Medical Sciences
University of Tehran

FACULTY OF PHARMACY

S.N.Ostad PharmD, PhD Professor in Toxicology & Pharmacology/Head f Cell & Animal Toxicity Lab

Cell and Animal Toxicology Laboratory

Report No: 1403-995

Version: English

Print date: 02/06/2024

Final Report

SKIN IRRITATION TEST

Study Program: 1403-995

Contract n: k25/Avi

Sponsor: Avijeh Daroo Co. LTD

Test substance: Argacida (animal fungicidal spray) Pilot batch

---

Study Director. Prof. S. N. Ostad

..... Released on: 02/06/2024.....

In the **skin irritation test** the test material was performed by direct contact of sample to the skin

0.5 ml of sample of test substance was applied on intact skin of 4 x 3 (three separate occasion tests) rabbits, in the dorsal region on the left and right side.
The back's right caudal and left cranial area of four tested animals has been treated with the suspension 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 inert vehicle only. In other separate animal groups (positive and independent negative group the 0.1% histamine and vehicle treated respectively).
The application lasted for 4 hours for simple irritation test and 3 days for repeated irritation test.
The skin reactions were evaluating 1, 24, 48 and 72 hours after the beginning of the treatment.
In all animals treated with the test material in physiological solution no edema or erythema was observed. The positive group show the edema and erythema as mentioned in table.
On the basis of the results, interpreted according to BS EN ISO 10993:10; 2016, the test substance **is none- irritant.**
The detailed procedure is reported in Experimental Report.

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

INTRODUCTION

This study has been carried out on behalf Avijeh Daroo Co. LTD to evaluate the biocompatibility of the test substance through the following test:

- Skin irritation test

The study was performed at the Cell and animal Toxicology Laboratory, Faculty of Pharmacy, University of Tehran Medical Sciences, Tehran P.O. Box: 14155/6451 I.R. IRAN

The **skin irritation test** started on May 7th, 2024 with Argacida (animal fungicidal spray) Pilot batch and was completed on May 16th, 2024.

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

TEST SUBSTANCE DESCRIPTION

The test substance is a solution intended to animal use in contact with the skin and mucosa

Test substance: Argacida (animal fungicidal spray) Pilot batch

ANALYSED SAMPLE

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

Name: Argacida (animal fungicidal spray) Pilot batch

Acceptance number: A2

Receiving number: BA2

Receiving date: May 1, 2024

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

Experimental Report 1403-995

SKIN IRRITATION TEST

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

EXPERIMENTAL PROCEDURE

1. TEST METHOD

1.1 Characterization

Specie: White rabbits
Strain: New Zealand
No.: 4 x 3 (separate occasion)
Sex: male
Weight: 1355-1412 g at the beginning of the test
Supplier: Pasteur Institute Tehran IRAN

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 Lab 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.

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

Four rabbits have been used to perform the test for each time.
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. Independent negative and positive control as mentioned in introduction has been tested.

2.1 Preparation of the assay sample

As mentioned above.

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 and for repeated skin irritation test are renewals.

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	0
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

In all animals treated with the test material in physiological solution no edema or erythema was observed after 48 hrs.

Results referring to the single animals as row data to each observation time are shown in appendix.

INDEX SKIN IRRITATION: 0.0

CONCLUSIONS

On the basis of the results, interpreted according ES BN ISO 10993:10; 2016, the test substance Argacida (animal fungicidal spray) Pilot batch must be considered **NONE-IRRITANT** for skin.

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

Sample: Argacida (animal fungicidal spray) Pilot batch
Company: Avijeh Daroo CO Ltd
The Animal Model: White Albino Rabbits
Number of Animals: four for each group
Serial No: 1a1

Positive Control	Negative Control	Observation +72 hrs	Observation +48 hrs	Observation +24 hrs	Observation +4 hrs	Animal NO
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	1
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	2
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	3
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	4

Er = erythema Ed = Edema

Starting Date: 07.05.2024
Finishing Date: 14.05.2024

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

Sample: Argacida (animal fungicidal spray) Pilot batch
Company: Avijeh Daroo CO Ltd
The Animal Model: White Albino Rabbits
Number of Animals: four for each group
Serial No: 1a2

Positive Control	Negative Control	Observation +72 hrs	Observation +48 hrs	Observation +24 hrs	Observation +4 hrs	Animal NO
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	1
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	2
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	3
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	4

Er = erythema Ed = Edema

Starting Date: 08.05.2024
Finishing Date: 15.05.2024

Medical Sciences
University of Tehran

FACULTY OF PHARMACY

Sample: Argacida (animal fungicidal spray) Pilot batch
Company: Avijeh Daroo CO Ltd
The Animal Model: White Albino Rabbits
Number of Animals: four for each group
Serial No: 1a3

Positive Control	Negative Control	Observation +72 hrs	Observation +48 hrs	Observation +24 hrs	Observation +4 hrs	Animal NO
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	1
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	2
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	3
Er ++++ Ed ++++	Er - Ed -	Er - Ed -	Er - Ed -	Er - Ed -	-	4

Er = erythema Ed = Edema

Starting Date: 09.05.2024
Finishing Date: 16.05.2024