

DATA EVALUATION RECORD

1. **CHEMICAL:** Pirate® (AC 303,630).
Shaughnessey No. 129093.
2. **TEST MATERIAL:** AC 303,630 technical; CAS No. 122453-73-0;
Lot No. AC-7504-59A; 94.5% active ingredient (ai); a tan powder.
3. **STUDY TYPE:** 141-1. Acute Contact LD₅₀ Test. Species Tested: Honey bee (*Apis mellifera*).
4. **CITATION:** Hoxter, K.A., S.P. Lynn, J.A. Gagne, and V.M. Canez. 1993. Acute Contact Toxicity Study with AC 303,630 in the Honey Bee (*Apis mellifera*). Laboratory Project ID No. 130-153A. Conducted by Wildlife International Ltd., Easton, MD. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 427702-33.
5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Mark A. Mossler*
Date: *7/9/93*
8/4/93
6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *7/9/93*
8/4/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: *Henry T. Craven*
Date: *8/6/93*
7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute contact study. A 48-hour LD₅₀ of 0.12 µg/bee (0.11 µg ai/bee) classifies the test material as highly toxic to honey bees (*Apis mellifera*). The NOEL was 0.02 µg/bee.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

A. **Test Animals:** Five days before test initiation, two frames of bee (*Apis mellifera*) pupae were placed in a life cycle room (34-35°C, 58% relative humidity). The bees were allowed to emerge as adults and were 1 to 5 days old at the initiation of the test. The bees appeared to be in good health at test initiation, at which time they were immobilized with nitrogen and placed in test chambers.

B. **Test System:** Bees were contained in one pint rolled paper containers (87 mm in diameter and 85 mm high). Each container was covered with a plastic petri plate in which a 20-ml glass vial containing 50% sugar/water solution was inserted. The vial opening was covered with cheesecloth to prevent leakage. This food source was available *ad libitum* throughout the test. A sponge inside the chamber was misted daily to increase the humidity.

Bees were kept in a room that was supplied with eight hours of light per day. The temperature was maintained at 22-23°C, while the mean relative humidity was 60%.

C. **Dosage:** Forty-eight-hour acute contact test. Eight treatment levels representing 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 µg/bee were tested in conjunction with a solvent control (2 µl acetone/bee) and a negative control.

The test material was dissolved in acetone and appropriately diluted to prepare each dosing solution. Samples of the dosing solutions were sent to the sponsor for analysis using high performance liquid chromatography.

D. **Design:** Two replicates of 25 bees each were indiscriminately selected for each treatment and control. The bees were again immobilized with nitrogen and laid out on paper. They were then dosed individually on the thorax and/or abdomen with 2 µl of test solution. Negative control bees were handled identically to treated bees, but were not dosed with any material. Solvent control bees received only acetone. Observations of mortality and toxic symptoms were recorded twice on day 0 and once on day 1 and day 2.

E. **Statistics:** An LD_{50} value and the associated 95% confidence interval (C.I.) were determined by binomial probability method.

12. **REPORTED RESULTS:** Cumulative mortalities of the test bees during the 48-hour exposure period are presented in Table 1 (attached). At test termination, mortality in the negative control and solvent control was 12 and 20%, respectively. A small number of bees in both groups were noted as immobile on day 1. The surviving bees were normal in appearance and behavior.

Mortality in the test groups ranged from 2 to 100%. Bees were noted as immobile in various groups during the study. Mortality was noted within 4 hours for bees at the six highest treatment levels.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 48-hour LD_{50} of the test material for honey bees was approximately 0.2 $\mu\text{g}/\text{bee}$ (95% C.I. = 0.1-0.4 $\mu\text{g}/\text{bee}$). The pattern of mortality at the two lowest treatment levels did not seem to be treatment-related, but mortality and signs of toxicity were treatment-related at the six highest treatment levels. The no observed effect dose was determined to be 0.025 $\mu\text{g}/\text{bee}$, based on a treatment-related increase in bee mortality and signs of toxicity at the six highest dosage levels.

The study director confirmed that this study was conducted in compliance with Good Laboratory Practice (GLP) standards (40 CFR Part 160). Quality Assurance and GLP statements were included in the report. A GLP compliance statement was included in the analytical appendix.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedures generally followed the protocols recommended by the SEP and Subdivision L guidelines.
- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to verify the LD_{50} (see attached printout). Although the solvent and negative control mortality was higher than is generally desired, the treatment groups demonstrated a well defined dose response. The value obtained by the reviewer using moving average angle analysis is more conservative than that of the authors, and will therefore be reported. The 48-hour LD_{50} and 95% C.I. are 0.12 $\mu\text{g}/\text{bee}$ and 0.10-0.14 $\mu\text{g}/\text{bee}$, respectively.

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 - ☐ Identity of product impurities.
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MOSSLER AC 303630 APIS MELLIFERA 6-29-30

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.4	50	50	100	0
.2	50	24	48	0
.1	50	15	30	0
.05	50	15	30	0
.025	50	4	8	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .2037356

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.0264405	.1194292	.1028788 .1399856

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.9756829	7.384596	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.120521

95 PERCENT CONFIDENCE LIMITS = 2.594113E-02 AND 4.215101

LC50 = .1270817

95 PERCENT CONFIDENCE LIMITS = 1.007766E-02 AND 25030.17

LC10 = 3.200175E-02

95 PERCENT CONFIDENCE LIMITS = 5.877472E-39 AND 8.039016E-02
