**SYN524464**

**SYN524464 FS (A16148F) – Acute Eye Irritation Study in Rabbits**

**Final Report**

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

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

**STUDY COMPLETION DATE:** August 27, 2008

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

**LABORATORY PROJECT ID:** Report Number: 11816-08
Study Number: 11816-08
Task Number: T001111-08

**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

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

27 Aug 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 (A16148F): 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 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 | 1 Apr 08 | 1 Apr 08 | 1 Apr 08 |
| Observations | 30 Apr 08 | 30 Apr 08 | 30 Apr 08 |
| Report/Data Audit | 30 May 08 | 30 May 08 | 30 May 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 | |
| Technical Staff: | Carol Morris, B.A | Paul Siemens, B.A. |
| | Hector Fuentes | Robert Preston |
| | Nancy Casajuana, L.A.T. | |
| Data Services: | Connie Pavatte, Report Preparation | |

## Study dates

Study initiation date: 14 Apr 08
Experimental start date: 28 Apr 08
Experimental termination date: 1 May 08

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## 1.0 EXECUTIVE SUMMARY

An acute eye irritation study was conducted on three albino rabbits using test substance SYN524464 FS (500) (A16148F). 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 | | | |
|---|---|---|---|---|
| | 1 | 24 | 48 | 72 |
| Cornea | | | | |
| Opacity | 0/3 | 0/3 | 0/3 | 0/3 |
| Iritis | 0/3 | 0/3 | 0/3 | 0/3 |
| Conjunctivae | | | | |
| Redness | 0/3 | 0/3 | 0/3 | 0/3 |
| Chemosis | 0/3 | 0/3 | 0/3 | 0/3 |

There were no positive effects exhibited in any eyes at any time after treatment, placing SYN524464 FS (500) in EPA Toxicity Category IV. Based on these results and according to the classification of Kay and Calandra (1962), SYN524464 FS (500) is rated non-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 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 14 Apr 08, the pre-dose experimental portion began on 27 Apr 08, and the animals were treated with the test substance between 1254 and 1256 on 28 Apr 08. The in-life portion of the study was terminated on 1 May 08.

## 3.0 MATERIALS AND METHODS

### 3.1 Test Substance

| | |
|---|---|
| Reference Name: | SYN524464 FS (500) |
| Label Identification: | SYN524464 FS (500)<br>ID 533158<br>A16148F |
| Date & Quantity Received: | 14 Apr 08; 3691g (Gr.Wt.) |
| Physical Description: | Pink opaque liquid |
| Storage: | Room temperature |
| Purity (w/w): | 45.6% 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 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: | 20 & 27 Jan 08 / 2 & 18 Apr 08 |
| Acclimation Period: | At least 5 days |
| Quantity & Sex: | 1 male and 2 females (nulliparous and non-pregnant) |
| Initial Body Weight: | Male: 3.250 kg; Females: 2.100-3.000 kg |
| Final Body Weight: | Male: 3.350 kg; Females: 2.250-3.050 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%<br>•12-hour light/dark cycle •10-12 air changes/hour |
| Actual Temp/Rel. Humidity: | 18-22° C / 51-96%<br>Protocol deviation: humidity was 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 6.02. 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 0, 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 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 any time 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 0.0, obtained after treatment, was used to rate SYN524464 FS (500) (A16148F) non-irritating. Fluorescein staining did not occur in any of the eyes.

## 5.0 CONCLUSIONS

There were no positive effects exhibited in any eyes at any time after treatment, placing SYN524464 FS (500) (A16148F) in EPA Toxicity Category IV. Based on the maximum average irritation score of 0.0 out of a possible 110, the test substance SYN524464 FS (500) is rated non-irritating. Irritation was not present at any time during the study.

# TABLES SECTION

## TABLE 1 Ocular Reactions

ACUTE EYE IRRITATION IN RABBITS
Test Substance: SYN524464 FS (500)

| | Rabbit No. 2530-M | | | | | | | | | | Rabbit No. 2531-F | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **B. Area** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **C. Fluorescein Staining** | - | 0 | - | - | | | | | | | - | 0 | - | - | | | | | | |
| **D. Stippling** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **SCORE** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **II. Iris** | | | | | | | | | | | | | | | | | | | | |
| **A. Grade** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **SCORE** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **III. Conjunctivae** | | | | | | | | | | | | | | | | | | | | |
| **A. Redness** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **B. Chemosis** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **C. Discharge** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **D. Necrosis or Ulceration** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **SCORE** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| **TOTAL SCORE** | 0 | 0 | 0 | 0 | | | | | | | 0 | 0 | 0 | 0 | | | | | | |

M – Male; F – Female
Duration of Study: 72 Hrs

## TABLE 1 Ocular Reactions (Continued)

ACUTE EYE IRRITATION IN RABBITS
Test Substance: SYN524464 FS (500)

| | Rabbit No. 2539-F | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hrs After Treatment | | | | Days After Treatment | | | | | |
| | 1 | 24 | 48 | 72 | 4 | 7 | 10 | 14 | 17 | 21 |
| **I. Cornea** | | | | | | | | | | |
| **A. Opacity** | 0 | 0 | 0 | 0 | | | | | | |
| **B. Area** | 0 | 0 | 0 | 0 | | | | | | |
| **C. Fluorescein Staining** | - | 0 | - | - | | | | | | |
| **D. Stippling** | 0 | 0 | 0 | 0 | | | | | | |
| **SCORE** | 0 | 0 | 0 | 0 | | | | | | |
| **II. Iris** | | | | | | | | | | |
| **A. Grade** | 0 | 0 | 0 | 0 | | | | | | |
| **SCORE** | 0 | 0 | 0 | 0 | | | | | | |
| **III. Conjunctivae** | | | | | | | | | | |
| **A. Redness** | 0 | 0 | 0 | 0 | | | | | | |
| **B. Chemosis** | 0 | 0 | 0 | 0 | | | | | | |
| **C. Discharge** | 0 | 0 | 0 | 0 | | | | | | |
| **D. Necrosis or Ulceration** | 0 | 0 | 0 | 0 | | | | | | |
| **SCORE** | 0 | 0 | 0 | 0 | | | | | | |
| **TOTAL SCORE** | 0 | 0 | 0 | 0 | | | | | | |

M – Male; F – Female

Duration of Study: 72 Hrs

## TABLE 2 Scores and Score Summary

ACUTE EYE IRRITATION IN RABBITS
Test Substance: SYN524464 FS (500)

| Time After Treatment | Rabbit Number | | | Average Score |
|---|---|---|---|---|
| | 2530-M | 2531-F | 2539-F | |
| Hour 1 | 0 | 0 | 0 | 0.0 |
| Hour 24 | 0 | 0 | 0 | 0.0 |
| Hour 48 | 0 | 0 | 0 | 0.0 |
| Hour 72 | 0 | 0 | 0 | 0.0 |
| Day 4 | | | | |
| Day 7 | | | | |
| Day 10 | | | | |
| Day 14 | | | | |
| Day 17 | | | | |
| Day 21 | | | | |
| Descriptive Rating from the Legend to Table 2: | | | | |
| Maximum Average Score: | | | 0.0 | Non-irritating |
| | | | | |
| M – Male; F – Female | Duration of Study: 72 Hrs | | | |

# 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

syngenta

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

Document 10350420.doc
Page 1 of 1

Certificate of Analysis
Study T000973-08