LIFE SCIENCE RESEARCH

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

M2036

ACUTE PERCUTANEOUS TOXICITY STUDY IN THE RABBIT

Protocol prepared

by

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

20 August 1986

1. INTRODUCTION AND OBJECTIVES

The acute toxicity of the test compound under the conditions of administration to the rabbit will be assessed from the results of tests of 14 days duration. The final report will detail the character and time-course of immediate, delayed or persistent toxic effects, identify the tissues or organs visibly changed at necropsy and relate these reactions to the administered dosages.

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). It comprises either a demonstration of the low toxicity of the test compound or a determination of the median lethal dosage (LD_{50}) with 95% confidence limits.

The rabbit has been selected as an appropriate test species.

2. METHODS

The study will be carried out, using the percutaneous route of administration, according to the Standard Operating Procedure ISTT 211 except:

8. NECROPSY (Third paragraph)

Macroscopic abnormalities from all animals will be preserved in fixative, at additional cost. These tissues may be examined histologically following consultation with the Sponsor (at additional cost).

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 : H A Cummins, B.Sc.
(Telephone no: 0379 4122)
(Telex no: 975389 LIFSCIG).

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

8. APPROVAL OF PROTOCOL

For LIFE SCIENCE RESEARCH LIMITED

Issued by : M D Smith Date : 22/8/86

Released by : J Rock Date : 22-8-86

For PENNWALT CORPORATION

Accepted by : Joel A. Seckar Date : 25/8/86

Ref. ISTT 211

ACUTE PERCUTANEOUS TOXICITY TEST IN THE RABBIT

(O.E.C.D. Regulations)

Standard Operating Procedure

of

Life Science Research
Eye, Suffolk, IP23 7PX
England

March 1986

1. ANIMAL SPECIES AND STRAIN

New Zealand White rabbits are used unless the Sponsor specifically requests an available alternative. The animals are two and a half to three months old on arrival and are purchased to be within the bodyweight range 2.0 - 2.6 kg and not more than ca four and one-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).

2. ANIMAL HUSBANDRY

2.1 Caging

The rabbits are housed individually in suspended stainless steel cages (Type RC10/L) mounted in 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.

2.2 Environmental control

Each room within the limited access building is assigned to one species alone. All 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 is set to achieve target values for temperature and humidity of 18°C (range 10°-25°C) and 55% R.H. (range 40%-75% 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 a power failure.

All personnel entering the building change into clean protective clothing and wear an additional gown, alternative footwear, 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, Witham, Essex, England). The manufacturer supplies analytical data with each batch of diet supplied. This includes the concentrations of selected nutritional components, aflatoxins and heavy metals, pesticides and micro-organisms. The diet contains no added antibiotic or other chemotherapeutic or prophylactic agent.

Animals have free access to tap water supplied to each cage by an automatic piped system. Mains supply is derived from a protected subterranean source and meets 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.

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 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. Stock labels are affixed specifying the date of arrival, supplier, sex of animal and Home Office licence number of the study supervisor.

On arrival each rabbit is inspected before being accepted and unfit individuals are rejected. Individual bodyweight is recorded for each stock animal on the day of receipt and at weekly intervals thereafter, until the rabbit begins treatment. A tag bearing a unique reference number is attached to the ear of each rabbit within 24 hours of arrival, the reference number is recorded on the cage label and stock records. An acclimatisation period of at least six days is allowed between arrival at the laboratory and administration of the test compound.

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

Each cage is re-labelled with details of the schedule number, sex, unique reference number, route of administration, treatment level, responsible licensee and day of dosing of the cage occupant.

The dorsum between the limb girdles is clipped free of hair as close to the skin as possible using Oster small animal electric clippers 24 hours before dosing. Chemical depilatories are not used. The treatment site is examined, any animal showing abnormality or irritation of the dermal test site is rejected and replaced by another acclimatised animal.

Bodyweight immediately before dosing is usually within the range 2.0 - 3.0 kg.

4. PREPARATION OF TEST COMPOUNDS

The identity, strength, stability and purity of the test material received and the stability of the test compound under the conditions of 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 material is stored at ambient temperature and assigned to Class 3 of the LSR test compound hazard classification system. Large quantities of the test material remaining after completion of the study are returned to the supplier.

Samples of stored test material are only sent to the Monitor if required by the Sponsor before commencement of the study. No tests of test compound absorption, stability or dose homogeneity are undertaken without the instructions of the Monitor and, in all cases, are at additional cost to the Sponsor.

Liquid test materials are administered without dilution and dosages are expressed volumetrically. Solid test materials are applied to the dorsum and then slightly moistened with a known quantity of distilled water to maximise contact with the skin, but dosages, expressed gravimetrically, are in terms of the dry weight of the test material.

5. ADMINISTRATION PROCEDURE

The required dose is determined for each animal according to bodyweight on the morning of dosing. The dose is administered on Day 1 of the test.

The dose is applied as a thin layer to the shaven area of dorsum and is covered with an unmedicated gauze dressing. The gauze is kept in place and protected by a sheet of aluminium foil and a bandage of waterproof plaster ("Sleek", Smith and Nephew Limited) wrapped twice around the trunk of the body with sufficient tension to ensure the dose remains securely in place and in contact with the skin. The use of an impermeable occlusive dressing maximises the absorbtion of test compound across the dermal barrier. The foil forms an inert layer preventing chemical interaction between test compound and bandage material and is non-irritant to the shaven skin. Solid materials are applied as dry powders but the dose is moistened with distilled water immediately before application of the occlusive dressing.

A uniform thickness of test compound is applied to the skin, increased dosages are achieved by spreading the dose across the same surface area of the dorsum, but with increased thickness.

After 24 hours, the bandage is removed, great care being taken to avoid damaging the test site. The exposed skin is gently wiped with wet disposable towels to reduce the risk of oral ingestion of the test compound through grooming.

6. STUDY DESIGN

When the potential toxicity of the test material is unknown or is suspected of being of slight or low toxicity the study is conducted according to Option A. When it is known or suspected that the test material is of moderate or high toxicity Option B is undertaken.

Option A

The maximum practicable dosage is taken as 2000 mg/kg or approximately 2.0 ml/kg for liquids, unless reduced by the physico-chemical characteristics of the test compound.

One group of five male and five female rabbits are subject to administration of the maximum practicable dosage. In the event that no death, or not more than two deaths, occur then no further investigation is performed.

When more than two deaths occur a minimum of three further groups of five males and five females are treated at dosages arranged in a geometric progression to span the median lethal dosage (LD50). The first of these additional dosage groups is treated with a dosage one or more log intervals below the maximum practicable dosage, in order to determine a minimal effect level. Two or more groups are then treated at appropriate intervals between the maximum practicable dosage and the minimal effect level in order to define the LD50 and 95% confidence interval.

Option B

Three groups of one male and one female rabbit are treated with a range of dosages considered to span the median lethal dosage. A minimum of four further groups of five male and five female animals are then treated at dosages arranged in a geometric progression to span the median lethal dosage (LD50).

7. OBSERVATION PERIOD

The animals are returned to their cages immediately after dosing. On Day 1 all animals are examined three times in the first hour after dosing and at intervals up to at least four hours after dosing. The study is inspected twice daily from Day 2 until termination. The type, time of onset and duration of reactions to treatment are recorded. Separate daily records are kept of the effects and reactions of the dermal test site following removal of the bandage 24 hours after dosing.

The period elapsing between the death of an animal and discovery of a carcase is minimised by technical staff inspecting all animal rooms for decedents at approximately 0900, 1200 and 1600 hours daily, except at weekends when two inspections are made each day.

Bodyweights are recorded on the day before dosing, on Day 1 and at seven day intervals thereafter. The test is terminated on Day 15 unless recovery from toxic effects is incomplete.

8. NECROPSY

Carcases are stored in a refrigerator at approximately 4°C until trained necropsy staff are available. All decedent animals are thoroughly examined at necropsy for abnormality of tissues or organs. All major body cavities are opened, larger organs are narrowly sectioned and the gastro-intestinal tract is opened at intervals for examination of the mucosal surfaces. Abnormalities are described or the normal appearance of the major organs confirmed for each animal. Sites of macroscopic percutaneous application of the test compound are examined by palpation, and in section to ascertain local effects of treatment.

Animals are killed at termination of the study by intravenous administration of Sodium pentobarbitone B.Vet.C. (Expiral-Ceva Limited) and are examined at necropsy by the same procedure used for decedents.

No macroscopically abnormal tissues are retained nor histopathological examination undertaken unless specifically requested by the Sponsor (additional cost). Tissue samples are preserved in 10% buffered formal saline and stored in this medium. After dehydration in a series of concentrations of alcohol and embedding in paraffin wax, histological sections approximately 5 u thick are cut, permanently mounted on glass slides and stained with haematoxylin and eosin. The sections are examined by a pathologist.

9. APPRAISAL OF DATA

The acute median lethal dosage (LD50), 95% confidence limits and slope of the dose-response curve are usually calculated by probit analysis (Finney, D. J., 1952, Probit Analysis, pp. 236-245, Cambridge University Press) or by Logit analysis (Finney, D. J., Statistical Method in Biological Assay, Griffin, London, 1971).

Alternatively, the LD50 and 95% confidence limits may also be calculated using the method of moving average interpolation after Thompson, 1947 (Biometrics, 8, 51-54) for comparison or where the log dosage versus probit of mortality is not adequately represented by linear regression.

Separate LD50 values are determined for either sex and for both sexes in combination wherever possible.

An appendix detailing bodyweight, mortality, signs of reaction to treatment and necropsy reports of each treated animal is included in the final report.

10. 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.

11. NAME AND ADDRESS OF FACILITY

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

12. RECORDS KEPT

Title	Recorded details
Animal receipt form	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	Daily record of the general condition at commencement of the study. Withdrawals of stock animals for use on specified schedule numbers.
Animal room day book	Routine occurrences of study, i.e. receipt, randomisation, weighing, food consumption and dosing in chronological sequence, excludes times of observations and removal of decedents. Excludes data specifically entered in experimental record.
Environmental control record	Daily maximum and minimum temperature recording, humidity record.
Formulation request	Concentration required for each group, quantities of test compound 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 compounds, vehicle, location of study, day of dosing. Observations of clinical signs and dermal reactions at specified times. Bodyweight record at weekly intervals. Reasons for premature sacrifices. Route of administration and dosing record. Despatch of animals to necropsy record.
Necropsy record	Individual reports on every animal examined by necropsy staff, giving carcase weight and describing all abnormalities or confirming normal appearance of major organs. Decedent and animals killed at termination are recorded in the same file.
Necropsy request	Where relevant, instructions to necropsy staff to take and preserve specified tissue samples from specified animals.
Histological request	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.

13. 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.

v) The test compound 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 compound 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 or statistician. 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.

LAF 15/4/86