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

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

**Antimicrobials Division (AD)**

Tuesday, April 05, 2011

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No.: 67619-EU
DP Barcode: D385824
Product Name: *Blondie*

From: Ian Blackwell, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Through: Karen Hicks, Team Leader
Chemistry and Toxicology Team
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To: Marshall Swindell, PM 33/ Abigail Downs
Regulatory Management Branch
Antimicrobials Division (7510P)

Applicant: Clorox Professional Products Company

FORMULATION FROM LABEL:

| Active Ingredient(s): | % by wt. |
|---|---|
| Hydrogen peroxide | 1.4 |
| Other Ingredient(s): | 98.6 |
| Total: | 100.00 |

"Have product container or label with you when calling a Poison Control Center or doctor for treatment advice."

"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 DERMAL TOXICITY TESTING (OPPTS 870.1200)

**Product Manager**: 33
**MRID No.**: 483360-04

**Reviewer**: CSC and Ian Blackwell
**Completion Date**: November 4, 2010
**Study No.**: 30106

**Testing Laboratory**: Eurofins | PSL, 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), 1989.

**Test Material**: Blondie F2010.0127, Batch #: 10ACE10 / Clear colorless 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: 236-263 grams; Females: 170-204 grams; at experimental start
**Source**: Ace Animals, Inc., Boyertown, PA
**Housing**: Temperature Range: 20-24°C
Humidity Range: 51-74%
Photoperiod: 12-hour light/12-hour dark cycle
**Acclimation**: 8 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)**: None

**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, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, dermal irritation, 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.

measurements are not within 10 percent of each other. Only one pre-test exposure trial was conducted. The laboratory conducted 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 |
| 2.06 | 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 |
| 2.06 | 1 | 1.9 | 2.13 | 0.0 | 2.5 | 7.9 | 23.8 | 60.6 | 77.3 | 86.6 | 92.4 | 97.1 |
| | 2 | 2.0 | 2.26 | 0.0 | 2.8 | 8.2 | 23.0 | 59.9 | 76.6 | 85.9 | 91.0 | 96.8 |

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

**Chamber Environment During Exposure**

| Exposure Level (mg/L) | 2.06 |
|---|---|
| Chamber Volume (L) | 6.7 |
| Average Total Airflow Volume (Lpm)[1] | 25.7 |
| Air Changes Per Hour | ~230 |
| Mean Oxygen Content (%) | not reported |
| Temperature Range (°C) | 21-23 |
| Relative Humidity Range (%) | 54-62 |

[1]Total air = filter air + compressed mixing air

**Clinical Observations:**
All animals survived exposure to the test atmosphere and gained body weight during the study. Immediately 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 any of the animals when necropsied at the conclusion of the 14-day observation period.

**Incidence of Irritation**

| Time Post Instillation | No. of Animals Testing "Positive" / No. of Animals Tested | | | Severity –Mean Score |
|---|---|---|---|---|
| | Corneal Opacity | Iritis | Conjunctivae | |
| 1 hour | 2 / 3 | 0 / 3 | 3 / 3 | 13.3 |
| 24 hours | 0 / 3 | 0 / 3 | 1 / 3 | 6.7 |
| 48 hours | 0 / 3 | 0 / 3 | 0 / 3 | 1.3 |
| 72 hours | 0 / 3 | 0 / 3 | 0 / 3 | 0.7 |
| Day 4 | 0 / 3 | 0 / 3 | 0 / 3 | 0 |

**Individual Scores for Ocular Irritation**

| Observations | Rabbit No. 3401 (Male) | | | | |
|---|---|---|---|---|---|
| | Time After Treatment | | | | |
| | 1 hour | 24 hours | 48 hours | 72 hours | Day 4 |
| I. Corneal Opacity | 1 | 0[1] | 0 | 0 | 0 |
| II. Iritis | 0 | 0 | 0 | 0 | 0 |
| III. Conjunctivae | | | | | |
| A. Redness | 2 | 2 | 1 | 1 | 0 |
| B. Chemosis | 1 | 1 | 0 | 0 | 0 |
| C. Discharge | 2 | 1 | 0 | 0 | 0 |
| **Observations** | **Rabbit No. 3402 (Male)** | | | | |
| | Time After Treatment | | | | |
| | 1 hour | 24 hours | 48 hours | 72 hours | Day 4 |
| I. Corneal Opacity | 1 | 0[1] | 0 | 0 | 0 |
| II. Iritis | 0 | 0 | 0 | 0 | 0 |
| III. Conjunctivae | | | | | |
| A. Redness | 2 | 1 | 1 | 0 | 0 |
| B. Chemosis | 1 | 1 | 0 | 0 | 0 |
| C. Discharge | 2 | 1 | 0 | 0 | 0 |
| **Observations** | **Rabbit No. 3403 (Female)** | | | | |
| | Time After Treatment | | | | |
| | 1 hour | 24 hours | 48 hours | 72 hours | Day 4 |
| I. Corneal Opacity | 0 | 0[1] | 0 | 0 | 0 |
| II. Iritis | 0 | 0 | 0 | 0 | 0 |
| III. Conjunctivae | | | | | |
| A. Redness | 2 | 1 | 0 | 0 | 0 |
| B. Chemosis | 1 | 1 | 0 | 0 | 0 |
| C. Discharge | 2 | 1 | 0 | 0 | 0 |

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

The Primary Dermal Irritation Index for Blondie F2010.0127 was calculated to be 0.5. [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, Blondie F2010.0127 is classified as slightly irritating to the skin.

**Incidence of Irritation**

| Time after Patch Removal | Erythema | Edema |
|---|---|---|
| **30-60 minutes** | 3 / 3 | 0 / 3 |
| **24 hours** | 2 / 3 | 0 / 3 |
| **48 hours** | 1 / 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** | 1 / 0 | 1 / 0 | 1 / 0 | 0 / 0 |
| **3502** | **F** | 1 / 0 | 1 / 0 | 0 / 0 | 0 / 0 |
| **3503** | **F** | 1 / 0 | 0 / 0 | 0 / 0 | 0 / 0 |
| **Total** | | 3 / 0 | 2 / 0 | 1 / 0 | 0 / 0 |
| **Mean** | | 1.0 / 0.0 | 0.7 / 0.0 | 0.3 / 0.0 | 0 / 0 |

**Summary of Skin Irritation Scores[1]**

| | Time After Patch Removal | | | |
|---|---|---|---|---|
| | 30-60 minutes | 24 hours | 48 hours | 72 hours |
| **Erythema** | 1.0 | 0.7 | 0.3 | 0.0 |
| **Edema** | 0.0 | 0.0 | 0.0 | 0.0 |
| **TOTAL (PDI)[2]** | 1.0 | 0.7 | 0.3 | 0.0 |

[1]Average values for three rabbits

[2]PDI = Average Erythema + Average Edema

- The guidelines state that, as a minimum, the erythema and edema must be graded. The laboratory only graded erythema.
- The guidelines state that the number of animals per cage is to be reported. The laboratory reported that "animals were group housed in suspended stainless steel caging." The laboratory did not report the number of animals per cage.

**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%, 18%, 12%, 6%, and 3%. Each concentration was applied (0.4 mL each) to a test site using an occlusive 25 mm Hill Top Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 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 a 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 3% w/w mixture in distilled water.

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 3% 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.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent

## Test Animal Group Skin Reaction Scores

| Treatment Phase | Induction | | | | | | Challenge | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | | |
| Concentration | 100% | | 100% | | 100% | | 3% | |
| Hours[1] | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 |
| Animal No. / Sex | | | | | | | | |
| **Test Group** | | | | | | | | |
| 3601 / M | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 0.5 | 0 | 0 |
| 3602 / M | 0 | 0.5 | 1 | 1 | 1 | 1 | 0.5 | 0 |
| 3603 / M | 1 | 1 | 1 | 0.5 | 1 | 0.5 | 0.5 | 0.5 |
| 3604 / M | 0.5 | 0.5 | 1 | 1 | 2 | 1 | 0 | 0 |
| 3605 / M | 1 | 0.5 | 1 | 1 | 1 | 0.5 | 0 | 0 |
| 3606 / M | 0 | 0.5 | 1 | 1 | 0.5 | 0 | 0.5 | 0.5 |
| 3607 / M | 0.5 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 3608 / M | 0.5 | 0.5 | 2 | 1 | 1 | 1 | 0.5 | 0 |
| 3609 / M | 0.5 | 1 | 1 | 1 | 0.5 | 0 | 0.5 | 0 |
| 3610 / M | 0.5 | 1 | 1 | 1 | 1 | 0.5 | 0.5 | 0 |
| 3611 / M | 0.5 | 0.5 | 1 | 1 | 0.5 | 0 | 0 | 0 |
| 3612 / M | 1 | 1 | 1 | 1 | 1 | 0.5 | 0.5 | 0.5 |
| 3613 / M | 0 | 1 | 1 | 0.5 | 1 | 1 | 0 | 0 |
| 3614 / M | 0.5 | 1 | 1 | 1 | 1 | 0.5 | 0 | 0 |
| 3615 / M | 1 | 1 | 1 | 1 | 1 | 1 | 0.5 | 0 |
| 3616 / M | 0.5 | 1 | 1 | 1 | 1 | 0.5 | 0 | 0 |
| 3617 / M | 0.5 | 1 | 1 | 0.5 | 1 | 1 | 0.5 | 0 |
| 3618 / M | 1 | 2 | 0.5 | 0.5 | 1 | 0.5 | 0 | 0 |
| 3619 / M | 0.5 | 1 | 1 | 1 | 1 | 1 | 0.5 | 0 |
| 3620 / M | 0.5 | 1 | 1 | 1 | 1 | 1 | 0.5 | 0 |
| **Naïve Control Group** | | | | | | | | |
| 3621 / M | -- | -- | -- | -- | -- | -- | 0 | 0 |
| 3622 / M | -- | -- | -- | -- | -- | -- | 0.5 | 0 |
| 3623 / M | -- | -- | -- | -- | -- | -- | 0 | 0 |
| 3624 / M | -- | -- | -- | -- | -- | -- | 0 | 0 |
| 3625 / M | -- | -- | -- | -- | -- | -- | 0 | 0 |
| 3626 / M | -- | -- | -- | -- | -- | -- | 0.5 | 0 |
| 3627 / M | -- | -- | -- | -- | -- | -- | 0.5 | 0 |
| 3628 / M | -- | -- | -- | -- | -- | -- | 0.5 | 0 |
| 3629 / M | -- | -- | -- | -- | -- | -- | 0.5 | 0.5 |
| 3630 / M | -- | -- | -- | -- | -- | -- | 0 | 0 |

[1]Hours after induction or challenge dose