AR226_3254

**TRADE SECRET**

***Study Title***

H-25357: Acute Eye Irritation Study in Rabbits

Laboratory Project ID: DuPont-11111

**TEST GUIDELINES:** U.S. EPA Health Effects Test Guidelines
OPPTS 870.2400 (1998)

OECD Guidelines for Testing of Chemicals
Section 4: Health Effects, No. 405 (1987)

Commission Directive 92/69/EEC
EEC Method B.5 (1992)

**AUTHOR:** Carol Finlay, B.A.

**STUDY COMPLETED ON:** August 9, 2002

**PERFORMING LABORATORY:** E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

[redacted]

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17, except for the item documented below. The item listed does not impact the validity of the study.

The test substance was characterized by the sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the characterization was done by an ISO 9000 certified lab and the accuracy of the data is considered sufficient for the purposes of this study.

Applicant / Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Carol Finlay 9-Aug-2002
Carol Finlay, B.A. Date
Staff Toxicologist

Applicant / Sponsor: _____ _____
DuPont Representative Date

## QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

25357

Dates of Inspections:

Protocol: June 24, 2002

Conduct: June 24, 2002

Records, Reports: August 1, 2002

Dates Findings Reported to:

Study Director: August 2, 2002

Management: August 2, 2002

Reported by: Matthew Frentzel 9-Aug-2002

Matthew Frentzel
Quality Assurance Auditor

Date

Company Sanitized. Does not contain TSCA CBI

## CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of the data obtained from this study.

**Reviewed by:** James Mackay II 6-Aug-2002
James C. Mackay II
Associate Scientist
Date

**Issued by Study Director:** Carol Finlay 9-Aug-2002
Carol Finlay, B.A.
Staff Toxicologist
Date

## STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: • H-25357
[redacted]

Submitter's Notebook Number(s): [redacted]

Haskell Number: 25357

CAS Registry Number: [redacted]

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Amber liquid

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: June 14, 2002 / (see report cover page)

In-Life Initiated/Completed: June 24, 2002 / June 27, 2002

## STUDY PERSONNEL

| | |
|---|---|
| Study Director: | Carol Finlay, B.A. |
| Management: | Scott E. Loveless, Ph.D. |
| Primary Technician: | James C. Mackay II |
| Toxicology Report Preparation: | Sean M. Callaghan, B.A. |
| Management: | Nancy S. Selzer, M.S. |
| Laboratory Veterinarian: | William Singleton, D.V.M., A.C.L.A.M. |

## SUMMARY

H-25357 was evaluated for acute eye irritation potential in 3 adult New Zealand White rabbits. An aliquot of 0.1 mL of test substance was administered to 1 eye of each test animal. The eyes remained unwashed following treatment. The conjunctiva, iris, and cornea of each treated eye were evaluated and scored according to a numerical scale approximately 1, 24, 48, and 72 hours following administration of the test substance.

Two rabbits pawed the treated eye after instillation of the test substance. The test substance produced conjunctival redness (score of 1) in the treated eyes of two rabbits and discharge (score of 1) in the treated eyes of 2 rabbits. Fluorescein stain examinations were negative for corneal injury in the treated eyes of all rabbits. The treated eyes of the rabbits were normal by 24 or 48 hours after instillation of the test substance. No clinical signs were observed. Body weight loss of approximately 4% of initial weight occurred by study termination (72 hours) in one rabbit.

Mean values were calculated for each animal separately by using numerical scores obtained from the quantitative evaluation of each ocular response observed in each rabbit approximately 24, 48, and 72 hours following treatment. These values are as follows:

| RABBIT NUMBER | CORNEAL OPACITY | IRITIS | CONJUNCTIVAL REDNESS | CONJUNCTIVAL CHEMOSIS |
|---|---|---|---|---|
| 35553 | 0.00 | 0.00 | 0.33 | 0.00 |
| 35502 | 0.00 | 0.00 | 0.00 | 0.00 |
| 35566 | 0.00 | 0.00 | 0.00 | 0.00 |

In accordance with Directive 67/548/EEC, H-25357 is not classifiable as irritant.

# INTRODUCTION

## A. Objective

The objective of this study was to evaluate the eye irritation potential and the reversibility of ocular effects of H-25357 following a single ocular application to albino rabbits. The results of the study were used to determine the appropriate toxicity classification and labeling requirements in accordance with EEC.

## B. Principles of the Methodology

Ocular effects are evaluated and scored approximately 1, 24, 48, and 72 hours after instillation of 0.1 mL of test substance (or a weight of not more than 100 mg of test substance) into the lower conjunctival sac of 1 eye of each of 3 rabbits. The reversibility of any effects is assessed for up to 21 days, if necessary. Ocular effects are scored according to the Draize Scale (Table 1).

# MATERIALS AND METHODS

## A. Test Guidelines

The study design complies with the following test guidelines:

- Office of Prevention, Pesticides and Toxic Substances (OPPTS) U.S. Environmental Protection Agency (EPA) (1998). OPPTS 870.2400 Acute Eye Irritation. *Health Effects Test Guidelines*.
- Organisation for Economic Co-Operation and Development (OECD) (1987). 405 Acute Eye Irritation/Corrosion. *Guideline for Testing of Chemicals*.
- European Economic Communities (EEC) (1992). Directive 92/69/EEC Annex V, Part B.5, Acute Toxicity (Eye Irritation). *Methods for the Determination of Toxicity*.

## B. Test Substance

The test substance, H-25357, was supplied by the sponsor as an amber liquid. The test substance was inverted to mix before each amount for dosing was removed. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

## C. Animal Husbandry

Adult HM:(NZW)fBR New Zealand White rabbits were received from Covance Research Products, Denver, Pennsylvania. The rabbits were housed singly in stainless steel, wire-mesh cages suspended above cage boards. Each rabbit was assigned a unique identification number which was recorded on a card affixed to the cage. The last 3 digits of the identification number were written on the inside of each rabbit's ear with a water insoluble marker. The rabbits were offered approximately 125 grams of PMI® Nutrition International, LLC Certified High Fiber Rabbit LabDiet® 5325 daily during the study. Water was available *ad libitum*.

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for total bacterial, spore, and fungal counts.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

Rabbits were weighed and observed for general health during the 7-day quarantine period. Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted for a temperature of 20°C ± 1°C and relative humidity of 50% ± 10%. Excursions outside these ranges were judged to have been of insufficient magnitude and/or duration to have adversely affected the validity of the study.

## D. Protocol

On the day of treatment, the eyes of 3 male New Zealand White rabbits were examined using illumination, magnification, and fluorescein stain. Animals used on this study were free of pre-existing corneal or conjunctival injury or irritation and were judged to be in good health. The rabbits weighed from 2082 to 2592 grams on the day of treatment.

An aliquot of 0.1 ml of H-25357 was introduced into the lower conjunctival sac of the right eye of each rabbit. The left eye of each rabbit was not treated with the test substance and served as a control. The treated and control eyes of all rabbits remained unwashed. Each rabbit was observed for approximately 30 to 60 seconds before being returned to its cage.

Approximately 1, 24, 48, and 72 hours after H-25357 was administered, the rabbits were examined for evidence of eye irritation and for clinical signs of toxicity. At each of these observation periods, eyes were examined using illumination and magnification and scored for ocular reactions according to the Draize Scale (Table 1). Any other adverse ocular reactions, particularly those indicative of corrosive action, were also noted. Control eyes were not scored. These untreated eyes were used for comparison and were considered "normal" relative to the treated eye. Fluorescein stain examinations were conducted at the 24-hour and each subsequent evaluation. The rabbits were weighed on the day of treatment and at the last ocular evaluation.

## E. Data Analysis and Interpretation of Results

For each animal, a mean value was calculated for each lesion (corneal opacity, iritis, conjunctival redness, and conjunctival chemosis) by averaging the scores obtained from the 24-, 48-, and 72-hour observations. The results were interpreted according to Directive 67/548/EEC relative to the general classification and labeling requirements for dangerous substances.

The test substance will be classified as "IRRITANT" and will require the symbol "Xi" and the indication of danger "IRRITANT" in accordance with the criteria below.

In addition, the following risk (R) phrases will be assigned to substances classified "IRRITANTS" as applicable, according to the following criteria:

R36 "IRRITATING TO EYES"

The risk phrase, R36 "IRRITATING TO EYES", will be assigned if, when applied to the eye of the test animal, the test substance causes significant ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours. Ocular lesions are significant if the individual animal mean values of the 24-, 48-, and 72-hour evaluations in 2 or more animals comply with any of the following criteria:

| | |
|---|---|
| - corneal opacity | equal to or greater than 2.0, but less than 3.0 |
| - iridial lesion | equal to or greater than 1.0, but less than 2.0 |
| - conjunctival redness | equal to or greater than 2.5 |
| - conjunctival chemosis | equal to or greater than 2.0 |

R41 "RISK OF SERIOUS DAMAGE TO THE EYES"

The risk phrase, R41 "RISK OF SERIOUS DAMAGE TO THE EYES", will be assigned if the test substance causes severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours. Ocular lesions are severe if the individual animal mean value of the 24-, 48-, and 72-hour evaluations in 2 or more animals comply with any of the following criteria:

- corneal opacity equal to or greater than 3.0
- iridial lesion equal to 2.0

R41 "RISK OF SERIOUS DAMAGE TO THE EYES" risk phrase is also required if:

- ocular lesions are still present 21 days following treatment
- the test substance causes irreversible coloration of the eyes

## RESULTS AND DISCUSSION

Two rabbits pawed the treated eye after instillation of the test substance. The test substance produced conjunctival redness (score of 1) in the treated eyes of two rabbits and discharge (score of 1) in the treated eyes of 2 rabbits. Fluorescein stain examinations were negative for corneal injury in the treated eyes of all rabbits. The treated eyes of the rabbits were normal by 24 or 48 hours after instillation of the test substance. No clinical signs were observed. Body weight loss of approximately 4% of initial weight occurred by study termination (72 hours) in one rabbit.

The ocular scores from individual animals with respect to observation time are presented in Table 2. Mean ocular scores calculated in accordance with EEC are presented in Table 3. Individual body weights and clinical signs of toxicity are presented in Table 4.

## CONCLUSION

In accordance with Directive 1999/45/EEC, H-25357 is not classifiable as irritant.

## RECORDS AND SAMPLE STORAGE

All data and records for analytical characterization conducted by the sponsor will be maintained at Chambers Works, Deepwater, New Jersey or at Iron Mountain Records Management, Wilmington, Delaware.

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

# TABLES

TABLE 1

DRAIZE[a] SCALE FOR SCORING OCULAR LESIONS

(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 one half, but less than three quarters ..... 3
Greater than three quarters, up to whole area ..... 4

(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

(3) **Conjunctiva**

(A) *Redness (refers to palpebral and bulbar conjunctiva excluding cornea and iris)*

Vessels normal ..... 0
Vessels definitely injected above normal ..... 1
More diffuse, deeper crimson red, individual vessels not easily discernible ..... 2
Diffuse beefy red ..... 3

TABLE 1 (Continued)

DRAIZE SCALE FOR SCORING OCULAR LESIONS

(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 |

a Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

TABLE 2

INDIVIDUAL RABBIT OCULAR IRRITATION SCORES FOLLOWING TREATMENT WITH H-25357

| READING | RABBIT NUMBER | CORNEA | | IRITIS | CONJUNCTIVA | | | FLUORESCEIN STAIN |
|---|---|---|---|---|---|---|---|---|
| | | OPACITY | AREA | | REDNESS | CHEMOSIS | DISCHARGE | |
| 1 HOUR | 35553 | 0 | 0 | 0 | 0 | 0 | 1 | NA |
| | 35502 | 0 | 0 | 0 | 0 | 0 | 1 | NA |
| | 35566 | 0 | 0 | 0 | 1 | 0 | 0 | NA |
| 24 HOURS | 35553 | 0 | 0 | 0 | 1 | 0 | 1 | NEG |
| | 35502 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| | 35566 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| 48 HOURS | 35553 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| | 35502 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| | 35566 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| 72 HOURS | 35553 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| | 35502 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |
| | 35566 | 0 | 0 | 0 | 0 | 0 | 0 | NEG |

NEG = negative for corneal injury

TABLE 3

MEAN SCORES[a] FOR OCULAR RESPONSES FOR INDIVIDUAL RABBITS

| RABBIT NUMBER | CORNEAL OPACITY | IRITIS | CONJUNCTIVAL REDNESS | CONJUNCTIVAL CHEMOSIS |
|---|---|---|---|---|
| 35553 | 0.00 | 0.00 | 0.33 | 0.00 |
| 35502 | 0.00 | 0.00 | 0.00 | 0.00 |
| 35566 | 0.00 | 0.00 | 0.00 | 0.00 |

a 24hr + 48hr + 72hr /3. Calculated according to EEC.

TABLE 4

INDIVIDUAL BODY WEIGHTS OF RABBITS

| RABBIT NUMBER | SEX | INITIAL WEIGHT (g) | FINAL WEIGHT (72 HOURS) (g) |
|---|---|---|---|
| 35553 | M | 2082 | 1989 |
| 35502 | M | 2592 | 2604 |
| 35566 | M | 2110 | 2120 |

CLINICAL SIGNS OF TOXICITY OBSERVED IN RABBITS

No clinical signs of toxicity were observed in any of the rabbits during the study.