REPORT

IRRITANCY AND SENSITISATION TESTS ON SIX TOPICAL FORMULATIONS IN 100 SUBJECTS

Cutest

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This is a confidential report prepared by Cutest
for Spirig Pharma Ltd
on 23rd April 2002

CONTENTS

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1. SUMMARY

Six topical formulations have been tested for their irritancy and sensitisation potential in a test panel of 104 normal healthy volunteer subjects.

The design of the study was that of a "repeat insult patch test" consisting of 6 applications to the lower back over a period of 14 consecutive days. After a rest period of seven days, a 48 hour challenge was carried out at a separate site with assessments made at 48 hours immediately after patch removal (day 24) and at 96 hours (day 26).

Irritancy Scores

There was one grade 1 reaction to *Karrer Gesichtstomick LO58/7.001* recorded at day 15 of the irritancy phase of the study. In addition four grade 2 reactions were recorded during the study. This result indicates *Karrer Gesichtstomick LO58/7.001* may be classified as a weak irritant under the conditions of this test.

Sensitisation Scores

There was one grade 1 reaction and one grade 2 to *Karrer Gesichtstomick LO58/7.001* recorded on day 24 of the challenge phase of the study. In addition one grade 1 reaction was recorded on day 26. This result indicates that *Karrer Gesichtstomick LO58/7.001* has a low or possibly moderate potential for sensitisation.

2. STUDY DETAILS

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Study Dates:
Start of study 4th February 2002
End of study 1st March 2002
Final report issued 23rd April 2002

Product	Product Description
1	[illegible]
2	[illegible]
3	[illegible]
4	*Karrer Gesichtstomick LO58/7.001*
5	[illegible]
6	[illegible]

The study materials were supplied by Spirig Pharma AG.

Personnel Involved:
Principal Investigator: Professor R Marks BSc, FRCP, FRCPath
Co-Investigators: P J Dykes PhD, A D Pearse MSc, MIBiol, CBiol, FIScT
Skin Assessments: Staff Nurse Mrs M Clancy SRN
Staff Nurse Mrs J Prescott RGN

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3. INTRODUCTION

This study was performed as described in Spirig Pharma Ltd protocol 10 and was designed to assess the skin irritancy and sensitisation potential of six topical formulations after their repeated application to the skin of normal healthy volunteer subjects. The study design was that of a repeat insult patch test consisting of 6 applications over a period of 14 consecutive days. After a rest period of seven days, a 48 hour challenge was carried out at a separate and distant site with assessments made at 48 hours, 10 minutes after patch removal (day 24) and 96 hours (day 26). This technique maximises the cutaneous insult from a topical preparation and is designed to reveal low orders of irritancy as well as providing information on sensitisation potential.

3.1 Regulatory Guidelines

The study was carried out on the premises of Cutest and was performed in accordance with The ICH Harmonised Tripartite Guidelines for Good Clinical Practice. In addition this study complies with the Guidelines for Medical Experiments in non-patient human volunteers which were initially published in the United Kingdom by the Association of the British Pharmaceutical Industry (ABPI) in March 1988 and further amended in May 1990.

4. THE STUDY

4.1 Test Panel

A total of 104 male and female subjects were recruited from the volunteer test panel of Cutest. Details of the age and sex of the subjects are given in Table 1.

There were 85 female subjects and 19 male subjects. The mean age of the female subjects tested was 42 years; range 20 to 65 years. The mean age of the male subjects tested was 46 years; range 29 to 65 years.

All subjects were deemed to be normal healthy volunteers who had previously been given a medical examination which included blood pressure reading, pulse rate determination, medical history and full examination of the skin before joining the test panel of Cutest. Each subject's medical history was also updated and recorded immediately prior to participation in this study by the study nurse.

In addition all subjects fulfilled the inclusion and exclusion criteria as detailed in the protocol, these were as follows:

Inclusion Criteria

1. Subjects within the age range 18 - 65 years.
2. Healthy with no significant concurrent illnesses.
3. Had signed the consent form and received the volunteer information after the nature of the study had been fully explained.

Exclusion criteria

1. Pregnant or lactating females or females of reproductive age who do not take steps to avoid becoming pregnant during the course of the study.
2. Use of any systemic or topical medication likely to interfere with the study. (e.g. Systemic anti-inflammatory drugs)
3. Use of experimental drug within the previous 30 days.
4. Had taken part in a study involving the test site during the previous 8 weeks (56 days).
5. History of skin disease or allergy likely to interfere with the study.
6. History or evidence of alcohol or drug abuse.

4.2 Ethical Considerations

Ethical approval for the study was obtained from the Bro Taf Local Research Ethics Committee of the Bro Taf Health Authority, Cardiff, UK.

All subjects had the nature of the study explained to them and were given written information concerning the study. They were informed that they were able to withdraw from the study at any stage without obligation and without being required to state a reason. All subjects gave their written, witnessed informed consent before starting the study.

4.3 Materials

The test materials were as follows:

Product	Product Description
1	
2	
3	
4	*Karrer Gesichtstomick LO58/7.001*
5	
6	

The test materials were supplied by Spirig Pharma AG.

Ingredient listings for the products are given in Appendix I.

4.4 Dosage

Each subject received all six test materials at the designated test sites on the back during the irritancy phase and on the outer upper arm during the challenge phase of the study. Approximately 0.1g was applied at each application to the appropriate sites by placing the study materials into 12mm aluminium Finn Chambers mounted on Scanpor® tape.

4.5 Test Procedures

The materials were applied, removed and re-applied repeatedly under occlusion, each to the same site, for a period of 14 days using the repeat insult schedule of applications and assessments as seen below. The materials were applied to six separate test sites on the lower back in an area between the waistline and the mid point of the back avoiding the area over the vertebrae. The six test chambers were applied vertically in two columns each of three test chambers, to one side of the back. Site number 1 was the upper test chamber in the left hand column and received product 1, site number 2 was the middle test chamber in the left hand column and received product 2 and site number 3 was the lower test chamber in the left hand column and received product 3. Site number 4 was the upper test chamber in the right hand column and received product 4, site number 5 was the middle test chamber in the right hand column and received product 5 and site number 6 was the lower test chamber in the right hand column and received product 6.

The schedule of applications and assessments was as follows:

Day 1	Monday	Apply material under occlusion
Day 3	Wednesday	Remove, wait 10 minutes, assess sites. Re-apply
Day 5	Friday	Remove, wait 10 minutes, assess sites. Re-apply
Day 8	Monday	Remove, wait 10 minutes, assess sites. Re-apply
Day 10	Wednesday	Remove, wait 10 minutes, assess sites. Re-apply
Day 12	Friday	Remove, wait 10 minutes, assess sites. Re-apply
Day 15	Monday	Remove, wait 10 minutes, assess sites. No re-application End of Irritancy/Induction phase

The test sites were not specially cleaned before application (including challenge sites). The test sites were inspected for any features such as moles or blemishes and the test materials were applied in such a way to avoid covering such features. Re-applications were made to the same test sites.

4.6 Assessments

4.6.1 Erythema

Re-applications were made to the same test sites. At each assessment time the sites were graded for erythema using an eight point ranking scale, as follows:-

0 = No reaction.

0.5 = Slight, patchy erythema.

1 = Slight uniform erythema.

2 = Moderate, uniform erythema.

3 = Strong erythema.

4 = Strong erythema, spreading outside patch.

5 = Strong erythema, spreading outside patch with either swelling or vesiculation.

6 = Severe reaction with erosion

The test materials were removed carefully. The sites were assessed after a minimum of 10 minutes to allow any reactions due to the Scanpor® tape to subside.

Subjects were requested not to interfere with the test site and avoid sitting against a chair back in order to prevent the test site being masked by pressure marks. Where a grade 2 or greater reaction was reached, the test material was not re-applied, in this situation the site was recorded as 'NR' (Not Re-applied) in the tables of results.

4.6.2 Clinical Signs

The following letters were appended to the numerical score if a clinical sign was noted at the test site.

OE = Oedema

V = Vesiculation

S = Scaling

C = Cracking or crazing

SC = Scabbing

P = Papules

SO = Reaction spreading outside area of application

G = Glazing

4.6.3 Subjective Observations

The letters BS (Burning or Stinging) were appended to the numerical score if reported by the volunteer subject.

4.7 Grading System for Degree of Irritancy in a 100 Subject Test

The category of irritant is determined primarily by the number of grade 2 or greater reactions that occur during the 14 day application period (Column A). The category of irritant may be increased to a higher one if the number of subjects reacting with a grade 1 reaction at day 15 exceeds the total number of reactions for that category description.

Classification of Irritancy for 100 subject panel

Column A No. of subjects reacting with a grade 2 or more reaction during the application period.	Column B No. of subjects reacting with a grade 1 reaction at the end of the 14 day application period (day 15).	Column C Total number of reactors. Column A plus Column B.	Category of irritant.
None	Up to 5 subjects	5 subjects or less	**Non irritant**
1 or 2 subjects	Up to 5 subjects	1 - 7 subjects	**Very Weak**
3 - 4 subjects	Up to 8 subjects	3 - 12 subjects	**Weak**
5 - 8 subjects	Up to 12 subjects	5 - 20 subjects	**Mild/moderate**
9 - 15 subjects	Up to 25 subjects	9 - 40 subjects	**Moderately Strong**
16 - 40 subjects	Up to 40 subjects	16 - 80 subjects	**Strong**
41 - 100 subjects	Up to 60 subjects	41 - 100 subjects	**Very Strong**

4.8 Sensitisation Testing.

After the sixth grading (Day 15) the sites were left untreated for the remainder of the study. At day 22 the materials were re-applied to new sites on the outer aspect of the upper arms in order to test for any delayed hypersensitivity reactions (allergy). The six test materials were applied to the right and left arm in a line at the mid point. Site number 1 was the upper test chamber on the left arm and received product 1, site number 2 was the middle test chamber on the left arm and received product 2 and site number 3 was the lower test chamber on the left arm and received product 3. Site number 4 was the upper test chamber on the right arm and received product 4, site number 5 was the middle test chamber on the right arm and received product 5 and site number 6 was the lower test chamber on the right arm and received product 6.

Any subject reacting with a grade 1 or grade 2 or more reaction to a test material during the first eight days of the study was not challenged with that product.

At day 24 the patches were removed and the sites assessed using the same eight point ranking scale used during the initial application period. A further assessment was carried out at day 26.

Grading System for Sensitisation.

The following categories of sensitisation potential were used:

No. of subjects reacting with a grade 2 or more reaction at day 24 and day 26.	No. of subjects reacting with a grade 1 reaction at day 24 and day 26.	Category of sensitisation potential.
None	Up to 2 subjects	**Low** potential
1 or 2 subjects	Up to 4 subjects	**Moderate** potential
3 or more subjects	5 or more subjects	**High** potential

5. RESULTS

5.1 Test Panel Attendance

All of the 104 subjects recruited for the study completed both the irritancy and sensitisation phase.

Subjects 30 and 40 both failed to attend the day 12 assessment, subject 56 failed to attend the day 10 assessment, subject 75 failed to attend the day 3 assessment and subject 100 failed to attend the day 5 assessment (See 5.2 Protocol Deviations). Subject 82 failed to attend the day 8 assessment due to illness (See 5.3 Adverse Events).

5.2 Protocol Deviations

Subject 30 and 40 failed to attend on day 12, subject 56 failed to attend on day 10, subject 75 failed to attend on day 3 and subject 100 failed to attend on day 8, all due to reasons unrelated to the study. In this situation the subjects continued wearing the patches which were removed assessed and re-applied at the subsequent visit.

5.3 Adverse Events

Two adverse events were reported during the study, both were unrelated to use of the study materials.

Subject 77

This subject developed menorrhagia due to fibroids and polycystic ovaries on day 12 of the study (17.02.02). Cyklokapion (50mg I TAB QDS 2/7) was prescribed.

This adverse event was mild in severity, was considered to be unrelated to use of the study materials and was resolved with treatment.

Subject 82

Subject 82 developed diarrhoea and vomiting on day 6 of the study (9.02.02). This adverse event was moderate in severity, was considered to be unrelated to use of the study materials and was resolved without treatment (12.02.02).

5.4 Irritancy and Sensitisation

Karrer Gesichtstomick LO58/7.001

There was one grade 1 reaction to *Karrer Gesichtstomick LO58/7.001* recorded at day 15 of the irritancy phase of the study. In addition four grade 2 reactions were recorded during the study. *Karrer Gesichtstomick LO58/7.001* had the letter S (Scaling) appended five times during the course of the study, the letters SO (Reaction spreading outside area of application) appended once during the study and the letter P (Papules) appended twice times during the course of the study. These papular reactions may be due to follicular occlusion. This result indicates *Karrer Gesichtstomick LO58/7.001* may be classified as a weak irritant under the conditions of this test.

There was one grade 1 reaction and one grade 2 reaction to *Karrer Gesichtstomick LO58/7.001* recorded on day 24 of the challenge phase of the study. In addition one grade 1 reaction was recorded on day 26. This result indicates that *Karrer Gesichtstomick LO58/7.001* has a low or possibly moderate potential for sensitisation.

DECLARATION AND SIGNATURES

The undersigned hereby declare that this study was performed under our direction and in accordance with the procedures and undertakings specified in the study protocol.
This report is a true and accurate record of the results obtained.

Professor R Marks ..... date 25th April 2002
Principal Investigator

Dr P J Dykes ..... date 24th April 2002
Co-Investigator

Mr A D Pearse ... . date 24th April 2002
Co-Investigator

Mrs V P Pearse ..... date 23-04-2002
Study Co-ordinator

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TABLE 10

Summary of Results

Results are expressed as the number of subjects reacting with an erythema score of 1 or ≥ 2 at each assessment time. Where application of a material has been stopped because of a grade 2 or more reaction, the site has continued to be scored as a grade 2 or more reaction i.e. the summary is cumulative for grade 2 or more reactions.

		Irritancy Phase						Challenge	
Product		**Day 3**	**Day 5**	**Day 8**	**Day 10**	**Day 12**	**Day 15**	**Day 24**	**Day 26**
4	Score of 1	4	9	3	2	3	1	1	1
	Score of ≥ 2	2	3	4	4	4	4	1	0

Product	Product Description
1	[redacted]
2	[redacted]
3	[redacted]
4	*Karrer Gesichtstomick LO58/7.001*
5	[redacted]
6	[redacted]

APPENDIX I

Ingredient Listings - Continued

4. *Karrer Gesichtstomick LO58/7.001*
   Aqua
   Alcohol
   Butylene Glycol
   Hamamelis Virginiana Folia Extract
   Sodium Lactate
   Cycloheptaamylose
   Salicylic Acid
   Allantoin
   Menthol