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

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

January 6, 2010

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. 64240-AL
Product Name: WC Complete
DP Barcode: D371361

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)

TO: Jacqueline McFarlane (acting PM#34)/Stacey Grigsby
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Combat Insect Control Systems

PRODUCT FORMULATION FROM LABEL:

PC Codes	Active Ingredient(s):	% by wt.
128929	Lactic Acid	4.0
	Other Ingredient(s):	96.0
	Total:	100.0

I) BACKGROUND:

The registrant has submitted a complete Acute Toxicity six pack of studies for registration of this product. This product is promoted for use as a ready to use household cleaner/disinfectant/deodorizer for use on hard non-porous surfaces. The product is applied as a spray or foam from a hand pump trigger sprayer.

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, any citations or data waivers meet EPA/OPP criteria, and is responsible for this memorandum.

II) FINDINGS: PSB findings are:

A. Each of the Acute Systemic Toxicity Studies (Oral, Dermal, and Inhalation) are acceptable as reported.

B. The Primary Eye Irritation study results are acceptable. This product is categorized as being mildly irritating to the eye.

C. The Primary Skin Irritation study results are acceptable. This product showed no significant irritation.

D. The Dermal Sensitization study is also acceptable. Employing this laboratory's Buehler method, this product was not found to be a dermal sensitizer. Also, individually none of the ingredients in this product are dermally sensitizing. It is noted that the laboratory begins the study using guinea pigs at 3 – 4 weeks old. The laboratory was called to confirm that this is their normal practice. They have found repeatable historic positive control results over time using animals of this age.

III) The acute toxicity profile for EPA File Symbol: 64240-AL is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	478898-03	IV	Acceptable
Acute Dermal Toxicity	478898-04	IV	Acceptable
Acute Inhalation Toxicity	478898-05	IV	Acceptable
Primary Eye Irritation	478898-06	III	Acceptable
Primary Skin Irritation	478898-07	IV	Acceptable
Dermal Sensitization	478898-08	Non-Sensitizer	Acceptable

IV) LABELING: Below is required labeling. Additional labeling may be added upon agreement of the Regulatory reviewer.

Keep Out of Reach of Children

A. The signal word for EPA File Symbol: 64240-AL is **Caution** based on the category III for Primary Eye Irritation.

B. Precautionary labeling:

Hazards to Humans and Domestic Animals:

Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Or using the toilet.

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 1-800-222-1222.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 34
MRID No.: 478898-03

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 9, 2009
Project #: MB 09-18011.01

Testing Laboratory: MB Research Laboratories, Spinnerstown, PA
Author: Daniel R. Cerven, M.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 was conducted in accordance with the Good Laboratory Practices regulations of the EPA 40 CFR 160 and 792, FDA 21 CFR 58, and as specified in Principles on Good Laboratory Practices, published by the Organization for Economic Cooperation & Development (OECD), 1997, with the following exception: Test article characterization was provided to the study director prior to study initiation but did not include purity, composition, uniformity and stability."

Test Material: WC Complete
Batch #: 3659-155 Lot #1 / Clear liquid

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

Species: 3 Rats; Wistar albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (10-11 weeks old)
Weight: 200-230 grams; pre-test (i.e., Day 0)
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: Temperature controlled
Humidity Range: Information not provided
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: At least 5 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

- No procedure deviations were reported.
- The laboratory reported the following deviation to the Good Laboratory Practices: "Although full test article characterization was not provided to the study director prior to study initiation, information on identity and strength was provided. The information provided should be adequate for the intents and purposes of this study."

- The guidelines state that the temperature of the experimental animal room should be 22±3°C and that the relative humidity of the animal room should be at least 30% and preferably not exceed 70%. The laboratory did not provide specific information on the temperature and humidity ranges. The laboratory only stated that the animal room was "temperature controlled."

Results:

Limit Test

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

S – Survival

Observations:
All animals survived the 5,000 mg/kg oral dose. Body weight changes were normal in two out of three animals. One animal lost weight during the second week of the observation period. A single instance of few feces was noted during the observation period.

Gross Necropsy Findings:
Necropsy results were normal.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 34
MRID No.: 478898-04

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 9, 2009
Project #: MB 09-18011.02

Testing Laboratory: MB Research Laboratories, Spinnerstown, PA
Author: Daniel R. Cerven, M.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 was conducted in accordance with the Good Laboratory Practice regulations of EPA 40 CFR 160 and 792, FDA 21 CFR 58, and as specified in Principles on Good Laboratory Practices, published by the Organization for Economic Cooperation & Development (OECD), 1997, with the following exception: Test article characterization was provided to the study director prior to study initiation but did not include purity, composition, uniformity and stability."

Test Material: WC Complete
Batch #: 3659-155 Lot # 1 / Clear liquid

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

Species: 10 Rabbits; New Zealand white
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Adult (17-23 weeks old)
Weight: Males: 2.6-3.1 kilograms; Females: 2.8-3.2 kilograms; pre-test (i.e., Day 0)
Source: Covance Research Products, Inc., Denver, PA
Housing: Temperature Range: Temperature controlled
Humidity Range: Information not provided
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: At least 5 days

Summary:

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

2. **The estimated acute dermal LD_{50} is** greater than 5,000 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

- The laboratory reported the following deviation to the protocol: "The 4 hour systemic observations for all animals were inadvertently not recorded. This deviation did not appear to have an impact on the outcome of the study since there was no mortality at this time."

- The laboratory reported the following deviation to the Good Laboratory Practices: "Although full test article characterization was not provided to the study director prior to study initiation, information on identity and strength was provided. The information provided should be adequate for the intents and purposes of this study."
- The guidelines state that the temperature of the experimental animal room should be 20±3°C for rabbits and that the relative humidity of the experimental animal room should be 30 to 70%. The laboratory did not provide specific information on the temperature and humidity ranges. The laboratory only stated that the animal room was "temperature controlled."

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 the 5,000 mg/kg dermal application. Dermal responses were absent to very slight at 24 hours and absent on Days 7 and 14. Body weight changes were normal in nine out of ten animals. Weight loss was noted in one out of ten animals between Days 7 and 14. No abnormal physical signs were noted during the observation period.

Gross Necropsy Findings:
Necropsy results were normal.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)
(WHOLE-BODY EXPOSURE)

Product Manager: 34
MRID No.: 478898-05

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 9, 2009
Project #: MB 09-18011.05

Testing Laboratory: MB Research Laboratories, Spinnerstown, PA
Author: Daniel R. Cerven, M.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 Good Laboratory Practice requirements of EPA 40 CFR parts 792 and 160, FDA 21 CFR 58, and as specified in The Testing of Chemicals, published by the Organization for Economic Cooperation & Development (OECD), 1997, with the following exception: Test article characterization was provided to the study director prior to study initiation but did not include purity, composition, uniformity and stability. The in study inspection of the in-life phase of the study was inadvertently not performed."

Test Material: WC Complete
Batch #: 3659-155 Lot # 1 / Clear liquid

Species: 10 Rats; Wistar albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8-9 or 8-11 weeks old) (*see procedure deviations)
Source: Ace Animals, Inc., Boyertown, PA
Weight: Males: 206-222 grams; Females: 202-220 grams; pre-test (i.e., Day 0)
Housing: Temperature Range: Temperature controlled
Humidity Range: Information not provided
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: At least 5 days

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.02	not provided

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 WC Complete is greater than 2.02 mg/L in male and female rats.**

3. **Average MMAD**: 2.45 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 deviations to the protocol:
  - "The date of receipt for the female animals was inadvertently not recorded. The date was either 03/17/09 or 03/31/09. This deviation did not appear to have an impact on the outcome of the study since the animals were within the proper age limits given the weight range."
  - "The in-life phase inspection of the study was inadvertently not performed. This exception did not affect the scientific validity of the study."
- The laboratory reported the following deviation to the Good Laboratory Practices: "Although full test article characterization was not provided to the study director prior to study initiation, information on identity and strength was provided. The information provided should be adequate for the intents and purposes of this study."
- The guidelines state that the temperature of the animal room should be 22±3°C and that the relative humidity should be 30-70 percent. The laboratory did not provide specific information on the temperature and humidity ranges of the animal room. The laboratory only stated that the animal room was "temperature controlled."
- The guidelines state that the animals should be acclimated and heat stressed minimized. The laboratory did not indicate whether animals were acclimated to exposure conditions and heat stress minimized.
- The laboratory did not provide specific information on pre-test trials. Three sample measurements were taken during testing, as recommended by the guidelines.
- The guidelines state that the relative humidity of the exposure chamber should be maintained between 30 and 70%. The lower limit of the humidity range of the exposure chamber was below the recommended limit.
- The guidelines state that body weight changes should be calculated and recorded when survival exceeds 1 day. Individual body weights of test animals were recorded; however, body weight changes were not reported.
- The guidelines state that the equipment for measuring temperature, humidity, particle size, and actual concentration should be reported. The laboratory did not provide this information.
- The guidelines state that the nominal concentration should be reported. The laboratory did not report the nominal concentration.

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]								
				0.4	0.7	1.1	2.1	3.3	4.7	5.8	9.0	10.0
2.02	1	3.4	3.22	2.84	9.08	27.8	37.8	45.6	52.7	58.5	77.1	99.9
	2	2.40	3.38	4.36	11.0	35.3	50.1	62.7	73.4	82.2	88.1	100.
	3	1.55	2.27	4.69	16.2	43.8	60.5	76.8	89.3	100	100	100

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

Chamber Environment During Exposure

Exposure Level (mg/L)	2.02
Chamber Volume (L)	100
Average Total Airflow Volume (Lpm)	36.9
Air Changes Per Hour	10-15
Mean Oxygen Content (%)	not reported
Temperature Range (°C)	22-24
Relative Humidity Range (%)	28-31

Clinical Observations:
All animals survived the 4-hour exposure at 2.02 mg/L. Instances of dyspnea, hunched posture, closed eyes, and coating of the fur with test article were noted during the exposure. One hour post exposure, instances of ataxia and dyspnea were noted. From Day 1 through Day 8, instances of few feces, emaciation, chromorhinorrhea, wetness of the anogenital area, and coating of the fur with test article were noted. All animals appeared normal from Day 9 through Day 14. Body weight changes were normal in eight out of ten animals. Weight loss was noted in 2 male animals during the first week of the observation period. By Day 14, body weight changes were normal.

Gross Necropsy Findings:
Necropsy results were normal.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 34
MRID No.: 478898-06

Reviewer: CSC and Earl Goad (CTT)
Completion Date: February 9, 2009
Project #: MB 08-17788.04

Testing Laboratory: MB Research Laboratories, Spinnerstown, PA
Author: Debra A. Hall, LATG

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 was conducted in accordance with the Good Laboratory Practice requirements of EPA 40 CFR parts 792 and 160, FDA 21 CFR 58 and as specified in Principles on Good Laboratory Practices, published by the Organization for Economic Cooperation & Development (OECD), 1997, with the following exception: Test article characterization was provided to the Study Director prior to study initiation but did not include purity, composition, uniformity and stability."

Test Material: WC Complete
Batch #: 3659-155 Lot # 1 / Clear liquid

Dosage: 0.1 mL (instilled as received)

Species: 3 Rabbits; New Zealand white
Sex: 1 Male and 2 Females.
[The laboratory did not report whether females were nulliparous and non-pregnant.]
Age: Adult (16-17 weeks old)
Weight: 2.3-2.7 kilograms; pre-test
Source: Covance Research Products, Inc., Denver, PA
Housing: Temperature Range: Temperature controlled
Humidity Range: Information not provided
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: At least 5 days

Summary:

1. **Toxicity Category**: III

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.

- No procedure deviations were reported.
- The laboratory reported the following Deviation to the Good Laboratory Practices: "Although full test article characterization was not provided to the study director prior to study initiation, information on identity was provided. The effect of the lack of test article characterization cannot be fully assessed."

- The guidelines state that the ambient temperature and humidity of caging conditions should be reported. The laboratory only stated that the animal room was "temperature controlled."

Results:
No corneal opacity or iritis was noted at any observation period. Conjunctival irritation, noted in three out of three eyes, cleared within 7 days. No abnormal physical signs were noted during the observation period. All animals were free of ocular irritation by Day 7 (study termination).

The Maximum Mean Total Score of WC Complete is 10.7. Under the conditions of this study, WC Complete is classified as mildly 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	0 / 3	0 / 3	2 / 3	8.7
24 hours	0 / 3	0 / 3	3 / 3	10.7
48 hours	0 / 3	0 / 3	1 / 3	5.3
72 hours	0 / 3	0 / 3	0 / 3	2.0
Day 7	0 / 3	0 / 3	0 / 3	0

Individual Scores for Ocular Irritation

Observations	Rabbit No. H2142 (Female)				
	Hours After Treatment				
	1 hour	24 hours	48 hours	72 hours	Day 7
I. Corneal Opacity	0	0	0	0	0
II. Iritis	0	0	0	0	0
III. Conjunctivae					
A. Redness	2	3	1	1	0
B. Chemosis	1	2	1	0	0
C. Discharge	2	2	1	1	0
Observations	**Rabbit No. H2145 (Female)**				
	Hours After Treatment				
	1 hour	**24 hours**	**48 hours**	**72 hours**	**Day 7**
I. Corneal Opacity	0	0	0	0	0
II. Iritis	0	0	0	0	0
III. Conjunctivae					
A. Redness	1	2	1	0	0
B. Chemosis	1	1	0	0	0
C. Discharge	1	1	0	0	0
Observations	**Rabbit No. H2161 (Male)**				
	Hours After Treatment				
	1 hour	**24 hours**	**48 hours**	**72 hours**	**Day 7**
I. Corneal Opacity	0	0	0	0	0
II. Iritis	0	0	0	0	0
III. Conjunctivae					
A. Redness	2	2	2	1	0
B. Chemosis	1	1	1	0	0
C. Discharge	2	2	1	0	0

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 34
MRID No.: 478898-07

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 10, 2009
Project #: MB 09-18011.03

Testing Laboratory: MB Research Laboratories, Spinnerstown, PA
Author: Laura J. DiDonato, 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 was conducted in accordance with the Good Laboratory Practice requirements of EPA, 40 CFR 160 and 792, FDA 21 CFR 58, and the OECD, Principles on Good Laboratory Practices, 1997, with the following exception: Test article characterization was provided to the study director prior to study initiation but did not include purity, composition, uniformity and stability."

Test Material: WC Complete
Batch #: 3659-155 Lot # 1 / Clear liquid

Dosage: 0.5 mL (applied as received)

Species: 3 Rabbits; New Zealand white
Sex: Females
Age: Adult (17 weeks old)
Weight: 2.4-2.9 kilograms; pre-test
Source: Covance Research Products, Inc., Denver, PA
Housing: Temperature Range: Temperature controlled
Humidity Range: Information not provided
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: At least 5 days

Summary:

1. **Toxicity Category**: IV

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.

- No procedure deviations were reported.
- The laboratory reported the following deviation to the Good Laboratory Practices: "Although full test article characterization was not provided to the study director prior to study initiation, information on identity and strength was provided. The information provided should be adequate for the intents and purposes of this study."
- The guidelines state that the ambient temperature and humidity of caging conditions should be reported. The laboratory only stated that the animal room was "temperature controlled."

Results:
No erythema was noted for any animal at 60 minutes following the 4-hour exposure. At 24 hours, 2 out of 3 animals had very slight erythema. No erythema was noted at 48 and 72 hours. One animal had very slight edema at 24 hours. No other edema was observed at any other time point. No abnormal physical signs were noted during the observation period. One animal lost weight. All other body weight changes were normal.

The Primary Dermal Irritation Index for WC Complete was calculated to be 0.25 [Scores for observations made during the first 60 minutes, 24, 48, and 72 hours were used in this calculation.] Under the conditions of this study, WC Complete is classified as not irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
60 minutes	0 / 3	0 / 3
24 hours	2 / 3	1 / 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			
		60 minutes	24 hours	48 hours	72 hours
H2440	**F**	0 / 0	1 / 1	0 / 0	0 / 0
H2441	**F**	0 / 0	1 / 0	0 / 0	0 / 0
H2442	**F**	0 / 0	0 / 0	0 / 0	0 / 0
Total		0 / 0	2 / 1	0 / 0	0 / 0
Mean		0 / 0	0.7 / 0.3	0 / 0	0 / 0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal			
	60 minutes	24 hours	48 hours	72 hours
Erythema	0	0.7	0	0
Edema	0	0.3	0	0
TOTAL (PDI)[2]	0	1.0	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.: 478898-08

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 10, 2009
Project #: MB 09-18011.06

Testing Laboratory: MB Research Laboratories, Spinnerstown, PA
Author: Debra A. Hall, LATG

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 was conducted in accordance with the Good Laboratory Practices regulations of the EPA 40 CFR 160 and 792, FDA 21 CFR 58, and as specified in Principles on Good Laboratory Practices, published by the Organization for Economic Cooperation & Development (OECD), 1997, with the following exception: Test article characterization was provided to the study director prior to study initiation but did not include purity, composition, uniformity or stability."

Test Material: WC Complete
Batch #: 3659-155 Lot # 1 / Clear liquid

Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA), tech. 85%
Historical data – Completed on May 11, 2009

Species: 30 Guinea pigs; Hartley, albino
Sex: Test Group: 10 Males and 10 Females
Naïve Control Group: 5 Males and 5 Females
[The laboratory did not report whether females were nulliparous and non-pregnant.]
Age: ~3 weeks old
Weight: Males: 306-355 grams; Females: 276-344 grams; pre-test
Source: Elm Hill Breeding Labs, Chelmsford, MA
Housing: Temperature Range: Temperature controlled
Humidity Range: Information not provided
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: At least 5 days

Method: Buehler Method

Summary:

1. **Based on these findings and on the evaluation system used, WC Complete is not considered to be a contact 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 deviations to the protocol:
  - "The systemic/mortality observations were not performed/recorded on day 14 of the study. This deviation did not appear to have an impact on the outcome of the study since the animals appeared normal the day before and after."
  - "The dose sites for Induction 3 were not re-clipped the day before dosing. They were however, re-clipped 35 minutes prior to dosing. This deviation did not appear to have an impact on the outcome of the study since the sensitization of the study is based on the challenge phase and not on the Induction 3 phase of the study."
- The laboratory reported the following deviation from the Good Laboratory Practices: "Although full test article characterization was not provided to the study director prior to study initiation, information on identity and strength was provided. The information provided should be adequate for the intents and purposes of this study."
- The guidelines state that young adult guinea pigs be used for the test. The laboratory used animals that were only approximately 3 weeks old on the first exposure to the test substance.
- The guidelines state that the temperature of the experimental animal room should be 20±3°C and that that the relative humidity of the experimental animal room should be 30-70%. The laboratory did not provide specific information on the temperature and humidity ranges. The laboratory only stated that the animal room was "temperature controlled."
- The guidelines state that female animals should be nulliparous and non-pregnant. The laboratory did not indicate whether the female animals used in the study were nulliparous and non-pregnant.
- The guidelines state that, as a minimum, the erythema and edema must be graded. The laboratory only graded erythema.

Procedure:

Site Preparation: The day prior to the first induction application, Site 1 (left shoulder area) of all animals was clipped free of hair with an electric clipper. The clipped area was approximately 5 x 10 cm. Any animal with skin irregularities or irritation was eliminated from the study. The treated sites were re-clipped the day prior to each induction application. Thirteen days after the last induction application, a naïve site (Site 3; left hip area) on each animal was clipped free of hair. Sites 2 and 4 (right shoulder and right hip areas, respectively) were reserved for alternate sites in the event that severe irritation was noted during the induction phase and/or a re-challenge was required.

Induction Phase:
Group 1, 100%: Ten males and ten females in Group 1 were dosed with 0.4 mL of the test article. The dose was applied to the left shoulder area (Site 1) using a 25 mm Hilltop Chamber which is designed to keep the test article on a 25 mm area of the site. The chamber contained a cotton pad used to facilitate contact of the liquid test article with the site. The chamber was covered with a strip of rubber dental dam sufficient to cover the treated sites. The torso was wrapped with non-irritating tape to provide occlusion. After 6 hours, the dams and test article were removed. Any residual test article was cleansed from the sites with distilled water and the sites were dried with soft toweling. This procedure was performed once/week on the same day each week for a three week period, a total of three 6-hour insults.

Group 2: Five males and five females were untreated for the three week induction period and served as the naïve control.

Challenge: Fourteen days after the last induction exposure, animals in Groups 1 and 2 were challenged using the same dosing procedure as in the induction phase. Based on the results of the induction application, 100% was chosen as the highest non-irritating concentration for the challenge. The doses were applied to a naïve site on the lower left dorsal area (Site 3).

Observations:
Induction: The treated sites of animals in Group 1 were examined and scored at 24 and 48 hours following each patch removal for each induction period.

Challenge: The challenge sites of animals in Groups 1 and 2 were scored at 24 and 48 hours following patch removal.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde, tech, 85%, as a positive control substance. The most recent validation, Project # MB 09-17975.06, was performed by MB Research Laboratories. Testing was completed on May 11, 2009. This test was conducted with Hartley albino guinea pigs from Elm Hill Breeding Labs, Inc. following induction and challenge procedures similar to those described above.

Results:
Body weight changes were normal. In Group I, one male was observed with soiling of the anogenital area and one female was observed with diarrhea and soiling of the anogenital area. In Group 2, one male was observed with wetness of the anogenital area. All other animals appeared normal.

Induction Phase:
Test Animals (100% test substance): Erythema was absent to very faint (0-0.5) after the first induction. Erythema was absent to moderate (0-2), with flaking skin, after the second induction. Erythema was very faint to strong (0.5-3), with eschar and flaking skin, after the third induction.

Historical Positive Control Animals (HCA applied undiluted): Erythema was absent to faint (0-1) after the first induction. Erythema was absent to moderate (0-2), with flaking skin, after the second induction. Erythema was absent to strong (0-3), with flaking skin, after the third induction.

Challenge Phase:
Test Animals (100% test substance): Erythema was absent (0) for all twenty test sites 24 hours (and 48 hours) after challenge.

Naïve Control Animals (100% test substance): Erythema was absent (0) for all ten naïve control site 24 hours (and 48 hours) after challenge.

Historical Positive Control Animals (50% mixture of HCA in acetone): Very faint to faint erythema (0.5-1) was noted for six of twenty test sites 24 hours after challenge. Very faint to faint erythema (0.5-1) was noted for seven of twenty test sites 48 hours after challenge.

Historical Naïve Control Animals (50% mixture of HCA in acetone): Very faint erythema (0.5) was noted for one naïve control site 24 hours after challenge. No erythema was noted at any naïve control site 48 hours after challenge.

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	0
Naïve Control Animals – Challenge	0 / 10	0 / 10	0	0

[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	
	1		2		3			
Concentration	100%		100%		100%		100%	
Hours[1]	24	48	24	48	24	48	24	48
Animal No. / Sex								
Test Group								
D3240 / M	0	0	0.5	0,f	1	1	0*	0
D3241 / M	0	0	0	0,f	2	3,e	0	0
D3242 / M	0	0	0	0,f	2	2,e	0*	0
D3243 / M	0.5	0	1	1,f	3	3,e	0	0
D3244 / M	0	0	2	1	1	1,f	0*	0
D3245 / M	0	0	0	0	1	2,e	0*	0
D3246 / M	0	0	0	0	0.5	1,e	0	0
D3247 / M	0	0	2	2	1	1,f	0	0
D3248 / M	0	0	2	1	3	3,e	0	0
D3249 / M	0	0	0	0	2	2,e	0	0
D3250 / F	0	0	1	1,f	2	2,f	0	0
D3251 / F	0	0	0	0	2	3,e	0*	0
D3252 / F	0	0	0	0	2	1	0	0
D3253 / F	0	0	1	1,f	2	2,e	0	0
D3254 / F	0	0	0	0	1	2,e	0	0
D3255 / F	0	0	0	0	1	2,e	0*	0
D3256 / F	0	0	1	0	3	3,e	0	0
D3257 / F	0	0	0	0	2	3,e	0	0
D3258 / F	0	0	2	2,f	2	2,f	0	0
D3259 / F	0	0	1	0	2	3,e	0	0
Naïve Control Group								
D3260 / M	--	--	--	--	--	--	0*	0
D3261 / M	--	--	--	--	--	--	0	0
D3262 / M	--	--	--	--	--	--	0	0
D3263 / M	--	--	--	--	--	--	0	0
D3264 / M	--	--	--	--	--	--	0	0
D3265 / F	--	--	--	--	--	--	0	0
D3266 / F	--	--	--	--	--	--	0	0
D3267 / F	--	--	--	--	--	--	0	0
D3268 / F	--	--	--	--	--	--	0	0
D3269 / F	--	--	--	--	--	--	0	0

[1]Hours after induction or challenge dose

* = reclipped; e = eschar; f = flaking skin