Report Title: Irritant Effects on the Rabbit Eye of E2414.01

Test Type: Ocular Irritation

Conducting Laboratory and Location: Huntingdon Research Centre, England

Test Substance(s): #E2414.01 – 1% Octopirox in shampoo. Undiluted material was tested.

Species: Rabbit

# of Animals: 3 to 6 rabbits tested

Test Conditions: Group 1: 0.01 ml, undiluted, eyes un-rinsed, six rabbits
Group 2: 0.01 ml, undiluted, eyes rinsed after 4 seconds, three rabbits

Results: Group 1: MAS = 11,test material is a moderate irritant
Group 2: MAS < 1

Study #: ECM BTS 930
Report Date: 11/30/84

Accession #: 31973

#1

BCM BTS : 930

BIOLOGICAL TEST FOR SAFETY : EUROPEAN OPERATIONS REQUEST DOCUMENT (EORD).

A

Originator: A. Cairns (Name) 17/5/84 (Date) — Originator's SH: T.C. Bell (Name) 24/5/84 (Date) — Originator's AD: R.E. Atkinson (Name) (Date)

Toxicologist: D.J.G. Muller (Name) 3/5/84 (Date) — P&RS SH: G. Calvin (Name) 7.5.84 (Date) — P&RS Manager: T.M. McCarthy (Name) 28/8/84 (Date)

Estimated Total Cost : £ 1400

Estimated Dates of : Availability of Analytical Data : _____

Receipt of Report : Summer 1984

Product Coordination Manager : J.B. Camden (Name) 27/7/84 (Date)

B

Name of Test Substance : Head & Shoulder AR w/ 1% Octopirox

Identification Number E-2414.01

Substances required by (date) _____

Agreed by PDP _____

TSCR Completion Date _____

			Panel/Blind Test	Ship Test	Test Market	National
Type of Consumer Exposure(s) for Which Safety Clearance is Requested	No. of Subjects	:	200			
	Starting Date	:	fall '84			
	Duration	:	6 weeks			
	Location	:	UK			

C

Safety Test Requirements (By P&RS)

Tests :

	Amount of Substance Needed	Cost
1. LD_{50} (rat), Up & Down method	200 g	£ 300
2. Eye Irritation (rabbit, low volume protocol)	20 g	£ 250
3. Skin Irritation (rabbit)	20 g	£ 100
4. Skin Sensitization (guinea Pig)	100 g	£ 750

D.J.G. Muller
Toxicologist

3-5-84
Date

/mp.w - 0229M

CONFIDENTIAL

B49B1D/P&G 1164/SE

IRRITANT EFFECTS ON THE RABBIT EYE OF S 2414.01

(Project ECM BTS 930)

Addressee:

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15 November 1984
Re-issued amended copies, 30 November 1984

84981D/RAC 1164/SE

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Michael P. Liggett,
Head of Unit - Acute Studies
Department of Industrial Toxicology

Brenda I. Parcell,
Assistant Scientific Officer,
Department of Industrial Toxicology

Sample designation: E 2414.01.

Examination for: Irritant effects on the rabbit eye.

1. INTRODUCTION

1.1. This study was designed to assess eye irritation potential.

The test substance may come into contact with the eye during handling or use.

1.2. The albino rabbit was chosen as it has been shown to be a suitable model for eye irritation studies and is the animal recommended in the test protocol.

1.3. The study plan was agreed by the Study Director on 19 October 1984 and the study was undertaken between 22 October and 5 November 1984.

2. TEST SUBSTANCE

2.1. E 2414.01, a pale blue viscous liquid was received on 12 October 1984 and was stored at ambient temperature.

2.2. The stability and absorption of the test substance were not determined.

2.3. The test substance was administered as supplied by the Sponsor.

3. EXPERIMENTAL PROCEDURE

3.1. Protocol

The experimental procedure used was based on Procter and Gamble Limited Standard Procedure No. C2B.

3.2. Animal management

3.2.1. Nine New Zealand White strain rabbits in the weight range 2.3 to 2.9 kg and approximately 10 to 13 weeks of age were obtained from Buxted Rabbits, Buxted, Sussex. The rabbits selected for the study were all acclimated to the laboratory environment.

3.2.2. Each animal was identified by a numbered aluminium tag placed through the edge of one ear. This number was unique within the HRC Industrial Toxicology Department throughout the duration of the study.

3.2.3. The animals were allocated to the following two treatment groups:

Group 1. E 2414.01, 0.01 ml, undiluted, eyes unrinsed - six rabbits.

Group 2. E 2414.01, 0.01 ml, undiluted, eyes rinsed after 4 seconds - three rabbits.

3.2.4. The rabbits were individually housed in metal cages with perforated floors in Building R 17 Room 6. They had free access to tap water and Grain Harvester Special Rabbit Diet 474.

3.2.5. Animal room temperature was maintained at approximately 19°C and relative humidity at 30-70%.

Air exchange was maintained at approximately 19 air changes per hour and lighting was controlled to give 12 hours of artificial light in each 24 hour period.

3.2.6. All animals were observed daily for signs of ill health or toxic signs.

3.3. Treatment procedure

3.3.1. The eyes of each animal were examined prior to instillation of the test substance to ensure that there was no pre-existing corneal damage or conjunctival inflammation.

3.3.2. A 0.01 ml aliquot of E 2414.01, undiluted, was placed directly onto the corneal surface of one eye of each animal.

The eyelids were then gently held together for one second before releasing. The contralateral eye remained untreated and served as a control.

3.3.3. Approximately 4 seconds after instillation of the test substance the treated eyes of animals in Group 2 were rinsed using 20 ml of lukewarm tap water.

3.4. Observations and scoring

3.4.1. Examination of the eyes was made 1, 2, 3, 4, 7 and 14 days after instillation. Observation of the eyes was aided by the use of a handheld torch.

3.4.2. Grading and scoring of the ocular lesions were performed based on the following numerical scoring system*:

3.4.2.1. 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 half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4

A x B x 5 Total maximum = 80

3.4.2.2. 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, haemorrhage, gross destruction (any or all of these)	2

A x 5 Total maximum = 10

* Draize J.H. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Assn. of Food and Drug Officials of the United States. p. 51 (1959).

3.4.2.3. 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 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

Score (A + B + C) x 2 Total maximum = 20

The total score for the eye (P&G score) is the sum of all the scores obtained for the cornea, iris and conjunctivae.

4. MAXIMUM AVERAGE SCORE

The maximum average score (MAS) was calculated by averaging the highest total P&G score on any one day.

5. MEDIAN RECOVERY TIME

The median number of days for the eyes to clear was calculated by placing the animals in sequential order with regard to persistence of the response to treatment. The average number of days of the eyes of the third and fourth animal in the series to become normal was then calculated to the nearest 0.1 day.

6. CLASSIFICATION

6.1. The test substance is considered to be non-irritant when no corneal damage, no iridial inflammation or conjunctival grades in excess of 1 are observed at the 24 hours reading.

6.2. A slight irritant is characterised by any of the following changes at the 24 hours or subsequent readings, which clear by Day 7:

6.2.1. Opacity of cornea (other than a slight dulling)

6.2.2. Iridial inflammation

6.2.3. Conjunctival grades 2 and above

6.2.4. Conjunctival grades of 1 persisting beyond 24 hours

6.3. A moderate irritant is characterised by changes/lesions in the ocular tissues persisting beyond 7 days but clearing by Day 21.

6.4. A severe irritant is a test substance that produces reactions persisting beyond Day 21 or that produces a necrotic lesion.

7. ARCHIVES

All specimens, raw data and other documents generated at HRC during the course of this study, together with a copy of this Final Report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

8. RESULTS

The numerical scores awarded to the ocular reactions elicited by E 2414.01 are given in Tables 1 and 2 and calculation of the MAS in Table 3.

E 2414.01, 0.01 ml, no rinse (Table 1)

Corneal opacities developed in two animals and dulling of the normal lustre of the cornea was seen in two other animals.

Temporary iritis was observed in two animals.

A diffuse crimson red colouration of the conjunctivae was seen in four animals and was accompanied in two animals by considerable swelling with partial eversion of the eyelids. Considerable discharge was seen in one animal.

Transient mild conjunctival inflammination was seen in one animal.

The MAS was calculated to be: 11

The median recovery time was calculated to be: 7 days.

E 2414.01, 0.01 ml, eyes rinsed after 4 seconds (Table 2)

No corneal damage or iridial inflammation was seen throughout the observation period.

Transient mild conjunctival inflammation was seen in one animal.

No reactions to treatment were seen in the remaining two animals during the observation period.

The MAS was calculated to be: <1.

9. CONCLUSION

E 2414.01 is considered to be a moderate irritant.

TABLE 1 849810/P&G 1164/SE

Ocular reactions elicited by E 2414.01, no rinse

Animal No./Sex	Region of eye		Day after instillation 1	2	3	4	7
1314♂	Area of corneal opacity						
	Cornea	Opacity	D	D	D	0	0
		Area	4	4	4	0	0
		Ulceration	0	0	0	0	0
		Stippling	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Iris	Value	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Conjunctiva	Redness	2	1	0	0	0
		Chemosis	1	0	0	0	0
		Discharge	1	0	0	0	0
		Necrosis	0	0	0	0	0
		P & G Score	8	2	0	0	0
	TOTAL P & G Score		8	2	0	0	0
1315♂	Area of corneal opacity						
	Cornea	Opacity	1	1	1	0	0
		Area	2	1	1	0	0
		Ulceration	0	0	0	0	0
		Stippling	0	0	0	0	0
		P & G Score	10	5	5	0	0
	Iris	Value	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Conjunctiva	Redness	1	2	1	1	0
		Chemosis	2	1	1	0	0
		Discharge	2	1	1	0	0
		Necrosis	0	0	0	0	0
		P & G Score	10	8	6	2	0
	TOTAL P & G Score		20	13	11	2	0
1316♂	Area of corneal opacity						
	Cornea	Opacity	0	0	0	0	0
		Area	0	0	0	0	0
		Ulceration	0	0	0	0	0
		Stippling	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Iris	Value	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Chemosis	0	0	0	0	0
		Discharge	0	0	0	0	0
		Necrosis	0	0	0	0	0
		P & G Score	2	0	0	0	0
	TOTAL P & G Score		2	0	0	0	0

(continued)

Animal No./Sex	Region of eye		Day after instillation 1	2	3	4	7	14
1325♂	Area of corneal opacity							
	Cornea	Opacity	0	0	0	0	0	0
		Area	0	0	0	0	0	0
		Ulceration	0	0	0	0	0	0
		Stippling	0	0	0	0	0	0
		P & G Score	0	0	0	0	0	0
	Iris	Value	0	0	0	0	0	0
		P & G Score	0	0	0	0	0	0
	Conjunctiva	Redness	2	2	1	1	0	0
		Chemosis	0	0	1	0	0	0
		Discharge	1	1	1	0	0	0
		Necrosis	0	0	0	0	0	0
		P & G Score	4	6	6	2	0	0
	TOTAL P & G Score		4	6	6	2	0	0
1327♂	Area of corneal opacity							
	Cornea	Opacity	0	D	D	D	0	0
		Area	0	4	3	4	0	0
		Ulceration	0	0	0	0	0	0
		Stippling	0	0	0	0	0	0
		P & G Score	0	0	0	0	0	0
	Iris	Value	0	1	1	1	0	0
		P & G Score	0	5	5	5	0	0
	Conjunctiva	Redness	1	1	1	1	0	0
		Chemosis	1	1	0	0	0	0
		Discharge	1	0	0	0	0	0
		Necrosis	0	0	0	0	0	0
		P & G Score	6	4	2	2	0	0
	TOTAL P & G Score		6	9	7	7	0	0
1329♂	Area of corneal opacity							
	Cornea	Opacity	1	1	1	1	0	0
		Area	2	3	3	3	0	0
		Ulceration	0	0	0	0	0	0
		Stippling	0	0	0	0	0	0
		P & G Score	10	15	15	15	0	0
	Iris	Value	1	1	1	0	0	0
		P & G Score	5	5	5	0	0	0
	Conjunctiva	Redness	2	2	2	2	1	0
		Chemosis	1	2	2	1	0	0
		Discharge	2	3	2	1	0	0
		Necrosis	0	0	0	0	0	0
		P & G Score	10	14	12	8	2	1
	TOTAL P & G Score		25	34	32	23	2	0

D = Dulling

TABLE 2

Ocular reactions elicited by S2414.01, eyes rinsed after 4 seconds

Animal No./Sex	Region of eye		Day after application 1	3	5	4	7
1330♂	Area of corneal opacity						
	Cornea	Opacity	0	0	0	0	0
		Area	0	0	0	0	0
		Ulceration	0	0	0	0	0
		Stippling	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Iris	Value	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Conjunctiva	Redness	0	0	0	0	0
		Chemosis	0	0	0	0	0
		Discharge	0	0	0	0	0
		Necrosis	0	0	0	0	0
		P & G Score	0	0	0	0	0
	TOTAL P & G Score		0	0	0	0	0
1331♂	Area of corneal opacity						
	Cornea	Opacity	0	0	0	0	0
		Area	0	0	0	0	0
		Ulceration	0	0	0	0	0
		Stippling	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Iris	Value	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Chemosis	0	0	0	0	0
		Discharge	0	0	0	0	0
		Necrosis	0	0	0	0	0
		P & G Score	2	0	0	0	0
	TOTAL P & G Score		2	0	0	0	0
1332♂	Area of corneal opacity						
	Cornea	Opacity	0	0	0	0	0
		Area	0	0	0	0	0
		Ulceration	0	0	0	0	0
		Stippling	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Iris	Value	0	0	0	0	0
		P & G Score	0	0	0	0	0
	Conjunctiva	Redness	0	0	0	0	0
		Chemosis	0	0	0	0	0
		Discharge	0	0	0	0	0
		Necrosis	0	0	0	0	0
		P & G Score	0	0	0	0	0
	TOTAL P & G Score		0	0	0	0	0

TABLE 3

Total P & G scores and maximum average scores (MAS) elicited by E 3414.01

Treatment	Animal Number/ Sex	Day after instillation					
		1	2	3	4	7	14
0.01 ml, no rinse	1314♂	8	2	0	0	0	
	1315♂	20	13	11	2	2	
	1316♂	2	0	0	0	0	
	1325♂	4	6	6	2	0	
	1327♂	6	9	7	7	0	
	1329♂	25	34	32	23	2	0
	Average	11	11	9	6	<1	0
	MAS	11					
0.01 ml, rinse after 4 seconds	1330♂	0	0	0	0	0	
	1331♂	2	0	0	0	0	
	1332♂	0	0	0	0	0	
	Average	<1	0	0	0	0	
	MAS	<1					