MRID No. 403566-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Triclopyr triethylamine.
Shaughnessey No. 116002.

2. **TEST MATERIAL:** Triclopyr acid; ((3,5,6-trichloro-2-pyridinyl)oxy) acetic acid; a white powder; 99.2 ±0.4% purity.

3. **STUDY TYPE:** Honey Bee Acute Contact LD_{50} test.
Species Tested: Honey bee (Apis mellifera).

4. **CITATION:** Dingledine, J. 1985. Triclopyr Acid. An Acute Contact Toxicity Study with Honey Bees. Final Report. Conducted by Wildlife International, Ltd., St. Michaels, MD. Project No. 103-239. Submitted by Agricultural Products Dept., Dow Chemical U.S.A. EPA MRID No. 403566-02.

5. **REVIEWED BY:**

James L. Nation, Ph.D.
Professor
Dept. of Entomology
and Nematology
University of Florida

Signature: James L. Nation
Date: 15 Jan 1991

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 21 Jan. 1991

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

Signature: Henry T. Craven
Date: 3/15/91

6 hrs

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a honey bee acute contact test. Triclopyr acid showed no toxic effects to honey bees at a dose of 100 µg active ingredient/bee. The contact LD50 value was determined to be greater than 100 µg/bee, the highest dosage tested. Triclopyr is considered relatively non-toxic to Honey bees (Apis mellifera).

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Seven days before initiation of testing, brood frames containing only pupae were removed from colonies on site at Wildlife International Ltd. and placed in a Marsh Roll-X automatic incubator. Apparently healthy, adult worker honey bees (Apis mellifera) that emerged during the 7 days prior to beginning the study were collected for testing. Test bees were from 1 to 7 days of age. Mean body weights of the bees were determined at the beginning of the study by weighing groups of 25 bees. The mean weight per bee was 102 mg. On the day of the test, all emerged adult bees were immobilized with carbon dioxide and at least 25 bees were placed into each test chamber.

B. **Test System:** The test chambers were disposable one pint rolled paper containers measuring approximately 90 mm diameter and 80 mm high. Each container was covered with a disposable 100-mm plastic petri dish through which a 10 ml glass vial containing a 50% sugar/water solution was inserted. The opening of the vial was covered with cheese cloth to prevent leakage. This food was continuously available to the test bees.

Each test solution was prepared by weighing calculated amounts of triclopyr acid into a 10 ml volumetric flask and bringing it to volume by adding pesticide grade acetone.

Test bees were maintained in the dark except during dosing and during observations. Temperatures at the time of observations ranged from 24.4°C to 27.8°C.

C. **Dosage:** Forty-eight-hour acute contact test. Five treatment levels were tested, as well as a solvent control and a negative control. The treatment levels were 13, 22, 36, 60, and 100 µg active ingredient/bee. Test concentrations were adjusted to approximately 100% active ingredient.

D. **Design:** Two replicates, each consisting of 25 bees in a test chamber and designated as Replicate A or Replicate B, were tested at each dosage level and as solvent and negative controls. Test chambers containing 25 or more bees were selected randomly for dosing.

The bees in each test chamber were again immobilized with carbon dioxide and laid out on paper. Each of 25 bees were individually dosed on the abdomen with 2 µl of the appropriate test solution dispensed from an Eppendorf Digital Pipette. Dosed bees were returned to the test chamber, while any surplus bees were discarded.

An additional replicate of 25 bees was designated as Replicate C and dosed with 100 µg triclopyr acid/bee to replace Replicate B, which was discarded after the occurrence of inadvertent physical injury to several of the bees in replicate B. The solvent control bees received a volume of acetone equal to the largest volume (2 µl per bee) used during the test. Negative control bees were treated in the same manner as all other bees except that they were not dosed with any solution or solvent.

Bees were observed for mortality and signs of toxicity immediately after dosing. Observations were made twice on the day of initiation of the tests and once on Day 1 and Day 2 after dosing.

E. **Statistics:** The data obtained did not allow an analysis by classical probit analysis. The LD_{50} value for triclopyr acid was determined by inspection to be greater than 100 µg/bee. The LD50 value was used to classify the toxicity of the test material according to categories established by Atkins (1976), who classified any material with an LD_{50} equal to or greater than 11 µg/bee as "relatively non-toxic." Thus, an LD_{50} value greater than 100 µg/bee would place triclopyr acid in the relatively non-toxic category.

12. **REPORTED RESULTS:** The group weight and mean individual

weight of bees were reported in a table by the study author. The average group weight reported was 2.55 ±0.288 grams and the average individual weight was 0.102 grams.

The cumulative mortality of the bees after 48 hours of exposure was also reported in a table by the author for each replicate among treatments and controls. The cumulative 48-hour mortality for combined replicates did not exceed 10% in any treatment or control. The greatest mortality in any single replicate was 5 dead/25 bees in replicate C at 100 µg/bee, followed by 4 dead/25 bees in replicate B at 22 µg/bee. The occurrence of 0 dead/25 bees was common in replicates, including replicate A at 100 µg/bee, as well as most of the other treatment dosages. The negative control and solvent control each showed mortality equal to 3 dead/50 bees.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Triclopyr acid was classified as relatively non-toxic to honey bees according to toxicity categories of Atkins (1976). The honey bee 48-hour contact LD50 value for triclopyr acid was determined by inspection of the data to be greater than 100 µg/bee. The no-observed-effect dosage was 100 µg/bee.

The study report contains a Quality Assurance statement of conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs (Federal Register, volume 48, No. 230, November 29, 1983, pages 53946-53969). The Quality Assurance statement was signed by Lee F. Doggett, Quality Assurance Officer for Wildlife International Ltd.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended in the SEP. One possible deviation was that the bees tested were known to be from 1 to as much as 7 days old, whereas the SEP recommends the use of worker bees of uniform age. This deviation is not regarded as a serious flaw by the reviewer since the age span was only 7 days.

B. **Statistical Analysis:** Statistical analysis was not used to estimate LD_{50} values because the materials tested caused no mortality even at the higher dosages. Combined replicate 48 hour mortality for triclopyr acid treatments ranged from a low of 2% (at 60 µg/bee) to 10% (at 22 and 100 µg/bee), while the negative control and solvent control each showed 6% mortality. These

mortalities do not seem to be related to the treatment with triclopyr acid.

C. **Discussion/Results:** This study is scientifically sound. Triclopyr acid may be classified as relatively non-toxic to honey bees, Apis mellifera based upon classifications by Atkins of substances as relatively non-toxic if LD_{50} values are equal to or greater than 11 µg/bee. In the case of triclopyr acid, the LD_{50} values would be very much higher than this value based upon the test reported.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, Jan 2, 1991.

UCHNCSSEY No. 116002 Chemical Name TRICLOPYR ACID Chemical Class ____ Page 1 of 1

dy/Species/Lab/ cession	Chemical % a.i.	Results	Reviewer/ Date	Validati Status
Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%)=		
cies	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		
Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)=		
cies	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		
y Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
cies	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		
y Dietary LC_{50}		LC50 = ppm (95% C.L) Contr. Mort.(%)=		
ies	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		
Hour LD_{50}		LC50 = >100 mg/bee (95% C.L. N/A) Contr. Mort.(%)= 6.0		
ies *Apis mellifera*		Slope= N/A # Animals/Level= 50 mg/bee Sol. Contr. Mort.(%)= 6.0		
WILDLIFE ERNATIONAL LTD.	99-2	48-Hour Dose Level mg/bee /(%Mortality) Temperature = 24.4 To 27.8 °C		
) HD3566-02		13 (4), 22 (10), 36 (4), 60 (2), 100 (10)		
		Comments:	JEN/01/09/91 COR	
our LC_{50}		LC50 = ppm (95% C.L.) Con. Mort(%)= Sol. Con. Mor.(%)=		
ies	____	Slope= # Animals/Level= Temp.=	____	____
		96-Hour Dose Level ppm /(%Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		
our LC_{50}		LC50 = (%) (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
ies	____	Slope= # Animals/Level= Temp.=	____	____
		96-Hour Dose Level ppm /(%Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		