Acute Ocular Irritation Test

with T-2960CoC

in Albino Rabbits

Experiment No.: 0880EB0598

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.,
St. Paul, Minnesota

Dates Conducted: October 21, 1980 to November 18, 1980

Conducted By: D. M. Markoe, Jr. 1/15/81
D. M. Markoe, Jr., BS Date
Toxicologist
Study Director

Reviewed By: K. L. Ebbens 2/16/81
K. L. Ebbens, BS Date
Supervisor, Acute Toxicology

dc: M. T. Case
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Summary

The results of the acute ocular irritation test conducted from October 21, 1980 to November 18, 1980 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-2960CoC is severely irritating (42.3/110.0) to the unwashed eye of albino rabbits. A five second and thirty second contact washed eye procedure were also conducted employing a 5 liter wash. The irritation ratings for T-2960CoC, using the limited contact procedures, were moderately irritating (37.7/110.0) for the five second and extremely irritating (62.3/110.0) for the thirty second contact procedure.

Slight corneal opacity with epithelial sloughing, iritis and severe conjunctivitis were produced during the unlimited contact procedure by the one hour evaluation. The corneal opacity persisted during the seven day test period and vascularization developed. The iritis subsided by the seven day evaluation and the conjunctivitis diminished to minimal.

T-2960CoC, when allowed both a five second and thirty second contact, produced slight corneal opacity, iritis, and severe conjunctivitis with hemorrhage by the one hour evaluation. Slight corneal opacity with vascularization, epithelial sloughing, and minimal conjunctivitis persisted through the seven day evaluation in the five second contact group.

Corneal opacity increased in severity with epithelial sloughing and vascularization by the seven day evaluation on the thirty second contact group. Mild conjunctivitis and iritis, noted in one animal, persisted through the seven day evaluation.

Introduction

The objective of this study[a] was to assess the acute ocular irritation potential of T-2960CoC to the washed and unwashed eye of albino rabbits. The study, which was initiated at Riker Laboratories, Inc., St. Paul, Minnesota on October 21, 1980 and completed on November 18, 1980, was not conducted to support a government submission or marketing permit and is therefore not regulated by the 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.

[a] Riker Toxicity Experiment No.: 0880EB0598, Test Method 602E

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 is 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] Dutchland, Inc., Denver, Pennsylvania
[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-2960CoC	6	liquid	0.1 ml	unlimited	none	1 Hour, 1, 2, 3, and 7 Days
T-2960CoC	3	liquid	0.1 ml	30	5 liter[a]	1 Hour, 1, 2, 3, and 7 Days
T-2960CoC	3	liquid	0.1 ml	5	5 liter[a]	1 Hour, 1, 2, 3, and 7 Days

[a] The wash procedure was conducted using a periflow pump (Technilab Instruments, Pequannock, NJ) at a flow rate of one liter/minute for five minutes.

TABLE 2

EYE IRRITATION TEST - ALBINO RABBITS

Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Draize Grade
Cornea	Opacity (A)	
	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
	Area of Cornea Involved (B)	
	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	Values (A)	
	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	

TABLE 2 (concluded)

EYE IRRITATION TEST - ALBINO RABBITS

Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Draize Grade
Conjunctiva	Redness (A)	
	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
	Chemosis (B)	
	Any swelling above normal (includes 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
	Discharge (C)	
	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	

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 $\leq$ 10 for 60% or more of the animals. Also, mean 7-day score must be $\leq$ 20. If 7-day mean score is $\leq$ 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 $\leq$ 30 for 60% or more of the animals. Also, mean 7-day score must be $\leq$ 40. If 7-day mean score is $\leq$ 40 but < 60% of the animals show scores $\leq$ 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-2960CoC

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS						Means
		OB 3273	OB 3323	OB 3309	OB 3303	OB 3305	OB 3306	
Cornea (D-A)	1 Hour	20(1-4)	20(1-4)E	20(1-4)E	20(1-4)E	15(1-3)E	20(1-4)	19.2
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)	18(3-3-3)	18(3-3-3)	18(3-3-3)	18(3-3-3)	18(3-3-3)	18.0
	Total	43	43	43	43	38	43	42.2
Cornea (D-A)	1 Day	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)E	20(1-4)E	20.0
Iris		5	5	5	5	5	0	4.2
Conjunctiva (RSD)		14(3-2-2)	16(3-3-2)	14(3-2-2)	14(3-2-2)	14(3-2-2)H	14(3-2-2)	14.3
	Total	39	41	39	39	39	34	38.5
Cornea (D-A)	2 Days	20(1-4)	40(2-4)E	20(1-4)	20(1-4)	20(1-4)	20(1-4)	23.3
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		14(3-2-2)	14(3-2-2)	14(3-2-2)	14(3-2-2)	14(3-2-2)H	14(3-2-2)H	14.0
	Total	39	59	39	39	39	39	42.3
Cornea (D-A)	3 Days	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20.0
Iris		5	5	0	0	0	0	1.7
Conjunctiva (RSD)		10(2-2-1)	8(2-1-1)	8(2-1-1)	8(2-1-1)	8(2-1-1)	8(2-1-1)	8.3
	Total	35	33	28	28	28	28	30.0
Cornea (D-A)	7 Days	40(2-4)EV	15(1-3)V	5(1-1)V	10(1-2)V	10(1-2)V	5(1-1)V	14.2
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		8(2-1-1)	2(1-0-0)	2(1-0-0)	4(1-1-0)	6(1-1-1)	2(1-0-0)	4.0
	Total	48	17	7	14	16	7	18.2

Key: Cornea: D=Density A=Area

Conjunctiva: R=Redness S=Swelling D=Discharge

E = Epithelial sloughing
H = Hemorrhages
V = Vascularization

TABLE 5

EYE IRRITATION TEST - ALBINO RABBITS
with T-2960CoC (five second contact)
RESULTS

Tissue	Examination Period	ANIMAL NUMBERS			MEANS
		OB 3413	OB 3416	OB 3491	
Cornea (D-A)	1 Hour	0	20(1-4)	20(1-4)	13.3
Iris		5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)	18(3-3-3)H	18(3-3-3)H	18.0
	Total	23	43	43	36.3
Cornea (D-A)	1 Day	10(1-2)E	20(1-4)	20(1-4)	16.7
Iris		5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)	18(3-3-3)	12(2-1-3)	16.0
	Total	33	43	37	37.7
Cornea (D-A)	2 Days	20(1-4)E	20(1-4)	20(1-4)E	20.0
Iris		5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)	10(2-1-2)	10(2-1-2)	12.7
	Total	43	35	35	37.7
Cornea (D-A)	3 Days	10(1-2)	20(1-4)E	15(1-3)E	15.0
Iris		0	5	5	3.3
Conjunctiva (RSD)		12(2-2-2)	10(2-1-2)	12(2-2-2)	11.3
	Total	22	35	32	29.6
Cornea (D-A)	7 Days	0	15(1-3)EV	15(1-3)EV	10.0
Iris		0	0	0	0.0
Conjunctiva (RSD)		0	10(2-1-2)	4(1-1-0)	4.7
	Total	0	25	19	14.7

E = Epithelial sloughing
V = Vascularization
H = Hemorrhage

Key: Cornea:
D=Density
A=Area

Conjunctivitis:
R=Redness
S=Swelling
D=Discharge

TABLE 6

EYE IRRITATION TEST - ALBINO RABBITS
with T-2960CoC (thirty second contact)
RESULTS

Tissue	Examination Period	ANIMAL NUMBERS			MEANS
		OB 3422	OB 3414	OB 3486	
Cornea (D-A)	1 Hour	20(1-4)	20(1-4)	20(1-4)	20.0
Iris		5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)H	18(3-3-3)H	18(3-3-3)H	18.0
	Total	43	43	43	43.0
Cornea (D-A)	1 Day	40(2-4)	40(2-4)	40(2-4)	40.0
Iris		5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)H	16(3-2-3)	18(3-3-3)H	17.3
	Total	63	61	63	62.3
Cornea (D-A)	2 Days	40(2-4)	40(2-4)	40(2-4)	40.0
Iris		5	5	5	5.0
Conjunctiva (RSD)		14(2-2-3)	10(2-1-2)	18(3-3-3)	14.0
	Total	59	55	63	59.0
Cornea (D-A)	3 Days	40(2-4)	20(1-4)E	40(2-4)	33.3
Iris		0	5	5	3.3
Conjunctiva (RSD)		12(2-2-2)	10(2-1-2)	16(3-2-3)	12.7
	Total	52	35	61	49.3
Cornea (D-A)	7 Days	40(2-4)EV	40(2-4)V	60(3-4)V	46.7
Iris		0	5	0	1.7
Conjunctiva (RSD)		12(2-2-2)	10(2-1-2)	12(2-2-2)	11.3
	Total	52	55	72	59.7

E = Epithelial sloughing
V = Vascularization
H = Hemorrhages

Key: Cornea:
D=Density
A=Area

Conjunctivitis:
R=Redness
S=Swelling
D=Discharge

Riker Experiment No.: [redacted]

APPENDIX I
PROTOCOL

TEST: Acute Ocular Irritation Test

SPONSOR: 3M [redacted] Division

CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota

TEST ARTICLE: [redacted]

CONTROL ARTICLE: [redacted]

PROPOSED STARTING/COMPLETION DATE OF TEST: 10/30 - 12/80

TEST SYSTEM AND SOURCE: Female New Zealand White Albino Rabbits, Pel-Freez, Inc., Rogers, Arkansas; [redacted], Inc., Denver, Pennsylvania

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 _____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 .1 ml with the contralateral eye of each animal serving as a control. At 1, [redacted] hours and [redacted] 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 with verbal approval of the requestor, washed eye procedures entailing a 5 and 30 second contact period with a 1 liter wash over a [redacted] period will be conducted using 3 animals per procedure. All raw data and the final report will be stored in the conducting laboratory's archives.

RECEIVED
OCT 6 1980
Safety Evaluation

[a] Purina Rabbit Chow, Ralston-Purina, St. Louis, Missouri
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics (1965)

[redacted] 10/6/80
Sponsor Date

[redacted] 10/8/80
Study Director Date

Form 19171-7-PWC

Riker Experiment No.: CR2DE82598

APPENDIX (Concluded)

Amendment to Protocol

13.

1. Due to availability male New Zealand White rabbits were used to conduct this study instead of female rabbits. (unlimited contact procedure only)

D.M. Marboe Sr. 10/21/80
Study Director Date

2. The wash will consist of 1 liter/minute for five minutes

D.M. Marboe Sr. 11/11/80
Study Director Date

3. Due to a delay in report processing the proposed completion date should be amended to 2/81

D.M. Marboe Sr. 1/12/81
Study Director Date

4.

Study Director Date

5.

Study Director Date

6.

Study Director Date

7.

Study Director Date

8.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
D. M. Markoe, Jr., BS	Acute Toxicology Toxicologist Study Director
K. D. O'Malley, BS	Advanced Toxicologist Technical Writer
K. L. Ebbens, BS	Supervisor Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore a statement signed and prepared by the Quality Assurance group is not applicable. This study was, however, audited by the Quality Assurance group.

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