DP Barcode: D209611 **MRID No.: 434207-02**

DATA EVALUATION RECORD
ACUTE CONTACT LD_{50} TOXICITY TEST WITH THE HONEY BEE
§ 141-1

1. **CHEMICAL: Propiconazole** PC Code No.: 122101

2. **TEST MATERIAL: TGAI** Purity: 90.5%

3. **CITATION**
Authors: Palmer, Susan J. and Beavers, Joann B.
Title: An Acute Contact Toxicity Study with the Honey Bee
Study Completion Date: August 23, 1994

Laboratory: Wildlife International LTD.
8598 Commerce Drive
Easton, Maryland 27419

Sponsor: Ciba Crop Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419

Laboratory Report ID: 108-373
MRID No.: 434207-02
DP Barcode: D209611

4. **REVIEWED BY:** Laura Dye, Agronomist, EEB, EFED

Signature: **Date:**

5. **APPROVED BY:** Norman Cook, Head, Section 2, EEB, EFED

Signature: **Date:**

6. **STUDY PARAMETERS**

Age of Test Organisms at Test Initiation: 1 to 6 days
Exposure Duration: 48 hours

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for an acute contact study with the honey bee. In an 48-hour acute contact test, the LD_{50} was determined to be greater than 25 micrograms of active ingredient per bee (µg ai/bee). Propiconazole is classified as practically nontoxic to honey bees.

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: This study is scientifically sound and meets

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

The study was conducted according to approved protocol, with the following exception: bees were maintained in an environmental chamber maintained at a mean temperature of 31 °C, rather than at ambient temperature.

10. SUBMISSION PURPOSE: Submitted to support the continued registration of propiconazole. This submitted study is Ciba's response to the Agency's Phase IV "Data-Call In" under the reregistration process.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species: Honey Bee (*Apis mellifera L.*)	Honey Bee (*Apis mellifera L.*)
Age at beginning of test: Worker bees of uniform age.	1 to 6 days old
Source	Wildlife International Apiary Easton, Maryland 21601
Were bees from diseased-free colonies?	Yes, obtained as captive brood from hives maintained by Wildlife International.
Were bees kept in conditions conforming to proper cultural practices?	Yes, honey bees were maintained according to honey bee husbandry practices recommended by the State of Maryland.

B. Test System

Guideline Criteria	Reported Information
Test Chambers	The test chambers were disposable one pint rolled paper containers measuring approximately 9 cm in diameter and 9 cm high.
Photoperiod	Bees were maintained in continuous darkness, except during periods of dosing and observations.
Temperature during exposure	Mean: 31 °C Range: 30.4 to 31.5 °C
Relative humidity during exposure	Mean: 69% Range: 50 to 88 %
Feeding	Each container was covered with a disposable plastic petri dish through which an inverted 20 ml glass vial was inserted. The vial contained a sugar/water solution (1:1). The opening of the vial was covered with gauze to prevent leakage, yet allowed the bees to feed throughout the test period.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No, dosages established using known toxicity data and information provided by the Sponsor.
Definitive Test **Nominal concentrations:** At least five, in a geometric scale, unless LD_{50} > 25 µg ai/bee	Geometric Series: 1.56, 3.13, 6.25, 12.5, and 25.0 µg ai/bee
Controls: Water control or vehicle control (if vehicle is used)	Solvent (acetone) and negative controls were maintained concurrently. In addition, two replicate test chambers were maintained in each treatment and control group.
Number of bees per chamber: at least 25 (strongly recommended)	A minimum of 25 bees were placed in each test chamber and two replicate tests were performed per dosing regime. (50 bees per experimental group)
Vehicle:	Acetone
Amount of vehicle per bee:	2 µl of acetone was applied to the thorax and/or abdomen of each bee.
Were bees immobilized prior to testing?	Yes, bees were immobilized with nitrogen twice: first, prior to removal from the acrylic holding boxes just before being placed in the holding containers, and then again, in the holding containers just prior to

Guideline Criteria	Reported Information
	dosing.
How were doses administered?	The five test doses were administered topically in a droplet to the abdomen and/or thorax of each nitrogen immobilized bee.
Were bees randomly or impartially assigned to test groups?	Yes
Preparation of Dosing Solutions	A calculated amount of propiconazole was mixed with sufficient pesticide grade acetone to represent the highest dosage, 25 µg ai/bee. Lower concentration dosing suspensions were then prepared by serial dilution.
Observations period 48 hours	Observations were recorded at the following intervals: .75, 1.5, 24 and 48 hours

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Were there no observed adverse effects on bees at the greatest aging interval?	No
Control Mortality	2 % in solvent group 6 % in negative control group
Were raw data included?	Yes
Were signs of toxicity (if any) described?	Yes, one bee appeared lethargic in the 12.5 µg ai/bee treatment group at 48 hours. However, because all

Guideline Criteria	Reported Information
	other surviving bees appeared normal throughout the test, the report's authors have concluded that these effects did not result from exposure to propiconazole, but rather the method used to immobilize and administer dosages. The 46 percent mortality rate at the 25.0 µg ai/bee dose, however, is considered to be related to contact exposure to propiconazole.

Mortality and Observations

Dosage (µg ai/bee)	Number of Bees Exposed	Number (Percent) Dead	Observations
Negative Control	50	3 (6 %)	Three bees died on Day 1 after dosing. All other bees appeared normal.
Solvent Control (acetone)	50	1 (2 %)	One bee died on Day 1 after dosing. All other bees appeared normal.
1.56	50	1 (2 %)	One bee died on Day 2 after dosing. All other bees appeared normal.
3.13	50	2 (4 %)	Two bees died on Day 2 after dosing. All other bees appeared normal.
6.25	50	1 (2 %)	One bee died on Day 1 after dosing. All other bees appeared normal.

Dosage (µg ai/bee)	Number of Bees Exposed	Number (Percent) Dead	Observations
12.5	50	3 (6 %)	Three bees died on Day 1 after dosing. One bee was exhibited lethargy on Day 2. All other bees appeared normal.
25.0	50	23 (46 %)	Twenty-two bees died on Day 1 after dosing. By Day 2, the cumulative number dead was 23. The 27 surviving bees appeared normal.

13. Reported Statistical Results

Statistical Method: None. The report's authors stated that the pattern of mortality did not facilitate the calculation of an LD_{50} value.

LD_{50}: >25 µg ai/bee, with 2%, 4%, 2%, 6%, and 46% mortality based on the following geometric series of doses: 1.56, 3.13, 6.25, 12.5 and 25.0 µg ai/bee.

14. VERIFICATION OF STATISTICAL RESULTS

The reviewer used EPA's Toxanal Program to determine the LD_{50} (see attached printout). A precise LD_{50} cannot be determined using binomial, moving average or probit methods. Significant "background noise" occurred in the test when comparing the negative to the solvent control, which the report states may be the result of the immobilization process. Because the number of test organisms used was so large, the 95 percent confidence intervals calculated for the binomial probability method are unreliable. In addition, the moving average method cannot be used with the above data set because no span which produces moving average angles that bracket 45 degrees also uses two percent dead between 0 and 100 percent. Lastly, the probit method is also not appropriate because the probability is less than

0.05. Therefore, based on visual inspection of the morbidity and mortality data, the LD_{50} is classified as greater than 25.0 µg ai/bee, practically nontoxic to honey bees.

15. **REVIEWER'S COMMENTS:** Although, the study deviated from approved protocols when bees were maintained in an environmental chamber at a mean temperature of 31 °C, the study is scientifically sound and meets core guideline requirements.