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Report Nº 1001-87-88
7 March 1995

Study title

FR-91

REPORT: CIC - 1001-87-88

**TESTING FOR TOXICITY BY REPEATED
INTRAMUSCULAR ADMINISTRATION TO
BEAGLE DOGS (SIX MONTHS STUDY)**

Author

Dr. J. Salas

Study completed on

17 January 1995

Performing Laboratory

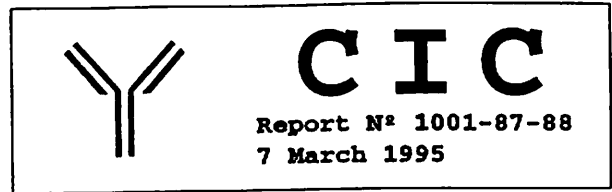
Centros Inmunológicos del Cáncer (CIC)

C/ Alejandro Villegas 23

E-28043, Madrid

Laboratory Project ID

Study Nº 1001-87-88



STATEMENT OF COMPLIANCE

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director *J. Salas* Date 05-03-95
Dr. J. Salas

Quality Assurance Unit *A. Fernández* Date 05-03-95
Dr. A. Fernández

FINAL REPORT

CIF: B80642804 Inscrita en el Registro Mercantil de Madrid, Tomo 6569, de la sección del libro de Sociedades, Folio 91, Hoja M-106910



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QUALITY ASSURANCE STATEMENT

Title: Testing for toxicity by repeated intramuscular administration to Beagle dogs
(six months study)

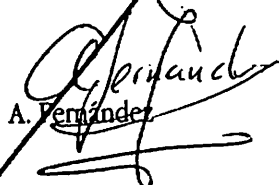
Date: 07/03/95

Study N°: CIC-1001-87-88

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below:

Inspection	Report
08.08.1994	08.08.1994
29.09.1994	29.09.1994
07.11.1994	07.11.1994
15.12.1994	15.12.1994
17.01.1995	17.01.1995
20.02.1995	20.02.1995
02.03.1995 - 07.03.1995	05.03.1995

CIC Research
Quality Assurance Unit


A. Fernández

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FR-91 TECHNICAL PRODUCT

**TOXICITY BY REPEATED INTRAMUSCULAR
ADMINISTRATION TO BEAGLE DOGS OVER SIX MONTHS**

SUMMARY

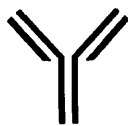
In a toxicity study, FR-91 technical product (code: lot 87-88) was administered by the intramuscular (i.m.) injections at three dose levels (0.5 / 1 / 2 ml/kg) to groups each consisting of five male and five female Beagle dogs (BIOCENRE S.A., sant feliu de Codines, Barcelona). A further group of the same size received physiological saline solution (Nacl 9 g/L) and served as a control.

All of the animals survived until the scheduled termination of the study.

After administration of three dose levels no product related toxic reactions were observed. No product-related toxic reactions were observed. No product-related changes or abnormalities could be detected on:

- Clinical observations and examinations,
- Food consumption,
- Body weight control,
- Neurological status,
- Ophtalmological examinations,
- Dental inspection,
- Laboratory examinations (clinical chemistry, Urianalysis)
- Postmortem examinations (Autopsies, organ weights, histology)

Regarding the haematology, no manifestation of any toxic effect was detected, only the white blood cells, (monocytes and neutrophils) increased significantly in the three groups compared to control group. The observed pharmacological effects seemed to be associated with the dose (group 2 > group 1 > group 0.5 ml/kg). The magnitude of the increase remained within normal limit values.



SYNOPSIS

Study Nº: CIC-1001-87-88

Sponsor: CHACON FARMACEUTICA, S.A.

Test Substance: FR-91 - Technical Product
Code: Lot 87, lot 88

Test species: Beagle Dog, BIOCENTRE, S.A.

Number of groups: 4

Number of animals per group: 5 males / 5 females

Route of Administration: Intramuscular injection

Dose Levels: 0 / 0.5 / 1 / 2 ml/kg

Vehicle: None

Duration of study: Six months

Number of Treatments:

Group I:	males: 129
	females: 130
Group II:	males: 130
	females: 133
Group III:	males: 133
	females: 134
Group IV:	males: 134
	females: 136

Start of acclimatisation: Animals were kept since birth under identical conditions (BIOCENTRE, S.A.)

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Start of study (1st treatment):
Group I: 06.07.1994
Group II: 07.07.1994
Group III: 08.07.1994
Group IV: 11.07.1994

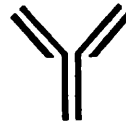
Last Treatment:
Group I: males: 02.01.1994
 females: 03.01.1994
Group II: males: 04.01.1995
 females: 09.01.1995
Group III: males: 10.01.1995
 females: 11.01.1995
Group IV: males: 12.01.1995
 females: 16.01.1995

Killing of animals:
Group I: males: 03.01.1994
 females: 04.01.1994
Group II: males: 05.01.1995
 females: 10.01.1995
Group III: males: 11.01.1995
 females: 12.01.1995
Group IV: males: 13.01.1995
 females: 17.01.1995

Responsibilities:
Study Director: Dr. J. Salas
Haematology: Dr. A. Sicilia
Clinical Chemistry: Dr. A. Gutiérrez
Dissection: Dr. F. Chadon

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Histology and Histopathology:

Dr. Niembro de la Rashe

Statistic:

Dr. F. García

Quality Assurance:

Dr. A. Fernández

Department of Toxicology:

Dr. F. Chadon

Testing facility and Archive:

Centros Inmunológicos del Cáncer (CIC)

Department of Toxicology

C/ Alejandro Villegas 23

28043 - Madrid, España

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OBJETIVE AND GUIDELINES

This cronic toxicity study was carried out in order to determine the toxicological profile of FR-91 when administered to Beagle dogs for a period of six months. The intramuscular route of administration was selected based on the therapeutic use in humans.

This study should provide a rational basis for a toxicological risk assessment in humans.

Rationale for dose selection:

The dose levels were selected on the basis of the previous toxicity studies in rats and dogs (reports from GRUPO INTERLAB and CIDA SAL). In this study, FR-91, was administered in daily concentrations (five days per week) of 0, 0.5, 1 and 2 ml/kg.

For the present chronic study, no toxic effects were expected to be produced by the dose levels. However the use of higher doses is not justified, since the highest dose was 2 ml/kg/day. The dosage to be use in humans is 2 ml per person and alternating days. Thus the dose used in beagle dogs is at least 30 times that propose for clinical use in humans.

Furthermore the volume injected daily is considerable and the use of larger volume in the experiment for determination of the therapeutic index is not justified.

Guidelines:

This study was conducted in compliance with the guidelines of authorities in the UE.

Test Guidelines

Pharmaceutical Substances

75/318/CEE Directive, 83/571/CEE

GLP guidelines

OECD Principles of Good Laboratory Practice, Annex 2,

OECD, Paris, June 1981



I TEST DESIGN

1.1 Test substance

Name: FR-91

Code: Lots 87 and 88

Identity: According to technical especifications, preclinic data from CHACON FARMACEUTICA, as stated in Certificate Analysis from GRUPO INTERLAB of FR-91, Lots 87 and 88.

Test Substance received: At start of study

Storage: At 2-8°C temperature

1.2 Test species and animal husbandry

Test animals: Pure-bred Beagle dogs from BIOCENTRE, were treated three times with ASCARICIDA-N-CANINO (endoparasitism prevention). The dogs were vaccinated from BIOCENTRE against Distemper, Hepatitis, Leptospirosis, Rabies and Parvovirus.

At the start of the study the males had a mean body weight of 12.3 Kg (9.3 - 13.4 Kg) and a mean age of 6 months, the females a mean body weight of 11.1 Kg (9.3 - 12.6 Kg) and a mean age also of 6 months.

The dogs were identified by a number tatoed in the left ear and a numbered collar.

Before the start of treatment, the animals were assigned at random to blocks according to body weights.



1.3 Housing and feeding

The dogs were kept in buildings at a steady temperature of about 19°C with separate Kennels (0.95 m x 1.0 m) for each dog and access to an outdoor exercise area (10.5 m x 10.0 m) for all animals of same group and sex. Kennels and outdoor exercise area were daily cleaned. The relative humidity was maintained between 40-70%.

The dogs received a mixed feed diet (Proplan, manufactured by AGRICENTRO, Pol. Industrial of Villalba, Madrid; for composition see appendix) in individual daily portions of 1000 g for the males and 800 g for the females. Due to their lower body weights, the daily feed ration for the females was one fifth lower than that given to the males.

Feeding took place at about 1.00 p.m. with the exception of weekends and public holidays when feeding was conducted at about 10.00 a.m.

Tap water (Canal de Isabel II, Madrid) was available ad libitum from automatic water dispensers. (for analysis see appendix).

1.4 Treatment

Duration of study and route of administration:

The test substance was administered by intramuscular injections (5 days per week) over a period of six months.

For the administration were determined 10 sites of injection:

Right thigh: 3 sites

Left thigh: 3 sites

Right Lumbar muscular mass: 2 sites

Left Lumbar muscular mass: 2 sites



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On each site of injection was administered 1/10 part of the total amount of FR-91 to administer.

Previously to injection, each area of injection was disinfected with etanol 96°.

Each injection was performed used an automatic "philips" syringe and 21G needle.

The amount of FR-91 administered to each animal was calculated according to weekly body weight.

The animals of the control group received the same volume of saline physiological solution (NaCl 9g/l) in the same proportion as the highest treatment group (2 ml/kg).

1.5 Assignment of animals to treatment groups

At the beginning of the acclimatisation period, 40 pure bred Beagle dogs were assigned to the following groups:

GROUP	FR-91 ml/kg	NUMBER OF DOGS		IDENTIFICATION Nos.	
		M	F	M	F
I	0	5	5	A11-A15	A01-B05
II	0.5	5	5	B11-C15	B01-D05
III	1	5	5	C11-E15	C01-C05
IV	2	5	5	D11-D15	D01-D05

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II TEST PROCEDURE

2.1 Clinical observations and examination

2.1.1 Deaths

Continual checks ad least twice daily.

2.1.2 General health check

Daily

2.1.3 Food consumption

Daily checks. Dogs which had not finished their feed after 2 hours were recorder;

2.1.4 Body weight control

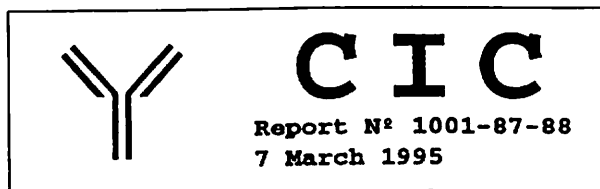
Once weekly

2.1.5 Behaviour

Daily observation, ad least twice

2.1.6 Neurological status

The flexor, patellar, anal, cutaneous, corneal, pupillary and blink reflexes, and also the placing (visual and tactile) and righting reactions were tested before the initial treatment (Groups I - IV), after 3 months and before the termination of the study (Groups I - IV).



2.1.7 Ophthalmological examinations

By means of an Okulus hand slit-lamp a Kowa RC2 fundus camera (direct ophthalmoscopy) and a Zeiss 100/16 slit-lamp microscope at same intervals as 2.1.6.

The following sections were examined:

Cornea, anterior chamber, iris/pupil, crystalline lens, vitreous body and fundus. The examinations were carried out in a darkened room, the pupils having been dilated beforehand by local administration of "Colircusi tropicamida" (manufactured by CISI, S.A. laboratory, Barcelona, Spain).

2.1.8 Dental inspection

At the same intervals as for 2.1.6

2.2 Laboratory examinations

The examinations were carried out in accordance with the methods in the List of Methods.

The blood samples for all examinations were taken from the vena cephalica antebrachii of the fasted animals about 16-20h after treatment.

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List of Methods

HAEMATOLOGY:

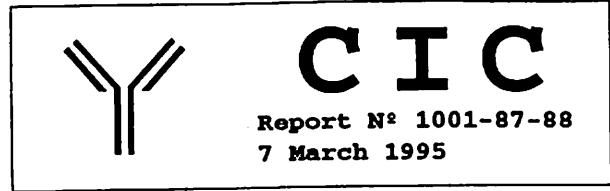
PARAMETER	METHOD
Erithrocytes	
Haematocrit	
Haemoglobin	
Leucocytes	H1 BAYER TECHNICON
Differential blood count	
Thrombocytes	
Prothrombin Time	

CLINICAL CHEMISTRY:

PARAMETER	METHOD
ASAT/GOT	AUTOANALYZER HITACHI 737
ALAT/GPT	AUTOANALYZER HITACHI 737
Alcaline Phosphatase	AUTOANALYZER HITACHI 737
Total proteins	AUTOANALYZER HITACHI 737
Albumine	Sulfosalic. Acid (578 nm) Lamda 2 - PERKIN ELMER Espectrophotometer
Sodium	
Potassium	Selective electrode-ion
Chloride	NOVA-13
Calcium	
inorganic Phosphorus	
Iron	

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Bilirubine	AUTOANALYZER HITACHI 737
Creatinine	AUTOANALYZER HITACHI 737
Glucose	AUTOANALYZER HITACHI 737
LDH	RA1000 THECNICON
Cholesterol	AUTOANALYZER HITACHI 737
Triglycerides	AUTOANALYZER HITACHI 737
Total Lipids	AUTOANALYZER HITACHI 737

URIANALYSIS

PARAMETER

Colour

pH

Specific gravity

Protein

Glucose

Haemoglobin

Bilirubin

Ketone Bodies

Urobilinogen

Nitrites

Sediment

METHOD

Microscopic observation

CLINITEX AMES

BAYER TECHNICON

CLINITEX AMES or if anomalous then Microscope

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2.2.1 Blood samples were taken and subjected to Haematological examinations before the start of the study (initial value), after 3 months (interim value), and before the termination of the study (terminal value).



Examination extended to determination and calculation of the following parameters:

PARAMETER	SI Unit
Erithrocytes	$10^{12}/L$
Haematocrit	%
Haemoglobin	g/L
Leucocytes	$10^9/L$
Differential blood count	%
Thrombocytes	$10^9/L$
Prothrombin Time	sec.
MCV	μ^3
MCH	pg
MCHC	g/dL

2.2.2 Clinical Chemistry

At the times stated in 2.2.1, the following parameters were determined in the serum (plasma LDH) of all animals from groups I - IV:

PARAMETER	SI Unit
ASAT/GOT	U/L
ASAT/GPT	U/L
Alcaline Phosphatase	U/L
Total proteins	g/L
Albumine	g/L
Sodium	mmol/L
Potassium	mmol/L
Chloride	mmol/L
Calcium	mmol/L
Inorganic Phosphorus	mmol/L
Iron	mmol/L
Bilirrubine	mg/dL

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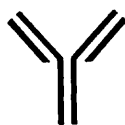
PARAMETER	SI Unit
Creatinine	mg/dL
Glucose	g/L
LDH	U/L
Cholesterol	mg/dL
Triglycerides	mg/dL
Total Lipids	g/L

2.2.3 At the times stated in 2.2.1, the collected urine of each animal separately was examined for the following parameters:

PARAMETER	
Colour	
pH	
Specific gravity	
Protein	g/L
Glucose	g/L
Haemoglobin	+/-
Bilirubin	+/-
Ketone Bodies	+/-
Urobilinogen	+/-
Nitrites	+/-
Sediment	

The sediment was examined for the detection:

- Leucocytes
- Erythrocytes
- Epithelial cells
- Microorganism (bacterial, etc...)
- Crystals
- Anomalous constituents



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2.3. Post mortem examinations

The animals were killed by an intravenous injection of sodium pentobarbital and by excision of axillar vascular packet. The animals of groups I-IV were killed on the day after the final treatment.

2.3.1 Autopsies

Dissection and macroscopic examination were carried out immediately after the animals had been killed.

All the animals of groups I-IV were necropsied.

2.3.2 Organ weights

The weights (groups I-IV) of the following organs were determined:

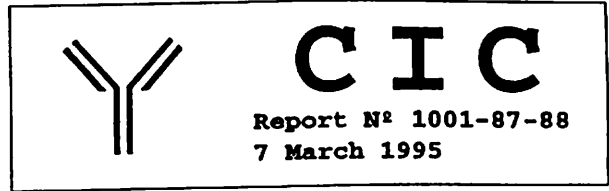
Heart, lungs, liver, kidneys, spleen, brain (without medulla), pituitary, pancreas, ovaries/testes, uterus/epididymides, prostate, thyroid with parathyroid, thymus and adrenals.

2.3.3 Histology

The following organs or parts of organs, were removed for microscopic examination and fixed in formaldehyde solution and/or Carnoy's fluid or Schaffer's solution:

Heart, lungs, liver, kidneys, spleen, adrenals, thyroid with parathyroid, pancreas, thymus, pituitary, cerebral cortex, brain stem, cerebellum (cortex and medulla), medulla, cervical, thoracic and lumbar regions of spinal cord, bone marrow (middle sternal segment), caput femoris, eyes (each with optic nerve), urinary bladder, testes/epididymides, ovaries/uterus, prostate, stomach, (Fundus and prepyloric region), intestine (duodenum, jejunum, ileum, caecum, colon and rectum), skeletal muscle (psoas), diaphragmatic muscle sample, gallbladder, tonsils, trachea, aorta (thoracic region), lymph nodes (cervical and iliac), sciatic nerve, skin with mammary gland, and salivary glands (parotid and mandibular).

Complete histological examination was performed on all above specified tissues from all dogs (groups I and IV) and all gross lesions.



2.4 Statistical evaluation

It is a general rule that statistical evaluation is carried out only if the number of test animals permits this, or if the measured parameters are non-sex-specific and thus provide a sufficiently wide data basis for calculation. The minimum requirement is either 4 animals per sex and group or, in the case of non-sex-specific (pooled) data, 4 individual values.

The following parameters were evaluated statistically:

- Body weight gains
- Haematology
- Clinical chemistry
- Urianalysis (specific gravity and pH value)
- Organ weights (absolute and relative)

The methods used for the individual parameters are given on the printouts of CIC Data Processing (computer printouts, excel/microsoft program). The individual dose groups were compared with the control group. The level of significance is given with an exactness of $p < 0.05$.

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III RESULTS

3.1 Clinical findings

(pp 264-275)

3.1.1 Deaths

No spontaneous deaths occurred. No intercurrent killing was necessary.

3.1.2 General health condition

All the dogs remained in good nutritional and health condition, and no product-related disturbances were observed.

3.1.3 Food consumption

No impairment of food consumption resulted from treatment. The dogs invariably consumed their feed without hesitation.

3.1.4 Body weight gains

(Individual body weight tables and calculations on tables and figures, pp 27-37, 177-193)

There was no disturbance of body weight gains.

3.1.5 Neurological Status

(for results see appendix, pp 276-289)

Testing for reflex excitability and postural reactions revealed no changes as compared with the initial status.

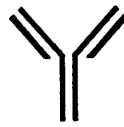
3.1.6 Ophthalmoscopic findings

(for observations see appendix, pp 290-305)

No product-related changes were seen.

3.1.7 Dental inspection

No abnormalities (see appendix, pp 306-315)



3.2. Laboratory examinations

3.2.1 Haematology

(pp 38-85, 194-217)

The white blood cells, (monocytes and neutrophils) increased (statistically significant) in the three groups compared to control group. The observed effects seemed to be associated with the dose (group 2 mg/kg > group 1 mg/kg > group 0.5 mg/kg). The white blood cells remained within normal values and no manifestation of any toxic effect was detected.

None of the restant measured parameters showed product related changes.

The results showing statistically significant deviations from the controls were in relation to initial values within the limits of normal physiological variation and are without toxicological relevance.

3.2.2 Clinical chemistry

(pp 86-113, 218-241)

None of the measured parameters showed product-related changes.

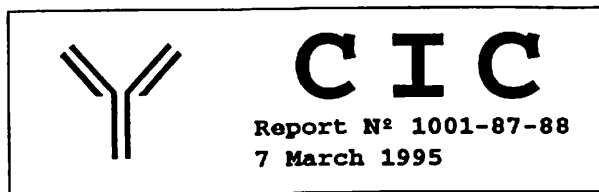
Any results showing statistically significant deviations from the controls were in relation to initial values within the limits of normal physiological variation and are without toxicological relevance.

3.2.3 Urinalysis

(pp 114-129, 242-245)

No product-related abnormalities could be detected.

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3.3 Post mort findings

3.3.1 Macroscopic organ findings

(Cf. report of Dr. Niembro, Pathology Services, HEMOS laboratory, Dr. Castelo 10, Madrid, 21 February 1995) (pp 152-159).

No product-related changes in any of the organs were found in the different groups (Groups I-IV).

3.3.2 Organ Weights

Absolute (pp 136-143, 246-253)

Relative (pp 144-151, 254-261)

(Individual organ weight tables and calculations on tables).

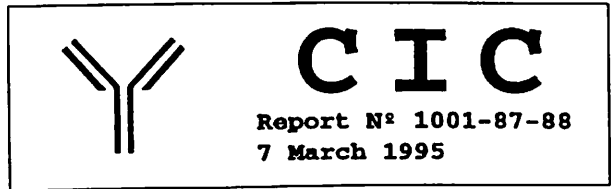
No product-related abnormalities could be detected.

3.3.3 Microscopic organ findings

(Cf. report of Dr. Niembro, Pathology Services, HEMOS laboratory, Dr. Castelo 10, Madrid, 21 February 1995) (pp 160-175).

All changes observed in this study are considered to be incidental, since they are commonly diagnosed in dogs of this breed and age.

No product-related abnormalities were seen.

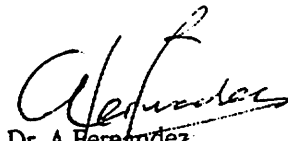



IV CONCLUDING ASESMENT


The analysis of the data obtained, under the conditions of the protocol described, offers the conclusion that the product under study, FR-91, provokes no toxicological changes compared with the control group, in any of the animals studied, at any dosage level tested.

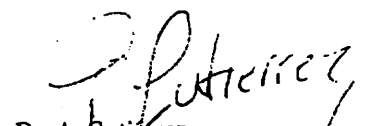
With respect to haematologic changes described, the white blood cells, monocytes and neutrophils, increased significantly in the three groups (dosage levels 0.5, 1 and 2 ml/kg) compared to the control group. The magnitude of the increase remained within normal laboratory range. The effect seemed to be associated with the dose.

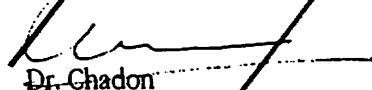
We declare that the results emitted in this report were obtained according to the protocol described.


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date 07/03/95


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FINAL REPORT