Ametryn/NOA449280

Ametryn/NOA449280 SC (A16361B) – 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, PhD, DABT

STUDY COMPLETION DATE: June 22, 2009

PERFORMING LABORATORY: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478 USA

LABORATORY PROJECT ID: Report Number: 12826-09
Study Number: 12826-09
Task Number: T007144-06

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, PhD, DABT
Study Director, STILLMEADOW, Inc.

22 Jan 09
Date

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

QUALITY ASSURANCE STATEMENT

Test Substance: Ametryn/NOA449280 SC (428.57/57.14)
Study Title: Ametryn/NOA449280 SC (A16361B): 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 7 May 09. 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 Feb 09	13 Feb 09	13 Feb 09
Dosing	29 Apr 09	29 Apr 09	29 Apr 09
Report/Data Audit	19 May 09	19 May 09	19 May 09

Richard L. Martin

Richard L. Martin, MS, CPhT
Quality Assurance, STILLMEADOW, Inc.

22 Jun 09
Date

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Janice O. Kuhn, PhD, DABT	Study Director
Carol Morris, BA	Quality Control
Paul Siemens, BA	Technician
Robert Preston	Technician
Nancy Casajuana, LAT	Technician
Connie Pavatte	Report Preparation

Study dates

Study initiation date: 2 Mar 09
Experimental start date: 15 Apr 09
Experimental termination date: 15 May 09

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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 Ametryn/NOA449280 SC (428.57/57.14) (A16361B) 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. 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 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 2 Mar 09, and the experimental range-finding began on 12 Apr 09. The animals were treated as follows and the study was terminated on 15 May 09:

Group	Induction Treatments		Challenge Treatment
	First	Last	
I Naive Control (5/sex)	--	--	13 May 09
II Test (10/sex)	15 Apr 09	29 Apr 09	13 May 09

3.0 MATERIALS AND METHODS

3.1 Test Substance

Reference Name:	Ametryn/NOA449280 SC (428.57/57.14)
Label Identification:	G34162/NOA449280 SC (428.57/057.14) ID 555418 A16361B
Date & Quantity Received:	16 Feb 09; 4609.9 g (GW)
Physical Description:	Tan liquid
Storage:	Room temperature
Purity (w/w):	38.2% Ametryn; 5.30% NOA449280
Stability:	Reassay: May 2010
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 & non-pregnant
Acclimation Period:	At least 5 days
Date Born/Date Received:	24 & 28 Feb 09 / 30 Mar 09
Animal Identification:	Ear punch
Weights When Tested:	Male: 342-401 g; Female: 304-398 g Protocol deviation: female weights outside protocol range but did not affect study outcome.

3.3 Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1-5 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:	17-22° C / 48-98% Protocol deviation: humidity 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:	alpha-Hexylcinnamaldehyde, tech. 85% Batch # 13102MO
Manufacturer:	Aldrich
Physical Description:	Clear yellow liquid
Storage:	Room temperature
Purity, Composition & Stability:	Certificate of Analysis available from manufacturer
Conc. Administered:	Induction: 0.4 mL undiluted Challenge: 0.4 mL undiluted

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; Wilmington, MA, 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. 11950-08
In-life started: 5 Jun 08; In-life completed: 5 Jul 08

Results: Data from this study are presented in Appendix A. A mean score of 1.2 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.

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, Ametryn/NOA449280 SC (428.57/57.14) (A16361B), 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: Ametryn/NOA449280 SC (428.57/57.14)

	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						
41-M					0	0
42-M					0	0
43-M					0	0
44-M					0	0
45-M					0	0
46-F					0	0
47-F					0	0
48-F					0	0
49-F					0	0
50-F					0	0
Group II – Test Group						
51-M	0t	0	0	0	0	0
52-M	0t	0	0	0	0	0
53-M	0t	0	0	0	0	0
54-M	0t	0	0	0	0	0
55-M	0t	0	0	0	0	0
56-F	0t	0	0	0	0	0
57-F	0t	0	0	0	0	0
58-F	0t	0	0	0	0	0
59-F	0t	0	0	0	0	0
60-F	0t	0	0	0	0	0
61-M	0t	0	0	0	0	0
62-M	0t	0	0	0	0	0
63-M	0t	0	0	0	0	0
64-M	0t	0	0	0	0	0
65-M	0t	0	0	0	0	0
66-F	0t	0	0	0	0	0
67-F	0t	0	0	0	0	0
68-F	0t	0	0	0	0	0
69-F	0t	0	0	0	0	0
70-F	0t	0	0	0	0	0

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

TABLE 2 Average Skin Reaction Scores

SKIN SENSITIZATION IN GUINEA PIGS
Test Substance: Ametryn/NOA449280 SC (428.57/57.14)

	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: Ametryn/NOA449280 SC (428.57/57.14)

Animal Number	Day of Study	
	Day 0	Day 31
Group I - Naive Control		
41-M	373	593
42-M	342	484
43-M	350	611
44-M	372	629
45-M	356	639
46-F	332	536
47-F	362	594
48-F	349	495
49-F	387	545
50-F	318	447
Group II – Test		
51-M	366	617
52-M	362	594
53-M	347	514
54-M	359	570
55-M	365	604
56-F	327	510
57-F	373	500
58-F	328	470
59-F	359	433
60-F	369	426
61-M	375	563
62-M	357	595
63-M	396	546
64-M	374	609
65-M	401	627
66-F	304	515
67-F	343	506
68-F	398	436
69-F	375	506
70-F	361	468

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: 11950-08
Positive Control Material: alpha-Hexylcinnamaldehyde

	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							
41-M						0	0
42-M						0	0
43-M						0.5	0
44-M						0	0
45-M						0.5	0
46-F						0.5	0
47-F						0	0
48-F						0	0
49-F						0	0
50-F						0	0
Group II – Test Group							
51-M	0	0	1	2		1	0
52-M	0	0	1	1		1	0
53-M	0	0	0	0.5		1	0.5
54-M	0	0	0	1		1	0
55-M	0	0	1	2		3	1
56-F	0	0	1	2		3	1
57-F	0	0	0	1		2	1
58-F	0	0	0	1		3	1
59-F	0	0	0	1		1	0
60-F	0	0	0	1		2	1

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: 11950-08
Positive Control Material: alpha-Hexylcinnamaldehyde

	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						
	0.0	0.0	0.4	1.2	1.8	0.6
Mean	0.0		0.4	1.2	1.2	

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

FIGURES SECTION

FIGURE 1 Irritation Screening

Date of Dosing: 13 Apr 09
Note: A dose of 0.4 mL/site was used for each concentration.
Each dilution was % v/v in deionized water.

31-M	
1	2
3	4

32-M	
4	1
2	3

33-F	
3	4
1	2

34-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	
31-M	346	0	0	0	0	0	0	0	0
32-M	342	0	0	0	0	0	0	0	0
33-F	367	0	0	0	0	0	0	0	0
34-F	354	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

syngenta

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

Certificate of Analysis

A16361B
555418 (GP-090113)

Batch Identification	555418
Product Design Code	A16361B
Product Denomination	G34162/NOA449280 SC (428.57/057.14)
Product by Common Name	Ametryn/NOA449280 SC (428.57/057.14)
Other Product Code(s)	GP-090113
Source	Technology & Projects, Syngenta Crop Protection, Inc.
Chemical Analysis (Active Ingredient Content)	
Identity of the Active Ingredient(s)*	Confirmed
Content of Ametryn*	38.2 (%wt/wt) or 427 g/L
Content of NOA449280*	5.30 (%wt/wt) or 59.3 g/L
Methodology Used for Characterization	HPLC
The Active Ingredient(s) content is within the FAO limits.	
Physical Analysis	
Appearance*	Tan liquid
Density*	1118 g/L
Stability:	
Storage Temperature	< 30°C
Expiration date	May 2010

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:

David Stubbs
Group Leader 1
Analytical & Product Chemistry Department

Febrary 5, 2009
Date