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SYN524464

SYN524464 FS (A16148F) – Skin Sensitization Study in Guinea Pigs

Final Report

DATA REQUIREMENT(S): OECD Guidelines for Testing of Chemicals, Procedure 406
EPA Health Effects Test Guidelines, OPPTS 870.2600

AUTHOR(S): Janice O. Kuhn, Ph.D., DABT

STUDY COMPLETION DATE: August 27, 2008

PERFORMING LABORATORY: STILLMEADOW, Inc.
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LABORATORY PROJECT ID: Report Number: 11818-08
Study Number: 11818-08
Task Number: T001113-08

SUBMITTER/SPONSOR: Syngenta Crop Protection, Inc.
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VOLUME 1 OF 1 OF STUDY

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS UNDER SPECIFIED FIFRA PROVISIONS

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) (discloses manufacturing or quality control processes), (B) (discloses the details of methods for testing, detecting or measuring the quantity of any deliberately added inert ingredient of a pesticide), or (C) (discloses the identity or percentage quantity of any deliberately added inert ingredient of a pesticide).

Company
Syngenta Crop Protection, Inc.

Company Agent: Adora Clark, Ph.D.

Adora Clark
U. S. Product Registration Manager

January 19, 2010
Date

Syngenta is the owner of this study. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the wording of our marking TRADE SECRET, this marking, by itself, conveys no supplemental claims of confidentiality under FIFRA Sections 10(a) or 10(b) (addressing protection of trade secrets and commercial and financial information). In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

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 — 27 Aug 08

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

Date

Performing Laboratory: STILLMEADOW, Inc.
12852 Park One Drive
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Odette Alexander — 8/19/10

Odette Alexander, M.S.
Representative of Submitter/Sponsor

Date

Submitter/Sponsor: Syngenta Crop Protection, Inc.
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QUALITY ASSURANCE STATEMENT

Test Substance: SYN524464 FS (500)
Study Title: SYN524464 FS (A16148F): Skin Sensitization Study in Guinea Pigs

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 25 Jul 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	1 Apr 08	1 Apr 08	1 Apr 08
Observations	12 Jun 08	12 Jun 08	12 Jun 08
Report/Data Audit	19 Aug 08	19 Aug 08	19 Aug 08

Richard L. Martin

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

27 Aug 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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	Robert Preston	Nancy Casajuana, L.A.T.
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Study dates

Study initiation date: 14 Apr 08
Experimental start date: 11 Jun 08
Experimental termination date: 11 Jul 08

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1.0 EXECUTIVE SUMMARY

A skin sensitization study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if test substance SYN524464 FS (500) (A16148F) produced a sensitizing reaction. Animals were assigned to each of two groups, designated Groups I and II. Group I animals (5/sex) remained untreated during the induction phase of the study and served as a naive control group. Group II animals (10/sex), the test group, were treated with 0.4 mL of undiluted test substance (selected from previous screening). The animals were treated once weekly for three weeks, for a total of three treatments. After a two-week rest period, all animals (Groups I and II) were challenged at a virgin test site with an application of 0.4 mL of undiluted test substance.

The test substance produced no irritation in the test animals (Group II) or the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitizing reaction in guinea pigs.

2.0 INTRODUCTION

The objective of this study was to determine the sensitizing potential of the test substance using a modification of the Buehler method (H.L. Ritz and E.V. Buehler, "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," Current Concepts in Cutaneous Toxicity, p. 25-42, Academic Press, NY, 1980), in accordance with OPPTS 870.2600, 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. SOP's. 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 the 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 14 Apr 08, and the experimental range-finding began on 8 Jun 08. The animals were treated as follows and the study was terminated on 11 Jul 08:

Group	Induction Treatments		Challenge Treatment
	First	Last	
I Naive Control (5/sex)	--	--	9 Jul 08
II Test (10/sex)	11 Jun 08	25 Jun 08	9 Jul 08

3.0 MATERIALS AND METHODS

3.1 Test Substance

Reference Name:	SYN524464 FS (500)
Label Identification:	SYN524464 FS (500) ID 533158 A16148F
Date & Quantity Received:	14 Apr 08; 3691 g (Gr.Wt.)
Physical Description:	Pink opaque liquid
Storage:	Room temperature
Purity (w/w):	45.6% active ingredient
Stability:	Reassay: Mar 2009
Conc. Administered:	Induction: 100% as received Challenge: 100% as received

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:	Guinea Pig; Hartley-Albino
Justification of Species:	The guinea pig is the preferred species for use in dermal sensitization testing.
Source:	Charles River Laboratories; Wilmington, MA
Quantity & Sex:	2/sex (Irritation Screening); 15/sex (Definitive Study); females nulliparous and non-pregnant
Acclimation Period:	At least 5 days
Date Born/Date Received:	22 & 26 Apr 08 / 27 May 08
Animal Identification:	Ear punch
Weight When Tested:	Males: 361-441 g; Females: 327-430 g

3.3 Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1-4 per cage (males separate from females)
Environmental Controls	
Set to Maintain:	•Temperature 20°C±3° •Relative Humidity 30-70% •12-hour light/dark cycle •10-12 air changes/hour
Actual Temp/Rel. Humidity:	17-20° C / 60-98% Protocol deviation: humidity was outside protocol range but did not affect study outcome.
Food:	PMI Feeds, Inc. Guinea Pig Diet 5025; available *ad libitum*
Water:	Municipal water supply analyzed by TCEQ Water Utilities Division, available *ad libitum* from water bowls

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, which would have interfered with or affected the results of the study.

3.4 Positive Control Material and Testing

Label:	1-CHLORO-2,4-DINITROBENZENE, 97% 138630-100G Batch: 10505DD
Manufacturer:	Aldrich
Physical Description:	White crystals
Storage:	Room temperature
Purity, Composition & Stability:	Certificate of Analysis available from manufacturer
Conc. Administered:	Induction: 0.4 mL of 1.0% w/v solution in ethanol Challenge: 0.4 mL of 0.45% w/v solution in acetone
Vehicle:	Ethanol and acetone

The sensitivity of guinea pigs to a positive control material is confirmed in this laboratory periodically. The positive control animals used to conduct this study were supplied by Charles River Laboratories, and were tested according to the Buehler Method (Ritz, H.L. and E.V. Buehler, "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests", Current Concepts in Cutaneous Toxicity, Academic Press, NY, 1980).

STILLMEADOW, Inc. Study No. 11662-08
In-life started: 26 Mar 08; In-life completed: 25 Apr 08

Results: Data from this study are presented in Appendix A. A mean score of 0.4 for the test group after challenge treatment, when compared with naive control group mean score of 0.1, confirmed the sensitivity of guinea pigs to the positive control material.

3.5 Irritation Screening

Two male and two female albino guinea pigs were selected for irritation screening (Figure 1) to determine both the maximum dose producing no more than moderate irritation, and the maximum non-irritating dose. Concentrations tested in the screening were 100% (undiluted), and 75%, 50% and 25% v/v dilutions in deionized water, with each animal receiving 0.4 mL of each concentration at different test sites.

3.6 Animal Preparation

Five males and five females were selected for Group I, and ten males and ten females were selected for Group II. Group I animals served as a naive control group and Group II animals were designated as the test group. On the day prior to each treatment, the animals were prepared by clipping the back of the trunk free of hair to expose a longitudinal area at least 8 x 10 cm on each animal. Individual body weights were recorded on Days 0 and 31.

3.7 Test Substance Administration

Based on the results of the irritation screening, the dose administered was an application of 0.4 mL of undiluted test substance. For each induction treatment, Group II animals were treated by introducing the test substance beneath a 4 ply, 2.5 x 2.5 cm surgical gauze patch. Each gauze patch was placed laterally from the midline of the back on the left front quadrant of the exposure area (Figure 2) and secured with a strip of non-irritating adhesive tape. A strip of clear polyethylene film was placed over the patch and securely taped. Each animal was then placed in a restrainer for approximately six hours. At the end of the exposure period, the animals were removed from the restrainers, the wrappings and patches were removed, and the animals were returned to their cages. Group II animals were treated once weekly for three weeks with 0.4 mL of undiluted test substance. Induction treatments were on Days 1, 8 and 15. The same treatment regimen and test site location was used for all three induction treatments. Group I animals remained untreated during the induction phase of the study.

3.8 Challenge Treatment

After a two week rest period, all animals (Groups I and II) were each challenged at a virgin test site with an application of 0.4 mL of undiluted test substance. The challenge treatment was on Day 29. The dose was applied in a manner identical to the induction treatments, except the test site was placed laterally on the right rear quadrant of the exposure area with the edge of the gauze pad adjacent to the midline of the back (Figure 2).

3.9 Observations and Scoring Methods

Observations for skin reactions at each test site were made approximately 24 hours after each treatment. In addition, observations for skin reactions were made approximately 48 hours after the first induction treatment and 48 hours after the challenge treatment. The scoring scale used to grade skin reactions is as follows:

Erythema	Score
No reaction	0
Very faint, usually nonconfluent	0.5
Faint, usually confluent	1
Moderate	2
Strong, with or without edema	3

An average score for each time period was obtained by adding all of the scores for each time period and dividing by the number of test sites scored for that time period. The test substance is considered a sensitizer if the mean irritation scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in the test group after the challenge treatment are appreciably greater than those for the naive challenge group.

3.10 Protocol Deviation

Only 3 rather than 9 treatments were performed during the induction phase of this study. A similar formulation (A16148C), differing only in the absence of the red colorant which is in SYN514464 FS (500) (A16148F), was tested undiluted in a 9-induction sensitization study. All scores during induction and challenge were zero. For this reason, it is concluded that this protocol deviation had no effect on the study outcome.

4.0 RESULTS AND DISCUSSION

Results of irritation screening are presented in Figure 1. Test site locations are presented in Figure 2. Skin reactions and average skin reaction scores during induction and challenge are presented in Tables 1 and 2, respectively. Individual body weights are presented in Table 3. The average skin reaction scores for each group at challenge are as follows:

Group	Mean Challenge Scores
Naive Control	0.0
Test	0.0

5.0 CONCLUSIONS

The test substance, SYN524464 FS (500) (A16148F), produced no irritation in the test animals (Group II) or the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitizing reaction in guinea pigs.

TABLES SECTION

TABLE 1 Skin Reaction Scores

SKIN SENSITIZATION IN GUINEA PIGS
Test Substance: SYN524464 FS (500)

	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
Animal #	24 hr	48 hr	24 hr	24 hr	24 hr	48 hr
Group I – Naive Control						
91-M					0	0
92-M					0	0
93-M					0	0
94-M					0	0
95-M					0	0
96-F					0	0
97-F					0	0
98-F					0	0
99-F					0	0
100-F					0	0
Group II – Test Group						
101-M	0	0	0	0	0	0
102-M	0	0	0	0	0	0
103-M	0	0	0	0	0	0
104-M	0	0	0	0	0	0
105-M	0	0	0	0	0	0
106-F	0	0	0	0	0	0
107-F	0	0	0	0	0	0
108-F	0	0	0	0	0	0
109-F	0	0	0	0	0	0
110-F	0	0	0	0	0	0
111-M	0	0	0	0	0	0
112-M	0	0	0	0	0	0
113-M	0	0	0	0	0	0
114-M	0	0	0	0	0	0
115-M	0	0	0	0	0	0
116-F	0	0	0	0	0	0
117-F	0	0	0	0	0	0
118-F	0	0	0	0	0	0
119-F	0	0	0	0	0	0
120-F	0	0	0	0	0	0

M – Male; F – Female; LF – Left Front test site; RR – Right Rear test site
Note: Observations were made for erythema.

TABLE 2 Average Skin Reaction Scores

SKIN SENSITIZATION IN GUINEA PIGS
Test Substance: SYN524464 FS (500)

	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
	24 h	48 h	24 h	24 h	24 h	48 h
Group I Naive Control					0.0	0.0
Mean					0.0	
Group II Test Group	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.0		0.0	0.0	0.0	

LF - Left Front test site
RR - Right Rear test site

TABLE 3 Body Weights

SKIN SENSITIZATION IN GUINEA PIGS
Test Substance: SYN524464 FS (500)

Animal Number	Day of Study	
	Day 0	Day 31
Group I - Naive Control		
91-M	397	584
92-M	441	580
93-M	365	630
94-M	363	624
95-M	408	590
96-F	344	518
97-F	412	564
98-F	381	550
99-F	358	571
100-F	331	416
Group II – Test		
101-M	428	662
102-M	378	694
103-M	407	554
104-M	415	564
105-M	403	688
106-F	367	461
107-F	335	404
108-F	374	427
109-F	352	497
110-F	357	460
111-M	361	621
112-M	394	634
113-M	423	632
114-M	425	552
115-M	411	631
116-F	341	423
117-F	430	521
118-F	361	454
119-F	373	468
120-F	418	430

M – Male; F – Female

Note: Body weights are in grams.

TABLE 4 Positive Control Study Skin Reaction Scores

SKIN SENSITIZATION IN GUINEA PIGS
Study No: 11662-08
Positive Control Material: DNCB

	Hours After Day of Treatment						
	Induction Treatments LF					Challenge RR	
	Day 1		Day 8	Day 15		Day 29	
Animal Number	24 h	48 h	24 h	24 h		24 h	48 h
Group I – Naive Control							
141-M						0	0
142-M						0.5	0
143-M						0	0
144-M						0	0
145-M						0	0
146-F						0	0
147-F						0	0
148-F						0.5	0
149-F						0.5	0
150-F						0	0
Group II – Test Group							
151-M	0.5	0	0.5	0.5		0.5	0
152-M	0.5	0	0.5	0.5		0.5	0
153-M	0	0	0.5	0.5		0.5	0.5
154-M	0.5	0	0.5	0.5		0.5	0.5
155-M	0	0	0.5	0.5		0.5	0
156-F	0.5	0	0.5	0.5		0.5	0.5
157-F	0	0	0.5	0.5		0.5	0
158-F	1	0	0.5	0.5		0.5	0
159-F	0	0	0	0.5		0.5	0
160-F	0	0	0	0.5		0.5	0

M – Male; F – Female
LF – Left Front test site; RR – Right Rear test site
Note: Observations were made for erythema

TABLE 5 Positive Control Study Average Skin Reaction Scores

SKIN SENSITIZATION IN GUINEA PIGS
Study No: 11662-08
Positive Control Material: DNCB

	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
	24 h	48 h	24 h	24 h	24 h	48 h
Group I Naive Control						
					0.2	0.0
Mean					0.1	
Group II Test Group						
	0.3	0.0	0.4	0.5	0.5	0.5
Mean	0.2		0.4	0.5	0.4	

LF - Left Front test site; RR - Right Rear test site

FIGURES SECTION

FIGURE 1 Irritation Screening

Date of Dosing: 9 Jun 08
Note: A dose of 0.4 mL/site was used for each concentration.
Each dilution was % v/v in deionized water.

81-M	
1	2
3	4

82-M	
4	1
2	3

83-F	
3	4
1	2

84-F	
2	3
4	1

1 = 100% 2 = 75% 3 = 50% 4 = 25%

Animal Number	Body Wt (g)	Observation Time After Treatment							
		24 Hour Score*				48 Hour Score*			
		Front Site		Rear Site		Front Site		Rear Site	
81-M	406	0	0	0	0	0	0	0	0
82-M	409	0	0	0	0	0	0	0	0
83-F	394	0	0	0	0	0	0	0	0
84-F	327	0	0	0	0	0	0	0	0

* - Observations made for erythema; M - Male; F - Female
Level Selected for Induction: 100% Level Selected for Challenge: 100%

FIGURE 2 Test Site Locations

HEAD OF ANIMAL	
LF	
	RR

LF - Left Front Test Site; RR - Right Rear Test Site

APPENDICES SECTION

APPENDIX 1 Analytical Report

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A16148F
Batch ID 533158 (GP-080305)

Batch Identification	533158
Product Design Code	A16148F
Product Denomination	SYN524464 FS (500)
Product by Common Name	SYN524464 FS (500)
Other Product Code(s)	GP-080305
Source	Technology & Projects, Syngenta Crop Protection, Inc.
Chemical Analysis (Active Ingredient Content)	
Identity of the Active Ingredient*	Confirmed
Content of SYN524464*	45.6% (wt/wt) or 534 g/L
Methodology Used for Characterization	HPLC
The Active Ingredient content is within the FAO limits.	
Physical Analysis	
Appearance*	pink opaque liquid
Density*	1171 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:

26 Mar 2008

Dorothea Jeffery
Group Leader I
Analytical & Product Chemistry Department

Date