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 Oral Toxicity of

NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

in Rats *(Rattus norvegicus)*

Guideline OECD Nº 425

Test number:

BIBR 6 – 30390

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

1MIC6100101299a

Consejo Profesional de Química

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

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Quality Assurance Unit Statement

GRUPO MICROQUIM

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A) STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICES

TITLE: ACUTE ORAL TOXICITY OF NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO IN RATS (*Rattus norvegicus*)

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

STUDY NUMBER: BIBR 6 – 30390

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: 08/07/10

B) PREFACE

B.1) GENERAL

Title: Acute oral toxicity of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO in rats *(Rattus norvegicus)*.

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 – 30390

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

Test system: Sprague Dawley Rat, strain Crl: CD®(SD) IGS-BR.

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.

B.2) STAFF

Technical Director:	Dr. Alejandro Lucini Chemistry Degree
Study Director:	Dr. Juan Manuel Catoyra Veterinarian
Animal Health Responsible	Dr. Juan Manuel Catoyra Veterinarian
Test Performance:	Silvana Sabatini Biotery Technician
Final Report Confection:	Gisele Keim María Ghea
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) : 03.19.10

Date of the first dose: 03.29.10

Observation period: 14 days after the administration for each doses.

Date of the end of the experimental phase: 04.21.10

B.4) TEST GUIDELINE

This test was carried out in agreement with the following method: Guideline for Chemical Tests N° 425 "Acute Oral Toxicity – Up-and-Down-Precedure" by the Organization for Economic Cooperation and Development (OECD). Adopted on 03.10.2008 and SOP: "Oral Toxicity in rats (425 Up and Down Proceduce)", (POE 172-BI/39). Doses not foreseen will be added to the guide when the regulations of each country require it

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 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.
- **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.

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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.

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C) SUMMARY

Title: Acute Oral Toxicity of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO in rats (*Rattus Norvegicus*).

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

Dose levels: 2000 mg/kg body weight

Median Lethal Dose >2000 mg/kg

Under present conditions, administration of 2000 mg/Kg body weight produced piloerection and lethargy

Animal mortality was not recorded during the observation period.

The animals gained the expected body weight throughout the entire study period.

Conclusion:

The median lethal dose **(LD_{50})** by oral administration of the test substance **NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO** in Sprague Dawley rats, strain Crl:CD®(SD)IGS-BR was **GREATER THAN 2000 mg/kg.**

D) PURPOSE

The aim of the study was the assessment of the acute toxicity of NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO when it is administered to rats by the oral route, followed by a 14 day observation period.
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: Sprague Dawley Rat, strain Crl:CD®(SD)IGS-BR.
Origin: Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Av. Chorroarín 280, C1427CWO - Buenos Aires, Argentina. Tel: 4524-8400.
Source: BIOMICRO. Costa Rica 4776, Malvinas Argentinas.
Animal Lot: R08/10
Number of test animals: 5 females .Females used were nulliparous and non-pregnant.
Age and weight at the start of the test: Young adult animals, between 8 and 12 weeks old. The weight variation in animals used in the test did not exceed ± 20% percent of the mean weight.
Identification: Marks on the tail with inerasable ink.
Acclimatization period: Animals were acclimatized to the laboratory conditions 10 days prior to the start of the test. After acclimatization healthy animals were randomized and assigned to treatment groups of 3 per cage (a same dose).

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 10-15 air changes per hour. The temperature range of the animals' room was 22 ± 3°C and the relative humidity 30-70 per cent, although the upper range for humidity may exceed during the cleaning of the room.

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: (41 al 50) 26.10.09, (1 al 4) 25.01.10.

The following diet was provided *ad libitum*: Balanced food Rat-Mouse Cooperaciòn supplied by Distribuidora Horacio Gilardoni. Lot number: 10007/09/38

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 SUBSTANCE

- Physical state of the test substance: Liquid
- Solvent used: water.
- Test substance concentration in the solvent: 20 % w/v

Doses were made immediately before the administration.

E.5) LIMIT TEST

A limit test of 2000 mg/Kg was performed. Since the test animals showed toxicological signs but no mortality, the test was finished.

E.6) DOSE LEVELS

A single oral dose of 2000 mg/kg body weight was performed. (Administered on 03.29.10, 03.31.10, 04.02.10, 04.05.10 and 04.07.10)

E.7) OBSERVATION PERIOD

The observation period was 14 days after the treatment.

F) TEST PERFORMANCE

F.1) DOSAGE

Animals fasted overnight prior to test substance administration.

Flexible K-30 probes were used for the administration.

The administration volume was 1 ml/100 g body weight.

After the test substance administration, food was withheld for a 3 hrs period.

Once the substance was administered observations were made and recorded systematically and individually for each animal.

The dosage was adjusted according to the guideline dictated by the Guideline recommendation (see Annex I).

F.2) CLINICAL EXAMINATION

The clinical examination was carefully made once a day and more often during the first day. Additional observations were made daily. Cage side observations were specific about changes in the skin, fur, eyes, mucous membranes, respiratory and circulatory system, autonomous and central nervous system, somatomotor activity and behaviour pattern.

Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleepiness and coma. Time of death, if it did occur, was recorded as precisely as possible.

F.3) ANATOMY - PATHOLOGY

The necropsy of all the test animals was carried out by the veterinarian and all gross pathological changes were recorded.

G) RESULTS

Results are summarized in a tabular form showing: tests groups, number of animals at the start of the test, number of animals displaying different toxicity signs, description of toxic effects and necropsy findings.

Clinical behaviour of the animals under treatment is provided in (TABLE N° 1). No mortality was recorded during the test period, following treatment with the test substance.

The body weight of the animals was within the physiological variability range of the test system, so it was observed an increase in the body weight curve. (TABLE N° 2).

Following 14 days exposure, the animals were sacrificed with carbon dioxide. No abnormalities were recorded at necropsy (TABLE N° 3).

The LD_{50} value is considered in agreement with the observed toxic effects and necropsy findings. The LD_{50} is a relative coarse measurement useful as a reference value for classification and labelling purposes and for an expression of toxicity of the test substance by the ingestion route.

H) CONCLUSION

The median lethal dose **(LD_{50})** by oral administration of the test substance **NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO** in Sprague Dawley rats, strain Crl:CD®(SD)IGS-BR was **GREATER THAN 2000 mg/kg body weight.**

I) REFERENCES

Good Laboratory Practice – ENV/MC/CHEM (98) 17 OECD.
Guideline for Chemical Tests N° 425 "Acute Oral Toxicity – Up-and-Down-Precedure" by the Organization for Economic Cooperation and Development (OECD). Adopted on 03.10.2008 and SOP: "Oral Toxicity in rats (425 Up and Down Proceduce)", (POE 172-BI/39).

The data contained in this report as well as the conclusion are the accurate reproduction of the raw data registered on the logbook **2.TOA (a) (b)** , pages 000536 to 000541.

MICROQUIM S.A.
JUAN MANUEL CATOYRA
Médico Veterinario
M.P. Nº 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:

ACUTE ORAL TOXICITY TEST - INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY

ANALYZED SAMPLE: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

SPONSOR: AJIM S.R.L

DATE OF THE END OF THE TEST: 04.21.10

ANALYSIS NUMBER: BIBR 6 – 30390

Dose mg/kg	*Animal N° and sex*		*Observation effects Post-administration*																
			Hours				*Days*												
			0	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2000	4	Female	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	Female	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	Female	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	Female	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8	Female	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes:

0: NO TOXIC SIGNS WERE OBSERVED

1: TOXIC SIGNS WERE OBSERVED

2: ANIMAL MORTALITY

2*: EUTHANASIED ANIMAL

SIGNS:

a.- MORTALITY DATA: No animal mortality was recorded during the observation period.

b.- CLINICAL OBSERVATIONS: Animals presented the following toxic sings: piloerection and lethargy.

c.- NECROPSIES: No abnormalities were observed.

TABLE 2:

ACUTE ORAL TOXICITY TEST - INDIVIDUAL BODY WEIGHTS

ANALYZED SAMPLE: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

SPONSOR: AJIM S.R.L

DATE OF THE END OF THE TEST: 04.21.10

ANALYSIS NUMBER: BIBR 6 – 30390

Dose mg/kg	*Animal N° and sex*		*Weight (g) at days*		
			0	**7**	**14**
2000	4	Female	208	220	233
	5	Female	213	224	234
	6	Female	216	226	237
	7	Female	210	222	231
	8	Female	212	224	233

TABLE 3:

ACUTE ORAL TOXICITY TEST - INDIVIDUAL NECROPSIES

ANALYZED SAMPLE: NES FUNGICIDA, ACARICIDA E INSECTICIDA SELECTIVO - LIQUIDO

SPONSOR: AJIM S.R.L

DATE OF THE END OF THE TEST: 04.21.10

ANALYSIS NUMBER: BIBR 6 – 30390

Dose mg/kg	*Animal N°* and sex		*Mortality time*	*Macroscopic Observations*
2000	4	Female	14 days (*)	No abnormalities were observed
	5	Female	14 days (*)	No abnormalities were observed
	6	Female	14 days (*)	No abnormalities were observed
	7	Female	14 days (*)	No abnormalities were observed
	8	Female	14 days (*)	No abnormalities were observed

(*) Sacrificed animal after the observation period

ANNEX I

AOT425StatPgm Model Developed by EPA

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program
Date/Time: Wednesday , April 21, 2010, 10:08:47 AM
Data file name: Nes Fungicida Acaricida e Insectisida Selectivo - Liquido.dat
Last modified: 4/21/2010 10:08:46 AM
Test/Substance: Nes Fungicida Acaricida e Insectisida Selectivo - Liquido
Test type: Limit Test
Limit dose (mg/kg): 2000
Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	4	2000	O	O
2	5	2000	O	O
3	6	2000	O	O
4	7	2000	O	O
5	8	2000	O	O

(X = Died, O = Survived)

Dose Recommendation: The limit test is complete.

Dose Recommendation: The limit test is complete.
SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
2000	5	0	5
All Doses	5	0	5

Statistical Estimates

The LD50 is greater than 2000 mg/kg

ANNEX II

Reference to the Toxicological Classification

Toxicological classification of the World Health Organization (WHO), stated in the Guidelines for the classification of pesticides (2004):

Class	WHO Classification according to hazard	LD_{50} (mg/Kg body weight)	
		Solid	Liquid
Ia	EXTREMELY HAZARDOUS	≤ 5	≤ 20
Ib	HIGHLY HAZARDOUS	5 to 50	20 to 200
II	MODERATELY HAZARDOUS	50 to 500	200 to 2000
III	SLIGHTLY HAZARDOUS	> 500	> 2000

Toxicological classification of the Argentine Resolution 350/99 published in the Official Bulletin N° 29.225 of 08.09.1999, as it is detailed in the following table:

Class	Hazard Classification	LD_{50} (mg/Kg)	
		Solid	Liquid
Ia	VERY TOXIC	≤ 5	≤ 20
Ib	TOXIC	> 5 to 50	> 20 to 200
II	HARMFUL	> 50 to 500	> 200 to 2000
III	CAUTION	> 500 to 2000	> 2000 to 3000
IV	GENERALLY NON HAZARDOUS	> 2000	> 3000

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

GHS: Globally Harmonized Classification System (mg/Kg body weight)	
CATEGORY 1	> 0 – 5
CATEGORY 2	> 5 – 50
CATEGORY 3	> 50 – 300
CATEGORY 4	> 300 – 2000
CATEGORY 5	> 2000 – 5000
CATEGORY 5 OR NO CLASSIFICATION	

ANNEX III
GLP Certificate

OAA
Organismo Argentino de Acreditación

Reconocido internacionalmente en los ámbitos de ILAC, IAF e IAAC

Av. Julio A. Roca 651 6° Sec. 8 y 9
(C1067ABB) Bs. As. Argentina
Telefax: 54-11 4349-3962 / 3 / 4
info@oaa.org.ar - www.oaa.org.ar

OAA- Organismo Argentino de Acreditación
F 13-(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.