HONEY BEE - ACUTE CONTACT & ORAL LC_{50} TEST
§141-1

1. **CHEMICAL:** Clothianidin TI-435 PC Code No.: 044309

2. **TEST MATERIAL:** TI-435 Technical Purity: 96.0%

3. **CITATION:**

Author: Weyman, G.S.

Title: TI-435 Technical: Acute Contact and Oral Toxicity to Honey Bees

Study Completion Date: March 13, 1998

Laboratory: Covance Laboratories, Ltd.
Otley Road, Harrogate
North Yorkshire, HG3 1PY, England

Sponsor: Takeda Chemical Industries, Ltd.
Development Department, Agro Company
13-10 Nihonbashi 2-chome
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Laboratory Report ID: 110049

DP Barcode: D278110

MRID No.: 45422426

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

Signature: Rebecca Bryan **Date:** 2/24/03

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

Signature: Teri Myers **Date:** 2/24/03

5. **Secondary Reviewer:** Gabe Patrick, Biologist, OPPTS/OPP/EFED/ERB 5

Signature: Gabe Patrick **Date:** 3/4/03

Secondary Reviewer: Valerie Hodge, MSc, Senior Evaluation Officer
Environmental Assessment Division, PMRA

Signature: Valerie Hodge **Date:** 3/20/03

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6. STUDY PARAMETERS:

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

Age or Size of Test Organism at Test Initiation: Not reported

Type of Concentrations: Measured

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

Honey bees, *Apis mellifera* L., were exposed to Clothianidin as TI-435 Technical for 48 hours in both oral and contact toxicity tests. In the oral test, nominal concentrations were 0.001024, 0.00256, 0.0064, 0.016, 0.04, and 0.1 µg a.i./bee, which corresponded to measured concentrations of 0.0009, 0.00254, 0.0062, 0.012, 0.022, and 0.065 µg a.i./bee. By 48 hours, mortality in the 0.0009, 0.00254, and 0.0062 µg a.i./bee treatment groups was 40, 57, 100%, respectively. The 0.015, 0.035, and 0.089 µg a.i./bee treatment groups had 100% mortality, compared to 0% mortality in the control group. In the contact test, bees were exposed to nominal concentrations of 0.00032, 0.0016, 0.008, 0.04, 0.2, and 1.0 µg a.i./bee, which corresponded to measured concentrations of 0.00039, 0.0019, 0.0095, 0.046, 0.22, and 1.14 µg a.i./bee. Percent mortality was 3, 10, 37, 93 and 100% in the 0.0019, 0.0095, 0.046, 0.22, and 1.14 µg a.i./bee, respectively, compared to 3% in the control group. **The LD_{50} value for the oral test was 0.00368 µg a.i./bee. The LD_{50} value for the contact test was 0.0439 µg a.i./bee. As a result, TI-435 Technical is categorized as highly toxic to bees on an acute oral and contact basis.**

This study is classified as Core. This study is scientifically sound and it satisfies the guideline requirements for an oral and contact toxicity test with honey bees (Subdivision L, §141-1).

Results Synopsis:

Oral Test

LD_{50}: 0.00368 µg a.i./bee — 95% C.I.: 0.00303 to 0.00445 µg a.i./bee
NOEL: 0.0009 µg a.i./bee — Probit Slope: N/A

Contact Test

LD_{50}: 0.0439 µg a.i./bee — 95% C.I.: 0.0296 to 0.0652 µg a.i./bee
NOEL: 0.0095 µg a.i./bee — Probit Slope: 1.78

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The age and size of the bees were not reported.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute oral and contact toxicity of TI-435 Technical to honey bees for the registration of this product.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	Worker bees
Supplier:	Peter Scott, The Bee Farm, Bramham, UK
All bees from the same source?	Yes, bees derived from a healthy colony.

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Cylindrical cage made of 3 mm stainless steel mesh(170 mm long x 45 mm diameter). One end closed by integral stainless steel plate and the other end by a 50 mm diameter foam bung.
Lighting:	Continuous darkness, except during assessments.
Temperature:	24.4-25.2°C
Relative humidity:	58.1-66.7%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	The nominal doses for the first rangefinder test were 0.1, 1, 10 and 100 µg a.i./bee for the oral test; and 0.16, 1.6, 16, and 32 µg a.i./bee for the contact test. The second range finding test lowered the doses to 0.0001, 0.001, 0.01, and 0.1 µg a.i./bee for the oral test; and 0.001, 0.01, 0.1, and 1.0 µg a.i./bee for the contact test.
Reference toxicant test?	The reference toxicant dimethoate(BASF Dimethoate 40 EC) was used. In the oral test, nominal dimethoate concentrations were 0.1, 0.15, and 0.2 µg a.i./bee. In the contact test, nominal dimethoate concentrations were 0.2, 0.4, and 0.8 µg a.i./bee.

Guideline Criteria	Reported Information
Method of administration:	Oral test: the test substance was mixed with a 50% sucrose solution. Contact test: the test substance was dissolved in acetone, and 1 µL of the test substance suspension was applied to the dorsal thorax of each bee using a microapplicator.
Nominal doses:	Oral test: 0.001024, 0.00256, 0.0064, 0.016, 0.01 and 0.1 µg a.i./bee. Contact test: 0.00032, 0.0016, 0.008, 0.04, 0.2, and 1.0 µg a.i./bee.
Controls: Negative control and/or diluent/solvent control	Diluent/solvent controls were used in both the oral and contact studies.
Number of colonies per group:	3 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Oral test: 50% aqueous sucrose solution Contact test: acetone
Feeding:	Oral test: Prior to test initiation, bees were starved for 2 to 3 hours. After test initiation, bees were supplied with a 50% aqueous sucrose solution, *ad libitum*. Contact test: a 50% aqueous sucrose solution was provided *ad libitum.*
Observations period:	Mortality and sublethal effects were monitored after 1, 2, 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% mortality by 48 hours. Contact test: 3% mortality by 48 hours.
Raw data included:	Yes
Signs of toxicity (if any) were described?	Category A (slight effect)-bees are hyperactive compared with controls, Category B (moderate effect)-partial paralysis and poor coordination of movement, Category C (severe effect)-almost complete paralysis, and Category D (dead)-no response at all to stimulation when touched or blown at.

Mortality - Oral Test

Measured and (Nominal) Dosage (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead				
			Hour of Study				
			1	2	4	24	48
Control Group	10	1	0	0	0	0	0
	10	2	0	0	0	0	1
	10	3	0	0	0	0	0
0.0009 (0.001024)	10	1	0	0	0	0	0
	10	2	0	0	1	1	1
	10	3	0	0	0	0	0
0.00254 (0.00256)	10	1	0	0	1	10	10
	10	2	0	0	0	0	0
	10	3	0	0	0	1	2

Measured and (Nominal) Dosage (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead				
			Hour of Study				
			1	2	4	24	48
0.0062	10	1	0	0	3	4	4
(0.0064)	10	2	0	0	3	8	8
	10	3	0	0	0	5	5
0.012	10	1	0	0	7	10	10
(0.016)	10	2	0	0	3	10	10
	10	3	0	0	4	10	10
0.022	10	1	0	0	5	10	10
(0.04)	10	2	0	0	9	10	10
	10	3	0	0	7	10	10
0.065	10	1	0	5	10	10	10
(0.1)	10	2	0	4	10	10	10
	10	3	0	5	10	10	10
Toxic Standard (Dimethoate):							
Control	30	3*	-	-	-	0	0
0.1	30	3	-	-	-	0	0
0.15	30	3	-	-	-	21	25
0.20	30	3	-	-	-	29	29
0.40	30	3	-	-	-	30	30
0.80	30	3	-	-	-	30	30

* Mortality data for the toxic standard were not reported for each replicate, but as the sum of the three replicates.

Observations: By 48 hours, mortality in the 0.0009, 0.00254, and0.0062 µg a.i./bee treatment groups was 40, 57, 100%, respectively. The 0.012, 0.022, and 0.065 µg a.i./bee treatment groups had 100% mortality, compared to 0% mortality in the control group. The only other toxic effects at 48 hours were moderate(partial paralysis/ poor coordination), and only present at the 0.00254 and 0.0062 µg a.i./bee doses.

The mortalities at 48 hours for dimethoate reference doses of 0.1, 0.15, and 0.2 µg a.i./bee were 0, 83, and 97 %, respectively.

Mortality - Contact Test

Measured and (Nominal) Dosage (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead				
			Hour of Study				
			1	2	4	24	48
Control	10	1	0	0	0	0	0
	10	2	0	0	0	0	0
	10	3	0	0	0	0	1
0.00039	10	1	0	0	0	0	0
(0.00032)	10	2	0	0	0	0	0
	10	3	0	0	0	0	0
0.0019	10	1	0	0	0	0	0
(0.0016)	10	2	0	0	0	0	0
	10	3	0	0	1	1	1
0.0095	10	1	0	0	0	1	1
(0.008)	10	2	0	0	2	2	2
	10	3	0	0	0	0	0
0.046	10	1	0	0	2	5	6
(0.04)	10	2	0	0	2	2	2
	10	3	0	0	0	5	5
0.22	10	1	0	0	3	9	9
(0.2)	10	2	0	0	3	10	10
	10	3	0	0	3	9	9
1.14	10	1	0	3	5	10	10
(1.0)	10	2	0	0	10	10	10
	10	3	0	0	7	10	10
Toxic Standard (Dimethoate):							
Control	30	3*	-	-	-	4	4
0.1	30	3	-	-	-	0	0
0.15	30	3	-	-	-	0	0
0.2	30	3	-	-	-	1	2

Measured and (Nominal) Dosage (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead				
			Hour of Study				
			1	2	4	24	48
0.4	30	3	-	-	-	16	16
0.8	30	3	-	-	-	26	27

* Mortality data for the toxic standard were not reported for each replicate, but as the sum of the three replicates.

Observations: By 48 hours, mortality was 3, 10, 37, 93 and 100% in the nominal 0.0019, 0.0095, 0.046, 0.22, and 1.14 µg a.i./bee, respectively, compared to 3% in the control group. There were moderately affected bees in the 0.0019, 0.0095, 0.046, and 0.22 µg a.i./bee treatment groups. One bee had almost complete paralysis in the 0.22 µg a.i./bee treatment group after 48 hours.

Statistical method: The LD_{50} value was calculated using mortality data and the PROBIT procedure in SAS 6.10. The NOEL was determined by the lethal and sublethal effects using ANOVA, Dunnett's Test, and Levene's Test.

Reported Statistical Results - Oral Test:

LD_{50}: 0.00379 µg a.i./bee 95% C.I.: 0.00229 to 0.00513 µg a.i./bee
NOEL: 0.001024 µg a.i./bee Probit Slope: Not reported.

Reported Statistical Results - Contact Test:

LD_{50}: 0.04426 µg a.i./bee 95% C.I.: 0.02781 to 0.06532 µg a.i./bee.
NOEL: 0.008 µg a.i./bee Probit Slope: Not reported.

13. VERIFICATION OF STATISTICAL RESULTS:

Method: The NOEC and LOEC values were determined using Fisher's Exact Test. The LD_{50} for the oral test was determined using the moving average angle method and the LC_{50} for the contact test was determined using the probit method. Values for both tests were estimated using the measured concentrations and computed using ToxAnal software.

Results - Oral Test

LD_{50}: 0.00368 µg a.i./bee	95% C.I.: 0.00303-0.0045 µg a.i./bee
NOEL: 0.0009 µg a.i./bee	Probit Slope: N/A

Results - Contact Test:

LC_{50}: 0.0439 µg a.i./bee	95% C.I.: 0.0296-0.0652 µg a.i./bee
NOEL: 0.0095 µg a.i./bee	Probit Slope: 1.78

14. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with those of the study author; however, the estimated NOEC and LD_{50}/LC_{50} values were slightly different. Differences were caused by the fact that the reviewer used the measured concentrations to calculate these values, while the study author used the nominal concentrations. The LD_{50} value for the oral test was 0.00368 µg a.i./bee. The LD_{50} value for the contact test was 0.0439 µg a.i./bee. As a result, TI-435 Technical is categorized as highly toxic to bees on an acute oral and contact basis.

Incomplete consumption of the test material occurred in the three highest treatment groups in the oral test. As a result, the nominally consumed doses of TI-435 technical were 0.0133, 0.025, and 0.073 µg a.i./bee for the nominal 0.016, 0.04, and 0.1 µg a.i./bee concentrations, respectively (Table 5, p. 23). These concentrations correspond to measured concentrations of 0.012, 0.022, and 0.065 µg a.i./bee.

The reviewer calculated measured dosages in units of µg a.i./bee using measured concentrations of µg TI-435 Technical/mL, nominal concentrations (µg/L), nominal dosages (µg a.i./bee), and in some cases, nominally consumed doses (Table 5, p. 23).

For the oral toxicity test, the LD_{50} of the toxic standard, dimethoate, was 0.131 µg a.i./bee.

For the contact toxicity test, the LD_{50} of the toxic standard, dimethoate, was 0.438 µg a.i./bee.

The age and size of the bees was not reported. Failure to report this information did not impact the classification of this study. However, this information should be reported in future acute oral and contact studies.

15. REFERENCES:

European and Mediterranean Plant Protection Organisation (EPPO).1992, No. 170. Guideline on test methods for evaluating the side-effects of plant protection products on honey bees. EPPO Bulletin 22, 203-215.