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TOXICITY STUDIES

**DELAYED HYPERSENSITIVITY TEST
IN GUINEA PIGS**

GPMT (Guinea pig maximization test)

Laboratory: INTERLAB

Test substance: FR-91

Submitted by: F. Chacon

Reference n°: T 0224

Starting date: 18/11/92

Finish date: 13/12/92





I.	INTRODUCTION	3
II.	METHODOLOGY	4
	a) Objectives	4
	b) Preparations	4
	1) Experimental animals	4
	2) Samples	4
	c) Species selected	4
	-Maintenance	5
	1. Lodging	5
	2. Experimental groups	6
	3. Experimental method	6
III.	RESULTS	8
	a) Evaluation table	8
IV.	DISCUSSION AND CONCLUSION	11





SKIN SENSITIZATION

Sensitization of the skin (allergic dermatitis) is an immunological cutaneous reaction to a substance.

After an initial exposure to the substance under study (the induction period), the animals are subjected, two weeks after the final inductive exposure, to an exposure of a challenge dose of this same substance with the objective of establishing whether or not a state of hypersensitivity has been induced. The sensitization is determined by examination of the cutaneous response to the challenge.

This assay has been carried out using the sample of FR-91, supplied to Interlab by F. Chacón, and is filed in Interlab under the reference number T 0224.

I INTRODUCTION

The sensitizing potential of the sample referred to above has been studied in the albino guinea pig (Dunkin-Hartley strain).

This type of study provides information on the potential health risks derived from repeated exposure to the same dosage. The results should be extrapolated using the due precaution.

The determination of the sensitizing properties of the test substances on mammalian skin constitutes an important initial step in the evaluation of the toxic properties of a substance.

This assay has been carried out according to the principles recommended by the OCDE in its guidelines for assays on chemical substances.





The following method and the assay in general have been carried out in accordance with the norms established by the EU in this area (Directive 79/831, Annex VIII part B, Toxicological Methods for the Evaluation of Chemical Substances).

II. METHODOLOGY

a) Objectives

The objective of the study is to evaluate the possible sensitizing effects of the sample on skin. The study is carried out over a period of 24 days.

b) Preparations

1) Experimental animals

The animals for this experiment, once they had been received in the laboratory, were examined by an expert veterinarian and separated into the distinct experimental groups according to weight and sex. The animals were kept in observation for a minimum of seven days, to assure an adequate state of health and acclimatization to the environmental conditions in which the assay is carried out.

The skin area to which the sample is to be applied is carefully shaved 24 hours before each application, taking care not to damage the skin.

2) Samples

The test sample, given its liquid nature, was used undiluted as supplied to Interlab.



c) Species selected

Male guinea pigs have been used for this study, with a weight between 274 and 402 g at the outset of the assay.

The extensive data available make the guinea pig an acceptable animal for this type of study, as its handling, laboratory conditions, pathology, behavior, etc. are known and standardized in protocols.

Studies in this type of animal are described by the EU in Directives 67/548 and 79/831, 84/449, 88/320.

The strain selected is the Dunkin-Hartley albino guinea pig.

-Maintenance:

The guinea pigs were inspected daily to discard those which presented signs of illness or any other anomaly.

1. Lodging

The conditions of the animal facility were controlled during the execution of the study.

-Temperature was maintained at $20^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

-Relative humidity was maintained between 40-70%.

-The photoperiodicity was 12/12 hours of light/darkness.

Water and food were available ad libitum for all animals. A





standard guinea pig diet was given.

2. Experimental groups

The groups were constituted of homogeneous animals as described previously. On termination of the acclimatization period, 30 guinea pigs were lodged and duly identified.

-10 animals (male) for the CONTROL GROUP

-20 animals (male) for the TREATED GROUP

The animals were supplied by the company IFFA-CREDO on 04/11/92. The quarantine period terminated 11/11/92.

The exclusive use of males for this assay is justified by the fact that no females were available at the outset of the study (EU protocols permit the use of males and/or females).

3. Experimental method

The animals were weighed before the induction phase and at the end of the assay. The procedure consisted of two phases:

-Induction phase

The animals are administered, by the intradermic route, three pairs of injections on either side of the midline, in the following manner:

*Day 0

TREATED GROUP





- 1°: 0.1 ml Freund's complete adjuvant (CFA)
- 2°: 0.1 ml assay substance
- 3°: 0.1 ml assay substance in CFA (1+1 proportion)

CONTROL GROUP

- 1°: 0.1 ml CFA

***Day 7**

TREATED GROUP

Hair is shaved in the area to be treated. Fragments of filter paper are saturated with the test substance and placed in contact with the shaved skin surface, and maintained in close contact for 48 hours using an adequate dressing.

CONTROL GROUP

As no vehicle is used for the test substance, no treatment is performed with this group.

-Challenge phase

*** Day 21**

The hair is shaved on the left flank of the treated and control groups, and the test substance applied on an appropriate dressing (as previously explained) for 24 hours.

*** Days 23 and 24**

Twenty-one hours after removal of the patches, the test area is cleaned and shaved. Three hours later (at 48 hours from the onset of the challenge phase), it is examined and the cutaneous reaction is recorded. A second reading is taken and recorded 24 hours later (at 72 hours from the onset of the challenge phase).



III. RESULTS

a) Evaluation table

The results expressed in this report have been evaluated according to the table of values in Annex I, according to the criteria of the OCDE and the EU for these types of study.

TABLE I. Evaluation of cutaneous reactions

	Value
No response	0
Slight erythema (barely perceptible)	±
Slight erythema (clearly defined)	1
Moderate erythema (well-defined)	2
Severe erythema (with or without edema)	3

Any induration (hardening due to prolonged congestion) should be evaluated as grade 3.





RESULTS TABLES

CUTANEOUS HYPERSENSITIVITY (Guinea pig maximization test) GPMT

Product: FR-91

Starting date: 18/11/92

Finish date: 13/12/92

CONTROL GROUP

Starting weight	Final weight	Sex	Observations	
			Day 23	Day 24
302	496	♂	0	0
364	648	♂	0	0
325	583	♂	0	0
381	534	♂	0	0
373	548	♂	0	0
318	546	♂	0	0
307	555	♂	0	0
392	514	♂	0	0
365	493	♂	0	0
376	505	♂	0	0





TREATED GROUP

Starting weight	Final weight	Sex	Observations	
			Day 23	Day 24
370	505	♂	0	0
380	521	♂	0	0
406	568	♂	0	0
274	414	♂	0	0
307	507	♂	0	0
365	586	♂	0	0
354	498	♂	0	0
401	525	♂	0	0
375	478	♂	0	0
358	548	♂	0	0
335	511	♂	0	0
394	587	♂	0	0
303	460	♂	0	0
355	449	♂	0	0
323	469	♂	0	0
327	465	♂	0	0
308	425	♂	0	0
337	458	♂	0	0
339	575	♂	0	0
402	591	♂	0	0





IV. DISCUSSION AND CONCLUSION

In accordance with this table, no erythematous or edematous lesions were observed in the guinea pigs in any of the treated or control areas, in any phase (either inductive or challenge) of this experiment.

All animals were scored with a score of 0 in both examinations.

In conclusion, we affirm that the sample FR-91, submitted by F. Chacón, with Interlab reference T 0224, did not generate delayed-type contact hypersensitivity under the conditions used in this study.

We declare that the results emitted in this report were obtained according to the protocol described.

A handwritten signature in dark ink, appearing to read "María José Benítez de Huelva".

MARÍA JOSÉ BENÍTEZ DE HUELVA
Study Director

Madrid, 15 December 1992

A handwritten signature in dark ink, appearing to read "Marta Malmierca".

MARTA MALMIERCA
Technical specialist

A handwritten signature in dark ink, appearing to read "Paloma Azpeitia".

~~PALOMA AZPEITIA~~
Head, Quality Control Unit