

US EPA ARCHIVE DOCUMENT

1-22-92

MRID No. 415651-42

**DATA EVALUATION RECORD**

- 1. **CHEMICAL:** Acetochlor.  
Shaughnessey No. 121601.
- 2. **TEST MATERIAL:** 1) Acetochlor technical, 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide; Batch No. P2; 89.4% w/w active ingredient. 2) Formulated acetochlor; (WF 2061) 768 g ai/l (68.6% ai w/w).
- 3. **STUDY TYPE:** Acute Contact LD<sub>50</sub> and Oral LD<sub>50</sub> Tests. Species Tested: Honey Bee (*Apis mellifera*).
- 4. **CITATION:** Gough, H.J., H.A. Yearsdon and G.B. Lewis. 1989. Acetochlor: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*). Laboratory Project ID No. 89JH251. Conducted by ICI Agrochemicals, Bracknell, Berkshire, UK. Submitted by ICI Americas Inc., Wilmington, DE. EPA MRID No. 415651-42.

5. **REVIEWED BY:**

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

Signature: *Mark Mossler*  
Date: *10/1/91*

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.  
Senior Scientist  
KBN Engineering and  
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Signature: *P. Kosalwat*  
Date: *10/1/91*

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

Signature: *Michael Doney*  
Date: *1-15-92*  
Signature: *Henry T. Craven*  
Date: *1-22-92*

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for an acute contact and oral study with the honey bee. Based on the EEB's memorandum dated April 18, 1984, a 48-hour contact LD<sub>50</sub> of  $\geq 11 \mu\text{g}$  ai/bee classifies both acetochlor technical and formulated acetochlor as relatively nontoxic to honey bees (*Apis mellifera*). The LD<sub>50</sub> value for both of these test materials was  $>200 \mu\text{g}$  ai/bee. The no-effect levels (NOELs) for the technical and formulated test materials were 200 and 100  $\mu\text{g}$

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ai/bee, respectively. The oral 48-hour LD<sub>50</sub> values for the technical and formulated test materials were >100 and >137 µg ai/bee, respectively. The oral no-effect levels (NOELs) for the technical and formulated test materials were 100 and 88.5 µg ai/bee, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

- A. **Test Animals:** Honey bees (*Apis mellifera*) were collected from a hive by sweeping them into a bucket. Drones were rejected. The same hive was used as a bee source for all tests.
- B. **Test System:** Bees were contained in cylindrical wire mesh cages (140 mm long and 40 mm in diameter). Each end of the container was sealed by corks. A glass feeding tube was inserted through one cork and projected a 2.5 mm feeding hole. The bees were fed a 50% sugar/water solution. This food source was available ad libitum throughout the test (except during oral dosing). The cages were kept in a controlled environment room at 23-25°C and 60-78% relative humidity.
- C. **Dosage:** Concentrations for the contact and oral studies were determined from preliminary rangefinding tests. For the contact study, a stock solution of 200 mg ai/ml was prepared for both acetochlor technical (in acetone) and the formulated product (in deionized water containing 500 mg/l 'Agral'). The doses applied to bees for both materials were 200, 100, 50, 20, 10, 5, and 1 µg ai/bee.

For the oral study, stock solutions of 5 and 200 mg ai/ml were prepared for acetochlor technical and the formulated product, respectively, in 50% sucrose solution. The doses that the bees ingested were 100, 50, 20, 10, 5, 2, and 1 µg ai/bee for the technical material and 200, 100, 50, 20, 10, 5, and 1 µg ai/bee for the formulated material.

In addition to the technical and formulated contact and oral tests, a reference toxicant, dimethoate, was used to determine if the bees were responding to the test materials normally.

- D. **Design:** The tests consisted of 7 treatment levels and a solvent control. Three replicates of 10 bees each were used for each treatment and control. For the contact study, randomly selected bees were immobilized with carbon-dioxide and laid out on filter paper. The bees were dosed individually on the thorax with 1  $\mu$ l of the appropriate test solution. Control bees were either treated with 1  $\mu$ l of acetone (technical acetochlor) or 1  $\mu$ l of deionized water that contained 500 mg/l "Agral" (the formulated product).

Oral exposure was accomplished by dissolving the test materials in a 50% sugar/water solution. Feeding was done by supplying 0.2 ml of the test solutions in the feeding tube for the ten bees per cage to feed upon. Control bees were given a 50% sucrose solution. When all the test solution had been ingested or after about 4 hours, whichever was sooner, the feeding tubes were replaced by tubes containing 50% sucrose solution. Any test material remaining was measured.

Observations were recorded at 1, 4, 24, and 48 hours after treatment. Sub-lethal effects were assessed by pre-determined categories. Category A: - bees are hyperactive compared with controls, and show the first signs of paralysis. Category B: - partial paralysis and poor coordination of movement. Category C: - almost complete paralysis.

- E. **Statistics:** The LD<sub>50</sub> values and 95% confidence limits were calculated using probit analysis. Adjustments were made for control mortality with a method similar to Abbott's correction. Mortality data were transformed using arcsine. One-way analysis of variance was conducted in conjunction with a t-test ( $p \leq 0.05$ ) to determine significant differences between treatments and controls. The no-observed-effect level (NOEL) was the level of treatment below the lowest level at which any effects (sub-lethal or mortality) were significantly different from the controls.

12. **REPORTED RESULTS:** After 48 hours, one mortality was witnessed in the 200, 20, 5, and control treatments and two mortalities were observed in the 50  $\mu$ g ai/bee treatment for the contact test with acetochlor technical (Table 3, attached). A similar trend was observed with the formulated material, however, more mortality was seen at the highest treatment level (200  $\mu$ g ai/bee) in which 10 of the thirty bees were dead after 48 hours (Table 4, attached). The LD<sub>50</sub> for acetochlor technical and as a formulated product was

therefore greater than 200  $\mu\text{g}$  ai/bee. No sublethal effects were observed and the subsequent NOELs were 200 and 100  $\mu\text{g}$  ai/bee for acetochlor technical and formulated acetochlor, respectively.

In the oral test with the formulated product, some of the doses were not completely consumed. The actual doses were calculated and reported in Table 7 (attached). After 48 hours, one mortality was witnessed in the 5 and 2  $\mu\text{g}$  ai/bee treatments and two mortalities were observed in the 20  $\mu\text{g}$  ai/bee and control treatments for the oral test with acetochlor technical (Table 6, attached). A similar trend was observed with the formulated material, however, more mortality was seen at the highest treatment level (96-137  $\mu\text{g}$  ai/bee) in which 4 of the thirty bees were dead after 48 hours (Table 7). The  $\text{LD}_{50}$  values for acetochlor technical and as a formulated product were therefore >100 and >137  $\mu\text{g}$  ai/bee, respectively. No sublethal effects were observed and the subsequent NOELs were 100 and 88.5 (due to incomplete ingestion)  $\mu\text{g}$  ai/bee for acetochlor technical and formulated acetochlor, respectively. At the higher concentrations of formulated acetochlor, bees were slow to feed on the test solution, indicating that they may have found formulated acetochlor distasteful.

Tests in which dimethoate was used as a reference toxicant demonstrated that the bees were reacting normally to acetochlor.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

"The results show that acetochlor is "virtually non-toxic" to honey bees. The 24-hour  $\text{LD}_{50}$  values were greater than the highest doses tested in all cases: Contact - >200  $\mu\text{g}$  ai/bee for both technical and formulated acetochlor; Oral - >100 and >137  $\mu\text{g}$  ai/bee for technical and formulated acetochlor, respectively. There were no sub-lethal effects attributable to the treatments. The 48-hour results from the test were similar to the 24-hour results indicating that there were no delayed effects."

A Quality Assurance statement was included in the report. However, the report also stated that the study was not subject to the requirements of 40 CFR Part 160.

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

- A. Test Procedure:** The test procedures generally follow the protocols recommended by the SEP and Subdivision L guidelines, except for the following:

The age of the bees was not given and it is not known whether all test bees were at a uniform age.

No true control group was included in the test.

- B. Statistical Analysis:** The LD<sub>50</sub> values were not calculated due to <50% mortality in all tests. The reviewer used ANOVA and Dunnett's test to determine the NOEL for formulated acetochlor contact toxicity data. The NOEL was found to be 100 µg ai/bee.
- C. Discussion/Results:** In the summary, the authors stated that 'Agral' is the formulated material. In the methods section, the authors stated that all solutions were made in deionized water that contained 500 mg/l 'Agral'. They also stated that control bees were treated with 1 µl of this solution (500 mg/l 'Agral'). If 'Agral' is indeed the formulated material, this would have added another 3.4 µg ai/bee to the test rates. However, the reviewer feels that 'Agral' is the formulating product (i.e., no acetochlor). Since the formulated material is being tested, the reviewer questions why the control bees were treated with anything other than deionized water. Since all test solutions contained this amount of 'Agral', the control reflected the toxicity inherent in the test treatments.

This study is scientifically sound and fulfills the requirements for acute contact and oral studies with the honey bee.

Based on the EEB's memorandum dated April 18, 1984, a 48-hour contact LD<sub>50</sub> of ≥11 µg ai/bee classifies both acetochlor technical and formulated acetochlor as relatively nontoxic to honey bees (*Apis mellifera*). The LD<sub>50</sub> value for both of these test materials was >200 µg ai/bee. The no-effect levels (NOELs) for the technical and formulated test materials were 200 and 100 µg ai/bee, respectively.

The oral 48-hour LD<sub>50</sub> values for the technical and formulated test materials were >100 and >137 µg ai/bee, respectively. The oral no-effect levels (NOELs) for the

technical and formulated test materials were 100 and 88.5  $\mu\text{g}$  ai/bee, respectively.

D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 9-20-91.

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ACETOCHLOR

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Pages   7   through  10  are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
  - Sales or other commercial/financial information.
  - A draft product label.
  - The product confidential statement of formula.
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contact toxicity for formulated acetabular to bees,

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
<i>Rate (µg ai/bee)</i>				
1 = control	3	.6667	.5774	86.6
2 1.0	3	.3333	.5774	173.2
3 5.0	3	.0000	.0000	.0
4 10	3	.3333	.5774	173.2
5 20	3	.3333	.5774	173.2
6 50	3	.6667	.5774	86.6
7 100	3	.3333	.5774	173.2
8* 200	3	3.3333	2.5166	75.5

NOEL = 100 µg ai/bee

Raw data from Table 4 (attached).

\*) the mean for this group is significantly greater than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minimum detectable difference for Dunnett's test = 2.133333  
 This difference corresponds to 320.00 percent of control

Between groups sum of squares = 23.833333 with 7 degrees of freedom.

Error mean square = 1.041667 with 16 degrees of freedom.

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 \* Warning - the test for equality of variances \*  
 \* could not be computed as 1 or more of the \*  
 \* variances is zero. \*  
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