

STILLMEADOW
I N C O R P O R A T E D

VOLUME __ OF __ OF SUBMISSION

ALKA VITA (ALKAHYDROXY®) (Concentrate)

AMENDED FINAL REPORT

RABBIT SKIN CORROSION (DOT) STUDY

DOT Regulation 49 CFR 173.137

AUTHOR:

Janice O. Kuhn, Ph.D., DABT

STUDY INITIATION DATE: 14 April 2005
STUDY COMPLETION DATE: 17 May 2005
STUDY AMENDMENT DATE: 20 June 2005

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

9018-05

VOLUME 1 OF 1 OF STUDY

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SUBMITTED TO:
Cisne Enterprises Inc.
3535 W. 16th Street
Odessa, TX 79763

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QUALITY ASSURANCE STATEMENT

Test Substance: ALKA VITA (ALKAHYDROXY®) (Concentrate)
Study Title: Rabbit Skin Corrosion (DOT) Study

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOP); however, no laboratory-phase inspection was conducted. The findings were reported to Study Director and Management as follows:

| Study Phase Inspected | Inspection Type | Date Inspected | Reported to Study Director | Reported to Management |
|-----------------------|-----------------|-------------------------|----------------------------|------------------------|
| Protocol Review | Study-based | 5 Apr 05 | 5 Apr 05 | 5 Apr 05 |
| Facility inspection | Facility-based | 29 Dec 04 – 6 Jan 05 | 6 Jan 05 | 6 Jan 05 |
| Report/Data Audit | Study-based | 16 May 05 | 16 May 05 | 16 May 05 |
| Amendment Audit | Study-based | 20 Jun 05 | 20 Jun 05 | 20 Jun 05 |


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


Date

SUMMARY

A skin corrosion/dermal irritation study was conducted on three albino rabbits using test substance ALKA VITA (ALKAHYDROXY®) (Concentrate) to determine the skin corrosion potential of the test substance. If the substance was non-corrosive by DOT criteria, the potential for primary dermal irritation was evaluated.

There were three intact skin test sites per animal. Each test site was treated with 0.5 ml of undiluted test substance. The test sites were dosed sequentially to permit removal of test substance and observation of the treated sites for dermal irritation and defects at several predetermined observation times. The first site was dosed, then washed and observed 3 minutes later. A second site was dosed, wrapped for 1 hour, and washed; then both the first and second test sites were observed. A third site was dosed and wrapped for 4 hours. One hour after unwrapping and washing of the third site, all three test sites were observed for signs of skin irritation and/or corrosion. Observations were conducted thereafter at 24, 48 and 72 hours after final unwrap.

Tissue destruction (necrosis or ulceration) was not observed in any animals during the evaluation period. Dermal irritation (erythema, edema or other) was not observed in any animals. The test substance is considered non-corrosive by DOT criteria when applied to the intact skin of albino rabbits.

INTRODUCTION

The objective of this study was to determine the skin corrosion and/or dermal irritancy potential of the test substance according to US Department of Transportation regulations (49 CFR, Part 173.137). This study was conducted for Cisne Enterprises Inc., according to the approved protocol and STILLMEADOW, Inc. SOP's. 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. The protocol, raw data, and this report are archived at STILLMEADOW, Inc. The animals were treated with the test substance between 0950 and 1101 on 19 Apr 05. The in-life portion of the study was terminated on 22 Apr 05.

TEST SUBSTANCE

| | |
|---------------------------|---|
| Label Identification: | ALKA VITA (Concentrate) Lot No. 008244 Purity: 99.99% |
| Date & Quantity Received: | 13 Apr 05; 209.4 g (Gr.Wt.) |
| Physical Description: | Clear liquid |
| Storage: | Room temperature |
| Purity & Composition: | Certificate of Analysis not provided by sponsor |
| Stability: | Not provided by sponsor |

Records pertaining to stability, characterization, identity, synthesis methods and location of documentation are the responsibility of the sponsor.

TEST SYSTEMExperimental Animals

Species & Strain: Albino Rabbit; New Zealand White
 Justification of Species: The rabbit is a representative species preferred by various regulatory agencies for use in skin corrosion testing.
 Source: Nichols Rabbitry Inc., Lumberton, TX
 Date Received: 13 Apr 05
 Quantity & Sex: 1 male and 2 females
 Quarantine Period: At least 5 days
 Animal Identification: Ear tags
 Initial Body Weight: Male: 2.275 kg; Females: 2.625-2.700 kg
 Date of Birth: 23 Jan 05

Animal Husbandry

Cage Type: Suspended, wire bottom, stainless steel
 Housing: One per cage
 Environmental Controls
 Set to Maintain: · Temperature Range 20°C±3° · Humidity Range 30-70%
 · 12-hour light/dark cycle · 10-12 air changes/hour
 Food: PMI Feeds Lab Rabbit Diet #5321, in measured amounts
 Water Type: 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 which would have interfered with or affected the results of the study.

PROCEDURESPreparation of Animals

Healthy albino rabbits were released from quarantine. Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. Care was taken to avoid abrading the skin. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. There were three intact skin test sites per animal.

Test Substance Application

Each test site was treated by applying 0.5 ml of undiluted test substance beneath a 4 ply, 2.5 x 2.5 cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. With the exception of dosing for the 3 minute observation, the entire trunk of each animal was then wrapped with clear plastic film to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. The wrappings were held in place with non-irritating adhesive tape.

Removal of Test Substance

The wrappings were removed at the end of the 1 and 4 hour exposure periods. All test sites (including the 3 minute exposure site) were washed with room temperature tap water and a clean cloth to prevent further exposure.

PROCEDURES (cont.)Observations and Scoring Method

The scoring scale used for the evaluation of skin reactions is presented in the Legend to Tables 1 and 2. Test sites were observed and scored for signs of skin irritation, necrosis, or other defects after patch removal at 3 minutes and 1 hour for the 3 minute and 1 hour exposure sites, respectively. All test sites were observed at 1, 24, 48 and 72 hours after last patch removal.

DOT Corrosion Evaluation

Corrosion under DOT criteria is considered to have occurred if the test substance in contact with the intact skin of the rabbits causes destruction or irreversible alteration of the skin on any rabbits tested at any time. Tissue destruction is considered to have occurred if there is ulceration or necrosis at any observation time. Tissue destruction does not include erythema, edema, flaking of the epidermis, or shallow lateral fissuring. The Packing Group is determined by the shortest exposure time producing tissue destruction (Legend to Tables 1 and 2).

RESULTS AND DISCUSSIONEvaluation

Observations for dermal corrosion/irritation through 24 hours after dosing of the 4 hour test site are presented in Table 1. Observations for dermal corrosion/irritation from 48 hours after final dosing through 72 hours are presented in Table 2. Erythema, edema and other signs of irritation were not observed in any animals at any time during the study. Tissue destruction (necrosis or ulceration) was not observed in any animals during the entire period.

CONCLUSION

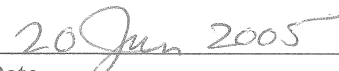
The test substance ALKA VITA (ALKAHYDROXY®) (Concentrate) is considered non-corrosive by DOT criteria when applied to the intact skin of albino rabbits.

Study Director:


 Janice O. Kuhn, Ph.D., DABT
 Senior Toxicologist, STILLMEADOW, Inc.

Date

Orig. Date: 17 May 05



STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
 Hector Fuentes

Paul Siemens, B.A.
 Robert Preston

Data Services: Connie Pavatte, Report Preparation

LEGEND TO TABLES 1 & 2
RABBIT SKIN CORROSION (DOT)

Evaluation of Skin Reactions

| <u>Erythema Formation</u> | <u>Score</u> |
|---|--------------|
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 |
| Maximum Possible | 4 |

| <u>Edema Formation</u> | <u>Score</u> |
|--|--------------|
| No edema | 0 |
| Very slight edema (barely perceptible) | 1 |
| Slight edema (edges of area well defined by definite raising) | 2 |
| Moderate edema (raised approximately 1 mm) | 3 |
| Severe edema (raised more than 1 mm and extending beyond the area of exposure) | 4 |
| Maximum Possible | 4 |

Classification of Test Substance

Classification of Corrosive Materials into DOT Packing Groups*:

Packing Group I - Substances that cause visible destruction or irreversible alterations of the skin tissue at the site of contact when tested on the intact skin of an animal for a period of not more than 3 minutes.

Packing Group II - Substances, other than those meeting Group I criteria, that cause visible destruction or irreversible alterations of the skin tissue at the site of contact when tested on the intact skin of an animal for a period of not more than 60 minutes.

Packing Group III - Substances, other than those meeting Group I or II criteria, that cause visible destruction or irreversible alterations of the skin tissue at the site of contact when tested on the intact skin of an animal for a period of not more than 4 hours.

* - 49 CFR 173.137 Class 8 - Assignment of packing group

TABLE 1
RABBIT SKIN CORROSION (DOT)
 Signs of Dermal Corrosion/Irritation
 Test Substance: ALKA VITA (ALKALYDROXY®) (Concentrate)

| Obs. time after treatment | Test site | Erythema | | | | | | Edema | | | | | | Other | | | |
|--|-----------|---------------|--------|--------|---------------|--------|--------|---------------|--------|--------|---------------|-------------|--------|--------|---------------|--------|--|
| | | Rabbit Number | | | Rabbit Number | | | Rabbit Number | | | Rabbit Number | | | 8606-M | 8605-F | 8607-F | |
| | | 8606-M | 8605-F | 8607-F | 8606-M | 8605-F | 8607-F | 8606-M | 8605-F | 8607-F | 8606-M | 8605-F | 8607-F | | | | |
| 3 min | RA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| 1 Hr | RA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| 1 Hr | RP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| 5 Hr | RA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| 5 Hr | RP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| 5 Hr | LA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| *24 Hr | RA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| *24 Hr | RP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| *24 Hr | LA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | |
| | | | | | | | | | | | | DOT CLASS = | | | Non-corrosive | | |
| Note: A dash (-) is used if there are no other signs of dermal irritation. | | | | | | | | | | | | | | | | | |
| RA - Right Anterior; RP - Right Posterior; LA - Left Anterior | | | | | | | | | | | | | | | | | |
| * - Observations were made 24 hours after final unwrap. | | | | | | | | | | | | | | | | | |
| M - Male; F - Female | | | | | | | | | | | | | | | | | |

TABLE 2
RABBIT SKIN CORROSION (DOT)
Signs of Dermal Corrosion/Irritation
Test Substance: ALKA VITA (ALKALYDROXY®) (Concentrate)

| Animal Number | Test site | Erythema | | | | | | Edema | | | | | | Other | | | | | |
|---------------|-----------|----------|----|------|----|------|------|-------|----|------|---|------|------|-------|----|------|----|------|------|
| | | Hours | | Days | | Days | Days | Hours | | Days | | Days | Days | Hours | | Days | | Days | Days |
| | | 48 | 72 | 7 | 10 | | | 14 | 48 | 72 | 7 | | | 10 | 14 | 48 | 72 | | |
| 8606-M | RA | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| | RP | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| | LA | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| 8605-F | RA | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| | RP | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| | LA | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| 8607-F | RA | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| | RP | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |
| | LA | 0 | 0 | | | | | 0 | 0 | | | | | - | - | | | | |

Note: A dash (-) is used if there is no other signs of irritation.

RA - Right Anterior; RP - Right Posterior; LA - Left Anterior

M - Male; F - Female Study Duration: 72 Hrs

APPENDIX A

ALKA VITA (ALKAHYDROXY®) (Concentrate)

Rabbit Skin Corrosion (DOT) Study

(DOT Regulation 49 CFR 173.137)

Study Number 9018-05

Sponsor: Cisne Enterprises Inc.

Final Report Amendment No. 1

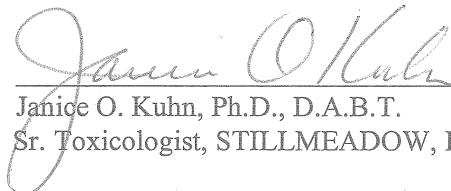
This amendment modifies the final report:

To change: Test substance reference name: from ALKA VITA (Concentrate)
To ALKA VITA (ALKAHYDROXY®) (Concentrate)

Reason for amendment: Sponsor request.

Amendment Approval:

Study Director:


Janice O. Kuhn, Ph.D., D.A.B.T.
Sr. Toxicologist, STILLMEADOW, Inc.

Date

20 Jun 05