With Eyes and Lips. To apply to clothing,** dispense the lotion into one hand, rub the hands together and brush lightly on socks, around collars, waist, sleeve and trouser cuffs and where clothing fits snugly such as over the shoulders, elbows, knees and buttocks. Repeat as necessary. Wipe hands after application.

May Damage certain synthetic fabrics, plastics, painted or varnished surfaces. Avoid smearing on plastic eyeglass frames, goggles, watch crystals, etc. **WILL NOT DAMAGE** nylon, cotton or wool fabrics.

Disposal: Do not reuse empty container. Wrap container and put in trash.

Personal Care Products/3M
3M Center
St. Paul, Minnesota 55144-1000

EPA Reg. No. XXX
EPA Est. No. XXXXX

2.1.10 In-Vitro Penetration/Evaporation Test

Personnel from PCP visited Letterman Army Institute of Research (LAIR) on February 5, 1986, to observe the operation of the in-vitro test procedure developed by Dr. William Reifenrath and co-workers[3] to measure the evaporation and penetration of DEET from pigskin. Initially, PCP chose to monitor the procedure with unlabeled DEET using a capillary gas chromatograph. Amounts as small as tenths of a microgram of DEET in a Tenax GC extraction solution have been determined. The exact procedure used is as follows:

One millimeter thick pig skin epidermis which had been stored frozen for one month was mounted on Laboratory Glass Apparatus evaporation-penetration chambers. The 3M candidate formulation, .0007 g, was applied to the first, third and fifth chambers using a stirring rod and weight differences. The second, fourth and sixth chambers were each treated with a dose of 252.2 ug/.782 cm^2 of N,N-diethyl-m-toluamide. The penetration and evporation jackets were maintained at 37°C. The flow rate of air at 23°C and 55% RH through the evaporation cells was 600 ml/minutes. Tubes containing Tenax absorbent were mounted in the evaporation cells to trap the evaporated DEET from the air stream. The tubes were replaced with fresh ones at 1, 2, 4, 6, 8, 10, 21, 23 and 25 hours. The Tenax absorbent in the tubes was extracted with 10 ml of methyl ethyl ketone and the amount of DEET determined by capillary-gas chromatography. A profile of the evaporation of DEET versus time is shown in Figure XXVIII for the two formulations. Ringers lactate solution with 1 ml of added gentamicin sulfate was pumped through the penetration chamber at 5 ml/hour. The solutions were pooled into a 0-10 hour sample and a 10-25 hour sample. These were extracted with ethyl ether, dried on a roto vac, reconstituted with 10 ml of methyl ethyl ketone (MEK), dried with a small amount of anhydrous magnesium sulfate, filtered and the amount of DEET determined by capillary-gas chromatography. At the end of the experiment, the evaporation cell was rinsed with MEK, the pig skin was cored with a cork bore and both the inner and outer sections were cut up and extracted with MEK. Problems were encountered when the skin pieces were digested with dry ice in a small blender; they weren't cut up in very small pieces. A better job was accomplished by physically cutting the skin with a razor blade.

3 - Procedures similar to G.S. Hawkins and W.G. Reifenrath, Fundam. Appl. Toxicol., 4, S133-144, (1984).

The data is summarized in Figure XXiX and a graph of the evaporation data is shown in Figure XXViii. The iatter shows the extension of the evaporation rate of DEET for ionger iengths of time for the 3M formuiation. it is this extension above the 1 ug/cm^2/hour minimum effective dose which is probabiy responsibie for the increased mosquito repeiiency seen for this formuiation.

The evaporation penetration experiment was repeated once more using the same conditions as above. The evaporation curves were very simiiar to those seen previousiy. The data is summarized in Figure XXXii. The evaporation rate curve is shown in Figure XXXi.

Personai Care Products did not run this test using the radioiabeied DEET. The resuits obtained using non-radioiabeied materiai seemed to fuifiii the requirements of the contract without PCP having to work with the iabeiied DEET.

2.1.11 User Training Package Deveiopment

The appropriate changes have been made in Section iii, Biting Insects, Use Insect Repeiient

Fieid Hygiene and Sanitation, Section 4. REPELLENTS 3.4.3.1 Personai Use or Skin Appiication

Miiitary Entomoiogy Operation Handbook, 91. Individuai Protective Measures, C. insect Repeiient for Personai Appiication (DEET)

to aiiow the use of PCP's new dispenser and arthropod repeiient iotion. The abridged sections foiiow:

Section iii. Biting insects

USE INSECT REPELLENT

Use on aii exposed skin: face, ears, neck, arms, and hands. Do not get repeiient in eyes or mouth.

Use where ciothing fits tightiy, such as upper back, buttocks, and ankies.

Appiy a thick coat immediately if you get wet or --

* Every 11-12 hours if you get sweaty, or
* Every 14-15 hours if you don't get wet or sweaty.

Read the iabel for directions and other precautions before using.

WEAR UNIFORM PROPERLY

Wear uniform as your commander directs.
Wear headgear to protect the top of your head.

3.4.3.1 Personai Use or Skin Appiication. Repelients for personai use are appiied directiy to the skin. Usualiy a smaii amount rubbed between the hands and spread evenly over the face, neck, hands and other exposed skin areas offer protection, for severai hours, depending upon the pest species concerned. An additional amount may be spread on the ciothing at the shoulders and other areas where the cloth fits tightly against the body. Be carefui to keep the chemicals out of the eyes and mouth. The chemical is lost from the skin by abrasion, absorption, and evaporation. The effectiveness of the material is iost more rapidly in hot, humid ciimates where profuse sweating occurs. Repeilents which are recommended for application on the skin may aiso be appiied by hand to the outside of the clothing if desired. However, severai speciai items have been developed for impregnation of ciothing to either repel or kiii mites, insects, or other pests. The repeiient for personai use is DEET (N,N-diethyi-m-toiuamide) iotion which provides protection against aii types of mosquitoes and other biting Diptera and against fleas. it is reiatively effective against ticks and chiggers.

c. insect Repelient for Personai Appiication (DEET). This insect repeiient is avaiiabie in a 2-ounce piastic tube. insect repeiient must be appiied to the hands and then rubbed on the face. With DEET on the exposed skin and with the uniform impregnated and worn correctiy (a and b above), good protection is provided against disease-carrying mosquitoes and other insects for 12 or more hours, provided the repeiient is not washed off or diiuted with perspiration. More frequent appiications may be necessary for soidiers engaged in strenuous activity. in an emergency DEET appiied to hands and then brushed on serves as a suppiementary repelient for clothing (b above). The DEET shouid be appiied around the ciothing openings such as the coiiar, waist, sieeve cuffs, and boot tops and to other parts which fit over the body snugiy such as over the shouider biades and buttocks.

2.1.12 Shelf Life Studies

Preliminary stability testing of the final formulation was started during the search for a better packaging material (see Section 2.4).

2.1.13 Deliverables

The following quantities of PCP/3M's Phase II arthropod repellent were sent to the following addresses:

Quantity	Location	Delivery Date
1650 Specimens	Product Manager for Arthropod Repellents ATTN: SGRD-UMB (Colonel Reinert) U.S. Army Medical Material Development Activity Building T-622 Fort Detrick, Frederick, MD 21701-5009	September 10, 1986
500 Specimens	Department of Cutaneous Hazards; Letterman Army Institute of Research ATTN: SGRD-UL-CH Building 1110 Presidio of San Francisco, CA 94129	September 8, 1986
450 Specimens	Insects Affecting Man and Animals Research Lab P.O. Box 14565, USDA ATTN: Repellent Section Gainesville, FL 32604	September 8, 1986
24 Specimens	Insects Affecting Man and Animals Research Lab (same address as above)	July 3, 1986

2.2 Formulation Refinement and Development

The preferred way to obtain increased mosquito repellency would be to use a formulation containing a higher level of DEET. During the Phase I contract, all attempts to prepare the continuous water emulsions containing levels of DEET greater than 30% were not very successful. The formulations were either unstable or cosmetically unacceptable. When the Phase I contract

ended, this effort was renewed without any expense to the Army.

2.2.1 Formulations Containing 40% DEET

The initial "successful" attempt at preparing a continuous-water arthropod repellent emulsion containing higher levels of DEET came when the Phase I submission was proportioned up so that the resultant formulation contained 40% DEET and, of course, less water. The emulsion looked reasonably good, so a statistical design was set up to optimize its stability and cosmetic appearance. The ingredients in the formulation were the independent variables which were studied to determine what effect they had on the emulsion and which ones contributed positively to the formulation. Based on this, second generation repellent formulations were prepared. The composition of one of the better 40% DEET, continuous water emulsion formulations is shown in Figure I.

While PCP was successful in making arthropod repellent lotions with higher levels of DEET, it was felt that these amounts were overkill when one considers the mosquito repellency data generated for PCP's Phase I 30% DEET submission. In addition, these higher levels of oil phase in a lotion generally contribute to less cosmetically acceptable formulations. Based on this, a repellent formulation containing 35% DEET seemed to be a nice compromise for future Phase II work.

2.2.2 First 35% DEET Formulation Design Experiment

The first design experiment of the Phase II contract to increase the mosquito repellency attributes and to improve the cosmetic acceptability of PCP's Phase I arthropod repellent formulation follows. The level of DEET was increased to 35% (from 30%) and the acryiate polymer to 5.83%. The aesthetics would be improved by properly balancing the amounts of the other ingredients in the formulation. The independent variables in the design were the raw materials (ingredients) used to make the formulations and were $\pm$ 20% of the centerpoint composition as dictated by the design matrix (see Figure II). The response (dependent) variables were the formulation resistance, formulation viscosity, formulation aesthetic evaluation and the 6-hour DEET substantivity. The form used for the aesthetic evaluation is shown in Figure III. Here a lower score indicates a better formulation. The resistance measurement is indicative of stability and shows whether the formulation is continuous water; a lower value indicates a water continuous system. The results of these tests for this design experiment are shown in Figure IV. The fifth

formulation in the design had the best aesthetic value, the lowest formulation resistance and an acceptable DEET retention (substantivity) value. Statistical analysis of the individual aesthetic response factors for rub-in, tackiness, etc., indicated which raw material affected the formulation the most. Less of the Varonic surfactants (independent variable 1 in Figure ii), less Lexemul AS (independent variable 2) and more Carbowax 400/Liponic EG-7 (variable 3) in the formulations were shown to improve the cosmetic characteristics. Formulations with less of the Varonic surfactants (1) also resulted in increased DEET substantivity on the skin.

2.2.3 Second 35% DEET Formulation Design Experiment

The second statistical design experiment was set up utilizing what was learned above. The preferred formulation from the first design (Figure V) serves as the center point for this study. The independent variables were those which were shown to have an affect in the first design. The design matrix and the independent variables are shown in Figure Vi.

2.2.3.1 Aesthetic and DEET Substantivity Studies

The data on the aesthetic evaluation, the formulation resistance and the 6-hour DEET substantivity test are shown in Figure Vii. Aesthetically, the formulations are improved over the best formulation from the first 35% design. Statistical analysis of the data indicated that formulations with more Lexemul AS (independent variable 3 in Figure Vi), less Carbowax 400 (independent variable 4) and more Liponic EG-7 (independent variable 5) were cosmetically more acceptable. The best formulations were Number 3, 2 and 6.

The DEET substantivity decreased slightly for these design formulations. This could have been experimental error. The formulation with the highest substantivity was Number 8. The best formulation combining the aesthetic values and DEET substantivity value was Number 6 (Figure Viii).

2.2.3.2 Comparison to Phase I Submission

The preferred formulations developed in Phase ii were compared to PCP's Phase i submission. Aesthetically, the 35% DEET formulations were

demonstrated to be better than the Phase I submission. Aiso, the 35% DEET formuiations retained more DEET on the skin's surface via the 6-hour DEET substantivity test.

2.2.3.3 Mosquito Repeiiency Test

A iaboratory mosquito repeiiency test was conducted at Hazieton Laboratories. Therein the preferred formuiations from the second 35% design aiong with PCP's Phase i candidate formuiation were evaiuated. The modified ASTM: E951-83 was used. The test conditions were variable high humidity: 78-95°F; 74-100% R.H. Fifteen 5-15 day oid femaie Aedes aegypti mosquitoes were used for each exposure. After exposure to the test sites, these mosquitoes were sacrificed and fresh ones used for the next exposure. The reserve mosquitoes were kept outside of the high temperature room, were transferred to the smaii cages, and then brought in approximateiy 1/2 - 3/4 hours prior to exposure. The formuiations tested are shown in Figure Xi and the repeiiency resuits in Figure Xii. The percent repeiiency vaiues at 12 hours and 14 hours demonstrate the differences quite niceiy. The formuiations containing the higher ieveis of DEET are better than formuiations containing 30% DEET (E&F).

2.2.4 Third 35% DEET Formuiation Design Experiment

During a site visit, Coionei J. Reinert expressed his concern that the aesthetic properties of the formuiations couid stiii be improved. Therefore Cabosii was inciuded as an aid to reduce the gioss and the oiiiness of the formuiations after appiication. A third design experiment was set up using the best formuiation to date as the center point. The independent variabies were the raw materiais -- Cabosii M-5 (fumed siiica), Liponic EG-7 (giycereth-7), Lexemui AS (giyceryi monostearate) and Carbowax 400 (poiyethyiene giycoi 400), (see Figure iX). These were varied at $\pm$ 20% in the formuiations as the design matrix dictated. The other non-varying raw materiais utiiized are aiso shown in Figure iX. The response variables for the design were the formuiation aesthetics, 6-hour DEET substantivity and viscosity. The aesthetic score expressed as a percentage and the substantivity vaiue were combined for each test formuiation and appear at the far right in Tabie iX.

Statisticai anaiysis of the data indicated that higher ieveis of Cabosii resuited in formuiations with improved aesthetic properties (reduced gioss and oiiiness) and that higher ievels of Carbowax 400 had the opposite effect. This is contrasted with the DEET substantivity vaiues where the Cabosii reduced the vaiues whiie Carbowax 400, Lexemui AS and Liponic EG-7 increased the vaiues. Looking at the combined scores in Figure iX, one can see that the best formuiation with respect to substantivity and aesthetic properties was #8 (49-18-6).

2.2.5 Fourth 35% DEET Formuiation Design Experiment

Since Cabosii had the biggest effect on improving the cosmetic acceptabiiity and Carbowax 400 seemed to improve the DEET substantivity the most, a fourth design was run to optimize these. The design matrix and response variabies are in Figure X. As before, Cabosii reduced the substantivity vaiues. However, Carbowax 400 aiso reduced it. Apparentiy the amount of effective Carbowax has been exceeded and a iower ievei is indicated. Formuiations from the previous design were better than these were.

A separate approach to improve the aesthetic properties of the most substantive formuiation from Design iii wouid be to use higher ieveis of Cabosii - 2.75% and 3.50%. These formuiations are 213-8-7 and 213-8-8 in Figure X. The aesthetic vaiues for the iatter were the best obtained to date. However, the substantivity vaiues were sacrificed too much.

2.2.6 Finai Seven 35% DEET Formuiations

The iong iead times required for toxicoiogy testing dictated that PCP choose their finai formuiation(s) by Aprii 1, 1986. Seven formuiations from the iast two designs were chosen based on aesthetic and 6-hour DEET substantivity scores as PCP's finai Phase ii formuiations. These were sent to be evaiuated for toxicity on animais at Hazieton Labs and on humans by Dr. Maibach in San Francisco, Caiifornia. They were evaiuated for aesthetic acceptabiiity via the fuii arm procedure and for mosquito repeiiency at Hazieton Labs. These two tests were used to par the preferred formuiations down to one, the finai Phase ii formuiation.

2.2.6.1 Aesthetic Evaiuation

The finai seven formulations were evaiuated for their cosmeticaiiy acceptabiiity. The participants were asked to rate the formuiation,

using the form in Figure iii during application and 1 and 10 minutes after application. They were instructed to apply the product (1 ml) over their "entire" forearm. The data (Figure XV) showed that formulation 49-19-2 had the best (lowest) rating with 11.8, followed by 49-18-7 with 12.0 and 49-18-6 with 12.2. Statistically the values are probably all equivalent.

The Phase I submission was included in this evaluation to see if aesthetic improvements had been made. It scored 14.0. As can be seen, the Phase II formulations which contain more DEET have lower scores and are cosmetically superior.

2.2.6.2 Mosquito Repellency Test

The same formulations were tested for mosquito repellency using the modified ASTM:E951-83 procedure. Fifteen fresh, 5-15 day old, female Aedes aegypti mosquitoes were used during each exposure. The variable high humidity basic climatic condition was used. Six of the formulations passed the 95% repellency requirement at 12 hours and 4 of the 35% DEET formulations were still effective at 16 hours (Figure XIV). Formulation 49-18-6 was the best with 99% repellency at 16 hours followed by 49-18-7, 213-8-3 and 49-19-2, with 97% repellency.

Personal Care Products' Phase I arthropod repellent formulation had 95% repellency at 12 hours when evaluated under the same conditions during Phase I. Its repellency fell below 95% after 12 hours. These data were duplicated during Phase II (see Figure XI and XII) when the Phase I formulation was used as a reference point. The current 35% DEET formulations with values of 97-99% at 16 hours demonstrates a marked improvement over the Phase I product. Personal Care Products was supposed to improve the repellency values in Phase II of the contract and have done so quite nicely.

2.2.6.3 Final Phase II Arthropod Repellent Selection

Formulation 49-18-6 was chosen as PCP's final Phase II arthropod repellent selection. It was felt that for a repellent product, its mosquito repellency attribute should be most important.

This formulation repelled mosquitoes the best, and its aesthetic properties were equivalent to those of the other cosmetically preferred formulations.

2.2.7 Final Attempted Formulation Aesthetic Improvement

During the evaluations reported above, there was always a person who wanted a formulation containing 45 percent water to perform cosmetically like a formulation containing 85 percent water -- which isn't going to happen. To address this, a number of formulations were prepared with reduced viscosities. This was achieved by reducing the levels of hydroxyethyl cellulose and magnesium aluminum silicate in the formulations. Also, one would think that the addition of a sticky acrylate polymer would impact greatly on the aesthetic properties of a formulation, and it generally does exactly this. So, formulations were prepared with 5.00% and 4.20% polymer instead of 5.83%. All of these were evaluated full arm for their aesthetic properties. None were better than 49-18-6. They actually were worse. Formulation 49-18-6 had been optimized with respect to the raw materials using design experiments. Apparently if one ingredient level is changed, it throws the whole formulation out of balance aesthetically.

One thing which did improve the aesthetics of a formulation was to reduce the total oil phase. Formulations containing 30 and 25% DEET were prepared by proportioning the 35% formulation (49-18-6) down. Aesthetic evaluation showed the 30% to be comparable to the 35% formulation while the 25% formulation was better with a score of 10.6. Again, what is the mosquito efficacy of these formulations?

A modified ASTM:E951-83 procedure was used to evaluate repellency in the variable high humidity climatic condition. The percent repellency data is shown in Figure XVIII. The preferred 35% DEET formulation (49-18-6) has 98% repellency at 16 hours. The other formulations containing 30, 25 and 30% DEET had 95% protection times of 13, 10 and 15 hours, respectively. Since the repellency had to be as good as it could be, and the aesthetic properties of the formulations were somewhat similar, 49-18-6 will remain the preferred and final formulation.

2.3 Toxicology Testing

The long lead times required for toxicology testing necessitated

PCP's selection of their final formulation by the first of April, 1986. The best seven formulations based on aesthetic evaluations, 6-hour DEET substantivity values and mosquito repellency data (when available) were submitted for testing. The animal tests were conducted by Hazleton Laboratories in Madison, Wisconsin, and the human test by Dr. Howard Malbach.

The seven formulations were very similar with respect to their compositions. They all used the same ingredients. The only difference between them was the amount of each ingredient. For example, one formulation would have 1.75% of an ingredient; the next would have 2.75% of the same ingredient, etc. Of the seven formulations tested, six easily met all of the toxicological requirements set forth by the EPA. The seventh had a problem with the eye irritation study in which "five of six unwashed eyes had all zero scores at 7 days, the sixth eye had all zero scores at 14 days". In a separate test where the eyes were rinsed after application, "two of three eyes had all zero scores at 7 days, but one had approximately 15% corneal epithelial peeling at 21 days" (Frank Griffith - draft summary of toxicity tests). This washed eye irritation study was repeated. This time, two eyes were all zero scores at 7 days and the third eye was all zero scores at 14 days. The EPA requirement is that all scores are zero at 21 days. This seventh formulation is PCP's preferred final Phase II repellent.

The specific data of only PCP's final Phase II repellent is attached (Appendix D). The other data is available from PCP if necessary.

2.4 Package Design

The Phase I package for PCP's arthropod repellent lotion was a 2 ounce low density polyethyiene tube with a flip-top cap. After aging for 3 months, there was a significant weight loss in the samples at 113°F and a smaller loss at room temperature (Figure XVI). The point of failure seemed to be at the seam on the sealed end of the tube. No leakage was seen through the flip-top cap (see high density polyethylene [HDPE] bottle with the flip-top cap). Colonel Reinert confirmed this problem with the samples sent to him, as well as the samples sent for repellency testing by USDA personnel. Basically it appeared that the sealed area was not heated enough to fuse the plastic or the area had been contaminated with formulation prior to the sealing process.

To test these hypotheses, additional tubes were filled and sealed. The pressure required to burst a tube or cause a seam to split was 251 $\pm$ 31 pounds per square inch for 15 tubes. Not one of the seams split open. The tubes all failed by rupturing at the edge of the seam on the tube side.

PCP's first choice for a package still remained the tube. However, we opted to order the high density polyethylene tubes because they are more resistant to penetration by an ingredient than the low density tubes (Figure XVI). Therefore, 10,000 barrier coated, olive-drab, high density polyethylene tubes were ordered for PCP's Phase II submission as well as 10,000 olive-drab push-up spouted caps.

2.5 Package Label

A completely new prototype label (Figure XX) for PCP's arthropod repellent formulation which is in compliance with Sections III through VII of the EPA Registration Standard and Section V of the Guidance Package was sent to Colonel Reinert and Mr. Louis Rutledge for their comments and suggestions. In the interim, more specific use directions were developed to insure that the required amount of repellent is dispensed each time to deliver the best protection (see Figure XXI).

The comments and suggestions received from Mr. Rutledge (LAIR) were incorporated into the label. The final Phase II label is shown in Figure XXVIIa and XXVIIb.

2.6 Shelf Life Studies

This deals with continued Phase I submission shelf life studies and those for Phase II.

2.6.1 Phase I Submission

Shelf life studies of PCP's Phase I submission after three months at 113°F were less than desirable. The formulation in glass looked very good (see Figure XVI). However, in low density polyethylene (LDPE) tubes, there was a significant weight loss. The problem was traced to leaks in the sealed end of the tubes (see Section 2.7). As pointed out before, this leakage is easily controlled if care is taken in the sealing step. The above study also tested HDPE (high density polyethylene) tubes. These were shown to have a lower weight loss with time and were selected as the tube of choice.

2.6.2 Phase II Submission

The HDPE tube was chosen as the tube of choice to package PCP's Phase II insect repellent lotion. This selection

was confirmed as being the best after aging the respective tube choices at 120°F for two months. The HDPE tube had the lowest percentage weight loss after two months. Again, the low density polyethylene tube was shown to be unacceptable (see Figure XXX).

3.0 SUMMARY

A Phase II Army contract was received by Personal Care Products/3M for the refinement and improvement of PCP's Phase I submission of a "controlled-release arthropod repellent formulation for topical application to a soldier's exposed skin areas that will provide extended protection against biting arthropods, be safe and agreeable to use, be compatible with other current and projected military materials and systems, and comply with registration requirements of the Environmental Protection Agency". The Army stated that the aesthetic properties and the repellency characteristics of PCP's Phase I formulation needed to be improved.

Increased repellency for the formulation could be obtained by increasing the percent DEET, by increasing the amount of acrylate polymer, or by increasing the molecular weight of the polymer. The last two would have a negative impact on aesthetic properties, so the first was chosen as the approach to take. The aesthetic properties of the formulation would be improved by balancing the various raw materials used to make up the formulation.

Statistical design experiments were used to optimize the aesthetic properties of the formulation. The independent variables were the ingredients used to make the formulation. The dependent variables, i.e., response variables, were: formulation resistance (a measure of stability), formulation viscosity, formulation appearance, aesthetic evaluations, 6-hour DEET substantivity and mosquito repellency.

Four design experiments using 35% DEET were run consecutively to study the effect of the formulation ingredients on the response variables listed above. In this manner, the amounts of ingredients were adjusted to optimize the formulation with respect to aesthetics, DEET substantivity, etc. Out of more than 60 formulations studied, seven were selected as the most preferred. At this time and prior to the final selection process, all seven of these formulations had to be sent out for human and animal toxicology assessments because of the long lead times required.

The selection process to determine the most preferred formulation consisted of evaluating the aesthetic properties more critically and determining the mosquito repellency attributes of the formulations. A full arm aesthetic evaluation panel was used to pick out the three best formulations (49-19-2, 49-18-7 and 49-18-6). Correspondingly, laboratory mosquito repellency tests showed that formulations 49-18-6, 49-18-7, 213-8-3 and 49-19-2 gave the best percent

repellency at 16 hours in the variable high humidity climatic condition. Since the aesthetic assessment of the three best formulations showed them to be statistically equivalent, the final selection was based on repellency data. Formulation 49-18-6 with 99% repellency at 16 hours was chosen as PCP's final Phase II arthropod repellent formulation. This formulation will be referred to as the Phase II submission hereafter.

The Phase II submission was evaluated for mosquito repellency in the laboratory and in the field. For the former, a modified ASTM:E951-83 method was used. The Phase II submission and a 75% DEET/alcohol formulation were evaluated at the use levels suggested by the label in the constant high humidity, the variable high humidity and the basic hot climatic conditions. The Phase II submission had 95% repellency protection times of 14-15 hours, 10-11 hours and 14-15 hours, respectively. The 75% DEET/alcohol formulation had 10-11 hours, 2-3 hours and 10-11 hours, respectively. The same formulations were evaluated in the same manner via a modified ASTM:E939-83 test method outdoors in Louisiana in late July. The climatic conditions were the variable high humidity conditions. Against Aedes sollicitans mosquitoes in a swampy area near the Gulf, complete protection times of 10.7 $\pm$ 2.6 hours and 5.5 $\pm$ 1.8 hours were determined for the Phase II submission and the 75% DEET/alcohol formulation respectively. The same formulations evaluated under similar conditions against Anopholes quadrimaculatus mosquitoes gave complete protection times of 12.4 $\pm$ 1.9 hours and 7.7 $\pm$ 1.8 hours, respectively.

The same two formulations were evaluated for cosmetic acceptability to men and women of military age. The test was run in the variable high humidity climatic condition in Texas. After being outdoors for ten minutes, 88% of the participants stated that they would be at least likely to use the PCP/3M Phase II submission. For the 75% DEET formulation, 91% stated the same. These values are not statistically different at 90% confidence. Both formulations exceed the 75% user acceptability requirement. Similarly, 46.5% of the people preferred the Phase II submission and 53% preferred the 75% DEET formulation. Again, these values aren't statistically different.

The odor comparison study, at a distance of 5 feet, showed the formulations as essentially the same. For the Phase II submission, 11.5% of the people stated they could detect an odor and 10% stated they could detect an odor for the 75% DEET formulation. Only 2% of the people detected an odor for both formulations.

The final package for the Phase II submission is a 2-ounce, olive-drab, HDPE tube with an olive-drab cap that has a push-up spout. This package is sufficiently different from the current military product to prevent the carryover of any negative opinion of the latter. This tube will easily fit into the blouse pocket of a battledress uniform. Initial stability testing of the Phase II submission in this package is very good. The weight loss is less than

that for the LDPE tubes proposed for Phase I.

A prototype label has been developed for the Phase II submission and has been affixed to samples sent to the Army using pressure sensitive adhesive technology. For Phase III, the label would be silk-screened directly onto the tubes. The label is in compliance with Sections III to VI of the EPA Registration Standard and Section V of the Guidance Package. The "Directions for Use" section was developed in conjunction with the Department of Cutaneous Hazards, LAIR.

An EPA Registration Data Package has been put together for the registration of PCP/3M's Phase II arthropod repellent submission. This was annexed to the Technical Data Package dealing with the manufacturing process, product specifications, raw material specifications, etc.

In-vitro penetration/evaporation studies of the Phase II submission were run. They demonstrated that the evaporation rate of DEET from the skin is maintained above the minimum effective dose for longer lengths of time for this formulation than for a DEET/alcohol formulation at the same concentration.

A user training package was put together for the Phase II submission.

4.0 CONCLUSION

Personal Care Products of the 3M Company has improved the mosquito repellency and aesthetic properties of a controlled-release personal use arthropod repellent developed in Phase I of this contract. The Phase II formulation contains 35% DEET and an acrylate terpolymer. The formulation is acceptable to 88% of men and women of military age when evaluated in the variable high humidity climatic condition. It repels Aedes aegypti mosquitoes for 14-15 hours, 10-11 hours and 14-15 hours in the constant high humidity condition, the variable high humidity condition and the basic hot climatic conditions respectively in the laboratory. The formulation had complete protection times of 10.7 $\pm$ 2.6 hours and 12.4 $\pm$ 1.9 hours against Aedes sollicitans and Anopholes quadrimaculatus mosquitoes in field testings conducted in the variable high humidity climatic condition. These protection times were always greater than the 75% DEET/ethanol formulations evaluated at the use dosages per the label directions. The PCP/3M formulation was shown to meet all the EPA requirements for toxicity testing and it is less irritating than the current Army repellent issue. The odor signal of the formulation was shown to be comparable to the Army issue at a distance of 5 feet. The formulation is as compatible with military camouflagic face paints and is less damaging to selected military materials than the Army's current formulation.

The package for the 35% DEET formulation is a 2-ounce, olive-drab, HDPE tube with an olive-drab cap with a push-up spout. A label in

compliance with Sections III to VI of the EPA Registration Standard and Section V of the Guidance Package was attached to the tube. The specific directions for use were developed in conjunction with the Department of Cutaneous Hazards, LAIR.

An EPA Registration Data Package and a Technical Data Package were also put together.

Neil A. Randen, Ph.D.
Principal Investigator

NAR:lme

APPENDIX A

FIGURE I

40% DEET FORMULA

INGREDIENTS (CTFA NAMES)	% BY WGT
Deionized Water	34.94
Polyethylene Glycol 400	1.50
Glycereth-7	1.54
Magnesium Aluminum Silicate	1.01
Hydroxyethyl Cellulose	1.01
PEG-82 Glyceryl Monotallowate	1.84
Glyceryl Monostearate	2.76
3M Polymer (85:7.5:7.5 mole ratio iso-octyl acrylate:stearyl methacrylate:acrylic acid)	6.67
DEET	40.00
Propylene Glycol Dicaprylate/Dicaprate	4.60
PPG-15 Stearyl Ether	1.23
Cetyl-Stearyl Alcohol	1.23
Cetyl Palmitate	.62
PEG-200 Glyceryl Monotallowate	.77
Diazolidinyl:Urea:Methyl Paraben:Propyl Paraben: Propylene Glycol	.24
	100.00

FIGURE

35% DEET OIL-IN-WATER FORMULATION DESIGN-I

DESIGN ORDER	INDEPENDENT VARIABLES						
	1	2	3	4(12)	5(13)	6(23)	7(123)
1	-	-	-	+	+	+	-
2	+	-	-	-	-	+	+
3	-	+	-	-	+	-	+
4	+	+	-	+	-	-	-
5	-	-	+	+	-	-	+
6	+	-	+	-	+	-	-
7	-	+	+	-	-	+	-
8	+	+	+	+	+	+	+
9a	0	0	0	0	0	0	0
9b	0	0	0	0	0	0	0
9c	0	0	0	0	0	0	0
9d	0	0	0	0	0	0	0

INDEPENDENT VARIABLES	CENTER POINT COMPOSITION*
1. PEG-200 GLYCERYL MONOTALLOWATE	.67%
PEG-82 GLYCERYL MONOTALLOWATE	1.61%
2. GLYCERYL MONOSTEARATE	2.42%
3. POLYETHYLENE GLYCOL 400	1.35%
GLYCERETH-7	1.35%
4. MAGNESIUM ALUMINUM SILICATE	.88%
HYDROXYETHYL CELLULOSE	.88%
5. PROPYLENE GLYCOL DICAPRYLATE/DICAPRATE	4.03%
6. CETYL PALMITATE	.54%
7. PPG 15 STEARYL ETHER	1.08%
CETYL-STEARYL ALCOHOL	1.08%

FI[illegible] III

SAMPLE _____ DATE _____ NAME _____

MOSQUITO REPELLENT AESTHETIC EVALUATION
(PUT ON OUTER FOREARM AREA, 0.1 ml)

A. APPEARANCE OF THE FORMULATION "PRIOR" TO APPLICATION (IN BOTTLE):

Appearance		Viscosity
2 = Not Very Good		2 = Too Thick
1 = Nice	+	1 = Too Thin -
0 = Very Nice		0 = Just Right

Score _____

B. RUB-IN (TIME IT TAKES FOR THE "EMULSION" [WHITENESS] TO DISAPPEAR ON THE SKIN):

4 = Very Long Time, Very Hard to Rub in
3 = Long Time, Hard to Rub in
2 = Acceptable Time, Not Hard, But Not Easy to Rub in
1 = Short Time, Easy to Rub in
0 = Very Short Time, VeryEasy to Rub in

Score _____

C. TACKINESS "DURING" APPLICATION:

3 = Very Tacky (Sticky)
2 = Tacky
1 = Slightly Tacky
0 = Not Tacky

Score _____

D. OILINESS "DURING" APPLICATION:

3 = Very Oily
2 = Oily
1 = Slightly Oily
0 = Not Oily

Score _____

TOTAL SCORE _____

E. APPEARANCE "AFTER" APPLICATION:

3 = Application Site Very Shiny
2 = Site is Shiny
1 = Site is Slightly Shiny
0 = Site is Not Shiny

	1 min.	5 min.	10 min.
Score			

F. TACKINESS "AFTER" APPLICATION:

3 = Very Tacky
2 = Tacky
1 = Slightly Tacky
0 = Not Tacky

	1 min.	5 min.	10 min.
Score			

G. OILINESS "AFTER" APPLICATION:

3 = Very Oily
2 = Oily
1 = Slightly Oily
0 = Not Oily

	1 min.	5 min.	10 min.
Score			

H. WOULD YOU USE THIS PRODUCT AS A REPELLENT IF YOU WERE GOING HIKING OR CAMPING IN AN AREA WITH LOTS OF FLIES, MOSQUITOS OR OTHER BITING INSECTS:

4 = Definitely Not
3 = Probably Not
2 = Would Use if Nothing Else
1 = Probably Would Use
0 = Definitely Would Use Score _____

FIGURE IV

35% DEET DESIGN-I RESPONSE FACTORS

DESIGN ORDER	"AVERAGE" TOTAL SCORE	AESTHETIC EVALUATION[1] DURING APPLICATION[1] RUB-IN	TACKINESS	OILINESS	AFTER APPLICATION[2] APPEARANCE	TACKINESS	OILINESS	WOULD USE	RESISTANCE	6-HOUR SUBSTANTIVITY
1	12.7	26	14	21	33	19	29	16	3900	84.9
2	16.7	34	21	25	63	18	46	21	3600	72.2
3	17.4	26	15	27	52	26	53	23	3400	87.5
4	17.9	32	21	29	63	23	56	24	3600	77.3
5	11.9	28	14	23	27	14	36	16	3200	83.2
6	13.8	25	15	23	36	26	44	16	4200	73.5
7	15.4	29	17	23	41	15	44	23	3800	79.3
8	17.9	31	18	27	58	25	54	26	3800	76.3
9a	15.7	32	17	23	44	17	36	20	3600	83.6
9b	15.4	34	15	26	42	22	41	20	3600	81.9
9c	16.4	35	15	23	57	24	48	22	3400	84.5
9d	17.9	35	20	27	61	27	52	24	3700	89.8
10[3]	14.4	38	13	21	24	8	27	40	650000	75.1

[1] n=15

[2] total of 15 evaluations

[3] no polymer

FIGURE V

PREFERRED FORMULATION FROM 35% DEET DESIGN
DESIGN ORDER (NUMBER)5 - FORMULATION 49-12-3

INGREDIENTS (CTFA NAMES)	% BY WEIGHT
DEIONIZED WATER	45.98
POLYETHYLENE GLYCOL 400	1.62
GLYCERETH-7	1.62
MAGNESIUM ALUMINUM SILICATE	1.06
HYDROXYETHYL CELLULOSE	1.06
PEG-82 GLYCERYL MONOTALLOWATE	1.54
GLYCERYL MONOSTEARATE	1.94
3M POLYMER (85:7.5:7.5 MOLE RATIO ISO-OCTYL ACRYLATE: STEARYL METHACRYLATE:ACRYLIC ACID	5.83
DEET	35.00
PROPYLENE GLYCOL DICAPRYLATE/DICAPRATE	3.22
PPG-15 STEARYL ETHER	1.30
CETYL-STEARYL ALCOHOL	1.30
CETYL PALMITATE	.43
PEG-200 GLYCERYL MONOTALLOWATE	1.29
DIAZOLIDINYL:UREA:METHYL PARABEN:PROPYL PARABEN: PROPYLENE GLYCOL	.24
	100.00

FIGURE VI

35% DEET OIL-IN-WATER FORMULATION DESIGN-II

DESIGN ORDER	INDEPENDENT VARIABLES 1	2	3	4(12)	5(13)
1	-	-	-	+	+
2	+	-	-	-	-
3	-	+	-	-	+
4	+	+	-	+	-
5	-	-	+	+	-
6	+	-	+	-	+
7	-	+	+	-	-
8	+	+	+	+	+
9a	0	0	0	0	0
9b	0	0	0	0	0
9c	0	0	0	0	0

INDEPENDENT VARABLES	CENTER POINT COMPOSITION
1. PEG-200 GLYCERYL MONOTALLOWATE	.54
2. PEG-82 GLYCERYL MONOTALLOWATE	1.29
3. GLYCERYL MONOSTEARATE	1.94 (2.90)
4. POLYETHYLENE GLYCOL 400	1.62
5. GLYCERETH-7	1.62

OTHER DESIGN POINTS ARE ± 20% OF THESE VALUES PER DESIGN

RESPONSE FACTORS (DEPENDENT VARIABLES)

FORMULATION RESISTANCE
FORMULATION VISCOSITY
FORMULATION AESTHETICS
6-HOUR SUBSTANTIVITY TEST

IN-VITRO EVAPORATION/PENETRATION
MOSQUITO REPELLENCY TEST

FIGURE VII

35% DEET DESIGN-II DEPENDENT VARIABLES

DESIGN ORDER	"AVERAGE" TOTAL SCORE	AESTHETIC EVALUATION[1]								
		DURING APPLICATION[1]			AFTER APPLICATION					
		RUB-IN	TACKINESS	OILINESS	APPEARANCE	TACKINESS	OILINESS	MOULD USE	RESIS[illegible]CE	6-HOUR SUBSTANTIVITY
1	19.6	34	19	28	66	29	54	25	5[illegible]	80.2
2	13.5	28	18	20	38	20	42	13	5100	78.7
3	12.6	27	14	21	34	19	46	15	4300	76.1
4	16.6	34	17	24	63	21	61	21	4600	68.5
5	19.9	35	12	32	70	30	66	26	4600	80.5
6	14.0	26	16	21	30	27	39	16	4200	77.8
7	16.6	26	18	25	70	27	50	19	4300	75.1
8	17.7	34	17	28	63	34	56	21	4600	88.9
9a	18.2	34	18	24	58	31	49	23	4600	85.4
9b	16.9	32	15	26	68	22	52	20	4000	76.2
9c	14.3	25	19	25	34	21	44	18	4200	83.9

[1] TOTAL OF 15 EVALUATIONS

FIGURE VIII

PREFERRED FORMULATION FROM 35% DEET DESIGN-II
DESIGN ORDER (NUMBER)6: FORMULATION 49-14-3

	% BY WEIGHT
DEIONIZED WATER	43.57
POLYETHYLENE GLYCOL 400	1.30
GLYCERETH-7	1.94
MAGNESIUM ALUMINUM SILICATE	.70
HYDROXETHYL CELLULOSE	.70
PEG-82 GLYCERYL MONOTALLOWATE	1.03
GLYCERYL MONOSTEARATE	3.48
3M POLYMER (85:7.5:7.5 MOLE RATIO ISO-OCTYL ACRYLATE:STEARYL METHACRYLATE:ACRYLIC ACID	5.83
DEET	35.00
PROPYLENE GLYCOL DICAPRYLATE/DICAPRATE	3.22
PPG-15 STEARYL ETHER	.86
CETYL-STEARYL ALCOHOL	.86
CETYL PALMITATE	.65
PEG-200 GLYCERYL MONOTALLOWATE	.65
DIAZOLIDINYL:UREA:METHYL PARABEN: PROPYL PARABEN:PROPYLENE GLYCOL	.24
	100.00

FIGURE IX

35% DEET OIL-IN-WATER FORMULATION DESIGN III

DESIGN ORDER	FORMULATION NUMBER	DESIGN MATRIX INDEPENDENT VARIABLES				DEPENDENT VARIABLES VISCOSITY	AESTHETIC SCORE		6-HR SUBSTANTIVITY		COMBINED SCORE
		1	2	3	4 (123)		total	percentage			
1	49-18-2	-	-	-	-	94,380	13.5	67.1	70.8		137.9
2	49-19-2	+	-	-	+	156,000	10.0	75.6	68.1		143.7
3	49-18-3	-	+	-	+	81,900	12.7	69.0	73.5		142.5
4	49-19-4	+	+	-	-	154,400	10.6	74.1	63.4		137.5
5	49-19-3	-	-	+	+	107,600	11.9	71.0	77.5		148.5
6	49-18-7	+	-	+	-	>156,000	9.2	77.6	63.0		140.6
7	49-18-4	-	+	+	-	103,700	11.0	73.2	72.6		145.8
8	49-18-6	+	+	+	+	>156,000	9.9	75.9	80.5		156.4
9a	49-18-5	0	0	0	0	113,900	11.7		73.1		
9b	49-18-1	0	0	0	0	91,300	12.2	71.3	71.8	72.5	143.8
9c	49-19-1	0	0	0	0	107,600	11.4		72.7		

Center Point Composition

Independent Variables

1 - Fumed Silica (Cabosil M-5) - 2.00%
2 - Glycereth-7 (Liponic EG-7) - 1.94%
3 - Glyceryl Monostearate (Lexemul AS) - 3.48%
4 - Polyethylene Glycol 400 (Carbowax 400) - 0.98%

Other (Constant Ingredients)

PEG-200 Glyceryl Monotallowate - 0.65%, PEG-82 Glyceryl Monotallowate - 1.03%, Magnesium Aluminum Silicate - 0.70%, Hydroxyethyl Cellulose - 0.50%, Propylene Glycol Dicaprylate/Dicaprate - 3.22%, Cetyl Palmitate - 0.65%, PPG-Stearyl Ether - 0.86%, Cetyl-Stearyl Alcohol - 0.86%, 85:7.5:7.5 mole ratio Iso-Octyl Acrylate:Stearyl Methacrylate Acrylic Acid - 5.83%, N,N-Diethyl-m-Toluamide - 35.00%, Preservative - 0.24%, Water qs to 100.00.

FIGURE X

35% DEET OIL-IN-WATER FORMULATION DESIGN IV

DESIGN ORDER	FORMULATION NUMBER	DESIGN MATRIX INDEPENDENT VARIABLES		DEPENDENT VARIABLES AESTHETIC SCORE		6-HR SUBSTANTIVITY	COMBINED SCORE
		1	2	TOTAL	PERCENT		
1	213-8-3	-	-	10.1	75.4	67.8	143.2
2	213-8-4	+	-	11.8	71.2	73.8	145.0
3	213-8-1	-	+	14.3	65.1	66.9	132.0
4	213-8-5	+	+	11.0	73.2	58.4	131.6
5a	213-8-2	0	0	11.2	72.7	63.3	136.0
5b	213-8-6	0	0	9.9	75.9	61.8	137.7
	213-8-7			11.7	71.5	68.3	139.8
	213-8-8			7.6	81.5	65.0	146.5

Design Center Point6 Composition & Variation From Independent Variables

1 - Fumed Silica - 2.75 + 0.75%
2 - Polyethylene Glycol 400 - 1.00 ± 0.33%

Other Ingredients

Glycerol Monostearate 4.06%, Glycereth-7 - 1.62%, rest of ingredients are the same as in Figure IX.

FIGURE XI

MOSQUITO REPELLENT FORMULATION

FORMULATION NUMBER / INGREDIENTS (CTFA NAMES)	49-14-1	49-14-2	49-14-3	49-14-4	49-14-6	49-15-1	49-15-2	49-15-4	49-15-5	PHASE I	49-7-3
DEIONIZED WATER	44.28	44.01	43.57	43.88	42.38	44.18	44.40	43.76	45.34	43.19	51.12
POLYETHYLENE GLYCOL	1.94	1.62	1.30	1.30	1.94	1.94	1.30	19.4	1.30	2.00	1.16
GLYCERETH-7	1.94	1.62	1.94	1.30	1.94	1.30	1.94	1.30	1.30	2.00	1.16
MAGNESIUM ALUMINUM SILICATE	.70	.70	.70	.70	.70	.70	.70	.70	.70	.88	.75
HYDROXYETHYL CELLULOSE	.70	.70	.70	.70	.70	.70	.70	.70	.70	.88	.75
PEG-82 GLYCERYL MONOTALLOWATE	1.03	1.29	1.03	1.55	1.55	1.55	1.55	1.03	1.03	2.40	1.38
GLYCERYL MONOSTEARATE	2.32	2.90	3.48	3.48	3.48	2.32	2.32	3.48	2.32	2.40	2.08
3M POLYMER (85:7.5:7.5 MOLE RATIO ISO-OCTYL ACRYLATE: STEARYL METHACRYLATE:ACRYLIC ACID	5.83	5.83	5.83	5.83	5.83	5.83	5.83	5.83	5.83	5.00	5.00
OEET	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	30.00	30.00
PROPYLENE GLYCOL DICAPRYLATE/ DICAPRATE	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	6.00	3.46
PPG-15 STEARYL ETHER	.86	.86	.86	.86	.86	.86	.86	.86	.86	1.60	.93
CETYL-STEARYL ALCOHOL	.86	.86	.86	.86	.86	.86	.86	.86	.86	1.60	.93
CETYL PALMITATE	.65	.65	.65	.65	.65	.65	.65	.65	.65	.80	.46
PEG-200 GLYCERYL MONOTALLOWATE	.43	.54	.65	.43	.65	.65	.43	.43	.65	1.00	.51
DIAZOLIDINYL:UREA:METHYL PARABEN: PROPYL PARABEN:PROPYLENE GLYCOL	.24	.24	.24	.24	.24	.24	.24	.24	.24	.25	.24
HAZLETON CODE			A		B		C		D	E	F

FIGURE XII

PERCENT MOSQUITO REPELLENCY DATA VERSUS TIME

EXPOSURE TIME(HOURS)[1]	FORMULATIONS *						# OF BITES ON CONTROL
	A[2]	B[2]	C[3]	D[4]	E[4]	F[4]	
8	100	98	98	100	100	98	51
10	100	97	100	100	100	100	83
12	100	94	96	98	94	92	53
13	100	100	100	100	96	96	25
14	100	95	95	97	90	85	39
15	94	94	100	100	94	85	26
16	100	100	100	100	100	100	38

* See Figure XI for specific formulations

1 - Hours from application; 20 mg/3.0 cm diameter site
2 - Replications - 13
3 - Replications - 14
4 - Replications - 15

FIGURE XIII

PHASE II SECOND REPELLENCY TEST FORMULATIONS

	CABOSIL M-5	VAROMIC LI420	VARONIC LI48	LEXEMUL AS	CARBOWAX 400	LIPONIC EG-7	VEEGUM	NATRASOL 250HR	LEXOL PG 865	MAXEMOL B16	ARLAMOL E	ADOL 63	POLYMER	DEET	GERMABEN II	WATER
49-14-3(c-II)	--	.65	1.03	3.48	1.30	1.94	.70	.70	3.22	.65	.86	.86	23.33	17.50	.24	43.57
49-18-6(s-III)	2.75	.65	1.03	4.06	.98	2.26	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-18-7(a-III)	2.75	.65	1.03	4.06	.33	1.62	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-19-2(c-III)	2.75	.65	1.03	2.90	.98	1.62	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-19-3(s-III)	1.25	.65	1.03	4.06	.98	1.62	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
213-8-3(c-IV)	2.00	.65	1.03	4.06	.66	1.62	---	.10	3.22	.65	.43	.86	23.33	17.50	.24	qs
213-8-8(a-IV)	3.50	.65	1.03	4.06	1.00	1.62	---	.10	3.22	.65	.43	.86	23.33	17.50	.24	qs

FIGURE XIV

PHASE II SECOND MOSQUITO REPELLENCY TEST DATA [1]
VARIABLE HIGH HUMIDITY CLIMATIC CONDITION

FORMULATION[2]	PERCENT MOSQUITO REPELLENCY[3] EXPOSURE TIME (HRS)						
	8	10	12	13	14[4]	15	16
49-14-3	98	100	97	100	83	100	90
49-18-6	100	100	100	100	95	100	99
49-18-7	100	100	98	100	95	100	97
49-19-2	98	97	98	100	97	100	97
49-19-3	100	98	99	100	85	100	86
213-8-3	97	100	99	100	90	100	97
213-8-8	100	100	94	91	80	96	90
Total Bites on Control[5]	61	62	86	65	59	47	87

1 - Modified ASTM:E951-83

3 - Equals $\frac{\text{Total Control Bites - Treatment Bites}}{\text{Total Control Bites}} \times 100$

2 - Formulations in Figure XIII

4 - Data run but not on hand at time report written

5 - Thigh - no treatment control on ten participants

FIGURE XV

FINAL SEVEN FORMULATIONS AESTHETIC EVALUATION[1]

FORMULA NUMBER[2]	FORMULA VISCOSITY (CPS	TOTAL AVER. SCORE	TOTAL OF SCORES OF ELEVEN PARTICIPANTS												
			FORMULATION		DURING APPLICATION			AFTER APPLICATION							WOULD YOU USE IT
								APPEARANCE		TACKINESS		OILINESS			
			APPEAR	VISC	RUB-IN TIME	TACK-INESS	OIL-INESS	1min	10min	1min	10min	1min	10min		
49-14-3	84,000	16.4	20	9	25	10	19	17	10	6	4	20	18		22
49-18-6	270,000	12.2	9	8	17	19	16	8	3	14	10	11	7		13
49-18-7	260,000	12.0	8	12	19	15	15	10	6	10	7	12	10		17
49-19-2	231,000	11.8	7	4	15	17	17	9	6	12	7	13	9		14
49-19-3	110,000	15.0	11	12	20	18	20	10	8	10	6	17	15		18
218-8-3	39,000	14.7	6	0	17	13	24	17	13	12	7	20	15		18
218-8-8	107,000	14.6	9	7	17	15	20	15	9	15	9	19	13		16
Phase I		15.2	8	5	24	15	20	15	10	8	4	20	19		19

1 - 1.0 ml to full forearm, evaluation form - Figure III

2 - See Figure XIII for specific formulations

FIGURE XVI

PHASE I AGING DATA

CONTAINER	% WEIGHT CHANGE			
	ROOM TEMPERATURE		113° F	
	1 MONTH	3 MONTHS	1 MONTH	3 MONTHS
LDPE[1] Bottle	-0.08	-0.12	-1.00	-3.38
HDPE[2] With Flip-Top Cap	0.0	-0.06	-0.21	-0.89
HDPE With Sure Snap Cap	0.0	-0.01	-0.20	-0.82
LDPE Tube	-0.06	-0.21	-1.53	-5.98
Glass Bottle With HDPE Cap	-0.07	-0.15	-0.25	-1.06

1 - Low density polyethylene
2 - High density polyethylene

FIGURE XVII

FORMULATIONS FOR THIRD MOSQUITO REPELLENCY TEST

NOTEBOOK NUMBER	49-18-6	49-27-1	49-28-1
Fumed Silica	2.75	2.35	1.96
Glycereth-7	2.26	1.94	1.62
Glyceryl Monostearate	4.06	3.48	2.90
Polyethylene Glycol	.98	.84	.70
PEG-200 Glyceryl Monotallowate	.65	.56	.47
PEG-82 Glyceryl Monotallowate	1.03	.88	.73
Magnesium Aluminum	.70	.60	.50
Hydroxyethyl Cellulose	.50	.42	.35
Propylene Glycol Dicaprylate/Dicaprate	3.22	2.76	2.30
Cetyl Palmitate	.65	.56	.47
PPG-15 Stearyl Ether	.43	.37	.31
Cetyl-Stearyl Alcohol	.86	.74	.62
85:7.5:7.5 Mole Ratio Iso-Octyl Acrylate:Stearyl Methacrylate: Acrylic Acid	5.83	5.00	4.17
N,N-Diethyl-m-Toluamide	35.00	30.00	25.00
Diazoledinyl:Urea:Methyl Paraben: Propyl Paraben:Propylene Glycol	.24	.24	.24
Water	qs to 100	qs to 100	qs to 100

NOTEBOOK NUMBER 49-29-1 same as 49-27-1, different process

FIGURE XVIII

THIRD PHASE II MOSQUITO REPELLENCY TEST - VARIABLE HIGH HUMIDITY CLIMATIC CONDITION

Formulation * % DEET	Percent Repellency[1] Exposure Time[2] - Hour 8	10	12	13	14	15	16
49-18-6 (35%)	100	100	100	100	98	100	98
49-27-1 (30%)	100	100	68[3]	100	87	90	98
49-28-1 (25%)	98	100	79	98	70	85	88
49-29-1 (30%)	100	100	100	100	92	100	90
Total Number of Control Bites	58	51	28	42	53	41	41

1 - [(No. Control Bites - No. Treatment Bites) No. Control Bites] x 100; 5 subjects, 10 replications; control 5 replications

2 - Time after application

3 - Bites were on the two lower sites on the inner forearm of one subject

* - See Figure XVII for specific formulations

FIGURE XIX

FINAL MOSQUITO REPELLENCY TESTS
PERCENT REPELLENCY OF 3M'S PHASE II CANDIDATE
FORMULATION VERSUS THE ARMY'S 75% DEET/ALCOHOL
FORMULATION EVALUATED IN THE THREE BASIC CLIMATIC CONDITIONS

Climatic Condition	Formulation	Percent Repellency[1] Exposure Time - Hours 0	2	4	6	8	10	12	14	16
Constant High Humidity	3M[2]	100	100	100	100	100	100	91	100	76
	75% DEET[3]	100	100	100	100	98[4]	99	46	38	-2
	No. Bites on Control[5]	NR[6]	NR	NR	NR	NR	68	56	42	46
Variable High Humidity	3M	100	100	100	94[7]	98[7]	95	93[8]	90	67
	75% DEET	100	100	83[7] (91)[9]	90[7] (95)[9]	81[7] (86)[9]	28[10]			
	No. Bites on Control	NR	NR	NR	NR	54[11]	79	70	96	90
Basic Hot	3M	100	100	100	100	100	100	97	96	91
	75% DEET	100	100	97[12]	98[12]	89	98	67	77[10]	
	No. Bites on Control	NR	NR	NR	(40)[11]	75	93	66	69	101

1 - Equals [(No. Control Bites - No. Treatment Bites) No. Control Bites] x 100
2 - Notebook 49-35-1
3 - 75% DEET in alcohol
4 - Use average of control bites received during hours of 10-16 of test = 53 bites
5 - Total bites of ten participants
6 - NR = Not Run
7 - Use average of control bites received during hours of 10-16 of test = 84 bites
8 - Questionable data entry and site location on tenth person. Also questionable activity of said person with respect to abrading inner

test site, therefore nine replicates only used

9 - Based on maximum number of bites that the control could theoretically obtain - 10 x 15 bites = 150

10 - Test stopped for this formulation

11 - Total bites on the control sites of five people

12 - Use average of control bites received during hours of 8-16 of test = 81 bites

FIGURE XX[a]- PROTOTYPE LABEL

Front Label

YYYY-YY-YYY-YYYY
INSECT REPELLENT LOTION (CREAM)
TYPE (XXX)
Federal Specification XXXXXXX
Contents: 2 Fluid Ounces

For use in tropical areas where pests occur. Repels biting flies, chiggers, deer flies, mosquitos, fleas, stable flies and terrestrial leeches.

Provides 95% protection against mosquitos for 12 or more hours under normal use conditions.

ACTIVE INGREDIENTS: N,N-Diethyl-m-toluamide 33.25%
Other isomers 1.75%; inert ingredients 65%.

FOR EXTERNAL USE ONLY
Keep out of reach of children. See additional cautions on back panel.

Contract No. DAMD17-85-C-5017

FIGURE XXb

Back Label

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Apply generously to all exposed skin. Spread evenly and completely. **To apply to face** squeeze lotion into palm of hand and spread on face and neck. **Avoid Contact With Eyes and Lips. To apply to clothing**, dispense the lotion into one hand, rub the hands together and brush lightly on socks, around collars, waist, sleeve and trouser cuffs and where clothing fits snugly such as over the shoulders, elbows, knees and buttocks. Repeat as necessary. Wipe hands after application.

Caution - Hazard to Humans: Harmful if swallowed. Avoid contact with eyes and lips. In case of contact, flush with plenty of water. Do not apply to excessively sunburned or damaged skin.

May Damage rayon, dynel, spandex or other synthetic fabrics; lacquer or enamel painted surfaces, plastic eyeglasses, watches or rifle stocks.

Disposal: Do not reuse empty container. Wrap container and put in trash.

Personal Care Products/3M
3M Center
St. Paul, Minnesota 55144-1000

EPA Reg. No. XXX
EPA Est. No. XXXXX

FIGURE XXI - IMPROVED BACK LABEL

Back Label

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Squeeze into one hand a 2-1/2" strip of repellent equal in length and width to the diagram on the side of the tube. Rub hands together and apply thoroughly in a thin layer to both forearms. Use additional lotion for upper arms. Repeat for other exposed areas. To apply to face squeeze lotion into palm of hand and spread on face and neck. Avoid Contact With Eyes and Lips. To apply to clothing, dispense the lotion into one hand, rub the hands together and brush lightly on socks, around collars, waist, sleeve and trouser cuffs and where clothing fits snugly such as over the shoulders, elbows, knees and buttocks. Repeat as necessary. Wipe hands after application.

Caution - Hazard to Humans: Harmful if swallowed. Avoid contact with eyes and lips. In case of contact, flush with plenty of water. Do not apply to excessively sunburned or damaged skin.

May Damage rayon, dynel, spandex or other synthetic fabrics; lacquer or enamel painted surfaces, plastic eyeglasses, watches or rifle stocks.

Disposal: Do not reuse empty container. Wrap container and put in trash.

Personal Care Products/3M
3M Center
St. Paul, Minnesota 55144-1000

EPA Reg. No. XXX
EPA Est. No. XXXXX

FIGURE XXII

FIELD REPELLENCY TEST AGAINST *AEDES SOLLICITANS* MOSQUITOES

EXPOSURE TIME-HOURS[1]	NUMBER OF BITES[2] PER EXPOSURE TIME PER FORMULATION												UN-TREATED CONTRL
	75% DEET/ALCOHOL REPLICATION NUMBER						3M FORMULATION REPLICATION NUMBER						
	1	2	3	4	5	6	1	2	3	4	5	6	
0	0	0	0	0	0	0	0	0	0	0	0	0	8
1	0	0	0	0	0	0	0	0	0	0	0	0	3
2	0	0	0	0	0	0	0	0	0	0	0	0	12
3	0	0	0	0	0	0	0	0	0	0	0	0	7
4	0	0	0	0	0	0	0	0	0	0	0	0	13
5	0	1	0	2	7	2	0	0	0	0	0	0	5
6	0	1	0				0	0	0	0	0	0	10
7	2	1	0				0	0	0	0	0	0	5
8		4	1				0	0	0	0	0	0	9
9			2				7	0	0	0	0	4	300
10								0	0	1	4		40
11								0	0	1			30
12								1	0	0			9
13								1	1	1			16
14								4	4	3			13

Complete Protection Time
5.5 ± 1.8 Hours

Complete Protection Time
10.7 ± 2.6 Hours

[1] - Time after application
[2] - If 2 or greater the site was closed to further mosquito exposure

FIELD MOSQUITO REPELLENCY TEST
VARIABLE HIGH HUMIDITY CLIMATIC CONDITION

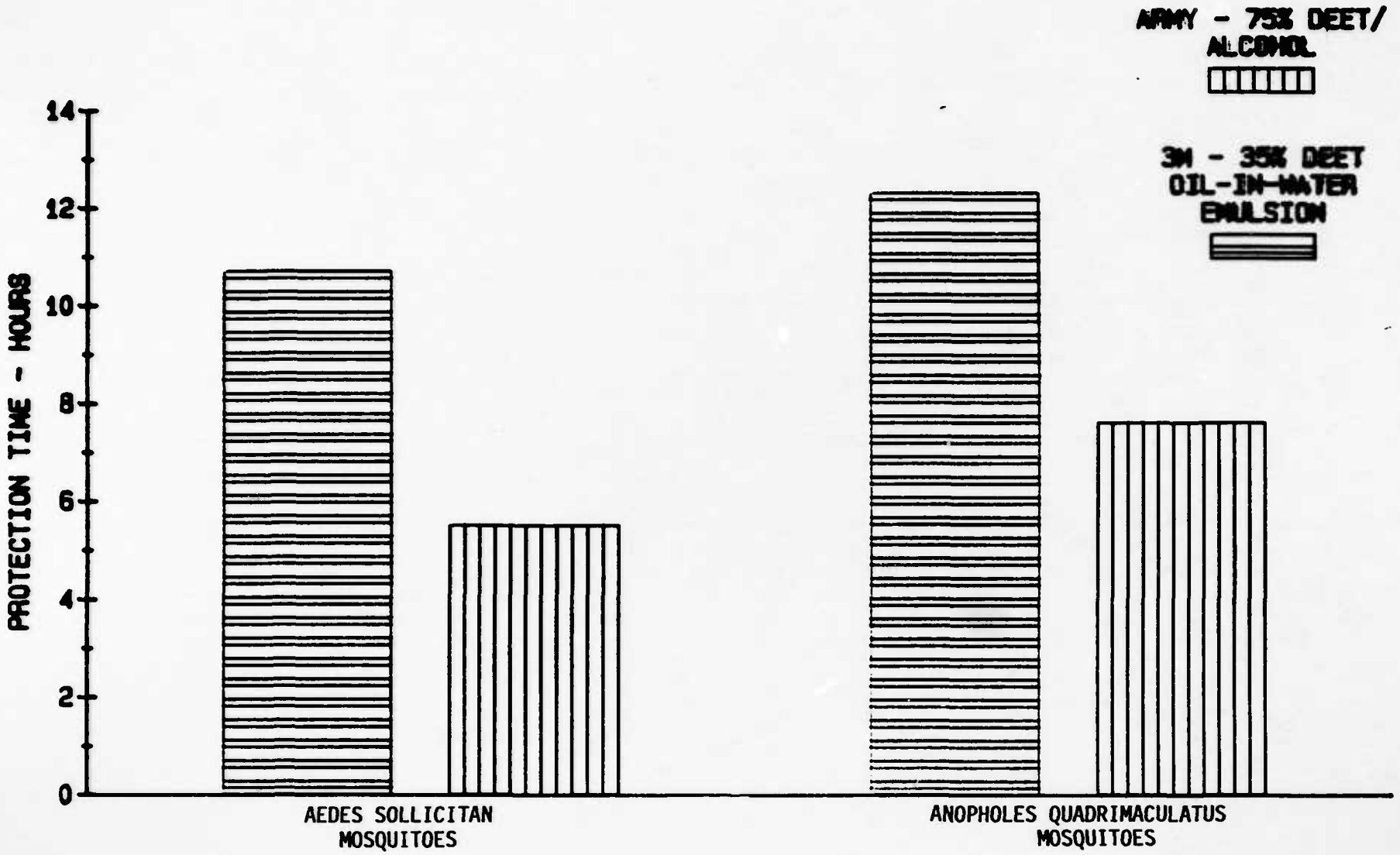


FIGURE XXIV

LABORATORY MOSQUITO REPELLENCY TEST IN THREE BASIC CLIMATIC CONDITIONS

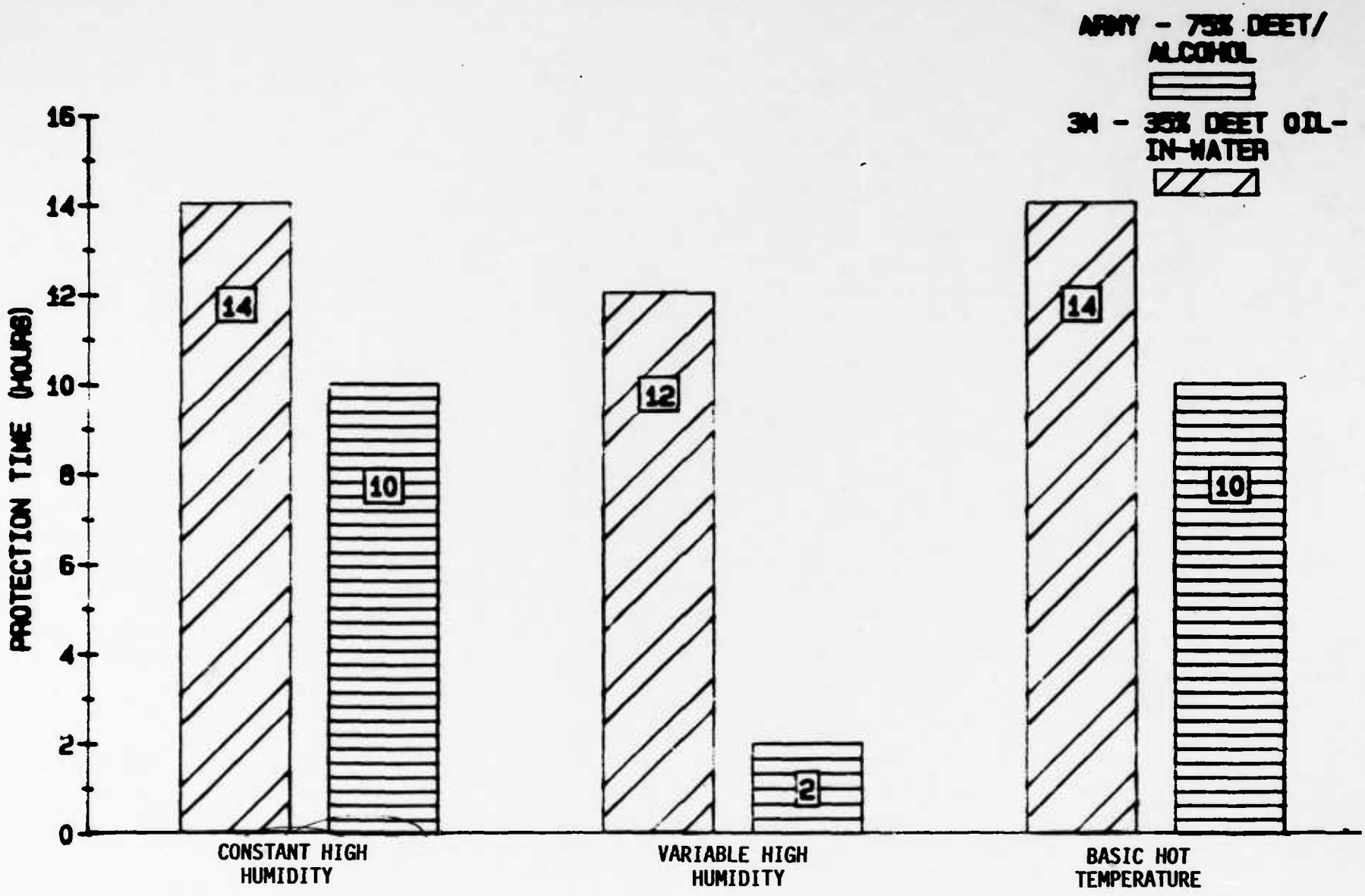


FIGURE XXV

PERCENT REPELLENCY FOR HYPOTHETICAL MOSQUITO REPELLENCY DATA[1]

A. TWICE THE NUMBER OF BITES PER EXPOSURE[2]

EXPOSURE TIME-HOURS	UNTREATED CONTROL BITES[3]	75% DEET/ALCOHOL REPLICATIONS 1	2	3	4	5	6	TOTAL BITES	PERCENT REPELLENCY[4]	3M FORMULATION REPLICATIONS 1	2	3	4	5	6	TOTAL BITES	PERCENT REPELLENCY
4	780	0	0	0	0	0	0	0	100.0	0	0	0	0	0	0		
5	300	0	1	0	2	7	2	12	96.0	0	0	0	0	0	0		
6	600	0	1	0	4*	14*	4*	23*	96.2	0	0	0	0	0	0		
7	300	2	1	0	8*	28*	8*	47*	84.3	0	0	0	0	0	0		
8	540	4*	4	1	16*	56*	16*	97*	82.0	0	0	0	0	0	0	0	100.0
9	18000	8*	8*	2	32*	112*	32*			7	0	0	0	0	4	11	99.9
10	2400	16*	16*	4*	64*	224*	64			14*	0	0	1	4	8*	27*	98.9
11	1800									28*	0	0	1	8*	16*	53*	97.1
12	540									56*	1	0	0	16*	32*	104*	86.5
13	960									112*	1	1	1	32*	64*	211*	78.0
14	780										4	4	3				

B. THREE TIMES THE NUMBER OF BITES PER EXPOSURE[5]

4	780	0	0	0	0	0	0	0	100.0								
5	300	0	1	0	2	7	2	12	96.0								
6	600	0	1	0	6*	21*	6*	34*	94.3								
7	300	2	1	0	18*	63*	18*	102*	66.0								
8	540	6*	4	1	54*	189*	54*	308*	43.0	0	0	0	0	0	0	0	100.0
9	18000			2						7	0	0	0	0	4	11	99.9
10	2400									21*	0	0	1	4	12*	38*	98.4
11	1800									63*	0	0	1	12*	36*	112*	93.8
12	540									189*	1	0	0	36*	108	334*	38.1
13	960										1	1	1				
14	780										4	4	3				

* Hypothetical data point, see footnotes 2 & 5

1 - Aedes sollicitans mosquitoes

2 - Assume each failed site will receive twice as many bites the next time if it had been exposed

3 - Bites on 1 person per minute x 10 minutes x 6 replications

4 - (Total bites on control - total bites on site ÷ total bites on control)x100

5 - Assume each failed site will receive three times as many bites the next time if the site had been exposed

FIGURE XXVI

FIELD REPELLENCY TEST AGAINST ANOPHOLES QUADRIMACULATUS MOSQUITOES

		NUMBER OF BITES PER EXPOSURE TIME													
		75% DEET/ALCOHOL REPLICATION NUMBER							3M FORMULATION REPLICATION NUMBER						UN-
MILITARY TIME	EXPOSURE TIME-HRS	1	2	3	4	5	6	EXPOSURE TIME-HRS	1	2	3	4	5	6	TRTD CTRL
10:00								0[1]	0	0	0	0	0	0	0
11:00								1	-[2]	-	-	-	-	-	
12:00								2	-	-	-	-	-	-	
13:00								3	-	-	-	-	-	-	
14:00	0[1]	0	0	0	0	0	0	4	-	-	-	-	-	-	
15:00	1	-	-	-	-	-	-	5	-	-	-	-	-	-	
16:00	2	-	-	-	-	-	-	6	-	-	-	-	-	-	
17:00	3	0	0	0	0	0	0	7	0	0	0	0	0	0	0
18:00	4	0	0	0	0	0	0	8	0	0	0	0	0	0	0
19:00	5	0	0	0	0	0	0	9	0	0	0	0	0	0	
20:00	6	0	0	0	0	0	13	10	0	0	0	0	0	0	0
	6.5	1	2	0	0	0		10.5	2	1	0	0	0	0	
21:00	7	0	2	0	0	0		11		2	0	0	0	0	
22:00	8	-		-	-	-		12			-	-	-	-	
	8.5	0		2	1	1		12.5			1	0	0	1	0
								12.75			-	-	0	0	
23:00	9	0			2	0		13			1	0	0	2	
	9.3	1				1		13.5				0	1		
	9.5	2				1		13.6				-	1		
24:00								14				0	0		2
								14.5				0			
01:00								15				0			
02:00	Complete Protection Time 7.7 ± 1.8 hrs.							16				2			5
									Complete Protection Time 12.4 ± 1.90 hrs.						

1 - staggered product application
2 - no entry

FIGURE XXVIIa

Front Label

YYYY-YY-YYY-YYYY
INSECT REPELLENT LOTION (CREAM)
TYPE (XXX)
Federal Specification XXXXXXX
Contents: 2 Fluid Ounces

Repels biting flies, chiggers, deer flies, mosquitoes, fleas and stable flies. Also repels terrestrial leeches in tropical areas where pest occurs.

Provides 95% or greater protection against mosquitoes for 12 or more hours under normal use conditions.

ACTIVE INGREDIENTS: N,N-Diethyl-m-toluamide 31.58%
Other isomers 1.58% inert ingredients 66.75%.

FOR EXTERNAL USE ONLY
Keep out of reach of children.

Caution - Avoid contact with eyes and lips. In case of eye contact, flush with plenty of water. Do not apply to excessively sunburned or damaged skin.

Contract No. DAMD17-85-C-5017

FIGURE XXVIIb

Back Label

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Squeeze into one hand a 2.5 ml strip of repellent, equal in length and width to the diagram on the side of the tube. Rub hands together and apply thoroughly in a thin layer to both forearms. Use additional lotion for upper arms. Repeat for other exposed areas. **To apply to face** squeeze lotion into palm of hand and spread on face and neck. **Avoid Contact With Eyes and Lips. To apply to clothing,** dispense the lotion into one hand, rub the hands together and brush lightly on socks, around collars, waist, sleeve and trouser cuffs and where clothing fits snugly such as over the shoulders, elbows, knees and buttocks. Repeat as necessary. Wipe *hands after application.*

May Damage certain synthetic fabrics, plastics, painted or varnished surfaces. Avoid smearing on plastic eyeglass frames, goggles, watch crystals, etc. **WILL NOT DAMAGE** nylon, cotton or wool fabrics.

Disposal: Do not reuse empty container. Wrap container and put in trash.

Personal Care Products/3M
3M Center
St. Paul, Minnesota 55144-1000

EPA Reg. No. XXX
EPA Est. No. XXXXX

Figure XXVIII. In Vitro Evaporation of 3M Formulation.

Study 213-26-31

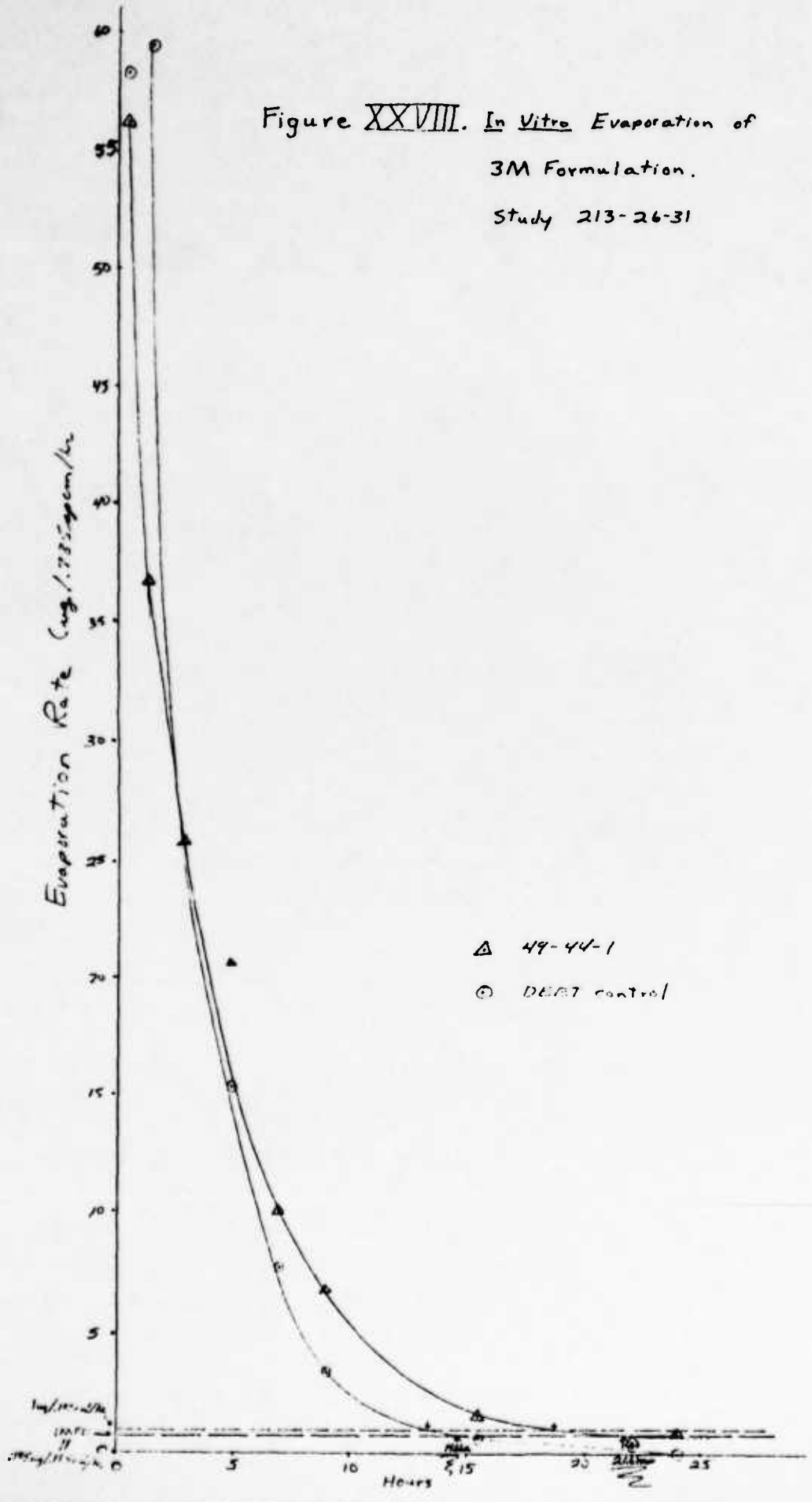


FIGURE XXIX

IN-VITRO PENETRATION AND EVAPORATION[1] OF THE 3M FORMULATION

	WEIGHT[2] AND PERCENTAGE OF APPLIED DEET					
FORMULATION	EVAPORATION	PERCUTANEOUS PENETRATION	SKIN WASH	SKIN DIGESTION	APPARATUS WASH	TOTAL RECOVERY
3M Formulation[3]	233.2/70.5%	69.1/20.9%	2.9/0.9%	5.1/1.5%	5.0/1.5%	315.3/95.3%
DEET Control[4]	230.3/91.3%	67.6/26.8%	4.3/1.7%	16.3/6.5%	4.3/1.7%	318.5/126%

1 - Hawkins and Reifenrath, Fundam. Appl. Toxicol., 4, 133-144, (1984)
2 - Micrograms
3 - a) 0.0007 g applied, theoretically = 245 ug DEET, same amount washed into glass vials gave 330.7 ug DEET
4 - 10 ul of a 2.5215% DEET/alcohol solution applied, theoretically = 252.2 ug DEET

FIGURE XXX

PACKAGE AGING DATA

PACKAGE COMPOSITION	PERCENT WEIGHT LOSS (2 MONTHS) ROOM TEMPERATURE	120°F
LDPE 1004 overcoat	.4%	7.6%
LDPE Phase I tube 1004 overcoat	.3%	5.6%
LDPE UV; TP-46	.2%	5.2%
HDPE UV; TP-46	.1%	1.8%

LDPE = low density polyethylene
HDPE = high density polyethylene

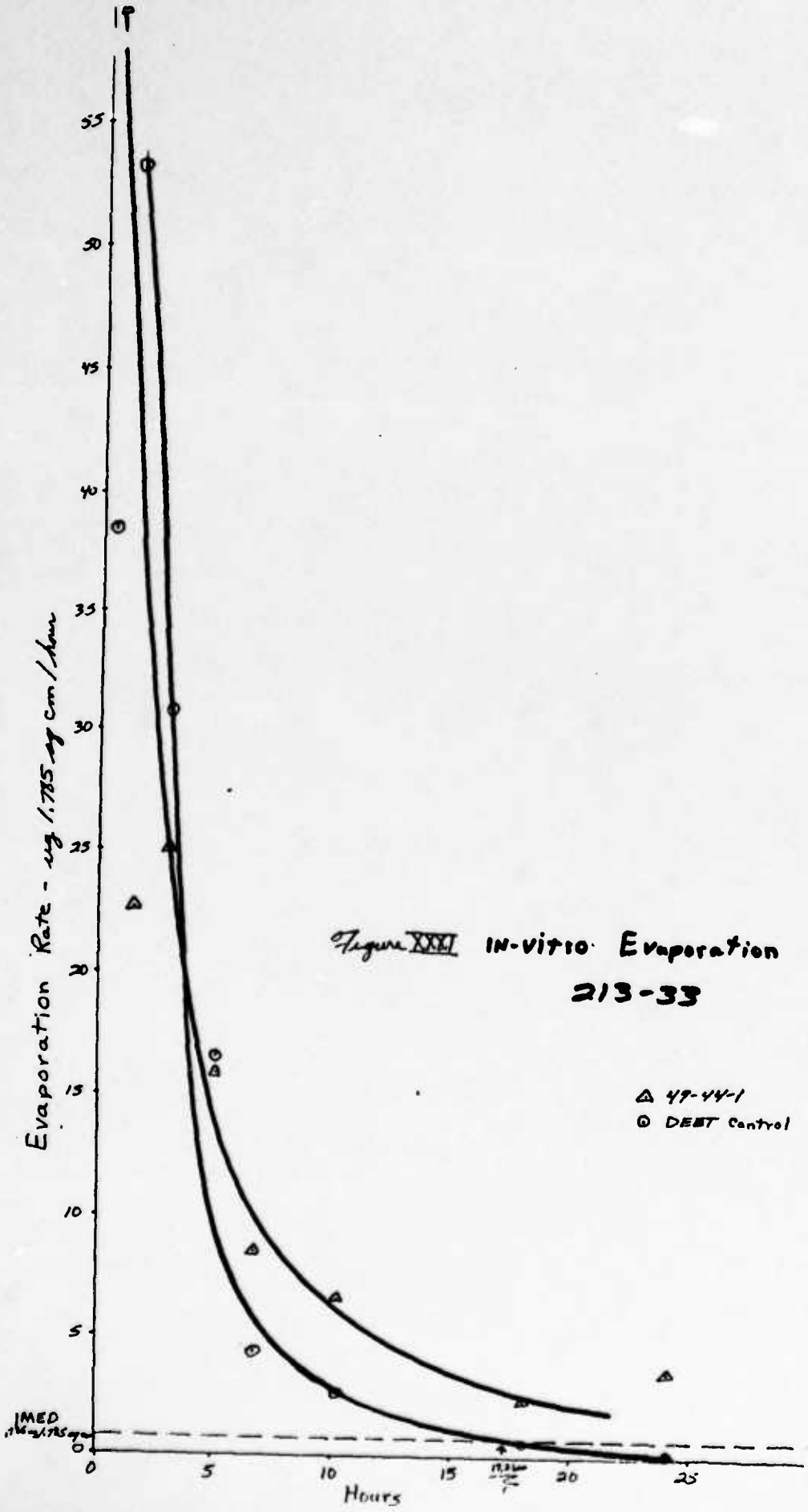


Figure XXXI IN-VITRO Evaporation Study

213-33

FIGURE XXXII

IN-VITRO PENETRATION AND EVAPORATION [1] OF THE 3M PHASE II FORMULATION - SECOND STUDY

	WEIGHT[2] and PERCENTAGE OF APPLIED DEET					
FORMULATION	EVAPORATION	PERCUTANEOUS PENETRATION	SKIN WASH	SKIN DIGESTION	APPARATUS WASH	TOTAL RECOVERY
3M Formulation[3]	249.3/88.4%	69.6/24.7%	14.8/5.2%	13.1/4.6%	10.5/3.7%	357.3/126.7%
DEET Control[4]	217.6/86.3%	54.6/21.6%	2.6/1.0%	19.7/7.8%	.4/.2%	294.9/117%

1 - Hawkins and Reifenrath, Fundam. Appl. Toxicol., 4, 133-144, (1984)
2 - Micrograms
3 - a) 0.0007 g applied to pig skin and extracted immediately - 282.0 + 29.3 ug DEET; 49-44-1
4 - 10 ul of a 2.5215% DEET/alcohol solution applied, theoretically = 252.2 ug DEET

FIGURE XXXIII

HYPOTHETICAL FIELD DATA AGAINST AEDES SOLLICITANS MOSQUITOES
BASED ON FAILURE RATE FOR AEDES AEGYPTI MOSQUITOES

		NUMBER OF BITES[2] PER EXPOSURE TIME PER FORMULATION												
		75% DEET/ALCOHOL[3] REPLICATION NUMBER							3M FORMULATION[5] REPLICATION NUMBER					
EXPOSURE TIME-HOURS[1]	UN-TREATED CONTROL	1	2	3	4	5	6	PERCENT REPELLENCY[4]	1	2	3	4	5	6	PERCENT REPELLENCY
0	8	0	0	0	0	0	0		0	0	0	0	0	0	
1	3	0	0	0	0	0	0		0	0	0	0	0	0	
2	12	0	0	0	0	0	0		0	0	0	0	0	0	
3	7	0	0	0	0	0	0		0	0	0	0	0	0	
4	13	0	0	0	0	0	0		0	0	0	0	0	0	
5	5	0	1	0	2	7	2	96.0	0	0	0	0	0	0	
6	10	0	1	0	15*	20*	15*	91.5	0	0	0	0	0	0	
7	5	2	1	0	13*	18*	13*	84.3	0	0	0	0	0	0	
8	9	14*	4	1	30*	35*	30*	78.9	0	0	0	0	0	0	
9	300	662*	394*	2	1204*	1207*	1204*	74.1	7	0	0	0	0	4	99.9
10	40								11*	0	0	1	4	8*	99.0
11	30								21*	0	0	1	7*	18*	97.4
12	9	Complete Protection Time 5.5 ± 1.8 Hours							14*	1	0	0	8*	11*	93.7
13	16								25*	1	1	1	17*	22*	93.0
14	13								26*	4	4	3	19*	23*	81.3
									Complete Protection Time 10.7 ± 2.6 Hours						

1 - Time after application
2 - If 2 or greater, the site was closed to further mosquito exposure
3 - Regression equation defining percent repellency once failure started in final laboratory testing y=96-9.0 x time, r=.82957
4 - Equals $\frac{\text{Control Bites - Treatment Bites}}{\text{Control Bites}} \times 100$
5 - Regression equation y=102 - 3.35 x time, r=.8528
* - Hypothetical data points = no. bites at failure + $\frac{(\text{control bites - \% repellency x control bites})}{100}$

% repellency via regression equations for times after site failed by 2 bite endpoint. Two bite endpoint is defined as time 0 for regression equations.

APPENDIX B

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

REPORT

Laboratory Testing of Two Mosquito Repellent Formulations

Study No. 6171-111

for

3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Chemical & BioMedical Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

September 8, 1986

INTRODUCTION

Insect repellents are volatile chemical agents that, when applied to the skin or clothing, vaporize to discourage the approach of insects and consequently protect the skin from insect bites. The ideal insect repellent should afford effective protection for several hours and withstand various environmental conditions. The objective of this study was to determine the repellent efficacy of one candidate mosquito repellent formulation against the standard mosquito repellent formulation offered by the U.S. Army.

SUBJECT POPULATION

The subject population consisted of six normal, healthy male and four nonpregnant, non-nursing female volunteers between the ages of 18 and 45 who, to the best of their knowledge, were not hypersensitive to insect bites. Other inclusion criteria were:

- Susceptibility to insect bites and to local erythema and edema at the site of the bite
- Willingness and ability to meet all requirements of the signed protocol
- Signing the informed consent form

Exclusion criteria were:

- Prior history of hypersensitivity to insect bites
- Prior history of hypersensitivity to insect repellents
- Females with known or suspected pregnancy and lactation
- Nonsusceptibility to insect bites
- Unwillingness to meet all requirements of the protocol
- Refusal to sign the informed consent form

STUDY DESIGN AND ROOM CONDITIONS

The protocol was designed to evaluate the repellent formulations under various temperature and humidity conditions (Tables 1 through 3).

Condition	Temperature (°F)	Relative Humidity (%)
A (Constant high humidity)	75	95-100
B (Variable high humidity)	78-95	74-100
C (Hot with low humidity)	86-110	14-44

MATERIALS

Test Cages

The rectangular test cage was 18 cm long x 5 cm wide x 4 cm high. The top of the cage was made of mosquito screening and the sides, ends, and bottom were made of 3.2-mm thick clear acrylic plastic.

Five 29-mm circular openings were drilled in line in the floor of the cage. The two sides and one of the ends of the cage were grooved and slotted to receive a flexible rectangular slide made of 0.012-in. (0.3 mm) thick cellulose acetate sheeting.

Two 2.5-cm by 30-cm belts equipped with fasteners were used to secure the test cage to the forearm.

Test Equipment

- o Hygrothermograph
- o Ivory® soap

Test Insect

The test insect was the yellow fever mosquito, Aedes aegypti. L.

TEST PROCEDURES

Before the test samples were applied, the forearms were washed with Ivory soap and warm water and then wiped dry with cloth toweling. The same procedures were repeated at the end of the test day. The surface area on subjects' forearms and palms were determined before the repellents were applied. The subjects applied the repellents to their forearms according to label directions (Table 4). The U.S. Army formulation was applied by placing six drops in the palm of the hand and then thoroughly rubbing the material over the opposite forearm. The 3M formulation was applied by putting the measured amount on a tongue depressor and applying the material to the subjects palm. The subjects rubbed the material thoroughly over the opposite forearm. The application followed a paired randomized design.

To evaluate repellent efficacy, five circular test areas on the flexor region of the forearms and on the outer surface of the forearms were exposed to biting mosquitoes. An equal, unprotected control area on the upper surface of one thigh was exposed during each exposure period. The subjects were exposed for 90 seconds every 2 hours for a 16-hour period. The treated areas were inspected for landings, bites, and feedings during and after each exposure. The exposure areas were alternated between the inner and outer forearms for each successive time point.

Once a site was bitten, it was closed from further exposure and the cage was removed. The arm was cleaned using Ivory liquid after the 16-hour exposure as in the pretest cleansing.

The mosquitoes were transferred from the stock cage to the test cages by aspiration and without CO_2 anesthesia. Fifteen adult nulliparous females were used per test cage. The exposed mosquitoes were sacrificed after exposure and replaced with fresh mosquitoes.

RESULTS

The results obtained under Environmental Condition A (constant high humidity) are in Table 5. The repellent efficacy results for the formulations under Condition B (variable high humidity) are in Table 6. The results under Condition C (hot with low humidity) are in Table 7.

APPROVAL

Curtis C. Dary, PhD, RPE
Staff Scientist
Metabolism

10-Sept-86
Date

Susana R. K. de Dennis, MD
Medical Director

9-10-86
Date

William Goetz
Consulting Entomologist

10-Sept-86
Date

by and for Hazleton Laboratories America, Inc.

(2759F/kk)

Table 1

Temperatures and Relative Humidities
Under Condition A (Constant High Humidity)

Time of Day	Temperature (°F)		Relative Humidity (%)	
	06/04/86	06/05/86	06/04/86	06/05/86
07:00	76	75	99	94
08:00	76	75	100	97
09:00	76	76	98	92
10:00	76	76	97	97
11:00	76	76	98	88
12:00	75	77	97	95
13:00	76	77	97	97
14:00	76	76	96	97
15:00	76	76	97	97
16:00	76	76	97	95
17:00	76	76	99	98
18:00	76	76	98	95
19:00	76	76	98	95
20:00	76	76	98	95
21:00	76	76	98	95
22:00	76	76	99	96
23:00	76	77	97	96
24:00	76	77	97	96
Mean	75.9	76.1	97.8	95.3
Standard deviation	0.2	0.6	1.0	2.3
Coefficient of variation (%)	0.31	0.76	1.03	2.41

Table 2

Temperatures and Relative Humidities
Under Condition B (Variable High Humidity)

Time of Day	Temperature (°F)		Relative Humidity (%)	
	06/09/86	06/10/86	06/09/86	06/10/86
07:00		72		72
08:00		73		72
09:00	72	73	58	72
10:00	80	80	95	81
11:00	82	86	96	88
12:00	84	90	80	90
13:00	75	86	82	94
14:00	78	85	78	80
15:00	85	85	80	83
16:00	86	88	79	79
17:00	82	92	74	74
18:00	88	93	80	79
19:00	90	96	79	81
20:00	92	92	74	83
21:00	87	92	80	85
22:00	90	92	82	85
23:00	88	92	94	80
24:00	88	90	81	91
Mean	84.2	86.5	80.7	81.6
Standard deviation	5.7	7.4	9.1	6.6
Coefficienct of variation (%)	6.77	8.56	11.25	8.05

Table 3

Temperatures and Relative Humidities
Under Condition C (Hot with Low Humidity)

Time of Day	Temperature (°F)		Relative Humidity (%)	
	06/12/86	06/13/86	06/12/86	06/13/86
07:00	83	89	48	46
08:00	83	89	48	46
09:00	83	89	47	46
10:00	83	92	47	47
11:00	84	90	46	46
12:00	88	92	42	42
13:00	93	94	38	38
14:00	94	95	37	36
15:00	95	97	38	35
16:00	95	97	34	35
17:00	102	98	33	36
18:00	102	98	33	37
19:00	104	100	34	34
20:00	103	100	34	35
21:00	103	100	34	35
22:00	103	100	34	37
23:00	102	101	36	36
24:00	100	101	38	38
Mean	94.4	95.7	38.9	39.2
Standard deviation	8.4	4.5	5.7	4.8
Coefficient of variation (%)	8.86	4.67	14.75	12.29

Table 4

Dosage of Repellent Applied to Forearm (g)

Subject Number	Surface Area (sq cm)	Condition A		Condition B		Condition C	
		3M	U.S. Army	3M	U.S. Army	3M	U.S. Army
J-05521	539	1.22	0.27	1.26	0.32	1.51	0.31
J-05522	489	1.12	0.28	1.03	0.28	1.03	0.31
J-05523	665	1.50	0.29	1.51	0.29	1.28	0.30
J-05524	441	1.03	0.29	1.12	0.32	1.23	0.33
J-05525	612	1.27	0.31	1.23	0.30	1.12	-
J-05526	643	1.30	0.33	1.47	0.30	1.66	0.30
J-05527	553	1.11	0.34	1.01	0.34	1.11	0.32
J-05528	829	1.68	0.30	1.66	0.29	1.47	0.36*
J-05529	503	1.01	0.30	1.12	0.28	1.01	0.32
J-05530	725	1.45	0.36	1.30	0.27	1.30	0.32
Mean	599.9	1.27	0.31	1.27	0.30	1.27	0.32
Standard deviation	118.9	0.23	0.03	0.22	0.02	0.22	0.02
Coefficient of variation (%)	19.82	17.23	9.22	17.05	7.30	17.09	5.75

- = No entry.

*Seven drops of repellent applied.

Table 5

Mosquito Repellency Test
with Aedes aegypti L. Under Controlled
Conditions of Constant High Humidity
(75°F, Relative Humidity 95% to 100%)

Subject Number	Sex	Formulation	Test Site*	Number of Bites Post-treatment (Hours) 0	2	4	6	8	10	12	14	16
J-05521	M	3M	LA	0	0	0	0	0	0	0	0	0
		U.S. Army	RA	0	0	0	0	0	0	7	6	7
		Control	LL	-	-	-	-	-	10	-	11	-
		Control	RL	-	-	-	-	-	-	11	-	4
J-05522	F	3M	LA	0	0	0	0	0	0	0	0	0
		U.S. Army	RA	0	0	0	0	0	0	2	2	5
		Control	LL	-	-	-	-	-	12	-	4	-
		Control	RL	-	-	-	-	-	-	5	-	2
J-05523	M	3M	RA	0	0	0	0	0	0	1	0	0
		U.S. Army	LA	0	0	0	0	0	1	4	5	6
		Control	RL	-	-	-	-	-	-	7	-	4
		Control	LL	-	-	-	-	-	12	-	3	-
J5524	F	3M	RA	0	0	0	0	0	0	0	0	0
		U.S. Army	LA	0	0	0	0	0	0	0	1	0
		Control	LL	-	-	-	-	-	8	-	1	-
		Control	RL	-	-	-	-	-	-	1	-	0
J-05525	M	3M	LA	0	0	0	0	0	0	0	0	2
		U.S. Army	RA	0	0	0	0	0	0	0	1	3
		Control	LL	-	-	-	-	-	11	-	6	-
		Control	RL	-	-	-	-	-	-	8	-	9
J-00526	M	3M	LA	0	0	0	0	0	0	2	3	12
		U.S. Army	RA	0	0	0	0	0	0	2	0	7
		Control	LL	-	-	-	-	-	1	-	2	-
		Control	RL	-	-	-	-	-	-	2	-	9
J-00527	F	3M	RA	0	0	0	0	0	0	2	0	0
		U.S. Army	LA	0	0	0	0	0	0	0	0	2
		Control	LL	-	-	-	-	-	5	-	2	-
		Control	RL	-	-	-	-	-	-	3	-	1

- = No entry.

*LA = left arm, RA = right arm, LL = left leg, RL = right leg.

Table 5 (Continued)

Mosquito Repellency Test
with Aedes aegypti L. Under Controlled
Conditions of Constant High Humidity
(75°F, Relative Humidity 95% to 100%)

Subject Number	Sex	Formulation	Test Site*	Number of Bites Post-treatment (Hours)								
				0	2	4	6	8	10	12	14	16
J-05528	M	3M	LA	0	0	0	0	0	0	0	0	0
		U.S. Army	RA	0	0	0	0	0	0	4	3	9
		Control	LL	-	-	-	-	-	3	-	0	-
		Control	RL	-	-	-	-	-	-	3	-	5
J-00529	F	3M	LA	0	0	0	0	0	0	0	0	2
		U.S. Army	RA	0	0	0	0	0	0	1	1	4
		Control	LL	-	-	-	-	-	2	-	7	-
		Control	RL	-	-	-	-	-	-	8	-	2
J-00530	M	3M	RA	0	0	0	0	0	0	0	0	0
		U.S. Army	LA	0	0	0	0	1	0	9	5	0
		Control	LL	-	-	-	-	-	4	-	5	-
		Control	RL	-	-	-	-	-	-	8	-	10

- = No entry.

*LA = left arm, RA = right arm, LL = left leg, RL = right leg.

Table 6

Mosquito Repellency Test
with Aedes aegypti L. Under Controlled
Conditions of Variable High Humidity
(78°F to 95°F, Relative Humidity 74% to 100%)

Subject Number	Sex	Formulation	Test Site*	Number of Bites Post-treatment (Hours)								
				0	2	4	6	8	10	12	14	16
J-05521	M	3M	LA	0	0	0	0	0	0	1	0	1
		U.S. Army	RA	0	0	0	0	0	3	5	2	8
		Control	LL	-	-	-	-	-	-	1	-	7
		Control	RL	-	-	-	-	-	9	-	3	-
J-05522	F	3M	LA	0	0	0	0	0	0	0	0	0
		U.S. Army	RA	0	0	0	0	0	0	0	1	5
		Control	LL	-	-	-	-	-	-	3	-	12
		Control	RL	-	-	-	-	-	4	-	9	-
J-05523	M	3M	RA	0	0	0	0	0	0	0	0	1
		U.S. Army	LA	0	0	0	0	0	0	0	0	1
		Control	LL	-	-	-	-	-	-	2	-	4
		Control	RL	-	-	-	-	-	7	-	0	-
05524	F	3M	RA	0	0	0	0	0	0	0	0	4
		U.S. Army	LA	0	0	0	0	0	1	5	1	5
		Control	LL	-	-	-	-	-	-	8	-	12
		Control	RL	-	-	-	-	-	5	-	7	-
J-05525	M	3M	LA	0	0	0	0	0	0	0	1	0
		U.S. Army	RA	0	0	0	0	0	3	8	1	3
		Control	LL	-	-	-	-	-	-	6	-	5
		Control	RL	-	-	-	-	-	4	-	2	-
J-05526	M	3M	RA	0	0	0	1	2	-	1	3	8
		U.S. Army	LA	0	0	6	0	7	15	-	-	-
		Control	LL	-	-	-	-	-	10	-	15	-
		Control	RL	-	-	-	-	13	-	9	-	7
J-05527	F	3M	RA	0	0	0	0	0	0	0	0	0
		U.S. Army	LA	0	0	0	0	2	9	0	0	0
		Control	LL	-	-	-	-	-	2	-	15	-
		Control	RL	-	-	-	-	7	-	11	-	9

- = No entry.

*LA = left arm, RA = right arm, LL = left leg, RL = right leg.

Table 6 (Continued)

Mosquito Repellency Test
with Aedes aegypti L. Under Controlled
Conditions of Variable High Humidity
(78°F to 95°F, Relative Humidity 74% to 100%)

Subject Number	Sex	Formulation	Test Site*	Number of Bites Post-treatment (Hours)								
				0	2	4	6	8	10	12	14	16
-05528	M	3M	LA	0	0	0	0	0	3	11?	4	8
		U.S. Army	RA	0	0	8	6	4	15	0?	0?	0?
		Control	LL	-	-	-	-	-	13	-	15	-
		Control	RL	-	-	-	-	15	-	5	-	9
-05529	F	3M	LA	0	0	0	0	0	1	1	1	5
		U.S. Army	RA	0	0	0	0	0	6	0	0	0
		Control	LL	-	-	-	-	-	14	-	15	12
		Control	RL	-	-	-	-	6	-	15	-	-
-05530	M	3M	RA	0	0	0	0	0	0	2	1	3
		U.S. Army	LA	0	0	0	0	8	5	0	0	0
		Control	LL	-	-	-	-	-	11	10	-	-
		Control	RL	-	-	-	-	13	-	-	15	13

- = No entry.

*LA = left arm, RA = right arm, LL = left leg, RL = right leg.

? Questionable data entries NAR 9-1-86

Table 7

Mosquito Repellency Test
with Aedes aegypti L. Under Controlled
Conditions of Hot with High Humidity
(86°F to 100°F, Relative Humidity 14% to 44%)

Subject Number	Sex	Formulation	Test Site*	Number of Bites Post-treatment (Hours)								
				0	2	4	6	8	10	12	14	16
J-05521	M	3M	LA	0	0	0	0	0	0	0	1	0
		U.S. Army	RA	0	0	0	0	4	0	2	2	3
		Control	LL	-	-	-	-	6	-	3	-	15
		Control	RL	-	-	-	-	-	4	-	15	-
J-05522	F	3M	LA	0	0	0	0	0	0	0	0	4
		U.S. Army	RA	0	0	0	0	0	0	1	1	0
		Control	LL	-	-	-	-	12	-	8	-	15
		Control	RL	-	-	-	-	-	15	-	13	-
J-05523	M	3M	RA	0	0	0	0	0	0	0	0	1
		U.S. Army	LA	0	0	0	0	0	1	0	2	4
		Control	LL	-	-	-	-	7	-	2	-	15
		Control	RL	-	-	-	-	-	9	-	8	-
J-05524	F	3M	RA	0	0	0	0	0	0	1	0	0
		U.S. Army	LA	0	0	0	0	0	0	0	0	1
		Control	LL	-	-	-	-	11	-	14	-	9
		Control	RL	-	-	-	-	-	-9	-	4	-
J-05525	M	3M	LA	0	0	0	0	0	0	0	1	0
		U.S. Army	RA	0	0	0	0	4	0	2	5	6
		Control	LL	-	-	-	-	12	-	6	-	8
		Control	RL	-	-	-	-	-	11	-	15	-
J-05526	M	3M	RA	0	0	0	0	0	0	0	0	0
		U.S. Army	LA	0	0	0	0	0	1	1	0	0
		Control	LL	-	-	-	-	3	-	9	-	8
		Control	RL	-	-	-	2	-	13	-	2	-
J-05527	F	3M	RA	0	0	0	0	0	0	0	0	0
		U.S. Army	LA	0	0	0	0	0	0	6	3	0
		Control	LL	-	-	-	-	1	-	2	-	11
		Control	RL	-	-	-	13	-	15	-	6	-

- = No entry.

*LA = left arm, RA = right arm, LL = left leg, RL = right leg.

Table 7 (Continued)

Mosquito Repellency Test
with Aedes aegypti L. Under Controlled
Conditions of Hot with High Humidity
(86°F to 100°F, Relative Humidity 14% to 44%)

Subject Number	Sex	Formulation	Test Site*	Number of Bites Post-treatment (Hours)								
				0	2	4	6	8	10	12	14	16
J-05528	M	3M	LA	0	0	0	0	0	0	1	1	2
		U.S. Army	RA	0	0	1	2	0	0	6	0	0
		Control	LL	-	-	5	-	7	-	3	-	6
		Control	RL	-	-	-	8	-	2	-	0	-
J-05529	F	3M	LA	0	0	0	0	0	0	0	0	0
		U.S. Army	RA	0	0	0	0	0	0	3	2	0
		Control	LL	-	-	-	-	10	-	12	-	13
		Control	RL	-	-	-	11	-	14	-	2	-
J-05530	M	3M	RA	0	0	0	0	0	0	0	0	2
		U.S. Army	LA	0	0	0	0	0	0	1	1	0
		Control	LL	-	-	-	-	5	-	2	-	1
		Control	RL	-	-	-	6	-	1	-	4	-

- = No entry.

*LA = left arm, RA = right arm, LL = left leg, RL = right leg.

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

REPORT

Field Testing of Candidate Mosquito Repellent Formulations

Study No. 6171-113

for

The 3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Chemical & BioMedical Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

September 10, 1986

STUDY IDENTIFICATION

Field Testing of Candidate Mosquito Repellent Formulations

Study No.	6171-113
Study Location	Hazleton Laboratories America, Inc. Chemical & BioMedical Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	Mosquito repellent formulation Personal Care Products/3M
Sponsor's Project Directors	Mr. Craig Sterling Neil A. Randen, PhD
Principal Investigators	Susana R.K. de Dennis, MD Curtis C. Dary, PhD, RPE
Proposed Study Timetable	
Starting Date	July 17, 1986
Completion Date	July 22, 1986

TABLE OF CONTENTS

	Page
STUDY IDENTIFICATION	1
STUDY PERSONNEL	iii
OBJECTIVE	1
EQUIPMENT AND TEST MATERIAL	1
Equipment	1
Subject Population	1
Test Populations of Mosquitoes	1
Test Areas	1
TEST PROCEDURE	2
Preliminary Procedures	2
Definitive Tests	2
RESULTS	3
APPROVAL	3
TABLE	
1 Estimations of Dosage (mg/cm^2) of Repellent Applied to Test Areas on Human Subjects	4
2 Mosquito Repellency Test with Aedes sollicitans (Walker)	5
3 Mosquito Repellency Test with Anopheles Sp.	6
4 Test Conditions During Subject Exposure to Anopheles Sp.	8
5 Test Conditions During Subject Exposure to Aedes sollicitans (Walker)	9

STUDY PERSONNEL

Investigators

Susana R.K. de Dennis, MD
Medical Director
Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704
(608) 241-7200

Curtis C. Dary, PhD, RPE
Staff Scientist
Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704
(608) 241-4471

Consultant Entomologists

Mr. William Goetz
232 High Point Road
Madison, Wisconsin 53719
(608) 833-2903

Mr. John S. Billodeaux
District Director
Jefferson Davis Parish Mosquito
Abatement District No. 1
1310 Airport Road
Jennings, Louisiana 70546

Monitor

Neil A Randen, PhD
Research Specialist
Personal Care Products Department
The 3M Company
Building 230-2
St. Paul, Minnesota 55144
(612) 733-3147

STUDY PERSONNEL (Continued)

Panel Coordinator and Secretary

Ms. Linda Speropulos
Senior Secretary
Clinical Research Unit
Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Recorder

Mr. Howard C. Miller
Laboratory Technician
Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

OBJECTIVE

The objective of this study was to compare the performance of a candidate mosquito repellent, manufactured by the Sponsor, to the current mosquito repellent developed by the U.S. Army under field conditions in the presence of biting populations of an Anopheles species (Anopheles Sp.) and a species of the genus Aedes.

EQUIPMENT AND TEST MATERIAL

Equipment

- Balance, two significant places
- Battery-operated aspirators
- Microscope
- Mosquito identification key
- Light meter
- Watch
- Insect collection vials
- Clothing for standardizing color (blue)
- Battery-operated head lamps
- Head nets
- Cotton gloves
- Chairs
- Table
- Notebook and data sheets
- Ivory® liquid soap and towels
- Water

Subject Population

The test subjects consisted of a group of healthy human volunteers (60:40 ratio of males to nonpregnant, non-nursing females) between 18 and 45 years old. The subjects were, to the best of their knowledge, not hypersensitive to insect bites or repellent formulations.

Test Populations of Mosquitoes

Field populations of mosquitoes, identified as Aedes sollicitans (Walker) and Anopheles crucians (Wiedemann) and A. quadrimaculatus (Say) were identified in the test areas.

Test Areas

Two test areas were chosen from four original study areas that were proposed by consulting entomologists in Southwestern Louisiana. These areas were chosen because of the avidity of the biting populations of mosquitoes. Tests

of repellency with exposure to Aedes sollicitans W. were conducted along a fresh water bayou canal in Section 14 (R1E, T16S) at 92°/20° latitude and 29°/40° longitude in Vermillion Parish, Louisiana.

Repellency tests with the Anopheles Sp. were conducted in a pasture adjacent to a rice field in Section 41 (R3W, T10S), at 92°/40° latitude and 30°/13° longitude in Jefferson Davis Parish, Louisiana. Tests required changes in location within the test area to assure proper landing and biting rates.

TEST PROCEDURES

Preliminary Procedures

The entomological staff at Hazleton Laboratories America, Inc. (HLA) conducted preliminary tests to confirm the presence of species of the genera Aedes and Anopheles Sp. in the proposed test area. These tests included a determination of the time of maximum biting activity. The data obtained were used to determine the testing schedule as well as the biting locations (e.g., arms, legs, etc.).

Definitive Tests

The test subjects wore uniformly colored garments (blue) to eliminate any variability in attraction. All portions of the body not treated with repellent were suitably covered. The test surfaces (arms and legs) were washed with Ivory liquid soap, rinsed with water, and towel dried without rubbing.

The repellents were applied according to label directions, as would be expected of the average user. The quantity of repellent applied was determined by weighing the repellent container before and after each application. The surface area of the treated portions of each subjects arms and legs was measured before application. The repellents were applied evenly to the forearms and lower legs of the subjects, by the subjects, according to the standard method (ASTM:E939-83). The choice of arms and legs to be tested with each formulation was determined randomly according to a paired design. The standard repellent was paired with the test repellent on opposite arms and with similar pairing on the legs. All untreated areas were covered. Sleeves were secured with Gauze-Tex® (General Bandage Inc., Morton Grove, Illinois.). Hands were covered with cotton twill gloves, Dickies®.

The test subjects (n = 3) remained in the test area throughout the study period. The subjects were exposed for 10 minutes on the hour following application. The exposure periods were extended beyond 10 minutes when the biting frequency of the target species of mosquito became lower than that of the nontarget species; this happened when testing Anopheles Sp.

Observations were made until the repellent failed. Failure was judged according to the standard method (ASTM:E939-83) where a first confirmed bite was followed by a second bite within 30 minutes of the first bite. A confirmed bite was considered to be a bite by the target species. Biting mosquitoes were identified on repellent-treated areas by the subjects and the recorders. When on-sight identification was difficult because of failing light, biting mosquitoes were aspirated and identified in the resting quarters.

Avidity of the target species was evaluated throughout the study period. Recorders were dressed similarly to the test subjects and were exposed on untreated areas of their arms and legs for 1 minute or until greater than 10 bites by the target species occurred. Frequent changes in location were required to accommodate the target species according to evaluations of avidity.

RESULTS

The doses of the repellents applied to the test sites are in Table 1. Exposure to mosquitoes was observed from Time 0 until the products failed to repell the target species (Tables 2 and 3). The environmental conditions of the test locations are in Tables 4 and 5.

APPROVAL

Susana R.K. de Dennis, MD
Medical Director

9-12-86
Date

Curtis C. Dary, PhD, RPE
Staff Scientist
Study Director

12-Sept-86
Date

by and for Hazleton Laboratories America, Inc.

(2755F/cls)

Study No. 6171-113

Table 1

Estimations of Dosage
of Repellent Applied to Test Areas
on Human Subjects

Subject Number	Test Site	Dosage (g)	Area (cm^2)	Dosage (mg/cm^2)
J-05775	LA	1.4	511.3	2.7
	RA	0.3*	511.3	0.6
	LL	0.4	800	0.5
	RL	1.8	800	2.3
J-05776	LA	0.2	600	0.3
	RA	1.9	600	3.2
	LL	3.6	1,015	3.5
	RL	0.5	1,015	0.5
J-05777	LA	1.6	443.9	3.6
	RA	0.4	443.9	0.9
	LL	0.3	864	0.3
	RL	8.0	864	9.3

LA = left arm.
RA = right arm.
LL = left leg.
RL = right leg.

*Estimated value.

Study No. 6171-113

Table 2

Mosquito Repellency Test
with Aedes sollicitans (Walker)

Subject Number	Sex	Formulation	Test Site	Number of Bites Post Treatment (Hours)															
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
J-05774	F	U.S. Army	RA	0	0	0	0	0	0	0	0	1	2	-	-	-	-	-
		3M	LA	0	0	0	0	0	0	0	0	0	0	1	1	0	1	3
		3M	RL	0	0	0	0	0	0	0	0	0	0	0	2	0	1	4
		U.S. Army	LL	0	0	0	0	0	2	-	-	-	-	-	-	-	-	-
J-05775	M	U.S. Army	RA	0	0	0	0	0	0	0	2	-	-	-	-	-	-	-
		3M	LA	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4
		3M	RL	0	0	0	0	0	0	0	0	0	7	-	-	-	-	-
		U.S. Army	LL	0	0	0	0	0	1	1	1	4	-	-	-	-	-	-
J-05776	M	3M	RA	0	0	0	1	1	0	0	0	0	4	-	-	-	-	-
		U.S. Army	LA	0	0	0	0	0	2	-	-	-	-	-	-	-	-	-
		U.S. Army	RL	0	0	0	0	7	-	-	-	-	-	-	-	-	-	-
		3M	LL	0	0	0	0	0	0	0	0	0	0	4	-	-	-	-

- = No entry.
RA = right arm.
LA = left arm.
RL = right leg.
LL = left leg.

Study No. 6171-113

Table 3

Mosquito Repellency Test
with Anopheles Sp.*

Subject Number	Sex	Formulation	Test Site	Number of Bites Post Treatment (Hours)													
				0	3.0	4.0	5.0	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	9.7	10
J-05774	F	U.S. Army	RA	0	0	0	0	0	0	0	-	0	2	-	-	-	-
		3M	LA	-	-	-	-	-	-	0	-	0	-	0	-	-	0
		3M	RL	-	-	-	-	-	-	0	-	0	-	0	-	-	0
		U.S. Army	LL	0	0	0	0	0	0	0	-	-	1	2	-	-	-
J-05775	M	U.S. Army	RA	0	0	0	0	0	1	0	-	-	0	0	1	2	-
		3M	LA	-	-	-	-	-	-	0	-	0	-	0	-	-	-
		3M	RL	-	-	-	-	-	-	0	-	0	-	0	-	-	-
		U.S. Army	LL	0	0	0	0	0	2	-	-	-	-	-	-	-	-
J-05776	M	3M	RA	-	-	-	-	-	-	0	-	0	-	0	-	-	0
		U.S. Army	LA	0	0	0	0	0	0	0	-	-	0	1	2	-	-
		U.S. Army	RL	0	0	0	0	13	-	-	-	-	-	-	-	-	-
		3M	LL	-	-	-	-	-	-	0	-	0	-	0	-	-	0

- = No entry.
RA = right arm.
LA = left arm.
RL = right leg.
LL = left leg.

*Biting species Anopheles quadrimaculatus (Say) and A. crucians (Wiedemann).

Study No. 6171-113

Table 3 (Continued)

Mosquito Repellency Test
with Anopheles Sp.*

Subject Number	Sex	Formulation	Test Site	Number of Bites Post Treatment (Hours)												
				10.5	11.0	11.5	12.0	12.5	13.0	13.15	13.5	13.65	13.75	14.0	15.0	16.0
J-05774	F	U.S. Army	RA	-	-	-	-	-	-	-	-	-	-	-	-	-
		3M	LA	0	0	-	-	1	2	-	-	-	-	-	-	-
		3M	RL	0	0	-	-	0	0	-	0	-	-	0	0	2
		U.S. Army	LL	-	-	-	-	-	-	-	-	-	-	-	-	-
J-05775	M	U.S. Army	RA	-	-	-	-	-	-	-	-	-	-	-	-	-
		3M	LA	2	-	-	-	-	-	-	-	-	-	-	-	-
		3M	RL	1	2	-	-	-	-	-	-	-	-	-	-	-
		U.S. Army	LL	-	-	-	-	-	-	-	-	-	-	-	-	
J-05776	M	3M	RA	0	0	0	0	0	0	0	-	0	1	-	-	-
		U.S. Army	LA	-	-	-	-	-	-	-	-	-	-	-	-	-
		U.S. Army	RL	-	-	-	-	-	-	-	-	-	-	-	-	-
		3M	LL	0	0	-	-	1	0	2	-	-	-	-	-	-

- = No entry.
RA = right arm.
LA = left arm.
RL = right leg.
LL = left leg.

*Biting species Anopheles quadrimaculatus (Say) and A. crucians (Wiedemann).

Study No. 6171-113

Table 4

Test Conditions During Subject Exposure to *Anopheles Sp.*

Time	Temperature (°F)	Relative Humidity (%)	Air (Wind) Speed (MPR)
1030	86	80	<5
1100	-	-	-
1200*	92	67	<5
1300	92	66	<5
1430	82	77	6
1500	-	-	-
1600	83	70	<5
1700	-	-	-
1800	-	-	-
1900	81	67	<5
2000	83	70	<5
2140	76	86	<5
2200	-	-	-
2315	76	90	<5
2418	73	89	<5
0138	74	92	<5

- = No entry.

*Noon.

Study No. 6171-113

Table 5

Test Conditions During Subject Exposure
to Aedes Sollicitans (Walker)

Time	Temperature (°F)	Relative Humidity (%)	Air (Wind) Speed (MPR)
1000	88	79	<5
1100	88	72	<5
1200*	92	70	<5
1300	92	68	<5
1400	88	70	<5
1500	85	80	<5
1600	92	66	<5
1700	91	68	<5
1800	88	74	6
1900	86	74	<5
2000	84	88	<5
2100	82	80	<5
2200	82	80	<5
2300	80	92	<5
2400	-	-	-
0100	79	93	<5

- = No entry.

*Noon.

APPENDIX C

ARTHROPOD REPELLENT PROJECT
USER ACCEPTABILITY TESTING
PHASE II RESULTS
CMR PROJECT #1570

Prepared for: Craig Sterling, Personal Care Products/3M

Prepared by: Peter A. Schamel, Corporate Marketing Research/3M

Date: August 1, 1986

Background

As part of a 3M development contract for the U.S. Army, contract number DAMD17-85-C-5017, Controlled-Release Personal Use Arthropod Repellent Formulation, Phase II, a user acceptability test was conducted by Corporate Marketing Research. A 3M formulation, containing DEET as active ingredient in a lotion base, was tested and compared with the current Army standard insect repellent. This report presents the findings of the five-foot odor detectability test and of absolute and relative acceptability tests of both products.

Methodology

Testing was conducted in Dallas, Texas. A sample of 200 respondents participated in the product tests. All were qualified as being between 18 and 35 years old and as not having any skin disease, dermatological problems or sensitivities to topical skin care products. In addition, female respondents who were pregnant were excluded. This sample was chosen to be demographically close to the current make-up of the U.S. Army. Of the 200, 20 (10%) were female and 60 (30%) were non-Caucasian.

Respondents first tested the detectability of the odor of each product. They were placed five feet away from a person who had applied one of the repellents to both his or her forearms. After approximately 10 seconds, they were asked if they could detect an odor. They were then exposed to the other product, on the arms of a different person, and asked if they could detect its odor. Respondents who could detect an odor were asked to rate the strength of the odor.

Next, respondents applied a small amount of the 3M formulation to one of their forearms and a small amount of the standard Army repellent to the other forearm. One half of the respondents tried the 3M product first and one half tried the military product first. They then were asked a short series of questions about their preferences. The respondents were then taken outdoors, and remained in that warm and humid environment for 10 minutes, after which they returned to the test facility and were questioned again about their preferences and likelihood to use each product. Temperatures ranged from 82° F to 100° F, with an average of 94° F. Humidity ranged from 9% to 78%, with and average of 27%.

Results

Of the 200 respondents, 11.5% (23) could detect the odor of the 3M product and 10% (20) could detect the odor of the military product. Four respondents (2%) detected the odor of both products.

To test absolute acceptability, respondents were asked whether or not they would be likely to use each formulation if they were involved in an outdoor activity, given that no other insect repellent was available. Immediately after application, 94.5% of respondents (189) stated that they would be at least somewhat likely to use the 3M formulation. After being outdoors for 10 minutes, 88% (176) stated that they would be at least somewhat likely to use the 3M formulation. Immediately after application, 96.5 % (193) stated that they would be at least somewhat likely to use the military standard formulation. After being outdoors for 10 minutes 91% (182) stated that they would be at least somewhat likely to use the military standard formulation. These results are projectable to the general population of military age personnel of similar demographics with accuracy of $\pm$ 6% at the 90% confidence level.

In comparative testing, which was conducted after respondents had been outdoors, respondents were asked which of the two repellents they would prefer to use if they were involved in an outdoor activity. 46.5% of respondents (93) preferred the 3M formulation and 53% (106) prefered the military standard product. A 12% difference would be statistically significant at the 90% confidence level (14% at 95% confidence), therefore no difference in preference between the products can be confirmed.

Conclusion

The 3M formulation far exceeds the 75% user acceptability requirement of the Army contract. There is no statistically significant difference between the acceptability of the 3M insect-repellent formulation and that of the military standard insect repellent. The 3M formulation has virtually the same odor detectability at five feet as the current military standard formulation.

PAS:dg

17403/483d

APPENDIX D

Summary of Toxicity Tests

Controlled Release Personal Use
Arthropod Repellant - Phase II

U.S. Army Contract No.
DAMD 2100414504

August 29, 1986

F. D. Griffith, Ph.D.
Manager, Toxicology Services
Medical Department/3M
3M Center
Bldg. 220-2E-02
St. Paul, MN 55144

(TS56.22)

Index

	Page
Introduction	1
Procedures	2
Eye Irritation	2
Primary Dermal Irritation	2
Acute Oral Toxicity	2
Acute Dermal Toxicity	2
Repeated Insult Human Patch Test	3
Results	4
Eye Irritation	4
Primary Skin Irritation	4
Acute Oral Toxicity	4
Acute Dermal Toxicity	4
Repeated Insult Human Patch Test	4
Appendix I. Identification of Formulations	i

Introduction

This is a summary of pertinent toxicity tests that were conducted in conjunction with this project. Original reports are in the 3M files.

Various formulations were tested but results from the control (the current standard of 75% DEET* and 25% ethanol) and the final 3M candidate are summarized here.

All formulations were assigned code numbers (T-Numbers) for ease of reference and security of composition. This procedure also aided in avoiding bias in the laboratory.

*N,N-diethyl-m-toluamide

Procedures

Eye Irritation

The test was conducted according to U.S. Environmental Protection Agency Guidelines for Testing Pesticides and Toxic Substances. The Draize procedure was followed using New Zealand white rabbits with a washed and an unwashed group. The washed eyes were flushed with lukewarm water for one minute beginning 30 seconds after instillation. Observations were recorded at 1, 24, 48, 72, and 96 hours then at 7, 14 and 21 days after treatment.

Primary Dermal Irritation

The test was conducted according to the U.S. Environmental Protection Agency Guidelines for Testing Pesticides and Toxic Substances. Test sites were prepared by clipping the hair from the backs and flanks of 6 New Zealand white rabbits. One-half (0.5) ml of sample was spread evenly over the intact skin of each test site, covered with a 2.5 x 2.5 cm gauze pad and secured in a semi-occlusive condition for 4 hours. Patches were then removed and the test sites washed with lukewarm water and paper towels. All sites were scored by the Draize method at 1/2, 24, 48 and 72 hours after patch removal.

Acute Oral Toxicity

The test was conducted according to the U.S. Environmental Protection Agency Guidelines for Testing Pesticides and Toxic Substances. Young adult Sprague-Dawley albino rats were divided into groups of five males and five females. Each animal received test material equivalent to 5 g/kg of body weight by gavage following an overnight fast. Observations were at 1, 2.5 and 4 hours following dosing, then twice daily for 14 days. The animals were weighed just before dosing, at 7 days and at 14 days then necropsied for gross observation.

Acute Dermal Toxicity

The test was conducted according to the U.S. Environmental Protection Agency Guidelines for Testing Pesticides and Toxic Substances. Test species and test site preparation were similar to those described above for Primary Dermal Irritation. The test material, 2 g/kg body weight, was applied to the backs of five male and five female rabbits then covered by a 4x4 inch gause patch and held in place in a semi-occlusive condition. Patches were removed after 24 hours and the sites washed with lukewarm water and paper towels. The animals were observed for signs of toxicity and mortality at 1, 2.5, and 4 hours after application then daily through the 14 day observation period. They were also observed for irritation at 1/2 hour after patch removal then on study days 3, 7, 10 and 14. Animals were weighed just before application then at 7 and 14 days. All animals were then subjected to a gross necropsy.

Repeated Insult Human Patch Test

In a modification of the Draize procedure, 0.2 ml of sample was applied to a Webril pad and secured with tape in an occlusive manner to the skin of human volunteers. During a 3 week induction period, patches were applied three times per week for 48-72 hours. After a two week rest period, patches were applied to naive sites for 72 hours, then scored at 24 and 48 hours after removal. Two hundred seven subjects completed all phases of the study.

This procedure was conducted instead of the guinea pig sensitization because the Repeated Insult Human Patch Test results are directly applicable to humans.

Results

Eye Irritation

T-3755 - Mild to moderate irritation in both the washed and unwashed eyes. Pain response in one of six animals in the unwashed group but none in the washed group. Conjunctival blanching and corneal epithelial peeling in all unwashed and one washed animal. Petite hemorrhage in some animals in the washed eyes. One unwashed eye had neo-vascularization at 7 days. Signs persisted at 7 days but not at 14 days. Washing alleviated but did not prevent serious damage.

T-3896 - Mild to moderate irritation in both washed and unwashed eyes. No pain response. Conjunctival blanching in all eyes. Corneal epithelial peeling in unwashed eyes and in two of three washed eyes. Petite hemorrhage in some unwashed eyes but none in the washed eyes. Five of six unwashed eyes had all zero scores at 7 days and one had all zero scores at 14 days. Two of three washed eyes had all zero scores at 7 days but one had approximately 15% corneal epithelial peeling at 21 days.

In a repeat of the wash procedure, two eyes were all zero scores at 7 days and one was all zero scores at 14 days.

Primary Dermal Irritation

T-3755 - No irritation reported.

T-3896 - Minimal erythema in three animals at 24 hours and two animals at 48 hours. Minimal edema in one animal at 24 and 48 hours.

Acute Oral Toxicity

T-3755 - Three males and all females died within one day following dosing. The rat acute oral LD50 is less than 5 g/kg body weight.

T-3896 - Red stained face on study days 1 and 2. No other signs. The rat oral LD50 is greater than 5 g/kg body weight.

Acute Dermal Toxicity

T-3755 - All appeared clinically normal. Irritation consisted of slight to severe erythema and edema, slight to marked atonia, desquamation, conaceousness and fissuring. The rabbit acute dermal LD50 is greater than 2 g/kg body weight.

T-3896 - One female had signs of diarrhea on days 4, 5 and 7. There was slight to severe erythema, slight to moderate edema, desquamation, fissuring and some subcutaneous hemorrhaging. The acute dermal LD50 in rabbits is greater than 2 g/kg body weight.

Repeated Insult Human Patch Test

T-3755 and T-3896 - Mild, transient irritation with no indication of sensitization.

APPENDIX I

PHASE II FINAL TEST FORMULATIONS

PCP No.	T-No.	CABOSIL M-5	VARONIC L1420	VARONIC L148	LEXEMUL AS	CARBOWAX 400	LIPONIC EG-7	VEEGUM	NATRASOL 250HR	LEXOL PG 865	WAXENOL 816	ARLAMOL E	ADOL 63	POLYMER	DEET	GERMABEN II	WATER
49-22-1	3895	--	.65	1.03	3.48	1.30	1.94	.70	.70	3.22	.65	.86	.86	23.33	17.50	.24	43.57
49-23-1	3896	2.75	.65	1.03	4.06	.98	2.26	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-23-2	3897	2.75	.65	1.03	4.06	.33	1.62	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-23-6	3901	2.75	.65	1.03	2.90	.98	1.62	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-23-3	3898	1.25	.65	1.03	4.06	.98	1.62	.70	.50	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-23-4	3899	2.00	.65	1.03	4.06	.66	1.62	---	.10	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-23-5	3900	3.50	.65	1.03	4.06	1.00	1.62	---	.10	3.22	.65	.43	.86	23.33	17.50	.24	qs
49-24-1	3755														75.00 + 25.00 g Ethanol		

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

FRANK GRIFFITH, PH.D.
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 60405110
SAMPLE ENTERED: 04/21/86
REPORT PRINTED: 06/26/86

T-3896

PURCHASE ORDER NUMBER: T757575-TBR, REL. # 604

ENCLOSED: ACUTE ORAL TOXICITY STUDY IN RATS - METHOD, SUMMARY, PATHOLOGY
QAU STATEMENT
RAW DATA/PROTOCOL APPENDIX

SIGNED: Steven M. Glaza ..... 7-1-86 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

T-3896

ACUTE ORAL TOXICITY

Objective: To determine the acute oral toxicity produced when a test material is administered by the oral route (gavage) to rats according to the U.S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances.

Test Material: T-3896
Physical Description: White cream
Purity and Stability: Sponsor assumes responsibility for purity and stability determinations.

Test Animal: Young adult male and female albino rats of the Sprague-Dawley strain were procured, maintained by sex in group cages in temperature- and humidity-controlled quarters, provided continuous access to Purina Rodent Chow and water, and held for an acclimation period of at least 7 days.

Acclimated animals were chosen at random for the study. Test animals were housed by sex in groups of five and identified by animal number and corresponding ear tag. Food and water were available ad libitum throughout the study, except for an overnight period just before test material administration when food, but not water, was withheld.

Reason for Species Selection: The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Method: Five male and five female rats weighing between 200 and 239 g were used for a single dosage level of 5.0 g/kg.

Preparation and Administration of Test Material: An individual dose was calculated for each animal based upon its fasted body weight and administered undiluted by gavage.

The dose volume of the test material was 5.15 ml/kg of body weight based upon an average bulk density of 0.97 g/ml.

Reason for Route of Administration: This is the method for administering a known quantity of test substance and has been the route of choice historically.

T-3896

ACUTE ORAL TOXICITY (CONTINUED)

Observations: The animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours after test material administration. The animals were observed daily thereafter for 14 days for clinical signs and twice daily for mortality.

All animals were weighed just before test material administration, at 7 days and at study termination.

Pathology: At study termination all animals were euthanized, subjected to a gross necropsy examination and all abnormalities were recorded.

T-3896

ACUTE ORAL TOXICITY (CONTINUED)

SUMMARY

Test Animal: Albino Rats - Sprague-Dawley strain
Source: Charles River Laboratories, Inc., Portage MI
Date Animals Received: 03/17 and 04/14/86
Temperature and Humidity of Animal Room: 19 to 23 Degrees C.; 38 to 66% Relative Humidity

Method of Administration: Oral Gavage

Date Test Started: 04/25/86 Date Test Completed: 05/09/86

Estimated Oral LD50: Male - Greater than 5.0 g/kg of body weight
Female - Greater than 5.0 g/kg of body weight

	Dosage Level (g/kg)	Average Body Weights (g) Initial	Day 7	Terminal	Mortality (Number Dead/Number Dosed)
Male	5.0	218	279	327	0/5
Female	5.0	208	245	248	0/5

Comments: Red-stained face was the only clinical sign observed during the study. All animals had returned to a normal appearance by Study Day 2.

Deviation from the protocol: During the study period, the temperature of the animal room ranged from 19 to 23 degrees C. rather than 20 to 24 degrees C. as stated in the protocol. This deviation is not considered to have had an effect on the validity of the study.

T-3896

ACUTE ORAL TOXICITY (CONTINUED)

PATHOLOGY

Dosage Level: 5.0 g/kg of body weight Date Dosed: 04/25/86

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C46892	M	-	14	No visible lesions.
C45395	M	-	14	No visible lesions.
C45377	M	-	14	No visible lesions.
C46894	M	-	14	No visible lesions.
C46895	M	-	14	No visible lesions.
C46863	F	-	14	No visible lesions.
C46841	F	-	14	No visible lesions.
C46828	F	-	14	No visible lesions.
C46865	F	-	14	No visible lesions.
C46864	F	-	14	No visible lesions.

References:

1. Hitch, R.K., "Acute Oral Toxicity Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substance Series 81-1, pp. 34-39, November 1982.

2. 40 CFR 160.

3. DHEW Publication No. (NIH 85-23 1985) Guide for the Care and Use of Laboratory Animals.

QUALITY ASSURANCE STATEMENT

Acute Oral Toxicity Study in Rats

Study No. 60405110

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 40 CFR 160.35 (b) (6) (7). It has been found to identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
4/30/86	Process Audit	5/05/86
6/01/86	Report Review	6/03/86

Susan Kramlich 6-10-86

Susan Kramlich
Inspector, Quality Assurance Unit

Date

ACUTE ORAL TOXICITY

Dose Administration/ Body Weight/ Mortality Record

Test Material: T-3896 Vehicle: NA MLA No.: 60405110

Bulk Density: 0.97 (g/mL) Species: Albino Rat Source: Charles River Date Received: 3-17-86, 4-14-86

Dose Level: 5.0 (g/kg) Fasted: Date 4-24-86 Time 5:30pm Technician Sam Room No. 8

Strain: Sprague Dawley Route of Administration: Oral Gavage

1986

Dose Volume 5.15 (mL/kg)	Sex: Male/Female					Dose Time: 1:30 P.M.		Technician	Date	Scale Used
Animal No. C4-	6891	6892	5395	5877	6894	6893	6895	GH	4-25	MA
Pretested Body Weight (g)	NA									→
Fasted Body Weight (g)	1948 ③	2048 ⑤	239	233	213	219	201	GH	4-25	KTRON 5228
Actual Dose (mL)	⑥ * 1.0	1.1	1.2	1.2	1.1	1.1	1.0	MP/GH	4-25	Verified by: MH 4-25-86
Day 7 Body Weight (g)	*	250	303	299	283	*	260	SD	5/2	KTRON 5228
Day 14 Body Weight (g)	*	297	341	352 ②	338	*	308	Sam	5/9	KTRON 15019
Dead Body Weight (g)										

1986

Dose Volume 5.15 (mL/kg)	Sex: Male/Female						Dose Time: 1:40 P.M.	Technician	Date	Scale Used
Animal No. C4-	6842 ④	6841	6828	6865	6864	6826		GH	4-25	MA
Pretested Body Weight (g)	NA									→
Fasted Body Weight (g)	207	209	201	200	223	213		GH	4-25	KTRON 5228
Actual Dose (mL)	1.1	1.1	1.0	1.0	1.1	1.1		MP/GH	4-25	Verified by: MH 4-25-86
Day 7 Body Weight (g)	244	252	232	237	258	*		SD	5/2	KTRON 5228
Day 14 Body Weight (g)	252	249	237	241	261	*		Sam	5/9	KTRON 15019
Dead Body Weight (g)										

MORTALITY (NO. DIED/NO. DOSED)

g/kg Dose Level	Sex	Hours 0 - 4	Study Day 1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM	11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM	Total
5.0	♂	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	MM
5.0	♀	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	MA
Technician		MP	MP	MP	MP	MP	GH	Sam	Sam	Sam	SD	Sam	Sam	Sam	SD	Sam	Sam	MP	MP	MP	MP	MP	Sam	Sam	Sam	Sam	Sam	SD	Sam	MA	MH
Date 1986		4/25	4/25	4/26	4/26	4/27	4/27	4/28	4/28	4/29	4/29	4/30	4/30	5/1	5/1	5/2	5/2	5/3	5/3	5/4	5/4	5/5	5/5	5/6	5/6	5/7	5/7	5/8	5/8	5/9	MA	5/12

NA - Not Applicable

* - Dosage calculated, but not administered unused animal returned to stock

Reviewed By ③ MH MH ③ Date 5-12-86 4-25-86

⑤ Entry error SD 5/2/86

⑥ Dose not administered due to low bodyweight. MP 5/9/86

① entry error GH 4-25-86

② entry error GH 4-25-86

③ entry error MH 4-25-86

④ #6842 is not an extra animal - it is on test 4-25-86 JP

GROSS CLINICAL OBSERVATIONS

Study Title: Acute Oral Toxicity

Test Material: T-3896 HLA No.: 60405110

Dose Level: 5.0 g/kg, Vehicle: NA

Species: Rat Sex: ♂

Number of Animals Affected/Observation Period

Observations	Pre dose	Hours			Study Day													
		1.0	2.5	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared Normal	5	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5
Red Stained face	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Deaths																		
Technician	NP	NP	NP	NP	[illegible]	[illegible]	[illegible]	[illegible]	SD	[illegible]	SD	[illegible]	NP	NP	[illegible]	[illegible]	[illegible]	[illegible]
Date 1986	4-25	4-25	4-25	4-25	4/26	4/27	4/28	4/29	4/30	5/1	5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9

NA = Not Applicable
— = Not Evident
★ = Found Dead, P.M. Check

Reviewed By: MM Date: 5-12-86

GROSS CLINICAL OBSERVATIONS

Study Title: Acute Oral Toxicity

Test Material: T-3896 HLA No.: 60405110

Dose Level: 5.0 g/kg Vehicle: NA

Species: Rat Sex: ♀

Number of Animals Affected/Observation Period

Observations	Pre dose	Hours			Study Day													
		1	2.5	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared Normal	5	2	2	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5
red stained face	0	3	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Deaths																		
Technician	RP	RP	RP	RP	RP	RP	RP	Sam	SD	Sam	SD	Sam	RP	RP	Sam	Sam	Sam	Sam
Date 1986	4-25	4/25	4/25	4/25	4/26	4/27	4/28	4/29	4/30	5/1	5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9

NA = Not Applicable
— = Not Evident
* = Found Dead, P.M. Check

Reviewed By: MH Date: 5-12-86

SAMPLE SUBMITTAL FORM

ENCLOSE WITH SAMPLES AND SEND TO:
HAZLETON LABORATORIES AMERICA, INC.
Chemical and BioMedical Sciences Division
3301 KINSMAN BOULEVARD
MADISON, WISCONSIN 53704
(608) 241-4471

Submitted By: F. D. GRIFFITH Date: 4-16-86

Company: 3M TOXICOLOGY SERVICES Invoice To: _____

P. O. Number _____

Type of Report: ___ All tests in one report
✓ One report for each test
20 Number of reports required

Full GLP compliance: ✓ yes
___ no

___ FDA (21 CFR 58)
___ EPA (TSCA - 40 CFR 792)
✓ EPA (FIFRA - 40 CFR 160)
___ OECD

T-3901

Sample Name: T-3755, T-3895, T-3896, T-3897, T-3898, T-3899, T-3900
Physical Description: T-3755 - CLEAR ALCOHOLIC SOLUTION; ALL OTHERS - WHITE LOTION

Storage Requirement: ✓ Room Temp. ___ Refrigerated ___ Other ___ 2018

Test — Acute Oral Toxicity in Rats

___ TP4207 Internal screen; No. of animals ___M ___F at _____
___ TP3206 FHSA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
✓ TP3013 EPA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
___ TP2069 OECD screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg

Special Instructions: _____

Test — Acute Dermal Toxicity in Rabbits

___ TP3207 FHSA screen; 5M-5F at 2.0 g/kg
✓ TP3016 EPA screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg
___ TP2070 OECD screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg

Special Instructions: _____

Disposal of test material:
___ Return to submitter.
✓ Dispose of according to HLA SOP.

Test — Primary Skin Irritation

___ TP4209 Internal screen; No. of animals _____
No. of sites/rabbit ___ Abraded
___ Intact MMMT 26
___ TP3208 FHSA; 6 rabbits-1 abraded/1 intact site per rabbit
✓ TP3014 EPA; 6 rabbits-1 intact site/rabbit
___ TP2071 OECD; 3 rabbits-1 intact site/rabbit
___ TP4206 DOT Corrosivity; 6 rabbits-1 intact site/rabbit

Special Instructions: _____

Test — Primary Eye Irritation

___ TP4208 Internal screen; No. of animals _____
___ TP3209 FHSA; 6 rabbits unwashed
✓ TP2012 1978 EPA; 6 rabbits unwashed-3 washed
___ TP3015 1982 EPA; 6 rabbits unwashed
___ TP2072 OECD; 3 rabbits unwashed
___ 3 Rabbits washed at 4 sec.
___ 3 Rabbits washed at 30 sec.

Special Instructions: _____

Test — Guinea Pig Sensitization

___ TP2017 EPA Magnusson-Kligman maximization
___ TP2008 EPA Buehler sensitization

Special Instructions: _____

FOR HLA USE

Additional Comments: CONDUCT ACCORDING TO THE ATTACHED PROTOCOL.
SG 4-23-86

This form is to be used when submitting a sample for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Toxicology Department at (608)-241-4471 Ext. 304 or the Client Services Center at Ext. 222.

COPY 1 — HAZLETON LABORATORIES • COPY 2 — RETAIN FOR RECORDS

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD. • P.O. BOX 7546 • MADISON, WI 53707 • (608) 241-4471 • TLX 703868 HAZRAL MDS UD

PROTOCOL TP3013

Acute Oral Toxicity Study in Rats
(1982 EPA Guidelines)

Study No. 60405110

for

The 3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

April 8, 1986

PROTOCOL TP3013

Acute Oral Toxicity Study in Rats
(1982 EPA Guidelines)

Study No.	60405110
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	(See sample submittal form)
Sponsor's Representative	F. D. Griffith, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of 4-21-86
Completion Date	Week of 5-5-86
Final Report Date	Week of 6-2-86

SG 4-23-86

PROTOCOL TP3013

1. Study Title	Acute Oral Toxicity Study in Rats (1982 EPA Guidelines)
2. Objective	To determine the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats
3. Test Material	
A. Identification	(See sample submittal form)
B. Physical Description	(See sample submittal form)
C. Purity and Stability	The Sponsor assumes responsibility for purity and stability determinations.
D. Storage Conditions	(See sample submittal form)
E. Retention	Any unused test material will be discarded 30 days after issuance of the final report unless directed otherwise by the Sponsor.
F. Safety Precautions	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.
4. Regulatory Compliance	All aspects of this study will conform to the U. S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances[1] and the U. S. Environmental Protection Agency's Good Laboratory Practice Standards.[2,3]
5. Quality Assurance	The conduct of this study and the final report will be audited by the Quality Assurance Unit in accordance with Standard Operating Procedures (SOPs) at Hazleton Laboratories America, Inc. (HLA).

6. Experimental Design

A. Animals

(1) Species	Albino rat
(2) Strain/Source	Sprague-Dawley/Charles River Laboratories, Portage, Michigan
(3) Age at Initiation	Young adult (approximately 8 weeks of age)
(4) Weight at Initiation	200 to 300 g (range must be ±20% of the mean weight)
(5) Number/Sex	Five/sex
(6) Identification	Each animal will be assigned a permanent identification number and will be identified with a metal ear tag. All data collected from an animal will be recorded and filed under its identification number.
(7) Husbandry	Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[4] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance.
(a) Housing	The animals will be separated by sex and group housed in screen-bottom stainless steel cages (heavy gauge) held on racks with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.
(b) Food	Purina Rodent Chow® will be provided ad libitum.
(c) Water	Water will be provided ad libitum.
(d) Contaminants	No contaminants are expected to be present in the feed or water which would interfere with and affect the results of the study.

(e) Environment of Animal Room	
o Temperature	22°C ±2°
o Relative Humidity	50% ±20%
o Air Change	At least 10 changes an hour of filtered 100% outside air
o Light Cycle	12 hours light/12 hours dark
(f) Acclimation	At least 7 days
(8) Selection of Test Animals	The animals will be selected based on health and body weight. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.
(9) Justification	The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

B. Procedures

(1) Experimental Design	Initially, a single dose of 5.0 g/kg will be administered to five males and five females. If no test material-related mortality is produced at this level, no further testing will be required. If any mortality occurs at the 5.0 g/kg dose level, additional dose levels may be added at the Sponsor's request. Each dose level will consist of five males and/or five females. Animals will be assigned to groups according to HLA Standard Operating Procedure OP-TOX 42.
(2) Preparation and Administration of Test Material	All animals will receive the same concentration of dosing solution per group. If a solid, the test substance will be suspended in an appropriate vehicle. If a liquid, the test substance will be dosed undiluted, using the specific density to determine the dose volume. If the material is an

aerosol it will be discharged into a beaker and administered as a liquid. Individual dosages will be calculated based upon the animal's body weight taken just before administration of the test material. The animals will have feed withheld for approximately 17 to 20 hours prior to test material administration.

(3) Reason for Route of Administration — This is the method for administering a known quantity of test substance and has been the route of choice historically.

C. Observation of Animals

(1) Clinical Observations — The animals will be observed for clinical signs and mortality at 1.0, 2.5, and 4 hours after test material administration. The animals will be observed daily thereafter for at least 14 days for clinical signs and twice daily (morning and afternoon) for mortality. The duration of observations may be extended when considered necessary.

(2) Body Weights — Individual body weights will be recorded just prior to study initiation and at 7 and 14 days following test material administration and at death (when survival exceeds 1 day).

D. Pathology — All test animals, whether dying during the study or sacrificed at termination, will be subjected to a gross necropsy examination and abnormalities will be recorded.

7. Report

At termination of the study, a report which includes the following information will be prepared and submitted:

- A description of the test material
- A description of the test system
- Dates of study initiation and termination
- A tabulation of mortality data
- A description of any toxic effects
- A tabulation of body weights by sex and dose level
- LD_{50} calculations for each sex with 95% confidence intervals (when applicable)
- Gross pathology findings

8. Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin.

REFERENCES

1. Hitch, R. K., "Acute Oral Toxicity Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U. S. Environmental Protection Agency Office of Pesticide and Toxic Substances Series 81-1, pp. 34-39 (November 1982).

2. 40 CFR 160.

3. 40 CFR 792.

4. DHEW Publications No. (NIH) 78-23 (1978).

APPLICABLE HLA STANDARD OPERATING PROCEDURES

OP-TOX 2	Acute Oral Toxicity Study (OECD/1982 EPA Guidelines)
OP-TOX 55	Quality Assurance Inspections of the Acute Toxicology Department
OP-GENB 36	Animal Arrival, Observations, and Release from Acclimation
OP-GENB 24	Unique Identification of Laboratory Animals and Their Cages and Identification Numbers for Medical Department Test Subjects
OP-TARC 230	Monitoring, Recording, and Reporting of Animal Room Environmental Conditions
OP-GEN 33	Archiving of Data

PROTOCOL APPROVAL

F. D. Griffith

4-15-86
Date

F. D. Griffith, PhD
Sponsor's Representative
The 3M Company

Steven M. Glaza

4-8-86
Date

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

(1278S/kk)

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

FRANK GRIFFITH, PH.D.
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 60405111
SAMPLE ENTERED: 04/21/86
REPORT PRINTED: 06/26/86

T-3896

PURCHASE ORDER NUMBER: T757575-TBR, REL. # 604

ENCLOSED: ACUTE DERMAL TOXICITY STUDY IN RABBITS - METHOD, SUMMARY, PATHOLOGY
QAU STATEMENT
RAW DATA/PROTOCOL APPENDIX

SIGNED: Steven M. Glaza ..... 7-1-86 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

T-3896

ACUTE DERMAL SCREEN

Objective: To assess the systemic toxicity and relative skin irritancy of a test substance when this substance is applied to the skin according to the U.S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances.

Test Material: T-3896
Physical Description: White cream
Purity and Stability: Sponsor assumes responsibility for purity and stability determinations.

Test Animal: Young adult male and female rabbits of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to water ad libitum and a measured amount of Purina High Fiber Rabbit Chow, and held for an acclimation period of at least 7 days. All animals were identified by animal number and corresponding ear tag.

Acclimated animals were chosen at random, treated, and maintained during the observation period as specified for the acclimation period. Approximately twenty-four hours before test material application, each rabbit's back was shaved with an electric clipper. The shaved area made up approximately 20% of the total body surface.

Reason for Species Selection: Historically, the New Zealand White albino rabbit has been the animal of choice due to the large amount of background information on this species.

Method: Five male and five female rabbits weighing between 2085 g and 2684 g were used for a single dosage level of 2.0 g/kg.

Preparation of Test Material: An individual dose of the undiluted test material was weighed out for each animal based upon its body weight at study initiation.

Treatment: The test material was applied to each animal's back and the area of application was covered with a 10 x 10-cm gauze patch secured with paper tape and overwrapped with Saran Wrap and Elastoplast tape. Twenty-four hours later the bandages were removed and the backs were washed with lukewarm tap water and disposable paper towels. Collars were applied to restrain the test animals during the 24-hour exposure period.

Reason for Route of Administration: Historically, the route of choice based on the method of Draize.

T-3896

ACUTE DERMAL SCREEN (CONTINUED)

Observations: The animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours after test material administration. Thirty minutes after removal of the test material the initial dermal irritation reading was made. Subsequent readings of dermal irritation were made on Study Days 4, 7, 10 and 14. The animals were observed daily for clinical signs and twice daily (morning and afternoon) for mortality. The animals were weighed just prior to test material application, at 7 days and at study termination.

Pathology: At study termination, all animals were euthanatized, subjected to a gross necropsy examination and all abnormalities were recorded.

T-3896

ACUTE DERMAL SCREEN (CONTINUED)

SUMMARY

Test Animal: Albino Rabbits - New Zeelend White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 04/08 and 04/22/86
Temperature and Humidity of Animal Room: 20 to 23 Degrees C.; 41 to 61% Relative Humidity

Date Test Started: 05/06/86 Date Test Completed: 05/20/86

Method of Administration: Dermal Application

Estimated Dermal LD50: Male - Greater than 2.0 g/kg of body weight
Female - Greater than 2.0 g/kg of body weight

	Dosage Level (g/kg)	Average Body Weights (g) Initial	Day 7	Terminal	Mortality (Number Dead/Number Dosed)
Male	2.0	2461	2657	2713	0/5
Female	2.0	2314	2559	2584	0/5

Comments: One female animal (F13390) exhibited diarrhea on Study Day 4 and soft stools on Study Days 5 and 7. All other animals appeared clinically normal throughout the study. Dermal irritation observed consisted of slight to severe erytheme, and slight to moderate edeme, desquemation and fissuring. Subcuteneous hemorrheging was also seen within the test site of six animals.

Deviation from the protocol: Dermal irritation readings were made on Study Day 4 rather than Study Day 3 as stated in the protocol. This deviation is not considered to have had an effect on the validity of the study.

T-3896

ACUTE DERMAL SCREEN (CONTINUED)

PATHOLOGY

Animal Number	Sex	Test Day Died	Sacrificed	Necropsy Comments
F13377	M	-	14	No visible lesions.
F13364	M	-	14	No visible lesions.
F13271	M	-	14	No visible lesions.
F13382	M	-	14	No visible lesions.
F13381	M	-	14	No visible lesions.
F13380	F	-	14	No visible lesions.
F13390	F	-	14	No visible lesions.
F13384	F	-	14	No visible lesions.
F13392	F	-	14	No visible lesions.
F13441	F	-	14	No visible lesions.

References:

1. Hitch, R.K., "Acute Dermal Toxicity Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substances Series 81-2, pp. 39-44, November, 1982.

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

3. 40 CFR 160.

4. DHEW Publication No. (NIH 85-23 1985) Guide for the Care and Use of Laboratory Animals.

QUALITY ASSURANCE STATEMENT

Acute Dermal Toxicity Study in Rabbits

Study No. 60405111

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 40 CFR 160.35 (b) (6) (7). It has been found to identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
4/30/86	Process Audit	5/05/86
6/20/86	Report Review	6/24/86

Susan Kramlich
Susan Kramlich
Inspector, Quality Assurance Unit

6-26-86
Date

ACUTE DERMAL APPLICATION (LD_{50}) IN RABBITS

TEST COMPOUND T-3896 RT NO. L0405111

Physical Description: White cream VEHICLE NA

DOSAGE LEVEL (g/kg) 2.0 DATE ANIMALS CLIPPED 5-5-86 TECH P.K.

DATE ANIMAL RECEIVED ③ ~~3-25-86~~ 4-8 and 4-22-86 SOURCE HRP ROOM NO. 161

Strain: New Zealand White

DOSAGE CALCULATIONS				COMPOUND PREPARATION WEIGHTS		
ANIMAL NUMBER	BODY WT (kg)	DOSE LEVEL (g/kg)	DOSE ANIMAL (g)	TARE WT (g)	TOTAL WT (g)	SAMPLE WT (g)
Fl-3377	2.665	2.0	5.33	9.24	14.57	5.33
3364	2.684		5.37	9.18	14.55	5.37
3271	2.339		4.68	9.31	~~14.09~~ 13.99	4.68
3382	2.361		4.72	9.25	13.97	4.72
3381	2.256		4.51	9.19	13.70	4.51
3380	2553		5.11	9.20	14.31	5.11
3390	2329		4.66	9.23	13.89	4.66
3384	2.311		4.62	9.27	13.89	4.62
3392	2.291		4.58	9.19	13.77	4.58
3441 ~~3381~~ ①	~~2.256~~		4.17	9.21	13.38	4.17

CALCULATED BY: [illegible] DATE: 5/6/86 CONDUCTED BY: Sam DATE 5-6-86

VERIFIED BY: Sam DATE: 5-6-86 APPROVED BY: [illegible] DATE 5/6/86

SCALE USED: Sartorius 2811 MP2

ANIMAL BODY WEIGHTS (g)

ANIMAL NUMBER / EAR TAG NUMBER	SKIN PREP	SEX	STUDY DAY 0	STUDY DAY 7	STUDY DAY 14
Fl-3377	I	♂	2665	2841	2834
3364	I	♂	2684	3014	2964
3271	I	♂	2339	2370	2514
3382	I	♂	2361	2573	2676
3381	I	♂	2256	2485	2576
3380	I	♀	2553	2693	2761
3390	I	♀	2329	2641	2711
3384	I	♀	2311	2494	2489
3392	I	♀	2291	2625	2627
3441 ~~339+~~ ①	I	♀	~~2685~~ 2476 ①	2350	2332
TECHNICIAN	Sam	Sam	MB	MB	MP
DATE 1986	5/6	5/6	5-6	5-13	5-20
SCALE USED	~~[illegible]~~ KTRCN		15019	5228	15019

① Animal replaced 5-6-86 Sam

I - INTACT ② Entry error 5/6/86

A - ABRADED ③ Entry error 5/7/86 MM

M - MALE

(1055A) F - FEMALE ④ entry error 5/22/86 M.B.

NA - NOT APPLICABLE

Reviewed by MM

Date 5-21-86

ACUTE DERMAL TOXICITY STUDY IN RABBITS - MORTALITY RECORD

TEST MATERIAL: T-3896 DOSAGE LEVEL (g/kg): 2.0 HLA NO. 60405111

ANIMAL NUMBER	OBSERVATION PERIOD (DAYS)																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
F1- 3377	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3364	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3271	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3382	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3381	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3380	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3390	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3384	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3392	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
©3441 ~~3391~~	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
TECHNICIAN	MH						MH	MH	MH	MH	MB		MB			MB	MB	MB												
DATE 1986	5/7	5/7	5/8	5/8	5/9	5/9	5/10	5/10	5/11	5/11	5/12	5/12	5/13	5/13	5/14	5/14	5/15	5/15	5/16	5/16	5/17	5/17	5/18	5/18	5/19	5/19	5/21	5/20		

©Animal replaced 5-6-86 SAM

NA - NOT APPLICABLE
X - DEAD
✓ - ALIVE

Reviewed by MH

Date 5-21-86

ACUTE DERMAL TOXICITY

Individual Clinical Observations

Test Material: T-3896 Dose Level: 2.0g/kg HLA No.: 60405111

(Male) Female

Animal Number	Observations	Pre dose	Hours 1	2.5	4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
3377	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3364	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3271	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3382	Appeared Normal	✓	✓	✓	✓	✓	✓	/	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3381	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Deaths																		
	Technician	SMM	SMM	SMM	SMM	MH	SMM	QD	MH	MH	MB	MB	N	MB	QH	QD	QD	QH	RB
	Date 1986	5/6	5/6	5/6	5/6	5/9	5/9	5/9	5/10	5/11	5/12	5/13	5/14	5/15	5/16	5/17	5-18	5/19	5/20

✓ = Sign present.
Sl = Sign present, slight.
- = Not evident.

Reviewed By: MH

Date: 5-21-86

ACUTE DERMAL TOXICITY

Individual Clinical Observations

Test Material: T-3896 Dose Level: 2.0g/kg HLA No.: 60405111

Male / Female

Animal Number	Observations	Pre dose	Hours 1	2.5	4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
3380	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3390 ②	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	NE	–	✓	–	✓	✓	✓	✓	✓	✓	✓
	Mucoid diarrhea	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–	–	–
	Soft stools	–	–	–	–	–	–	–	–	✓	–	✓	–	–	–	–	–	–	–
3384	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3392	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3441 3391 ①	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Deaths																		
	Technician	SMM	SMM	SMM	SMM	MM	SMM	VP	MM	MM	MB	MB	VP	MB	DH	VP	VP	DH	MB
	Date 1986	5/6	5/6	5/6	5/6	5/7	5/8	5/9	5/10	5/11	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20

① Animal replaced 5-6-86 SMM ② entry error 5/10/86 MM

✓ = Sign present.
SI = Sign present, slight.
NE = – = Not evident. slh 6-25-86

Reviewed By: MM

Date: 5-21-86

ACUTE DERMAL IRRITATION RECORD

Test Material: T-3896 HLA No.: 60405111
Dosage Level: 2.0 g/kg

Observation Period (Days)

	Males					Females				
	1	③ 3 4	7	10 ~~10~~ ~~11~~	④ 14	1	③ 3 4	7	10	14
	5/7	5/9 5/10 ⑤	5/13	5/16 ④	5/20	5/7	5/9 5/10 ⑤	5/13	5/14	5/20
Animal No.: F1 3377				Intact	Abraded	**Animal No.: F1 3380**			Intact	Abraded
Erythema	0	2	0	0	0	0	3A	1	0	0
Edema	0	1	0	0	0	0	2	1	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	1	1	1	1	0	1	1	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	1	1	1	0	0	1	1	0	0
Animal No.: F1 3364				Intact	Abraded	**Animal No.: F1 3390**			Intact	Abraded
Erythema	0	3A	2	0	0	0	2	0	0	1
Edema	0	2	1	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	1	1	0	0	0	0	1	1	2
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	2	1	0	0	0	1	1	1	1
Animal No.: F1 3271				Intact	Abraded	**Animal No.: F1 3384**			Intact	Abraded
Erythema	0	2A	1	0	0	0	2A	1	0	0
Edema	0	2	1	0	0	0	2	0	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	1	1	0	0	0	1	1	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	2	1	0	0	0	1	1	0	0
Animal No.: F1 3382				Intact	Abraded	**Animal No.: F1 3392**			Intact	Abraded
Erythema	0	2A	0	0	0	0	2	0	0	0
Edema	0	2	0	0	0	0	2	1	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	1	1	0	0	0	1	1	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	2	0	0	0	0	1	1	0	0
Animal No.: F1 3381				Intact	Abraded	**Animal No.: F1 ~~3391~~ 3441** ①			Intact	Abraded
Erythema	0	2A	1	0	0	0	② 1	0	0	0
Edema	0	2	1	0	0	0	1	0	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	1	1	0	0	0	1	1	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	1	1	0	0	0	1	1	0	0
Technician	MN	MN	SH	SH	NP	MN	MN	SH	SH	NP
Date 1986	5/7	5/10	5/13	5/16	5/20	5/7	5/10	5/13	5/16	5/20

A - Subcutaneous hemorrhage
B - Blanching
C - Scab formation
D - Eschar
E - Exfoliation

① Animals replaced 5-6-86 sam
② entry error 5/10/86 MN

Reviewed by MN Date 5-21-86

③ Form Change 5/10/86 MN
④ Entry errors 5/10/86 MN
⑤ CORRECTION OF ENTRY 6-25-86 slh

SAMPLE SUBMITTAL FORM

ENCLOSE WITH SAMPLES AND SEND TO:
HAZLETON LABORATORIES AMERICA, INC.
Chemical and BioMedical Sciences Division
3301 KINSMAN BOULEVARD
MADISON, WISCONSIN 53704
(608) 241-4471

Submitted By: F. D. GRIFFITH Date: 4-16-86

Company: 3M TOXICOLOGY SERVICES Invoice To: _____

P. O. Number _____

Type of Report: ___ All tests in one report
✓ One report for each test
20 Number of reports required

Full GLP compliance: ✓ yes
___ no

___ FDA (21 CFR 58)
___ EPA (TSCA - 40 CFR 792)
✓ EPA (FIFRA - 40 CFR 160)
___ OECD

Sample Name: T-3755, T-3895, T-3896, T-3897, T-3898, T-3899, T-3900, T-3901
Physical Description: T-3755- CLEAR ALCOHOLIC SOLUTION; ALL OTHERS - WHITE LOTION

Storage Requirement: ✓ Room Temp. ___ Refrigerated ___ Other ___ 2018

Test — Acute Oral Toxicity in Rats

___ TP4207 Internal screen; No. of animals ___M ___F at _____
___ TP3206 FHSA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
✓ TP3013 EPA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
___ TP2069 OECD screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg

Special Instructions: _____

Test — Acute Dermal Toxicity in Rabbits

___ TP3207 FHSA screen; 5M-5F at 2.0 g/kg
✓ TP3016 EPA screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg
___ TP2070 OECD screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg

Special Instructions: _____

Disposal of test material:
___ Return to submitter.
✓ Dispose of according to HLA SOP.

Test — Primary Skin Irritation

___ TP4209 Internal screen; No. of animals _____
No. of sites/rabbit ___ Abraded
___ Intact MMMT 26
___ TP3208 FHSA: 6 rabbits-1 abraded/1 intact site per rabbit
✓ TP3014 EPA: 6 rabbits-1 intact site/rabbit
___ TP2071 OECD: 3 rabbits-1 intact site/rabbit
___ TP4206 DOT Corrosivity: 6 rabbits-1 intact site/rabbit

Special Instructions: _____

Test — Primary Eye Irritation

___ TP4208 Internal screen; No. of animals _____
___ TP3209 FHSA: 6 rabbits unwashed
✓ TP2012 1978 EPA: 6 rabbits unwashed-3 washed
___ TP3015 1982 EPA: 6 rabbits unwashed
___ TP2072 OECD: 3 rabbits unwashed
___ 3 Rabbits washed at 4 sec.
___ 3 Rabbits washed at 30 sec.

Special Instructions: _____

Test — Guinea Pig Sensitization

___ TP2017 EPA Magnusson-Kligman maximization
___ TP2008 EPA Buehler sensitization

Special Instructions: _____

FOR HLA USE

Additional Comments: CONDUCT ACCORDING TO THE ATTACHED PROTOCOL.
SG 4-23-86

This form is to be used when submitting a sample for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Toxicology Department at (608)-241-4471 Ext. 304 or the Client Services Center at Ext. 222.

COPY 1 - HAZLETON LABORATORIES • COPY 2 — RETAIN FOR RECORDS

HAZLETON LABORATORIES AMERICA, INC.

3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP3016

Acute Dermal Toxicity Study in Rabbits (1982 EPA Guidelines)

Study No. 6040511

for

The 3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

April 8, 1986

PROTOCOL TP3016

Acute Dermal Toxicity Study in Rabbits
(1982 EPA Guidelines)

Study No.	60405111
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	(See sample submittal form)
Sponsor's Representative	F. D. Griffith, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of 5-5-86
Completion Date	Week of 5-19-86
Final Report Date	Week of 6-16-86 SC 5-6-86

PROTOCOL TP3016

1. Study Title	Acute Dermal Toxicity Study in Rabbits (1982 EPA Guidelines)
2. Objective	To assess the systemic toxicity and relative skin irritancy of a test substance when applied to the skin
3. Test Material	
A. Identification	(See sample submittal form)
B. Physical Description	(See sample submittal form)
C. Purity and Stability	The Sponsor assumes responsibility for purity and stability determinations.
D. Storage Conditions	(See sample submittal form)
E. Retention	Any unused test material will be discarded 30 days after issuance of the final report unless directed otherwise by the Sponsor.
F. Safety Precautions	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.
4. Regulatory Compliance	All aspects of this study will conform to the U. S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances[1] and the U. S. Environmental Protection Agency's Good Laboratory Practice Standards.[2,3]
5. Quality Assurance	The conduct of this study and the final report will be audited by the Quality Assurance Unit in accordance with Standard Operating Procedures (SOPs) at Hazleton Laboratories America, Inc. (HLA).

6. Experimental Design

A. Animals

(1)	Species	Rabbit
(2)	Strain/Source	New Zealand White/Hazleton Research Products, Inc.
(3)	Age at Initiation	Young adult (approximately 14 weeks of age)
(4)	Weight at Initiation	2.0 to 3.0 kg
(5)	Number/Sex	Five/sex
(6)	Identification	Each animal will be assigned a permanent identification number and will be identified with a metal ear tag. All data collected from an animal will be recorded and filed under its identification number.
(7)	Husbandry	Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[4] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance.
	(a) Housing	The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.
	(b) Food	A measured amount of Purina High Fiber Rabbit Chow® will be provided.
	(c) Water	Water will be provided ad libitum.
	(d) Contaminants	No contaminants are expected to be present in the feed or water which would interfere with and affect the results of the study.

(e) Environment of Animal Room	
o Temperature	21°C ±2°
o Relative Humidity	50% ±20%
o Air Change	At least 10 changes an hour of filtered 100% outside air
o Light Cycle	12 hours light/12 hours dark
(f) Acclimation	At least 7 days
(8) Selection of Test Animals	The animals will be selected based on health and body weight. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.
(9) Justification	Historically, the New Zealand White albino rabbit has been the animal of choice due to the large amount of background information on this species.

B. Procedures

(1) Experimental Design	Initially a single dose of 2.0 g/kg will be administered to 10 animals (five males and five females) with intact skin. If no test material-related mortality is produced at this level, no further testing will be required. If any mortality occurs at the 2.0 g/kg level, additional dose levels may be added at the Sponsor's request. Each dose level will consist of five males and/or five females. Animals will be assigned to groups according to HLA Standard Operating Procedure OP-TOX 42.
(2) Preparation of Exposure Area	The hair will be removed from the back of each rabbit with an electric clipper approximately 24 hours before test material application. Not less than 10% of the total body surface area will be shaved.

(3) Administration of Test Material	All animals will receive a single administration of test material. The dosage will be calculated based upon the animal's body weight taken just before administration of the test material. The area of application will be covered with as thin and uniform a layer as possible. If a solid, the test material will be moistened with 0.9% saline prior to application. The area of application will be wrapped with a gauze bandage secured with paper tape around all edges, overwrapped with Saran Wrap®, and secured with Elastoplast® tape. The rabbits will be collared during the 24-hour application period.
(4) Reason for Route of Administration	Historically, this is the route of choice based on the method of Draize.[4]
(5) Removal of Test Material	Twenty-four hours following test material application the bandages will be removed and the residual test substance will be removed using water or an appropriate solvent, if necessary.

C. Observation of Animals

(1) Reading of Dermal Irritation	Approximately 30 minutes following bandage removal, the initial dermal irritation reading will be taken. Additional dermal irritation readings will be made on Study Days 3, 7, 10, and 14. Individual dermal irritation records will be maintained for each animal (see Appendix A).
(2) Body Weights	Body weights will be determined just prior to test material application on Days 7 and 14, and at death (when survival exceeds 1 day).
(3) Clinical Observations	The animals will be observed for clinical signs and mortality at 1.0, 2.5, and 4 hours after test material administration. The rabbits will be observed daily thereafter for clinical

signs and for mortality twice daily (morning and afternoon) for a period of at least 14 days. The duration of observations may be extended when considered necessary.

D. Pathology

All test animals, whether dying during the study or sacrificed at study termination, will be subjected to a gross necropsy examination and abnormalities will be recorded.

7. Report

At termination of the study, a report which includes the following information will be prepared and submitted:

- A description of the test material
- A description of the test system
- Dates of study initiation and termination
- Response data for mortality
- A description of any toxic effects
- Body weights by sex and dose levels
- LD_{50} values by sex with 95% confidence limits (when applicable)
- Gross necropsy findings

8. Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin.

REFERENCES

1. Hitch, R. K., "Acute Dermal Toxicity Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U. S. Environmental Protection Agency Office of Pesticide and Toxic Substances Series 81-2, pp. 39-44 (November 1982).

2. 40 CFR 160.

3. 40 CFR 792.

4. DHEW Publications No. (NIH) 78-23 (1978).

5. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

APPLICABLE HLA STANDARD OPERATING PROCEDURES

OP-TOX 8	Acute Dermal Toxicity Study (OECD/1982 EPA Guidelines)
OP-TOX 55	Quality Assurance Inspections of the Acute Toxicology Department
OP-GENB 36	Animal Arrival, Observations, and Release from Acclimation
OP-GENB 24	Unique Identification of Laboratory Animals and Their Cages and Identification Numbers for Medical Department Test Subjects
OP-TARC 230	Monitoring, Recording, and Reporting of Animal Room Environmental Conditions
OP-GEN 33	Archiving of Data

PROTOCOL APPROVAL

F. D. Griffith, PhD
Sponsor's Representative
The 3M Company

4-15-86
Date

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

4-8-86
Date

(1277S/kk)

SCALE FOR SCORING SKIN REACTIONS

Erythema

0 - None
1.0 - Slight
2.0 - Moderate (well defined)
3.0 - Severe (beet red)

Edema

0 - None
1.0 - Slight (barely perceptible to well defined by definite raising)
2.0 - Moderate (raised approximately 1 mm)
3.0 - Severe (raised more than 1 mm)

Atonia

0 - None
1.0 - Slight (slight impairment of elasticity)
2.0 - Moderate (slow return to normal)
3.0 - Marked (no elasticity)

Desquamation

0 - None
1.0 - Slight (slight scaling)
2.0 - Moderate (scales and flakes)
3.0 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1.0 - Slight (decrease in pliability)
2.0 - Moderate (leathery texture)
3.0 - Marked (tough and brittle)

Fissuring

0 - None
1.0 - Slight (definite cracks in epidermis)
2.0 - Moderate (cracks in dermis)
3.0 - Marked (cracks with bleeding)

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

FRANK GRIFFITH, PH.D.
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 60405112
SAMPLE ENTERED: 04/21/86
REPORT PRINTED: 06/26/86

T-3896

PURCHASE ORDER NUMBER: T757575-TBR, REL. # 604

ENCLOSED: PRIMARY DERMAL IRRITATION STUDY IN RABBITS - METHOD, SUMMARY
QAU STATEMENT
RAW DATA/PROTOCOL APPENDIX

SIGNED: Steven M. Glaza ..... 7-1-86 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

T-3896

PRIMARY SKIN IRRITATION

Objective: To determine the reletive level of primery skin irritetion of e test meterial on rebbits under semioccluded conditions according to the U.S. Environmentel Protection Agency's Guidelines for Testing Pesticides end Toxic Substences.

Test Materiel: T-3896
Physical Description: White creem
Purity and Stability: Sponsor essumes responsibility for purity end stability determinetions.

Test Animal: Young adult rebbits of the New Zealand White strein were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to weter ad libitum and a measured amount of Purina High Fiber Rabbit Chow, end held for an acclimation period of at least 7 days.

Six acclimated animals, weighing from 2615 to 2995 g, were chosen et random for the test, treated, and mainteined during the observetion period as specified for the acclimation period. Test animels were identified by enimal number and corresponding eer teg. Within 24 hours prior to treetment the heir was clipped from the beck end flanks of each animal.

Reason for Species Selection: Historicelly, the New Zeeland White elbino rabbit has bean the enimel of choice for evelueting the effect of chemicals on the skin.

Preparation end Concentretion of Test Meterial: The semple wes dosed as received. The pH was not determined.

Treatment: The test material was epplied to the intect skin of each rabbit in the amount of 0.5 ml. The treated area wes covered with e 2.5 x 2.5-cm gauze patch secured with peper tape, loosely overwrapped with Saran Wrap and secured with Elastoplest tepe to provide a semi-occlusive dressing. Collers were used to restrein the animels for the 4-hour exposure period.

Reeson for Route of Administration: Historically, the route of choice based on the method of Dreize.

T-3896

PRIMARY SKIN IRRITATION (CONTINUED)

Observations: After the exposure period, the patches were removed and the test sites were washed with lukewarm tap water and disposable paper towels. Care was taken to remove the test material as thoroughly as possible without irritating the skin. Thirty minutes following removal of the test material, the degree of erythema and edema was read according to the Draize technique. Subsequent examinations were made at 24, 48 and 72 hours after patch removal.

Individual body weights were taken just prior to study initiation.

Pathology: At study termination all animals were euthanatized and discarded.

T-3896

PRIMARY SKIN IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino Rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 03/25/86
Temperature and Humidity of Animal Room: 18 to 24 Degrees C.; 35 to 60% Relative Humidity

Date Test Started: 04/30/86 Date Test Completed: 05/03/86

Individual Dermal Irritation Scores
Test Material: T-3896

Animal Number	Sex	Erythema Score Hours 4	24	48	72	Edema Score Hours 4	24	48	72
F13124	M	0	2	1	0	0	1	1	0
F13125	M	0	0	0	0	0	0	0	0
F13126	M	0	1	0	0	0	0	0	0
F13133	F	0	0	0	0	0	0	0	0
F13134	F	0	0	0	0	0	0	0	0
F13135	F	0	1	1	0	0	0	0	0
Mean		0.0	0.7	0.3	0.0	0.0	0.2	0.2	0.0

Deviation from the protocol: During the study period, the temperature of the animal room ranged from 18 to 24 degrees C. rather than 19 to 23 degrees C. as stated in the protocol. This deviation is not considered to have had an effect on the validity of the study.

T-3896

PRIMARY SKIN IRRITATION (CONTINUED)

References:

1. Hitch, R.K., "Primary Dermal Irritation Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substances Series 81-5, pp. 55-59 (November 1982).

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

3. 40 CFR 160.

4. DHEW Publication No. (NIH 85-23 1985) Guide for the Care and Use of Laboratory Animals.

QUALITY ASSURANCE STATEMENT

Primary Dermal Irritation Study in Rabbits

Study No. 60405112

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 40 CFR 160.35 (b) (6) (7). It has been found to identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
4/30/86	Process Audit	5/05/86
5/22/86	Report Review	5/28/86

Susan Kramlich
Susan Kramlich
Inspector, Quality Assurance Unit

5-30-86
Date

PRIMARY DERMAL IRRITATION STUDY
(4 Hour Exposure)

Test Material: T-3896
Physical Description: white cream
HLA Number: 60405112
Dose: 0.5 ml Per Site NA Moistened with 0.9% Saline
pH Result: 4 with Fisher Accumet
Source: HRP
pH Meter No.: NA
Date Animals Received: 3-25-86 Species/Strain: New Zealand White Rabbit Room Number: 161C
Date Animals Clipped: 4-29-86 Technician: SAM Initiated by: SAM Date: 4-30-86
Skin Preparation: ✓ Intact NA Abraded (with a clipper blade) Reviewed by: [signature] Date: 4/30/86

FI- Animal Number/Sex		3124 ♂	3125 ♂	3126 ♂	3133 ♀	3134 ♀	3135 ♀	Technician	Recorded by	1986 Date	KTron Scale used:
Initial Body Weight (g)		2615	2818	2936	2990	2717	2995	NP	SD	4/30	15019
7-Day Body Weight (g)											
14-Day Body Weight (g)											
Observation Period											Dermal Irritation Score
4 Hours	Erythema	0	0	0	0	0	0	SAM	SAM	4-30	0.0 ✓ MN 5-5-86 SLH 4-30-86
	Edema	0	0	0	0	0	0				
24 Hours	Erythema	2	0	1	0	0	1	4H	NP	5-1	0.8 ✓ MN 5-5-86 SLH 5-2-86
	Edema	1	0	0	0	0	0				
48 Hours	Erythema	1	0	0	0	0	1	SAM	SAM	5-2	0.5 ✓ MN 5-5-86 SLH 5-2-86
	Edema	1	0	0	0	0	0				
72 Hours	Erythema	0	0	0	0	0	0	SAM	SAM	5-3	0.0 ✓ SLH 5-9-86 MN 5-5-86
	Edema	0	0	0	0	0	0				
96 Hours	Erythema										
	Edema										
7 Days	Erythema										
	Edema										
14 Days	Erythema										
	Edema										

NA - Not applicable.
A - Subcutaneous hemorrhage.
B - Blanching.
N - Possible necrotic area.
U - Unable to determine pH.

All animals appeared normal just prior to dosing. Technician NP Date 4-30-86

Reviewed by: MN Date: 5-5-86

(4253A)

SAMPLE SUBMITTAL FORM

ENCLOSE WITH SAMPLES AND SEND TO:
HAZLETON LABORATORIES AMERICA, INC.
Chemical and BioMedical Sciences Division
3301 KINSMAN BOULEVARD
MADISON, WISCONSIN 53704
(608) 241-4471

Submitted By: F. D. GRIFFITH Date: 4-16-86

Company: 3M TOXICOLOGY SERVICES Invoice To: _____

P. O. Number _____

Type of Report: ___ All tests in one report
✓ One report for each test
20 Number of reports required

Full GLP compliance: ✓ yes ___ no

___ FDA (21 CFR 58)
___ EPA (TSCA - 40 CFR 792)
✓ EPA (FIFRA - 40 CFR 160)
___ OECD

T-3901

Sample Name: T-3755, T-3895, T-3896, T-3897, T-3898, T-3899, T-3900
Physical Description: T-3755 - CLEAR AQUEOUS SOLUTION; ALL OTHERS - WHITE LIQUID

Storage Requirement: ✓ Room Temp. ___ Refrigerated ___ Other ct. # 2018

Test — Acute Oral Toxicity in Rats

___ TP4207 Internal screen; No. of animals ___M ___F at _____
___ TP3206 FHSA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
✓ TP3013 EPA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
___ TP2069 OECD screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg

Special Instructions: _____

Test — Acute Dermal Toxicity in Rabbits

___ TP3207 FHSA screen; 5M-5F at 2.0 g/kg
✓ TP3016 EPA screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg
___ TP2070 OECD screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg

Special Instructions: _____

Disposal of test material:
___ Return to submitter.
✓ Dispose of according to HLA SOP.

Test — Primary Skin Irritation

___ TP4209 Internal screen; No. of animals _____
No. of sites/rabbit ___ Abraded
___ Intact MMMT 26
___ TP3208 FHSA: 6 rabbits-1 abraded/1 intact site per rabbit
✓ TP3014 EPA: 6 rabbits-1 intact site/rabbit
___ TP2071 OECD: 3 rabbits-1 intact site/rabbit
___ TP4206 DOT Corrosivity: 6 rabbits-1 intact site/rabbit

Special Instructions: _____

Test — Primary Eye Irritation

___ TP4208 Internal screen; No. of animals _____
___ TP3209 FHSA; 6 rabbits unwashed
✓ TP2012 1978 EPA; 6 rabbits unwashed-3 washed
___ TP3015 1982 EPA; 6 rabbits unwashed
___ TP2072 OECD; 3 rabbits unwashed
___ 3 Rabbits washed at 4 sec.
___ 3 Rabbits washed at 30 sec.

Special Instructions: _____

Test — Guinea Pig Sensitization

___ TP2017 EPA Magnusson-Kligman maximization
___ TP2008 EPA Buehler sensitization

Special Instructions: _____

FOR HLA USE

Additional Comments: CONDUCT ACCORDING TO THE ATTACHED PROTOCOL.
SG 4-23-86

This form is to be used when submitting a sample for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Toxicology Department at (608)-241-4471 Ext. 304 or the Client Services Center at Ext. 222.

COPY 1 — HAZLETON LABORATORIES • COPY 2 — RETAIN FOR RECORDS

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP3014

Primary Dermal Irritation Study in Rabbits (1982 EPA Guidelines)

Study No. 60405112

for

The 3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

April 8, 1986

PROTOCOL TP3014

Primary Dermal Irritation Study in Rabbits
(1982 EPA Guidelines)

Study No.	60405112
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	(See sample submittal form)
Sponsor's Representative	F. D. Griffith, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of 4-28-86
Completion Date	Week of 5-12-86
Final Report Date	Week of 6-9-86

SG 4-30-86

PROTOCOL TP3014

1. Study Title	Primary Dermal Irritation Study in Rabbits (1982 EPA Guidelines)
2. Objective	To determine the relative level of primary skin irritation of a test material on rabbits under semioccluded conditions
3. Test Material	
A. Identification	(See sample submittal form)
B. Physical Description	(See sample submittal form)
C. Purity and Stability	The Sponsor assumes responsibility for purity and stability determinations.
D. Storage Conditions	(See sample submittal form)
E. Retention	Any unused test material will be discarded 30 days after issuance of the final report unless directed otherwise by the Sponsor.
F. Safety Precautions	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.
4. Regulatory Compliance	All aspects of this study will conform to the U. S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances[1] and the U. S. Environmental Protection Agency's Good Laboratory Practice Standards.[2,3]
5. Quality Assurance	The conduct of this study and the final report will be audited by the Quality Assurance Unit in accordance with Standard Operating Procedures at Hazleton Laboratories America, Inc. (HLA).

6. Experimental Design

A. Animals

(1)	Species	Rabbit
(2)	Strain/Source	New Zealand White/Hazlaton Research Products, Inc.
(3)	Age at initiation	Adult
(4)	Weight at Initiation	2.0 to 3.5 kg
(5)	Number/Sex	Six/either sex
(6)	Identification	Each animal will be assigned a permanent identification number and will be identified with a metal ear tag. All data collected from an animal will be recorded and filed under its identification number.
(7)	Husbandry	Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[4] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance.
	(a) Housing	The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.
	(b) Food	A measured amount of Purina High Fiber Rabbit Chow® will be provided.
	(c) Water	Water will be provided <u>ad libitum</u>.
	(d) Contaminants	No contaminants are expected to be present in the feed or water which would interfere with and affect the results of the study.

(e) Environment of animal room	
o Temperature	21°C ±2°
o Relative humidity	50% ±20%
o Air change	At least 10 changes an hour of filtered 100% outside air
o Light cycle	12 hours light/12 hours dark
(f) Acclimation	At least 7 days
(8) Selection of test animals	The animals will be selected based on health and body weight. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.
(9) Justification	Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

B. Procedures

(1) Preparation of Exposure Area	Within 24 hours prior to test material administration, the hair will be clipped from the the back and flanks of each animal. The treatment sites will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals.
(2) Administration of Test Material	The test material will be applied to the test area (approximately 6 cm^2) on each rabbit, in the amount of 0.5 mL in the case of liquids and 0.5 g in the case of solids. Solid test materials will be moistened with 0.9% saline. The treated area will be covered with a 2.5-cm x 2.5-cm gauze patch secured with paper tape and loosely overwrapped with Saran Wrap® and Elastoplast® tape to provide a semiocclusive dressing. Collars will be used to restrain the animals during the 4-hour exposure period.

(3) Reason for Route of Administration	Historically, the route of choice based on the method of Draize.[5]
(4) Removal of Test Material	After the 4 hours of exposure, the patches and test material will be removed as thoroughly as possible using water and/or an appropriate solvent without irritating the skin.
C. Observation of Animals	Dermal irritation readings and body weights will be recorded in a study notebook.
(1) Reading of Dermal Irritation	Thirty minutes after removing the patches, the degree of erythema and edema will be recorded according to the Draize technique (Attachment 1). The intact skin of each animal will serve as its own control. Subsequent readings will be taken at 24, 48, and 72 hours after patch removal. Further observations may be recorded, as necessary, to establish reversibility. If irritation is increasing in severity at the 72-hour examination, observations may be repeated at 96 hours, and at 7, 14, and 21 days, if applicable.
(2) Body Weights	Body weights will be taken just prior to test material administration and at weekly intervals throughout the study.
(3) Clinical Observations	Any abnormal clinical signs will be recorded in the study folder.
D. Pathology	All animals, whether dying on test or sacrificed at study termination, will be discarded.

7. Report

At termination of the study, a report which includes the following information will be prepared and submitted:

- A description of the test material
- A description of the test system
- Dates of study initiation and termination
- A tabulation of irritation data
- A description of any toxic effects other than dermal irritation

8. Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin.

REFERENCES

1. Hitch, R. K., "Primary Dermal Irritation Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U. S. Environmental Protection Agency Office of Pesticide and Toxic Substances Series 81-5, pp. 55-59 (November 1982).

2. 40 CFR 160.

3. 40 CFR 792.

4. DHEW Publications No. (NIH) 78-23 (1978).

5. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

APPLICABLE HLA STANDARD OPERATING PROCEDURES

OP-TOX 4	Primary Dermal Irritation Study (OECD/1982 EPA Guidelines)
OP-TOX 55	Quality Assurance Inspections of the Acute Toxicology Department
OP-GENB 36	Animal Arrival, Observations, and Release from Acclimation
OP-GENB 24	Unique Identification of Laboratory Animals and Their Cages and Identification Numbers for Medical Department Test Subjects
OP-TARC 230	Monitoring, Recording, and Reporting of Animal Room Environmental Conditions
OP-GEN 33	Archiving of Data

PROTOCOL APPROVAL

F. D. Griffith

4-15-86

F. D. Griffith, PhD
Sponsor's Representative
The 3M Company

Date

Steven M. Glaza

4-8-86

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

Date

(1279S/kk)

ATTACHMENT I

PRIMARY SKIN IRRITATION SCORING SCALE

1. Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

2. Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

FRANK GRIFFITH, PH.D.
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 60405113
SAMPLE ENTERED: 04/21/86
REPORT PRINTED: 06/26/86

T-3896

PURCHASE ORDER NUMBER: T757575-TBR, REL. # 604

ENCLOSED: PRIMARY EYE IRRITATION STUDY IN RABBITS - METHOD, SUMMARY
QAU STATEMENT
RAW DATA/PROTOCOL APPENDIX

SIGNED: Steven M. Glaza ..... 7-1-86 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

T-3896

EYE IRRITATION

Objective: To determine the reletive level of irritetion produced following a single exposure of e test meterial to one eye of elbino rabbits eccording to the U.S. Environmentel Protection Agency's Guidelines for Testing Pesticides end Toxic Substences.

Test Meteriel: T-3896
Physicel Description: White creem
Purity end Stebility: Sponsor essumes responsibility for purity end stebility determinetions.

Test Animel: Young edult rebbits of the New Zeelend White strein were procured, maintained individuelly in screen-bottom ceges in temperature- end humidity-controlled quarters, provided eccess to water ad libitum end e measured emount of Purine High Fiber Rabbit Chow, end held for en acclimetion period of at least 7 days.

Nine ecclimated enimels, weighing from 2357 to 3030 g, were chosen at rendom for the test. The enimels' eyes were exemined epproximately 24 hours befora test material administration using sodium fluorescein dye procedures. Only those enimels with no sign of oculer injury or irritetion were used on the test. Test enimels were identified by enimel number end corresponding ear tag. The rebbits were divided into two groups consisting of six rebbits in Group I end three rabbits in Group II.

Reason for Species Selection: The New Zeeland White albino rebbit is the enimel of choice besed upon its lerge orbit end nonpigmented iris.

Preperetion of Test Meteriel: The sample wes dosed as received. The pH wes not determined.

Treetment: Eech rebbit received 0.1 ml of the test material placed on the everted lower lid of one eye, with the contralateral eye serving as the untreeted control. The upper and lower lids were held together for one second to prevent loss of meteriel end then released. Group II enimels hed the treeted eye flushed for one minute with lukewarm water, sterting 30 seconds after test meteriel instillation. The eyes of the rebbits in Group I remeined unflushed.

Reeson for Route of Administration: Historically, this is the route of choice based on the method of Draize.

T-3896

EYE IRRITATION (CONTINUED)

Observations: Readings of ocular irritation in the treated eyes of both groups were made at 1, 24, 48, 72 and 96 hours, and at 7 and 14 days after treatment. Observations continued on Day 21 for the Group II animals.

At the 72-hour and 7, 14 and 21-day readings, sodium fluorescein was used to aid in revealing possible corneal injury. Irritation was graded and scored according to the Draize technique.

Animals were weighed just prior to test material administration and at weekly intervals during the study.

Pathology: At study termination all animals were euthanatized and discarded.

T-3896

EYE IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 03/11/86

Temperature and Humidity of Animal Room: 18 to 23 Degrees C.; 38 to 63% Relative Humidity

Date Test Started: 04/24/86 Date Test Completed: 05/15/86

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	Group I 6 Rabbit Mean 0.1 ml (Unwashed)	Group II 3 Rabbit Mean 0.1 ml (Washed)
1 Hour:	39.0	39.0
24 Hours:	34.7	28.7
48 Hours:	35.7	27.3
72 Hours:	26.2	12.3
96 Hours:	26.0	12.7
7 Days:	3.7	3.0
14 Days:	0.0	4.3
21 Days:	---	3.0

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals in each group (6 or 3) at each observation period.

Deviation from the protocol: During the study period, the temperature of the animal room ranged from 18 to 23 degrees C. rather than 19 to 23 degrees C. as stated in the protocol. This deviation is not considered to have had an effect on the validity of the study.

T-3896

EYE IRRITATION (CONTINUED)

Table 1
Individual Eye Irritation Scores
Group I - (unwashed)

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F12909	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	1	4	20	1	5	2	2	0	8
	48 Hours	1	4	20	1	5	2	2	0	8
	72 Hours	1	4	20	1	5	2	1	0	6
	96 Hours	1	4	20	1	5	2	1	0	6
	7 Days	1	4	20	0	0	1	0	0	2
	14 Days	0	0	0	0	0	0	0	0	0
F12947	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	1	4	20	1	5	2	2	1	10
	48 Hours	1	4	20	1	5	2	2	1	10
	72 Hours	1	3	15	1	5	1	2	0	6
	96 Hours	2	1	10	0	0	1	0	0	2
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
F12942	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	1	4	20	1	5	2	2	2	12
	48 Hours	1	4	20	1	5	2	2	1	10
	72 Hours	1	2	10	1	5	2	3	2	14
	96 Hours	2	1	10	0	0	2	1	0	6
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
F12943	1 Hour	1	4	20	1	5	2	2	3	14
	24 Hours	1	4	20	1	5	3	3	2	16
	48 Hours	1	4	20	1	5	3	3	2	16
	72 Hours	1	3	15	1	5	2	2	2	12
	96 Hours	2	3	30	1	5	3	2	0	10
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0

T-3896

EYE IRRITATION (CONTINUED)

Table 1 (continued)
Individual Eye Irritation Scores
Group I - (unwashed)

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F12944	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	1	4	20	1	5	2	2	0	8
	48 Hours	1	4	20	1	5	2	2	0	8
	72 Hours	1	3	15	1	5	2	2	0	8
	96 Hours	1	4	20	1	5	2	1	0	6
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
F12930	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	2	1	10	1	5	3	2	2	14
	48 Hours	1	4	20	1	5	3	2	1	12
	72 Hours	1	1	5	0	0	2	1	0	6
	96 Hours	2	1	10	1	5	2	1	0	6
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0

Table 2
Sodium Fluorescein Examination
Group I

Animal Number	Pre-initiation	Observation Period 72 Hours	7 Days	14 Days
F12909	NEG	POS (100%)	NEG	NEG
F12947	NEG	POS (45%)	NEG	NEG
F12942	NEG	POS (30%)	NEG	NEG
F12943	NEG	POS (60%)	NEG	NEG
F12944	NEG	POS (75%)	NEG	NEG
F12930	NEG	POS (20%)	NEG	NEG

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

T-3896

EYE IRRITATION (CONTINUED)

Comments:

Group 1 (unwashed) -

No pain response (vocalization) was elicited from any animal following instillation of the test meterial.

Blanching of the conjunctivae was seen in all six animals at 1 and 24 hours, in four animals at 48 and 72 hours, and in two animals at 96 hours.

Petita hamorrhage of the conjunctivae was exhibited by three animals at 24 hours, by one animal at 48 and 72 hours, and by two animals at 96 hours.

Corneal apithelial peeling was observed in three animals at 1 hour, in one animal at 24 hours, in all six animals at 72 hours, and in four animals at 96 hours.

Corneal neovascularization was seen in two animals at 96 hours, and in one animal at Day 7.

T-3896

EYE IRRITATION (CONTINUED)

Table 3
Individual Eye Irritation Scores
Group II - (washed)

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F12854	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	1	4	20	1	5	3	2	2	14
	48 Hours	1	4	20	1	5	3	2	1	12
	72 Hours	1	1	5	1	5	2	2	1	10
	96 Hours	1	1	5	0	0	2	1	0	6
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
	21 Days	0	0	0	0	0	0	0	0	0
F12915	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	2	1	10	1	5	2	2	2	12
	48 Hours	2	1	10	1	5	2	2	1	10
	72 Hours	1	1	5	0	0	2	2	1	10
	96 Hours	1	2	10	1	5	2	2	1	10
	7 Days	1	1	5	0	0	1	1	0	4
	14 Days	1	1	5	0	0	1	2	1	8
	21 Days	1	1	5	0	0	1	1	0	4
F12916	1 Hour	1	4	20	1	5	2	3	2	14
	24 Hours	2	1	10	0	0	2	2	1	10
	48 Hours	1	1	5	1	5	2	2	1	10
	72 Hours	0	0	0	0	0	1	0	0	2
	96 Hours	0	0	0	0	0	1	0	0	2
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
	21 Days	0	0	0	0	0	0	0	0	0

Table 4
Sodium Fluorescein Examination
Group II

Animal Number	Pre-initiation	Observation Period 72 Hours	7 Days	14 Days	21 Days
F12854	NEG	POS (20%)	NEG	NEG	NEG
F12915	NEG	POS (20%)	POS (10%)	POS (15%)	POS (15%)
F12916	NEG	NEG	NEG	NEG	NEG

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

T-3896

EYE IRRITATION (CONTINUED)

Comments:

Group II (washed) -

No pain response (vocelization) was elicited from any animal following instillation of the test meterial.

Blenching of the conjunctivae was exhibited by all three animals at 1 and 24 hours.

Corneal epithelial peeling was observed in two animals at 24 hours, in two animals at 72 and 96 hours, and in one animal at Days 7, 14 and 21.

References:

1. Environmental Protection Agency, Proposed Guidelines for Pesticide Registration, Federal Register, Vol. 43, No. 173, Section 163.81-2 pp. 37,356-37,357 (August 22, 1978).

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

3. 40 CFR 160.

4. DHEW Publication No. (NIH 85-23 1985) Guide for the Care and Use of Laboratory Animals.

QUALITY ASSURANCE STATEMENT

Primary Eye Irritation Study in Rabbits

Study No. 60405113

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 40 CFR 160.35 (b) (6) (7). It has been found to identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
4/30/86	Process Audit	5/05/86
6/05/86	Report Review	6/10/86

Susan Kramlich
Susan Kramlich
Inspector, Quality Assurance Unit

6-13-86
Date

Primary Eye Irritation Test

Initial Sodium Fluorescein Exam and Animal Body Weights

Test Material: T-38960 HLA No. 60405113

Physicel Description: White cream pH Result: U with Fisher Accumet pH Meter No. NA

Dose: 0.1ml/eye Room No. 161A

Date Animals Received: 3-11-86 Source: HRP

Review of Folder Preparation By: SAM Date: 4-24-86

SPECIES: RABBIT
STRAIN: NEW ZEALAND WHITE

Animal No.	Sex	Initial SF*	Vocalization Following Dosing	Animal Body Weights (g) Initiation	Day 7	Day 14	Day 21
F1- 2909	♀	NEG	N	2975	3180	3392	NA
2947	♀	NEG	N	3030	3236	3340	NA
2942	♂	NEG	N	2784	2883	3017	NA
2943	♂	NEG	N	2763	2885	2990	NA
2944	♂	NEG	N	2879	3008	3136	NA
2930	♂	NEG	N	2609	2759	2900	NA
2854	♂	NEG	N	2357	2519	2685	2692 2660
2915	♀	NEG	N	2897	3006	3158	3198
2916	♀	NEG	N	2685	2881	3148	3200
TECHNICIAN	QH/SG	QH/SG	NP	QH	SAM	QH	QH
DATE 1986	4-23	4-23	4-24	4-23 ① 24	5-1-86	5-8-86	5/15
SCALE USED				Ktron 5228	KTRON 15019	Ktron 5228	Ktron 5228

① entry error QH 4-24-86
② entry error 5-15-86 NP

*Sodium Fluorescein Examination
NEG = Negative
POS = Positive
NA = Not Applicable
U = Unable to determine pH
Y = Yes
N = No

✓ Dosed in the conjunctival sac
NA Dosed directly on the cornea
Time of Dosing 2:10 PM
Technician NP Date 4-24-86
Time of first observation 3:10 PM
Technician NP Date 4-24-86

All animals appeared normal just prior to dosing. Technician: QH Date: 4-24-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 1 hour

Animal No. FI-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	1 S=90%	1	1 S=90%	1 S=80%
Area	4	4	4	4	4	4
Iris	1 I	1 I	1 I	1 I	1 I	1 I
Conjunctivae - Redness	2 B	2 B	2 B	2 B	2 B	2 B
Chemosis	3	3	3	2	3	3
Discharge	2 C	2 C	2 C	3 C	2 C	2 C
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician MP

Date 4-24-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 39.0 SLL 4-27-86 ✓MN 5-19-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 24 hours

Animal No. F1-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	1	1	1	2 J=10%
Area	4	4	4	4	4	1
Iris	1 I	1 I	1 I	1 I	1 I	1 I
Conjunctivae -	AB	B	B	AB	B	AB
Redness	2	2	2	3	2	3
Chemosis	2	2	2	3	2	3
Discharge	0	1 C	2 C	2 D	0	2 D
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician SG

Date 4-25-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 34.7 24 42 96

✓MN 5-19-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 48 hours

Animal No. F1-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	1	1	1	1
Area	4	4	4	4	4	4
Iris	1 I	1 I	1 I	1 I	1 I	1 I
Conjunctivae -	AB		B	B	B	
Redness	2	2	2	3	2	3
Chemosis	2	2	2	3	2	2
Discharge	0	1 C	1	2 D	0	1 D
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician [signature]

Date 4-26-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MH Date: 5-19-86 Eye Irritation Score: 35.7 sk 4-27-86
✓ MH 5-19-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 72 hours

Animal No. F1-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	S=100% / I	S=45% / I	S=30% / I	S=60% / I	S=75% / I	S=20% / I
Area	4	3	2	3	3	1
Iris	/ I	/ I	/ I	/ I	/ I	0
Conjunctivae -	BA		B	B	B	
Redness	2	1	2	2	2	2
Chemosis	1	2	3	2	2	1
Discharge	0	0	0	0	0	0
Sodium Fluorescein Exam	Pos 100%	Pos 45%	Pos 30%	Pos 60%	Pos 75%	Pos 20%

Technician SLH/pp

Date 4/27/86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MM Date: 5-19-86 Eye Irritation Score: 26.7 SLH 4-27-86 ✓MM 5-19-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 96 hours

Animal No. F1-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	J=100% 1	2 N	J=15% 2 N	J=70% 2	J=100% 1	2
Area	4	1	1	3	4	1
Iris	1 I	0	0	1 I	1 I	1 I
Conjunctivae - Redness	2 AB	1	2	3 B	2	2 A
Chemosis	1	0	1	2	1	1
Discharge	0	0	0	0	0	0
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician sam/sc

Date 4-28-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 0.0 24 4796

✓MN 5-19-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 7 days

Animal No. F1-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	IN	0	0	0	0	0
Area	4	0	0	0	0	0
Iris	0	0	0	0	0	0
Conjunctivae - Redness	1	0	0	0	0	0
Chemosis	0	0	0	0	0	0
Discharge	0	0	0	0	0	0
Sodium Fluorescein Exam	NEG	NEG	NEG	NEG	NEG	NEG

Technician SMM

Date 5-1-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MH Date: 5-19-86 Eye Irritation Score: 3.7 5-28-86 ✓MH 5-19-86

(1253A)

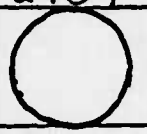
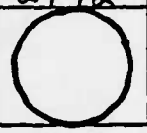
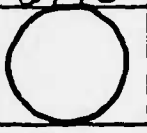
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 1

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 14 DAYS

Animal No. F1-	2909	2947	2942	2943	2944	2930
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	0	0	0	0	0
Area	0	0	0	0	0	0
Iris	0	0	0	0	0	0
Conjunctivae -						
Redness	0	0	0	0	0	0
Chemosis	0	0	0	0	0	0
Discharge	0	0	0	0	0	0
Sodium Fluorescein Exam	NEG	NEG	NEG	NEG	NEG	NEG

Technician JP

Date 5-8-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MH Date: 5-19-86 Eye Irritation Score: 0.0 MH 5-8-86
✓ MH 5-19-86

(4253A)

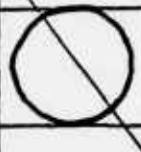
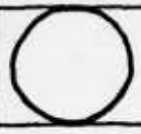
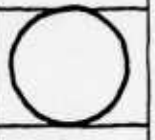
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 1 hour

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	1			
Area	4	4	4			
Iris	1 I	1 I	1 I			
Conjunctivae - Redness	2 B	2 B	2 B			
Chemosis	3	3	3			
Discharge	2 C	2 C	2 C			
Sodium Fluorescein Exam	NA	NA	NA			

Technician MP

Date 4-24-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 29 D&H 4-29-86
✓MN 5-19-86

(4253A)

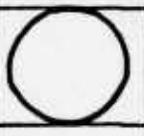
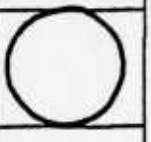
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 24 hours

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head		 J=10%	 J=5%			
Cornea - Opacity	1	2	2			
Area	4	1	1			
Iris	1 I	1 I	0			
Conjunctivae - Redness	3 B ①	2 B ①	2 B ①			
Chemosis	2	2	2			
Discharge	2 C	2 C	1 D			
Sodium Fluorescein Exam	NA	NA	NA			

Technician SG

Date 4-25-86

① ENTRY ERRORS SG 4-25-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MM Date: 5-19-86 Eye Irritation Score: 28.7 SG 4-27-86 ✓MM 5-19-86

(4253A)

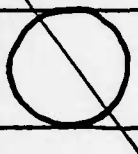
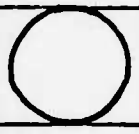
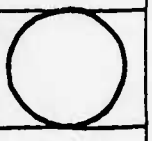
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 48 hours

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	2	1			
Area	4	1	1			
Iris	1 I	1 I	1 I			
Conjunctivae -						
Redness	3	2	2			
Chemosis	2	2	2			
Discharge	1 C	1 C	1 C			
Sodium Fluorescein Exam	NA	NA	NA			

Technician JP

Date ~~4/5~~ 4-26-86

① entry error 4-26 eg JP

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MA Date: 5-19-86 Eye Irritation Score: 27.3 JP 4-27-86
✓ MA 5-19-86

)(4253A)

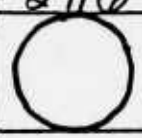
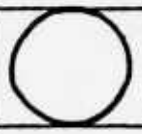
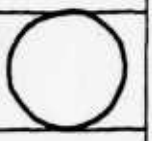
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 72 hours

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	5=20% 1	5=20% 1	0			
Area	1	1	0			
Iris	1 I	0	0			
Conjunctivae - Redness	2	2	1			
Chemosis	2	2	0			
Discharge	1 C	1 C	0			
Sodium Fluorescein Exam	Pos 20%	Pos 20%	Neg.			

Technician slh

Date 4-27-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 12.3 slh 4-27-86 ✓ MN 5-19-86

)4253A)

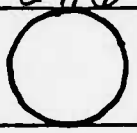
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 96 hours

Animal No. FI-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	0			
Area	1	2	0			
Iris	0	1I	0			
Conjunctivae -						
Redness	2	2	1			
Chemosis	1	2	0			
Discharge	0	1C	0			
Sodium Fluorescein Exam	NA	NA	NA			

Technician SAM/SG

Date 4-28-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MH Date: 5-19-86 Eye Irritation Score: 12.7 MH 4-29-86 ✓ MH 5-19-86

(4253A)

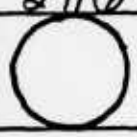
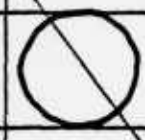
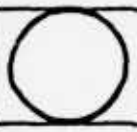
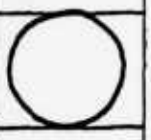
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: DAY 7

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	1 S=10%	0			
Area	0	1	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	.1	0			
Chemosis	0	1	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NEG	POS 10%	NEG			

Technician SAM

Date 5-1-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MM Date: 5-19-86 Eye Irritation Score: 3.0 5-2-86 / MM 5-19-86

(4253A)

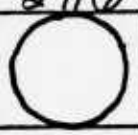
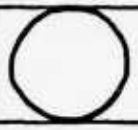
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: DAY 1

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	1 S=15%	0			
Area	0	1	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	1	0			
Chemosis	0	2	0			
Discharge	0	1 D	0			
Sodium Fluorescein Exam	Neg.	POS 15%	Neg			

Technician JP

Date 5-8-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 4.3 dlh 5-8-86 ✓MN 5-19-86

(4253A)

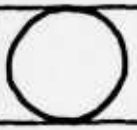
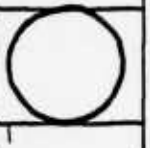
Primary Eye Irritation Test Observations

Test Material: T-38896 HLA No. 60405113

Test Eye: Right Group: 2

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 21 DAYS

Animal No. F1-	2854	2915	2916			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	1 S=15%	0			
Area	0	1	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	1	0			
Chemosis	0	1	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	Neg	Pos 15%	Neg			

Technician GD

Date 5-15-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 5-19-86 Eye Irritation Score: 3.0 GD 5-15-86 ✓ MN 5-19-86

(4253A)

SAMPLE SUBMITTAL FORM

ENCLOSE WITH SAMPLES AND SEND TO:
HAZLETON LABORATORIES AMERICA, INC.
Chemical and BioMedical Sciences Division
3301 KINSMAN BOULEVARD
MADISON, WISCONSIN 53704
(608) 241-4471

Submitted By: F. D. GRIFFITH Date: 4-16-86

Company: 3M TOXICOLOGY SERVICES Invoice To: _____

P. O. Number _____

Type of Report: ___ All tests in one report
✓ One report for each test
20 Number of reports required

Full GLP compliance: ✓ yes ___ no

___ FDA (21 CFR 58)
___ EPA (TSCA - 40 CFR 792)
✓ EPA (FIFRA - 40 CFR 160)
___ OECD

T-3901

Sample Name: T-3755, T-3895, T-3896, T-3897, T-3898, T-3899, T-3900
Physical Description: T-3755- CLEAR ALCOHOLIC SOLUTION; ALL OTHERS - WHITE LOOSE

Storage Requirement: ✓ Room Temp. ___ Refrigerated ___ Other ct.# 2018

Test — Acute Oral Toxicity in Rats

___ TP4207 Internal screen; No. of animals ___M ___F at _____
___ TP3206 FHSA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
✓ TP3013 EPA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
___ TP2069 OECD screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg

Special Instructions: _____

Test — Acute Dermal Toxicity in Rabbits

___ TP3207 FHSA screen; 5M-5F at 2.0 g/kg
✓ TP3016 EPA screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg
___ TP2070 OECD screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg

Special Instructions: _____

Disposal of test material:
___ Return to submitter.
✓ Dispose of according to HLA SOP.

Test — Primary Skin Irritation

___ TP4209 Internal screen; No. of animals _____
No. of sites/rabbit ___ Abraded
___ Intact MMMT 26
___ TP3208 FHSA; 6 rabbits-1 abraded/1 intact site per rabbit
✓ TP3014 EPA; 6 rabbits-1 intact site/rabbit
___ TP2071 OECD; 3 rabbits-1 intact site/rabbit
___ TP4206 DOT Corrosivity; 6 rabbits-1 intact site/rabbit

Special Instructions: _____

Test — Primary Eye Irritation

___ TP4208 Internal screen; No. of animals _____
___ TP3209 FHSA; 6 rabbits unwashed
✓ TP2012 1978 EPA; 6 rabbits unwashed-3 washed
___ TP3015 1982 EPA; 6 rabbits unwashed
___ TP2072 OECD; 3 rabbits unwashed
___ 3 Rabbits washed at 4 sec.
___ 3 Rabbits washed at 30 sec.

Special Instructions: _____

Test — Guinea Pig Sensitization

___ TP2017 EPA Magnusson-Kligman maximization
___ TP2008 EPA Buehler sensitization

Special Instructions: _____

FOR HLA USE
Additional Comments: CONDUCT ACCORDING TO THE ATTACHED PROTOCOL. SG 4-23-86

This form is to be used when submitting a sample for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Toxicology Department at (608)-241-4471 Ext. 304 or the Client Services Center at Ext. 222.

COPY 1 — HAZLETON LABORATORIES • COPY 2 — RETAIN FOR RECORDS

HAZLETON LABORATORIES AMERICA, INC.

3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP2012

Primary Eye Irritation Study in Rabbits (1978 EPA Guidelines)

Study No. 60405113

for

The 3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

April 8, 1986

PROTOCOL TP2012

Primary Eye Irritation Study in Rabbits
(1978 EPA Guidelines)

Study No.	60405113
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	(See sample submittal form)
Sponsor's Representative	F. D. Griffith, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of 4-21-86
Completion Date	Week of 5-12-86
Final Report Date	Week of 6-9-86

SG 4-23-86

PROTOCOL TP2012

1. Study Title	Primary Eye Irritation Study in Rabbits (1978 EPA Guidelines)
2. Objective	To determine the relative level of irritation produced following a single exposure of a test material to one eye of albino rabbits
3. Test Material	
A. Identification	(See sample submittal form)
B. Physical Description	(See sample submittal form)
C. Purity and Stability	The Sponsor assumes responsibility for purity and stability determinations.
D. Storage Conditions	(See sample submittal form)
E. Retention	Any unused test material will be discarded 30 days after issuance of the final report unless directed otherwise by the Sponsor.
F. Safety Precautions	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.
4. Regulatory Compliance	All aspects of this study will conform to the U. S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances[1] and the U. S. Environmental Protection Agency's Good Laboratory Practice Standards.[2,3]
5. Quality Assurance	The conduct of this study and the final report will be audited by the Quality Assurance Unit in accordance with Standard Operating Procedures at Hazleton Laboratories America, Inc. (HLA).

6. Experimental Design

A. Animals

(1)	Species	Rabbit
(2)	Strain/Source	New Zealand White/Hazleton Research Products, Inc.
(3)	Age at initiation	Adult
(4)	Weight at Initiation	2.0 to 3.5 kg
(5)	Number/Sex	Nine/either sex (Group 1 - six animals, Group 2 - three animals)
(6)	Identification	Each animal will be assigned a permanent identification number and will be identified with a metal ear tag. All data collected from an animal will be recorded and filed under its identification number.
(7)	Husbandry	Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[4] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance.
(a)	Housing	The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.
(b)	Food	A measured amount of Purina High Fiber Rabbit Chow® will be provided.
(c)	Water	Water will be provided ad libitum.
(d)	Contaminants	No contaminants are expected to be present in the feed or water which would interfere with and affect the results of the study.
(e)	Environment of animal room	
	o Temperature	21°C ±2°

o Relative humidity	50% ±20%
o Air change	At least 10 changes an hour of filtered 100% outside air
o Light cycle	12 hours light/12 hours dark
(f) Acclimation	At least 7 days
(8) Selection of test animals	The animals will be selected based on health and body weight. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The rabbits' eyes will be examined using fluorescein dye procedures within 24 hours prior to test material administration. Only animals with no sign of corneal injury or eye abnormalities will be utilized.
(9) Justification	Historically, the New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

B. Procedures

(1) Administration of Test Material	Each rabbit will receive 0.1 mL of liquid or 0.1 g (or the weight equivalent of 0.1 mL) of solid test material. If necessary, solid test materials will be finely ground into a dust or powder. The test material will be placed into the everted lower lid of the rabbit's eye. The upper and lower lids are then to be gently held together for 1 second before releasing to prevent loss of material. If an aerosol, the test eye will be held open and the test material administered in a single burst of about 1 second from a distance of 10 cm directly in front of the eye. The eyes of the Group 1 rabbits will remain unflushed for 24 hours following instillation of the test material. After 24 hours, a washout may be used if considered appropriate. The eyes of the Group 2

	animals will be flushed with lukewarm tap water for 1 minute starting 30 seconds after test material instillation. One eye of each animal will be treated with the test material and the other eye will serve as the untreated control.
(2) Reason for Route of Administration	Historically, the route of choice based on the method of Draize.[5]
C. Observation of Animals	Ocular irritation observations and body weights will be recorded in a study notebook.
(1) Reading of Ocular Irritation	The treated eyes of all animals will be examined for ocular irritation at 1, 24, 48, and 72 hours after treatment. If no irritation or injury is present at 72 hours, the study will be terminated. If irritation is present at 72 hours, additional observations will be made at 96 hours and at 7, 14, and 21 days. If at any of these time points there is no irritation within a group, that group will be terminated. If injury is still present at 21 days, the Sponsor will be contacted to determine whether the study should continue or be terminated. After recording the 24-hour observations, sodium fluorescein may be used to aid in revealing possible corneal injury. Irritation will be graded and scored using the Draize technique (Attachment 1).[5] All eye abnormalities will be recorded. All animals that have a damaged eye producing undue stress or discomfort will be sacrificed for humane reasons after consulting with the Sponsor.
(2) Body Weights	Body weights will be recorded prior to test material administration and at weekly intervals throughout the study.
(3) Clinical Observations	Any abnormal clinical signs will be recorded in the study folder.
D. Pathology	All animals, whether dying on test or sacrificed at study termination, will be discarded.

7. Report

At termination of the study, a report which includes the following information will be prepared and submitted:

- A description of the test material
- A description of the test system
- Dates of study initiation and termination
- A summary table showing the irritation data at each observation period
- Any special observations that were recorded

8. Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin.

REFERENCES

1. Environmental Protection Agency, Proposed Guidelines for Pesticide Registration, Federal Register, Vol. 43, No. 173, Section 163.81-2, pp. 37, 356-37, 357 (August 22, 1978).

2. 40 CFR 160.

3. 40 CFR 792.

4. DHEW Publications No. (NIH) 78-23 (1978).

5. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

APPLICABLE HLA STANDARD OPERATING PROCEDURES

OP-TOX 3	Primary Eye Irritation Study (OECD/1982 EPA Guidelines)
OP-TOX 55	Quality Assurance Inspections of the Acute Toxicology Department
OP-GENB 36	Animal Arrival, Observations, and Release from Acclimation
OP-GENB 24	Unique Identification of Laboratory Animals and Their Cages and Identification Numbers for Medical Department Test Subjects
OP-TARC 230	Monitoring, Recording, and Reporting of Animal Room Environmental Conditions
OP-GEN 33	Archiving of Data

PROTOCOL APPROVAL

F. D. Griffith, PhD
Sponsor's Representative
The 3M Company

4-15-86
Date

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

4-8-86
Date

(13033/emm)

PROTOCOL - ATTACHMENT 1

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity ——— 0
Scattered or diffuse area, details of iris clearly visible ——— 1
Easily discernible translucent areas, details of iris slightly obscured ——— 2
Opalescent areas, no details of iris visible, size of pupil barely discernible ——— 3
Opaque, iris invisible ——— 4

(B) Area of cornea involved

One quarter (or less), but not zero ——— 1
Greater than one quarter, but less than half ——— 2
Greater than half, but less than three quarters ——— 3
Greater than three quarters, up to whole area ——— 4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values

Normal ——— 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) ——— 1
No reaction to light, hemorrhage, gross destruction (any or all of these) ——— 2

A x 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral conjunctivae only)

Vessels normal ——— 0
Vessels definitely injected above normal ——— 1
More diffuse, deeper crimson red, individual vessels not easily discernible ——— 2
Diffuse beefy red ——— 3

(B) Chemosis

No swelling ——— 0
Any swelling above normal (includes slight swelling of fold) ——— 1
Obvious swelling with partial eversion of lids ——— 2
Swelling with lids about half closed ——— 3
Swelling with lids about half closed to completely closed ——— 4

(C) Discharge

No discharge ——— 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) ——— 1
Discharge with moistening of the lids and hairs just adjacent to lids ——— 2
Discharge with moistening of the lids and hairs, and considerable area around the eye ——— 3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

HAZLETON LABORATORIES AMERICA, INC
Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

F. D. GRIFFITH, PH.D.
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 60801575
SAMPLE ENTERED: 08/11/86
REPORT PRINTED: 09/22/86

SAMPLE: T-3896

PURCHASE ORDER NUMBER: T757575 REL. #

ENCLOSED: PRIMARY EYE IRRITATION STUDY IN RABBITS - METHOD, SUMMARY
QAU STATEMENT
RAW DATA/PROTOCOL APPENDIX

SIGNED: Steven M. Glaza ..... 9-23-86 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

SAMPLE: T-3896

EYE IRRITATION

Objective: To determine the level of ocular irritation produced following a single exposure of a test substance to one eye of albino rabbits according to the U.S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances.

Test Material: T-3896
Physical Description: White cream
Purity and Stability: Sponsor assumes responsibility for purity and stability determinations.

Test Animal: Young adult rabbits of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to water ad libitum and a measured amount of Purina High Fiber Rabbit Chow, and held for an acclimation period of at least 7 days. If variations from the prescribed environmental conditions existed, they were documented and were considered not to have an adverse effect on the study outcome.

Three acclimated animals, weighing from 2236 to 2401 g, were chosen at random for the test. The animals' eyes were examined approximately 24 hours before test material administration using sodium fluorescein dye procedures. Only those animals with no sign of ocular injury or irritation were used on the test. Test animals were identified by animal number and corresponding ear tag.

Reason for Species Selection: The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Preparation of Test Material: The sample was dosed as received. The pH was not determined.

Treatment: Each rabbit received 0.1 ml of the liquid test material placed on the everted lower lid of one eye, with the contralateral eye serving as the untreated control. The upper and lower lids were held together for one second to prevent loss of material and then released. The treated eyes of the rabbits were flushed for one minute with lukewarm tap water, starting 30 seconds after test material instillation.

Reason for Route of Administration: Historically, this is the route of choice based on the method of Draize.

SAMPLE: T-3896

EYE IRRITATION (CONTINUED)

Observations: The treated eyes were observed for ocular irritation at 1, 24, 48, 72 and 96 hours, and at 7 and 14 days after treatment.

At the 72-hour, and 7 and 14-day readings, sodium fluorescein was used to aid in revealing possible corneal injury. Irritation was graded and scored according to the Draize technique.

Animals were weighed just prior to test material administration, at 7 days and at study termination.

Pathology: At study termination all animals were euthanatized and discarded.

SAMPLE: T-3896

EYE IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 08/05/86

Temperature and Humidity of Animal Room: 21 to 26 Degrees C.;
40 to 66% Relative Humidity

Date Test Started: 08/15/86 Date Test Completed: 08/29/86

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	3 Rabbit Mean 0.1 ml (Washed)
1 Hour:	45.0
24 Hours:	28.0
48 Hours:	41.3
72 Hours:	37.0
96 Hours:	34.3
7 Days:	1.7
14 Days:	0.0

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals (3) at each observation period.

SAMPLE NUMBER: 60801575

SAMPLE: T-3896

EYE IRRITATION (CONTINUED)

Table 1
Individual Eye Irritation Scores

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F14606	1 Hour	1	4	20	1	5	3	4	3	20
	24 Hours	3	1	15	0	0	2	1	0	6
	48 Hours	1	4	20	1	5	2	2	2	12
	72 Hours	1	4	20	1	5	2	2	1	10
	96 Hours	1	4	20	1	5	2	1	0	6
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
F14607	1 Hour	1	4	20	1	5	3	4	3	20
	24 Hours	1	4	20	0	0	2	2	0	8
	48 Hours	1	3	15	1	5	2	1	1	8
	72 Hours	2	1	10	1	5	2	1	0	6
	96 Hours	2	1	10	1	5	2	1	0	6
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
F14608	1 Hour	1	4	20	1	5	3	4	3	20
	24 Hours	1	4	20	1	5	2	1	2	10
	48 Hours	4	2	40	1	5	2	2	3	14
	72 Hours	2	4	40	1	5	2	2	1	10
	96 Hours	2	4	40	1	5	2	1	0	6
	7 Days	1	1	5	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0

Table 2
Sodium Fluorescein Examination

Animal Number	Observation Period Pre-initiation	72 Hours	7 Days	14 Days
F14606	NEG	POS (100%)	NEG	NEG
F14607	NEG	POS (15%)	NEG	NEG
F14608	NEG	POS (100%)	NEG	NEG

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

SAMPLE NUMBER: 60801575

SAMPLE: T-3896

EYE IRRITATION (CONTINUED)

Comments:

No pain response (vocalization) was elicited from any animal following instillation of the test material.

Blanching of the conjunctivae was exhibited by all three animals at 1 and 24 hours and in one animal at 48, 72 and 96 hours.

Petite hemorrhaging the conjunctivae was seen in one animal at 48 hours, in two animals at 72 hours and in all three animals at 96 hours.

Corneal epithelial peeling was observed in one animal at 24 hours, in all three animals at 72 hours and in one animal at 96 hours.

References:

1. Environmental Protection Agency, Proposed Guidelines for Pesticide Registration, Federal Register, Vol. 43, No. 173, Section, 163.81-2 pp. 37, 356-37, 357 (August 22, 1978).

2. Draize J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the United States, pp. 46-59 (1975).

3. 40 CFR 160.

4. DHHS Publication No. (NIH 85-23 1985) Guide for the Care and Use of Laboratory Animals.

QUALITY ASSURANCE STATEMENT

Primary Eye Irritation Study in Rabbits

Study No. 60801575

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 40 CFR 160.35 (b) (6) (7). It has been found to identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
7/24-25/86	Process Audit	7/28/86
9/17/86	Report Review	9/19/86

Susan Kramlich
Susan Kramlich
Inspector, Quality Assurance Unit

9-22-86
Date

Primary Eye Irritation Test

Initial Sodium Fluorescein Exam and Animal Body Weights

Test Material: T-3896 HLA No. 60801575

Physical Description: White Cream pH Result: NA with Fisher Accumet pH Meter No. NA

Dose: 0.1 ml/eye Room No. 161C, ③ 161B

Date Animals Received: 8-5-86 Source: HRP

Review of Folder Preparation By: GMK Date: 8-15-86

Species/Strain: NZW Rabbit

Animal No.	Sex	Initial SF*	Vocalization Following Dosing	Animal Body Weights (g) Initiation	Day 7	Day 14	Day 21
F1-4606	♀	Neg	N	2236	2402	2417	
4607	♀	Neg	N	2242	2390	2498	
4608	♂	Neg	N	2401	2589	2596	
TECHNICIAN	GS	~~GS~~ NP	NP	GS	SAM	SG	
DATE 19R	8-13 2nd ①	~~8-14~~ 8-15	8-15	8-15 2nd ①	8-22	8-29	
SCALE USED			K-TRON	15019	15019	5228	

*Sodium Fluorescein Examination
NEG = Negative
POS = Positive
NA = Not Applicable
U = Unable to determine pH
Y = Yes
N = No

✓ Dosed in the conjunctival sac the upper and lower lids were gently held together for one second NP 9-12-86
NA Dosed directly on the cornea
Time of Dosing 11:55 AM
Technician NP Date 8-15-86
Time of first observation 12:55 PM
Technician MM Date 8-15-86

All animals appeared normal just prior to dosing. Technician: NP Date: 8-15-86

(4253A)

① Entry error GS 8-15-86

② Entry error GS 9-2-86

③ THE ANIMALS WERE MOVED FROM ROOM 161C TO ROOM 161B ON 8-26-86. 9-9-86 sh

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 1 hour

Animal No. F1-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	1			
Area	4	4	4			
Iris	1 I	1I	1 I			
Conjunctivae -						
Redness	3 B	3 B	3 B			
Chemosis	4	4	4			
Discharge	3 D	3 D	3 D			
Sodium Fluorescein Exam	NA	NA	NA			

Technician MN/MP

Date ① ~~X~~ 8-15-86

① entry error ~~8-15-86~~ ② MN

② write-over error 8-15-86 MN

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: ③ MN 9-2-86 Date: ③ ~~MN~~ 9-2-86 Eye Irritation Score: 45.0 slw 8-18-86 ✓MN 9-2-86

(4253A)

③ entry errors 9-2-86 MN

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 24 hours

Animal No. F1-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	① 3 A J-10%	1	1			
Area	1	4	4			
Iris	0	0	1 I			
Conjunctivae - Redness	2 B	2 B	2 B			
Chemosis	1	2	1			
Discharge	0	0	2 C			
Sodium Fluorescein Exam	NA	NA	NA			

Technician MN

Date 8-11-86

① entry error 8-16-86 MN

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 9-2-86 Eye Irritation Score: 28.0 8-18-86 ✓MN 9-2-86

(4253A)

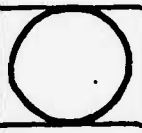
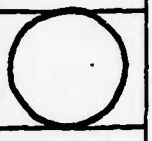
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 48 hours

Animal No. F/-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	1	4			
Area	4	3	2			
Iris	1 I	1 I	1 I			
Conjunctivae -						
Redness	2	2	2 A B			
Chemosis	2	1	2			
Discharge	2 C	1 C	3 C			
Sodium Fluorescein Exam	NA	NA	NA			

Technician MN

Date 9-17-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 9-2-86 Eye Irritation Score: 41.3 sdw 8-18-86 ✓MN 8 9-2-86 ①

(4253A) ①entry error 9-2-86 MN

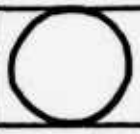
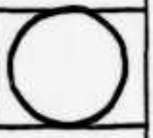
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 72 hours

Animal No. F1-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	J=100% 1	J=15% 2	J=100% 2			
Area	4	1	4			
Iris	1 I	1 I	1 I			
Conjunctivae -						
Redness	2 A	2	2 AB			
Chemosis	2	1	2			
Discharge	1 C	0	1 D			
Sodium Fluorescein Exam	POS 100%	POS 15%	POS 100%			

Technician MM/MP

Date 8-18-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MM Date: 9-2-86 Eye Irritation Score: 37.0 sm 8-18-86 / MM 9-2-86

(4253A)

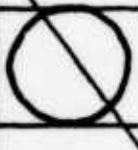
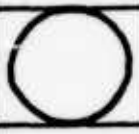
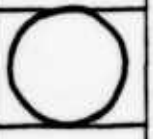
Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 96 hours

Animal No. F1-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	1	2 J45%	2			
Area	4	1	4			
Iris	1I	1I	1I			
Conjunctivae -						
Redness	2A	2A	2AB			
Chemosis	~~2~~ 1 ①	1	1			
Discharge	0	0	0			
Sodium Fluorescein Exam	NA	NA	NA			

Technician sam ① Recording error 8-19-86 sam

Date 8-19-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: mm Date: 9-2-86 Eye Irritation Score: 3 1 3 1 4 8-20-86
✓ mm 9-2-86

(4253A).

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 7 days

Animal No. F1-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head	○	○	◑	○	○	○
Cornea - Opacity	0	0	1			
Area	0	0	1			
Iris	0	0	0			
Conjunctivae -						
Redness	0	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NEG	NEG	NEG			

Technician Sam

Date 8-22-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MN Date: 9-2-86 Eye Irritation Score: 1 8-25-86

✓ MN 9-2-86

(4253A)

Primary Eye Irritation Test Observations

Test Material: T-3896 HLA No. 60801575

Test Eye: Right Group: NA

✓ Washed: 30 seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for 60 seconds. NA Unwashed

OBSERVATION PERIOD: 14 DAYS

Animal No. F1-	4606	4607	4608			
Location of Corneal Lesions Tail ------ Head	◯	◯	◯	◯	◯	◯
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NEG	NEG	NEG			

Technician SG

Date 8-29-86

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

✓ 9-9-86

Reviewed By: MH Date: 9-2-86 Eye Irritation Score: 0 MH 9-2-86

(4253A)

60801575

SAMPLE SUBMITTAL FORM

ENCLOSE WITH SAMPLES AND SEND TO:
HAZLETON LABORATORIES AMERICA, INC.
Chemical and BioMedical Sciences Division
3301 KINSMAN BOULEVARD
MADISON, WISCONSIN 53704
(608) 241-4471

Submitted By: E.D. GRIFFITH Date: 7.6.76

Company: 3M '2 Invoice To: _____

P. O. Number _____

Type of Report: ✓ All tests in one report
_____ One report for each test
_____ Number of reports required

Full GLP compliance: ✓ yes
_____ no

_____ FDA (21 CFR 58)
_____ EPA (TSCA - 40 CFR 792)
✓ EPA (FIFRA - 40 CFR 160)
_____ OECD

Sample Name: T-3896
Physical Description: white liquor

Storage Requirement: ✓ Room Temp. _____ Refrigerated _____ Other _____

Test — Acute Oral Toxicity in Rats

_____ TP4207 Internal screen; No. of animals ____M ____F at _____
_____ TP3206 FHSA screen; 5M-5F at 5.0 g/kg
_____ Conduct defined study if death occurs at 5.0 g/kg
_____ TP3013 EPA screen; 5M-5F at 5.0 g/kg
_____ Conduct defined study if death occurs at 5.0 g/kg
_____ TP2069 OECD screen; 5M-5F at 5.0 g/kg
_____ Conduct defined study if death occurs at 5.0 g/kg

Special Instructions: _____

Test — Acute Dermal Toxicity in Rabbits

_____ TP3207 FHSA screen; 5M-5F at 2.0 g/kg
_____ TP3016 EPA screen; 5M-5F at 2.0 g/kg
_____ Conduct defined study if death occurs at 2.0 g/kg
_____ TP2070 OECD screen; 5M-5F at 2.0 g/kg
_____ Conduct defined study if death occurs at 2.0 g/kg

Special Instructions: _____

Disposal of test material:
✓ Return to submitter.
_____ Dispose of according to HLA SOP.

Test — Primary Skin Irritation

_____ TP4209 Internal screen; No. of animals _____
No. of sites/rabbit _____ Abraded
_____ Intact
_____ TP3208 FHSA; 6 rabbits-1 abraded/1 intact site per rabbit
_____ TP3014 EPA; 6 rabbits-1 intact site/rabbit
_____ TP2071 OECD; 3 rabbits-1 intact site/rabbit
_____ TP4206 DOT Corrosivity; 6 rabbits-1 intact site/rabbit

Special Instructions: _____

Test — Primary Eye Irritation

_____ TP4208 Internal screen; No. of animals _____
_____ TP3209 FHSA; 6 rabbits unwashed
✓ TP2012 1978 EPA; 6 rabbits unwashed-3 washed
_____ TP3015 1982 EPA; 6 rabbits unwashed
_____ TP2072 OECD; 3 rabbits unwashed
_____ 3 Rabbits washed at 4 sec.
_____ 3 Rabbits washed at 30 sec.

Special Instructions: 3 washed eyes first the 6 unwashed

Test — Guinea Pig Sensitization

_____ TP2017 EPA Magnusson-Kligman maximization
_____ TP2008 EPA Buehler sensitization

Special Instructions: _____

FOR HLA USE

Additional Comments: CONDUCT ACCORDING TO THE ATTACHED PROTOCOL.
NOTE: RUN 3 RABBIT WITH WASHED PROCEDURE ONLY.
SL 3.13.80

This form is to be used when submitting a sample for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Toxicology Department at (608)-241-4471 Ext. 304 or the Client Services Center at Ext. 222.

COPY 1 — HAZLETON LABORATORIES • COPY 2 — RETAIN FOR RECORDS

HAZLETON LABORATORIES AMERICA, INC.

3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP2012

Primary Eye Irritation Study in Rabbits (1978 EPA Guidelines)

Study No. 60801575

for

The 3M Company
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

April 8, 1986

PROTOCOL TP2012

Primary Eye Irritation Study in Rabbits
(1978 EPA Guidelines)

Study No.	60801575
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	(See sample submittal form)
Sponsor's Representative	F. D. Griffith, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of 8-11-86
Completion Date	Week of 9-1-86
Final Report Date	Week of 9-22-86 SG 8-13-86

PROTOCOL TP2012

1.	Study Title	Primary Eye Irritation Study in Rabbits (1978 EPA Guidelines)
2.	Objective	To determine the relative level of irritation produced following a single exposure of a test material to one eye of albino rabbits
3.	Test Material	
	A. Identification	(See sample submittal form)
	B. Physical Description	(See sample submittal form)
	C. Purity and Stability	The Sponsor assumes responsibility for purity and stability determinations.
	D. Storage Conditions	(See sample submittal form)
	E. Retention	Any unused test material will be discarded 30 days after issuance of the final report unless directed otherwise by the Sponsor.
	F. Safety Precautions	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.
4.	Regulatory Compliance	All aspects of this study will conform to the U. S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances[1] and the U. S. Environmental Protection Agency's Good Laboratory Practice Standards.[2,3]
5.	Quality Assurance	The conduct of this study and the final report will be audited by the Quality Assurance Unit in accordance with Standard Operating Procedures at Hazleton Laboratories America, Inc. (HLA).

6. Experimental Design

A. Animals

(1)	Species	Rabbit
(2)	Strain/Source	New Zealand White/~~Hazleton~~ Research Products, Inc.
(3)	Age at initiation	Adult
(4)	Weight at Initiation	2.0 to 3.5 kg
(5)	Number/Sex	~~Nine~~ THREE/either sex ~~(Group 1 — six animals~~, Group 2 1 - three animals) PROTOCOL REVISIONS SW 8-13-86
(6)	Identification	Each animal will be assigned a permanent identification number and will be identified with a metal ear tag. All data collected from an animal will be recorded and filed under its identification number.
(7)	Husbandry	Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[4] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance.
	(a) Housing	The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.
	(b) Food	A measured amount of Purina High Fiber Rabbit Chow® will be provided.
	(c) Water	Water will be provided ad libitum.
	(d) Contaminants	No contaminants are expected to be present in the feed or water which would interfere with and affect the results of the study.
	(e) Environment of animal room	
	o Temperature	21°C ±2°

o Relative humidity	50% ±20%
o Air change	At least 10 changes an hour of filtered 100% outside air
o Light cycle	12 hours light/12 hours dark
(f) Acclimation	At least 7 days
(8) Selection of test animals	The animals will be selected based on health and body weight. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The rabbits' eyes will be examined using fluorescein dye procedures within 24 hours prior to test material administration. Only animals with no sign of corneal injury or eye abnormalities will be utilized.
(9) Justification	Historically, the New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

B. Procedures

(1) Administration of Test Material	Each rabbit will receive 0.1 mL of liquid or 0.1 g (or the weight equivalent of 0.1 mL) of solid test material. If necessary, solid test materials will be finely ground into a dust or powder. The test material will be placed into the everted lower lid of the rabbit's eye. The upper and lower lids are then to be gently held together for 1 second before releasing to prevent loss of material. If an aerosol, the test eye will be held open and the test material administered in a single burst of about 1 second from a distance of 10 cm directly in front of the eye. ~~The eyes of the Group 1 rabbits will remain unflushed for 24 hours following instillation of the test material. After 24 hours, a washout may be used if considered appropriate.~~ The eyes of the Group 8.1

PROTOCOL REVISIONS SG 8-13-86

	animals will be flushed with lukewarm tap water for 1 minute starting 30 seconds after test material instillation. One eye of each animal will be treated with the test material and the other eye will serve as the untreated control.
(2) Reason for Route of Administration	Historically, the route of choice based on the method of Draize.[5]
C. Observation of Animals	Ocular irritation observations and body weights will be recorded in a study notebook.
(1) Reading of Ocular Irritation	The treated eyes of all animals will be examined for ocular irritation at 1, 24, 48, and 72 hours after treatment. If no irritation or injury is present at 72 hours, the study will be terminated. If irritation is present at 72 hours, additional observations will be made at 96 hours and at 7, 14, and 21 days. If at any of these time points there is no irritation within a group, that group will be terminated. If injury is still present at 21 days, the Sponsor will be contacted to determine whether the study should continue or be terminated. After recording the 24-hour observations, sodium fluorescein may be used to aid in revealing possible corneal injury. Irritation will be graded and scored using the Draize technique (Attachment 1).[5] All eye abnormalities will be recorded. All animals that have a damaged eye producing undue stress or discomfort will be sacrificed for humane reasons after consulting with the Sponsor.
(2) Body Weights	Body weights will be recorded prior to test material administration and at weekly intervals throughout the study.
(3) Clinical Observations	Any abnormal clinical signs will be recorded in the study folder.
D. Pathology	All animals, whether dying on test or sacrificed at study termination, will be discarded.

7. Report

At termination of the study, a report which includes the following information will be prepared and submitted:

- A description of the test material
- A description of the test system
- Dates of study initiation and termination
- A summary table showing the irritation data at each observation period
- Any special observations that were recorded

8. Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin.

REFERENCES

1. Environmental Protection Agency, Proposed Guidelines for Pesticide Registration, Federal Register, Vol. 43, No. 173, Section 163.81-2, pp. 37, 356-37, 357 (August 22, 1978).

2. 40 CFR 160.

3. 40 CFR 792.

4. ~~DHEW~~ DHHS Publications No. (NIH) ~~78-23~~ 85-23 (~~1978~~) 1985. REFERENCE UPDATE SG 8-13-86

5. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

APPLICABLE HLA STANDARD OPERATING PROCEDURES

OP-TOX 3	Primary Eye Irritation Study (OECD/1982 EPA Guidelines)
OP-TOX 55	Quality Assurance Inspections of the Acute Toxicology Department
OP-GENB 36	Animal Arrival, Observations, and Release from Acclimation
OP-GENB 24	Unique Identification of Laboratory Animals and Their Cages and Identification Numbers for Medical Department Test Subjects
OP-TARC 23C	Monitoring, Recording, and Reporting of Animal Room Environmental Conditions
OP-GEN 33	Archiving of Data

PROTOCOL APPROVAL

F. D. Griffith, PhD
Sponsor's Representative
The 3M Company

7-15-86
Date

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

4-8-86
Date

(13033/emm)

PROTOCOL - ATTACHMENT 1

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(B) Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral conjunctivae only)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

MODIFIED DRAIZE SKIN SENSITIZATION STUDY

STUDY #HIM 86-3M-D-1
3M

PURPOSE: To evaluate for irritation and sensitization in a repeat insult patch test on human subjects, the test materials listed below.
The method is that of Draize.

TEST MATERIALS: Test and control articles, as indicated, are furnished by the sponsor. They are identified:

T-3896

The sponsor assumes responsibility for any necessary evaluations for purity, strength, and stability.

STORAGE CONDITIONS: Room Temperature (68-72° F)

PREPARATION FOR DOSING: as is

SPONSOR: 3M, St. Paul, MN 55144

TESTING FACILITY: Howard I. Maibach, M.D.
San Francisco, CA 94143

PROPOSED STARTING DATE: 4-28-86

COMPLETION DATE: 6-13-86

SUBJECTS: Approx. 220 adult subjects (over 18 years of age) who, prior to commencement of the study, were examined and deemed to be free of any active skin pathology. Medical histories and consent forms are obtained from all subjects.

STUDY MONITOR: Dr. Frank Griffith

METHODS: The study is performed by modification of the procedure set forth by Draize.* The test patches are moistened with approximately 0.2 gm of the test material and adequately secured to the skin by means of occlusive bandage (Blenderm tape). The pad is Webril.

Patches of the test materials are applied to the upper arms or backs of all panelists. The samples are applied to the patches shortly before application to the panelists' skin.

The study is performed in approximately a six-week period for each subject. During the first three weeks, or the induction period, patches are applied thrice weekly for 48-72 hours. The panelists are instructed to leave the patches on and keep them dry following each application.

All applications of samples are made to the same site (unless severe reactions make this inadvisable. In these cases, applications would be made to a previously unpatched adjacent site. If strong reactions reoccur on these fresh sites, the sample will be omitted until challenge application.

Should an unusually high percentage of panelists exhibit high degrees of irritation to any of the test materials during the induction period, the study monitor will be notified as soon as possible. Recommendations, if any, will be made at that time.)

Approximately two weeks after the sensitization phase, the challenge or elicitation applications are made. The patch is applied to a previously unpatched site. The challenge patches are removed 72 hours following applications. Reactions to the challenge applications are scored at 96 hours following applications.

The scoring scale employed for all evaluations is as follows:

I = minimal glazing, such as in the "peau d'orange"

0 = negative

± = equivocal reaction

1 = erythema

2 = erythema and induration

3 = erythema, induration and vesicles

4 = erythema, induration and bullae

REPORT: The report will include incidence and severity of sensitization.

NOTE: This study is run according to the anticipated principals of GCP. If after the study is underway it becomes necessary to make changes in the approved protocol, the revisions and reasons for change will be documented.

DATA RETENTION: The raw data and the original of the final report will be an file at the laboratory for not less than two years. Unused test articles will be returned to sponsor unless otherwise requested.

REFERENCE: *Marzulli, F. and Maibach, H. CONTACT ALLERGY: PREDICTIVE TESTING IN HUMANS.
Advances in Modern Toxicology 4:353-372,1977.

RESULTS: These are attached.

COMMENT: There was evidence of cumulative irritancy. One subject had an equivocal retest (#69); although this morphologically appeared to be irritant, he had a provocative use test performed. This consists of two applications per day to the cubitol fasse - for seven days. This was negative.

Thus there was no evidence of induction of allergic contact dermatitis.

JDS 10-16-86
Date of Sponsor Approval

Study Director — October 7, 1986 DATE

STUDY:HIM 86-3M-D-1

DATE:4/28/86-6/13/86

DRAIZE TEST

SITE DESCRIPTION

3 T-3896

LABORATORY TEST SHEET - CODE

H - HEALING
T - TAPE REACTION
M - MISSED MEDICATION
∂ - TAPE MOVED
G - GLAZE
D - DISCONTINUED

RACIAL CODE

A = ASIAN
B = BLACK
C = CAUCASIAN
O = OTHER

DR. MAIBACH UCSF

SUMMARY OF RESPONSE TO SITE#3 DESCR:3 T-3896

	INDUCTION									ELICITATION		1ST RETEST		2ND RETEST	
!	1	2	3	4	5	6	7.	8	9!	72HR!	96HR!	24HR!	48HR!	24HR!	48HR!
0!	210	178	164	144	127	121	111	102	102!	176 !	205 !	0 !		1 !	
+!	4	33	43	63	80	85	95	104	104!	31 !	2 !	2 !		1 !	
1!	0	0	0	0	0	1	1	1	1!	0 !	0 !	0 !		0 !	
2!	0	0	0	0	0	0	0	0	0!	0 !	0 !	0 !		0 !	
3!	0	0	0	0	0	0	0	0	0!	0 !	0 !	0 !		0 !	
4!	0	0	0	0	0	0	0	0	0!	0 !	0 !	0 !		0 !	
TTL!	214	211	207	207	207	207	207	207	207!	207 !	207 !	2 !		2 !	

(NOTE: SUBJECT SCORES OF G = 0 AND D = 2)

NUMBER OF SUBJECTS STARTING STUDY: 220

NUMBER OF SUBJECT NOT COMPLETING ALL PHASES: 13

SUMMARY/INTERPRETATION:

H.I. MAIBACH, M.D.
STUDY DIRECTOR

MODIFIED DRAIZE SKIN SENSITIZATION STUDY

STUDY #HIM 86-3M-D-1
3M

PURPOSE:	To evaluate for irritation and sensitization in a repeat insult patch test on human subjects, the test materials listed below. The method is that of Draize.
TEST MATERIALS:	Test and control articles, as indicated, are furnished by the sponsor. They are identified: T-3755 The sponsor assumes responsibility for any necessary evaluations for purity, strength, and stability.
STORAGE CONDITIONS:	Room Temperature (68-72° F)
PREPARATION FOR DOSING:	as is
SPONSOR:	3M, St. Paul, MN 55144
TESTING FACILITY:	Howard I. Maibach, M.D. San Francisco, CA 94143
PROPOSED STARTING DATE:	4-28-86
COMPLETION DATE:	6-13-86
SUBJECTS:	Approx. 220 adult subjects (over 18 years of age) who, prior to commencement of the study, were examined and deemed to be free of any active skin pathology. Medical histories and consent forms are obtained from all subjects.
STUDY MONITOR:	Dr. Frank Griffith
METHODS:	The study is performed by modification of the procedure set forth by Draize.* The test patches are moistened with approximately 0.2 gm of the test material and adequately secured to the skin by means of occlusive bandage (Blenderm tape). The pad is Webril.

Patches of the test materials are applied to the upper arms or backs of all panelists. The samples are applied to the patches shortly before application to the panelists' skin.

The study is performed in approximately a six-week period for each subject. During the first three weeks, or the induction period, patches are applied thrice weekly for 48-72 hours. The panelists are instructed to leave the patches on and keep them dry following each application.

All applications of samples are made to the same site (unless severe reactions make this inadvisable. In these cases, applications would be made to a previously unpatched adjacent site. If strong reactions reoccur on these fresh sites, the sample will be omitted until challenge application.

Should an unusually high percentage of panelists exhibit high degrees of irritation to any of the test materials during the induction period, the study monitor will be notified as soon as possible. Recommendations, if any, will be made at that time.)

Approximately two weeks after the sensitization phase, the challenge or elicitation applications are made. The patch is applied to a previously unpatched site. The challenge patches are removed 72 hours following applications. Reactions to the challenge applications are scored at 96 hours following applications.

The scoring scale employed for all evaluations is as follows:

I = minimal glazing, such as in the "peau d'orange"

0 = negative

$\pm$ = equivocal reaction

1 = erythema

2 = erythema and induration

3 = erythema, induration and vesicles

4 = erythema, induration and bullae

MODIFIED DRAIZE SKIN SENSITIZATION STUDY
continued. . . p.3

REPORT: The report will include incidence and severity of sensitization.

NOTE: This study is run according to the anticipated principals of GCP. If after the study is underway it becomes necessary to make changes in the approved protocol, the revisions and reasons for change will be documented.

DATA RETENTION: The raw data and the original of the final report will be on file at the laboratory for not less than two years. Unused test articles will be returned to sponsor unless otherwise requested.

REFERENCE: *Marzulli, F. and Maibach, H. CONTACT ALLERGY: PREDICTIVE TESTING IN HUMANS.
Advances in Modern Toxicology 4:353-372,1977.

RESULTS: These are attached.

COMMENT: There was evidence of cumulative irritancy. One subject had an equivocal retest (#69); although this morphologically appeared to be irritant, he had a provocative use test performed. This consists of two applications per day to the cubital fossa - for seven days. This was negative.

Thus there was no evidence of induction of allergic contact dermatitis.

10-16-86
Date of Sponsor Approval

October 7, 1986
Study Director **DATE**

STUDY:HIM 86-3M-D-1

DATE:4/28/86-6/13/86

DRAIZE TEST

SITE DESCRIPTION
---
1 T-3755

LABORATORY TEST SHEET - CODE

H - HEALING
T - TAPE REACTION
M - MISSED MEDICATION
Ø - TAPE MOVED
G - GLAZE
D - DISCONTINUED

RACIAL CODE

A = ASIAN
B = BLACK
C = CAUCASIAN
O = OTHER

DR. MAIBACH UCSF

SUMMARY OF RESPONSE TO SITE#1 DESCR:1 T-3755

	INDUCTION									ELICITATION		1ST RETEST		2ND RETEST	
!	1	2	3	4	5	6	7	8	9!	72HR!	96HR!	24HR!	48HR!	24HR!	48HR!
0!	207	162	147	126	97	93	86	83	82!	175 !	204 !		1 !	2 !	
+!	7	47	55	70	91	90	93	94	95!	29 !	1 !		1 !	1 !	
1!	0	2	5	11	18	21	25	25	25!	3 !	1 !	1 !	0 !		
2!	0	0	0	0	1	3	3	5	5!	0 !	1 !		0 !	0 !	
3!	0	0	0	0	0	0	0	0	0!	0 !	0 !		0 !	0 !	
4!	0	0	0	0	0	0	0	0	0!	0 !	0 !		0 !	0 !	
TL!	214	211	207	207	207	207	207	207	207!	207 !	207 !		3 !	3 !	

(NOTE: SUBJECT SCORES OF G = 0 AND D = 2)

NUMBER OF SUBJECTS STARTING STUDY: 220

NUMBER OF SUBJECT NOT COMPLETING ALL PHASES: 13

SUMMARY/INTERPRETATION:

H.I. MAIBACH, M.D.
STUDY DIRECTOR

APPENDIX E

TABLE I
COMPATIBILITY TEST - FABRICS

FABRIC MATERIAL USED	NO TREATMENT	TENSILE STRENGTH/ELONGATION (LBS/INCH/PERCENT) FORMULATION: 75% DEET/Alcohol 5 g/m² ROOM TEMPERATURE 1HR	6HR	24HR	71°C 1HR	6HR	24HR	Immersion RT 1HR	6HR	24HR	Immersion 71°C 1HR	6HR	24HR
Nylon: Cotton 50:50 (BOU)	42/43	---	42/43*	39/45	38/50	32/50	41/48	42/48	44/48	44/45	43/45	47/43	41/48
Cotton[1]	9/8	7/10	6/13	9/10	4/10*	11/8	6/25	4/25	9/8	12/8	5/8	7/20	10/10
Nylon	27/36	19/45	26/43	25/43	25/40	24/43	22/43	19/48	22/45	25/45	24/43	24/48	24/43
Polyester	28/40	18/38	27/40	26/40	25/40	21/35*	25/40	22/33*	27/40	24/38	21/35	22/35*	25/38
Kevlar	64/6	64/10*	69/10*	73/10*	72/10*	73/10*	60/8*	71/10*	76/10*	75/13*	62/10*	80/10*	74/10*
Vinyl	16/218	13/135	14/110	15/110	16/195	---	16/245	12/125	10/95	11/120	12/105	11/135	14/135
100% Cotton[2] (BOU)	30/14	28/15	31/15	29/15	23/16	26/10	30/15	37/15	35/18	31/18	34/18	27/15	33/20

FABRIC MATERIAL USED	NO TREATMENT	3M Phase II Formulation 5 g/m² RT 1HR	6HR	24HR	71°C 1HR	6HR	24HR	Immersion RT 1HR	6HR	24HR
Nylon: Cotton 50:50 (BOU)	42/43	38/43	43/48	41/43	39/48	44/40	34/48	37/45*	25/45	41/45
Cotton[1]	9/8	6/10	9/10	8/20	3/15	10/10	9/5	4/13	7/10	11/8
Nylon	27/36	22/45	24/40	24/43	21/43	24/45*	24/45	24/40	24/43	28/45
Polyester	28/40	29/43	26/38	20/33*	19/40	27/40	25/40	22/33	23/38	25/40
Kevlar	64/6	54/5*	73/10*	67/10*	57/5*	75/10*	69/8*	66/10*	57/10*	56/10*
Vinyl	16/218	13/190	17/175	15/210	15/205	15/240	15/230	13/140	17/130	16/135
100% Cotton[2] (BOU)	30/14	25/15	30/15*	32/15	24/15	32/15	32/18	14/15	31/15	34/15

	Immersion 71°C 1HR	6HR	24HR
Nylon: Cotton 50:50 (BOU)	32/48	37/50*	45/50*
Cotton[1]	5/10	9/10	7/10
Nylon	25/40	23/40	23/43
Polyester	24/43	26/35	22/33
Kevlar	66/10*	77/10*	56/10*
Vinyl	12/115	14/155	14/260
100% Cotton[2] (BOU)	31/15	30/15*	27/15*

* Broke outside of treated area
1 - Cheesecloth
1 - Supplied by Army

TABLE II

REPELLENT COMPATIBILITY WITH PAINTED SURFACES - 24 HOURS

	71°C (160°F)				ROOM TEMPERATURE			
	PHASE II SUBMISSION		75% DEET/ALCOHOL		PHASE II SUBMISSION		75% DEET/ALCOHOL	
PAINT TYPE	5 g/m^2	SATURATED	5 g/m^2	SATURATED	5 g/m^2	SATURATED	5 g/m^2	SATURATED
Enamel	No Effect[1]	100% Lifted[2]	Slight Discoloration	80% Lifted	Very Slight Discoloration	5% Lifted	Slight Discoloration	100% Lifted
Urethane	No Effect[2]	100% Lifted[2]	No Effect	100% Lifted	Very Slight Discoloration	100% Lifted	10% Lifted	100% Lifted
Acrylic	Very Slight Discoloration, Soften[2]	Discolored[2]	No Effect	Discolored	Very Slight Discoloration	90% Lifted	Discolored	90% Lifted
Lacquer (Vehicle Paint)	Soften[2]	Formulation[2] Stack to Film	Orange Peeled	Discolored	Soften to No Effect	100% Removal[3]	No Effect[2]	Partial Removal[3]

1 - Saturated
2 - Formulation present on surface of paint
3 - When surface wiped

TABLE III

REPELLENT COMPATIBILITY WITH PLASTIC MATERIALS - 24 HOURS

PLASTIC MATERIAL	SHORE HARDNESS TYPE D											
	71°C (160°F)						ROOM TEMPERATURE					
	PHASE II SUBMISSION			75% DEET/ALCOHOL			PHASE II SUBMISSION			75% DEET/ALCOHOL		
	INITIAL	5 g/m^2	SAT'D.[1]	INITIAL	5 g/m^2	SAT'D.	INITIAL	5 g/m^2	SAT'D.	INITIAL	5 g/m^2	SAT'D.
Polycarbonate Glasses	80	81	81	80	80	81	86	78	85	82	75	82
Eyeglass Frame	75	75	67	75	70	70	75	75	75	75	75	75
Eyeglass Plastic Lens	85	85	81	80	79	80	75	72	78	85	83	83
Polyethylene	64	64	64	64	64	64	63	63	63	64	62	64
SAW Plastic Grip	82	82	80	80	80	78	80	78	74	80	78	65
Kevlar Helmet	70	76	75	76	76	74	80	82	80	78	78	75

1 - Saturated

TABLE IV

REPELLENT COMPATIBILITY WITH RUBBER MATERIALS AND LEATHER - 24 HOURS

	71 C (160 F)				ROOM TEMPERATURE			
	PHASE II SUBMISSION		75% DEET/ALCOHOL		PHASE II SUBMISSION		75% DEET/ALCOHOL	
COMPOSITION	5 g/m^2	SATURATED	5 g/m^2	SATURATED	5 g/m^2	SATURATED	5 g/m^2	SATURATED
Silicon Rubber	No Effect[1]	No Effect[1]	No Effect[3]	No Effect[3]	No Effect[1]	Slight Plasticizing	No Effect	Slight Plasticizing
Natural Rubber	No Effect[1]	Tacky[1]	No Effect[2]	No Effect[2]	Slightly Tacky	Slightly Tacky[1]	Slightly Tacky	Slightly Tacky
Neoprene	No Effect[1]	Tacky[1]	No Effect[2]	No Effect[2]	No Effect[1]	No Effect[1]	Slight Swelling	Slight Swelling
Buty Rubber	No Effect[1]	No Effect[1]	No Effect	No Effect	No Effect	No Effect[1]	No Effect	No Effect
Leather	---------	Slight Stain[1]	--------	No Effect	Slight Stain[1]	Slight Stain[1]	Slight Stain	Stained

1 - Formulation present on surface
2 - Point of application visible
3 - Doesn't wet out on surface

TABLE V

REPELLENT COMPATIBILITY WITH CHEMICAL PROTECTIVE SUIT

	71°C (160°F)		ROOM TEMPERATURE	
PART	PHASE II SUBMISSION SATURATED	75% DEET/ALCOHOL SATURATED	PHASE II SUBMISSION SATURATED	75% DEET/ALCOHOL SATURATED
Rubber Inner Pocket	Dried Formulation Residue	Very Slight Stain	Very Slight Stain	Very Slight Stain
Carbon Black Lining	Dried Formulation Residue	No Effect	Slight Stain	Slight Stain Swelled Back Side
Outer Shell	Dried Formulation Residue	Very Slight Stain	Slight Stain	No Effect

APPENDIX F - PACKAGE SPECIFICATIONS

HDPE TUBE

Color:	Olive Drab
Material:	High Density Polyethylene
Size:	1-1/2" x 3-1/2" Tube
Neck Finish:	22/400
Orifice:	0.500
Decorating:	Plain
Internal Laquer:	None
External Coat:	#1004 Barrier Coat
Cap:	Olive Drab Polytop Dispenser Cap, Polyethylene, 22/400

APPENDIX G

Front Label

YYYY-YY-YYY-YYYY
INSECT REPELLENT LOTION (CREAM)
TYPE (XXX)
Federal Specification XXXXXXX
Contents: 2 Fluid Ounces

Repels biting flies, chiggers, deer flies, mosquitoes, fleas and stable flies. Also repels terrestrial leeches in tropical areas where pest occurs.

Provides 95% or greater protection against mosquitoes for 12 or more hours under normal use conditions.

ACTIVE INGREDIENTS: N,N-Diethyl-m-toluamide 31.58%
Other isomers 1.58% inert ingredients 66.75%.

FOR EXTERNAL USE ONLY
Keep out of reach of children.

Caution - Avoid contact with eyes and lips. In case of eye contact, flush with plenty of water. Do not apply to excessively sunburned or damaged skin.

Contract No. DAMD17-85-C-5017

Back Label

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Squeeze into one hand a 2.5 ml strip of repellent, equal in length and width to the diagram on the side of the tube. Rub hands together and apply thoroughly in a thin layer to both forearms. Use additional lotion for upper arms. Repeat for other exposed areas. **To apply to face** squeeze lotion into palm of hand and spread on face and neck. **Avoid Contact With Eyes and Lips. To apply to clothing,** dispense the lotion into one hand, rub the hands together and brush lightly on socks, around collars, waist, sleeve and trouser cuffs and where clothing fits snugly such as over the shoulders, elbows, knees and buttocks. Repeat as necessary. Wipe hands after application.

May Damage certain synthetic fabrics, plastics, painted or varnished surfaces. Avoid smearing on plastic eyeglass frames, goggles, watch crystals, etc. **WILL NOT DAMAGE** nylon, cotton or wool fabrics.

Disposal: Do not reuse empty container. Wrap container and put in trash.

Personal Care Products/3M
3M Center
St. Paul, Minnesota 55144-1000

EPA Reg. No. XXX
EPA Est. No. XXXXX