

DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT & ORAL LC₅₀ 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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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 LC₅₀ value for the oral test was estimated as >105.25 µg a.i./bee, the highest concentration of intake. The LD₅₀ 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	95% C.I.: N/A
NOEL: 105.25 µg a.i./bee	Probit Slope: N/A
LOEL: >105.25 µg a.i./bee	

Reported Statistical Results - Contact Test:

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

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 (<i>Apis mellifera</i> , <i>Megachile rotundata</i> , or <i>Nomia melanderi</i>)	<i>Apis mellifera</i> 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 <u>oral test</u> , the applied concentrations of dimethoate were 0.08, 0.10, 0.13, and 0.17 µg a.i./bee. In the <u>contact test</u> , the applied concentrations of dimethoate were 0.11, 0.14, 0.18, and 0.23 µg a.i./bee.
Method of administration:	<u>Oral test</u> : 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. <u>Contact test</u> : 2 µL of test substance in solvent (acetone) was applied to the ventral thorax of each bee using a microapplicator.
Nominal doses:	<u>Oral test</u> : 100.00 µg a.i./bee (Actual oral concentration was 105.25 µg a.i./bee.) <u>Contact test</u> : 100.00 µg a.i./bee.

Guideline Criteria	Reported Information
Controls: Negative control and/or diluent/solvent control	<u>Oral test:</u> negative and solvent control <u>Contact test:</u> negative and solvent control
Number of colonies per group:	<u>Oral test:</u> 5 replicates; 10 bees/replicate <u>Contact test:</u> 5 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Acetone
Feeding:	<u>Oral test:</u> Prior to test initiation, bees were starved for 2 hours. After treatment, bees were supplied with a pure untreated 50% aqueous sucrose solution, <i>ad libitum</i> . <u>Contact test:</u> A 50% aqueous sucrose solution was provided <i>ad libitum</i> .
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:	<u>Oral test:</u> 0.0% negative control mortality and 2.0% solvent control mortality by 48 hours. <u>Contact test:</u> 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 $\mu\text{g a.i./bee}$ (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 $\mu\text{g a.i./bee}$ 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 $\mu\text{g a.i./bee}$ treatment group. No behavioral differences were observed during the test.

Mortality - Contact Test

Dosage $\mu\text{g a.i./bee}$	No. of bees	Percent Mortality (%)		Corrected Mortality (%)	
		Hour of Study		Hour of Study	
		24	48	24	48
Test Substance (Thidiazuron): ^a					

Dosage $\mu\text{g a.i./bee}$	No. of bees	Percent Mortality (%)		Corrected Mortality (%)	
		Hour of Study		Hour of Study	
		24	48	24	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 $\mu\text{g a.i./bee}$ 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 $\mu\text{g a.i./bee}$ 95% C.I.: N/A
 NOEL: 105.25 $\mu\text{g a.i./bee}$ Probit Slope: N/A
 LOEL: >105.25 $\mu\text{g a.i./bee}$

Reported Statistical Results - Contact Test:

LD_{50} : >100.0 $\mu\text{g a.i./bee}$ 95% C.I.: N/A
 NOEL: 100.0 $\mu\text{g a.i./bee}$ Probit Slope: N/A
 LOEL: >100.0 $\mu\text{g a.i./bee}$

13. VERIFICATION OF STATISTICAL RESULTS:

The LC₅₀/LD₅₀ and NOEC estimates could be determined visually.

Results - Oral Test:

LD₅₀: >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:

LD₅₀: >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 LC₅₀ value for the oral test was estimated as >105.25 µg a.i./bee, the highest concentration of intake. The LD₅₀ 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 LD₅₀ was calculated by means of probit analysis using statistic program SAS release V8. In the oral test, the dimethoate LD₅₀ 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 LD₅₀ 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.

OECD, 1998 Guideline for the testing of chemicals; Honey bees; acute oral toxicity test; 213.

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

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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.