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Chlorothalonil/Acibenzolar

Chlorothalonil/Acibenzolar SC (A16422A) – Acute Oral Toxicity Up-and-Down Procedure in Rats

Final Report

DATA REQUIREMENT(S): EPA Health Effects Test Guidelines, OPPTS 870.1100
OECD Guidelines for Testing of Chemicals, Test No. 425

AUTHOR(S): Jennifer Durando, B.S.

STUDY COMPLETION DATE: August 4, 2008

PERFORMING LABORATORY: Eurofins | Product Safety Laboratories
2394 Highway 130
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LABORATORY PROJECT ID: Report Number: 24535
Study Number: 24535
Task Number: T008659-06

SUBMITTER/SPONSOR: Syngenta Crop Protection, Inc.
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

1) *The following statement applies to submissions to regulatory agencies in the United States of America.*

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

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

Company: Syngenta Crop Protection, Inc.

Company Representative: Fred Pearson

Title: N

Signature: Fred Pearson Date: Aug 22 2008

These data are the property of Syngenta Crop Protection, Inc. and, as such, are considered to be confidential for all purposes other than compliance with the regulations implementing FIFRA Section 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other provision of common law or statute or in any other country.

2) *The following statement applies to submissions to regulatory agencies other than in the United States of America.*

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO SYNGENTA LIMITED.

It should not be disclosed in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of this product or any other product without the written permission of Syngenta Limited.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98)17, OECD, Paris, 1998 and 11-Nousan-No. 6283, 1 October, 1999: JMAFF. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Jennifer Durando — 8/4/08

Jennifer Durando, B.S. — Date
Study Director, Eurofins | Product Safety Laboratories

Performing Laboratory: Eurofins | Product Safety Laboratories
2394 US Highway 130
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Merrill Tisdel — August 22 2008

Merrill Tisdel, B.S. — Date
Representative of Submitter/Sponsor

Submitter/Sponsor: Syngenta Crop Protection, Inc.
410 Swing Road
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Greensboro, NC 27419-8300 USA

QUALITY ASSURANCE STATEMENT

The Eurofins | Product Safety Laboratories' Quality Assurance Unit reviewed this study for adherence to EPSL's Standard Operating Procedures, the study protocol, and all applicable GLP standards. This final report was found to be an accurate representation of the work conducted. Records of QA findings are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	Jul 31, 2007[1]; June 14, 2008	Jul 31, 2007; June 14, 2008
In-process inspections; *Day 9 in-life observations for Animal #3101*	April 10, 2008	June 14, 2008
Raw data audit	June 14, 2008	June 14, 2008
Draft report review	June 14, 2008	June 14, 2008

Annamarie LaPorte, RQAP-GLP
Quality Assurance Auditor
Eurofins | Product Safety Laboratories

Aug 4, 2008
Date

[1] The protocol used for this study was reviewed by Quality Assurance on this date.

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Jennifer Durando, B.S.
Study Director

Study dates

Study initiation date: March 13, 2008
Experimental start date: April 1, 2008
Experimental termination date: May 20, 2008

Deviations from the Guidelines: None

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

An acute oral toxicity test (Up and Down Procedure) was conducted with rats to determine the potential for Chlorothalonil/Acibenzolar SC (718.75/001.47) (A16422A) to produce toxicity from a single dose via the oral route. Under the conditions of this study, the acute oral LD_{50} of the test substance is estimated to be 3,045 mg/kg (based on maximum likelihood) of body weight in female rats with a 95% PL Confidence interval of 0 mg/kg (lower) to greater than 20,000 mg/kg (upper).

An initial limit dose of five thousand milligrams of the test substance per kilogram of body weight was administered to one healthy female rat by oral gavage. Due to the absence of mortality in this animal, two additional females received the same dose level, simultaneously. Both these animals died, therefore a third female was administered the test substance at 5,000 mg/kg. Since three animals died at this dose level, the study proceeded to the Main Test. Using the default starting level of 175 mg/kg and following the Up and Down procedure, eight additional females were dosed at levels of 175, 550, 1,750 or 5,000 mg/kg. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing or until death occurred. Body weights were recorded prior to administration and again on Days 7 and 14 (termination) following dosing or after death. Necropsies were performed on all animals.

2.0 INTRODUCTION

To provide information on health hazards likely to arise from a short-term exposure to Chlorothalonil/Acibenzolar SC (718.75/001.47) (A16422A) by the oral route.

3.0 MATERIALS AND METHODS

3.1 Test substance

The test substance was identified as: Chlorothalonil/Acibenzolar SC (718.75/001.47)
A16422A
Batch ID 530458

It was received on March 11, 2008 and was further identified with EPSL Reference Number 080311-15H. The test substance was gray liquid and was stored at room temperature. The sample was administered as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

Characterization of the test substance was provided to Eurofins | Product Safety Laboratories by the Sponsor was:

Purity: Content of Chlorothalonil 53.3% (wt/wt) or 720 g/L
Content of Acibenzolar 0.10% (wt/wt) or 1.40 g/L

Solubility: Not provided

Stability: Test substance was expected to be stable for the duration of testing

Expiration Date: February 2009

3.2 Animals

Number of Animals: 12

Sex: Female, nulliparous and non-pregnant
.
Species/Strain: Rat/Sprague-Dawley derived, albino.

Age/Body weight: Young adult (9-11 weeks)/173-216 grams at experimental start.

Source: Received from Ace Animals, Inc., Boyertown, PA on March 25, April 1, 8 and 15, 2008.

Justification of Test System and Route of Exposure: The rat was the system of choice because, historically, it has been the preferred and most commonly used species for acute oral toxicity tests. The oral route of administration was used because human exposure may occur via this route.

3.3 Husbandry

Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH).* Litter paper was placed beneath the cage and was changed at least three times per week.

Animal Room Temperature and Relative Humidity Ranges: 19-21ºC and 36-61%, respectively.

Photoperiod: 12-hour light/dark cycle

Acclimation Period: 7-23 days

Food: Purina Rodent Chow #5012

Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.

Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Eurofins | Product Safety Laboratories.

3.4 Identification

Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study number 24535, constituted unique identification.

3.5 Selection of Animals

Prior to each dosing, experimentally naive rats were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed. Twelve healthy female rats were selected for test.

3.6 Dose Calculations

Individual doses were calculated based on the initial body weights, taking into account the specific gravity (determined by EPSL) of the test substance.

3.7 Dosing

The test substance was administered using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after test substance administration.

Individual animals were dosed as follows:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5,000	S	S
2	3102	5,000	D	D
3	3103	5,000	D	D
4	3104	5,000	D	D

Main Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3105	175	S	S
2	3106	550	S	S
3	3107	1,750	S	S
4	3108	5,000	D	D
5	3109	1,750	S	S
6	3110	5,000	D	D
7	3111	1,750	D	D
8	3112	550	S	S

S – Survival D – Death

Initially, four animals received a limit dose of 5,000 mg/kg. Due to the mortality in three of these animals, a Main Test was conducted. For the Main Test, the test substance was administered in sequence to the animals as described above. The decision to proceed with the next animal was based on the survival of the previous animal following dosing. Dose progressions and stopping criteria were determined using the statistical program described in Section 3.11.

3.8 Body Weights

Individual body weights of the animals were recorded prior to test substance administration (initial-Day 0) and again on Days 7 and 14 (termination) following dosing or after death.

3.9 Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes within the first several hours post-dosing and at least once daily thereafter for up to 14 days after dosing or until death occurred. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma.

3.10 Necropsy

Surviving rats were euthanized via CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all decedents and euthanized animals. Tissues and organs of the thoracic and abdominal cavities were examined.

3.11 Statistical Analysis

The *Acute Oral Toxicity (Guideline 425) Statistical Program* (Weststat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations and/or LD_{50} and confidence limit calcuations.

3.12 Study Conduct

This study was conducted at Eurofins | Product Safety Laboratories, 725 Cranbury Road, East Brunswick, NJ 08816. The primary scientist for this study was Jacek Ochalski, D.V.M. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 40 CFR 160: U.S. EPA GLP Standards: Pesticide Programs (FIFRA)
- OECD Principles of GLP (as revised in 1997) published in ENV/MC/CHEM (98)17, OECD, Paris, 1998
- 11-Nousan-No. 6283, 1 October, 1999: JMAFF GLP Standards

and based on the following testing guidelines

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)
- OECD Guidelines for Testing of Chemicals, Test No. 425 (2006)

In the opinion of the Sponsor and the Study Director, this study did not unnecessarily duplicate any previous work.

3.13 Quality Assurance

The final report was audited for agreement with the raw data records and for compliance with the protocol, Eurofins | Product Safety Laboratories Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

3.14 Amendments to Final Protocol

None

3.15 Deviations from Final Protocol

None

3.16 Records to be Maintained

The original signed final report and an electronic copy (pdf.) of the final report, including the signed QA and GLP Compliance pages will be sent to the Sponsor. A copy of the signed report, together with the protocol (P320.UDP SYN) and all raw data generated at Eurofins | Product Safety Laboratories, is maintained in the Eurofins | Product Safety Laboratories Archives in Notebook No. 08-113: pages 21-39. EPSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by EPSL.

4.0 RESULTS AND DISCUSSION

Individual body weights/weight gains, doses, and mortalities are presented in Table 1. Individual cage-side and necropsy observations are presented in Tables 2 and 3, respectively.

175 mg/kg (1 animal) and 550 mg/kg (2 animals)

All animals from the above dose levels survived test substance administration, appeared active and healthy and gained body weight over the course of the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

1,750 mg/kg (3 animals)

One animal died within one day of test substance administration. There were no toxic signs noted in the decedent prior to death. Apart from piloerection noted for one surviving rat, 5 hours post-dosing, both survivors appeared active and healthy and gained body weight over the 14-day observation period. There were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior noted in either survivor. Gross necropsy of the decedent revealed discoloration of the intestines. No gross abnormalities were noted for either of the euthanized animals necropsied at the conclusion of the 14-day observation period.

5,000 mg/kg (6 animals)

Five animals died within one day of test substance administration. Toxic signs noted in the decedents prior to death included hypoactivity, piloerection and/or ano-genital staining. Following administration, the surviving rat exhibited clinical signs including soft feces and ano-genital staining. The survivor recovered from the above symptoms by Day 6 and appeared active and healthy for the remainder of the study, gaining body weight over the 14-day observation period. Gross necropsy of the decedents revealed discoloration of the intestines. No gross abnormalities were noted for the euthanized animal necropsied at the conclusion of the 14-day observation period.

5.0 CONCLUSIONS

Under the conditions of this study, the acute oral LD_{50} of Chlorothalonil/Acibenzolar SC (718.75/001.47) (A16422A) is estimated to 3,045 mg/kg (based on maximum likelihood) of body weight in female rats with a 95% PL[1] Confidence interval of 0 mg/kg (lower) to greater than 20,000 mg/kg (upper).

[1] PL = Profile-likelihood based confidence interval

TABLES SECTION

TABLE 1 Individual Body Weights/Weight Gains, Doses, and Mortalities

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)					Dose[1] ml	Mortality	
			Day 0 Weight	Day 7 Weight	Gain*	Day 14 Weight	Gain*		Day	Weight (g)
3105	F	175	210	226	16	254	44	0.027	E	-
3106	F	550	182	200	18	244	62	0.076	E	-
3112	F		210	234	24	258	48	0.088	E	-
3107	F	1,750	182	200	18	239	57	0.24	E	-
3109	F		216	236	20	257	41	0.29	E	-
3111	F		206	-	-	-	-	0.26	1	180
3101	F	5,000	185	200	15	247	62	0.70	E	-
3102	F		193	-	-	-	-	0.73	1	184
3103	F		174	-	-	-	-	0.66	1	160
3104	F		197	-	-	-	-	0.74	1	189
3108	F		173	-	-	-	-	0.65	1	161
3110	F		181	-	-	-	-	0.68	1	172

* - Body weight gain from Day 0.
E - Euthanized via CO_2 inhalation after weighing on Day 14

[1] The test substance was administered as received. Specific Gravity – 1.323 g/ml.

TABLE 2 Individual Cage-Side Observations

Animal Number	Findings	Day of Occurrence
175 mg/kg		
3105	Active and healthy	0-14
550 mg/kg		
3106, 3112	Active and healthy	0 -14
1,750 mg/kg		
3107	Active and healthy	0-14
3109	Active and healthy Piloerection	0 (1-3 hrs), 1-14 0 (5 hrs)
3111	Active and healthy Dead	0 (1-5 hrs) 1
5,000 mg/kg		
3101	Active and healthy Ano-genital staining Soft feces	0 (1-5 hrs), 6-14 1-5 3
3102, 3103	Active and healthy Ano-genital staining Dead	0 (1 hr) 0 (3-5 hrs) 1
3104	Active and healthy Ano-genital staining Hypoactive, piloerection Dead	0 (1 hr) 0 (3-6 hrs) 0 (6 hrs) 1
3108	Active and healthy Ano-genital staining Dead	0 (1-3 hrs) 0 (5 hrs) 1
3110	Active and healthy Hypoactive, ano-genital staining Dead	0 (1 hr) 0 (3-5 hrs) 1

TABLE 3 Individual Necropsy Observations

Animal Number	Tissue	Findings
175 mg/kg		
3105	All tissues and organs	No gross abnormalities
550 mg/kg		
3106, 3112	All tissues and organs	No gross abnormalities
1,750 mg/kg		
3107, 3109	All tissues and organs	No gross abnormalities
3111	Intestines	Red
5,000 mg/kg		
3101	All tissues and organs	No gross abnormalities
3102, 3103, 3104, 3108, 3110	Intestines	Red

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

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Syngenta Crop Protection, Inc.
Technology & Projects
Analytical & Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

A16422A
530458 (GP-080203)

Batch Identification	530458
Product Design Code	A16422A
Product Denomination	R44686/CGA245704 SC (718.75/001.47)
Product by Common Name	Chlorothalonil/Acibenzolar SC (718.75/001.47)
Other Product Code(s)	GP-080203
Source	Technology & Projects, Syngenta Crop Protection, Inc.
Chemical Analysis (Active Ingredient Content)	
Identity of the Active Ingredient(s)*	Confirmed
Content of Chlorothalonil*	53.3% (wt/wt) or 720 g/L
Content of Acibenzolar*	0.10% (wt/wt) or 1.40 g/L
Methodology Used for Characterization	GC
The Active Ingredient(s) content is within the FAO limits.	
Physical Analysis	
Appearance*	Grey liquid
Density*	1351 g/L
Stability:	
Storage Temperature	< 30°C
Expiration date	February 2009

The stability of this test substance will be determined concurrently through reanalysis of material held in inventory under GLP conditions at Syngenta Crop Protection, Inc., Greensboro, NC.

This Certificate of Analysis is summarizing data (marked with an asterisk) from a study that has been performed in compliance with Good Laboratory Practices per 40 CFR Part 160. Raw data, documentation, protocols, any amendments to study protocols and reports pertaining to this study are maintained in the Syngenta Crop Protection Archives in Greensboro, NC.

Authorization:

Susan Grunenwald — 5-March 2008

Susan Grunenwald
Senior Chemist
Analytical & Product Chemistry Department

Date

MAR.13'2008 07:26 3366327581 SYNGENTA #0401 P.002/004