Thiamethoxam

Thiamethoxam FS (A9700J) - Acute Eye Irritation/Corrosion Study in Rabbits (*Oryctolagus cuniculus*)

Final Report

DATA REQUIREMENT(S):	OECD 405, 2017.
AUTHOR(S):	Ana Lúcia Borges Shimada (PhD)
COMPLETION DATE:	19 March 2018
PERFORMING LABORATORY:	TECAM Tecnologia Ambiental São Roque Ltda Estrada do Carmo, 3001 CEP: 18130-970 São Roque/SP - Brazil
LABORATORY PROJECT ID:	Report Number: RL12915/2017IO-B Study Number: 12915/2017IO Task Number: TK0308848
SPONSOR (S):	Syngenta Proteção De Cultivos Ltda Av. Das Nações Unidas, 18.001 - 4º Andar Cep: 04795-900 São Paulo/Sp- Brazil

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Title: Thiamethoxam FS (A9700J) – Acute Eye Irritation/Corrosion Study in Rabbits (*Oryctolagus cuniculus*)

Study Number: 12915/2017IO

This study was conducted under my responsibility in accordance to NIT-DICLA-035 (INMETRO, Sep/11, Rev.02) and its complementary documents and the Good Laboratory Practice Principles as published by the OECD (N° 1 [ENV/MC/CHEM (98) 17]) which meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160].

This study was conducted in accordance to the written study plan authorized by the Sponsor and TECAM Management and to TECAM standard operating procedures. This report represents a true and accurate record of the obtained results. There were no major known circumstances that may have affected the quality or integrity of the study.

All original raw data, including any storage medium for electronically recorded data, documentation, the signed study plan, the protocol amendments, the final report and a sample of the test substance will be retained in the GLP Archives at TECAM Tecnologia Ambiental.

Ana Lúcia Shimada

19 March 2018

Ana Lúcia Borges Shimada (PhD) — Date

Study Director

Performing Laboratory: TECAM Tecnologia Ambiental São Roque Ltda
Estrada do Carmo, 3001
CEP: 18130-970
São Roque/SP - Brazil

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Title: Thiamethoxam FS (A9700J) – Acute Eye Irritation/Corrosion Study in Rabbits (*Oryctolagus cuniculus*)

Study Number: 12915/2017IO
Based on a quality assurance review, it was concluded that the final report is a true reflection of the raw data.

The final report was examined with respect to the study plan, standard operating procedures and raw data. Proceedings of the present study were inspected by process-based inspections. The inspections were carried out according to the standard operating procedures of the Quality Assurance of TECAM Tecnologia Ambiental.

Dates of inspections and the dates on which the findings were reported to the Study Director and Management are given below. These reports are kept in the GLP Archives at TECAM Tecnologia Ambiental.

Inspection	Date of inspection	Reporting Dates	
		To Study Director	GIT
Study plan	29 August 2017	29 August 2017	29 August 2017
Experimental phase	07 November 2017	20 November 2017	20 November 2017
Raw data	29 November 2017	29 November 2017	29 November 2017
Draft report	29 November 2017	29 November 2017	29 November 2017
Final report	19 March 2018	19 March 2018	19 March 2018
English Version	19 March 2018	19 March 2018	19 March 2018

Liliian Mion
Quality Assurance
TECAM Tecnologia Ambiental

19 March 2018
Date

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Ana Lúcia Borges Shimada (PhD)	Study Director, Pharmacist.
Cynthia B. Pestana (PhD)	Test Facility Manager.
Lilian Polakiewicz (MSc)	Deputy Test Facility Manager.
Lilian Mion	Quality Assurance.
Éder da Silva Xavier	Biomédico.
Iasmin Ferreira de Araújo	Bióloga.
Ana Beatriz de Souza Pereira	Technical Support.
Lucimara de Souza Pereira	Technical Support.
Fábio Barcelos	Technical Support.
Merielen Garcia Pontes	Syngenta Study Manager.

Study dates

Study Initiation Date:	06 September 2017.
Experimental Starting Date:	13 November 2017 (animal 1); 20 November 2017 (animals 2 and 3).
Experimental Termination Date:	16 November 2017 (animal 1); 23 November 2017 (animals 2 and 3).
Study Completion Date:	19 March 2018.
English Version:	19 March 2018.

Performing laboratory

The present study was performed at TECAM Tecnologia Ambiental, located at Estrada do Carmo, 3001. São Roque, SP – Brazil.
The physico-chemical analysis in water was subcontracted and monitored by the Quality Assurance of TECAM.

Study plan adherence

No deviations were recorded to the study plan.
The amendment registered in the study plan was:
Amendment Nº01: Due to the updating of the guideline OECD 405, 2017, this will be the methodology followed in the study. This alteration had no impact on the outcome of study.

Archives

All the original raw data and records of this study are the property of the Sponsor. Data will be properly registered, signed and stored in TECAM´s archives for five years. Test item will be properly stored during the test and after that will be returned to the Sponsor. When possible a sample will be retained for two years or until the expiry date.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The present study was performed to evaluate Thiamethoxam FS (A9700J) (supplied by Syngenta Proteção De Cultivos Ltda) to provide information about ocular irritation/corrosion in rabbits. The guideline followed was OECD 405, 2017. Three New Zealand young adult and healthy albino male rabbits (*Oryctolagus cuniculus*) were used. Test substance (0.1 mL) was applied in single dose and undiluted into the conjunctival sac of the left eye of the animals. The right eye was untreated and used as control. The eyes of the rabbits were then evaluated for reactions in the eye cornea, iris and conjunctiva at 1h, 24h, 48h and 72h after application according with reversibility of clinical signs observed. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48, 72 hours. The degree of ocular reactions was evaluated according to the method of Draize.

1.2 Results

No systemic or abnormality in body weight changes were detected during the observation period. All animals showed ocular reactions one hour after the treatment. Iritis, conjunctival redness and chemosis reverted by 24h. Evaluations from 24 hours after treatment to the end of the test were performed with the aid of fluorescein. No retention was observed in any of evaluations (24h, 48h and 72h). Due to the reversion of clinical signs of ocular irritation the test was finalized in 72h for all animals.

1.3 Conclusion

Under the test conditions, test substance Thiamethoxam FS (A9700J) showed very slight ocular reactions such as iritis, conjunctival redness and chemosis reverted by 24 hours.

2.0 INTRODUCTION

2.1 Study Purpose

This study was performed to evaluate irritation/corrosion potential of the test substance Thiamethoxam FS (A9700J) in the rabbit's eyes (*Oryctolagus cuniculus*).

2.2 Study Guidelines

The study was performed according to:
OECD Guideline for the testing of chemicals. Acute Eye Irritation/Corrosion 405, 19p., 2017.

2.3 Weight of Evidence Analysis

All available data relevant to the potential irritation/corrosion of the test substance were evaluated in a weight-of-evidence analysis prior to conducting this study, for reasons of animal welfare. This testing strategy comprised a stepwise approach including the evaluation of existing human and/or animal data showing effects on the skin or mucous membranes, measurement of pH (pH<2.0 or pH>11.5, the test substance not be tested due to corrosive potential) and the evaluation of data on systemic toxicity before *in vivo* testing in rabbits.

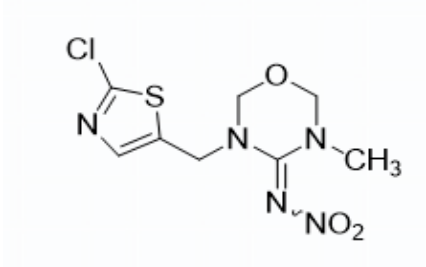
2.4 Animal Welfare

Animals are maintained in the test facility according to local and international requirements described in the current SOPs (Standard Operating Procedures), based on the *Guide for the Care and Use of Laboratory Animals* (ILAR-NRC, 2011). Animals showing continuing signs of severe distress and/or pain at any stage of the test are humanely killed and the test substance assessed. Procedures for animal care and criteria for making the decision to humanely kill moribund and severely suffering animals are described in detail on the SOPs, based on the Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation (OECD 19, 2000).

3.0 MATERIAL AND METHODS

3.1 Test Substance

Identification:	Thiamethoxam FS (A9700J)
Synonymy:	A9700J
TECAM Test substance number:	1716282
RET Number:	003105
Received on:	14 August 2017
Batch Nº:	RPJ 001-003-001
Study number:	12915/2017IO
Active ingredient (a.i.):	Thiamethoxam
Declared concentration of active ingredient:	350 g/L
Analysed concentration of a.i.:	360.44 g/L * (Appendix 1)

IUPAC name of active ingredient:	(EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine ***
Number CAS of active ingredient:	153719-23-4 ***
Class:	Insecticide **
Structural formula:	 Thiamethoxam ***
Molecular formula:	$C_8H_{10}ClN_5O_3S$ ***
Molecular weight:	291.7 g/mol ***
Manufactured on:	July 2017
Expiry date:	July 2019
Formulation:	Flowable concentrate for seed treatment (FS)
Physical state:	Liquid
Homogeneity:	Homogeneous (Visual Inspection) *
Stability:	Stable under use conditions **
Test substance supplied by:	Syngenta Proteção de Cultivos Ltda

* Analysis performed by TECAM Tecnologia Ambiental

** Sponsor information

*** The Online Pesticide Manual (17th ed., 2015).

3.2 Test System

Species:	*Oryctolagus cuniculus*.
Strain:	New Zealand (albino rabbits).
Justification for the test system:	The albino rabbit is the species recommended by various regulatory agencies and has been shown to be sensitive to irritant/corrosive effects of a variety of drugs and chemicals. Historical information concerning New Zealand albino rabbits is available in published literature.
Number and sex:	3 male rabbits.
Received on:	07 November 2017 (animal 1); 14 November 2017 (animals 2 and 3).
Age and body weight range:	Young adult rabbits (at least 1.5 kg prior to dosing); born on 07 September 2017 (animal 1) and on 03 September 2017 (animals 2 and 3).
Source:	Animals were acquired from an external supplier (Anilab Animais de Laboratório Criação e Comércio Ltda. EPP., Paulínia -

	SP) and it was performed complete clinical examination for presence, but not limited to: prostration, neurological and gastrointestinal alterations, bleeding, nasal and oral secretions, alterations of skin and fur, presence of ectoparasites, lesions in body and dehydration.
Acclimatization:	Animals were acclimatized for five days prior to dosing in a controlled room and they were evaluated immediately before the start of the study; animals exhibiting abnormal signs during this period were not used for the study.
Housing:	Animals were housed individually in cages for the species.
Identification:	Cage cards displaying animal number, date of entrance in the animal facility, sample code, dose and study dates were fixed to each cage.

3.3 Animal Health and Environmental Monitoring Program

As a program of animal health and environmental monitoring, the following procedures are performed periodically to ensure that contaminant levels are below those that might impact the scientific integrity of the study:

Feeding:	Pelleted commercial diet for the species (Do Sítio Coelhão - Guabi) was provided *ad libitum* throughout acclimatization and test periods; food id analyzed periodically by TECAM/SP for aerobic bacteria, molds, yeasts and *Salmonella* spp. The commercial food was considered not to contain any contaminant at levels that might have affected the purpose or integrity of the study.
Drinking water:	Filtered water provided *ad libitum* throughout acclimatization and test was analyzed periodically for chemicals and microbiological contaminants. The drinking water did not contain any contaminant at levels that might have affected the purpose or integrity of the study.

3.4 Environmental Conditions

The environmental conditions in the animal's room were monitored and recorded daily. The average temperature and relative humidity was 20.3ºC and 57.9% (animal 1) and 21.7ºC and 61.2% (animals 2 and 3). Animals were provided with an automatically controlled light cycle of 12 hours light (only artificial light from 7:00 a.m. to 7:00 p.m.) and 12 hours dark with a minimum of 10-12 air changes per hour.

3.5 In vitro Study Results

An *in vitro* study, Bovine Corneal Opacity Permeability assay (TECAM code: 12916/2017 BCOP) suggests that the test item is not classified as severe irritant and not classified as non-irritant. It was concluded that an in vivo study was required for proper classification.

3.6 Identification of pH

Test substance in aqueous solution 1% (V/V) was initially measured in pH 7.15 using a pHmeter, permitting the test item to be used in the animal studies.

3.7 Procedures

Due to prior data available about properties for eye irritation/corrosion to the rabbit's eyes, and for animal welfare reasons, the in vivo eye irritation/corrosion test was performed initially with one single animal. As no severe ocular lesions and/or injuries were observed in the first animal, two additional animals were tested.

3.7.1. Preparation of Animals: Both eyes of each animal were previously examined within 24 hours to check for preexisting abnormalities, eye irritation or injury to the cornea before application of the test substance with the aid of fluorescein (Allergan®, Batch F54712). Animals did not show any abnormality that might interfere with rabbit's ocular evaluation. Animals were weighed prior to exposure of the test substance and at the end of the test.

3.7.2. Anesthesia Protocol: About sixty minutes before the application of the test substance, a dose of 0.1 mg/kg bw butorphanol tartrate (Torbugesic®, Batch 001/16) was administered via subcutaneous injection, to preventively provide a therapeutic level of systemic analgesia in the animals. A local anesthetic 0.5% proxymetacaine hydrochloride (Anestalcon®, Batch 64772 and 65697), that is not expected potential interaction with test substance, was instilled in the left and right (control) eye of each animal approximately five minutes before the application of the test substance. The local anesthetic did not showed interference with the study. Four hours after application of the test substance, the same systemic analgesic (butorphanol tartrate) in a dose of 0.1 mg/kg bw was administered to animals for the maintenance of therapeutic levels performed pre-treatment. The use of butorphanol tartrate instead of buprenorphine, the analgesic suggested by OECD 405, 2017 is justified by its shorter half-time life and the possibility of its administration just four hours after test substance application. Eight hours after test substance exposure, animals received meloxicam (Maxicam®, Batch 0005/16) at dose level 0.5 mg/kg bw.

3.7.3. Application of the Test Substance: On the treatment day, the test substance (0.1 mL) was applied in a single dose and undiluted into the conjunctival sac of the left eye of the animals after pulling the lower lid away from the eyeball. Following application, the lids were held together for few seconds in order to prevent loss of the test substance. The untreated right eye was used as the control of the experiment. The treated eye of the animals was washed with demineralized water 24-hour after application of the test substance.

From the evaluation 24 hours until completion of the drop test was used fluorescein to aid ocular evaluation. After instillation of fluorescein eyes were washed with demineralized water and then the evaluation was made.

3.8 Clinical Observations

In addition to observation for ocular reactions, a clinical examination was performed by a trained technician and responsible for the study to verify local and/or systemic toxic effects.

3.9 Evaluation of Ocular Reactions Results

The eyes of the animals were examined at various time points after application (1h, 24h, 48h and 72h) for signs of irritation in the iris (iritis), cornea (opacity and area of the opacity) and conjunctivae (chemosis and conjunctival redness) according to the Draize method (OECD, 2017). The grading of eye reactions was summarized in a table.

3.10 Evaluation Criteria

- If no ocular reactions are observed 72 hours after treatment or ocular reactions are reverted within 72 hours, the test is concluded.
- If an ocular reaction persists after 72 hours, observations are performed on days 7, 14 and 21 in order to determine the progress of the lesions and their reversibility or irreversibility.

- If the following severe eye lesions are observed at any stage such as corneal perforation or significant corneal ulceration; blood in the anterior chamber of the eye; score 4 corneal opacity; absence of a light reflex (iridial response score 2); ulceration of the conjunctival membrane; necrosis of the conjunctivae or nictitating membrane and *pannus*, the animal is humanely killed at any stage of the test, it is not expected reversibility of these lesions.

3.11 Euthanasia

At the end of the observation period, the animals were euthanized. The animals received an intramuscular general anesthesia containing xylazine 2% (5-10 mg/kg b.w.) and ketamine 10% (35-50 mg/kg b.w.). When it was registered that the reflex ceased, a lethal dose of the same combination of anesthetics was administered (4 - 10 times the dose of general anesthetic) intravenously. Total volume was calculated based on the body weight of each animal at the time of euthanasia. No necropsy was conducted on animals.

4.0 RESULTS AND DISCUSSION

4.1 Mortality and Clinical Signs

Neither mortality nor clinical signs were observed among the tested animals. Individual clinical observation is presented on Table 1. Due to the absence of clinical signs of pain and/or distress the analgesic protocol was discontinued.

4.2 Body Weight

Individual body weights (g) initial and final of the animals during the testing period and the change in body weight are shown in Table 1. At the end of the test, all animals showed body weight gain within physiologic variability.

4.3 Eye Evaluation

Data of the individual scores at 1, 24, 48 and 72 hours are summarized in Table 2. Animal 1 presented iritis (score 1), conjunctival redness (score 1) and chemosis (score 1) at 1h time point evaluation reversibility was registered at 24h and decrease of normal lustre on the evaluation at 1h. Animals 2 and 3 showed conjunctival redness (score 1) and chemosis (score 1) at 1h time point evaluation. These ocular reactions were reversible by 24h and decrease of normal lustre on the evaluation at 1h. Evaluations from 24 hours after treatment to the end of the test were performed with the aid of fluorescein Absence of fluorescein retention was recorded in the eye evaluations 24h, 48h and 72h was observed in animals (Table 3). Due to the reversion of clinical signs of ocular irritation the test was finalized at 72h time point evaluation.

5.0 CONCLUSION

Under the test conditions, test substance Thiamethoxam FS (A9700J) showed very slight ocular reactions such as iritis, conjunctival redness and chemosis reverted by 24 hours.

6.0 REFERENCES

Draize, J. H. Woodward, G., and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmaco. Exp. Ther.*1944; 82:377-390.

ILAR. Institute for Laboratory Animal Resources - National Research Council. Guide for care and use of laboratory animals. Washington: National Academy Press, 2011. 220p.

INMETRO: NIT-DICLA-035 – Good Laboratory Practices – GLP, Rev. 02, September/2011 and it´s complementary documents.

OECD Environmental Health and Safety Publications, Series on Testing and Assessment. Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. No. 19.Paris, 2000. 39p.

OECD Guideline for the testing chemicals. Acute Eye Irritation/Corrosion. 405, 19p., 2017.

Plumb, D. C. Veterinary Drug Handbook (Pocket Edition). 4th ed. Ames, IA: Iowa State Press, 2002.

TABLES SECTION

TABLE 1 Body Weight (g) and Clinical Observations

Rabbit Nº	Body weight (g)			Clinical observations (days)		
	Initial	Final	Variation	1	2	3
1	2106	2204	+ 98	NA	NA	NA
2	2500	2502	+ 2	NA	NA	NA
3	2328	2424	+ 96	NA	NA	NA

NA: No alteration.

TABLE 2 Grading of Eye Reactions

Rabbit Nº	Time	Opacity	Area	Iritis	Conjunctival redness	Chemosis
	1h*	0	0	1	1	1
	24h	**0**	**0**	**0**	**0**	**0**
1	**48h**	**0**	**0**	**0**	**0**	**0**
	72h	**0**	**0**	**0**	**0**	**0**
	Mean[1]	**0.0**	**-**	**0.0**	**0.0**	**0.0**
	1h*	0	0	0	1	1
	24h	**0**	**0**	**0**	**0**	**0**
2	**48h**	**0**	**0**	**0**	**0**	**0**
	72h	**0**	**0**	**0**	**0**	**0**
	Mean[1]	**0.0**	**-**	**0.0**	**0.0**	**0.0**
	1h*	0	0	0	1	1
	24h	**0**	**0**	**0**	**0**	**0**
3	**48h**	**0**	**0**	**0**	**0**	**0**
	72h	**0**	**0**	**0**	**0**	**0**
	Mean[1]	**0.0**	**-**	**0.0**	**0.0**	**0.0**

[1] Mean values calculated as the mean scores of readings at 24h, 48h and 72h.
* Decrease of normal lustre.

TABLE 3 Fluorescein Retention

Rabbit Nº	Time	Fluorescein retention
1	24h	-
	48h	-
	72h	-
2	24h	-
	48h	-
	72h	-
3	24h	-
	48h	-
	72h	-

-: Absence of fluorescein retention.

APPENDIX SECTION

APPENDIX 1 Certificate of Analysis for Thiamethoxam FS (A9700J)

TECAM

CERTIFICATE OF ANALYSIS N°: CA1716282-B

Sponsor: SYNGENTA PROTEÇÃO DE CULTIVOS LTDA

Address: AV. DAS NAÇÕES UNIDAS, 18.001 - 2° Andar. CEP: 04795-900 - SÃO PAULO

1. TEST SUBSTANCE INFORMATION

Identification:	Thiamethoxam FS (A9700J).
Synonym:	A9700; A9700J.
TECAM code:	12992/2017CA.
Batch number:	RPJ 001-003-001.
Manufactured on:	July 2017.
Expiry date:	July 2019.
Active ingredient (a.i.):	Thiamethoxam.
Declared concentration of a.i.:	350 g/L.
IUPAC name:	(EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine.

2. EXPERIMENTAL

Equipment: High Performance Liquid Chromatograph (HPLC) 1200 Series AGILENT TECHNOLOGIES – TECAM N° 000225.

3. DATES

Initial date: 21 September 2017.

Final date: 29 September 2017.

4. RESULTS

Certificate of Analysis	Result
CA1716282-B	360.44 g/L

5. METHODOLOGY

TECAM Analytical Method: Amendment N° FQ 022/42 - Ver. 1 – Determinação do Teor de Thiamethoxam.

6. SIGNATURES

Damaris M. Picolo — 29-September-2017
Damaris Molino Picolo — Date
Pharmacist
CRF/SP 37.248

Carolina Satie Hayashida — 29-September-2017
Carolina Satie Hayashida — Date
Quality Assurance

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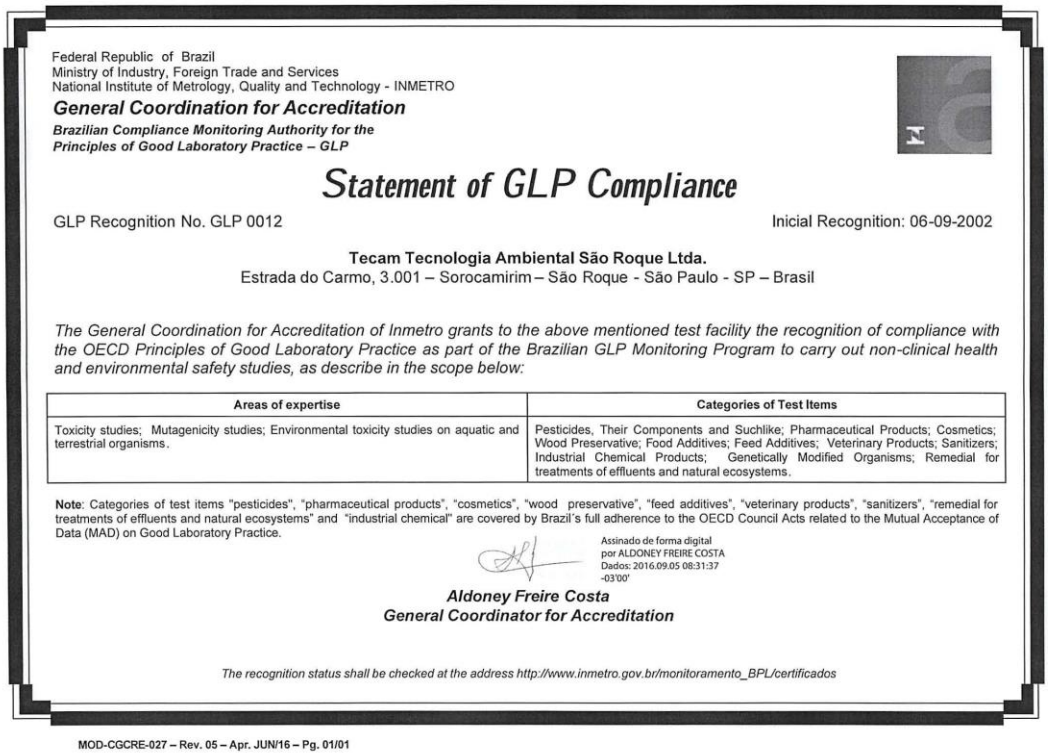
TECAM Tecnologia Ambiental Ltda. - Rua Fábia, 59 - CEP: 05051-030 - São Paulo - SP - Brazil

APPENDIX 2 Recognition of Compliance With The Principles on Good Laboratory Practice



Federal Republic of Brazil
Ministry of Industry, Foreign Trade and Services
National Institute of Metrology, Quality and Technology - INMETRO

General Coordination for Accreditation

Brazilian Compliance Monitoring Authority for the Principles of Good Laboratory Practice – GLP

Statement of GLP Compliance

GLP Recognition No. GLP 0012 — Inicial Recognition: 06-09-2002

Tecam Tecnologia Ambiental São Roque Ltda.
Estrada do Carmo, 3.001 – Sorocamirim – São Roque - São Paulo - SP – Brasil

The General Coordination for Accreditation of Inmetro grants to the above mentioned test facility the recognition of compliance with the OECD Principles of Good Laboratory Practice as part of the Brazilian GLP Monitoring Program to carry out non-clinical health and environmental safety studies, as describe in the scope below:

Areas of expertise	Categories of Test Items
Toxicity studies; Mutagenicity studies; Environmental toxicity studies on aquatic and terrestrial organisms.	Pesticides, Their Components and Suchlike; Pharmaceutical Products; Cosmetics; Wood Preservative; Food Additives; Feed Additives; Veterinary Products; Sanitizers; Industrial Chemical Products; Genetically Modified Organisms; Remedial for treatments of effluents and natural ecosystems.

Note: Categories of test items "pesticides", "pharmaceutical products", "cosmetics", "wood preservative", "feed additives", "veterinary products", "sanitizers", "remedial for treatments of effluents and natural ecosystems" and "industrial chemical" are covered by Brazil's full adherence to the OECD Council Acts related to the Mutual Acceptance of Data (MAD) on Good Laboratory Practice.

Assinado de forma digital por ALDONEY FREIRE COSTA
Dados: 2016.09.05 08:31:37 -03'00'

Aldoney Freire Costa
General Coordinator for Accreditation

The recognition status shall be checked at the address http://www.inmetro.gov.br/monitoramento_BPL/certificados

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