CONFIDENTIAL

86/PTC013/634

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RC9927 : ACUTE ORAL TOXICITY IN THE RAT

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

PENNWALT CORPORATION
CHEMICALS • EQUIPMENT • HEALTH PRODUCTS

900 First Avenue, P.O. Box C, King of Prussia, Pennsylvania 19406-0018 • (215) 337-6500

SAFETY, HEALTH AND ENVIRONMENTAL AFFAIRS

SUMMARY OF TOXICOLOGY STUDY

PERFORMED FOR THE VENTURE GROUP

Test Material:	FR-45B
Product Code:	RC 9927
Study Type:	Acute oral toxicity in the rat
Testing Laboratory:	Life Science Research Elm Farm Eye, Suffolk IP23 7PX, England
Summary of Results:	No mortality or toxic effects seen after single oral administration of 5,000 mg/kg.
Storage:	The report is filed in the Technical Records Center at King of Prussia under Master No. 22227.
Information for MSDS:	LD50 > 5,000 mg/kg (no symptoms or mortality)

Signatures

J. F. Jadlocki — 3-11-87

Joseph F. Jadlocki, Jr.
Manager, Product Safety

Date

Joel A. Seckar — 3/11/87

Joel A. Seckar, Ph.D.
Manager, Toxicology

Date

RC9927 :
ACUTE ORAL TOXICITY IN THE RAT

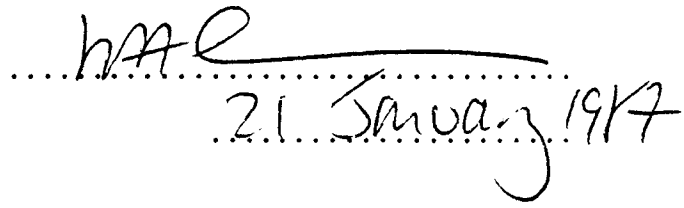
LSR Report No: 86/PTC013/634

I 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, the protocol did not require analysis of the dose form and no analysis of the test material was received from the Sponsor.

The Study Director fulfilled the responsibilities required by these regulations.

H. A. Cummins, B.Sc.
Study Director
(Head, Sub-Department of
Short-Term Toxicology)



RC9927 :
ACUTE ORAL TOXICITY IN THE RAT

LSR Report No: 86/PTC013/634

QUALITY ASSURANCE INSPECTIONS

	Dates (Day/Month/Year)		
	Inspection	Report to study Director	Report to Management
PROTOCOL			
Inspection of protocol was made in accordance with LSR Standard Operating Procedure QAU/020. Dates for inspection of protocol amendments in accordance with this SOP are not quoted	24.9.86	26.9.86	26.9.86
DATA			
Inspection of data generated on this type of study was made in accordance with LSR Standard Operating Procedure QAU/050	13.10.86		14.10.86
PROCEDURES			
Inspection of procedures on this type of study was made in accordance with LSR Standard Operating Procedure QAU/040	10.9.86 10.9.86 16.9.86 17.9.86 23.9.86		10.9.86 10.9.86 17.9.86 23.9.86 24.9.86

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
.....

CONTENTS

TABLES

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1. SUMMARY

1.1 The acute oral toxicity of RC9927 was investigated in a group of five male and five female rats of the Charles River CD strain at a dosage of 5000 mg/kg. The test material was administered at a volume-dosage of 10 ml/kg in maize oil. Mortality and signs of reaction to treatment were recorded during a subsequent 14-day period of observation. The animals were killed on Day 15 and subjected to necropsy.

1.2 No death occurred and there were no signs of reaction to treatment with RC9927.

All animals achieved anticipated bodyweight gains during the study and necropsy findings on Day 15 were unremarkable.

1.3 It is concluded that, under the conditions of this study, the acute oral median lethal dosage (LD50) of the test material was greater than 5000 mg/kg. Accordingly, RC9927 was assigned into the class 'low oral toxicity'.

2. INTRODUCTION

The objective of this study was to determine the acute median lethal dosage, 95% confidence limits and slope of the dose response curve of the test material, or to demonstrate its low toxicity at the maximum practicable dosage following a single oral administration to rats; to identify any target organs or systems; to assess the time course of response; and to identify any delayed or irreversible effects resulting from sub-lethal dosages.

The study was 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).

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

17 September - 1 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. The test material 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

Young adult rats of the CD strain (remote Sprague-Dawley origin), supplied by Charles River (U.K) Limited, were about one month old on arrival. The animals were bred under barriered conditions and travelled from the supplier to the animal-holding room in sealed boxes with filter protected air-vents. The albino rat was selected for this study as it has been widely accepted as the standard laboratory species for use in acute toxicity tests. The strain has been used for toxicological purposes since its establishment under S.P.F. conditions in 1955. There is extensive knowledge of the biology of the individual animal.

The animals were housed in Type RC1 cages consisting of a high density polypropylene body, measuring 56 x 38 x 18 cm, with stainless steel grid floors and tops (North Kent Plastics Limited). The grid floor ensured rapid removal of waste material to undertrays which were cleaned as necessary. Five animals of the same sex were housed in each cage. The cages were held in mobile tubular steel racks.

4.2 Husbandry

The animals were held in a limited-access rodent facility. All rooms were kept at slight positive pressure relative to the outside and each had its own filtered air supply giving approximately 17 complete air changes per hour without re-circulation. The maximum and minimum temperatures of the previous 24 hour period and the relative humidity were recorded at the beginning of each working day. Environmental control equipment was set to achieve target values of 21°C (range 18°-25°C) and 55% R.H. (range 40%-70% R.H.), respectively.

Electric time-switches regulated a lighting cycle of 12 hours of artificial light per day. An emergency generator was available to maintain the electricity supply in the event of a power failure. All personnel entering the building changed into clean protective clothing and wore an additional gown, gloves, plastic over-shoes, and face mask to service animal-holding areas.

A commercially-available complete pelleted rodent diet (Laboratory Animal Diet No. 1 from Labsure, Manea, Cambridgeshire, England) was fed without restriction, except for the removal of food for approximately 18 hours before administration of the test material.

The manufacturer supplied analytical data with each batch of diet which included concentrations of nutritional components, aflatoxins and selected heavy metals, pesticides and microorganisms. The diet contained no added antibiotic or other chemotherapeutic or prophylactic treatment.

Animals had free access to tap water supplied in two bottles per cage and re-filled as required. The water was supplied by the East Anglian Water Company from a protected subterranean source to meet World Health Organisation European Standards for quality of drinking water. Reports from the local Water Authority recorded the chemical and bacteriological quality of the water. There was no known information to indicate that normal levels of common contaminants or any specific contaminants, would influence the outcome of the study.

4.3 Pre-exposure period

Clean cages were prepared the day before delivery of stock animals. Stock labels were affixed specifying the date of arrival, requested bodyweight range, strain, number and sex of cage occupants.

On arrival, each animal was inspected before being accepted. All animals were weighed on arrival and the range of bodyweight was recorded. Five rats of the same sex were allocated to each cage. An acclimatisation period of at least six days was allowed between arrival at the laboratory and administration of the test material. Ear-marks identifying each individual within the cage were made within one day of delivery. The sex of each animal was checked at the same time. A daily check on the general condition of the animals was recorded by the technical staff and this record was consulted before each cage of animals was accepted for use in the study.

Food was removed from the hoppers at approximately 1700 hours on the day before dosing to ensure the stomach was void of food at the time of dosing. Each cage was re-labelled with details of the schedule number, unique cage reference, treatment regime, ear-mark numbers and sex of occupants, responsible licensee and date of administration of the test material.

Pre-fasted bodyweight was recorded and ranged for males from 113 - 127 g and for females from 106 - 138 g. At the time of administration of the test material males were within the bodyweight range 101 - 116 g and females 96 - 122 g. The animals were about five weeks old at this time.

4.4 Preparation of test material

The test material was prepared as a 50% w/v solution in maize oil.

Dosages were calculated and expressed gravimetrically in terms of the material as received. A fresh preparation of the test material was prepared on the morning of administration and any surplus remaining after dosing was destroyed on the same day.

No attempt was made to measure the stability, dose homogeneity or absorption of the test material from the solvent system employed.

4.5 Treatment groups and sizes

A single group of five male and five female rats was given the maximum practicable dosage of RC9927 at a volume-dosage of 10 ml/kg. Since no rats died as a result of treatment the low toxicity of RC9927 was demonstrated and no further groups of animals were employed.

4.6 Administration of test material

Dose-volume was determined for animals according to the fasted bodyweight on the morning of dosing. Dosing commenced at 11.24 hours on Day 1.

A flexible catheter (8-choke) was passed down the oesophagus allowing instillation of the dose into the lumen of the stomach. Each animal was returned to its cage and food hoppers were refilled approximately three hours after dosing.

4.7 Observation period

The animals were returned to their cages immediately after dosing. Three separate inspections were made during the first hour after administration and at intervals up to five hours after dosing. From Day 2 onwards the animals were inspected twice daily. The type, time of onset and duration of reactions to treatment were recorded.

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

Bodyweight of each animal was recorded on the day before dosing and Days 1, 8 and 15. The test was terminated on Day 15.

4.8 Necropsy

Animals were killed at termination of the study by carbon dioxide asphyxiation. Each animal was weighed and thoroughly examined at necropsy for abnormality of tissues or organs. All body cavities were opened, larger organs were sectioned and the gastro-intestinal tract was opened at intervals for examination of the mucosal surfaces. All abnormalities were described or the normal appearance of major organs was confirmed.

All macroscopically abnormal tissues were preserved in 4% buffered formaldehyde saline and retained for future possible histopathological examination.

4.9 Treatment of data

An adaptation of the classification of Hodge and Sterner (1949) was used in assessing the toxicity rating of the compound. The acute toxicity is expressed descriptively according to the LD50 value, as follows:

TEXT TABLE 1 Classification of acute toxicity

LD50 (mg/kg)	Classification
< 5	extreme toxicity
5 - 50	high toxicity
50 - 500	moderate toxicity
500 - 5000	slight toxicity
> 5000	low toxicity

4.10 Name and address of facilities

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

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

4.11 Reference

HODGE, H. C. and STERNER, J. H. (1949). American Industrial Hygiene Association Quarterley, 10, 93.

5. RESULTS

5.1 Mortality (Tables 1 and 2)

No animals died during the course of the study.

5.2 Signs (Table 2)

There were no signs of reaction to treatment with RC9927.

5.3 Bodyweight (Table 3)

All animals achieved anticipated bodyweight gains during the 14-day observation period.

5.4 Macroscopic pathology (Table 4)

Necropsy findings on Day 15 were confined to single observations of occasional dark areas on the thymus and fluid distension of the uterus. Neither of these lesions were considered to reflect an effect of treatment with RC9927.

6. CONCLUSION

Under the conditions of this study, the acute oral median lethal dosage (LD50) of the test material was greater than 5000 mg/kg. Accordingly, RC9927 was assigned into the class 'low oral toxicity'.

TABLE 1

Mortality in a group of male and female rats given a single oral dosage of RC9927 at a volume-dosage of 10 ml/kg in maize oil

Dosage (mg/kg)	Mortality		
	Male	Female	Combined
5000	0/5	0/5	0/10

TABLE 2

Distribution of signs among a group of male and female rats given a single oral dosage of RC9927 at a volume-dosage of 10 mg/kg in maize oil

Dosage: 5000 mg/kg

Signs of reaction to treatment	Cage, animal number and sex L971 Male	Number showing effect at time after dosing																																				
		Hour					Day																																	
	71 72 73 74 75	1/4	1/2	1	3	5	2 a	2 p	3 a	3 p	4 a	4 p	5 a	5 p	6 a	6 p	7 a	7 p	8 a	8 p	9 a	9 p	10 a	10 p	11 a	11 p	12 a	12 p	13 a	13 p	14 a	14 p	15 a					
CLINICAL SIGNS																																							
Decedents																																						
None																																						
Animals surviving at Day 15																																						
No abnormality detected		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5					
Total number of survivors: 5																																						

a = am : p = pm

TABLE 2 - continued

Distribution of signs among a group of male and female rats given a single oral dosage of RC9927 at a volume-dosage of 10 mg/kg in maize oil

Dosage: 5000 mg/kg

Signs of reaction to treatment	Cage, animal number and sex L972 Female	Number showing effect at time after dosing
		Hour: 1/4	1/2	1	3	5 — Day: 2 a	2 p	3 a	3 p	4 a	4 p	5 a	5 p	6 a	6 p	7 a	7 p	8 a	8 p	9 a	9 p	10 a	10 p	11 a	11 p	12 a	12 p	13 a	13 p	14 a	14 p	15 a
CLINICAL SIGNS																																
Decedents																																
None																																
Animals surviving at Day 15																																
No abnormality detected	76 77 78 79 80	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Total number of survivors: 5																																

a = am : p = pm

TABLE 3

Individual bodyweights in a group of male and female rats given a single oral dosage of RC9927 at a volume-dosage of 10 ml/kg in maize oil

Dosage (mg/kg)	Bodyweight (g)	Animal number and sex				
		71M	72M	73M	74M	75M
5000	Day -1	114	127	113	116	113
	Day 1	103	116	102	104	101
	Day 8	192	204	186	182	185
	Day 15	249	265	236	232	242
	Increment	135	138	123	116	129
	Mean of Increment					128
		76F	77F	78F	79F	80F
	Day -1	120	138	106	106	110
	Day 1	110	122	96	96	102
	Day 8	167	188	149	142	149
	Day 15	184	216	163	165	161
	Increment	64	78	57	59	51
	Mean of Increment					62

TABLE 4

Necropsy observations among a group of male and female rats given a single oral dosage of RC9927 at a volume-dosage of 10 ml/kg in maize oil

Dosage: 5000 mg/kg

Animal number and sex	Died or Sacrificed	Time of death Day	Necropsy observation
71M	Sacrificed	15	External No significant lesion Internal No significant lesion
72M	Sacrificed	15	External No significant lesion Internal No significant lesion
73M	Sacrificed	15	External No significant lesion Internal No significant lesion
74M	Sacrificed	15	External No significant lesion Internal No significant lesion
75M	Sacrificed	15	External No significant lesion Internal No significant lesion

TABLE 4 - continued

Necropsy observations among a group of male and female rats given a single oral dosage of RC9927 at a volume-dosage of 10 ml/kg in maize oil

Dosage: 5000 mg/kg

Animal number and sex	Died or Sacrificed	Time of death Day	Necropsy observation
76F	Sacrificed	15	External No significant lesion Internal No significant lesion
77F	Sacrificed	15	External No significant lesion Internal No significant lesion
78F	Sacrificed	15	External No significant lesion Internal Thymus: Occasional dark areas
79F	Sacrificed	15	External No significant lesion Internal Uterus: Fluid distension
80F	Sacrificed	15	External No significant lesion Internal No significant lesion

ACUTE ORAL TOXICITY STUDY IN THE RAT

Protocol prepared

by

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

20 August 1986

1. INTRODUCTION AND OBJECTIVES

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).

The acute toxicity of the test material under the conditions of administration to the rat 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 will comprise 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.

2. METHODS

The study will be carried out using the oral route of administration, according to the Standard Operating Procedure ISTT 180i 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 LIFSCI G)

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

8. APPROVAL OF PROTOCOL

For LIFE SCIENCE RESEARCH LIMITED

Issued by : ..... Date : 22/8/86.....

Approved by : ..... Date : 22-8-86.....

For PENNWALT CORPORATION

Accepted by : ..... Date : 25/8/86.....

Ref: ISTT 180i

ACUTE TOXICITY STUDIES IN THE RAT

Standard Operating Procedures

of

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

August 1985

1. ANIMAL SPECIES AND STRAIN

Albino rats of the Charles River CD strain (remote Sprague-Dawley origin) are used unless the Sponsor specifically requests an available alternative. These are bred under barriered conditions and travel from supplier to animal-holding room in sealed boxes with filter-protected air-vents. The supplier is Charles River (U.K.) Limited, Margate, Kent.

As an experimental animal the albino rat is amenable to manipulation, is available in large numbers at short notice and is widely accepted as the standard laboratory species for use on acute toxicity studies. The strain has been widely used for toxicological purposes since its establishment under SPF conditions in 1955, and there is extensive knowledge of the biology of the individual animal.

2. HUSBANDRY

2.1 Caging

Rats are housed in high density polypropylene cages measuring 56 x 38 x 18 cm, with stainless steel grid floors and tops (North Kent Plastics Limited). The grid floors ensure rapid removal of waste material to undertrays which are cleaned out as necessary. A minimum floor area of 250 cm^2 per rat is provided by grouping no more than six animals of the same sex in each cage. Mobile tubular steel racks hold 21 suspended cages each.

2.2 Environmental control

The animals are housed in a limited access building. All rooms are kept at slight positive pressure relative to the outside and each has it's own filtered air supply giving approximately 17 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 rat room has target values for temperature of 21°C (range 18°-25°C) and humidity of 55% R.H. (range 40% - 70%). Electric time-switches control a lighting cycle of 12 hours artificial light per day, there is no source of natural light. A stand-by generator maintains electricity supply in the event of mains power failure.

All personnel entering the building change into clean protective clothing and wear an additional gown, alternative footwear, gloves and face mask to service animal-holding areas.

2.3 Food and water

A commercially available complete, pelleted rodent diet, (LAD 1, from Labsure, K and K Greeff Ltd., Croydon, CR9 3QL) is provided. The manufacturer supplies analytical data with each batch of diet which includes the concentrations of nutritional components, aflatoxins and selected heavy metals, pesticides 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 in polythene bottles with sipper tubes. Mains water 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 or 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 study.

3. PRE-EXPOSURE PERIOD

Clean cages are prepared the day before a delivery of stock animals. Stock labels are affixed specifying the date of arrival, requested bodyweight range, strain, number and sex of cage occupants. On arrival each animal is inspected before being accepted for use and unfit individuals are culled. An appropriate number of healthy animals of the same sex is assigned to each cage by use of a random number sequence. Ear-marks identifying each individual within a cage are made within one day of delivery. At the same time the sex of each animal is confirmed and it's bodyweight recorded.

A daily check on the general condition of the animals is recorded by the technical staff and this record is consulted before each cage of animals is accepted for use on study. An acclimatisation period of at least six days is allowed between arrival at the laboratory and administration of the test compound.

3.1 Percutaneous test

For percutaneous toxicity studies, 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 200 - 280 g. The animals are about eight weeks old at this time.

3.2 Oral test

Food is removed from the hoppers of rats assigned to oral toxicity studies at approximately 1700 hours on the day before dosing to ensure the stomach is void of food at the time of dosing.

Bodyweight immediately before dosing is usually within the range 100 - 140 g and the animals are approximately 5 weeks old at this time.

3.3 Intraperitoneal, intramuscular, subcutaneous or intravenous tests

Sites of injection are shaven, where appropriate, on the day before dosing. Any animal showing abnormality or irritation of the site of injection is rejected and replaced by another acclimatised animal.

Bodyweight immediately before commencement of dosing is usually within the range 100 - 140 g and the animals are approximately five weeks old at this time.

4. PREPARATION OF TEST COMPOUNDS

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

Proof of absorption of the test compound from the vehicle, homogeneity and achieved concentration of the material administered or stability of the test compound under the conditions of storage, may be necessary to fulfil the requirements of G.L.P. Samples of body fluids, test doses or stored test compound are sent to the Monitor at intervals specified by the Monitor before commencement of the study. No tests of 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.

Solid test compounds may be subject to dry milling (17-40 Ultra-centrifugal Mill, Glen Creston Limited) to improve the homogeneity of prepared suspensions.

Fresh solutions or suspensions of the test compound are prepared on the morning of administration. Any dose remaining at completion of administration is disposed of on the same day.

Dosages, in terms of the material as received, are normally expressed gravimetrically. When volumetric units are used for liquids, the density of the test compound is reported so that equivalent gravimetric units can be calculated.

4.1 Percutaneous test

Liquid test compounds are administered without dilution and dosages are expressed volumetrically. Solid test compounds 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 compound.

4.2 Oral, intraperitoneal, subcutaneous or intramuscular test

Solid test substances are finely ground in a mortar and pestle. Physiologically compatible vehicles are used to produce overtly homogenous suspensions or solutions suitable for parenteral administration, these are commonly maize oil, distilled water or 0.5% w/v methylcellulose in water. Less usual solvent systems are used only after consultation with the Sponsor. Vehicles may be requested or provided by the Sponsor where necessary.

4.3 Intravenous test

Physiologically compatible vehicles such as water for injection (BP) or physiological saline are used to produce solutions suitable for intravenous perfusion.

5. ADMINISTRATION PROCEDURES

5.1 Oral administration

Dosages are given at a constant volume-dosage of 10 ml/kg or 20 ml/kg where an aqueous vehicle is used. Dose-volume is determined for each rat according to bodyweight on the morning of dosing. The first dose is administered on Day 1 of the study.

A flexible catheter is passed down the oesophagus allowing instillation of test compound into the lumen of the stomach. Where the test compound is suspected of reacting with the plastic of the syringe and catheter, a glass syringe and rigid metal cannula are used.

Food-hoppers are re-filled approximately three hours after dosing is completed.

5.2 Intravenous administration

Prepared solutions of the test compound are administered at a constant volume-dosage of 10 ml/kg. Dose-volume is determined according to individual bodyweight on the morning of dosing. The dose is administered into a tail vein on Day 1 of the study.

Injection into the tail vein is made with the bevelled edge of the hypodermic needle uppermost. Care is taken to exclude air-bubbles from the dose and to avoid subcutaneous administration as indicated by blanching at the injection site. The tail is cleansed and the blood vessels dilated by immersion is warm water before injection. The injection site is swabbed with aqueous ethanol and the dose administered. Excess blood is wiped from the tail before replacing the animal in the appropriate cage. The dose is administered at a constant rate of 2 ml per minute by use of an infusion catheter (Portex Limited) coupled to a syringe pump (Sage Series 355, Orion Research Inc., Cambridge, Mass., USA). A new catheter is used for every 5 animals or each cage as appropriate.

5.3 Intraperitoneal administration

Dose-volume is determined for each animal according to bodyweight on the morning of dosing. The dose is administered on Day 1 and at a constant volume-dosage of 10 ml/kg or 20 ml/kg where an aqueous vehicle is used.

The rat is grasped firmly by the skin fold overlying the nape and the scapulae. The hind-limbs and tail are also restrained so that the ventral surface of the animal is uppermost. The injection site, a point approximately 10 mm to one side of the ventral mid-line and mid-way between the diaphragm and vulva is prepared by swabbing with aqueous ethanol. The hypodermic needle is introduced at a shallow angle and passed towards the anterior of the animal before the measured dose is injected into the intraperitoneal cavity.

5.4 Subcutaneous administration

Dose-volume is determined for each animal according to bodyweight on the morning of dosing. The dose is administered on Day 1 and at a constant volume-dosage of 10 ml/kg.

The rat is restrained and the skin overlying the scapulae is swabbed with aqueous ethanol. The dorsum is gently pinched to raise the cleansed area of skin. Insertion of a hypodermic needle through the base of the skin-fold and parallel to the spinal column allows subcutaneous injection of test material.

5.5 Intramuscular administration

Dosages are given at a constant volume-dosage of 4 ml/kg on Day 1 of the study. Dose-volume is determined according to individual bodyweight on the morning of dosing.

The rat is restrained with one hind-limb extended. Gentle pressure is applied on the outer and inner aspects of the thigh to force the musculature firmly against the dermis. The injection site is swabbed with aqueous ethanol before a fine gauge hypodermic needle is inserted into the muscle-bed and the dose is administered. Excess blood is wiped from the injection site before the rat is returned to the appropriate cage.

5.6 Percutaneous administration

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 dosing the rats are housed individually in type RM2 cages measuring 38 x 25 x 18 cm (North Kent Plastics Ltd.) to prevent cage-mates interfering with the occlusive dressing or ingesting the test compound.

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. The rats are returned to their original cages, with up to five animals per cage, after the bandages have been removed.

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 5000 mg/kg except in the case of percutaneous tests, when the maximum practicable dosage is taken as 2000 mg/kg, unless reduced by the physico-chemical characteristics of the test compoud.

One group of five male and five female rats 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 to span the median lethal dosage (LD_{50}). 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 LD_{50} and 95% confidence interval.

Option B

Three groups of one male and one female rats 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, as far as possible, in a geometric progression to span the median lethal dosage (LD_{50}).

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. In the case of percutaneous toxicity studies, separate daily records are kept of the effects and reactions of the dermal test site following removal of the bandage 24 hours after dosing. Local reactions at the sites of injection are also recorded, where appropriate.

The period elapsing between the death of an animal and discovery of 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 injection or 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 CO_2 asphyxiation 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 4% 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 μ 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 (LD_{50}), 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 LD_{50} 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 LD_{50} 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.

The classification of Hodge and Sterner, 1949 (American Industrial Hygeine Association Quarterley, 10, 93.) is usually used in assessing the toxicity rating of compounds administered by the oral route. Other classifictory systems may be used as appropriate or as indicated by the Sponsor. These will be documented 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. (Record maintained by Department of Animal Management).
Observations during acclimatisation	Daily record of the general condition at commencement of the study. Withdrawals of stock animals for use on specified schedule numbers. (Record maintained by Department of Animal Management).
Animal room day book	Routine occurences 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. (Record maintained by Department of Animal Management).
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. (Record of preparation of doses are maintained by Short-Term Toxicology Department).

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. (Records of necropsy tissue preservation, histological processes and the report of the Pathologist are maintained by the Dept. of Pathology).
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.

BJH 28/2/86