Final Report SAM2817i-3

SKIN IRRITATION TEST

Study Program: SAM2817

Contract n.: E05/0137.4MI

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

Study Director.....
(Dr. P. Consonni)

Released on: .....

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- Datos de partida issued on November 12th, 2002 by AndroMedical (4 pages)

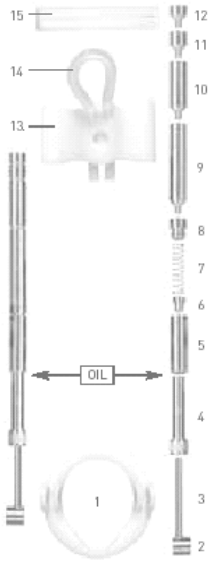
SUMMARY

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

- Skin irritation 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



In the **skin irritation 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 a weight/volume ratio of:

- 0.2 g/ml for the PLASTIC (1 and 13)
- 0.2 g/ml for the FOAM (15)

and a surface/volume ratio of:

- 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.
0.5 ml of each eluates of test substance were applied on intact skin of 6 rabbits, in the dorsal region on the left and right side.

The back's right caudal and left cranial area of 3 tested animal has been treated with the extract of examined substance into physiological solution , while the left caudal area and right cranial area of the back has been used as control, treated with the eluent only.

The back's right caudal and left cranial area of other 3 tested animal has been treated with the extract of examined substance into vegetable oil , while the left caudal area and right cranial area of the back has been used as control, treated with the eluent only

The application lasted for 4 hours.
The skin reaction were evaluate 1, 24, 48 and 72 hours after the beginning of the treatment.

60 minutes after the treatment, in all animals treated with eluate of the test material in vegetable oil, slight erythema without edema was observed.
The erythema has totally disappeared in all animals treated with eluate of the test material in vegetable oil 24 hours after the treatment.

In all animals treated with eluate of the test material in physiological solution no edema or erythema was observed.

On the basis of the results, interpreted according to ISO 10993-10:2002, the test substance ANDRO-PENIS **did not cause any irritant effects on skin**.

The detailed procedure is reported in Experimental Report SAM2817i.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:

- skin irritation test

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

The **skin irritation test** started on October 7th, 2005 with eluates preparation and was completed on October 13th, 2005.

BIBLIOGRAPHY

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

FILING

All raw data are filed in the archives of BIOLAB S.p.A for ten years after the issuing of the final report.
No control sample has been be kept.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the materials 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-3.A1

SKIN IRRITATION TEST

PRIMARY INVESTIGATOR: P. Consonni

EXPERIMENTAL PROCEDURE

1. TEST METHOD

1.1 Characterization

Specie: White rabbits
Strain: New Zealand
No.: 3
Sex: male
Weight: 3090-3140 g at the beginning of the test
Supplier: Conelli Arona (NO) - Italy

1.2 Caging

Each rabbit was caged in stainless steel cages of cm 48.2x63x37 h equipped with automatic washing cycle.
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 cleaning and disinfecting were performed periodically. The cages were provided with automatic washing equipment.

1.4 Feeding

The animals were fed with standard pellet complete diet supplied by the authorised breeder Harlan.

1.5 Watering

Filtered tap water from local network was supplied ad libitum.

1.6 Animal identification

A numbered plastic tag placed through the edge of the right ear identified the animals selected for the study.
A label identified the cages.

1.7 Quarantine

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

1.8 Animal selection

The animals used for this study were selected randomly from those suitable, available at that time.

2. EXPERIMENTAL DESIGN

Three rabbits have been used to perform the test.
The back's right caudal and left cranial area of each tested animal has been treated with the examined substance, while the non-treated left tail and right cranial area of the back has been used as control.

2.1 Preparation of the assay sample

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 a weight/volume ratio of:

- 0.2 g/ml for the PLASTIC (1 and 13)
- 0.2 g/ml for the FOAM (15)

and a surface/volume ratio of:

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

3. TREATMENT

3.1 Skin preparation

Approximately 24 hours before the test, the fur was removed from an area approximately 240 cm^2 wide by clipping and shaving the dorsal and flank zones of the animals.
An area of the back, about 6 cm^2 wide, was designed for the application of the test sample.

3.2 Application

25 x 25 mm of the test substance were applied directly to the skin on cranial site of each rabbit.

The application sites were covered with non-occlusive dressing and the wrap the application sites with a semi-occlusive bandage.

3.3 Removal of the patches

The patches were removed 4 hours after the application.

4. OBSERVATIONS

General conditions of the animals were verified daily. Reactions were evaluated following the removal of the patches and were evaluated again at 24, 48, 72 hours after exposure.
Skin irritation was scored and recorded according to the scores reported in the following table.

GRADING VALUES

Erythema and eschar formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness with slight eschar formation; injuries in depth)	4

Edema formation

No edema	
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

INTERPRETATION OF RESULTS

For acute exposure, determine the Primary Irritation Index (PII) as follows.

For each animal, add together the Primary Irritation Scores for the test substance for both erythema and edema at each time specified and divide by the total number of observations. When vehicle controls are used, calculate the Primary Irritation Score for the vehicle controls and subtract that score from the score for the test substance to obtain the Primary Irritation Score.

Only use 24 hours, 48 hours and 72 hours observations for calculations. Observations made prior to dosing or after 72 hours, to monitor recovery, are not used in the determination.

Add the scores for each animal and divide the total by number of animals. This value is the Primary Irritation Index.

Number and description in following table characterise the Primary Irritation Index:

Response category	Mean score
Negligible	0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

RESULTS

60 minutes after the treatment, in all animals treated with eluate of the test material in vegetable oil, slight erythema without edema was observed.
The erythema has totally disappeared in all animals treated with eluate of the test material in vegetable oil 24 hours after the treatment.

In all animals treated with eluate of the test material in physiological solution no edema or erythema was observed.

Results referring to the single animals and mean values of erythema and edema referring to each observation time are shown in appendix n. 1.

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CONCLUSIONS

On the basis of the results, interpreted according ISO 10993-10:2002, the test substance ANDRO-PENIS must be considered **NOT IRRITANT** for skin.

SKIN IRRITATION TEST

APPENDIX

APPENDIX N.1: Skin reaction

Physiological solution

REACTION	TIME AFTER REMOVAL OF PATCHES	RABBIT N.											
		310				315				317			
		Treated		Control		Treated		Control		Treated		Control	
		Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx
Erythema	60 minutes	0	0	0	0	0	0	0	0	0	0	0	0
	24 hours	0	0	0	0	0	0	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0	0	0	0	0	0	0
	72 hours	0	0	0	0	0	0	0	0	0	0	0	0
Edema	60 minutes	0	0	0	0	0	0	0	0	0	0	0	0
	24 hours	0	0	0	0	0	0	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0	0	0	0	0	0	0
	72 hours	0	0	0	0	0	0	0	0	0	0	0	0

Vegetable oil

REACTION	TIME AFTER REMOVAL OF PATCHES	RABBIT N.											
		319				403				404			
		Treated		Control		Treated		Control		Treated		Control	
		Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx	Cr dx	Ca sx
Erythema	60 minutes	1	1	0	0	1	1	0	0	1	1	0	0
	24 hours	0	0	0	0	0	0	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0	0	0	0	0	0	0
	72 hours	0	0	0	0	0	0	0	0	0	0	0	0
Edema	60 minutes	0	0	0	0	0	0	0	0	0	0	0	0
	24 hours	0	0	0	0	0	0	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0	0	0	0	0	0	0
	72 hours	0	0	0	0	0	0	0	0	0	0	0	0

SKIN IRRITATION TEST

ADDENDUM

(4 Pages)