



TOXICITY STUDIES

**ACUTE TOXICITY BY THE
INTRAMUSCULAR ROUTE**

Laboratory: INTERLAB

Test substance: FR-91

Submitted by F. Chacon



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**ACUTE TOXICITY
BY THE INTRAMUSCULAR ROUTE**

Test substance FR-91

Report: A/50001-P/0A

DECLARATION OF GOOD LABORATORY PRACTICES

This study has been carried out in accordance with the norms set out in Annex II of the guidelines of the OCDE on non-clinical assays with chemical substances and in conformity with the European Directives 87/18 and 88/320 which regulate this matter.

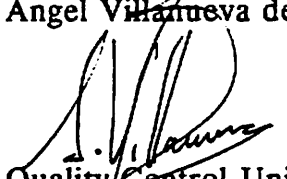
The following periodic inspections have been carried out:

Reports:

Arrival of animals	14 July
Lodging and established conditions	20 July
Manipulation processes and dose administration	21 July
Programmed autopsies	5 August

As the result of these inspections, strict observance of the standardized working protocols and the overall study plan was appreciated.

Angel Villanueva del Río



Quality Control Unit



ACUTE TOXICITY: 14 days, rat, intramuscular route

Report: A/50001-P/0A

Test substance: FR-91

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Report date:	26 August 1991



ACUTE TOXICITY

Acute toxicity study (limiting dose assay) of the product FR-91 by the intramuscular route in rat, filed in INTERLAB Laboratories under the reference n° A/50001-P/0A.

I. INTRODUCTION

The sample referred to above has been studied in acute toxicity in the rat.

This type of study provides information on the potential health risks derived from exposure to a single dose, using due care in the extrapolation of results.

Acute toxicity is a measure of the short-term effects on the health provoked by the administration of a substance or mixture of substances. This type of assay is carried out according to Good Laboratory Practices in accordance with with 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, B1 Toxicological Methods, for the evaluation of chemical substances, in accordance with the norms established in Directive 75/319 and 87/19 on pharmaceutical preparations.

In accordance with the guidelines established, if in a limiting toxicity study (single limiting dose study), the atotoxicity of the test substance is demonstrated for this study, in these circumstances it is not necessary to carry out an acute toxicity study using lower doses. In this manner, the unnecessary use of experimental animals is avoided.



II. METHODOLOGY

a) Objectives

The objective of the study is to evaluate the possible acute toxic effects of the sample mentioned above. The study is carried out over a period of 14 days. When this period has terminated, the surviving animals are sacrificed. Necropsy and macroscopic examination are performed.

b) Preparations

-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 ideal weight and state of health. The animals were acclimatized to laboratory conditions for a period of five days; when finished, those animals considered unsuitable for the experiment by the veterinarian were withdrawn from the study.

-Samples

The sample was administered at a single dosage level of 5000 mg/Kg, limit assay.

The dose was calculated by volume/animal weight, taking into account the correction for the density of the preparation ($\delta = 1.007$ g/cm³).

c) Species selected

The rat has been used in this study, given that it is a standardized laboratory animal, used to evaluate acute toxicity for numerous chemical substances.



The large quantity of data available and accumulated make the rat an acceptable animal for this type of study, given that its handling, laboratory conditions, pathology, behavior, etc. are known and standardized in protocols. Studies in rodents are described by the EU in Directives 67/548 and 79/831, 84/449, 88/320.

In accordance with the methodology, the extrapolation of the results to the human species is facilitated, always with the due limitations, considering the qualitative and quantitative aspects between the two species.

The strain selected is Wistar albino of uniform characteristics. At the outset of this study, the rats weighed between 180 and 250 grams in the case of the females and 240 and 350 grams in the case of the males.

The animals were supplied by IFFA-CREDO, France, through their Spanish distributor J. Alonso Villamañana, c/ Sepúlveda, 13, Ed. Indubuilding (Madrid) on 14 July 1991.

-Maintenance:

The rats were examined daily to evaluate possible pathological alterations which might arise. When the acclimatization period had terminated, five healthy males and five healthy females were selected and assigned to a single study group.

1. Lodging

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



-Temperature was maintained at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

-Relative humidity was maintained between 50-65%.

-The photoperiodicity was 12/12 hours of light/darkness with an intensity of 200 lux.

Water and food were available ad libitum for all animals. The diet was standard and tested by the supplier for this type of animal (supplied by PanLab, Ref. A-04).

Each group was identified by a metallic cage card, with an individual number for each cage.

2. Experimental groups

The group is constituted of homogeneous animals as described above. The distribution in relation to the samples was the following:

Group: Dose of 5000 mg/Kg 5 males

Group: Dose of 5000 mg/Kg 5 females

3. Experimental method

The sample was administered intramuscularly, undiluted, in the interior face of the animals thigh.

The animals received the correct volume of the sample relative to their body weight (see Table), in a single injected dose administered on day 1 of the experiment. The observation period extended over a 14-day period.



4. Observations

Examination was made for mortality, morbidity, behavior and signs of toxicity on the first, second and sixth hours after administration of the sample, and afterwards twice daily.

On the fourteenth day, surviving animals were sacrificed and submitted for autopsy.

III. RESULTS

a) Mortality

Dose: 5000 mg/Kg

MALES			OBSERVATIONS
Starting weight	Final weight	Volume / animal	
240 g	270 g	1200 μ l	Nothing unusual
325 g	340 g	1625 μ l	"
340 g	364 g	1700 μ l	"
350 g	367 g	1750 μ l	"
350 g	352 g	1650 μ l	"

FEMALES			OBSERVATIONS
Starting weight	Final weight	Volume / animal	
235 g	250 g	1175 μ l	Nothing unusual
215 g	240 g	1075 μ l	"
200 g	232 g	1000 μ l	"
180 g	205 g	900 μ l	"
250 g	250 g	1250 μ l	"



b) Morbidity

Neither pathological signs nor physiological disorders were observed in any of the animals (see Table).

c) Necropsy

All animals were examined post mortem under the supervision of an expert veterinarian. All animals were sacrificed according to standard norms.

Following the external examination, a macroscopic post mortem examination was performed on the thoracic and abdominal organs, with the following result:

Nothing unusual observed in:

- | | |
|----------------|---------------------|
| -Lung | -Colon |
| -Trachea | -Spleen |
| -Larynx | -Liver |
| -Heart | -Pancreas |
| -Aorta | -Associated glands |
| -Esophagus | -Kidney |
| -Stomach | -Urinary bladder |
| -Duodenum | -Prostate (males) |
| -Jejunum | -Testicles (males) |
| -Sciatic nerve | -Epididymus (males) |
| -Ileum | -Uterus (females) |
| -Cecum | -Ovaries (females) |



IV. DISCUSSION AND CONCLUSION

From the analysis of the data obtained, the conclusion is extracted that the product under study, FR-91, submitted by F. Chacón, shows no toxic effects due to the administration of the product by the intramuscular route at the maximum admissible dose for acute toxicity studies.

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

Madrid, 26 August 1991

STUDY DIRECTOR

A handwritten signature in cursive script, appearing to read "L. Fuente".

Luis de la Fuente Ramírez

LABORATORY DIRECTOR

A handwritten signature in cursive script, appearing to read "José Miguel Sicilia Socias".

José Miguel Sicilia Socias

QUALITY CONTROL UNIT

A handwritten signature in cursive script, appearing to read "Angel Villanueva del Río".

Angel Villanueva del Río