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# SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

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# SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

## 1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 8). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 19 December 2000 and 01 February 2001.

## 2. TEST MATERIAL

### 2.1 Description, Identification and Storage Conditions

| | | |
|---|---|---|
| Sponsor's identification | : | TPP |
| Description | : | white granular solid |
| Batch number | : | F21022 |
| Date received | : | 17 November 2000 |
| Storage conditions | : | room temperature in the dark |

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

### 2.2 Preparation of Test Material

For the purpose of this study the test material was freshly prepared in arachis oil BP. The concentrations used are discussed in the procedure section.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

## 3. METHODS

### 3.1 Animals and Animal Husbandry

Twenty-one male albino Dunkin Hartley guinea pigs were supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK and Harlan UK Ltd., Blackthorn, Bicester, Oxon, UK. After an acclimatisation period of at least five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen. At the start of the main study the animals weighed 345 to 410g, and were approximately eight to twelve weeks old.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Guinea Pig FD1 Diet, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

### 3.2 Procedure

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, J. Invest. Dermatol. (1969) 52: 268 - 276.

### 3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

#### *3.2.1.1 Selection of Concentration for Intradermal Induction*

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of two guinea pigs, using concentrations of 1% and 5% w/w in arachis oil BP. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the scale shown in Appendix 7. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

#### *3.2.1.2 Selection of Concentration for Topical Induction*

Two guinea pigs (intradermally injected with Freund's Complete Adjuvant six days earlier) were treated with four preparations of the test material (75%, 50%, 25% and 10% w/w in arachis oil BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

#### *3.2.1.3 Selection of Concentration for Topical Challenge*

Four preparations of the test material (75%, 50%, 25% and 10% w/w in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

### 3.2.2 Main Study

A group of fifteen guinea pigs was used for the main study, ten test and five control. The bodyweight of each animal was recorded at the start and end of the study and are presented in Appendix 6.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

#### *3.2.2.1 Induction*

**Induction of the Test Animals:** Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line into a 20 mm x 40 mm area. The injections were:

a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1

b) a 5% w/w formulation of the test material in arachis oil BP

c) a 5% w/w formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie. injection site b) was evaluated according to the scale shown in Appendix 7.

On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A filter paper patch (WHATMAN No.4: approximate size 40 mm x 20 mm) loaded with the test material formulation (75% w/w in arachis oil BP) as a thick, even layer was applied to the prepared skin and held in place with a strip of surgical adhesive tape covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale shown in Appendix 7.

Any other reactions were also recorded.

**Induction of the Control Animals:** The intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

### *3.2.2.2 Challenge*

Shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch (WHATMAN No.4: approximate size 20 mm x 20 mm) loaded with a thick, even layer of test material at the maximum non-irritant concentration (75% w/w in arachis oil BP) was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 50% w/w in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully removed and discarded. The challenge sites were swabbed with cotton wool soaked in distilled water to remove residual material. The position of the treatment sites was identified by using a black indelible marker-pen.

Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

Any other reactions were also recorded.

### 3.3 Interpretation of Results

Skin reactions noted at the challenge sites of the test group animals will be attributed to skin sensitisation, providing that reactions of equal severity are not seen at the corresponding challenge sites of the control group animals.

If skin reactions are seen at the challenge sites of the control group animals, these will be due to skin irritation, and therefore only skin reactions of greater severity in the test group animals will be attributed to skin sensitisation.

Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.

The sensitisation potential of the test material will be classified as follows:

| Percentage of sensitised animals | Classification |
|---|---|
| 0 | non-sensitiser |
| >0 – 8 | weak sensitiser |
| >8 – 28 | mild sensitiser |
| >28 – 64 | moderate sensitiser |
| >64 – 80 | strong sensitiser |
| >80 – 100 | extreme sensitiser |

## 4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

## 5. RESULTS

### 5.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the test and control group animals are presented in Appendix 4.

Discrete or patchy to moderate and confluent erythema was noted at the intradermal induction sites of test group animals.

### 5.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the test and control group animals are presented in Appendix 5.

Discrete or patchy to moderate and confluent erythema was noted at the topical induction sites of test group animals.

### 5.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of the test and control group animals are given in Table 1.

No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations.

## 6. CONCLUSION

The test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin under the conditions of the test.

The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 93/21/EEC. No symbol and risk phrase are required.