syngenta

Ametryn/NOA449280

Ametryn/NOA449280 SC (A16361B) – Acute Eye Irritation Study in Rabbits

Final Report Amendment

DATA REQUIREMENT(S): OECD Guidelines for Testing of Chemicals, Procedure 405
EPA Health Effects Test Guidelines, OPPTS 870.2400

AUTHOR: Janice O. Kuhn, PhD, DABT

STUDY COMPLETION DATE: May 7, 2012 (amended)
June 22, 2009 (original)

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

LABORATORY PROJECT ID: Report Number: 12824-09
Study Number: 12824-09
Task Number: T007143-06

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

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.

07 May 12
Date
Orig. Date 22 Jun 09

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): Acute Eye Irritation Study in Rabbits

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 13 Jan 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
Observation	18 Mar 09	18 Mar 09	18 Mar 09
Report/Data Audit	9 Apr 09	9 Apr 09	9 Apr 09
Amendment Audit	7 May 12	7 May 12	7 May 12

Richard L. Martin

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

07 MAY 12
Date
Orig. Date 22 Jun 09

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
Jeanne Poorman, BS	Report Preparation

Study dates

Study initiation date: 02 Mar 09
Experimental start date: 16 Mar 09
Experimental termination date: 20 Mar 09

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1.0 EXECUTIVE SUMMARY

An acute eye irritation study was conducted on three albino rabbits using test substance Ametryn/NOA449280 SC (428.57/57.14) (A16361B). The undiluted test substance (0.1 mL) was placed into the conjunctival sac of the right eye of each animal selected for testing. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation. The number of animals testing "positive" for each parameter (according to the Legend to Table 1) over the number of animals tested is presented below.

	Time After Treatment				
	Hours				Day
	1	24	48	72	4
Cornea					
Opacity	3/3	3/3	0/3	0/3	0/3
Iritis	3/3	2/3	0/3	0/3	0/3
Conjunctivae					
Redness	3/3	3/3	0/3	0/3	0/3
Chemosis	3/3	1/3	0/3	0/3	0/3

There were no positive effects exhibited in any eyes at 48 hours after treatment, placing Ametryn/NOA449280 SC (428.57/57.14) in EPA Toxicity Category III. Based on these results and according to the classification of Kay and Calandra (1962), Ametryn/NOA449280 SC (428.57/57.14) is rated moderately irritating.

2.0 INTRODUCTION

The objective of this study was to assess the relative level of eye irritation following a single exposure of the test substance to rabbits in accordance with US EPA OPPTS 870.2400, 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 that 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 02 Mar 09, the pre-dose experimental portion began on 15 Mar 09, and the animals were treated with the test substance between 1130 and 1132 on 16 Mar 09. The in-life portion of the study was terminated on 20 Mar 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):	Ametryn 38.2 %; NOA449280 5.30 %
Stability:	May 2010

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 rabbit; New Zealand White
Justification of Species:	The rabbit is preferred by various regulatory agencies for use in eye irritation testing.
Source:	Nichols Rabbitry Inc., Lumberton, TX
Date Born/Date Received:	07 Dec 08 / 25 Feb 09
Acclimation Period:	At least 5 days
Quantity & Sex:	2 males and 1 female (nulliparous and non-pregnant)
Initial Body Weight:	Males: 2.750 – 2.900 kg; Female: 2.450 kg
Final Body Weight:	Males: 2.825 – 3.050 kg; Female: 2.650 kg
Group/Animal Identification:	Cage cards/Ear tag

3.3 Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel	
Housing:	1 per cage	
Environmental Conditions		
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:	16°-22° C / 50-78% Protocol deviation: Temperature and relative humidity were outside protocol range but did not affect study outcome.	
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily	
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

Prior to starting the study, the pH of the test substance was determined to be 7.49. Healthy albino rabbits were released from quarantine. Both eyes of each animal were carefully examined within 24 hours prior to treatment with a fluorescein sodium ophthalmic solution, and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing.

On Day 1, a dose of 0.1 mL of the undiluted test substance as received was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls.

3.5 Observations

The treated eyes of all animals were examined without magnification under white room lighting provided by daylight-type fluorescent ceiling fixtures, and (if needed) an additional source of white light affixed to the examination table or using a handheld flashlight. The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours and at 4 days after treatment. The corneas of all treated eyes were examined immediately after the 24-hour observation with a fluorescein sodium ophthalmic solution. A Finoff ocular transilluminator with cobalt blue filter (Welch Allyn, Skaneateles Falls, NY) was utilized to enhance visualization of fluorescein staining. Any of the corneas that exhibited fluorescein staining at the 24-hour observation were re-examined with the fluorescein sodium ophthalmic solution at each consecutive observation until fluorescein staining of the cornea no longer occurred. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

3.6 Irritation Scoring Method

Individual irritation scores for each animal at each scheduled observation were determined using the grading scale given in the Legend to Table 1. An average irritation score for each scheduled observation for all eyes was then determined, based on the number of animals tested. A maximum average irritation score was derived from the observation yielding the highest average irritation score. The maximum average irritation score was used to rate the test substance according to the ratings presented in the Legend to Table 2. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

4.0 RESULTS AND DISCUSSION

4.1 Evaluation

The number of animals with "positive" findings at each observation period is presented in the summary section of this report. There were no positive effects exhibited in any eyes at 48 hours after treatment. Ocular reactions are presented in Table 1. A summary of irritation scores is presented in Table 2.

The maximum average irritation score of 39.0, obtained at 1 hour after treatment, was used to rate Ametryn/NOA449280 SC (428.57/57.14) (A16361B) moderately irritating. Fluorescein staining was observed in 3 eyes at 24 hours and was not observed in any eyes at 48 hours after treatment.

5.0 CONCLUSIONS

There were no positive effects exhibited in any eyes at 48 hours after treatment, placing Ametryn/NOA449280 SC (428.57/57.14) (A16361B) in EPA Toxicity Category III. Based on the maximum average irritation score of 39.0 out of a possible 110, the test substance Ametryn/NOA449280 SC (428.57/57.14) is rated moderately irritating. Irritation was no longer present on Day 4.

TABLES SECTION

TABLE 1 Ocular Reactions

ACUTE EYE IRRITATION IN RABBITS
Test Substance: Ametryn/NOA449280 SC (428.57/57.14)

	Rabbit No. 3644-M										Rabbit No. 3652-M									
	Hrs After Treatment				Days After Treatment						Hrs After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	1	1	0	0	0						1	1	0	0	0					
B. Area	4	3	0	0	0						4	4	0	0	0					
C. Fluorescein Staining	-	PA	0	-	-						-	PA	0	-	-					
D. Stippling	0	0	0	0	0						0	0	0	0	0					
SCORE	20	15	0	0	0						20	20	0	0	0					
II. Iris																				
A. Grade	1	1	0	0	0						1	1	0	0	0					
SCORE	5	5	0	0	0						5	5	0	0	0					
III. Conjunctivae																				
A. Redness	2	2	1	0	0						2	2	1	0	0					
B. Chemosis	2	2	1	0	0						2	1	0	0	0					
C. Discharge	3	1	0	0	0						3	2	0	0	0					
D. Necrosis or Ulceration	0	0	0	0	0						0	0	0	0	0					
SCORE	14	10	4	0	0						14	10	2	0	0					
TOTAL SCORE	39	30	4	0	0						39	35	2	0	0					

M – Male; F – Female

Duration of Study: 4 Days

TABLE 1 Ocular Reactions (Continued)

ACUTE EYE IRRITATION IN RABBITS
Test Substance: Ametryn/NOA449280 SC (428.57/57.14)

	Rabbit No. 3653-F									
	Hrs After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21
I. Cornea										
A. Opacity	1	1	0	0	0					
B. Area	4	3	0	0	0					
C. Fluorescein Staining	-	PA	0	-	-					
D. Stippling	0	0	0	0	0					
SCORE	20	15	0	0	0					
II. Iris										
A. Grade	1	0	0	0	0					
SCORE	5	0	0	0	0					
III. Conjunctivae										
A. Redness	2	2	1	1	0					
B. Chemosis	2	1	0	0	0					
C. Discharge	3	2	0	0	0					
25 D. Necrosis or	0	0	0	0	0					
SCORE	14	10	2	2	0					
TOTAL SCORE	39	25	2	2	0					

M – Male; F – Female

Duration of Study: 4 Days

TABLE 2 Scores and Score Summary

ACUTE EYE IRRITATION IN RABBITS
Test Substance: Ametryn/NOA449280 SC (428.57/57.14)

Time After Treatment	Rabbit Number			Average Score
	3644-M	3652-M	3653-F	
Hour 1	39	39	39	39.0
Hour 24	30	35	25	30.0
Hour 48	4	2	2	2.7
Hour 72	0	0	2	0.7
Day 4	0	0	0	0.0
Day 7				
Day 10				
Day 14				
Day 17				
Day 21				
Descriptive Rating from the Legend to Table 2:				
		Maximum Average Score:	39.0	Moderately irritating
M – Male; F – Female		Duration of Study: 4 Days		

APPENDICES SECTION

APPENDIX 1 Legends to Tables

ACUTE EYE IRRITATION IN RABBITS
Table 1 Grading Scale

I. Cornea

A. Opacity - degree (area most dense taken for reading)

No opacity	0
Slight dulling of normal luster	+
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous area, no details of iris visible, size of pupil barely discernible.	3*
Opaque cornea, iris not discernible through the opacity	4*

B. Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

C. Fluorescein Staining - appearance of yellow-green staining of cornea

Cornea not examined with fluorescein	-
No fluorescein staining	0
Positive fluorescein staining	P
Area of cornea involved	
One quarter (or less), but not zero	A
Greater than one quarter, but less than half	B
Greater than half, but less than three quarters	C
Greater than three quarters, up to whole area	D

D. Stippling - appearance of pinpoint roughening

No stippling	0
Presence of stippling	S
Area of cornea involved	
One quarter (or less), but not zero	A
Greater than one quarter, but less than half	B
Greater than half, but less than three quarters	C
Greater than three quarters, up to whole area	D

A X B X 5 Total Maximum = 80

* - Reaction indicates a positive effect.

APPENDIX 1 Legends to Tables (Continued)

ACUTE EYE IRRITATION IN RABBITS
Table 1 Grading Scale (cont.)

II. Iris

A. Grades

Normal 0

Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia or injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive)..... 1*

No reaction to light, hemorrhage, gross destruction (any or all of these)..... 2*

A X 5 Total Maximum = 10

III. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Blood vessels normal..... 0

Some blood vessels definitely hyperemic (injected) 1

Diffuse, crimson color, individual vessels not easily discernible..... 2*

Diffuse beefy red 3*

B. Chemosis: lids and/or nictitating membrane

No swelling..... 0

Any swelling above normal (includes nictitating membrane)..... 1

Obvious swelling with partial eversion of lids 2*

Swelling with lids about half closed..... 3*

Swelling with lids more than half closed..... 4*

C. Discharge

No discharge..... 0

Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)..... 1

Discharge with moistening of the lids and hairs just adjacent to lids..... 2

Discharge with moistening of the lids and hairs, and considerable area around the eye..... 3

D. Necrosis or Ulceration of the palpebral and bulbar conjunctivae or nictitating membrane

No necrosis or ulceration..... 0

Presence of necrosis or ulceration ..... N

(A + B + C) X 2 Total Maximum = 20

* - Reaction indicates a positive effect.

APPENDIX 1 Legends to Tables (Continued)

ACUTE EYE IRRITATION IN RABBITS
Table 2 Rating of Test Substance Based on Eye Irritation*

Rating	Maximum Average Score	Definition
Non-irritating	0.0-0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically non-irritating	>0.5-2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally irritating	>2.5-15.0	To maintain this rating, all scores at the 72-hour reading must be zero; otherwise, increase rating one level.
Mildly irritating	>15.0-25.0	To maintain this rating, scores at the 7 day reading must be zero; otherwise, increase rating one level.
Moderately irritating	>25.0-50.0	To maintain this rating, scores at the 7 day reading must be less than or equal to 10 for 60% or more of the animals; also, the 7 day mean score must be less than or equal to 20. If the 7 day mean score is less than or equal to 20, but less than 60% of the animals show scores less than or equal to 10, then no animal with a score greater than 10 can exceed a score of 30 if rating is to be maintained; otherwise, increase rating one level.
Severely irritating	>50.0-80.0	To maintain this rating, scores at the 7 day reading must be less than or equal to 30 for 60% or more of the animals; also, the 7 day mean score must be less than or equal to 40. If the 7 day mean score is less than or equal to 40, but less than 60% of the animals show scores less than or equal to 30, then no animal with a score greater than 30 can exceed a score of 60 if rating is to be maintained; otherwise, increase rating one level.
Extremely irritating	>80.0-110.0	

NOTE: The rating of the test substance is not to be increased more than one level above its maximum average score.

* - Slightly modified from: Kay, J.H. and Calandra, J.C. (1962) Interpretation of Eye Irritation Tests. J. Soc. Cosmetic Chemists 13:281-289.

APPENDIX 2 Analytical Report

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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

Febrny 5, 2019
Date

APPENDIX 3 Amendment

Acute Eye Irritation in Rabbits
(OECD 405 and OPPTS 870.2400)

Ametryn/NOA449280 SC (428.57/57.14)

Study 12824-09

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

Final Report Amendment 1

This amendment modifies the final report Table 1, animal #3652, 24 hour Total Score from 30 to 35.

This amendment modifies the final report Table 2, animal #3652, 24 hour individual score from 30 to 35.

This amendment modifies the final report Table 2, 24 hour Average Score from 28.3 to 30.0.

Reason for amendment: Miscalculations

Amendment Approval:

Janice O. Kuhn

Janice O. Kuhn, PhD, DABT
Study Director, STILLMEADOW, Inc.

07 May 12
Date