SYN524464

SYN524464 FS (A16148C) – Acute Oral Toxicity Study in Rats

Final Report

DATA REQUIREMENT(S): EPA Health Effects Test Guidelines,
OPPTS 870.1100
OECD Guidelines for Testing of Chemicals,
Procedure 425

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STUDY COMPLETION DATE: June 27, 2008

PERFORMING LABORATORY: STILLMEADOW, Inc.
12852 Park One Drive
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LABORATORY PROJECT ID: Report Number: 11740-08
Study Number: 11740-08
Task Number: T012079-05

SPONSOR: Syngenta Crop Protection, Inc.
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STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory and was conducted in compliance with:

- United States Environmental Protection Agency FIFRA: Good Laboratory Practice Standards, 40 CFR 160
- United States Environmental Protection Agency TSCA 40 CFR 792
- Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(98)17
- Japan Ministry of Agriculture, Forestry and Fisheries, Notification 11-Nousan-6283, Director- General of Agricultural Production Bureau

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

Janice O Kuhn

Janice O. Kuhn, Ph.D., DABT
Study Director, STILLMEADOW, Inc.

27 Jun 08
Date

Performing Laboratory: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478 USA

QUALITY ASSURANCE STATEMENT

Test Substance: SYN524464 FS (500)
Study Title: SYN524464 FS (A16148C): Acute Oral Toxicity Study in Rats

The study report and data have been audited in accordance with Good Laboratory Practice Standards and STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The final report accurately reflects the study data. The Quality Assurance Unit has not been involved in the actual conduct of this study.

The Quality Assurance Unit performed a recent facility inspection on 30 Apr 08. All findings were reported to Management, and the report and responses are kept in the Quality Assurance files.

The findings from any study inspections and audits were reported to the Study Director and Management as follows:

Critical Phase Inspected	Date Inspected	Reported to Study Director	Reported to Management
Protocol Review	13 Mar 08	13 Mar 08	13 Mar 08
Observations/Body Wt	15 Apr 08	15 Apr 08	15 Apr 08
Report/Data Audit	3 Jun 08	3 Jun 08	3 Jun 08

Richard L. Martin, M.S., C.Ph.T.
Quality Assurance, STILLMEADOW, Inc.

27 Jun 08
Date

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Study Director:	Janice O. Kuhn, Ph.D., DABT	
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	Hector Fuentes	Robert Preston
	Nancy Casajuana, L.A.T.	
Data Services:	Connie Pavatte, Report Preparation	

Study dates

Study initiation date: 24 Mar 08
Experimental start date: 1 Apr 08
Experimental termination date: 7 May 08

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1.0 EXECUTIVE SUMMARY

The test substance, SYN524464 FS (500) (A16148C), was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg. Since the test substance failed the limit test, the main test was conducted following the up-and-down procedure (UDP) at 175, 550, 1750 and 5000 mg/kg. The study was terminated following the stopping rules of this procedure. No mortality occurred at the 175 and 550 mg/kg levels. Three animals were humanely sacrificed during the study and are considered as "died on test". There were no clinical signs of toxicity in survivors during the study. Activity decrease, diarrhea, emaciation, hypothermia, lacrimation, piloerection, unresposiveness and salivation were observed in animals that died on test. There was no effect on body weight gain in survivors. Abnormal necropsy findings occurred only in the animals dying on test, and pertained to fur, eyes, lungs, and contents of the gastrointestinal tract and abdomen. The acute oral LD_{50} was estimated to be 2975 mg/kg.

2.0 INTRODUCTION

The objective of this study was to assess the acute oral toxicity potential of the test substance when administered by gavage to rats in accordance with US EPA OPPTS 870.1100, which is intended to meet testing requirements of FIFRA 7 USC 136, *et seq*, and TSCA 15 USC 2601. This study was conducted for Syngenta Crop Protection, Inc., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures in this study are in compliance with Animal Welfare Act Regulations. In the opinion of the sponsor, the study did not unnecessarily duplicate any previous work. The protocol, raw data, this report and a sample of test substance are archived at STILLMEADOW, Inc. The study was initiated on 24 Mar 08, the pre-dose experimental portion began on 31 Mar 08, and the animals were treated as follows:

Dose Level (mg/kg)	Treatment		Animal Number	In-life Termination Date
	Date	Time		
5000	1 Apr 08	1014	151	3 Apr 08
175	3 Apr 08	1015	152	17 Apr 08
550	4 Apr 08	0930	153	18 Apr 08
1750	8 Apr 08	1041	154	22 Apr 08
5000	9 Apr 08	0920	155	10 Apr 08
1750	11 Apr 08	1000	156	25 Apr 08
5000	16 Apr 08	1133	157	30 Apr 08
5000	18 Apr 08	0959	158	18 Apr 08
5000	22 Apr 08	1045	159	23 Apr 08
1750	23 Apr 08	1000	160	7 May 08
1750	24 Apr 08	1145	161	27 Apr 08

3.0 MATERIALS AND METHODS

3.1 Test Substance

Reference Name:	SYN524464 FS (500)
Label Identification:	SYN524464 FS (500) ID 531363 A16148C
Date & Quantity Received:	14 Mar 08; 4819 g (Gr.Wt.)
Physical Description:	White liquid
Storage:	Room temperature
Density:	1.1265 g/mL
Purity (w/w):	45.4% active ingredient
Stability:	Reassay: Mar 2009

Records pertaining to stability, characterization, identity, synthesis methods and location of documentation are the responsibility of the sponsor. A copy of the sponsor's Analytical Report is retained in the study file.

3.2 Experimental Animals

Species & Strain:	Albino rat; Sprague-Dawley
Justification of Species:	The rat is a representative rodent species preferred by various regulatory agencies for use in an acute oral study.
Source:	Texas Animal Specialties, Humble, TX
Date Born/Date Received:	4 & 25 Feb 08 / 27 Mar & 18 Apr 08
Quarantine Period:	5 days
Quantity & Sex:	11 females (nulliparous and non-pregnant)
Animal Identification:	Ear punch
Day -1 Wt/Day 0 (fasted) Wt:	181-233 g / 165-223 g

3.3 Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	•Temperature 22°C±3° •Relative Humidity 30-70% •12-hour light/dark cycle •10-12 air changes/hour
Actual Temp/Rel. Humidity:	20-25° C / 34-93% Protocol deviation: humidity was outside protocol range but did not affect study outcome.
Food:	PMI Feeds Inc.™ Formulab #5008; available *ad libitum* except for approximately 16 hours before dosing
Water:	Municipal water supply analyzed by TCEQ Water Utilities Division; available *ad libitum* from automatic water system

Animal husbandry and housing at STILLMEADOW, Inc. comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals" (NRC Publ.). No contaminants were expected to have been present in the feed or water that would have interfered with or affected the results of the study.

3.4 Test Substance Administration

The test substance was administered as received and was not diluted. An individual dose was calculated for each animal based on its fasted body weight and administered by gavage at a volume ranging from 0.155 mL/kg at the 175 mg/kg level to 4.44 mL/kg at the 5000 mg/kg level. Each dose was administered using an appropriately sized syringe and stainless steel ball-tipped intubation needle. The animals were returned to their cages immediately after dosing.

3.5 In-life Observations

Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14, or at the time of discovery after death.

3.6 Post-mortem Observations

On Day 14 after dosing, each surviving animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

3.7 Statistical Analysis

The LD_{50} value with 95% confidence interval was calculated using the AOT425 Stat Program supplied by the EPA.

4.0 RESULTS AND DISCUSSION

4.1 Mortality/Estimated Lethality Values

Individual mortality data, including time of death, are presented in Table 1. A summary of the mortality/survival incidence is presented below.

Main Test Sequence	Animal Number	Dose (mg/kg)	Results	Main Test Sequence	Animal Number	Dose (mg/kg)	Results
1	152	175	O	6	157	5000	O
2	153	550	O	7	158	5000	X
3	154	1750	O	8	160	1750	O
4	155	5000	X	9	159	5000	X
5	156	1750	O	10	161	1750	X

X = died; O = survived; Note: Animal 151 dosed for limit test (5000 mg/kg) died.

The acute oral LD_{50} for female rats was estimated to be 2975 mg/kg, with 95% confidence interval of 844 - greater than 20000 mg/kg.

4.2 Body Weights

Individual body weights are presented in Table 1. Body weight gain in surviving animals was unaffected by the administration of the test substance.

4.3 Clinical Signs

Clinical signs are presented in Table 2. All surviving animals appeared normal for the duration of the study. Clinical signs in animals that died on test included activity decrease, diarrhea, emaciation, hypothermia, lacrimation, piloerection, unresposiveness and salivation.

4.4 Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy on animals that died on test revealed wet/stained fur; swelling around eyes; discolored lungs and contents of gastrointestinal tract/abdomen; and empty stomach/intestines. The gross necropsy on animals surviving to termination of the study revealed no observable abnormalities.

5.0 CONCLUSIONS

The test substance, SYN524464 FS (500) (A16148C), was evaluated for its acute oral toxicity potential when administered to albino rats. The acute oral LD_{50}, as indicated by the data, is estimated to be 2975 mg/kg in females.

TABLES SECTION

TABLE 1 Body Weights, Time of Death and Gross Necropsy

ACUTE ORAL TOXICITY: UP & DOWN PROCEDURE (UDP) IN RATS
Test Substance: SYN524464 FS (500)

Dose Level: 175 mg/kg (0.155 mL/kg)

Animal Number	Dose Amt (mL)	Date of Dosing	Body Weights (g)			Time of Death*	Gross Necropsy Findings
			Day 0	Day 7	Final		
152	0.029	3 Apr 08	188	225	236	Day 14	NOA

Dose Level: 550 mg/kg (0.488 mL/kg)

Animal Number	Dose Amt (mL)	Date of Dosing	Body Weights (g)			Time of Death*	Gross Necropsy Findings
			Day 0	Day 7	Final		
153	0.087	4 Apr 08	178	215	236	Day 14	NOA

Dose Level: 1750 mg/kg (1.55 mL/kg)

Animal Number	Dose Amt (mL)	Date of Dosing	Body Weights (g)			Time of Death*	Gross Necropsy Findings
			Day 0	Day 7	Final		
154	0.272	8 Apr 08	175	204	223	Day 14	NOA
156	0.289	11 Apr 08	186	201	218	Day 14	NOA
160	0.337	23 Apr 08	217	242	246	Day 14	NOA
161	0.256	24 Apr 08	165	---	156	Day 3	NOA

Dose Level: 5000 mg/kg (4.44 mL/kg)

Animal Number	Dose Amt (mL)	Date of Dosing	Body Weights (g)			Time of Death*	Gross Necropsy Findings
			Day 0	Day 7	Final		
151	0.799	1 Apr 08	180	---	166	Day 2	Clear fluid on muzzle; swelling & fluid around eyes; brown fluid on abdomen & tail; lungs pale; stomach & intestines empty.
155	0.990	9 Apr 08	223	---	220	Day 1	Stomach full of yellow paste; sm intestine empty; lg intestine full of green paste.
157	0.803	16 Apr 08	181	215	235	Day 14	NOA
158	0.755	18 Apr 08	170	---	167	2-4 Hrs	Stomach & sm intestine full of yellow fluid; lg intestine full of green paste.
159	0.897	22 Apr 08	202	---	195	Day 1	White discharge on muzzle; brown fluid in abdominal cavity; fluid in sm intestine.

* - Indicates time of discovery after death (Day of dosing is Day 0; Day 14 is terminal sacrifice). If discovery was between scheduled observations, the time of death was recorded under the next scheduled observation.
NOA - No Observable Abnormalities

TABLE 2 Pharmacologic and/or Toxicologic Signs

ACUTE ORAL TOXICITY: UP & DOWN PROCEDURE (UDP) IN RATS
Test Substance: SYN524464 FS (500)

		Time After Treatment																	
Dose Level: 175 mg/kg (0.155 mL/kg)		DAY 0			DAYS														
Animal No.	Reaction and Severity	1st	2nd	3rd	1	2	3	4	5	6	7	8	9	10	11	12	13	14
152	Appeared normal at each observation.																	
Dose Level: 550 mg/kg (0.488 mL/kg)																		
153	Appeared normal at each observation.																	
Dose Level: 1750 mg/kg (1.55 mL/kg)																		
154	Appeared normal at each observation.																	
156	Appeared normal at each observation.																	
160	Appeared normal at each observation.																	
161	Activity decrease	-	e	e	e	e												
	Piloerection	-	m	m	m	m												
	Death	-	-	-	-	-	D											

v = very slight; s = slight; m = moderate; e = extreme; p = present; - = observation not present; D = death
Note: Time of death indicates time of discovery after death. If discovery was between scheduled observations, death is presented under next observation time.

TABLE 2 Pharmacologic and/or Toxicologic Signs (Continued)

ACUTE ORAL TOXICITY: UP & DOWN PROCEDURE (UDP) IN RATS
Test Substance: SYN524464 FS (500)

Dose Level: 5000 mg/kg (4.44 mL/kg)

		Time After Treatment																	
		DAY 0			DAYS														
Animal No.	Reaction and Severity	1st	2nd	3rd	1	2	3	4	5	6	7	8	9	10	11	12	13	14
151	Activity decrease	-	-	m	m	m												
	Piloerection	-	-	-	m	e												
	Diarrhea	-	-	-	-	e												
	Lacrimation	-	-	-	-	m												
	Emaciation	-	-	-	-	p												
	Salivation	-	-	-	-	m												
	Death	-	-	-	-	D*												
155	Activity decrease	-	-	m	e													
	Piloerection	-	-	-	e													
	Death	-	-	-	D*													
157	Appeared normal at each observation.																	
158	Activity decrease	m	e															
	Unresponsive	-	p															
	Hypothermia	-	p															
	Death	-	D*															
159	Activity decrease	-	m	e														
	Piloerection	-	s	s														
	Death	-	-	-	D													

v = very slight; s = slight; m = moderate; e = extreme; p = present; - = observation not present; D = death; * - Humanely sacrificed following observations.
Note: Time of death indicates time of discovery after death. If discovery was between scheduled observations, death is presented under next observation time.

TABLE 3 LD_{50} Analysis

ACUTE ORAL TOXICITY: UP & DOWN PROCEDURE (UDP) IN RATS
Test Substance: SYN524464 FS (500)

Test Type: Main Test
Assumed LD_{50} : Default
Limit Dose: 5000 mg/kg
Assumed sigma: 0.5 mg/kg

Recommended dose progression (mg/kg): 5000, 1750, 550 and 175

Test Sequence	Animal Number	Dose (mg/kg)	Short Term Results*	Long Term Results
1	152	175	O	O
2	153	550	O	O
3	154	1750	O	O
4	155	5000	X	X
5	156	1750	O	O
6	157	5000	O	O
7	158	5000	X	X
8	160	1750	O	O
9	159	5000	X	X
10	161	1750	X	X

X = died; O = survived

Dose Recommendation: Main Test Complete
Stopping Criteria Met: LR criterion

Summary of Results

Dose	O	X	Total
175	1	0	1
550	1	0	1
1750	3	1	4
5000	1	3	4
All Doses:	6	4	10

Estimated LD_{50} = 2975 mg/kg with 95% confidence interval of 844 – greater than 20000 mg/kg.

Based on AOT425statpgm (Version 1.0) Test Results and Recommendations Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program
* - At ~24-48 hrs after dosing that animal or when next dose is selected.

APPENDICES SECTION

APPENDIX 1 Analytical Report

syngenta

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

Certificate of Analysis

A16148C
Batch ID 531363 (GP-080206)

Batch Identification	531363
Product Design Code	A16148C
Product Denomination	SYN524464 FS (500)
Product by Common Name	SYN524464 FS (500)
Other Product Code(s)	GP-080206
Source	Technology & Projects, Syngenta Crop Protection, Inc.
Chemical Analysis (Active Ingredient Content)	
Identity of the Active Ingredient*	Confirmed
Content of SYN524464*	45.4% (wt/wt) or 533.5 g/L
Methodology Used for Characterization	HPLC
The Active Ingredient content is within the FAO limits.	
Physical Analysis	
Appearance*	a white liquid
Density*	1175 g/L
Stability:	
Storage Temperature	< 30°C
Expiration date	March 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:

Dorothea Jeffery
Group Leader I
Analytical & Product Chemistry Department

10 Mar 2008
Date