CONFIDENTIAL

CODE NUMBER: 329179 (50 ppm):

ACUTE EYE IRRITATION

TEST IN THE RABBIT

PROJECT NUMBER 28/6

AUTHOR: P.P. Tomlinson

STUDY SPONSOR:

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QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm Laboratories is carried out as a continuous process designed to encompass all major phases of each study type once per month. Dates of the most recently completed series of monthly inspections relevant to the study type(s) in this report are given below.

Date(s) of Inspection and Reporting:

02/12/92, 15/12/92

This report has been audited by Safepharm Laboratories Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

Date of Report Audit:

14/01/93

J.R. Pateman C. Biol., M.I. Biol.
FOR SAFEPHARM QUALITY ASSURANCE UNIT ..... J.R. Pateman ....

DATE: ..... 20/01/93 .....

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated in the study, and the work described was performed in compliance with the following principles of Good Laboratory Practice.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health 1989.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

P. Tomlinson ..... DATE: 18.1.93

P.P. Tomlinson B.Sc. (Hons)
Study Director
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SUMMARY

STUDY SPONSOR	:	CENTRILAB
PROJECT NUMBER	:	28/6
TEST MATERIAL	:	CODE NUMBER: 329179 (50 ppm)

1. A study was performed to assess the irritancy potential of the test material to the eye of the New Zealand White rabbit. The method used followed that described in the OECD Guidelines for Testing of Chemicals (1987) No. 405 "Acute Eye Irritation/Corrosion" referenced as Method B5 in Commission Directive 84/449/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

   The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 91/325/EEC).

2. A single instillation of the test material to the non-irrigated eye of three rabbits produced no adverse ocular effects.

3. The test material produced a maximum group mean score of 0.0 and was classified as non-irritating (Class 1 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

   The test material was also classified as non-irritant according to EEC labelling regulations. No symbol and risk phrase are required.

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ACUTE EYE IRRITATION

TEST IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following a single application to the rabbit eye (Safepharm Standard Method Number OECD 5). The method used followed the recommendations of the OECD Guidelines for Testing of Chemicals (1987) No. 405 "Acute Eye Irritation/Corrosion" referenced as Method B5 in Commission Directive 84/449/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 91/325/EEC).

The test system was chosen because the rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely eye irritancy potential of the test material to man.

The study was conducted in accordance with the internationally accepted general principles of Good Laboratory Practice and Safepharm Standard Operating Procedures.

The study was performed between 29 December 1992 and 2 January 1993.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

The test material was supplied by the study sponsor as follows:

Sponsor's label	:	HEATSAUR
Sample number	:	329179
Date received	:	8 December 1992
Description	:	colourless liquid with white crystals
Container	:	brown glass bottle
Storage conditions	:	room temperature

2. TEST MATERIAL (contd)

2.1 Description, Identification and Storage Conditions (contd)

Data relating to the identity, purity and stability of the test material are the responsibility of the sponsor.

2.2 Method of Preparation

For the purpose of this study a 50 ppm dilution of the test material was prepared in distilled water.

3. TEST SYSTEM

3.1 Specification

Three New Zealand White rabbits were supplied by David Percival Ltd., Moston, Sandbach, Cheshire, U.K. At the start of the study the animals weighed 2.93 - 3.12 kg and were approximately twelve to sixteen weeks old. After a minimum acclimatisation period of five days each animal was given a number unique within the study which was written with a black indelible marker-pen on the inner surface of the ear and on a cage label.

3.2 Husbandry

The animals were individually housed in suspended metal cages. Free access to mains drinking water and food (Spillers Rabbit Diet, Dalgety Agriculture Ltd., Almondsbury, Bristol, U.K.) was allowed throughout the study.

The animal room was maintained at a temperature of 17 - 20°C and relative humidity of 41 - 50%. The rate of air exchange was approximately 15 changes per hour and the lighting was controlled by a time switch to give 12 hours light and 12 hours darkness.

4. PROCEDURE

Immediately before the start of the test, both eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect with the aid of a light source from a standard ophthalmoscope. Animals showing evidence of ocular lesions were rejected and replaced.

4. PROCEDURE (contd)

One rabbit was initially treated. A volume of 0.1 ml of the test material was instilled into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about one second immediately after instillation, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes. Immediately after administration of the test material, an assessment of the initial pain reaction was made.

After consideration of the ocular responses produced in the first treated animal, two additional animals were treated.

Assessment of ocular damage/irritation was made approximately 1 hour and 24, 48 and 72 hours following treatment, according to the numerical evaluation given in Appendix I, (from Draize J.H. 1959, Association of Food and Drug Officials of the United States, Austin, Texas, "The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics").

Any other ocular effects were also noted. Examination of the eye was facilitated by use of the light source from a standard ophthalmoscope.

5. INTERPRETATION OF RESULTS

The numerical values corresponding to each animal, tissue and observation time were recorded. The data relating to the conjunctivae were designated by the letters A (redness), B (chemosis) and C (discharge), those relating to the iris designated by the letter D and those relating to the cornea by the letters E (degree of opacity) and F (area of opacity). For each tissue the score was calculated as follows:

$$\text{Score for conjunctivae} = (A + B + C) \times 2$$

$$\text{Score for iris} = D \times 5$$

$$\text{Score for cornea} = (E \times F) \times 5$$

5. INTERPRETATION OF RESULTS (contd)

Using the numerical data obtained a modified version of the system described by Kay J.H. and Calandra J.C., J. Soc. Cosmet. Chem., 1962 13 281-289 (see Appendix II) was used to classify the ocular irritancy potential of the test material. This was achieved by adding together the scores for the cornea, iris and conjunctivae for each time point for each rabbit. The group means of the total scores for each observation were calculated. The highest of these group means (the maximum group mean score) together with the persistence of the reactions enabled classification of the eye irritancy potential of the test material.

The results were also interpreted according to Commission Directive 91/325/EEC which adapts Council Directive 67/548/EEC on the regulations relating to the classification, packaging and labelling of dangerous substances, as follows:

i) Interpretation according to Annex VI Part II (B) Eye Irritation Criteria

The test material will be classified as irritant and will require the appropriate "Xi" symbol if ocular lesions occur within 72 hours after exposure, persist for at least 24 hours and correspond to one or more of the following mean values in two or more animals:

- corneal opacity	2 or more
- iridial lesion	1 or more
- redness of conjunctivae	2.5 or more
- chemosis of conjunctivae	2 or more

The 24, 48 and 72-hour readings for each animal will be used to calculate the mean values.

If these criteria are not satisfied the test material will be classified as non-irritant.

5. INTERPRETATION OF RESULTS (contd)

ii) Interpretation according to Annex VI Part II (D)

In addition the following risk (R) phrases will be assigned to the test material, if appropriate, according to the criteria indicated below:

R 36 "IRRITATING TO EYES"

If, when applied to the eye of three rabbits, significant ocular lesions are caused which are present 24 hours or more after the instillation of the test material in two or more animals. Ocular lesions are significant if the mean of the 24, 48 and 72-hour readings comply with any of the following criteria:

- corneal opacity	equal to or greater than 2 but less than 3
- iridial lesion	equal to or greater than 1
- conjunctival redness	equal to or greater than 2.5
- conjunctival chemosis	equal to or greater than 2

R 41 "RISK OF SERIOUS DAMAGE TO EYES"

If, when applied to the eye of three rabbits, severe ocular lesions are caused in two or more animals which are present 24 hours or more after instillation of the test material. Ocular lesions are severe if the mean of the 24, 48 and 72-hour readings comply with either of the following criteria:

- corneal opacity	equal to or greater than 3
- iridial lesion	equal to 2

6. ARCHIVES

Unless instructed otherwise by the sponsor, all original data and a copy of the final report will be retained in the archives of Safepharm Laboratories for a period of ten years. After this period, the sponsor's instructions will be sought.

7. RESULTS

Individual and group mean scores for ocular irritation are given in Tables 1 and 2. The individual mean scores as required for the EEC labelling regulations are presented in Table 3.

No adverse ocular effects were noted during the study.

8. CONCLUSION

The test material, CODE NUMBER: 329179 (50 ppm), produced a maximum group mean score of 0.0 and was classified as NON-IRRITATING (CLASS 1 ON A 1 TO 8 SCALE) to the rabbit eye according to a modified Kay and Calandra classification system.

The test material did not produce positive criteria in any rabbit according to the EEC labelling regulations and was classified as NON-IRRITANT to the rabbit eye. No symbol and risk phrase are therefore required.

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TABLE 1 INDIVIDUAL SCORES AND INDIVIDUAL TOTAL SCORES FOR OCULAR IRRITATION

Rabbit Number and Sex (Bodyweight Kg)	IPR = 0 95 Female (3.12)				IPR = 0 110 Female (2.93)				IPR = 0 109 Female (3.00)			
Time After Treatment	1 hr	24 hr	48 hr	72 hr	1 hr	24 hr	48 hr	72 hr	1 hr	24 hr	48 hr	72 hr
CORNEA												
E = Degree of Opacity	0	0	0	0	0	0	0	0	0	0	0	0
F = Area of Opacity	0	0	0	0	0	0	0	0	0	0	0	0
Score (E x F) x 5	0	0	0	0	0	0	0	0	0	0	0	0
IRIS												
D	0	0	0	0	0	0	0	0	0	0	0	0
Score (D x 5)	0	0	0	0	0	0	0	0	0	0	0	0
CONJUNCTIVAE												
A = Redness	0	0	0	0	0	0	0	0	0	0	0	0
B = Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C = Discharge	0	0	0	0	0	0	0	0	0	0	0	0
Score (A + B + C) x 2	0	0	0	0	0	0	0	0	0	0	0	0
Total Score	0	0	0	0	0	0	0	0	0	0	0	0

Key: hr = hour(s) IPR = initial pain reaction

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T A B L E 2 INDIVIDUAL TOTAL SCORES AND GROUP MEAN SCORES FOR OCULAR IRRITATION

Rabbit Number and Sex	Individual Total Scores At: 1 Hour	24 Hours	48 Hours	72 Hours
95 Female	0	0	0	0
110 Female	0	0	0	0
109 Female	0	0	0	0
Group Total	0	0	0	0
Group Mean Score	0.0 1 Hour	0.0 24 Hours	0.0 48 Hours	0.0 72 Hours

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TABLE 3 INDIVIDUAL & MEAN SCORES FOR CORNEA, IRIS & CONJUNCTIVAE REQUIRED FOR EEC LABELLING REGULATIONS

Rabbit Number & Sex (Bodyweight Kg)	Time After Treatment	Corneal Opacity	Iridial Inflammation	Conjunctival Redness	Conjunctival Chemosis
95 Female (3.12)	24 Hours	0	0	0	0
	48 Hours	0	0	0	0
	72 Hours	0	0	0	0
Total		0	0	0	0
Mean		0.0	0.0	0.0	0.0
110 Female (2.93)	24 Hours	0	0	0	0
	48 Hours	0	0	0	0
	72 Hours	0	0	0	0
Total		0	0	0	0
Mean		0.0	0.0	0.0	0.0
109 Female (3.00)	24 Hours	0	0	0	0
	48 Hours	0	0	0	0
	72 Hours	0	0	0	0
Total		0	0	0	0
Mean		0.0	0.0	0.0	0.0

A P P E N D I C E S

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A P P E N D I X I

DRAIZE SCALE FOR SCORING OCULAR IRRITATION

1. CONJUNCTIVAE

(A) Redness (refers to palpebral and bulbar conjunctivae 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

(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 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 a considerable area around the eye	3

THE TOTAL SCORE = (A + B + C) x 2 MAXIMUM TOTAL = 20

2. IRIS

(D) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris stil reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

THE TOTAL SCORE = D x 5 MAXIMUM TOTAL = 10

3. CORNEA

(E) Degree of Opacity (most dense area used)

No opacity	0
Scattered or diffuse areas, 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

(F) 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

THE TOTAL SCORE = (E x F) x 5 MAXIMUM TOTAL = 80

MAXIMUM TOTAL SCORE POSSIBLE = 110

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APPENDIX II

MODIFIED KAY AND CALANDRA INTERPRETATION OF EYE IRRITATION TEST

MAXIMUM MEAN SCORE	PERSISTENCE OF SCORE	DESCRIPTION RATING (AND CLASS)	
0.0 to 0.5	Group mean score at 24 hours = 0	Non-irritating	(1)
	Group mean score at 24 hours > 0	Practically non-irritating	(2)
0.5 to 2.5	Group mean score at 24 hours = 0	Non-irritating	(1)
	Group mean score at 24 hours > 0	Practically non-irritating	(2)
2.5 to 15	Group mean score at 48 hours = 0	Minimal irritant	(3)
	Group mean score at 48 hours > 0	Mild irritant	(4)
15 to 25	Group mean score at 72 hours = 0	Mild irritant	(4)
	Group mean score at 72 hours > 0	Moderate irritant	(5)
25 to 50	Group mean score at 7 days — 20 or less — {more than half of the individual {total scores at 7 days 10 or less	Moderate irritant	(5)
	Group mean score at 7 days — 20 or less — {more than half of the individual {total scores at 7 days > 10 but {no individual total score at {7 days > 30	Moderate irritant	(5)
	Group mean score at 7 days — 20 or less — {more than half of the individual {total scores at 7 days > 10 {and any individual score at {7 days > 30	Severe irritant	(6)
	Group mean score at 7 days > 20	Severe irritant	(6)
50 to 80	Group mean score at 7 days — 40 or less — {more than half of the individual {total scores at 7 days 30 or less	Severe irritant	(6)
	Group mean score at 7 days — 40 or less — {more than half of the individual {total scores at 7 days > 30 but {no individual total score at {7 days > 60	Severe irritant	(6)
	Group mean score at 7 days — 40 or less — {more than half of the individual {total scores at 7 days > 30 {and any individual score at {7 days > 60	Very severe irritant	(7)
	Group mean score at 7 days > 40	Very severe irritant	(7)
80 to 100	Group mean score at 7 days — 80 or less — {more than half of the individual {total scores at 7 days 60 or less	Very severe irritant	(7)
	Group mean score at 7 days — 80 or less — {more than half of the individual {total scores at 7 days > 60 but {no individual total score at {7 days > 100	Very severe irritant	(7)
	Group mean score at 7 days — 80 or less — {more than half of the individual {total scores at 7 days > 60 {and any individual score at {7 days > 100	Extremely severe irritant	(8)
	Group mean score at 7 days > 80	Extremely severe irritant	(8)
100 - 110	Group mean score at 7 days 80 or less	Very severe irritant	(7)
	Group mean score at 7 days > 80	Extremely severe irritant	(8)

APPENDIX III

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Laboratories Limited
PO Box 45
Derby
DE1 2BT

DATE OF INSPECTION

17 March 1992

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

D.F. Moore

11/6/92. D. F. Moore
Director
UK GLP Monitoring Unit