

Eye Irritation/Corrosion Effects
in Rabbits (*Oryctolagus cuniculus*) of
NES FUNGICIDA, ACARICIDA E
INSECTICIDA SELECTIVO - LIQUIDO

Guideline OECD N° 405

Test number:

BIBR 6 – 30394

ID **50907**

Ref. 6-2151/M

Date:

May 23th, 2010

Sponsor:

AJIM S.R.L Y JORGE NATALIO FELIPE PEISAJOVICH
GALANTE - AGROSERVICIOS NES - MEXICO

Av. Borrini 225

Resistencia, CP (3500), Chaco

Argentina

Study conducted by

MICROQUIM S.A.

Department of Biological Studies

5MIC6100101301a

Consejo Profesional de Química



Study N° BIBR 6 – 30394

Page 1 of 18

MICROQUIM S.A.
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Biological Studies

Index

	Pages
Title page	1
Index	2
A. Statement of Compliance with Good Laboratory Practices	3
B. Preface	4
C. Summary	8
D. Purpose	9
E. Test System and Materials	9
F. Test Performance	12
G. Results	12
H. Conclusion	13
I. References	13

Table

1. Determination of the Primary Eye Irritation Index	14
2. Individual Body Weights of the Animals at the Start and at the End of the Test	15

Annex

I –Reference to the Toxicological Classification	16
II- GLP certificate	18

Quality Assurance Unit Statement

A) STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICES

TITLE: EYE IRRITATION/CORROSION EFFECTS IN RABBITS (*Oryctolagus cuniculus*) OF NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

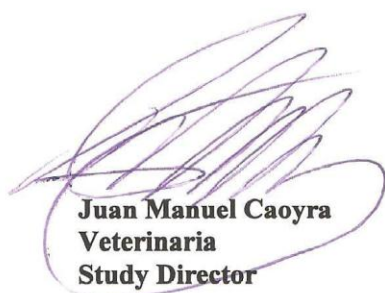
TEST SUBSTANCE: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

STUDY NUMBER: BIBR 6 – 30394

This study was conducted according to the OECD series on principles of Good Laboratory Practices and compliance monitoring, N°1, ENV/MC/CHEM (98) 17 OECD and pursuant to the written study plan, authorized by the Sponsor and the Technical Management of MICROQUIM. S.A. following the Standard Operating Procedures (SOP) stated in the Procedures of MICROQUIM S. A.

This report is a true and accurate record of the results obtained, and there were no known circumstances that could have affected the quality and integrity of the data. This certificate can only be reproduced with the approval of the laboratory.

The results obtained, as well as any storage medium for electronically recorded data, all documentation, study plan and final report are retained in the corresponding archives at MICROQUIM S.A.



Juan Manuel Caoyra
Veterinaria
Study Director

Date: 08/07/10

Study N° BIBR 6 – 30394

Page 3 of 18

MICROQUIM S.A.
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B) PREFACE

B.1) GENERAL

Title: Eye Irritation/Corrosion effects in rabbits (*Oryctolagus cuniculus*) of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO.

Sponsor: AJIM S.R.L Y JORGE NATALIO FELIPE PEISAJOVICH GALANTE - AGROSERVICIOS NES – MEXICO, Av. Borrini 225, Resistencia, CP (3500), Chaco, Argentina

Test number: BIBR 6 – 30394

Test substance: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

Test system: Albino rabbit (*Oryctolagus cuniculus*).

Testing Institution: MICROQUIM S.A., Av. Triunvirato 3447, (1427) Buenos Aires, Argentina.

Test facility: BIOMICRO S.A., Costa Rica 4776, Malvinas Argentinas.

Address of the Study Director: BIOMICRO S.A., Costa Rica 4776, Malvinas Argentinas.

Principal investigator: Jorge Maeyoshimoto.

Phase delegated: Determination of pH

Address of Principal investigator: MICROQUIM S.A., Av. Triunvirato 3447, (1427) Buenos Aires, Argentina.

B.2) STAFF

Technical Director:

Dr. Alejandro Lucini
Chemistry Degree

Study Director:

Dr. Juan Manuel Catoyra
Veterinarian

Principal Investigator:

Jorge Maeshoyimoto

Animal Health
Responsible

Dr. Juan Manuel Catoyra
Veterinarian

Study N° BIBR 6 – 30394

Page 4 of 18

MICROQUIM S.A.
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Test Performance:	Silvana Sabatini Biotery Technician
Final Report Confection:	Gisele Keim Romina Nostrala
Archive Responsible:	Silvina López

B.3) SCHEDULE

Date of entrance of the sample: 11.10.09
Start of the experimental phase (beginning of the acclimatization): 03.31.10
Date of the First dose: 04.05.10
Observation period: 7days after the administration.
Date of the end of the experimental phase: 04.12.10

B.4) TEST GUIDELINE

This test was performed in agreement with the following method: Guideline for Chemical Tests (1981) N° 405 “Acute Eye Irritation/Corrosion” by the Organization for Economic Cooperation and Development (OECD). Adopted on 24.04.2002 and SOP: “Acute Ocular Irritation/Corrosion in rabbits (POE 113-BM/04)”. In the phase delegated to the principal investigator it was realized in conformity by the following method: IT “Determination of pH” (POE 44-QG/11).

B.5 GOOD LABORATORY PRACTICES

This study will assure the performance of the standard operation procedures. The Quality Assurance Unit will periodically inspect test procedures and inspection dates will be included in the report.

This study will be performed according to the OECD principles of good laboratory practices, 1998. Established by SENASA Resolution No. 230/2000.

B.6) CERTIFICATIONS, REGISTRATION, ACCREDITATIONS AND REGISTERS

- **GLP** Certification in accordance with: EPA 40 CFR PART 160 “Principles of Good Laboratory Practice and Compliance Monitoring” ENV/MC/CHEM (98) 17 OECD. Study N° BIBR 6 – 30394

Page 5 of 18

MICROQUIM S.A.
Department of
Biological Studies

SENASA 230/2000 Resolution. Commission Directive 2004/10/EC of de European Parliament. Issued by **Bureau Veritas Certification**.

- **ISO 9001:2008** Certification for the Quality Management System, issued by **Bureau Veritas Certification** with accreditation of:

OAA (Argentina)

ANAB (U.S.A.)

UKAS (United Kingdom)

INMETRO (Brazil)

- **COFILAB** (Laboratories Control Committee)
- **CALIBA** (Argentine Chamber of Independent Laboratories of Bromatological, Environmental and Other Related Analysis)
- **SENASA** Accreditation (National Health Service and agro food quality) LR 0060 as laboratory of agrochemicals analysis to perform physicochemical, toxicological studies and determination of pesticide residues in vegetal matrixes.
- Animal facility subscribed at **SENASA** (National Health Service and agro food quality) according to regulations of Resolution 617/02 for the production of toxicological and ecotoxicological data.
- **AACyTAL** (Argentinian Asociation of Science and Technology of Laboratory animals) No. 09-0076
- **Colombian Agricultural Institute (ICA)**, part of the Treaty of the Andean Pact, Resolution No. 03431 as a Quality Control Laboratory of chemical pesticides for agricultural use.
- **EPA** (Environmental Protection Agency) assigned laboratory code number 955079.
- **Environment Aptitude certificate** issued by the Government of the Autonomous City of Buenos Aires Res. 077 A.A. 123/2000 Law.
- **Provincial Agency for Sustainable Development** Accreditation as Laboratory of Industrial Analysis according to the provisions of Resolution No. 504/01. Registration No. 31.

- **SEDONAR** (Secretaryship of Programs to prevent the drug addiction and fight against the drug trafficking) N° RN 858 PQ

B.7) AMENDMENT PROCEDURE

This final report can be amended by the Study Director and the Sponsor by the Sponsor in the event that his request. The Study Director will sign detailed descriptions of all amendments. The amendment will be effective at the time of Study Director's signature.

B.8) ARCHIVES

The laboratory will preserve the following data at least for 6 years: study plan, report and original data, in the general archive situated at Triunvirato 3447, (1427) Buenos Aires, Argentina. During that period no data will be discarded without the Sponsor's consent.

B.9) COMMITMENT OF CONFIDENTIALITY

The signatories of this final report are committed to safeguarding the confidentiality of all information involved in this study, both delivered by the Sponsor as that generated by this laboratory.

B.10) SAFETY PRECAUTIONS

Gloves, cap, mask with filters and protective goggles (if required) will be used to ensure proper safety and personal health and avoid inhalation and skin contact with the substance of test. In case of contact with eyes, wash them thoroughly with water and will seek medical treatment. In case of contact with skin, wash with soap and water with subsequent medical help.

C) SUMMARY

Title: Eye Irritation/Corrosion effects in rabbits (*Oryctolagus cuniculus*) of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

Test substance: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

Dose level: 0.1 ml/ per animal

Observation: Rabbits behaved normally and their external appearance was not influenced by the treatment.

Redness, secretion and chemosis of conjunctivae were noted but the effects decreased after 72 hours.

No ophthalmologic effects were recorded at the observation carried out 7 days after instillation of the test material.

Conclusion:

The grade of ocular irritation resulting from the application of **NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO** in the conjunctival sac of New Zealand rabbits was **12.67** (over a maximum of 110).

D) PURPOSE

The aim of the acute eye irritation study was to evaluate the possible irritation potential when applying a single dose of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO on the eye of the rabbit.

This test provided a rational basis for the hazard evaluation associated with the use of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO.

E) TEST SYSTEM AND MATERIALS

E.1) TEST ANIMALS

Test system: Albino rabbit

Source: DIPAGA Rabbit House – Ruta 8, Km 94 – 2764 Solís – San Andrés de Giles - Buenos Aires - Argentina. T.E: (15) 4405-7097 / (15) 4418-8745.

Animal Lot Number: C03/10/(BM)

Number of test animals: Three animals were used. The tests were performed on healthy adult animals.

Weight and age at the start of the test: young adult animals.

Identification: By cage number, by sex and labels in the ears.

Acclimatization: Animals were acclimatized to the laboratory conditions 5 days prior to the start of the test. After acclimatization healthy animals were randomized in individual cages.

E.2) HOUSING AND FEEDING CONDITIONS

Animals were housed under standard laboratory conditions.

The animals' test room was provided with conditioned air, filtered by HEPA filters, with 10 to 15 air changes per hour. The temperature of the animals' room was $20 \pm 3^{\circ}\text{C}$ and the relative humidity 30-70 per cent, although the higher relative humidity range may have exceeded during the cleaning of the room.

Animals were provided with photoperiods of 12 hours light- 12 hours dark and placed into individual cages made of steel with litter of autoclaved wood shavings. Lot

Number: (45 al 49) 26.10.09

Study N° BIBR 6 – 30394

Page 9 of 18

MICROQUIM S.A.
Department of
Biological Studies

The following diet was provided *ad libitum*: Ganave, Balanced food for rabbits supplied by Distribuidora Horacio Izaguirre. Lot Number: 10013-03-10

Tap water was used *ad libitum*.

E.3) IDENTIFICATION AND CHARACTERISTICS OF THE TEST SUBSTANCE

(According to information provided by the Sponsor)

- Label: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO
- Valuation of the active principle: AZUFRE: 13.68 % w/w; 14.91 % w/v, Batch N°: 025/2009
- Product description: Yellow-greens liquit
- Storage conditions: Room temperature.
- Container: Plastic bottle

The sampling was made by the sponsor

E.4) PREPARATION OF THE TEST SOLUTION

- Physical state of the test substance: Liquid
- pH of the test substance: 10.2 (Method: CIPAC MT 75 to 1% in aqueous solution)
- Solvent used: None.
- Dose: 0.1 ml /animal

E.5) EXPOSURE/OBSERVATION PERIOD

The observation period was 7 days.

E.6) EVALUATION OF RESULTS

The eye irritation will be graded according to Table 1, OECD 405.

1. Cornea	
Opacity:	
- Degree of density (area most dense taken for reading). No ulceration or opacity	0
- Scattered or diffuse areas of opacity, (other than slight dulling of normal luster), details of iris clearly visible	*1
- Easily discernible translucent area, details of iris slightly obscured	*2
- Nacrous area, no details or iris visible, size of pupil barely discernible	*3
- Opaque cornea, iris not discernible through the opacity	*4

2. Iris	
- Normal	0
- Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	*1
- No reaction to light, hemorrhage, gross destruction (any or all of these)	*2

3. Conjunctivae	
Redness: (refers to palpebral and bulbar conjunctivae, excluding cornea and iris)	
- Blood vessels normal	0
- Some blood vessels definitely hyperemic (injected)	1
- Diffuse, crimson color, individual vessels not easily discernible	*2
- Diffuse beefy red	*3
Chemosis (refers to lids and/or nictitating membranes)	
- No swelling	0
- Any swelling above normal (includes nictitating membranes)	1
- Obvious swelling with partial eversion of lids	*2
- Swelling with lids about half closed	*3
- Swelling with lids more than half-closed	*4

*Starred figures indicate positive grades

F) TEST PERFORMANCE

F.1) DOSAGE

The test substance was placed in one conjunctival eye sac of each animal, under the lower lid.

The lids were held together for one second in order to prevent loss of the substance. The other eye, which remained untreated, served as a control.

F.2) CLINICAL EXAMINATION

Eyes were examined at 60 minutes, 24, 48, 72 hours and 7 days. As there weren't irreversible irritant processes the test ended 7 days after it had started.

Observations of the cornea, iris and conjunctivae, and any other visible injuries, were recorded. Suitable devices such as a binocular loupe and slit lamp were used to verify the irritation and/or corrosion of the animals' eyes.

F.3) ANATOMY - PATHOLOGY

Deaths were not recorded during the observation period. Therefore the necropsy of the animals was not necessary.

G) RESULTS

Data were summarized in a tabular form, showing for each of the individual animals the irritation scores at the different designated times, a description of the irritation degree, the presence of serious injuries as well as any other ocular effect that could have been observed.

The ocular irritation scores were evaluated, in conjunction with the nature and reversibility or irreversibility of the observed effects. The irritation grades assessed individually will be used as reference values, and they will only be meaningful concerning the irritant properties of the test substance when supported by a complete evaluation.

The extrapolation of results of the ocular irritation/corrosion studies in animals to man is valid only within certain limit measures.

Study N° BIBR 6 – 30394

Page 12 of 18

MICROQUIM S.A.
Department of
Biological Studies

In most cases, rabbits are more sensitive to irritant and ocular corrosive agents than humans. Similar results in tests on other species can give more weight to extrapolation from animal studies to man.

H) CONCLUSION


The grade of ocular irritation resulting from the application of **NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO** in the conjunctival sac of New Zealand rabbits was **12.67** (over a maximum of 110).

I) REFERENCES


Good Laboratory Practice – ENV/MC/CHEM (98) 17 OECD.

Guideline for Chemical Tests (1981) N° 405 “Acute Eye Irritation/Corrosion” by the Organization for Economic Cooperation and Development (OECD). Adopted on 24.04.2002 and SOP: “Acute Ocular Irritation/Corrosion in rabbits” (POE 26-BI/22) and IT “Determination of pH” (POE 113-BM/04).

The data contained in this report as well as the conclusion are the accurate reproduction of the raw data registered on the logbook **5.EICO**, pages 001853 to 001854.



MICROQUIM S.A.
JUAN MANUEL CATOYRA
Médico Veterinario
M.P. N° 7553
Director de Estudio
Study Director



MICROQUIM S.A.
Dr. ALEJANDRO D. LUCINI
Director Técnico
Technical Director
M.N.7174/M.P. 4765

TABLE 1: DETERMINATION OF THE PRIMARY EYE IRRITATION INDEX

Observation time: 60 minutes

ANIMAL	GENDER	CORNEA		IRIS	CONJUNCTIVAE			
		Opacity	Area		Redness	Chemosis	Secretion	Is
28	Female	0	0	0	2	3	2	14.00
29	Female	0	0	0	2	3	1	12.00
30	Female	0	0	0	2	3	1	12.00

Score: 12.67

Observation time: 24 hours

ANIMAL	GENDER	CORNEA		IRIS	CONJUNCTIVAE			
		Opacity	Area		Redness	Chemosis	Secretion	Is
28	Female	0	0	0	2	2	1	10.00
29	Female	0	0	0	2	2	1	10.00
30	Female	0	0	0	2	2	1	10.00

Score: 10.00

Observation time: 48 hours

ANIMAL	GENDER	CORNEA		IRIS	CONJUNCTIVAE			
		Opacity	Area		Redness	Chemosis	Secretion	Is
28	Female	0	0	0	2	2	1	10.00
29	Female	0	0	0	2	2	1	10.00
30	Female	0	0	0	2	2	1	10.00

Score: 10.00

Observation time: 72 hours

ANIMAL	GENDER	CORNEA		IRIS	CONJUNCTIVAE			
		Opacity	Area		Redness	Chemosis	Secretion	Is
28	Female	0	0	0	1	1	0	4.00
29	Female	0	0	0	1	1	0	4.00
30	Female	0	0	0	1	1	0	4.00

Score: 4.00

Observation time: 7 days

ANIMAL	GENDER	CORNEA		IRIS	CONJUNCTIVAE			
		Opacity	Area		Redness	Chemosis	Secretion	Is
28	Female	0	0	0	0	0	0	0.00
29	Female	0	0	0	0	0	0	0.00
30	Female	0	0	0	0	0	0	0.00

Score: 0.00

Evaluation of the results according to “Modified NAS Classification Method” Toxicologist’s Pocket Handbook. Michael J. Derelanko

EYE IRRITATION GRADE: 12.67

TABLE 2: INDIVIDUAL BODY WEIGHT OF THE ANIMALS AT THE START AND AT THE END OF THE TEST

Number	Gender	Body weight during the test (gr.)		
		Day 0	Day 3	Day 7
28	Hembra	2412	2425	2436
29	Hembra	2482	2500	2521
30	Hembra	2636	2649	2661

CALCULUS METHOD OF PRIMARY EYE IRRITATION GRADE:

Cornea score = Opacity x area x 5

Iris score = Iris x 5

Conjunctivae score = (Redness + chemosis + secretion) x 2

Individual score (Is) = Cornea score + Iris score + Conjunctivae score

$$\text{TOTAL SCORE (TS)} = \frac{\text{Is animal N}^\circ 1 + \text{Is animal N}^\circ 2 + \text{Is animal N}^\circ 3}{\text{Total number of treated animals}}$$

ANEXO I

Reference to the Toxicological Classification

WHO (World Health Organization)

Class	Signal Word	Eye Effects
I	DANGER	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days
II	WARNING	Corneal involvement or irritation clearing in 8-21 days
III	CAUTION	Corneal involvement or irritation clearing in 7 days or less
IV	CAUTION	Minimal effects clearing in less than 24 hours

Kay and Calandra, Toxicologist's Pocket Handbook, 2000. by Michael J. Derelanko.

<u>EYE IRRITATION INDEX</u>	
0 – 0,5	Non irritant
> 0,5 – 2,5	Practically non irritant
> 2,5 – 15	Minimally irritant
> 15 – 25	Mildly irritant
> 25 – 50	Moderately irritant
> 50 – 80	Severely irritant
> 80 – 100	Extremely irritant
> 100 – 110	Maximally irritant

GHS (Harmonized Classification System for Chemicals Substances and Mixtures). By OECD, UN Comité Experts on Transport of Dangerous Goods, ILO and IOMC.

An eye irritant Category 1 (irreversible effects on the eye) is a material that produces:

at least in one animal, effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of (normally) 21 days;

and/or

at least in 2 of 3 tested animals, a positive response of:

corneal opacity ≥ 3 and/or

iritis > 1.5 ,

calculated as the mean score following grading at 24, 48 and 72 hours after application of the test material

An eye irritant Category 2A (irritating to eyes)

at least in 2 of 3 tested animals, a positive response of:

corneal opacity ≥ 1 and/or

iritis ≥ 1 and/or

conjunctival redness ≥ 2 and/or

conjunctival edema ≥ 2 ,

calculated as the mean score following grading at 24, 48 and 72 hours after application of the test material, and

which fully reverses within an observation period of (normally) 21 days.

An eye irritant Category 2B (irritating to eyes)

Within this category, an eye irritant is considered to be “Mildly irritating to eyes” when the effects listed above are fully reversible within 7 days of observation.

ANNEX II
CertificateGLP

Página 1 de 1

OAA ✓

Organismo
Argentino de
Acreditación

Notificación
informacionalmente
al OAA, en el marco
de la Ley N° 14.741

Av. Julio A. Roca 551 5° Sec. 8 y 9
(C1067ABE) Bk. Ar. Argentina
Tel/fax: 54-11 4549-3362 / 3 / 4
info@oaa.org.ar • www.oaa.org.ar

**CERTIFICATE OF COMPLIANCE WITH OECD
PRINCIPLES OF GOOD LABORATORY
PRACTICE**

Granted to the Testing Facility

MICROQUIM S.A.
División Biomicro

The Argentine Accreditation Body -OAA- declares that according to the requirements of its Good Laboratory Practice Monitoring Program, the Test Facility MICROQUIM S.A. DIVISIÓN BIOMICRO carries out the non-clinical studies mentioned in this certificate in compliance with the Principles of Good Laboratory Practice of the Organisation for Economic Co-operation and Development -OECD- (1998).

REGISTRATION OF COMPLIANCE WITH GPL No 02

AUTHORIZED REPRESENTATIVE: Lic. Alejandro LUCINI

Area of expertise:

- 2- toxicity testing
- 4- environmental toxicity studies on aquatic and terrestrial organisms

These types of non-clinical studies are performed in AGROCHEMICAL.

Carried out in: MICROQUIM S.A., DIVISIÓN BIOMICRO

Address of the Facility: Costa Rica 4776 – Partido de Malvinas Argentinas

Inspection Date:

October 5 and 6, 2009

Inspection and study audit

President
Higinio B. Ridolfi

This certificate was issued in Buenos Aires on January 4, 2010.

Study N° BIBR 6 – 30394

Page 18 of 18

MICROQUIM S.A.
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Biological Studies

QUALITY ASSURANCE UNIT STATEMENT

TITLE: Eye Irritation/Corrosion Effects in Rabbits (*Oryctolagus cuniculus*) of NES
 FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

TEST SUBSTANCE: NES FUNGICIDA, ACARICIDA E INSECTICIDA
 SELECTIVO - LIQUIDO

STUDY NUMBER: BIBR 6 - 30394

This study was audited during its different stages. For this study, the final report was compared with the study plan and the Standard Operating Procedures (SOP).

The report is in accordance with the obtained data.

The audit was carried out according to the Standard Operating Procedures (SOP) established in the Procedures Manual of MICROQUIM S.A.

The audit report was remitted to Direction and the Study Director, filing a copy of it in the "Internal Audits" archive of the Quality Assurance Unit of MICROQUIM S.A.

Audit N°	Audited area / Details	Audit Date
8643.a	Audit's Study Plan	03.18.10
8643.b	Audit's Study	04.08.10
8643.c	Final report revision.	07.16.10
8643.d	Audit's Report to Direction and the Study Director	07.19.10


Dr. Ana Inés Chanfreau
Veterinarian
GLP Quality Assurance Unit