UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA United States Environmental Protection Agency **Office of Pesticide Programs**

Wednesday, May 06, 2009

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. No.: 42048-R
Product Name: Glyco-san
DP Barcode: D362641

FROM: Earl Goad, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Earl Goad 5/6/2009

THRU: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

THRU: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

TO: Tracy Lantz -acting PM#34/Stacey Grigsby
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Celeste Industries Corporation
7978 Industrial Park Rd.
Easton, MD 21601

PRODUCT FORMULATION FROM LABEL:

PC Codes	Active Ingredient(s):	% by wt.
128929	L-Lactic acid	12.00
	Other Ingredient(s):	88.00
	Total:	100.00

I) BACKGROUND:

The registrant has submitted a full 6-pack of original studies to support the Acute Toxicity data requirements for registration of EPA file symbol: 42048-R (Glyco-san).

This is a commercial end use product proposed as a cleaner and disinfectant for potable water supply systems for aircraft. It is used both neat and at use dilutions depending on the application.

A primary review of these original studies was conducted by the Product Science Branch (PSB)/Antimicrobials Division (AD) contractor: Computer Sciences Corporation (CSC). The Chemistry and Toxicology Team (CTT) conducted a brief secondary review to assure that the studies meet EPA/OPP criteria, and is responsible for this memorandum.

II) FINDINGS: PSB findings are:

A. The systemic Acute Toxicity studies, including Oral, Dermal, and Inhalation all are classified as category IV. Also they were all conducted and reported in an acceptable manner

B. The Primary Eye Irritation study resulted in a category III and is also acceptable.

C. The Primary Skin Irritation is an acceptable category IV.

D. The Dermal Sensitization study reported some procedural irregularities. However appropriate accommodations were made to compensate for the errors. The results are fully acceptable; Glyco-san was not shown to be a dermal sensitizer.

III) The acute toxicity profile for 42048-R (Glyco-san) is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	476814-03	IV	Acceptable
Acute Dermal Toxicity	476814-04	IV	Acceptable
Acute Inhalation Toxicity	476814-05	IV	Acceptable
Primary Eye Irritation	476814-06	III	Acceptable
Primary Skin Irritation	476814-07	IV	Acceptable
Dermal Sensitization	476814-08	Non-Sensitizer	Acceptable

IV) LABELING:

Keep Out of Reach of Children

A. The signal word for EPA Reg. 42048-R (Glyco-san) is **CAUTION** based on the category III for Eye Irritation.

B. Precautionary labeling:

Hazards to Humans and Domestic Animals:

Causes eye irritation. Avoid contact with eyes, skin, or clothing. Wear eyewear and gloves when handling. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco.

C. First Aid Statements:

If in eyes:

- Hold eye open and rinse slowly and gently with water for 15-20 minutes.
- Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
- Call a poison control center or doctor for treatment advice.

If on skin:

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a poison control center or doctor for treatment advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment.

For emergency information on [product, use, etc.], call the **National Pesticides Information Center** at 1-800-858-7378, 6:30 AM to 4:30 PM Pacific time (PT), seven days a week. During other times, call the poison control center 1-800-222-1222.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 34
MRID No.: 476814-03

Reviewer: CSC and Earl Goad (CTT)
Completion Date: November 25, 2008
Study No.: 25508

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: GLYCO-SAN
Batch #: 8163 / Water-clear liquid

Dosage: Limit Test: 5,000 mg/kg (administered as received)

Species: 3 Rats; Sprague-Dawley derived, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (9-10 weeks old)
Weight: 180-204 grams at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 19-21°C
Humidity Range: 63-87%
Photoperiod: 12-hour light/dark cycle
Acclimation: 10 or 14 days

Conclusion:

1. **Acute Oral LD_{50} (mg/kg)**: Female Rats: >5,000 mg/kg

2. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1100): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was above the targeted upper limit of 70% during the study due to exceptionally high, seasonal humidity. Portable dehumidifiers were used to lower the humidity levels during this time."
- The laboratory reported the following protocol amendments: (1) the composition of the product was changed to read: Active: 12% L-Lactic Acid and Other: 88% Inert Ingredients; (2) the pH of the product was changed to read 2.8; and (3) the study director was changed in order to finalize the report in a timely manner.
- The guidelines state that the animals are to be observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours, and daily thereafter. The animals were observed during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing.

Results:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5,000	S	S
2	3102	5,000	S	S
3	3103	5,000	S	S

S - Survival

Observations:
All animals survived, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

Gross Necropsy Findings:
No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 34
MRID No.: 476814-04

Reviewer: CSC and Earl Goad (CTT)
Completion Date: November 25, 2008
Study No.: 25509

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: GLYCO-SAN
Batch #: 8163 / Water-clear liquid

Dosage: 5,000 mg/kg (applied as received)

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8-9 weeks old)
Weight: Males: 239-270 grams; Females: 187-216 grams; at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 21-23°C
Humidity Range: 61-80%
Photoperiod: 12-hour light/dark cycle
Acclimation: 9 days

Summary:

1. **Acute Dermal LD_{50} (mg/kg)**: Male and Female Rats: >5,000 mg/kg

2. **The estimated acute dermal LD_{50} is** greater than 5,000 mg/kg in male and female rats.

3. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1200): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was above the targeted upper limit of 70% during the study due to exceptionally high seasonal humidity. Portable dehumidifiers were used to lower the humidity levels during this time."
- The laboratory reported the following protocol amendments: (1) the composition of the product was changed to read: Active: 12% L-Lactic Acid and Other: 88% Inert Ingredients; (2) the pH of the product was changed to read 2.8; and (3) the study director was changed in order to finalize the report in a timely manner.
- The guidelines state that body weight changes should be calculated and recorded when survival exceeds one day. Individual body weights of test animals were recorded; however, body weight changes were not reported.

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
5,000	0 / 5	0 / 5	0 / 10

Observations:
All animals survived exposure to the test substance and gained body weight during the study. Other than the dermal irritation noted at six dose sites between Days 1 and 7, there were no other clinical findings recorded for any animal over the course of the study.

Gross Necropsy Findings:
No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)
(NOSE-ONLY EXPOSURE)

Product Manager: 34
MRID No.: 476814-05

Reviewer: CSC and Earl Goad (CTT)
Completion Date: November 25, 2008
Study No.: 25510

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: GLYCO-SAN
Batch #: 8163 / Water-clear liquid

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (10-11 weeks old)
Source: Ace Animals, Inc., Boyertown, PA
Weight: Males: 312-360 grams; Females: 218-256 grams; at experimental start
Housing: Temperature Range: 21-24°C
Humidity Range: 63-79%
Photoperiod: 12-hour light/dark cycle
Acclimation: 20 days

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.02	36.81

Summary:

1. **LC_{50} (mg/L) 4-hr exposure**: >2.02 mg/L in male and female rats

2. **The estimated 4-hr acute inhalation LC_{50} of GLYCO-SAN is greater than 2.02 mg/L in male and female rats.**

3. **Average MMAD**: 2.5 µm

4. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1300): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was above the targeted limit of 70% during the study due to exceptionally high, seasonal humidity. Portable dehumidifiers were used to lower the humidity levels during this time."

- The laboratory reported the following protocol amendments: (1) the composition of the product was changed to read: Active: 12% L-Lactic Acid and Other: 88% Inert Ingredients; (2) the pH of the product was changed to read 2.8; and (3) the study director was changed in order to finalize the report in a timely manner.
- The guidelines state that the animals should be acclimated and heat stressed minimized. The laboratory does not indicate whether animals were acclimated to exposure conditions and heat stress minimized.
- The guidelines state that three to four measurements should be taken during exposure if chamber concentrations and MMAD values taken during the trial run measurements are not within 10 percent of each other. Chamber concentrations ranged from 1.51 to 3.18 mg/L. MMAD values were reported for one of the three trial runs. The laboratory conducted only two sample measurements during the test, instead of the three to four measurements recommended in the guidelines.

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number Dead / Number Tested		
	Males	Females	Combined
2.02	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exp. Conc. (mg/L)	Sample	MMAD (µm)	GSD (µm)	Cumulative % of Particles < Effective Cutoff Diameter (µm)[1]								
				0.0	0.4	0.7	1.1	2.1	3.3	4.7	5.8	9.0
2.02	1	2.5	1.83	0.0	0.6	1.8	6.6	42.6	70.4	84.3	89.4	95.2
	2	2.5	1.85	0.0	0.3	1.3	7.0	39.7	68.1	82.8	88.0	95.0

[1]Percent of particles smaller than corresponding effective cutoff diameter

Chamber Environment During Exposure

Exposure Level (mg/L)	2.02
Chamber Volume (L)	6.7
Average Total Airflow Volume (L/min)	25.7
Air Changes Per Hour	230
Mean Oxygen Content (%)	not reported
Temperature Range (°C)	21-23
Relative Humidity Range (%)	68-72

Clinical Observations:
All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. Following exposure and throughout the 14-day observation period, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

Gross Necropsy Findings:
No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 34
MRID No.: 476814-06

Reviewer: CSC and Earl Goad (CTT)
Completion Date: November 25, 2008
Study No.: 25511

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: GLYCO-SAN
Batch #: 8163 / Water-clear liquid

Dosage: 0.1 mL (instilled as received)

Species: 3 Rabbits; New Zealand, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (specific age not provided)
Weight: Information not provided (and not required)
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature Range: 19-21°C
Humidity Range: 59-83%
Photoperiod: 12-hour light/dark cycle
Acclimation: 14 days

Summary:

1. Toxicity Category: **III (moderately irritating)**

2. Classification: **Acceptable**

Procedure (Deviations from 870.2400): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was above the targeted upper limit of 70% during the study due to exceptionally high, seasonal humidity. Portable dehumidifiers were used to lower the humidity levels during this time."
- The laboratory reported the following protocol amendments: (1) the composition of the product was changed to read: Active: 12% L-Lactic Acid and Other: 88% Inert Ingredients; (2) the pH of the product was changed to read 2.8; and (3) the study director was changed in order to finalize the report in a timely manner.
- The guidelines recommend that testing be performed using healthy adult albino rabbits. Testing was performed using young adult albino rabbits (specific age not provided).

Results:
All animals appeared active and healthy during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis, and "positive" conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by Day 7 (study termination). The Maximum Mean Total Score of GLYCO-SAN is 20.0. Under the conditions of this study, GLYCO-SAN is classified as moderately irritating to the eye.

Incidence of Irritation

Time Post Instillation	No. of Animals Testing "Positive" / No. of Animals Tested			Severity – Mean Score
	Corneal Opacity	Iritis	Conjunctivae	
1 hour	3 / 3	3 / 3	3 / 3	20.0
24 hours	2 / 3	3 / 3	3 / 3	19.7
48 hours	2 / 3	3 / 3	3 / 3	18.3
72 hours	0 / 3	2 / 3	2 / 3	10.7
Day 4	0 / 3	2 / 3	0 / 3	9.3
Day 7	0 / 3	0 / 3	0 / 3	0.0

Individual Scores for Ocular Irritation

Observations	Rabbit No. 3401 (Female)					
	Time After Treatment					
	Hours				Days	
	1	24	48	72	4	7
I. Corneal Opacity	1	0[1]	0	0	0	0
II. Iris	1	1	1	1	1	0
III. Conjunctivae						
A. Redness	2	2	2	2	1	0
B. Chemosis	1	1	1	1	1	0
C. Discharge	2	2	2	1	1	0
Observations	**Rabbit No. 3402 (Female)**					
	Time After Treatment					
	Hours				**Days**	
	1	**24**	**48**	**72**	**4**	**7**
I. Corneal Opacity	1	1[1]	1	0[1]	0	0
II. Iris	1	1	1	1	1	0
III. Conjunctivae						
A. Redness	2	2	2	2	1	0
B. Chemosis	1	2	1	1	1	0
C. Discharge	2	2	2	1	1	0
Observations	**Rabbit No. 3403 (Female)**					
	Time After Treatment					
	Hours				**Days**	
	1	**24**	**48**	**72**	**4**	**7**
I. Corneal Opacity	1	1[1]	1	0[1]	0	0
II. Iris	1	1	1	0	0	0
III. Conjunctivae						
A. Redness	2	2	2	1	1	0
B. Chemosis	1	2	1	1	1	0
C. Discharge	2	2	2	1	1	0

[1]2% ophthalmic fluorescein sodium used to evaluate the extent or verify the absence of corneal opacity

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 34
MRID No.: 476814-07

Reviewer: CSC and Earl Goad (CTT)
Completion Date: November 25, 2008
Study No.: 25512

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: GLYCO-SAN
Batch #: 8163 / Water-clear liquid

Dosage: 0.5 mL (applied as received)

Species: 3 Rabbits; New Zealand, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (specific age not provided)
Weight: Information not provided (and not required)
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature Range: 19-21°C
Humidity Range: 61-80%
Photoperiod: 12-hour light/dark cycle
Acclimation: 6 days

Summary:

1. Toxicity Category: **IV (slightly irritating)**

2. Classification: **Acceptable**

Procedure (Deviations from 870.2500): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was above the targeted upper limit of 70% during the study due to exceptionally high, seasonal humidity. Portable dehumidifiers were used to lower the humidity levels during this time."
- The laboratory reported the following protocol amendments: (1) the composition of the product was changed to read: Active: 12% L-Lactic Acid and Other: 88% Inert Ingredients; (2) the pH of the product was changed to read 2.8; and (3) the study director was changed in order to finalize the report in a timely manner.
- The guidelines recommend that testing be performed using healthy adult animals. Testing was performed using young adult animals (specific age not provided).

Results:
All animals appeared active and healthy during the study. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

From 30-60 minutes to 24 hours after patch removal, all three treated sites exhibited well-defined erythema and very slight to slight edema. The overall incidence and severity of irritation decreased thereafter. All animals were free of dermal irritation by 72 hours.

The Primary Dermal Irritation Index for GLYCO-SAN was calculated to be 1.9. Under the conditions of this study, GLYCO-SAN is classified as slightly irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
30-60 minutes	3 / 3	3 / 3
24 hours	3 / 3	3 / 3
48 hours	3 / 3	0 / 3
72 hours	0 / 3	0 / 3

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema			
		Time After Patch Removal			
		30-60 minutes	24 hours	48 hours	72 hours
3501	**F**	2 / 1	2 / 1	1 / 0	0 / 0
3502	**F**	2 / 2	2 / 2	1 / 0	0 / 0
3503	**F**	2 / 1	2 / 1	1 / 0	0 / 0
Total		6 / 4	6 / 4	3 / 0	0 / 0
Mean		2.0 / 1.3	2.0 / 1.3	1.0 / 0	0 / 0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal			
	30-60 minutes	24 hours	48 hours	72 hours
Erythema	2.0	2.0	1.0	0
Edema	1.3	1.3	0	0
TOTAL (PDI)[2]	3.3	3.3	1.0	0

[1]Average values for three rabbits
[2]PDI = Average Erythema + Average Edema

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(BUEHLER METHOD)

Product Manager: 34
MRID No.: 476814-08

Reviewer: CSC and Earl Goad (CTT)
Completion Date: November 25, 2008
Study No.: 25513

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), with the following exception: "The stability, uniformity of mixture, and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Eurofins | Product Safety Laboratories historical positive control study were not determined."

Test Material: GLYCO-SAN
Batch #: 8163 / Water-clear liquid

Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)
Historical data – Completed on July 10, 2008

Species:	49 Guinea pigs; Hartley, albino	
Sex:	Range-Finding:	9 Males
	Test Group:	20 Males
	Naïve Control Group – Challenge:	10 Males
	Naïve Control Group – Rechallenge:	10 Males
Age:	Young adult (specific age not reported)	
Weight:	Test and Naïve Control Groups: 358-564 grams at experimental start	
Source:	Elm Hill Breeding Labs, Chelmsford, MA	
Housing:	Temperature Range:	18-21°C
	Humidity Range:	60-79%
	Photoperiod:	12-hour light/dark cycle
Acclimation:	6-18 days	

Method: Buehler Method

Summary:

1. Based on these findings and on the evaluation system used, GLYCO-SAN **is not considered to be a contact derma sensitizer.**

2. Classification: **Acceptable**

Procedure (Deviations from 870.2600): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was above the targeted upper limit of 70% during the study due to exceptionally high, seasonal humidity. Portable dehumidifiers were used to lower the humidity levels during this time."
- The laboratory reported the following protocol amendments: (1) the composition of the product was changed to read: Active: 12% L-Lactic Acid and Other: 88% Inert Ingredients; (2) the pH of the product was changed to read 2.8; and (3) the study director was changed in order to finalize the report in a timely manner.
- The laboratory reported the following deviations:
    1. Due to a technical error, HNIC animal #3680 was wrapped for 21 hours instead of 6 hours as required by the protocol. The animals were challenged at a concentration of 50%, which may have been too low. Therefore, an additional HNIC at 100% on 4 animals was conducted.
    2. Due to a technical error (deviation #1), the test animals were challenged at 50% instead of 100% as indicated by the HNIC of 100%. The study test animals were rechallenged with the test substance at 100%. Since the test substance was found to be a non-sensitizer at challenge and rechallenge, this deviation does not adversely affect the outcome of this study.
- The guidelines state that the individual body weights of the animals at the start of the test and at the conclusion of the test should be reported. The laboratory did not provide the body weights at the conclusion of the test for the animals used for the naïve control group (re-challenge).
- The guidelines state that, as a minimum, the erythema and edema must be graded. The laboratory only graded erythema.

Procedure:
Preliminary Irritation Testing: A group of animals was used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was applied neat (100%) and also diluted with distilled water to yield w/w concentrations of 75%, 50%, 25%, and 12%. Each concentration was applied (0.4 mL) to a test site using an occlusive 25 mm Hill Top Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 or 21 hours of exposure, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 hours after application, each site was evaluated for local reactions (erythema) according to the scoring system provided in the laboratory report.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. The HNIC selected for the challenge phase was a 50% w/w mixture in distilled water.

A second HNIC was conducted to select an appropriate concentration to rechallenge using the same procedure as above and test substance at 100% and also diluted with

distilled water to yield w/w concentrations of 75%, 50%, and 25%. The HNIC selected for the re-challenge phase was 100%.

Preparation and Selection of Animals: On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy, naïve animals (not previously tested) without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

Induction Phase: Once each week for three weeks, four-tenths of a milliliter of the undiluted test substance was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Twenty-seven days after the first induction dose, four tenths of a milliliter of a 50% w/w mixture of the test substance in distilled water (HNIC) was applied to a naïve site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the scoring system.

In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the "naïve control" group.

Re-challenge Phase: Due to a technical error, HNIC animal #3680 was wrapped for 21 hours instead of 6 hours as required by the protocol. As a result, the animals were challenged at a concentration of 50%, which may have been too low. Therefore, an additional HNIC at 100% on 4 animals was conducted, which indicated the need to re-challenge the test group at 100%. Seven days after the primary challenge, the re-challenge was conducted using 100% of the test substance. Four-tenths of a milliliter of the undiluted test substance was applied to a naïve site on the right side of each animal as a re-challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the re-challenge application according to the scoring system. An additional group of ten guinea pigs was placed on test at re-challenge to serve as a naïve control group and was treated with the test substance as described above.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation, EPSL Study #25331, was performed by Eurofins | Product Safety Laboratories. Testing was completed on July 10, 2008. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described above.

Results:
Induction Phase:
Test Animals (undiluted test substance): Very faint erythema (0.5) was noted for most test sites during the induction phase.

Historical Positive Control Animals (100% of HCA): Very faint to faint erythema (0.5-1) was noted for all positive control test sites during the induction phase.

Challenge Phase:
Test Animals (50% w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was noted for four of twenty test sites 24 hours after challenge. Similar irritation persisted at one site at 48 hours.

Naïve Control Animals (50% w/w mixture of the test substance in distilled water): There was no dermal irritation noted for any of the naïve control sites 24 and 48 hours after challenge.

Historical Positive Control Animals (100% of HCA): Four of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge. Similar indications persisted at two sites through 48 hours.

Historical Naïve Control Animals (100% of HCA): Very faint erythema (0.5) was noted for one naïve control site 24 hours after challenge. Irritation cleared from the affected site by 48 hours.

Re-challenge Phase:
Test Animals (undiluted test substance): Very faint erythema (0.5) was noted for eight of twenty test sites 24 hours after re-challenge. Similar irritation persisted at two of these sites 48 hours after re-challenge.

Naïve Control Animals (undiluted test substance): Very faint erythema (0.5) was noted for five of ten naïve control sites 24 hours following the re-challenge application. Similar irritation persisted at one site at 48 hours.

Sensitization Response Indices (Erythema)

	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals: Challenge	0 / 20	0 / 20	0.10	0.03
Naïve Control Animals: Challenge	0 / 10	0 / 10	0.00	0.00
Test Animals:Re-challenge	0 / 20	0 / 20	0.20	0.05
Naïve Control Animals: Re-challenge	0 / 10	0 / 10	0.25	0.05

[1]Animals with scores greater than 0.5
[2]Sum of the erythema scores divided by the number of animals evaluated

Test Animal Group Skin Reaction Scores

Treatment Phase	Induction						Challenge		Re-challenge	
	1		2		3					
Concentration	Undiluted		Undiluted		Undiluted		50%[1]		Undiluted[2]	
Hours[3]	24	48	24	48	24	48	24	48	24[4]	48
Animal No. / Sex										
Test Group										
3601 / M	0	0	0.5	0.5	0.5	0.5	0.5	0	0.5	0
3602 / M	0	0	0	0	0.5	0.5	0	0	0	0
3603 / M	0	0	0.5	0.5	0.5	0.5	0	0	0	0
3604 / M	0	0	0.5	0.5	0.5	0.5	0	0	0	0
3605 / M	0	0	0	0	0	0	0	0	0.5	0
3606 / M	0	0	0	0	0	0	0.5	0.5	0	0
3607 / M	0	0	0	0	0.5	0.5	0	0	0	0
3608 / M	0	0	0.5	0	0	0	0	0	0	0
3609 / M	0	0	0	0	0	0	0	0	0.5	0.5
3610 / M	0	0	0	0	0	0	0	0	0	0
3611 / M	0	0	0.5	0	0.5	0	0	0	0.5	0
3612 / M	0	0	0	0	0	0	0	0	0.5	0
3613 / M	0	0	0.5	0.5	0.5	0.5	0.5	0	0.5	0.5
3614 / M	0	0	0.5	0.5	0.5	0.5	0	0	0.5	0
3615 / M	0	0	0	0.5	0	0	0	0	0	0
3616 / M	0	0	0.5	0	0.5	0	0	0	0	0
3617 / M	0	0	0.5	0	0.5	0.5	0	0	0	0
3618 / M	0	0	0.5	0.5	0.5	0.5	0	0	0	0
3619 / M	0	0	0	0	0	0	0.5	0	0	0
3620 / M	0	0	0	0	0	0	0	0	0.5	0

[1]Four-tenths of a milliliter of a 50% w/w mixture of the test substance in distilled water was applied.
[2]Four-tenths of a milliliter of the undiluted test substance was applied.
[3]Hours after induction or challenge.
[4]All dose sites relocated due to re-challenge.

Challenge / Re-challenge Control Group Skin Reaction Scores

Treatment Phase	Induction						Challenge		Re-challenge	
	1		2		3					
Concentration	Undiluted		Undiluted		Undiluted		50%[1]		Undiluted[2]	
Hours[3]	24	48	24	48	24	48	24	48	24	48
Animal No. / Sex										
Naïve Group										
3621 / M	--	--	--	--	--	--	0	0	--	--
3622 / M	--	--	--	--	--	--	0	0	--	--
3623 / M	--	--	--	--	--	--	0	0	--	--
3624 / M	--	--	--	--	--	--	0	0	--	--
3625 / M	--	--	--	--	--	--	0	0	--	--
3626 / M	--	--	--	--	--	--	0	0	--	--
3627 / M	--	--	--	--	--	--	0	0	--	--
3628 / M	--	--	--	--	--	--	0	0	--	--
3629 / M	--	--	--	--	--	--	0	0	--	--
3630 / M	--	--	--	--	--	--	0	0	--	--
Naïve Group										
3631 / M	--	--	--	--	--	--	--	--	0.5	0
3632 / M	--	--	--	--	--	--	--	--	0	0
3633 / M	--	--	--	--	--	--	--	--	0	0
3634 / M	--	--	--	--	--	--	--	--	0	0
3635 / M	--	--	--	--	--	--	--	--	0.5	0.5
3636 / M	--	--	--	--	--	--	--	--	0.5	0
3637 / M	--	--	--	--	--	--	--	--	0	0
3638 / M	--	--	--	--	--	--	--	--	0	0
3639 / M	--	--	--	--	--	--	--	--	0.5	0
3640 / M	--	--	--	--	--	--	--	--	0.5	0

[1]Four-tenths of a milliliter of a 50% w/w mixture of the test substance in distilled water was applied.
[2]Four-tenths of a milliliter of the undiluted test substance was applied.
[3]Hours after challenge.