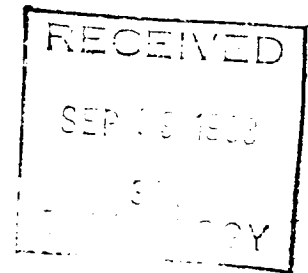


Acute Ocular Irritation Test
with T-3421
in Albino Rabbits



Experiment No.:

0883EB0286

Conducted At:

Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted:

June 21, 1983 to August 1, 1983

Conducted By:

D. M. Markoe, Jr. 5/15/83
D. M. Markoe, Jr., BS Date
Toxicologist
Study Director

Reviewed By:

Karen D O'Malley 5/23/83
K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

K. L. Ebbens 5/24/83
K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: M. T. Case
F. D. Griffith

~~W. J. McCormick~~

Summary

The results of the acute ocular irritation test conducted from June 21, 1983 to August 1, 1983 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-3421 is moderately irritating (40.0/110.0) to the unwashed eye of the female albino rabbit. A five second and thirty second contact washed eye procedure were also conducted employing a five liter wash. The irritation ratings for T-3421, using the limited contact procedures, were mildly irritating (15.7/110.0) for the five second and mildly irritating (18.3/110.0) for the thirty second contact procedure.

Minimal corneal opacity, iritis and moderate conjunctivitis were produced during the unlimited contact procedure by the one hour evaluation. The irritation subsided to minimal corneal opacity with vascularization (two animals) and slight conjunctivitis (three animals) by the day seven evaluation.

T-3421, when allowed a five and thirty second contact, produced iritis and mild conjunctivitis by the one hour evaluation. All irritation subsided by the day two evaluation. Corneal opacity was not observed in any animal of either treatment group.

Introduction

The objective of this study was to assess the acute ocular irritation properties of T-3421 when instilled into the washed and unwashed eye of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rabbits of the New Zealand breed^a were used to evaluate the ocular irritating properties of the test article. The test method was modeled after that of Draize et al^b.

The test article was instilled into the conjunctival sac of the right eye of each rabbit according to the treatment procedure presented in Table 1 with the left eye of each animal serving as a control. At each scoring interval, the cornea, iris and palpebral conjunctiva were examined and graded for irritation and injury according to a standard scoring system^b. The maximum possible score at any one examination and scoring period 110 points, which indicates maximal irritation and damage to all three ocular tissues (cornea, iris, conjunctiva) while a score of zero indicates no irritation (Table 2). In this scoring system, special emphasis is placed upon irritation or damage to the cornea, while less emphasis is placed upon damage to the iris and conjunctiva.

After completion of the test, the scores were analyzed, and a descriptive eye irritation rating was assigned to the test article. The criteria used for assignment of the descriptive rating were the frequency, the extent and the persistence of irritation or damage which occurred to the three ocular tissues (Table 3). The individual results are presented in Tables 4-6.

^a Hazleton Dutchland, Inc., Denver, PA

^b Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

The rating is arrived at by selecting the maximum mean irritation score at one hour, one, two or three days after instillation. If the rate of dissipation of injury does not meet the requirements defined for the descriptive rating appropriate for a particular numerical score, the descriptive rating is raised by one or more levels. The rating system is presented in Table 3. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Eye Irritation Test - Albino Rabbits

Treatment Procedure

Test Article	Number of Animals Evaluated	Form Administered	Quantity of Test Article Administered	Contact Period (seconds)	Volume of Wash (tap water)	Evaluation Time Post Dose Administration
T-3421	6	waxy solid	0.1 gm	unlimited	none	1 Hour, 1, 2, 3 and 7 Days
T-3421	3	waxy solid	0.1 gm	5 seconds	none	1 Hour, 1, 2, 3 and 7 Days
T-3421	3	waxy solid	0.1 gm	30 seconds	none	1 Hour, 1, 2, 3 and 7 Days

Eye Irritation Test - Albino Rabbits

Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Draize Grade
Conjunctiva	<u>Redness (A)</u>	
	Redness (refers to palpebral conjunctiva only). Vessels definitely injected above normal.	1
	More diffuse, deeper crimson red, individual vessels not easily discernible.	2
	Diffuse beefy red.	3
	<u>Chemosis (B)</u>	
	Any swelling above normal (included nictitating membrane).	1
	Obvious swelling with partial eversion of the lids.	2
	Swelling with lids about half-closed.	3
	Swelling with lids about half-closed to completely closed.	4
	<u>Discharge (C)</u>	
	Any amount different from normal (Does not include small amount observed in inner canthus of normal animals).	1
	Discharge with moistening of the lids and hairs just adjacent to the lids.	2
	Discharge with moistening of the lids and hairs and considerable area around eye.	3
	Score (A + B + C) x 2 Total maximum = 20	
Cornea	<u>Opacity (A)</u>	
	Opacity - Degree of density (area which is most dense is taken for reading).	
	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
	<u>Area of Cornea Involved (B)</u>	
	One quarter (or less) but not zero.	1
	Greater than one-quarter, but less than one-half.	2
	Greater than one-half, but less than three-quarters.	3
	Greater than three-quarters, up to whole area.	4
	Score equals A x B x 5 Total maximum = 80	
Iris	<u>Values (A)</u>	
	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
	Score equals A x 5 Total maximum = 10	

Note: The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva.

TABLE 3

EYE IRRITATION TEST - ALBINO RABBITS

Classification of Test Materials
Based on Eye Irritation Properties

Rating	Range	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores by the one day reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	> 0.5 - 2.5	To maintain this rating, all scores by the one day reading must be zero; otherwise, increase rating one level.
Minimally Irritating	> 2.5 - 15.0	To maintain this rating, all scores by the three day reading must be zero; otherwise, increase rating one level.
Mildly Irritating	> 15.0 - 25.0	To maintain this rating, all scores by the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	> 25.0 - 50.0	To maintain this rating, scores by 7 days must be ≤ 10 for 60% or more of the animals. Also, mean 7-day score must be ≤ 20 . If 7-day mean score is ≤ 20 but $< 60\%$ of animals show scores < 10 , then no animal among those showing scores > 10 can exceed a score of 30 if rating is to be maintained; otherwise, raise rating one level.
Severely Irritating	> 50.0 - 80.0	To maintain this rating, scores by 7 days must be ≤ 30 for 60% or more of the animals. Also, mean 7-day score must be ≤ 40 . If 7-day mean score is ≤ 40 but $< 60\%$ of the animals show scores ≤ 30 , then no animal among those showing scores > 30 can exceed a score of 60 if rating is to be maintained; otherwise, raise rating one level.
Extremely Irritating	> 80.0 - 110.0	

TABLE 4

EYE IRRITATION TEST - ALBINO RABBITS

with T-3421

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS						Means
		3B988	3B981	3B1012	3B1014	3B995	3B962	
Cornea (D-A)	1 Hour	10 (1-2)	15 (1-3)	5 (1-1)	5 (1-1)	10 (1-2)	5 (1-1)	8.3
Iris		5	0	5	0	5	0	2.5
Conjunctiva (RSD)		16 (2-3-3)	10 (2-1-2)	14 (2-2-3)	14 (2-2-3)	10 (2-1-2)	14 (2-2-3)	13.0
	Total	31	25	24	19	25	19	23.8
Cornea (D-A)	1 Day	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	20.0
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		18 (3-3-3)	12 (2-2-2)	16 (3-2-3)	12 (2-2-2)	18 (3-3-3)	14 (2-2-3)	15.0
	Total	43	37	41	37	43	39	40.0
Cornea (D-A)	2 Days	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	10 (1-2)	18.3
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		12 (2-2-2)	12 (2-2-2)	14 (2-2-3)	10 (2-1-2)	12 (2-2-2)	12 (2-2-2)	12.0
	Total	37	37	39	35	37	27	35.3
Cornea (D-A)	3 Days	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	20 (1-4)	10 (1-2)	18.3
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		8 (2-1-1)	8 (2-1-1)	12 (2-2-2)	8 (2-1-1)	8 (2-1-1)	8 (2-1-1)	8.7
	Total	33	33	37	33	33	23	32.0
Cornea (D-A)	7 Days	10 (1-2) V	10 (1-2) V	0	0	0	0	3.3
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		8 (2-1-1)	8 (2-1-1)	2 (1-0-0)	0	0	0	3.0
	Total	18	18	2	0	0	0	6.3

Key: Cornea: Conjunctiva:
 D=Density R=Redness V=Vascularization
 A=Area S=Swelling
 D=Discharge

TABLE 5

EYE IRRITATION TEST - ALBINO RABBITS
with T-3421 (5 Second Contact)

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS			MEANS
		3B1161	3B1164	3B1148	
Cornea (D-A)	1 Hour	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva		12 (2-2-2)	12 (2-2-2)	8 (2-1-1)	10.7
(RSD) Total		17	17	13	15.7
Cornea (D-A)	1 Day	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva		8 (2-1-1)	6 (1-1-1)	6 (2-1-0)	6.7
(RSD) Total		13	11	11	11.7
Cornea (D-A)	2 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD) Total		0	0	0	0.0
Cornea (D-A)	3 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD) Total		0	0	0	0.0
Cornea (D-A)	7 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD) Total		0	0	0	0.0

Key: Cornea: D = Density
A = Area

Conjunctiva: R = Redness
S = Swelling
D = Discharge

TABLE 6

EYE IRRITATION TEST - ALBINO RABBITS
with T-3421 (30 Second Contact)

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS			MEANS
		3B1170	3B1162	3B1165	
Cornea (D-A)	1 Hour	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva		12 (2-2-2)	16 (3-3-2)	12 (2-2-2)	13.3
(RSD)		17	21	17	18.3
Cornea (D-A)	1 Day	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva		8 (2-1-1)	10 (2-2-1)	10 (2-2-1)	9.3
(RSD)		13	15	15	14.3
Cornea (D-A)	2 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD)		0	0	0	0.0
Cornea (D-A)	3 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD)		0	0	0	0.0
Cornea (D-A)	7 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD)		0	0	0	0.0

Key: Cornea: D = Density
A = Area

Conjunctiva: R = Redness
S = Swelling
D = Discharge

APPENDIX I
PROTOCOL

10.

TEST: Acute Ocular Irritation TestSPONSOR: **3M** Commercial Chemical

Division

CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, MinnesotaTEST ARTICLE: T-3421CONTROL ARTICLE: NonePROPOSED STARTING/COMPLETION DATE OF TEST: 5/83 - 6/83TEST SYSTEM: Female New Zealand White Albino Rabbits

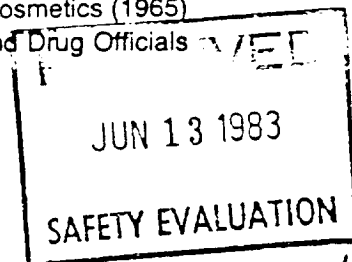
SOURCE:

OBJECTIVE: The objective of this test will be to determine the irritation potential of the test article to the ocular tissues (cornea, iris and conjunctiva) of 5 albino rabbits. Rabbits were selected as the test system for their sensitivity to irritants, historical use, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food^a and water offered *ad libitum*. Each animal will be assigned a numbered ear tag which will correspond to a card affixed to the outside of the cage. The test article will be instilled into the conjunctival sac of the right eye at a dose of 0.1 with the contralateral eye of each animal serving as a control. At 1 hours and 1 days (additional scoring intervals may be added to further characterize the ocular reactions), the tissues will be examined and graded for irritation and injury according to a standard scoring system of Draize *et al.*^b. After completion of the test, the scores will be analyzed, and a descriptive eye irritation rating assigned to the test article. Eye examinations may be carried out with the aid of sodium fluorescein. If deemed necessary by the study director, washed eye procedures entailing a 5 and 30 second contact period with a 1X 1/4-in wash over a 5-in period will be conducted using 3 animals per procedure. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

^a Purina Rabbit Chow, Ralston-Purina, St. Louis, Missouri

^b Draize: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics (1965)
Published by the Editorial Committee of The Association of Food and Drug Officials
of the United States.



W. C. McCormick
W. C. McCormick

Sponsor

6-9-83

Date

D. M. [Signature]
Study Director

6/17/83
Date

1. The source for the test animals is Hazelton-Dutchland Labs., Denver, PA.

Study Director 6/21/83
Date

2. _____

Study Director Date

3. _____

Study Director Date

4. _____

Study Director _____ Date _____

5. _____

Study Director Date

APPENDIX IIPrincipal Participating Personnel Involved in the Study

<u>Name</u>	<u>Function</u>
D. M. Markoe, Jr., BS	Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Toxicology Testing
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

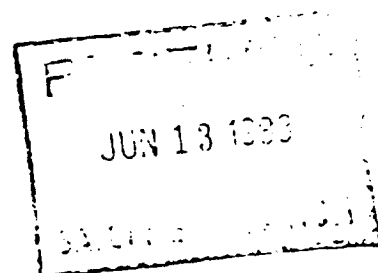
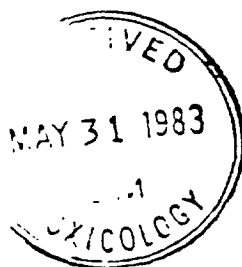
for
KTZ-15 (CC 834-4)
 T-3421

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 25 May 83 *J. H. Miller*
2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.
 yes ✓ no
3. The stability of the test and/or control substances have been determined or will be determined as of 25 June 83 *and end of Test* *Aug*

The above information and documentation are located in the sponsor's records.

D. Rucker 5/25/83
 Sponsor Date

cc L.D. Winter 236-2B
W.C. McCormick 220-2E
 W.H. Pearson 223-65E
 D. Pauby 236-1



APPENDIX IVQUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0883EB0286

This short term study was audited by Compliance Audit and the final report examined against the raw data on August 29, 1983. The results of the audit were reported to the study director and to management on August 30, 1983.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.



Compliance Audit

August 30, 1983
Date