Final Report SAM2817i-2

DELAYED-TYPE HYPERSENSIVITY TEST

Study Program: SAM2817

Contract No.: E05/0137.4MI

Sponsor: ANDROMEDICAL S.L.
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Test substance: ANDRO-PENIS

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Released on: .....

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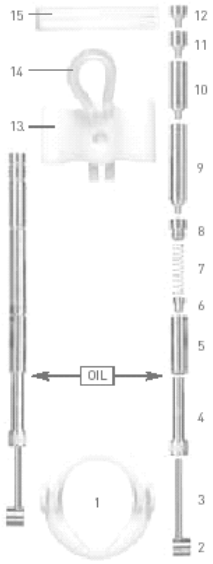
SUMMARY

A toxicological study was performed to evaluate the biocompatibility of the test substance ANDRO-PENIS, at this purpose the following test was carried out:

- delayed-type hypersensivity test

The analytical test was accomplished on the different materials which constitute the device and are in contact with the human skin:

1. Plastic base ring	Plastic
2. Rod (for the articulated screw)	Brass and Nickel
3. Articulated screw	Brass and Nickel
4. Adjustable bar screw	Brass and Nickel
5. Metal bar	Brass and Nickel
6. Screw	Brass and Nickel
7. Spring	Brass and Nickel
8. Screw to ground the spring	Brass and Nickel
9. Large 4 cm axis	Aluminium alloy
10. Medium 2 cm axis	Aluminium alloy
11. Small 0.5 cm axis	Aluminium alloy
12. Minimum 0.3 cm axis	Aluminium alloy
13. Superior plastic support	Plastic
14. silicone band	Silicon
15. Andro-Top	Foam



Two eluates of the test substance were prepared both in vegetable oil and in physiological solution in order to perform the delayed-type hypersensivity test.
In static condition the eluates of the test material were performed by immersing the test material in both physiological solution and vegetable oil in order to reach weight/volume ratios and surface/volume ratios of:

- 0.2 g/ml for the PLASTIC (1 and 13)
- 0.2 g/ml for the FOAM (15)
- 6 cm2/ml for the ALLUMINUM ALLOY (9-12)
- 3 cm2/ml for the SILICON (14)
- 6 cm2/ml for the BRASSED and NICKELED COMPONENTS (2-8)

The test sample was then incubated for 72 hours at 37°C ±1°C, after this period, has been done a pool of eluates.
For each elution 15 guinea pigs were used, 10 treated with the eluate of the test substance and 5 using as control treated with extraction liquid only.

The skin sensitisation test had 2 phases, induction phase and challenge phase.
During the induction phase the group of 10 treated guinea pigs were treated with 3 double intradermal injections as follows:
1st Freud Complete Adjuvant in distilled water (ratio 1:1)
2nd Elution of the test substance
3rd Elution of the test substance and FCA (ratio 1:1)
The control animals received the same pairs of injections, but in the 2nd injection only extraction liquid was administered (physiological solution or vegetable oil).
In the third injection, extraction liquid + FCA (ratio 1:1) was used.
After 6 days from the beginning of treatment on the all animals, a topical application, with slight massage, of 0.5 ml of Sodium Lauril Solfatum 10% was performed.
After 7 days from the intradermal injections, the test substance was applied (at a dose of 0.5 ml/animal). The application lasted 48 hours.
The same treatment was used on control guinea pigs using only extraction liquid.
After 21 days from the beginning of treatment the challenge phase was performed by applying 0.5 ml of the eluate on the left side and 0.5 ml of the eluates on the right side. The bandage was left on for 24 hours.

24, 48 and 72 hours after the beginning of this phase, the tested and the control animals were observed.

Neither edema nor erythema were observed in the animals treated with the test substance eluate prepared in physiological solution. and in vegetable oil.
No abnormalities were observed in the animals used as control.

On the basis of the results obtained for all tested components, interpreted according to ISO 10993-10:2002 the test substance ANDRO-PENIS must be considered **NOT SENSITIZING**.

The detailed procedure is described in Experimentation Report SAM2817i-2.A1

INTRODUCTION

This study has been carried out on behalf of ANDROMEDICAL S.L. to evaluate the biocompatibility of the test substance through the following test:

- delayed-type hypersensivity test

The study was performed at the Assay Centre Biolab S.p.A. of Vimodrone (MI) – via B. Buozzi n. 2 (Italy).

The **delayed-type hypersensivity test** started with eluates preparation on September 9th, 2005 with eluates preparation and was completed on October 8th, 2005.

BIBLIOGRAPHY

1. ISO 10993-10:2002
   Biological evaluation of medical devices
   Part 10: Tests for irritation and delayed-type hypersensivity

FILING

The study program, all raw data and a copy of the final report are filed in the archives of BIOLAB S.p.A. for ten years after the issuing of the final report.
No retained sample will be kept .
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the substances for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the Biolab Procedures Manual.

TEST SUBSTANCE DESCRIPTION

The test substance is a device consisting of different parts made of plastic and metallic materials intended to human use in contact with the skin.

Name: ANDRO-PENIS

ANALYSED SAMPLE

The analysed sample, representative of the test substance, is identified by the following numbers:

Name: ANDRO-PENIS

Acceptance number: 05.16494

Receiving number: R03758.05

Receiving date: August 22th, 2005

Experimental Report SAM2817i.A1

DELAYED-TYPE HYPERSENSIVITY TEST

SENIOR RESEARCHER: P. Consonni

EXPERIMENTAL PROCEDURE

1. TEST METHOD

1.1 Characterisation

Species:	Albino guinea pigs
Strain:	Hartley
N.:	30
Weight:	300 - 400 g at the arrival at the Centre
Sex:	female
Supplier:	Bettinardi - Momo (NO)

1.2 Caging

The animals were caged, in groups of ten, in transparent polycarbonate cages (dimensions: 590x385x200h mm).
The housing room was lighted with fluorescent lamps 12 hours for day.
Room temperature and humidity were regulated by a conditioning plant and were monitored daily.
Recordings of the housing conditions are being retained in Biolab S.p.A. files.

1.3 Cleaning and disinfection

The cages and the housing room were cleaned before the animals were accommodated, then disinfected periodically.

1.4 Feeding

Animals have been fed with standard pellet complete diet supplied by the authorized breeder Harlan.

1.5 Watering

Filtered tap water from local network was supplied ad libitum from an automatic watering system.

1.6 Quarantine

Before allocation to the study, the animals were kept in quarantine for one week.
During this period they were observed daily.
At the end of the quarantine period the animals were carefully examined in order to evaluate their suitability for the study.

2. TEST SAMPLE PREPARATION

The eluates of the test substance were prepared in static conditions by immersing:

- 1 piece (11.8 g) of PLASTIC (1 and 13) into 59 ml of both eluants in order to reach a weight/volume ratio of 0.2 g/ml.
- 2 g of FOAM (15) into 10 ml of both eluants in order to reach a weight/volume ratio of 0.2 g/ml.
- 6 pieces (75 cm2) of ALLUMINUM ALLOY (9-12) into 59 ml of both eluants in order to reach a surface/volume ratio of 6 cm2/ml.
- 1 piece (39 cm2) of SILICON (14) into 13 ml of both eluants in order to reach a surface/volume ratio of 3 cm2/ml.
- 2 pieces (47.1 cm2) of BRASSED and NICKELED COMPONENTS (2-8) into 7.8 ml of both eluants in order to reach a surface/volume ratio of 6 cm2/ml.

The test samples were then incubated for 72 hours at 37°C ±1°C, after this period, two pools of eluates have been done.

3. EXPERIMENTAL DESIGN

Experimental design consisted of two groups (treated) of 10 animals treated with extract in vegetable oil and in physiological solution of test substance (group 1-2) and two group (control) of 5 control animals treated with only vegetable oil or physiological solution (group 3-4).
The animals were divided in groups as follows:

GROUP	INDUCTION		CHALLENGE TOPIC APPLICATION
	Intradermal injection	**Topic application**	
1	1° Extract in physiological solution 2° Extract in physiological solution + FCA 3°FCA	Extract in physiological solution	Right side: Extract in physiological solution
			Left side: Physiological solution
2	1°Extract in freshly refined vegetable oil 2° Extract in freshly refined vegetable oil + FCA 3° FCA	Extract in vegetable oil	Right side: Extract in vegetable oil
			Left side: Vegetable oil
3	1° Physiological solution 2° Physiological solution + FCA 3°FCA	Physiological solution	Right side: Extract in physiological solution
			Left side: Physiological solution
4	1°Freshly refined vegetable oil 2° Freshly refined vegetable oil + FCA 3° FCA	Vegetable oil	Right side: Extract in vegetable oil
			Left side: Vegetable oil

The animals allocated to the study were selected randomly from those suitable, available at that time.

4. TREATMENT

4.1 Skin preparation

24 hours before testing, fur was removed by shaving a 50 cm^2 wide area on the back of the animals.

4.2 Administration

The test consisted of an induction phase and a challenge phase.

Induction phase

Day 0 - treated group
Three pairs of 0,1 ml intradermal injections were made in the intrascapolar region of each animal, on each side of the midline, according to the following scheme:
1) FCA in distilled water (ratio 1:1)
2) Elution of test substance
3) Elution of test substance + FCA (ratio 1:1)

Day 0 - control group
Three pairs of 0,1 ml intradermal injections were made in the intrascapolar region of each animal, on each side of the midline. The content was:
1) FCA in distilled water
2) Extraction liquid
3) Extraction liquid + FCA (ratio 1:1)

Day 6 - treated group and control group
After 6 days the beginning of treatment on the all animals a topical application, with slight massage of 0,5 ml of Sodium Lauril Solfatum 10%, was made.

Day 7 - treated group
Seven days after the intradermal injections had been made, 0,5 ml of the elution of the test substance were applied to each animal and held in place with an occlusive patch. The application was made on area caudally to the area of injection.
The dressing was left in place for 48 hours.

Day 7 - control group
The same treatment was performed on the control group, using vegetable oil and physiological solution instead of the test substance.

Challenge

Day 21 - treated and control groups
An occlusive patch with 0,5 ml of eluate of the test substance was applied to the right side and to the solvent to the left side.
The dressing was left in place for 24 hours.

OBSERVATIONS

On the 23rd day (24 hours after removal the patch), and the 24th day (48 hours after removal the patch) and the 25th day (72 hours after removal the patch) of tests all the animals treated and controlled were evaluated for a skin reaction.
The intensity of erythema and/or edema were evaluated according to the following scale:

Reaction	**Grade**
Erythema	
No erythema	0
Slight erythema	1
Well defined erythema	2
Moderate erythema	3
Severe erythema to slight eschar formation	4
Edema	
No edema	0
Slight edema	1
Well defined edema	2
Moderate edema	3
Severe edema	4

INTERPRETATION OF RESULTS

Both frequency and intensity of response were evaluated.
In case of positive reaction only in treated animals, the frequency of sensitisation was considered, without taking into account the intensity of the response.

RESULTS

Neither edema nor erythema were observed in the animals treated with the test substance eluates prepared in physiological solution and in vegetable oil.
No abnormalities were observed in the animals used as control.

% sensitising guinea pigs treated with extract in physiological solution: 0%
% sensitised guinea pigs treated with extract in vegetable oil: 0%

The data concerning every single animal are reported in appendices 1 and 2.

CONCLUSIONS

On the basis of the results obtained for all tested components, interpreted according to ISO 10993-10:2002 the test substance ANDRO-PENIS must be considered **NOT SENSITIZING**.

DELAYED-TYPE HYPERSENSIVITY TEST

APPENDICES

Appendix n.1: Skin reactions in treated animals with eluate

ANIMAL N.	TIME AFTER CHALLENGE APPLICATION					
	physiological solution					
	48 hours		72 hours		96 hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0

ANIMAL N.	TIME AFTER CHALLENGE APPLICATION					
	vegetable oil					
	48 hours		72 hours		96 hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0

Erythema

0= No erythema

Edema

0= No edema

Appendix n.2: **Skin reactions in control animals treated with vegetable oil and physiological solution**

ANIMAL N.	TIME AFTER CHALLENGE APPLICATION					
	physiological solution					
	48 hours		72 hours		96 hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0

ANIMAL N.	TIME AFTER CHALLENGE APPLICATION					
	vegetable oil					
	48 hours		72 hours		96 hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0

Erythema

0= No erythema

Edema

0= No edema

DELAYED-TYPE HYPERSENSIVITY TEST

ADDENDUM

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