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

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

Thursday, July 24, 2008

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. No.: 777-RNI
Product Name: Gattuso GP
DP Barcode: D354309

Earl Goad 7/24/08

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

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

Chris Jiang for KPH
7/24/08

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

TO: Adam Heyward PM#34/Stacey Grigsby
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
Morris Corporate Center IV
399 Interpace Parkway
Parsippany, NJ 07054

PRODUCT FORMULATION FROM LABEL:

PC Codes	Active Ingredient(s):	% by wt.
021801	Citric Acid	3.50
214900	Formic Acid	2.85
	Other Ingredient(s):	93.65
	Total:	100.00

I) BACKGROUND:

The registrant is submitting six original studies to satisfy the Acute Toxicity data requirements for registration of EPA file symbol: 777-RNI Gattuso GP.

This is an end use product (EUP) to be used as a cleaner and disinfectant for residential as well as commercial facilities. It is meant to be used for non-food contact on hard non-porous surfaces. The registrant plans to market in two use configurations:

- Trigger spray bottle.
- Pourable container for use as refill to sprayer.

This product is intended to be sprayed on and subsequently wiped off following normal label directions.

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 Acute Oral Toxicity study was performed using the Up Down Procedure, resulting in an acceptable toxicity category IV.

B. The Acute Dermal Toxicity study also was of low toxicity. This limit dose study is also acceptable category IV

C. The Acute Inhalation Toxicity study, though only performed at one dose level, is also acceptable category IV.

D. The Primary Eye Irritation was category II (severely irritating to the eye, with corneal involvement – clearing within 17 days) is acceptable. The pH of this product is less than 2.5.

E. The Primary Dermal Irritation is acceptable and graded as category IV with a slight degree of irritancy observed in only one of three rabbits used.

F. The Skin Sensitization Study was accepted as a non-sensitizer. There was so little dermal irritancy of the test material, even when 100% product was used, no irritancy whatsoever was observed with induction or challenge exposures.

III) The acute toxicity profile for File Symbol 777-RNI Gattuso GP is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	474557-12	IV	Acceptable
Acute Dermal Toxicity	474557-13	IV	Acceptable
Acute Inhalation Toxicity	474557-14	IV	Acceptable
Primary Eye Irritation	474557-15	II	Acceptable
Primary Skin Irritation	474557-16	IV	Acceptable
Dermal Sensitization	474557-17	Non-Sensitizer	Acceptable

IV) LABELING:

Keep Out of Reach of Children

A. The signal word for EPA Reg. 777-RNI is **WARNING** based on the category II for Eye Irritation.

B. Precautionary labeling:

Hazards to Humans and Domestic Animals:

Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear protective eyewear (goggles or face shield). Avoid contact with skin or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

Note to PM and or registrant: additional precautionary (for inhalation) and first aid labeling (skin and oral exposure) provided on the draft label is optional and acceptable since it doesn't represent significant over-labeling for study data provided. What is represented here is what is required based on the results of the studies.

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.

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 t-800-222- t222.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)
(UP AND DOWN PROCEDURE)

Product Manager: 34
MRID No.: 474557-12

Reviewer: Earl Goad(CTT)
Completion Date: October 26, 2007
Study No.: 11186-7

Testing Laboratory: Stillmeadow Inc., Sugar Land Texas
Author: Janice O. Kuhn, Ph.D.,DABT

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: Gattuso GP, Formula 1262-144A
Batch #: 1333-023 / Clear water white liquid

Dosage: Limit Test: 5,000 mg/kg (administered as received)
Animals Dosed Sequentially at 5,000 mg/kg

Species: Albino Rats; Sprague-Dawley
Sex: 3 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8 weeks old)
Weight: 192-208 grams at experimental start
Source: Texas Animal Specialties, Humble Texas
Housing: Temperature Range: 20-25°C
Humidity Range: 46-100%
Photoperiod: 12-hour light/dark cycle
Acclimation: 5 days(Quarantine): overnight fast before dosing

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): No procedural deviations or guideline deficiencies noted except humidity range was higher than recommended 30 – 70%.

Results:

Dosing and Weights

Animal No.	Dose Date	Dose Level (mg/kg)	Day 0- (g)	Day 7- (g)	Day 14–(g)
61	25-Sept 07	5,000	179	216	231
62	27-Sept 07	5,000	t92	200	203
63	27-Sept 07	5,000	177	204	237

Observations:
5,000 mg/kg (1 animal-limit dose) and 5,000 mg/kg Dose Level (2 animals): All animals survived test substance administration, appeared active and healthy, and gained body weight over the course of the study. There were no signs of gross toxicity, adverse clinical signs, or abnormal behavior. Animal number 62 exhibited a respiratory chirp on Day 14.

Gross Necropsy Findings:
Following necropsy on day 14, animal number 62 had pale lungs. No other gross abnormalities were noted for any of the animals.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager:34
MRID No.: 474557-13

Reviewer: Earl Goad (CTT)
Completion Date: October 26, 2007
Study No.: 11187-07

Testing Laboratory: Stillmeadow Inc., Sugar Land Texas
Author: Janice O. Kuhn, Ph.D., DABT

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: Gattuso GP, Formula 1262-144A
Batch #: 1333-023 / Clear water white liquid

Dosage: 5,050 mg/kg (applied as received)

Species: Albino Rabbit; New Zealand White
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (10.2 weeks on day of dosing)
Weight: Males: 2.400-2.750 kg; Females: 2.050-2.550kg at experimental start
Source: Nichols Rabbitry Inc.; Lumberton, Texas
Housing: Temperature Range: 19-24°C
Humidity Range: 41-84%
Photoperiod: 12-hour light/dark cycle
Acclimation: 5 days(Quarantine)

Summary:

1. **Acute Dermal LD_{50} (mg/kg)**: Male and Female Rabbits: >5,050 mg/kg

2. **The estimated acute dermal LD_{50} is** greater than 5,050 mg/kg in male and female rabbits

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.

- Temperature and relative humidity were outside of the protocol range, reported not to have effect on study outcome.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is dosed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female groups simultaneously.

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
5,050	0 / 5	0 / 5	0 / 10

Animal Number	Body Weights (kg)		
	Day 0	Day 7	Day 14 (Final)
1876-M	2.750	2.825	2.800
1878-M	2.750	2.875	2.800
1880-M	2.450	2.550	2.750
1882-M	2.500	2.800	2.850
1884-M	2.400	2.575	2.650
1851-F	2.350	2.425	2.600
1855-F	2.500	2.775	2.800
1859-F	2.300	2.500	2.600
1861-F	2.550	2.700	2.950
1867-F	2.050	2.175	2.300

Observations:
All animals survived the study. All animals appeared normal for the duration of the study. Body weight gain was unaffected by the test exposure except for two males (#1876-M and #1878-M) lost weight during the last week of the study. There were no signs of dermal irritation noted during the study.

Gross Necropsy Findings:
Upon termination of study (Day 14) all animals were euthanized. On necropsy no gross abnormalities was observed.

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

Product Manager:34
MRID No.: 474557-14

Reviewer: Earl Goad (CTT)
Completion Date: December 7, 2007
Study No.: 11188-07

Testing Laboratory: Stillmeadow, Inc., Sugar Land Texas
Author: Janice O. Kuhn, Ph.D., DABT

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: Gattuso GP, Formula 1262-144A
Batch #: 1333-023 / Clear water white liquid

Species: 10 Rats; Sprague-Dawley
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult: (9.3 weeks old)
Weight: Males: 319-338 grams; Females: 196-237 grams; at experimental start
Source: Texas Animal Specialties, Humble Texas
Housing: Temperature Range: 20-25°C
Humidity Range: 46-100%
Photoperiod: 12-hour light/dark cycle
Acclimation: 5 days (Quarantine)

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.23	24.9

Summary:

1. **LC_{50} (mg/L) 4-hr exposure**: > 2.23 mg/L in rats

2. **Average MMAD**: 2.2 µm at the 2.23 mg/L exposure level

3. **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 only protocol deviation recorded was temperature and humidity being outside of protocol range, reported not to affect study outcome.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is exposed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female groups simultaneously.
- 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 concentration values and MMAD values taken during the trial run measurements are not within t0 percent of each other. The laboratory reported 3 trial runs with chamber concentration values ranging from t.75-2.29 mg/L. In addition, only one MMAD value were reported during the trial run. The laboratory conducted only two sample measurements during the test, instead of the three to four measurements recommended in the guidelines.
- The guidelines state that changes in (body) weight should be calculated and recorded when survival exceeds 1 day. Individual body weights of test animals were recorded; however, changes in body weights were not reported.

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number Dead / Number Tested		
	Males	Females	Combined
2.23	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	0.3	0.5	0.9	1.7	2.6	4.3	t0.7	17.9
2.23	1(2hr)	1.9	7.4	0.0	25	25	31.3	37.5	50.0	68.75	87.5	100
	2(3hr)	2.4	4.5	0.0	0.0	6.5	9.7	35.5	67.7	74.2	90.3	93.6

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

Chamber Environment During Exposure

Exposure Level (mg/L)	2.23
Chamber Volume (L)	500
Average Total Airflow (Lpm)[1]	184
Number of Air Changes Per Hour	22.1
Mean Oxygen Content (%)	not reported
Mean Temperature (°C)	19.7-20.4
Mean Relative Humidity (%)	71.2-71.6

[1]Total air = compressed air + diluent air

Animal Number	Body Weights (g)		
	Day 0	Day 7	Day 14 (Final)
111-M	323	333	348
112-M	338	350	373
113-M	333	351	369
114-M	319	325	360
115-M	332	345	365
116-F	237	252	270
117-F	210	221	236
118-F	223	239	260
119-F	207	216	231
120-F	196	211	223

Clinical Observations:
After inhalation exposure at 2.23 mg/L: All animals survived the inhalation exposure until the study was terminated at day 14. Both males and females exhibited very slight to slight piloerection and decrease in activity observed within a half hour, two hours on to 24 hours after the end of exposure. All rats were asymptomatic at Day 2 and gained body weight over the 14-day observation period.

Gross Necropsy Findings:
The study was terminated on Day 14. Necropsy revealed no observable abnormalities.

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager:34
MRID No.: 474557-t5

Reviewer: Earl Goad (CTT)
Completion Date: November 7, 2007
Study No.: 11189-07

Testing Laboratory: Stillmeadow, Inc., Sugar Land Texas
Author: Janice O. Kuhn, Ph.D., DABT

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: Gattuso GP, Formula 1262-144A
Batch #: 1333-023 / Clear water white liquid

Dosage: 0.1 mL (instilled as received)

Species: 3 Rabbits; New Zealand, albino
Sex: 1 Male and 2 Females. Females were nulliparous and non-pregnant.
Age: Young adult (15 -17 wk)
Body Weight: Initial: Male: 3.600 kg; Females: 2.000-2.525 kg
Final: Male: 3.800 kg; Females: 2.200-2.900 kg
Source: Nichols Rabbitry Inc., Lumberton, Texas
Housing: Temperature Range: 19-25°C
Humidity Range: 41-98 %
Photoperiod: 12-hour light/dark cycle
Acclimation: 5 Days Quarantine

Summary:

1. **Toxicity Category**: II (Severely Irritating to Eye), corneal involvement and other eye irritation, clearing within 17 days.

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.

- Temperature and humidity readings were outside the protocol range. It was also noted that this did not affect the study outcome.
- There were no observations reports regarding any non-ocular events or symptoms post exposure.

Results:

A pH of 2 was reported for the test material. All treated eyes were washed free of test material for 1 minute with room temperature water after recording the 24 hour observation. The maximum irritation scoring was 57.7 obtained 24 hours after treatment

Within 24 hours after test substance instillation, all three treated eyes exhibited corneal opacity, iritis and "positive" conjunctivitis. The overall severity of irritation decreased until Day 17, all rabbits exhibited no observable irritation.

Severity of Irritation

Time Post Instillation	Mean Score
1 hour	30.7
24 hours	57.7
48 hours	49.0
72 hours	49.0
Day 4	29.3
Day 7	28.0
Day 10	11.3
Day 14	10.0
Day 17	0.0

Incidence of Irritation

Time Post Instillation	No. of Animals Testing "Positive" / No. of Animals Tested		
	Corneal Opacity	Iritis	Conjunctivitis
1 hour	1 / 3	3 / 3	3 / 3
24 hours	3 / 3	3 / 3	3 / 3
48 hours	3 / 3	3 / 3	3 / 3
72 hours	3 / 3	3 / 3	3 / 3
Day 4	2 / 3	2 / 3	3 / 3
Day 7	2 / 3	2 / 3	3 / 3
Day 10	1 / 3	0 / 3	2 / 3
Day 14	1 / 3	0 / 3	2 / 3
Day 17	0 / 3	0 / 3	0 / 3

Individual Scores for Ocular Irritation (all irritation cleared by Day 17)

Observations	Rabbit No. 1807-M (Male)							
	Hours After Treatment				Days After Treatment			
	1	24	48	72	4	7	t0	14[1]
I. Corneal Opacity	1	2	2	2	0	0	0	0
II. Iris	2	1	1	1	0	0	0	0
III. Conjunctivae								
A. Redness	2	2	3	3	1	1	0	0
B. Chemosis	2	3	3	3	1	1	0	0
C. Discharge	2	3	3	3	0	0	0	0
Observations	Rabbit No. 1869-F (Female)							
	Hours After Treatment				Days After Treatment			
	1	24	48	72	4	7	10	14[1]
I. Corneal Opacity	+	1	1	1	1	1	1	1
II. Iris	2	2	1	1	1	1	0	0
III. Conjunctivae								
A. Redness	2	2	2	2	2	2	2	1
B. Chemosis	2	3	3	3	3	3	2	1
C. Discharge	3	3	3	3	3	3	1	1
Observations	Rabbit No. t875-F (Female)							
	Hours After Treatment				Days After Treatment			
	1	24	48	72	4	7	10	14[1]
I. Corneal Opacity	+	2	1	1	1	1	0	0
II. Iris	2	2	1	1	1	1	0	0
III. Conjunctivae								
A. Redness	2	2	3	3	3	3	1	1
B. Chemosis	3	3	3	3	3	2	1	1
C. Discharge	3	3	3	3	3	2	0	0

[1]All Eye Irritation cleared to zero score by Day 17.

DATA REVIEW FOR PRIMARY DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager:34
MRID No.: 474557-16

Reviewer: Earl Goad (CTT)
Completion Date: November 7, 2007
Study No.: 11190-07

Testing Laboratory: Stillmeadow, Inc., Sugar Land Texas
Author: Janice O. Kuhn, Ph.D., DABT

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: Gattuso GP, Formula 1262-144A
Batch #: 1333-023 / Clear water white liquid

Dosage: 0.5 mL (applied as received)

Species: 3 Rabbits; New Zealand, albino
Sex: 3 Males.
Age: Young adult (9.9 weeks)
Body Weight: Initial: Male: 3.200-3.500 kg
Final: Male: 3.300-3.650kg
Source: Nichols Rabbitry Inc., Lumberton, Texas
Housing: Temperature Range: 19-21°C
Humidity Range: 50-76%
Photoperiod: 12-hour light/dark cycle
Acclimation: 5 days (Quarantine)

Summary:

1. **Toxicity Category**: IV (slightly irritating to the skin)

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.

- Humidity was slightly outside protocol, but was reported not to have an affect on study outcome.

Results:
Apart from the skin irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

One hour after patch removal, only one rat exhibited very slight erythema yet no observable edema. This animal showed no remaining irritation after 24 hours. The Primary Dermal Irritation Index for the test substance was calculated as 0.2. [Scores for observations made during the first 30-60 minutes, 24, 48, and 72 hours were used in this

calculation.] Under the conditions of this study, Gattuso is considered only slightly irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
30-60 minutes	1 / 3	0 / 3
24 hours	1 / 3	0 / 3
48 hours	0 / 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	Day 7
1804	M	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
1805	M	1 / 0	1 / 0	0 / 0	0 / 0	0 / 0
1806	M	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Total		1 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Mean[1]		0.3 / 0	0.3 / 0	0 / 0	0 / 0	0 / 0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal				
	30-60 minutes	24 hours	48 hours	72 hours	PDI[2]
Erythema	1	1	0	0	2 / 4 =0.5
Edema	0	0	0	0	0.0
TOTAL					0.5 / 3 = 0.2

[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.: 474557-17

Reviewer: Earl Goad (CTT)
Completion Date: November 7, 2007
Study No.: 11191+-07

Testing Laboratory: Stillmeadow, Inc., Sugar Land Texas
Author: Janice O. Kuhn, Ph.D., DABT

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: Gattuso GP, Formula 1262-144**A**
Batch #: 1333-023 / Clear water white liquid
Administered as 100% for both induction and challenge.

Positive Control Material:
1-Chloro-2,4-Dinitrobenzene, 97%
Induction: 0.4 mL of 1.5% w/v solution in alcohol
Challenge: 0.4 mL 0.5 % w/v solution in alcohol
(Historical positive control test completed on September 21, 2007.)

Species: 34 Guinea pigs; Hartley, albino
Sex: Range-Finding Group: 2 Males and 2 Females
Test Group: 10 Males and 10 Females
Naïve Control Group: 5 Males and 5 females
Age: Young adult: 7 – 8 weeks at start of dosing.
Weight: Males: 340 – 425 g
Females: 332 – 398 g
Source: Charles River Laboratories, Wilmington, MA
Housing: Temperature Range: 18-23°C
Humidity Range: 32-93%
Photoperiod: 12-hour light/dark cycle
Acclimation: 3-13 days

Method: Buehler Method

Summary:

1. **Based on these findings and on the evaluation system used, Gattuso GP is not considered to be a skin sensitizer.**

   Note: Due to the lack of irritancy of the test material no significant skin reactions were observed using a 100% product for induction and challenge treatments.

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.

- Female body weights were outside protocol range.
- Humidity was outside of protocol range.

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%. Each concentration was applied (0.4 mL) to a test site beneath a 4-ply, 2.5 x 2.5 cm surgical gauze patch, and secured with a strip of non-irritating adhesive tape, then covered with clear polyethylene film and securely taped. Each animal was restrained for 6 hours. Upon removal from restrainers, wrappings, patches were removed and animals returned to cages for observation. At 24 and 48 hours after application, each site was evaluated for local reactions (erythema) according to a scoring system provided in the laboratory report. Since due to low irritancy of this material a slight response (0.5) was only seen in one female at 24 hours with 100%. From these results, the HNIC (the highest concentration that produced responses) defaulted to 100% test material. The substance being tested was used neat (100%) for both induction and challenge doses.

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 animals 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 the same method as for preliminary irritation testing. After the 6-hour exposure period, the wrapping and gauze 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 according to the scoring system.

Challenge Phase: Twenty-seven days after the first induction dose, four tenths of a milliliter of the test material was applied neat(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.

Historical Positive Control: The procedures used in this study were validated using 1-Chloro-2, 4-Dinitrobenzene as a positive control substance. The cpositive control study was completed on September 21, 2008, which is well within the required 6 months. The test animals used were Hartley strain albino guinea pigs from Charles

River Laboratories, Wilmington. The induction and challenge procedures involving the positive control material are similar to those with the main test.

Results:

Induction Phase:
Test Animals (100% Test Substance): In the range finding study only one of four test animals showed a faint reaction at 24 hour. All of the 20 test animals (10 males and 10 females) showed no reaction whatsoever. This includes inductions 1 – 3 at all observed times.

Historical Positive Control Animals (DNCB 1.5 % w/v alcohol): Very faint to faint erythema (0.5-1) was noted for all positive control sites during the induction phase.

Challenge Phase:
Test Animals (100% Test Substance): No reaction was observed in any of the 20 test animals after the challenge dose within 24 or 48 hrs after Day 29 challenge.

Naïve Control Animals (100% Test Substance): No irritation was noted on any naïve control sites 24 or 48 hours after challenge. One animal was found dead at time of unwrapping.

Historical Positive Control Animals (DNCB 0.5% w/v alcohol) Nine of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 and 48 hours after challenge. Very faint erythema (0.5) was noted for the remaining test animal.

Historical Naïve Control Animals (DNCB 0.5% w/v alcohol): All ten naïve controls showed no erythema (0) at 24 and 48 hours after challenge.

Weights: All animals showed normal weight gains over the course of the 31 day study.