GRUPO MICROQUIM

MICROQUIM S.A.
LABORATORIO DE INVESTIGACIONES CIENTIFICAS POR CONTRATO
Av. Triunvirato 3447 • (C 1427 AAH) Buenos Aires, Argentina
Tel.: (5411) 4554-1415 • Fax: (5411) 4554-7860
E-mail: info@microquim.com • http://www.microquim.com

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

Guideline OECD Nº 404

Test number:

BIBR 6 – 30393

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

- Biomicro Division -

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Quality Assurance Unit Statement

GRUPO MICROQUIM

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A) STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICES

TITLE: ACUTE DERMAL 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 – 30393

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 Catoyra
Veterinarian
Study Director

Date: 08/04/10

B) PREFACE

B.1) GENERAL

Title: Acute Dermal 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 – 30393

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

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) : 03.25.10

Date of the First dose: 03.30.10

Observation period: : 72 hours after the administration.

Date of the end of the experimental phase: 04.02.10

B.4) TEST GUIDELINE

This test was performed in agreement with the following method: Test Guideline, N° 404, "Acute Dermal Irritation/Corrosion" by the Organization for Economic Cooperation and Development (OECD). Adopted on 24.04.2002 and SOP: "Acute Dermal Irritation/Corrosion in rabbits" (POE 112-BM/03). 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

- Certification in accordance with principles of Good Laboratory Practices (1998), issued by OAA.

- **GLP** Certification in accordance with: EPA 40 CFR PART 160 "Principles of Good Laboratory Practice and Compliance Monitoring" ENV/MC/CHEM (98) 17 OECD. 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 ac

  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.
- **SEDRONAR** (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: Acute Dermal 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: 0.5 ml per animal

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

Skin irritation effects were not recorded on the rabbits after the exposure to the test substance, during the first 72 hours.

Conclusion:

The Primary Dermal Irritation grade obtained by Patch Test of the test substance NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO was **0.00** (over a maximum of 8).

D) PURPOSE

The aim of the acute dermal irritation study was to evaluate the possible irritation potential when applying a single dose of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO on the skin of rabbits throughout 4 hours.
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 at the start of the test: Young adult animals.

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

Acclimatization: Animals were acclimatized to laboratory conditions for 5 days prior to the start of the test.

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 ± 3°C and the relative humidity 30-70 per cent.
Animals were provided with photoperiods of 12 hours light- 12 hours darkness and placed into individual cages made of steel with litter of autoclaved wood shavings. Lot number: (43 al 46) 26.10.09.

The following diet was provided *ad libitum*: Cooperaciòn, Balanced food for rabbits supplied by Distribuidora Horacio Gilardoni. Lot number: 10013-13-09

Well 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 Nº: 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 at 1% in aqueous solution)
- Solvent used: None.
- Application area: Approximately 6 cm^2 of skin.
- Application method: On the gauze patch
- Contact treatment: By means of a suitable semi-occlusive gauze patch, held in place with non-irritant tape.
- Exposure period: 4 hours, at the end of the period the residual substances were removed with water.
- Dose: 0.5 ml /animal

E.5) EXPOSURE/OBSERVATION PERIOD

The exposure time to the test substance was 4 hours.

The observation period was not rigidly fixed, but it was enough to evaluate the reversibility or irreversibility of the observed reactions.

E.6) EVALUATION OF RESULTS

The dermal irritation was graded according to OECD Nro. 404. Adopted on 24.04.2002, p.4.

1. ERYTHEMA AND ESCHAR FORMATION

No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet red) to slight eschar formation	4

Maximum possible score per erythema 4

2. EDEMA FORMATION

No edema	0
Very slight edema	1
Slight edema	2
Moderate edema	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Maximum possible score per edema 4

Maximum possible score (1+2) = 8

F) TEST PERFORMANCE

F.1) DOSAGE

Approximately 24 hours before the test, fur was shaved from a 10 cm x 10 cm surface of the trunk's dorsal area of the animals, avoiding abrading the skin. Only animals with healthy intact skin were used.

F.2) CLINICAL EXAMINATION

Skin reaction was observed, and responses were recorded at 60 minutes, and then at 24, 48 and 72 hours after removing the patch.

F.3) ANATOMY - PATHOLOGY

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

G) RESULTS

Each test animal was examined for signs of erythema and oedema and responses were scored at 60 minutes and at 24, 48 and 72 hours after the patch removal (See TABLE N°1).

The body weight of the animals was maintained within the physiological variability range of the test system (TABLE N° 2).

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

The extrapolation of results of the dermal irritation/corrosion studies in animals to man allowed to obtain values within the limit values. In most cases, rabbits are more sensitive to dermal irritants than human beings. Similar results obtained in tests on other species can give more weight to extrapolation from animal studies to man.

H) CONCLUSION

The Primary Dermal Irritation grade obtained by Patch Test of the test substance NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO was **0.00** (over a maximum of 8).

I) REFERENCES

Good Laboratory Practice – ENV/MC/CHEM (98) 17 OECD.
Test Guideline, N° 404, "Acute Dermal Irritation/Corrosion" by the Organization for Economic Cooperation and Development (OECD). Adopted on 24.04.2002 and SOP: "Acute Dermal Irritation/Corrosion in rabbits" (POE 10-BI/06) and IT "Determination of pH" (POE 112-BM/03).

The data contained in this report as well as the conclusion are the accurate reproduction of the raw data registered on the logbook **6.EICP,** pages 001834 to 001835.

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 DERMAL IRRITATION GRADE

Test animal: New Zealand rabbits

Quantity: 3

Observation time: 60 minutes

ANIMAL	SEX	ERYTHEMA AND ESCHARS	OEDEMA	Is
10	Female	0	0	0
11	Female	0	0	0
12	Female	0	0	0

Observation time: 24 hours

ANIMAL	SEX	ERYTHEMA AND ESCHARS	OEDEMA	Is
10	Female	0	0	0
11	Female	0	0	0
12	Female	0	0	0

Observation time: 48 hours

ANIMAL	SEX	ERYTHEMA AND ESCHARS	OEDEMA	Is
10	Female	0	0	0
11	Female	0	0	0
12	Female	0	0	0

Observation time: 72 hours

ANIMAL	SEX	ERYTHEMA AND ESCHARS	OEDEMA	Is
10	Female	0	0	0
11	Female	0	0	0
12	Female	0	0	0

P1 = 0/3
P2 = 0/3
P3 = 0/3
Pt: (0.00+ 0.00 + 0.00) / 3 = **0.00**

PRIMARY DERMAL IRRITATION GRADE: 0.00

CALCULUS METHOD OF PRIMARY DERMAL IRRITATION GRADE:

A= Erythema and eschars

B= Oedema

	60 min	24 hs	48 hs	72 hs
Animal N° 1	$(A+B)_1$	$(A+B)_1$	$(A+B)_1$	$(A+B)_1$
Animal N° 2	$(A+B)_2$	$(A+B)_2$	$(A+B)_2$	$(A+B)_2$
Animal N° 3	$(A+B)_3$	$(A+B)_3$	$(A+B)_3$	$(A+B)_3$

$$\text{Individual score 1 (Is1)} = \frac{\sum (A+B)_1}{\text{Total readings}}$$

$$\text{Individual score 2 (Is2)} = \frac{\sum (A+B)_2}{\text{Total readings}}$$

$$\text{Individual score 3 (Is3)} = \frac{\sum (A+B)_3}{\text{Total readings}}$$

$$\text{TOTAL SCORE} = \frac{Is1 + Is2 + Is3}{\text{Total number of test animals}}$$

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

Number	Sex	Body weight during the test (gr.)	
		Day 0	Day 3
10	Hembra	2118	2135
11	Hembra	2115	2142
12	Hembra	2135	2166

ANNEX I

Reference to the Toxicological Classification

Toxicology Classification of: Appraisal of Safety of Chemicals in Food and Cosmetics Association of Food and Drugs Officials of the United States Tex 1965.

DERMAL IRRITATION GRADE	
0	Non irritant
> 0 – 2	Mildly irritant
> 2 – 5	Moderately Irritant
> 5 – 8	Severely irritant

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

Corrosion:

Corrosive Category (category 1)	**Corrosive ≥ 1 of 3 animals**

Irritation:

Category	**Criteria**
Irritant **(Category 2)**	(1) Values of ≥ 2.3 - < 4.0 for erythema/eschar or for edema in at least 2 of 3 tested animals from grading at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grading on 3 consecutive days after the onset of skin responses; or (2) Inflammation that persists to the end of the observation period, normally 14 days, in at least 2 animals, particularly taking into account alopecia (in limited area), hyperkeratosis, hyperplasia and scaling; or (3) In some cases where there is pronounced variability of

	response among animals and where very definite positive effects that are related to chemical exposure but are less than the criteria above are observed in a single animal.
Mild irritant **(Category 3)**	Values of ≥ 1.5 - < 2.3 for erythema/eschar or for edema in at least 2 of 3 tested animals from grading at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grading on 3 consecutive days after the onset of skin responses (when not included in the irritant category above).

ANNEX II
CertificateGLP

OAA
Organismo Argentino de Acreditación

Reconocido internacionalmente ante ámbitos ILAC, IAF e IAAC

Av. Julio A. Roca 651 8° Sec. 8 y 9
(C1067ABB) Bs. As., Argentina
Tel/fax: 54-11 4349-8902 / 3 / 4
info@oaa.org.ar | www.oaa.org.ar

OAA- Organismo Argentino de Acreditación
F13-(PRO-BPL) v2, F.e.V.= 01-diciembre-2009

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.

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QUALITY ASSURANCE UNIT STATEMENT

TITLE: Acute Dermal 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 - 30393

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**
8642.a	Audit´s Study Plan	03.18.10
8642.b	Audit´s Study	04.01.10
8642.c	Final report revision.	07.16.10
8642.d	Audit´s Report to Direction and the Study Director	07.19.10

Dr. Ana Inés Chanfreau
Veterinarian
GLP Quality Assurance Unit