

Report n°: CD-89/13297T

Determination of acute toxicity by route endovenous in rats (Innocuousness Test)

Substance to testing: Vacuna Probios

October 1989

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SUMMARY

The substance VACUNA PROBIOS has proved to possess, when it is administered by endovenous route, a DL_{50} higher than 5000 mg/Kg .

No treated rats died with the dose previously mentioned.

The study animals treated with the substance for testing did not show alterations related to the treatment and their ponderal evolution, throughout observation period was normal.

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INTRODUCTION

The proposal of this study has been to assess the acute toxicity of the substance “VACUNA PROBIOS”, when it is administered by endovenous route in Wistar rats.

This substance is a partial hydrolyzed from constituent enzymes of probios. .

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EXPERIMENTAL METHOD

1.1. Animals

A total of 14 albino Wistar rats (7 males and 7 females), 90-100 Kg body weight and the 4 weeks age approximately, were used in this study.

The animals were supplied for PANLAB, S.L. (Barcelona), on 31st August 1989.

At their arrival to the CIDA, they were lodged in cages "Makrolon" Type IV (47 x 22.5 x 14.5 cm) with shaving beds until a maximum of 6 rats/cage and they were put to a period of previous observation and acclimatization not lower than one week. During this period, they were put to a veterinary inspection with the purpose of rejecting those animals which did not comply with health requirements necessary for the beginning of this study.

The animals involved in this preliminary study weighted at the beginning of the same one between 114 -121 g.

The treated rats weighted, at the administration moment, 104-134g (males), and 97-123 g (females).

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1.2 Identification

Animals were individually identified with a punch-hole code, in their ears, according to Standard Procedures of CIDA.

Rats were immediately marked after administration.

The number of rats was included in the identification card.

1.3 Group size

The Preliminary study of the substance for testing was carried out with a group constituted for 2 males and 2 females.

The main study was carried out with a group constituted for 5 males and 5 females.

1.3.1.- Preliminary study

Substance tested: Vacuna Probios

Group size: 4

Number of males: 1, 2

Number of females: 3, 4

Doses mg/Kg: 5000

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1.3.2.- Main study

Substance tested: Vacuna Probios

Group size: 10

Number of males: 5-9

Number of females: 10-14

Doses mg/Kg: 5000

1.4 Lodging

Rats were lodged in cages “Makrolon” Type IV (47 x 22.5 x 14.5 cm) with shaving beds until a maximum of 6 rats per cage. During the observation and acclimatization period each cage lodged 6 rats at the most.

Every cage lodged 2 animals with the same sex - in the preliminary study- and 5 animals in the main study.

The shaving beds were replaced by a metallic grating in the fasting period prior to administration.

The identification of cages was made by means of a card in which the study number, number of animals, sex code, level of dose, date of arrival, arrival and administration date, and study director were indicated.

During the study, the lodging place temperature was kept to 22°C. The relative humidity ranged between 50 and 75 %, coming occasionally to 45 % .

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The maintenance of lodging place was carried out according to Standard Procedures of CIDA.

The rats had free access to a standard diet for rats and had water ad libitum supplied by means of bottles. The used water is analyzed from time to time in order to detect the presence of possible pollutants.

1.5 Substance for testing

On July 18, 1989 a box was received in the CIDA (Centro de Investigación y Desarrollo Aplicado, S.A.L) containing three ampoules of transparent glass, each one with approximately 20 ml of the substance to testing: VACUNA PROBIOS

The above mentioned substance offered as a brown liquid

There was stored in freezer to 2-10 °C

The remainder of the mentioned substance was kept in the files of CIDA for a period of three months, from the date of this report. After the above mentioned period, it was get back to the customer.

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1.6 Administration of substance for testing

The product was administered, without dilution, by endovenous route, into the lateral tail vein of animals.

The administration was performed in one shot to a volume of 5 mg/kg.

The study was divided in two phases:

- Preliminary study performed over a group of two males and two females,
- Main study performed over 5 males and 5 females.

The aim of the preliminary study was to get information about the mortality rate induced because the chosen dose in order to choose an adequate level of dose for the main study.

2.- OBSERVATION

2.1 Preliminary study

Rats were at least observed twice a day for 14 days and after they were sacrificed.

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2.2 Main study

Rats were observed twice a day for 14 days. After this period of observation, the animals were sacrificed and subjected to terminal procedures described in the point 2.4

The observation included, changes in the respiratory and circulatory systems, central and autonomic nervous systems, somatome activity and behavior in order to record an eventual clinic respond.

2.3 Body weight

Rats were weighted before administration, in the middle of observation period and before slaughtering

2.4 Slaughtering and post mortem procedures

At the end of the observation period, all rats were slaughtering by inhalation of CO₂, . Necropsy was performed in all rats belonging to Main study.

The necropsy included the revision of untouched animals and all superficial tissues, followed by the observation of the entrails of the thoracic and abdominal cavities in situ and after evisceration

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3.- FILES

All the data obtained during the study were kept in the CIDA Files, for 10 years, at least.

The material related to this study will not be destroyed without notification by writing to the customer.

4.- STUDY FACILITIES

The study was performed in the facilities of Toxicology Department of CIDA S.A.L., Centro Industrial Santiga, C/ Argentera 6, 08130- Santa Perpétua de Mogoda (Barcelona).

5.- STUDY DATES

The length of this study has been the following:

- Date of acceptance of Protocol: 10th August 1989
- Date of reception of animals: 31st August 1989
- Date of beginning of experimental work: 7th September 1989
- Date of conclusion of experimental work: 22nd September 1989
- Date of Final report: October 1989

6.- DEVIATIONS OF PROTOCOL

During the development of the study, the following deviations of the experimental protocol have been produced:

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- The relative humidity ranged between 50 and 75 %, coming occasionally to 45%. This margin is superior to the specified one in the experimental protocol (60-10%).

7.-RESULTS

7.1.- Preliminary study

Mortality did not take place among animals of the preliminary study (2 males and 2 females) treated to the dose of 5000 mg/Kg.

Decrease of motor activity and piloerection were registered in all animals in the first hours after administration.

In addition, tonic convulsions were observed in the same period.

7.1.- Main study

Mortality did not take place among animals (5 males and 5 females) treated to the dose of 5000 mg/Kg.

Clonic convulsions and shivering were observed in all animals during the first hour after administration.

The evolution of body weight was normal. (Table 11).

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Macroscopic alterations were not recorded in the necropsies performed.

The DL_{50} of substance called VACUNA PROBIOS has turned out to be higher than 5000 mg/Kg where it is administered by endovenous route.

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TABLE N° 1

Substance: VACUNA PROBIOS

Specie: Wistar rat

BODY WEIGHT (in gram). MAIN STUDY

Dose level	N° of animals	Sex	Day		
			1	8	15
	5	M	109	185	240
	6	M	104	173	211
	7	M	114	187	234
	8	M	134	224	294
	9	M	112	188	247
5000	10	H	107	162	172
	11	H	97	143	170
	12	H	119	172	199
	13	H	123	176	201
	14	H	106	155	172

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UNIT OF QUALITY GUARANTEE

Inspection of report n° CD-89/1329T

In accordance to regulations of “good Laboratory Practice” of OCDE, this study has been inspected and its report reviewed together with Procedures standard of work of Q.A.U.

The inspection dates are detailed below:

Date	Phase	N° Inspection Q.A.U.	Reports to Direction
08.08.89	Protocol	2074	08.08.89
08.09.89	I.V Administration Main study	2128	08.09.89
22.09.89	Necropsies	2166	22.09.89
04.10.89	Final Report	2194	04.10.89

M^a Jesús Lázaro
Unit of quality guarantee