LIFE SCIENCE RESEARCH

LSR Enquiry No. ZZZ/1288D
LSR Schedule No.

M2038

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 dalays. 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 Manager : D. J. Ford, Ph.D.

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 R. Jackson 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 provided 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)

Reaction of animal to instillation of test compound	Class	Descriptive rating
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

Cornea	Grade
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.

Iris	Grade
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

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

Chemosis (lids and/or nictitating membrane)	Grade
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)

Cornea	Grade
Area of cornea affected by lesion	
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

Conjunctivae	Grade
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 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 quiteness, 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:

	Limit value
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 Programes 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 subjeted 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

Title	Recorded details
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 occurences 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 state-ment 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.

JAB 18/6/86