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Technical Division  
Safety, Health & Environmental Affairs  
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File \_\_\_\_\_

86/PTC016/686

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IMAGINE

RC9927 :  
ACUTE EYE IRRITATION/CORROSION  
TEST IN THE RABBIT

PENNWALT CORPORATION  
Technical Division  
FEB 11 1987  
Technical Records Center

To:  
Pennwalt Corporation  
900, 1st Avenue  
P.O. Box C  
King of Prussia  
Pennsylvania 19406-00181

From:  
Life Science Research Limited  
Eye  
Suffolk IP23 7PX  
England



RC9927 :  
ACUTE EYE IRRITATION/CORROSION  
TEST IN THE RABBIT

LSR Report No: 86/PTC016/686

We the undersigned hereby declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study. The aspects of the study conducted by Life Science Research were performed essentially in accordance with current OECD Good Laboratory Practice Principles and current EPA (TSCA) Good Laboratory Practice Standards, relating to non-clinical studies.

In line with normal practice in this type of study, no analysis of the test material was received from the Sponsor.

The Study Director fulfilled the responsibilities required by these regulations.

K. D. Smith, B.Sc.  
Study Director

*K D Smith*  
.....  
...21 January 1987

H. A. Cummins, B.Sc.  
Head, Sub-Department of  
Short-Term Toxicology

*HAC*  
.....  
...21 January 1987



RC9927 :  
ACUTE EYE IRRITATION/CORROSION  
TEST IN THE RABBIT

LSR Report No: 86/PTC016/686

QUALITY ASSURANCE INSPECTIONS

Dates (Day/Month/Year)			
Inspection		Report to Study Director	Report to Management
<u>PROTOCOL</u>			
Inspection of protocol was made in accordance with LSR Standard Operating Procedure QAU/020. Dates for inspection of protocol amendments in accordance with this S.O.P. are not quoted		25.9.86	26.9.86
<u>DATA</u>			
Inspection of data generated on this type of study was made in accordance with LSR Standard Operating Procedure QAU/050		16.10.86	16.10.86
<u>PROCEDURES</u>			
Inspection of procedures on this type of study was made in accordance with LSR Standard Operating Procedure QAU/040		11.9.86 23.9.86 30.9.86 4.11.86	22.9.86 23.9.86 30.9.86 27.11.86

Other routine procedures used in this type of study, and facilities were inspected regularly and reports made in accordance with LSR Standard Operating Procedure QAU/040.

This report has been reviewed by the LSR Quality Assurance Unit employing methods laid down in LSR Standard Operating Procedure QAU/060. The reported methods and procedures were found to describe those used and the results to constitute an accurate representation of the data recorded.

This review was completed on: 19 January 1987

D. J. Ford, B.Sc., Ph.D.  
(Head of Quality Assurance Unit)

.....  
.....20 January 1987

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## SAFETY, HEALTH AND ENVIRONMENTAL AFFAIRS

## SUMMARY OF TOXICOLOGY STUDY

## PERFORMED FOR THE VENTURE GROUP

Test Material: FR-45B

Product Code: RC 9927

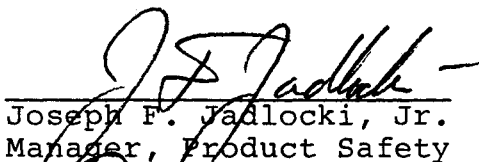
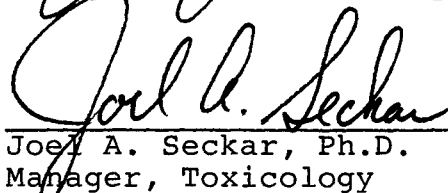
Study Type: Primary Eye Irritation

Testing Laboratory: Life Science Research  
Elm Farm  
Eye, Suffolk IP23 7PX, England

Summary of Results: Conjunctival irritation at one hour in all rabbits together with discharge in 4 of 6 rabbits. Effects recovered within 24 hour.

Storage: The report is filed in the Technical Records Center at King of Prussia under Master No. 22227.

Information for MSDS: Slight conjunctival irritation resolved within 24 hours.

Signatures  
Joseph F. Jadlocki, Jr.  
Manager, Product Safety3-11-87  
Date  
Joel A. Seckar, Ph.D.  
Manager, Toxicology3/11/87  
Date

11/72

1. SUMMARY

- 1.1 The potential of RC9927 to cause damage to the conjunctivae, iris or cornea was assessed in six New Zealand White rabbits, each subject to single ocular instillation of 0.1 ml of the test material. Ocular reactions were examined 1, 24, 48 and 72 hours after treatment.
- 1.2 A crimson-red appearance to the conjunctiva or slight injection of the conjunctival blood vessels and an ocular discharge, were observed among the rabbits one hour after instillation of RC9927. No other change was apparent.  
  
All ocular irritation responses had resolved by the 24-hour examination.
- 1.3 Practically none or no initial pain response was observed among the rabbits following instillation of the test material.
- 1.4 Under the conditions of this test, RC9927 was 'very slightly irritating' to the eye.

## 2. INTRODUCTION

The objective of the Acute Eye Irritation/Corrosion Test was the prediction of the potential of the test material to cause damage to the conjunctivae, cornea or iris after a single exposure of indeterminate duration.

The study was designed to conform with Section 4, sub-section 405 of the O.E.C.D. Guidelines for Testing of Chemicals (1981) and the EPA Toxic Substances Control Act Test Guidelines (1985).

The experimental work was carried out at the Elm Farm Laboratories of Life Science Research during the period :

13 October - 17 October 1986

## 3. MATERIAL

A consignment of 502 g (stated nett) RC9927, a slightly viscous, light yellow liquid, was received at the Elm Farm Laboratories on 1 September 1986. It was further identified by the code No. 6159-199-3.

The material was kept at ambient temperature, in the original container.

The identity, strength and purity of the test material received, and its stability under the storage conditions above, were the responsibility of the Sponsor.

#### 4. METHODS

##### 4.1 Animals and husbandry

Young albino rabbits of an outbred strain of New Zealand White were supplied by Ranch Rabbits, Crawley Down, Sussex, England. The animals used on this study were within the bodyweight range 2.10 - 2.55 kg on arrival. The rabbits were housed individually in suspended stainless steel cages in batteries manufactured by Cope and Cope Limited. The cages measured 0.6 x 0.6 x 0.4 m high and were fitted with mesh floors and automatic watering. A sledge running the length of the battery removed waste matter as required. Animals had free access to food hoppers containing pelleted diet, S.Q.C. Rabbit Diet (Special Diet Services Limited, Witham, Essex). The manufacturer supplied analytical data with each batch of diet supplied. This included the concentration of nutritional components, aflatoxins and selected heavy metals, pesticides and micro-organisms. The diet contains no added antibiotic or other chemotherapeutic or prophylactic treatment. The rabbits had free access to tap water supplied to each cage by an automatic piped system. The water was derived from a protected subterranean source to meet World Health Organisation European Standards for quality of drinking water. Reports from the local Water Authority record the chemical and bacteriological quality of the water. There was no information that normal levels of common contaminants, or any specific contaminants, would influence the outcome of the study.

##### 4.2 Environmental control

The animals were housed in a lagomorph room within a limited access building. The room was kept at a slight positive pressure relative to the outside and had its own filtered air supply giving approximately 12 complete air changes per hour without re-circulation.

The maximum and minimum temperature of the previous 24-hour period and relative humidity values were recorded at the beginning of each working day. Environmental control equipment was set to achieve target values of 18°C (range 15°-23°C) and 55% R.H. (range 40%-70% R.H.), respectively. Electric time-switches controlled a lighting cycle of 14 hours artificial light per day. An emergency generator was available to maintain electricity supply in the event of a power failure.

All personnel entering the building changed into clean protective clothing and wore an additional gown, plastic over-shoes, gloves and a face mask to service animal-holding areas.



#### 4.3 Pre-exposure

Clean cages were prepared on the day before delivery of the animals. Labels were affixed specifying the date of arrival, supplier, sex of the animal and Home Office licence number of the study supervisor. On arrival, each rabbit was inspected before being accepted. Individual bodyweight was recorded for each stock animal on the day of receipt and at regular intervals thereafter, until the rabbit began treatment. A tag bearing a unique reference number was attached to the ear of each rabbit within 24 hours of arrival, the reference number was recorded on the cage-label and stock records. An acclimatisation period of at least seven days was allowed between assignment to stock and administration of the test material.

A daily check on the general condition of the animals was recorded and the record consulted before each animal was accepted for use on study.

Both eyes of each animal were examined on the day before administration for signs of pre-existing irritation, reaction or abnormality which would eliminate it from the study. Each cage was re-labelled with details of the schedule number, ear-tag number, sex, route of administration, treatment level, responsible licensee and day of dosing the cage occupant.

Bodyweights on the day of dosing were within the range 2.74 - 3.54 g. The rabbits were at least three months old at this time.

#### 4.4 Constitution of test groups

Six acclimatised rabbits (four males and two females) were allocated to this study.

#### 4.5 Preparation of test material

An appropriate quantity of the test material, as provided by the Sponsor, was dispensed on the morning of Day 1.

#### 4.6 Administration of test material

The animal was gently restrained. The dose was instilled into the right eye of each animal by pulling the lower eyelid away from the eyeball to form a cup into which 0.1 ml of the test material was dropped. The eyelids were gently held together for one second and then released. The left eye remained untreated.

#### 4.7 Observation period

The behaviour of the rabbit was observed for several minutes immediately following instillation of the test material to allow assessment of the initial pain response by the criteria detailed in section 4.8.

The animals were returned to their cages and checked at least twice during the first hour after dosing and at regular intervals throughout the day to ensure no severe injury passed unnoticed. Ocular reactions to treatment were assessed one hour, 24, 48 and 72 hours after treatment, according to the criteria detailed in sections 4.9 and 4.10.

The untreated eye was used as a comparison with the treated eye during assessment of ocular lesions.

All animals were checked daily to ensure the treated eyes were not subject to infection or causing distress.

#### 4.8 Criteria for assessment of pain response (not a component of the OECD or EPA test)

<u>Reaction of animal to instillation of test material</u>	<u>Class</u>	<u>Descriptive Rating</u>
No response	0	No initial pain
A few blinks only, normal within one or two minutes	1	Practically no initial pain
Rabbit blinks and tries to open eye but the reflexes close it	2	Slight initial pain
Rabbit holds eye shut and puts pressure on lids; may rub eye with paw	3	Moderate initial pain
Rabbit holds eye shut vigorously, may squeal	4	Severe initial pain
Rabbit holds eye shut vigorously, may squeal, claw at eye and try to escape	5	Very severe initial pain

#### 4.9 Criteria for assessment of ocular lesions

<u>Cornea</u>	<u>Grade</u>
No ulceration or opacity	0
Scattered or diffuse areas of opacity, (other than slight dulling of normal lustre) details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Complete corneal opacity, iris not discernible pannus formation or ulceration	4

The presence or absence of ulceration or stippling of the cornea was designated by '+' : positive or '-' : negative.

<u>Iris</u>	<u>Grade</u>
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or any combination of them), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

<u>Conjunctivae</u>	<u>Grade</u>
<u>Redness</u> (refers to palpebral and bulbar conjunctivae, excluding cornea and iris)	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson-red, individual vessels not easily discernible	2*
Diffuse beefy-red	3

\* These figures are the lowest grades considered 'positive' under the OECD and EPA regulations.

<u>Chemosis</u>	<u>Grade</u>
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4
* These figures are the lowest grades considered 'positive' under the OECD and EPA regulations.	

4.10 Criteria for assessment of ocular lesions  
(not a component of OECD and EPA regulations)

<u>Cornea</u>	<u>Grade</u>
<u>Area of cornea affected by lesion</u>	
One-quarter or less, but not zero	1
Greater than one-quarter, less than one-half	2
Greater than one-half, less than three-quarters	3
Greater than three-quarters, up to whole area	4
 <u>Conjunctivae</u>	
<u>Discharge</u>	
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 the lids	2
Discharge with moistening of the lids and hairs and affecting a considerable area around the eye	3

4.11 Apparatus available to facilitate inspection of eyes

A pencil beam torch and ophthalmoscope were used to facilitate inspection of the eyes.

4.12 Mortality and termination procedure

Animals were sacrificed by intravenous injection of sodium pentobarbitone B.Vet.C (Expiral-Ceva Limited) once resolution of the effects of treatment had been confirmed.

4.13 Name and address of facilities

Life Science Research Limited  
Eye, Suffolk, IP23 7PX  
England

A full list of apparatus, diets, etc., and the names and addresses of the suppliers concerned is maintained by the Chief Animal Technician, Department of Animal Management, Life Science Research, Eye, Suffolk.

Original data pertaining to this study are held in the archives of Life Science Research.

5. RESULTS (Tables 1 and 2)

A crimson-red appearance to the conjunctiva or slight injection of the conjunctival blood vessels was observed in all six rabbits, and an ocular discharge in four animals, one hour after instillation of 0.1 ml RC9927. No other change was apparent.

All ocular irritation responses had resolved by the 24-hour examination.

Practically none or no initial pain response was observed among the rabbits following instillation of the test material.

6. CONCLUSION

Under the conditions of this test, RC9927 was 'very slightly irritating' to the eye.

TABLE 1

Mean values for ocular lesions, one hour,  
24, 48 and 72 hours after treatment<sup>+</sup>

Type of response	Time after instillation			
	1 hour	24 hours	48 hours	72 hours
Corneal opacity	0.0	0.0	0.0	0.0
Iridial lesions	0.0	0.0	0.0	0.0
Redness of conjunctivae	1.2	0.0	0.0	0.0
Chemosis	0.0	0.0	0.0	0.0

+ The mean values were calculated using the individual scores for all rabbits on each occasion of examination

TABLE 2

Grades for ocular irritation responses\*  
following instillation of 0.1 ml RC9927

Animal No: 12TX 848		Pain evaluation response: 1			
Region of the eye	Response	Grade of response at time after instillation			
		1	24	48	72
Cornea	Opacity	0	0	0	0
	Area	0	0	0	0
	Ulceration	-	-	-	-
	Stippling	-	-	-	-
Iris	Value	0	0	0	0
Conjunctiva	Redness	2	0	0	0
	Chemosis	0	0	0	0
	Discharge	2	0	0	0
	Necrosis	-	-	-	-
	Ulceration	-	-	-	-

\* Criteria documented in Sections 4.8 to 4.10



TABLE 2 - continued

Grades for ocular irritation responses\*  
following instillation of 0.1 ml RC9927

Animal No: 12TX 850		Pain evaluation response: 1			
Region of the eye	Response	Grade of response at time after instillation			
		Hour			
		1	24	48	72
Cornea	Opacity	0	0	0	0
	Area	0	0	0	0
	Ulceration	-	-	-	-
	Stippling	-	-	-	-
Iris	Value	0	0	0	0
Conjunctiva	Redness	1	0	0	0
	Chemosis	0	0	0	0
	Discharge	1	0	0	0
	Necrosis	-	-	-	-
	Ulceration	-	-	-	-

\* Criteria documented in Sections 4.8 to 4.10

TABLE 2 - continued

Grades for ocular irritation responses\*  
following instillation of 0.1 ml RC9927

Animal No: 12TX 851		Pain evaluation response: 0			
Region of the eye	Response	Grade of response at time after instillation			
		Hour			
		1	24	48	72
Cornea	Opacity	0	0	0	0
	Area	0	0	0	0
	Ulceration	-	-	-	-
	Stippling	-	-	-	-
Iris	Value	0	0	0	0
Conjunctiva	Redness	1	0	0	0
	Chemosis	0	0	0	0
	Discharge	1	0	0	0
	Necrosis	-	-	-	-
	Ulceration	-	-	-	-

\* Criteria documented in Sections 4.8 to 4.10

TABLE 2 - continued

Grades for ocular irritation responses\*  
following instillation of 0.1 ml RC9927

Animal No: 12TX 863		Pain evaluation response: 0			
Region of the eye	Response	Grade of response at time after instillation			
		Hour			
		1	24	48	72
Cornea	Opacity	0	0	0	0
	Area	0	0	0	0
	Ulceration	-	-	-	-
	Stippling	-	-	-	-
Iris	Value	0	0	0	0
Conjunctiva	Redness	1	0	0	0
	Chemosis	0	0	0	0
	Discharge	0	0	0	0
	Necrosis	-	-	-	-
	Ulceration	-	-	-	-

\* Criteria documented in Sections 4.8 to 4.10

TABLE 2 - continued

Grades for ocular irritation responses\*  
following instillation of 0.1 ml RC9927

Animal No: 12TX 864		Pain evaluation response: 0			
Region of the eye	Response	Grade of response at time after instillation			
		1	24	48	72
Cornea	Opacity	0	0	0	0
	Area	0	0	0	0
	Ulceration	-	-	-	-
	Stippling	-	-	-	-
Iris	Value	0	0	0	0
Conjunctiva	Redness	1	0	0	0
	Chemosis	0	0	0	0
	Discharge	1	0	0	0
	Necrosis	-	-	-	-
	Ulceration	-	-	-	-

\* Criteria documented in Sections 4.8 to 4.10

TABLE 2 - continued

Grades for ocular irritation responses\*  
following instillation of 0.1 ml RC9927

Animal No: 12TX 865		Pain evaluation response: 0			
Region of the eye	Response	Grade of response at time after instillation			
		Hour			
		1	24	48	72
Cornea	Opacity	0	0	0	0
	Area	0	0	0	0
	Ulceration	-	-	-	-
	Stippling	-	-	-	-
Iris	Value	0	0	0	0
Conjunctiva	Redness	1	0	0	0
	Chemosis	0	0	0	0
	Discharge	0	0	0	0
	Necrosis	-	-	-	-
	Ulceration	-	-	-	-

\* Criteria documented in Sections 4.8 to 4.10



File

LSR Enquiry No. ZZZ/12880  
LSR Schedule No.

M2038

**IMAGED**

**INDEXED**

ACUTE EYE IRRITATION TEST IN THE RABBIT

Protocol prepared

by

Life Science Research Limited  
Eye, Suffolk, IP23 7BX  
England

20 August 1986

1. INTRODUCTION AND OBJECTIVES

The objective of the acute Eye Irritation/Corrosivity Test is to determine the effect of the test material on the conjunctivae, cornea or iris of the rabbit after a single exposure. The study is designed to conform with Section 4, sub-section 401 of the OECD Guidelines for Testing of Chemicals (1981) and the EPA Toxic Substances Control Act Test Guidelines (1985).

## 2. METHODS

The study will be carried out according to the Standard Operating Procedure ISTT 133k except:

### 4. CONSTITUTION OF TEST GROUP

The test group will comprise six adult rabbits of either sex.

## 10. INTERPRETATION OF RESULTS

### 10.1 Ocular irritation

The assessment of the irritant properties of the test material will be based on the observations per se. The whole of Section 10.1 is deleted.

## 3. SCHEDULED TIME-PLAN

The study will be performed and reported to a time schedule designed to minimise delays. Due to the short duration of the test, no detailed time-plan will be issued unless specifically requested.

4. COMPOUND IDENTITY : FR 45D

5. SPONSOR : Pennwalt Corporation  
900, 1st Avenue  
P.O. Box C  
King of Prussia  
Pennsylvania 19406-00181

6. MONITOR : Dr. J. Seckar.

## 7. STUDY MANAGEMENT

7.1 Study director : K. Smith, B.Sc.  
(Telephone no: 0379 4122)  
(Telex no: 975389 LIFSCI G)

7.2 Quality assurance : D. J. Ford, Ph.D.  
Manager

8. APPROVAL OF PROTOCOL

For LIFE SCIENCE RESEARCH LIMITED

Issued by : D. C. Lodge Date : 22/8/86  
Approved by : M. D. Smith Date : 22/8/86

For PENNWALT CORPORATION

Accepted by : Joel E. Seckman Date : 25/8/86





ISTT 133k

## EYE IRRITATION TEST IN THE RABBIT (O.E.C.D. Regulations)

Standard Operating Procedure

of

Life Science Research  
Eye, Suffolk, IP23 7PX  
England

June 1986

### 1. ANIMAL SPECIES AND STRAIN

New Zealand White rabbits are used unless the Sponsor specifically requests an available alternative. The test rabbits are drawn from a pool of stock animals maintained for primary ocular irritation studies. The animals are two-and-half to three months old on arrival and are purchased to be within the bodyweight range 2.2 - 2.8 kg, and not more than ca four-and-half months old at the time of treatment.

The techniques of rabbit husbandry are well established and a commercial supply of good quality animals is available (Froxfield SPF Rabbits, Broadway Farm, Froxfield, Hampshire, England). The albino rabbit of the New Zealand White strain is widely accepted as the standard laboratory species for use on eye irritation studies. The structure is particularly suitable because the corneal surface and bulbar conjunctival areas are large and easy to inspect and the iris is unpigmented allowing accurate assessment of treatment-related changes of iridial structure.

## 2. ANIMAL HUSBANDRY

### 2.1 Caging

The rabbits are housed individually in suspended stainless steel cages (Type RC10/L) mounted in mobile batteries (Modular Systems and Development Co. Ltd). The cages measure 61 x 76 x 46 cm high and are fitted with perforated countersunk floor panels. An undertray beneath the floor is lined with absorbent crepe paper which is changed daily.

OR

The rabbits are housed individually in suspended galvanised steel cages mounted in batteries (Cope and Cope Limited). The cages measure 55 x 58 x 40 cm high and are fitted with mesh floors. An undertray beneath the mesh floors is cleared of waste matter daily by means of a rubber-lined sledge drawn along the length of the battery.

### 2.2 Environmental control

Each room within the limited access building is assigned to one species alone. All animal rooms are kept at slight positive pressure relative to the outside and each has a filtered forced air supply giving approximately 12 air changes per hour without re-circulation.

The maximum and minimum temperature of the previous 24 hour period and relative humidity are recorded at the beginning of each working day. Environmental control equipment in each rabbit room have target values for temperature and humidity of 18°C (range 15° - 23°C) and 55% R.H. (range 40% - 70% R.H.), respectively. Electric time-switches control a lighting cycle of 14 hours artificial light per day, there is no source of natural light. An emergency generator maintains electricity supply in the event of power failure.

All personnel entering the building change into clean protective clothing and wear additional gown/protective suit, overshoes, gloves and a face mask to service animal-holding areas.

### 2.3 Food and water

Rabbits have free access to food hoppers containing pelleted diet, S.Q.C. Rabbit Standard (Special Diet Services Ltd., Witham, Essex). The manufacturer supplies analytical data with each batch of diet supplied. This includes the concentration of nutritional components, aflatoxins, and selected pesticides, heavy metals and micro-organisms. The diet contains no added antibiotic or other chemotherapeutic or prophylactic treatment.

Animals have free access to tap water supplied to each cage by an automatic piped system. The water is derived from a protected subterranean source and meets the World Health Organisation European Standard. Reports from the local Water Authority record the chemical and bacteriological quality of the water.

The Sponsor is requested to provide information concerning any contaminants and their concentrations in diet and water which may influence the outcome of the study. Specific assays for such contaminants may be conducted at the Sponsors request, at additional cost. In the absence of such information it is assumed that normal levels of common contaminants will not influence the outcome of the study.

### 3. PRE-EXPOSURE PERIOD

Clean cages are prepared on the day before a delivery of stock animals. Labels are affixed specifying the date of arrival, supplier, sex of the animal and initials of the study supervisor who placed the order.

On arrival each animal is inspected, any unfit individuals rejected and the remainder allocated into the pool maintained for ocular irritation studies. A tag bearing a unique reference number is attached to the ear of each rabbit within 24 hours of arrival. This reference number is recorded on the cage label and stock records. The pool of stock animals may contain rabbits previously used for a single primary dermal irritation study, providing such an individual presents no significant dermal or systemic reaction to treatment. Individual bodyweight is recorded for each animal on assignment to stock and at weekly intervals thereafter until the rabbit begins treatment. An acclimatisation period of at least seven days is allowed between assignment to stock and administration of the test material.

A daily check on the general condition of stock animals is recorded by the technical staff and this record is consulted before any animal is accepted for use on study.

No rabbit is accepted for use if either of its eyes are seen to have abnormalities or indications of a current state of irritation at this examination.

Bodyweight is recorded before dosing on Day 1. Each cage is re-labelled with details of the schedule number, ear-tag number, sex, route of administration, treatment level, responsible licensee and day of dosing of the cage occupant.

#### 4. CONSTITUTION OF TEST GROUP

The test group comprises three young adult rabbits of either sex.

The test material is first administered to a single animal, allowing at least 24 hours for irritation responses to be assessed. In the event of the test material eliciting severe ocular effects, no further animals are employed.

#### 5. PREPARATION OF TEST MATERIAL

The identity, strength, stability and purity of the test material received and the stability of the test material under the conditions of storage and formulation, here described is the responsibility of the Sponsor. Information concerning necessary storage conditions or known hazards should be included with any consignment, otherwise the test compound is stored at ambient temperature and assigned to Class 3 of the LSR test material hazard classification system. Large quantities of the test material remaining after completion of the study are returned to the supplier.

Liquid test materials are used as supplied unless specific instructions concerning dilution or other preparation are given by the Sponsor. The pH of test materials in liquid form is recorded. The Sponsor is informed of any liquid in the ranges pH 1-2 or pH 11.5- 14, which are usually regarded as ocular irritants without animal testing being necessary. Materials which have demonstrated corrosivity or severe changes in a dermal irritation study are not tested for eye irritation. It may be presumed such substances will produce similarly severe effects in the eye.

Solid test materials are comminuted by a physical method, usually mortar and pestle, to a fine powder. When 0.1 ml of a test compound in flake, granule or powder form weighs less than 100 mg, doses are administered by volume e.g. 0.1 ml of compacted test compound (compacted without crushing or otherwise altering the individual particles). The weight of any such dose is recorded.

Samples of test doses or stored test material are only sent to the Monitor if requested by the Sponsor. No tests of compound stability or dose homogeneity are undertaken without the instructions of the Monitor.

## 6. ADMINISTRATION OF TEST MATERIAL

The animal is gently restrained. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which 0.1 ml/100 mg of the test material is dropped. The eyelids are gently held together for one second and then released.

Where rabbits in the first test group produce no "positive" irritation responses within 72 hours of treatment, no further investigations are necessary.

Where "positive" responses are seen and after consultation with the Sponsor, a second test group of six animals may be subjected to this procedure followed by flushing of the treated eye four seconds (three rabbits) or 30 seconds (three rabbits) after administration of the test material (at additional cost).

Flushing is carried out by directing a stream of lukewarm water into the ocular sac for five minutes.

The test material is first administered on Day 1 of the test.

## 7. OBSERVATION PERIOD

The behaviour of the rabbit is observed immediately following instillation of the test material. The criteria for assessment of pain response are given in Section 8.1.

The animals are returned to their cages and checked at least twice during the first hour after dosing and at regular intervals throughout the day to ensure no severe injury passes unnoticed. Ocular reactions to treatment are assessed 1, 24, 48 and 72 hours after instillation, according to the criteria detailed in sections 8.2 and 8.3. Rabbits showing unresolved lesions on Day 4 may be re-examined on Day 8 and at seven-day intervals for up to 21 days (at additional cost), providing there is some improvement in the condition of the eye and that the animal is not suffering. The condition of all treated eyes is checked daily to ensure the animal is not subject to infection or undue distress.

Animals showing severe effects which seem likely to continue and cannot be alleviated, are withdrawn immediately from the study and killed painlessly.

8. ASSESSMENT OF REACTIONS TO TREATMENT

8.1 Criteria for assessment of pain response  
(not a component of the O.E.C.D. test)

<u>Reaction of animal to</u> <u>instillation of test compound</u>	<u>Class</u>	<u>Descriptive</u> <u>rating</u>
No response	0	No initial pain
A few blinks only; normal within one or two minutes	1	Practically no initial pain
Rabbit blinks and tries to open eye but reflexes close it	2	Slight initial pain
Rabbit holds eye shut and puts pressure on lids, may rub eye with paw	3	Moderate initial pain
Rabbit holds eye shut vigorously, may squeal	4	Severe initial pain
Rabbit holds eye shut vigorously, may squeal, claw at eye and try to escape	5	Very severe initial pain

8.2 Criteria for assessment of ocular lesions

<u>Cornea</u>	<u>Grade</u>
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Complete corneal opacity, iris not discernible pannus formation or ulceration	4

\* These figures indicate lowest grades considered "positive"  
under O.E.C.D. regulations.

<u>Iris</u>	<u>Grade</u>
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or any combination of them), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

<u>Conjunctivae</u>	<u>Grade</u>
Redness (refers to palpebral and bulbar <u>conjunctivae, excluding cornea and iris</u> )	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3

<u>Chemosis</u> (lids and/or nictitating membrane)	<u>Grade</u>
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

\* These figures indicate lowest grade considered "positive"  
under O.E.C.D. regulations.

8.3 Criteria for assessment of ocular lesions  
(not a component of O.E.C.D. test)

<u>Cornea</u>	<u>Grade</u>
<u>Area of cornea affected by lesion</u>	
One-quarter or less, but not zero	1
Greater than one-quarter, less than one-half	2
Greater than one-half, less than three-quarters	3
Greater than three-quarters, up to whole area	4
<u>Conjunctivae</u>	<u>Grade</u>
<u>Discharge</u>	
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 the lids	2
Discharge with moistening of the lids and hairs and affecting a considerable area around the eye	3

The following effects are considered severe by the British Home Office :

Animals showing (1) general or localised behavioural signs of pain or distress, e.g. vocalisation, hunch posture, abnormal head position, or abnormal quietness, or (2) severe ocular or systemic effects. Severe ocular effects are defined for this purpose:-

- a) dense corneal opacity involving more than three quarters of the area (Grade 3/4)+ or other severe corneal damage.
  - b) Persisting conjunctival inflammation - with eyelids half to completely closed (Grade 3/4)+, and/or extensive mucoid discharge (Grade 3)+ particularly if blood-stained with diffuse conjunctival vascular effects (Grade 3)+.
  - c) Haemorrhage within the eye, or absence of a reaction to light in conjunction with other severe effects.
- + OECD Guidelines for Testing of Chemicals - Acute Eye Irritation/Corrosion Adopted 12 May 1981.



#### 8.4 Apparatus available to facilitate inspection of eyes

A pencil-beam torch and ophthalmoscope are available to facilitate inspection of the eyes.

At any time from 24 hours after instillation the assessment of low grade corneal lesions can be confirmed by the use of fluorescein. One drop of Fluorescein Sodium B.P. (Smith and Nephew Limited) is dropped directly onto the cornea. The rabbit is allowed to blink before the excess fluorescein is flushed out of the eye with physiological saline. Corneal damage is confirmed by distinctive yellow colouration of injured area when viewed under ultra-violet illumination.

The untreated eye is used as a comparison with the treated eye during assessment of ocular lesions.

#### 9. MORTALITY AND TERMINATION PROCEDURE

If all animals are recovered from the irritant effects 72 hours after treatment the study is terminated on Day 4. Otherwise, the animals are retained until ocular irritation responses are resolved or are considered to be irreversible. Animals showing severe pain or ocular effects are withdrawn immediately from the study and killed.

Animals killed on humane grounds or surviving the observation period are not subject to necropsy. In all cases sacrifice is by intravenous injection of sodium pentobarbitone B.P. (Vet) (Expiral-Civa Ltd.)

All animals found dead during the observation period or killed in extremis are examined at necropsy to establish, where possible, the cause of death or illness.

An assessment of the tissue changes found by histopathological examination is made only at the request of the Sponsor prior to commencement of the study.

## 10. INTERPRETATION OF RESULTS

### 10.1 Ocular irritation

An animal is considered as exhibiting a positive reaction (under O.E.C.D. guidelines) if the test compound produces at any of the 24, 48 or 72 hour post-instillation observations any of the following changes:

- i) ulceration of the cornea other than a fine stippling.
- ii) opacity of the cornea other than a slight dulling of the normal lustre.
- iii) inflammation of the iris other than a slight deepening of the iridial folds or a slight circumcorneal injection of the blood vessels.
- iv) obvious swelling of the conjunctivae with partial eversion of the lids.
- v) diffuse crimson-red appearance of the conjunctivae with individual vessels not easily discernible.

The Official Journal of the European Communities (L257/8, Vol 26., 16/09/83 and L257/19, Vol 26., 16/09/83) contains the following criteria for classification of ocular irritants.

A substance or preparation is considered irritant if, when applied to the eye of the animals, significant ocular lesions are caused which are present 24 hours or more after instillation of the test material.

Ocular lesions are considered significant if two or more of the rabbits have mean values at or above the limit values following:

	<u>Limit value</u>
Corneal opacity	2
Iris Lesions	1
Redness of conjunctivae	2.5
Chemosis	2

Mean values are calculated using all scores recorded 24, 48 and 72 hours after treatment. [If the limit values for corneal opacity or iridial lesions equal or exceed 3 or 2 respectively, the substance is considered to have potential to cause serious damage to the eye].

### 10.2 Assessment of pain response on instillation

The observed pain response following instillation of the test compound is reported for each animal individually.

## 11. REFERENCES

1. Draize, J. H. 1959. In "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics". P.49. Association of Food and Drug Officials of the United States, Austin, Texas.
2. Journal Officiel de la Republique Francaise. Year 103, No. 93, p.3863, 21 April 1971.
3. U.S. Federal Register, 191.12, 17 September 1964.
4. U.S. Federal Register, 191.12, 28 April 1972.
5. U.S. Federal Register, Environmental Protection Agency Pesticide Programmes Part II, p.37359, 22 August 1978.
6. Final report of the O.E.C.D. Short Term and Long Term Toxicology Groups, pp.40-44, 31 December 1979.
7. U.K. P.S.P.S. Regulations (1981 Revision)
8. Official Journal of European Communities, L257/8, Volume 26, 16/09/83.

## 12. QUALITY ASSURANCE

This study is conducted in accordance with current internationally recognised Good Laboratory Practice Regulations and is subjected to the following quality assurance procedures.

- the protocol is inspected for compliance
- procedures and data as used and produced on this type of study are periodically inspected
- the final report is reviewed to ensure that it accurately describes the methods and relevant Standard Operating Procedures and that the results are in accord with the primary data.

Periodic reports on these activities are made to management and the Study Director.

All raw data pertaining to the study are available for inspection by the study monitor (for scientific monitoring) or the Quality Assurance Unit of the Sponsor (compliance monitoring). In addition, specified scientists designated by the Sponsor may, upon appointment, examine any set of data.

13. NAME AND ADDRESS OF FACILITIES

Life Science Research Limited  
 Eye, Suffolk, IP23 7PX  
 England

14. RECORDS KEPT

<u>Title</u>	<u>Recorded details</u>
Animal receipt	Date of delivery, supplier and mode of transport. Numbers and sex of animals ordered and received. Weight of animals ordered and range of bodyweight from a sample weighed on receipt. Comments on the general condition of the animals on arrival. Anticipated allocation of animals to specified schedule numbers. Order number.
Observations during acclimatisation (Stock record)	Daily record of the general condition of the animals before commencement of the study. Withdrawals of stock animals for use on specified schedule numbers. Bodyweight record during acclimatisation and before dosing on Day 1.
Animal room day book	Routine occurrences of study, i.e. weighing, food consumption and dosing in chronological sequence, excludes times of observations and removal of decedents. Excludes data specifically recorded on experimental record.
Environmental control record	Daily maximum and minimum temperature recording, humidity record.
Dose receipt record	Weight of dispensed doses used during dosing each day. Confirmation of correct transcription from formulation to dose container.
Formulation request	Dosages and concentration of test material required. Quantities of test material and vehicle to be used in formulating doses.  Special precaution, hazards of measurements to be taken by formulation staff.

Experimental record	Study identity, study supervisor, protocol identity and concentration of test materials, vehicle, location of study, day of dosing. Observations of ocular reactions at specified times.  Reasons for premature sacrifices or extension of study.
Necropsy record	Individual reports on every animal examined by necropsy staff.
Necropsy request	Where relevant, instructions to necropsy staff to take and preserve specified tissue samples from specified animals.
Histological report	Where relevant, instructions to histology staff to process and have interpreted histological slides of specified animal tissues.
Quality assurance records	Records of protocol check for compliance, inspection records of procedures used and data generated on this type of study and the final report review.

A full list of apparatus, diets etc., and the name and address of the suppliers concerned is maintained by the Chief Technician, Department of Animal Management, Life Science Research.

## 15. REPORTING

This study is conducted according to the precepts of Good Laboratory Practice and the following information and data are included in the final report.

- i) Name and address of the facility performing the study and the initiation and termination dates.
- ii) Objectives and procedures stated in the approved protocol, including any changes subsequently made.
- iii) Empirical data generated while conducting the study, including any transformations, calculations or operations performed on the data. Tabulated mean values and standard deviations where appropriate.
- iv) Statistical methods employed for analysing the data (if any).
- v) The test material identified by name and or code number, strength, stability and purity, as instructed by the Sponsor. Physical nature and, where applicable, concentration and pH value of the test substance.

- vi) The Sponsors information regarding stability of the test material under the conditions of administration.
- vii) Methods used.
- viii) Animals used. The number in the study, sex, bodyweight range, source of supply, species, strain or sub-strain, age and procedure used for unique identification and where appropriate, randomisation of the animals. Duration of acclimatisation period. Controlled parameters of environment (photoperiod, temperature, humidity, diet, water, bedding and contaminants).
- ix) Dosage, dosage regime, route of administration and duration.
- x) Frequency and modes of observation. Observations recorded.
- xi) Any unforeseen circumstances which may have affected the quality or integrity of the study.
- xii) The name of the study director.
- xiii) A summary of the data, an analysis of the data and a statement of the conclusions drawn from the analysis.
- xiv) The reports of each of the individual scientists or other professionals involved in the study, e.g. pathologist. The dated signature of the study director and of all scientists and other professionals on their respective segments of the report.
- xv) The location where all raw data and the final report are to be stored.
- xvi) A statement by the Quality Assurance Unit.

Corrections or additions to a final report are in the form of an amendment by the study director. The amendment clearly identifies that part of the final report that is being added to or corrected and the reasons for the correction, or addition, and is signed and dated by the person responsible.