

CONFIDENTIAL

CODE NUMBER: 329179 (50 ppm):

ACUTE DERMAL IRRITATION

TEST IN THE RABBIT

PROJECT NUMBER: 28/5

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QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm Laboratories is carried out as a continuous process designed to encompass all major phases of each study type once per month. Dates of the most recently completed series of monthly inspections relevant to the study type(s) in this report are given below.

**Date(s) of Inspection and Reporting:**

02/11/92, 10/11/92, 17/11/92

This report has been audited by Safepharm Laboratories Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

**Date of Report Audit:**

04/01/93

J.R. Pateman C. Biol., M.I. Biol.  
FOR SAFEPHARM QUALITY ASSURANCE UNIT

*J. Pateman*

DATE:

.....05/01/93.....

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated in the study, and the work described was performed in compliance with the following principles of Good Laboratory Practice.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health 1989.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

P. Tomlinson ..... DATE: 5.1.93 .....

P.P. Tomlinson B.Sc. (Hons)  
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S U M M A R Y

STUDY SPONSOR : CENTRILAB

PROJECT NUMBER : 28/5

TEST MATERIAL : CODE NUMBER: 329179 (50 ppm)

1. A study was performed to assess the irritancy potential of a dilution of the test material to the skin of the New Zealand White rabbit. The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 404 "Acute Dermal Irritation/Corrosion" referenced as Method B4 in Commission Directive 84/449/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 91/325/EEC).

2. A single 4-hour, semi-occluded application of the diluted test material to the intact skin of three rabbits produced no skin reactions.
3. The diluted test material produced a primary irritation index of 0.0 and was classified as a non-irritant to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

The diluted test material was also classified as non-irritant according to EEC labelling regulations. No symbol and risk phrase are required.

CODE NUMBER: 329179 (50 ppm):

ACUTE DERMAL IRRITATION

TEST IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of a dilution of the test material following a single, 4-hour, semi-occluded application to the intact rabbit skin (Safepharm Standard Method Number OECD 4). The method used followed the recommendations of the OECD Guidelines for Testing of Chemicals (1981) No. 404 "Acute Dermal Irritation/Corrosion" referenced as Method B4 in Commission Directive 84/449/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 91/325/EEC).

The test system was chosen because the rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely skin irritancy potential of the test material to man.

The study was conducted in accordance with the internationally accepted general principles of Good Laboratory Practice and Safepharm Standard Operating Procedures.

The study was performed between 16 December 1992 and 19 December 1992.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

The test material was supplied by the study sponsor as follows:

Sponsor's label	: HEATSAUR
Sample number	: 329179
Date received	: 8 December 1992
Description	: colourless liquid with white crystals
Container	: brown glass bottle

2. TEST MATERIAL (contd)

2.1 Description, Identification and Storage Conditions (contd)

Data relating to the identity, purity and stability of the test material are the responsibility of the sponsor.

2.2 Method of Preparation

For the purpose of this study a 50 ppm dilution of the test material was prepared in distilled water.

3. TEST SYSTEM

3.1 Specification

Three New Zealand White rabbits were supplied by David Percival Ltd., Moston, Sandbach, Cheshire, U.K. At the start of the study the animals weighed 2.74 - 3.07 kg and were twelve to sixteen weeks old. After a minimum acclimatisation period of five days each animal was given a number unique within the study which was written with a black indelible marker-pen on the inner surface of the ear and on the cage label.

3.2 Husbandry

The animals were individually housed in suspended metal cages. Free access to mains drinking water and food (RABMA Rabbit Diet, Special Diet Services Ltd., Witham, Essex, U.K.) was allowed throughout the study.

The animal room was maintained at a temperature of 17 - 19°C and relative humidity of 43 - 61%. The rate of air exchange was approximately 15 changes per hour and the lighting was controlled by a time switch to give continuous 12 hours light and 12 hours darkness.

4. PROCEDURE

On the day before the test each of a group of three rabbits was clipped free of fur from the dorsal flank area using veterinary clippers. Only rabbits with a healthy intact epidermis by gross observation were

4. PROCEDURE (contd)

On the day of the test a suitable test site was selected on the back of each rabbit. A quantity of 0.5 ml of the diluted test material was introduced under a 2.5 cm x 2.5 cm gauze patch and placed in position on the shorn skin. The patch was secured in position with a strip of surgical adhesive tape (BLENDERM: approximate size 2.5 cm x 4.0 cm). To prevent the animals interfering with the patches, the trunk of each rabbit was wrapped in an elasticated corset (TUBIGRIP) and the animals were returned to their cages for the duration of the exposure period.

Four hours after application the corset and patches were removed from each animal and any residual test material removed by gentle swabbing with cotton wool soaked in distilled water.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale from Draize J.H. (1959) Association of Food and Drug Officials of the United States, Austin, Texas, "The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics":-

EVALUATION OF SKIN REACTIONS

<u>Erythema and Eschar Formation</u>	<u>Value</u>
No erythema .....	0
Very slight erythema (barely perceptible) .....	1
Well-defined erythema .....	2
Moderate to severe erythema .....	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) .....	4

<u>Oedema Formation</u>	<u>Value</u>
No oedema .....	0
Very slight oedema (barely perceptible) .....	1
Slight oedema (edges of area well-defined by definite raising) ....	2
Moderate oedema (raised approximately 1 millimetre) .....	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure) .....	4

5. INTERPRETATION OF RESULTS

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the three test rabbits (12 values) and this total was divided by 6 to give the primary irritation index of the test material. The test material was classified according to the following scheme:

<u>Primary Irritation Index</u>	<u>Classification of Irritancy</u>
0	Non-irritant
> 0 - 2	Mild irritant
> 2 - 5	Moderate irritant
> 5 - 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which may include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

The results were interpreted according to the EEC Commission Directive of 91/325/EEC which adapts Council Directive 67/548/EEC on the regulations relating to the classification, packaging and labelling of dangerous substances as follows:

i) Interpretation according to Annex VI Part II (B)

The test material will be classified as irritant or corrosive to the skin according to the following criteria:

a) Corrosion

The test material will be considered to be corrosive and will require the appropriate "C" symbol if it produces full thickness destruction of the skin tissue on at least one animal. If this criterion is not satisfied, the test material will be classified as non-corrosive.

5. INTERPRETATION OF RESULTS (contd)

i) Interpretation according to Annex VI Part II (B) (contd)

b) Irritation

The test material will be considered to be irritant and will require the appropriate "Xi" symbol if it causes inflammation of the skin, which persists for at least 24 hours, and if either erythema/eschar formation or oedema formation equivalent to a mean value of two or more, calculated for each animal separately is observed in two or more animals. The 24, 48 and 72-hour readings for each animal will be used to calculate the mean values. If these criteria are not satisfied the test material will be classified as non-irritant.

ii) Interpretation according to Annex VI, Part II (D)

In addition, the following risk (R) phrases will be assigned to the test material, if appropriate, according to the criteria indicated below:

Corrosive

R 35 "CAUSES SEVERE BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to three minutes exposure.

R 34 "CAUSES BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to four hours exposure.

Irritant

R 38 "IRRITATING TO SKIN"

If, when applied to healthy intact skin for up to four hours, significant inflammation is caused which is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean score for either erythema/eschar formation or oedema formation, observed in two or more animals, is

at 24, 48 and 72 hours

6. ARCHIVES

Unless instructed otherwise by the sponsor, all original data and a copy of the final report will be retained in the archives of Safepharma Laboratories for a period of ten years. After this period, the sponsor's instructions will be sought.

## 7. RESULTS

The individual scores for erythema/eschar and oedema, are given in Table 1. Mean values required for EEC labelling regulations are given in Table 2.

No skin reactions were noted during the study.

## 8. CONCLUSION

A 50 ppm dilution of the test material, CODE NUMBER: 329179, produced a primary irritation index of 0.0 and was classified as a NON-IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

The diluted test material did not produce positive criteria in any rabbit according to the EEC labelling regulations and was classified as NON-IRRITANT to rabbit skin. No symbol and risk phrase are therefore required.

CODE NUMBER: 329179 (50 ppm) : ACUTE DERMAL IRRITATION TEST IN THE RABBIT

INDIVIDUAL SKIN REACTIONS

T A B L E 1

Skin Reaction	Observation Time (Hours)	Individual Scores - Rabbit Number and Sex (Bodyweight Kg)		Total
		32 FEMALE (3.07)	81 MALE (2.74)	
Erythema/Eschar Formation	1	0	0	( 0 )
	24	0	0	0
	48	0	0	( 0 )
	72	0	0	0
Oedema Formation	1	0	0	( 0 )
	24	0	0	0
	48	0	0	( 0 )
	72	0	0	0
Sum of 24 and 72-Hour Readings (S)	:	0		
Primary Irritation Index (S/6)	:	0/6 = 0.0		
Classification	:	NON-IRRITANT		

( ) = Total values not used for calculation of primary irritation index

CODE NUMBER: 329179 (50 ppm) : ACUTE DERMAL IRRITATION TEST IN THE RABBIT

T A B L E 2  
INDIVIDUAL DAILY AND INDIVIDUAL MEAN SCORES  
FOR DERMAL IRRITATION FOLLOWING 4-HOUR EXPOSURE REQUIRED FOR EEC LABELLING REGULATIONS

Skin Reaction	Reading (Hours)	Individual Scores - Rabbit Number and Sex (Bodyweight Kg)	
		32 FEMALE (3.07)	81 MALE (2.74) 83 FEMALE (2.87)
Erythema/Eschar Formation	24 48 72	0 0 0	0 0 0
Total		0	0
Mean Score		0.0	0.0
Oedema Formation	24 48 72	0 0 0	0 0 0
Total		0	0
Mean Score		0.0	0.0

APPENDIX 1



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT  
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE  
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY  
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PO Box 45  
Derby  
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DATE OF INSPECTION

17 March 1992

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

11/6/92

D. F. Moore