

**DATA EVALUATION RECORD  
HONEY BEE - ACUTE CONTACT LC<sub>50</sub> TEST  
§141-1**

1. **CHEMICAL:** Thidiazuron formulation PC Code No.: 120301

2. **TEST MATERIAL:** AE B049537 00 SC42 A204 Purity: 41.9%

3. **CITATION:**

Author: Waltersdorfer, A.

Title: Contact toxicity (LD50) to the honey bees (*Apis mellifera* L.), Thidiazuron, Water miscible suspension concentrate 500 g/L

Study Completion Date: October 21, 2002

Laboratory: Bayer CropScience GmbH, Ecotoxicology  
Industriepark Hochst  
D-65926 Frankfurt am Main  
Federal Republic of Germany

Sponsor: Bayer CropScience GmbH, Ecotoxicology  
D-65926 Frankfurt am Main  
Federal Republic of Germany

Laboratory Report ID: CW02/046

DP Barcode: D294536

MRID No.: 46203518

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

**Signature:**

**Date:** 4/28/04

**APPROVED BY:** Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

**Signature:**

**Date:** 4/28/04

5. **APPROVED BY:** William Evans, Biologist, EFED/ERB-1

**Signature:**



**Date:** 11/16/04



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**6. STUDY PARAMETERS:**

**Scientific Name of Test Organism:** *Apis mellifera* L.

**Age or Size of Test Organism at Test Initiation:** Worker honey bee

**Type of Concentrations:** Nominal

**Definitive Study Duration:** 72 hours

**7. CONCLUSIONS:**

The honey bee, *Apis mellifera*, was exposed to a Thidiazuron formulation for 72 hours in a contact test. The nominal concentrations were 23.4, 58.5, 117.1, 175.6, and 234.2 µg product/bee. By 48 hours, percent mortality was 4, 2, 4, 0, and 2% in the 23.4, 58.5, 117.1, 175.6, and 234.2 µg product/bee groups, respectively. There was 2% mortality in the negative control. **The LC<sub>50</sub> value was estimated as >234.2 µg product/bee (>98.1 µg a.i./bee), the highest test concentration. As a result, Thidiazuron is categorized as practically nontoxic to honeybees on an acute contact basis.** The NOEL was 234.2 µg product/bee (98.1 µg a.i./bee).

**This acute contact study is classified as Core.** This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020).

**Reported Statistical Results:**

|  |                   |
|--|-------------------|
| LD <sub>50</sub> : >234.2 µg product/bee (>98.1 µg a.i./bee) | 95% C.I.: N/A     |
| NOEL: 234.2 µg product/bee (98.1 µg a.i./bee)                | Probit Slope: N/A |
| LOEL: >234.2 µg product/bee (>98.1 µg a.i./bee)              |                   |

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Core

**B. Rationale:** This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020).

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:**

1. The test material (and reference substance) was mixed with drinking water as the

diluent. Water is not an acceptable solvent to use in contact studies; however, the test material was a water miscible suspension concentrate, so this deviation was not believed to have impacted the results of this study. Furthermore, there was significant toxicity shown in the reference group, which also used water as a diluent.

2. The TGAI was not tested, but rather a formulation. The purity of the active ingredient (41.9%) was used to express the toxicity values in units of µg a.i./bee.

**10. SUBMISSION PURPOSE:** This study was submitted to provide data on the acute contact toxicity of a Thidiazuron formulation to honeybees for the purpose of chemical reregistration.

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

| Guideline Criteria   | Reported Information     |
|--|--------------------------|
| <b>Species:</b><br>Species of concern ( <i>Apis mellifera</i> , <i>Megachile rotundata</i> , or <i>Nomia melanderi</i> ) | <i>Apis mellifera</i> L. |
| <b>Age at beginning of test:</b>   | Worker honey bee         |
| <b>Supplier:</b>   | Laboratory colonies      |
| <b>All bees from the same source?</b>  | Yes                      |

**B. Test System**

| Guideline Criteria         | Reported Information  |
|----------------------------|---|
| <b>Cage size adequate?</b> | The cylindrical cages were made of wire mesh screening and closed on each end with a cork plug. Cage dimensions were 12-13 cm high and 5 cm diameter. |
| <b>Lighting:</b>           | Continuous darkness   |
| <b>Temperature:</b>        | 23-25.5°C   |
| <b>Relative humidity:</b>  | 58-70%  |

**C. Test Design**

| Guideline Criteria  | Reported Information   |
|---|--|
| <b>Range finding test?</b>  | No range finding study was conducted.  |
| <b>Reference toxicant test?</b>   | The reference toxicant was Triazophos, 40.9% w/w.<br>The applied concentrations were 0.2, 0.3, and 0.4 µg product/bee. |
| <b>Method of administration:</b>  | 1.0 µL of test substance was applied to the ventral thorax of each bee using a microapplicator.                        |
| <b>Nominal doses:</b>   | 23.4, 58.5, 117.1, 175.6, and 234.2 µg product/bee.  |
| <b>Controls:</b><br>Negative control and/or diluent/solvent control   | Negative control   |
| <b>Number of colonies per group:</b>  | 5 replicates; 10 bees/replicate  |
| <b>Solvent:</b><br>The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol. | Water was used as a diluent.   |
| <b>Feeding:</b>   | A 50% w/v sugar solution was provided <i>ad libitum</i> .  |
| <b>Observations period:</b>   | 24, 48, and 72 hours.  |

**12. REPORTED RESULTS:**

| Guideline Criteria  | Reported Information                       |
|---|--|
| <b>Quality assurance and GLP compliance statements were included in the report?</b> | Yes  |
| <b>Control performance:</b>   | 2% negative control mortality by 72 hours. |
| <b>Raw data included:</b>   | Replicate data were provided.              |
| <b>Signs of toxicity (if any) were described?</b>                                   | No signs of toxicity were described.       |

**Mortality**

| Dosage<br>µg product/bee      | No. of<br>bees | Percent Mortality (%) |    |    |
|-------------------------------|----------------|-----------------------|----|----|
|                               |                | Hour of Study         |    |    |
|                               |                | 24                    | 48 | 72 |
| Test Substance (Thidiazuron): |                |                       |    |    |
| Negative Control              | 50             | 2                     | 2  | 2  |
| 23.4                          | 50             | 4                     | 4  | 4  |
| 58.5                          | 50             | 0                     | 0  | 2  |
| 117.1                         | 50             | 0                     | 2  | 4  |
| 175.6                         | 50             | 0                     | 0  | 0  |
| 234.2                         | 50             | 2                     | 2  | 2  |
| Toxic Standard (Triazophos):  |                |                       |    |    |
| 0.2                           | 50             | 0                     | 0  | 0  |
| 0.3                           | 50             | 72                    | 72 | 74 |
| 0.4                           | 50             | 92                    | 92 | 92 |

Observations: By 48 hours, percent mortality was 4, 2, 4, 0, and 2% in the 23.4, 58.5, 117.1, 175.6, and 234.2 µg product/bee groups, respectively. There was 2% mortality in the negative control.

Statistical method: The LD<sub>50</sub> value was not calculated due to less than 50% mortality in all treatment groups. The NOEL and LOEL were determined based on mortalities.

**Reported Statistical Results:**

LD<sub>50</sub>: >234.2 µg product/bee      95% C.I.: N/A  
 NOEL: 234.2 µg product/bee Probit Slope: N/A  
 LOEL: >234.2 µg product/bee

**13. VERIFICATION OF STATISTICAL RESULTS:**

The LD<sub>50</sub> and NOEC estimates could be determined visually.

**Results:**

LD<sub>50</sub>: >234.2 µg product/bee (>98.1 µg a.i./bee)      95% C.I.: N/A  
NOEL: 234.2 µg product/bee (98.1 µg a.i./bee)      Probit Slope: N/A  
LOEL: >234.2 µg product/bee (>98.1 µg a.i./bee)

**14. REVIEWER'S COMMENTS:**

The reviewer's conclusions were similar to the study author's. **The LC<sub>50</sub> value was estimated as >234.2 µg product/bee (>98.1 µg a.i./bee), the highest test concentration. As a result, Thidiazuron is categorized as practically nontoxic to honeybees on an acute contact basis.**

The test was conducted in compliance with the OECD Principles of Good Laboratory Practice, adopted November 26, 1997 [(C97) 186/Final] (p. 3).

The reference LD<sub>50</sub> was calculated using SAS probit-analysis. In the contact test, the Triazophos LD<sub>50</sub> was 0.283 µg product/bee.

**15. REFERENCES:**

Guideline on test methods for evaluating the side-effects of plant protection products on honeybees.  
EPPO Bulletin 22, 203-215 (1992) No. 170.

OECD Guidelines for the Testing of Chemicals, 214; Honeybees, Acute Contact Toxicity Test;  
Adopted 21<sup>st</sup> September 1998

The SAS System for Windows, Release 8.01 TS Level 01MO, 1999-2000.