DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT & ORAL LC_{50} TEST
§141-1 and Non-guideline

1. **CHEMICAL:** Thidiazuron Technical — PC Code No.: 120301

2. **TEST MATERIAL:** AE B 049537 00 1D99 0003 — Purity: 99.5%

3. **CITATION:**

Author: Kling, A.

Title: Assessment of Side Effects of AE B 049537 00 1D99 0003 (Thiadiazuron, Techn,) to the Honey Bee, *Apis mellifera* L. in the Laboratory

Study Completion Date: March 2, 2003

Laboratory: Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytick GmbH
D-75223 Niefer-Oschelbronn

Sponsor: Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Laboratory Report ID: C029693

DP Barcode: D294536

MRID No.: 46203501

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:** 11/17/04

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5. APPROVED BY:

Signature: **Date:**

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera carnica*

Age or Size of Test Organism at Test Initiation: Young adult worker bee

Type of Concentrations: Nominal

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

The honey bee, *Apis mellifera*, was exposed to Thidiazuron for 48 hours in the oral test and contact test. The oral and contact nominal concentration was 100.00 µg a.i./bee. The actual oral intake concentration of Thidiazuron in the oral toxicity test was 105.25 µg a.i./bee. By 48 hours in the oral test, there was 4.0% mortality in the 100.00 µg a.i./bee treatment group. There was 0.0% and 2.0% mortality in the negative and solvent control, respectively. The corrected mortality at 48 hours was 2.0% for the 100.00 µg a.i./bee treatment group. By 48 hours in the contact test, there was 0.0% mortality in the 100.00 µg a.i./bee treatment group. There was 0.0% and 2.0% mortality in the negative and solvent control, respectively. No behavioral differences were observed during the test. No behavioral differences were observed during the tests. **The LC_{50} value for the oral test was estimated as >105.25 µg a.i./bee, the highest concentration of intake. The LD_{50} value for the contact test was >100.00 µg a.i./bee. As a result, Thidiazuron is categorized as practically nontoxic to honeybees on both an acute oral and contact basis.** The NOELs for the oral and contact tests were 105.25 and 100.00 µg a.i./bee, respectively.

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). **The acute oral study is scientifically sound and is classified as Supplemental.**

Reported Statistical Results - Oral Test:

LD_{50}: >105.25 µg a.i./bee
NOEL: 105.25 µg a.i./bee
LOEL: >105.25 µg a.i./bee

95% C.I.: N/A
Probit Slope: N/A

Reported Statistical Results - Contact Test:

LD_{50}: >100.0 µg a.i./bee
NOEL: 100.0 µg a.i./bee
LOEL: >100.0 µg a.i./bee

95% C.I.: N/A
Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: 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). The acute oral study is scientifically sound and is classified as Supplemental.

B. Rationale: This acute oral study is scientifically sound and is classified as Supplemental because the study is a non-guideline study and does not fulfill an OPP guideline requirement.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

No deviations noted.

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

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera* L.
Age at beginning of test:	Young adult worker bee
Supplier:	Laboratory colonies (originally from Mr. Berthold Nengel, Rheinland-Pfalz, Germany)
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	The cages were made of high grade steel with a transparent front pane and perforated board bottom. Cage dimensions were 10 cm wide, 5.5 cm depth, and 8.5 cm height.
Lighting:	Continuous darkness
Temperature:	23.5-25.0°C
Relative humidity:	60.0-80.0%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No range finding study was conducted.
Reference toxicant test?	The reference toxicant was dimethoate, Perfekthion. In the oral test, the applied concentrations of dimethoate were 0.08, 0.10, 0.13, and 0.17 µg a.i./bee. In the contact test, the applied concentrations of dimethoate were 0.11, 0.14, 0.18, and 0.23 µg a.i./bee.
Method of administration:	Oral test: The test chemical was dissolved in acetone for the stock solution and mixed with 50% aqueous sucrose solution. Then 250 µL of the mixture was provided to each cage for consumption. The food was offered in cages for 6 hours of uptake. Contact test: 2 µL of test substance in solvent (acetone) was applied to the ventral thorax of each bee using a microapplicator.
Nominal doses:	Oral test: 100.00 µg a.i./bee (Actual oral concentration was 105.25 µg a.i./bee.) Contact test: 100.00 µg a.i./bee.

Guideline Criteria	Reported Information
Controls: Negative control and/or diluent/solvent control	Oral test: negative and solvent control Contact test: negative and solvent control
Number of colonies per group:	Oral test: 5 replicates; 10 bees/replicate Contact test: 5 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Acetone
Feeding:	Oral test: Prior to test initiation, bees were starved for 2 hours. After treatment, bees were supplied with a pure untreated 50% aqueous sucrose solution, *ad libitum*. Contact test: A 50% aqueous sucrose solution was provided *ad libitum*.
Observations period:	4, 24, and 48 hours.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	Oral test: 0.0% negative control mortality and 2.0% solvent control mortality by 48 hours. Contact test: 0.0% negative control mortality and 2.0% solvent control mortality by 48 hours.
Raw data included:	Replicate data were provided.
Signs of toxicity (if any) were described?	No signs of toxicity were described.

Mortality - Oral Test

Dosage µg a.i./bee (actual intake)	No. of bees	Percent Mortality (%)		Corrected Mortality (%)	
		Hour of Study		Hour of Study	
		24	48	24	48
Test Substance (Thidiazuron):[a]					
Negative Control	50	0.0	0.0	--	--
Solvent Control	50	2.0	2.0	--	--
100.0 (105.25)	50	4.0	4.0	2.0	2.0
Toxic Standard (Dimethoate):[b]					
0.08 (0.09)	50	8.0	16.0	8.0	16.0
0.10 (0.12)	50	36.0	50.0	36.0	50.0
0.13 (0.14)	50	44.0	70.0	44.0	70.0
0.17 (0.18)	50	78.0	84.0	78.0	84.0

[a] The mortality was corrected with the solvent control mortality data.
[b] The mortality was corrected with the negative control mortality data.

Observations: By 48 hours, there was 4.0% mortality in the 100.00 µg a.i./bee treatment group. There was 0.0% and 2.0% mortality in the negative and solvent control, respectively. The corrected mortality at 48 hours was 2.0% for the 100.00 µg a.i./bee treatment group. No behavioral differences were observed during the test.

Mortality - Contact Test

Dosage µg a.i./bee	No. of bees	Percent Mortality (%)		Corrected Mortality (%)	
		Hour of Study		Hour of Study	
		24	48	24	48
Test Substance (Thidiazuron):[a]					

Dosage µg a.i./bee	No. of bees	Percent Mortality (%) Hour of Study 24	Percent Mortality (%) Hour of Study 48	Corrected Mortality (%) Hour of Study 24	Corrected Mortality (%) Hour of Study 48
Negative Control	50	0.0	0.0	--	--
Solvent Control	50	0.0	2.0	--	--
100.0	50	0.0	0.0	0.0	-2.0
Toxic Standard (Dimethoate):[b]					
0.11	50	0.0	4.0	0.0	4.0
0.14	50	18.0	28.0	18.0	28.0
0.18	50	50.0	60.0	50.0	60.0
0.23	50	96.0	96.0	96.0	96.0

[a] The mortality was corrected with the solvent control mortality data.
[b] The mortality was corrected with the negative control mortality data.

Observations: By 48 hours, there was 0.0% mortality in the 100.00 µg a.i./bee treatment group. There was 0.0% and 2.0% mortality in the negative and solvent control, respectively. No behavioral differences were observed during the test.

Statistical method: The LD_{50} values in the oral and contact toxicity tests were not calculated due to less than 50% mortality in all treatment groups. The NOEL and LOEL were determined based on mortalities. The mortalities were corrected according to the formula of Schneider-Orelli (1947, p. 13).

Reported Statistical Results - Oral Test:

LD_{50}: >105.25 µg a.i./bee
NOEL: 105.25 µg a.i./bee
LOEL: >105.25 µg a.i./bee
95% C.I.: N/A
Probit Slope: N/A

Reported Statistical Results - Contact Test:

LD_{50}: >100.0 µg a.i./bee
NOEL: 100.0 µg a.i./bee
LOEL: >100.0 µg a.i./bee
95% C.I.: N/A
Probit Slope: N/A

13. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50}/LD_{50} and NOEC estimates could be determined visually.

Results - Oral Test:

LD_{50}: >105.25 µg a.i./bee
NOEL: 105.25 µg a.i./bee
LOEL: >105.25 µg a.i./bee

95% C.I.: N/A
Probit Slope: N/A

Results - Contact Test:

LD_{50}: >100.0 µg a.i./bee
NOEL: 100.0 µg a.i./bee
LOEL: >100.0 µg a.i./bee

95% C.I.: N/A
Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. **The LC_{50} value for the oral test was estimated as >105.25 µg a.i./bee, the highest concentration of intake. The LD_{50} value for the contact test was >100.00 µg a.i./bee. As a result, Thidiazuron is categorized as practically nontoxic to honeybees on both an acute oral and contact basis.**

The test was conducted in compliance with the U.S. EPA Good Laboratory Compliance Standards 40 CFR 160 (p. 1c).

The toxic standard LD_{50} was calculated by means of probit analysis using statistic program SAS release V8. In the oral test, the dimethoate LD_{50} was 0.12 µg a.i./bee with 95% confidence interval of 0.11 to 0.13 µg a.i./bee. In the contact test, the dimethoate LD_{50} was 0.16 µg a.i./bee with 95% confidence interval of 0.16 to 0.17 µg a.i./bee.

15. REFERENCES:

OECD Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17 Organisation for Economic Co-operation and Development (OECD), Environmental Health and Safety Publications, Paris 1998.

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OECD, 1998 Guideline for the testing of chemicals; Honey bees; acute contact toxicity test; 214.

SAS Institute Inc. (Ed.): SAS/STAT User's Guide Version 8, Fourth Editions; Cary, NC, USA.

Schneider-Orelli, O. (1947): Entomologisches Praktikum. Aarau, 2. Auflage.