TRADE SECRET

Study Title

H-25134: Acute Eye Irritation/Corrosion Study in Rabbits

Laboratory Project ID: DuPont-9104

TEST GUIDELINES: 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: January 3, 2002

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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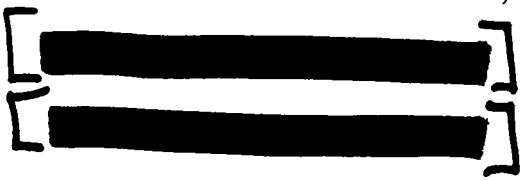

CERTIFICATION

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

Reviewed by: James Mackay II 3-Jan-2002
James C. Mackay II
Associate Scientist
Date

Issued by Study Director: Carol Finlay 3-Jan-2002
Carol Finlay, B.A.
Staff Scientist
Date

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STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: [redacted]
H-25134
[redacted]

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

Haskell Number: 25134

CAS Registry Number: [redacted]

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Dark brown solid

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

Study Initiated/Completed: November 28, 2001 / (see report cover page)

In-Life Initiated/Completed: December 10, 2001 / December 13, 2001

STUDY PERSONNEL

Study Director: Carol Finlay, B.A.
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SUMMARY

H-25134 was evaluated for acute eye irritation potential in 3 adult New Zealand White rabbits. Approximately 100 mg 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.

No irritation was observed in the treated eyes of 2 of the 3 rabbits. Conjunctival redness (score of 1) and discharge (score of 1) were observed one hour after treatment in the treated eye of the remaining rabbit. No irritation was observed 24, 48, or 72 hours after treatment. Fluorescein stain examinations were negative for corneal injury in the treated eyes of all rabbits. No clinical signs were observed, and no body weight loss occurred.

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
35417	0.00	0.00	0.00	0.00
35408	0.00	0.00	0.00	0.00
35414	0.00	0.00	0.00	0.00

Based on the mean values for each rabbit calculated according to the guide for the labeling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), H-25134 is not classified 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-25134 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 (Directive 93/21).

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). For each animal, a mean value is calculated for each ocular response by averaging the scores obtained from the 24-, 48-, and 72-hour observations. Classification of ocular irritancy potential is based on the mean values for the lesions observed as well as the persistence of the lesions observed.

MATERIALS AND METHODS

A. Test Guidelines

The study design complies with the following testing 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-25134, was supplied by the sponsor as a dark brown solid. 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, Inc. 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 quarantined, weighed, and observed for general health for 7 days. 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 1635 to 2104 grams on the day of treatment.

The container of test substance was heated in a 64°C waterbath until the test substance was liquefied. The test substance was then stirred. The weight of a 0.1 mL aliquot of the liquefied test substance was determined to be 119.4 mg. Therefore, approximately 100 mg of test substance was weighed out for each animal. The aliquot of test substance designated for each rabbit was pulverized before dosing.

Approximately 100 mg of H-25134 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 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-25134 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

The numerical values corresponding to each animal tissue (cornea, iris, conjunctiva) and time were recorded. 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 Annex VI of the EEC Commission Directive 93/21 relative to the general classification and labeling requirements for dangerous substances.

Interpretation according to Annex VI - Eye Irritation Criteria:

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

No irritation was observed in the treated eyes of 2 of the 3 rabbits. Conjunctival redness (score of 1) and discharge (score of 1) were observed one hour after treatment in the treated eye of the remaining rabbit. No irritation was observed 24, 48, or 72 hours after treatment. Fluorescein stain examinations were negative for corneal injury in the treated eyes of all rabbits. No clinical signs were observed, and no body weight loss occurred.

The ocular scores from individual animals with respect to observation time are presented in Table 2. Mean ocular scores calculated in accordance with EEC Directive 93/21 are summarized in Table 3. Individual body weights and clinical signs of toxicity are presented in Table 4.

CONCLUSIONS

Based on the mean values for each rabbit calculated according to the guide for the labeling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), H-25134 is not classified as irritant.

RECORDS AND SAMPLE STORAGE

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-25134

READING	RABBIT NUMBER	CORNEA		IRITIS	CONJUNCTIVA		
		OPACITY	AREA		REDNESS	CHEMOSIS	DISCHARGE
1 HOUR	35417	0	0	0	1	0	1
	35408	0	0	0	0	0	0
	35414	0	0	0	0	0	0
24 HOURS	35417	0	0	0	0	0	0
	35408	0	0	0	0	0	0
	35414	0	0	0	0	0	0
48 HOURS	35417	0	0	0	0	0	0
	35408	0	0	0	0	0	0
	35414	0	0	0	0	0	0
72 HOURS	35417	0	0	0	0	0	0
	35408	0	0	0	0	0	0
	35414	0	0	0	0	0	0

TABLE 3

MEAN SCORES[a] FOR OCULAR RESPONSES FOR INDIVIDUAL RABBITS

RABBIT NUMBER	CORNEAL OPACITY	IRITIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS
35417	0.00	0.00	0.00	0.00
35408	0.00	0.00	0.00	0.00
35414	0.00	0.00	0.00	0.00

a 24hr + 48hr + 72hr /3. Calculated according to EEC Directive 93/21.

TABLE 4

INDIVIDUAL BODY WEIGHTS OF RABBITS

RABBIT NUMBER	SEX	INITIAL WEIGHT (g)	FINAL WEIGHT (72 HOURS) (g)
35417	Male	2028	2192
35408	Male	1635	1752
35414	Male	2104	2208

CLINICAL SIGNS OF TOXICITY OBSERVED IN RABBITS

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