

***ACUTE ORAL TOXICITY STUDY
(UP-AND-DOWN PROCEDURE)
IN RATS
OF
PLANT GUARD™***

FOR

***ALPHA AGRI-PRODUCTS INC.
41656 Harriston Road
Bluevale, ON
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PROJECT NO.: 177764

/lp

**ACUTE ORAL TOXICITY STUDY (UP-AND-DOWN PROCEDURE)
OF PLANT GUARD™ IN RATS**

This study was conducted at Nucro-Technics over the period from December 8, 2006 to January 5, 2007, in compliance with the OECD Principles of Good Laboratory Practice (OECD, 1998), the United States Environmental Protection Agency, Title 40, Code of Federal Regulations Part 160 (2004), and the U.S. FDA Good Laboratory Practice for Non-clinical Studies, 21 CFR, Part 58 (2004). Stability, characterization, identity and verification of the test article as received and tested were the responsibility of the Study Sponsor.

NUCRO-TECHNICS

Nada Arulnesan, D.V .M.
Study Director

Date

John C. Fanaras, B.Sc.
President

Date

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: ALPHA AGRI-PRODUCTS INC.

Company Agent:

Name

Title

Signature

Date

STATEMENT OF GLP

This study was conducted according to EPA Good Laboratory Practice Standards, as detailed in 40 CFR Part 160.

STUDY SPONSOR:

Arnold Wiegersma
President
Alpha Agri-Products Inc.

Date

STUDY SUBMITTER:

Agent for Alpha Agri-Products Inc.

Date

STUDY DIRECTOR:

Nada Arulnesan, D.V .M.
Study Director
Nucro-Technics

Date

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QUALITY ASSURANCE STATEMENT

Test Article Name: Plant Guard™

Inspections of the Acute Oral Toxicity Study in Rats of the aforementioned test article were conducted on the following phases by the Quality Assurance Unit of Nucro-Technics.

Test Article Audit:	December 13, 2006
Animal Receipt / Acclimatization Records:	December 13, 2006
Examination of Animals:	December 13, 2006
Audit of Raw Data:	December 13, 2006; January 22, 2007
Audit of Report:	January 22, 2007

Written reports of the inspections were submitted to the Study Director and Management. To the best of my knowledge, there was no deviation from the Good Laboratory Practice Regulations which affected the quality or integrity of the study.

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (1998), the United States Environmental Protection Agency, Title 40, Code of Federal Regulations Part 160 (2004), and the U.S. FDA Good Laboratory Practice for Non-clinical Studies, 21 CFR, Part 58 (2004). Stability, characterization, identity and verification of the test article as received and tested were the responsibility of the study Sponsor.

Aldona Kenthol
Quality Assurance Associate

Date

SUMMARY

An Acute Oral Toxicity Study of the test article, Plant Guard™, was carried out at Nucro-Technics from December 8, 2006 to January 5, 2007. The study was conducted according to Protocol No. AAP/177764.

Since the test article was a liquid, it was used on an “as is” basis, taking into account its specific gravity of 1.02 for calculating doses.

The first animal was dosed at 5000 mg/kg (4.9 mL/kg by volume of test article). Since this first animal survived, four additional animals were dosed at approximately 48 to 72 hour intervals. A total of 5 female CD® rats were dosed. All animals received the test article by oral gavage using a feeding cannula.

The animals were observed for a 14-day period after dosing. Body weights were recorded before initiation of the treatment, on Day 7, Day 13 and at the end of the study.

No effects of toxicity or mortalities were observed post dosing and during the 14-day observation period in any of the animals. All five rats gained body weight by Day 7 and at the end of the study. At the end of the 14-day observation period, each animal was sacrificed and submitted for gross necropsy. No gross pathological findings were observed in any rat during necropsy.

Based on the foregoing results, the acute oral LD₅₀ in rats of the test article, Plant Guard™ was found to be in excess of 5000 mg/kg. Therefore, the test article is considered not to present a significant acute toxic risk if swallowed.

| The Globally Harmonized System of Classification and Labelling of Chemicals classifies compounds in which the estimated LD₅₀ is greater than 2000 mg/kg with no deaths or evidence of toxicity as being Category 5 chemicals.

The Office of Prevention, Pesticides and Toxic Substances classifies compounds in which the estimated LD₅₀ is greater than 5000 mg/kg with no deaths or evidence of toxicity as being Category IV chemicals.

STUDY INFORMATION

STUDY PURPOSE

To determine the toxic potential of the test article by oral ingestion. This study permitted an estimation of the LD₅₀ of the test article and the results allowed for ranking and classification of the test article according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), and OPPTS toxicity categories guidelines.

NUCRO-TECHNICS' STUDY NUMBER

177764

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QUALITY ASSURANCE Aldona Kenthol

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STUDY DESIGN

I. Method

The method used for conducting this study is the accepted standard described in OECD Guideline for the Testing of Chemicals, Acute Oral Toxicity (Up-and-Down Procedure), Section 425, (OECD, 2006), and OPPTS 870.1100, EPA, December 2002.

II. Justification for Selection of Test System

The test system is internationally accepted for use in acute oral toxicity studies.

III. Test System

Test Animal:	<i>Rattus norvegicus</i>
Strain:	CD [®] [CrI:CD [®] (SD)BR]
Source:	Charles River Canada, Montreal, Quebec
Number and Sex:	5 female rats were used.
Body Weight Range:	200-300 g before fasting. The weight variation in animals at the start of the study did not exceed ± 20 percent of the mean weight.
Acclimatization Period:	8 days
Age at Study Start:	Approximately 10 – 11 weeks
Animal Identification:	Body colour coding, cage labels

Animal Housing and Maintenance:

Female rats were individually housed in separate quarters in solid bottom cages. Individual animals were identified by colour coding, the animal number and group number also appeared on the outside of each cage to preclude mix-up. The animal room environment was controlled (targeted ranges: temperature 19°C to 25°C, relative humidity 30-70%) and monitored daily. The photo-cycle was 12 hours light and 12 hours dark. Upon arrival all animals were submitted to a general physical examination and all were found healthy and were admitted. Teklad Rodent Diet and water were offered ad libitum throughout the acclimatization and study periods. The cage cleaning schedule, air filtration and recirculation, health checks and facility maintenance were carried out in accordance with the applicable Nucro-Technics' Standard Operating Procedures, and such activities were recorded in the animal room records.

Animals were housed and maintained according to the AAALAC International Guide for the Care and Use of Laboratory Animals, CCAC Guidelines for Care and Use of Experimental Animals and Nucro-Technics' Standard Operating Procedures.

Animal Selection/Randomization:

The test population of animals was selected from newly arrived, previously unused rats. The method of randomization was based upon the random selection of numbers generated from a set of numbers without replacement.

Preparation of Animals:

All animals used for the Limit Test were fasted over-night. Food but not water was withheld from 5:00 p.m. on the day preceding dosing. In order to minimize stress caused by fasting, animals were offered a 10% w/v aqueous solution of glucose during this period.

IV. Test Article*

Identity:	Plant Guard™
Synonyms:	Plant Guard Plus™ / Field Guard Plus™
Color/Form:	Red liquid
Lot No.:	Formula
Composition:	Citronella 2.00%, pyrethrins 0.02%, others 97.98%
Storage Conditions:	Room temperature (15 - 30°C)
pH:	7.5
Solubility in Water:	Miscible
Specific Gravity:	1.02
Handling Precautions:	Refer to MSDS (Appendix V)
Supplier:	Alpha Agri-Products Inc.

* The Sponsor was responsible for determining the homogeneity, concentration and stability of the test substance.

V. **Experimental Procedures**

1. **Limit Test:**

The chemical composition indicated that the test article was likely to be non-toxic, therefore, a decision was made to proceed with the Limit Test.

The first animal was dosed at 5000 mg/kg. Since this animal survived, four additional animals were sequentially dosed at approximately 48 to 72 hours intervals. A total of five animals were tested.

2. **Test Article Preparation**

Since the test article was a liquid, it was used on an “as is” basis, taking into account its specific gravity of 1.02 for calculating doses.

3. **Dose Administration:**

The animals were dosed at 5000 mg/kg (4.9 mL/kg by volume). The individual doses of the test article were individually calculated for each animal based on the body weight of the animal. All doses were administered orally using a feeding cannula, inserted into the stomach of the animals.

4. **Observations During In-Life Phase:**

The animals were individually observed once during the first 30 minutes after dosing and periodically during the first 48 hours following dosing (with special attention given during the first 4 hours). Observations once daily were carried out for the remainder of the study. The animals were observed for 14 days after the dosing. Cageside observations were directed towards any changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Particular attention was directed to any observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and/or coma. Any symptoms of toxicity and deaths were recorded daily for the entire study period using the Acute Toxicity software module (Innovative Programming Associates, Version 2.2), and the entries were monitored by the Study Director. The body weights of the animals were determined prior to test article administration (i.e. Day 0), on Day 7, Day 13 and again on Day 14. Body weight gains were calculated. For calculation of LD₅₀, results were entered into the Acute Oral Toxicity statistical program V.1.0.

5. Post Mortem Examination:

Gross necropsy was performed on each rat at the end of the 14-day observation period and necropsy included an examination of: external surfaces of the body; all orifices; cranial cavity; external surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; thoracic, abdominal, and pelvic cavities and viscera.

6. Interpretation of Results and GHS* and OPPTS Classification:**

The results were interpreted as per GHS Classification (Table 1) and OPPTS Classification (Table 2):

Table 1: GHS Classification

<u>RESULTS</u> ESTIMATED LD₅₀ (mg/kg)	<u>CLASSIFICATION</u>
≤ 5	Category 1
> 5 ≤ 50	Category 2
> 50 ≤ 300	Category 3
> 300 ≤ 2000	Category 4
> 2000 with no deaths or evident toxicity	Category 5

* GHS = Globally Harmonized System of Classification and Labelling of Chemicals

Table 2: OPPTS Classification

Study	Category I	Category II	Category III	Category IV
LD ₅₀	Up to and including 50 mg/kg	> 50 through 500 mg/kg	> 500 through 5000 mg/kg	> 5000 mg/kg

** OPPTS = Office of Prevention, Pesticides and Toxic Substances

OBSERVATIONS AND RESULTS

No effects of toxicity or mortalities were observed post dosing and during the 14-day observation period in any of the 5 animals. All animals gained body weight by Day 7 and at the end of the 14-day observation period. Mean group body weights are presented in Table 3.

Table 3: Body Weight Summary

Mean Group Weight (g) ± S.D. (g); n = 5				Mean Weight Gain (g); n = 5
Initial	Day 7	Day 13	Day 14	–
247.7 ± 9.8	271.5 ± 9.5	285.5 ± 5.2	286.1 ± 5.4	38.4 ± 7.9

Each animal was sacrificed at the end of the 14-day observation period, and full gross necropsy was performed. No gross pathological findings were observed at the time of necropsy.

Detailed information on dosing, clinical signs and body weights are presented in the Individual Animal Records, Appendix II.

The Individual Necropsy Report and the Body Weights Summary Report are presented in Appendix III.

| [The AOT Statistical program report sheet is included in Appendix IV](#)

CONCLUSION

Based on the foregoing results, the acute oral LD₅₀ in rats of the test article, Plant Guard™ was found to be in excess of 5000 mg/kg. Therefore, the test article is considered not to present a significant acute toxic risk if swallowed.

The Globally Harmonized System of Classification and Labelling of Chemicals classifies compounds in which the estimated LD₅₀ is greater than 2000 mg/kg with no deaths or evidence of toxicity as being Category 5 chemicals.

The Office of Prevention, Pesticides and Toxic Substances classifies compounds in which the estimated LD₅₀ is greater than 5000 mg/kg with no deaths or evidence of toxicity as being Category IV chemicals.

REFERENCES

- 1) OECD Guideline for the Testing of Chemicals, Acute Oral Toxicity (Up-and-Down Procedure), Section 425, 2006.
- 2) Environmental Protection Agency, Code of Federal Regulations, Title 40, Part 160 (2004).
- 3) Handbook of In Vivo Toxicity Testing. (Ed.: Arnold, D.L., Grice, H.C. and Krewski, D.R.), Academic Press Inc., 1990.
- 4) Paget, G.E.: Methods in Toxicology, Blackwell Scientific Publications, 1970.
- 5) Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme Version 1.0, 2001.
- 6) Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.1100 Acute Oral Toxicity, 2002.
- 7) Plant Guard; MSDS: Alpha Agri-Products Inc., Nov. 9, 2006.
- 8) OECD Principles of Good Laboratory Practice, OECD, 1998.
- 9) United States Food and Drug Administration, 21 CFR, Part 58, Good Laboratory Practice for Non-clinical Laboratory Studies, 2004.
- 10) Globally Harmonized System of Classification and Labelling of Chemicals, United Nations, New York and Geneva, First revised edition, 2005.

Appendix I

Protocol No. AAP/177764

Appendix II

Individual Animal Records

Appendix III

Individual Necropsy Report and Body Weights Summary Report

Appendix IV

AOT Statistical Program Report Sheet

Appendix V

Material Safety Data Sheet