

DATA EVALUATION RECORD
HONEY BEE - ACUTE ORAL LD₅₀ TEST
No OPP Guideline Applicable - Acute Oral

1. **CHEMICAL**: Clothianidin (TI-435)

PC Code No.: 044309

2. **TEST MATERIAL**: TI-435 Metabolite TZNG

Purity: 98.6%

3. **CITATION**:

Author: Wilkins, P.

Title: TI-435 Metabolite TZNG: Acute Oral Toxicity to Honey Bees
(*Apis mellifera*)

Study Completion Date: January 27, 2000

Laboratory: National Bee Unit
Central Science Laboratory
Sand Hutton, York YO41 1LZ, England

Sponsor: Takeda Chemical Industries Ltd
Development Department, Agro Company
13-10 Nihonbashi 2-chome, Chuo-Ku
Tokyo 103-8668, Japan

Laboratory Report ID: 110057

DP Barcode: D278110

MRID No.: 45422430

4. **Secondary Reviewer**: Gabe Patrick, Biologist, EPA/OPPTS/OPP/EFED/ERB5

Signature: Gabe Patrick

Date:

Secondary Reviewer: Mike Rexrode, Ph.D., Senior Scientist,
EPA/OPPTS/OPP/EFED/ERB5

Signature: M. Rexrode

Date: 3/5/03

5. **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*

Age or Size of Test Organism at Test Initiation: Adult worker bees, age not specified.

Type of Concentrations: Nominal

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

The honey bee, *Apis mellifera*, was exposed to TZNG, a metabolite of TI-435, for 48 hours at nominal consumed concentrations 0.89, 2.1, 3.1, 6.3, 16, and 36 µg a.i./bee. Percent mortality was 0, 20, 33, 93, 97, and 93% in the 0.89, 2.1, 3.1, 6.3, 16, and 36 µg a.i./bee treatment groups, respectively. Mortality in the control was 0 %. Response of mortality to TZNG followed a dose-dependent pattern. No sub-lethal effects were observed by 48 hours. **The LD₅₀ was 3.95 µg a.i./bee for the honey bee, *Apis mellifera*, on an acute oral basis. Significant mortality was shown at levels ≥2.1 µg a.i./bee, so the NOAEL was 0.89 µg a.i./bee.**

This study is scientifically sound and is classified as **Supplemental** for a non-guideline study.

Results Synopsis:

LD₅₀: 3.95 µg a.i./bee

NOAEL: 0.89 µg a.i./bee

95% C.I.: Could not be determined

Probit Slope: 2.72

8. ADEQUACY OF THE STUDY:

A. Classification: 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 (as compared to 141-1 or 850.3020) DEVIATIONS:

None noted.

- 10. SUBMISSION PURPOSE:** This study was submitted to provide data on the acute oral toxicity of the TI-435 metabolite, TZNG, to honey bees for registration of TI-435 Technical (Clothianidin).

11. MATERIALS AND METHODS:

A. Test Organisms

Criteria	Reported Information
Species: Species of concern (<i>Apis mellifera</i>)	<i>Apis mellifera</i>
Age at beginning of test:	Adult worker bees, age not specified.
Supplier:	Central Science Laboratory's National Bee Unit, York, England
All bees from the same source?	Yes, all bees from a single colony.

B. Test System

Criteria	Reported Information
Cage size adequate?	Cylindrical mesh cages, no size given.
Lighting:	Darkness, except during observation periods.
Temperature:	25 ± 1°C
Relative humidity:	65 ± 5%

C. Test Design

Criteria	Reported Information
Range finding test?	Bees were exposed to TZNG at nominal concentrations of 0.129, 1.29, 12.9, and 129 µg ai/bee., with mortality rates of 0, 0, 63, and 97%, respectively. The definitive nominal concentrations were based upon these results.
Reference toxicant test?	Dimethoate, at concentrations of 0.063, 0.125, 0.25, and 0.5 µg ai/bee; 3 replicates with 10 bees/replicate.
Method of administration:	- Mixed with diet (50% w/v aqueous sucrose) - Doses offered to bees within 2 h after preparation - 200 µL/treatment group offered in glass feeder
Nominal doses:	1.25, 2.5, 5, 10, 20, and 40 µg a.i./bee
Controls: Negative control and/or diluent/solvent control	50% w/v aqueous sucrose control.
Number of colonies per group:	3 replicates per treatment group, 10 bees per replicate.
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	50% w/v aqueous sucrose
Feeding:	Bees were starved for 1.5 to 2 hours prior to test initiation. After the first, four hour observations, the food solution containing TZNG was removed and fresh aqueous sucrose (50% w/v) was provided <i>ad libitum</i> .

Criteria	Reported Information
Observations period:	Mortality and behavior observations occurred after 4 hours of test substance introduction, and 24 and 48 hours after treated diet was removed from cages.

12. REPORTED RESULTS:

Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	0% mortality by 48 hours.
Raw data included:	Yes.
Signs of toxicity (if any) were described?	Knocked down (alive but immobile) and stumbling (moving but in a poorly coordinated manner) bees were observed early on, in both the range-finding and definitive tests at the higher concentrations. These sub-lethal effects disappeared by 24 hours in both tests.

Mortality - TZNG Oral Test

Dosage ^a (μ g ai/bee)	Mean Nominally Consumed Dosage (μ g ai/bee)	No. of bees	Rep.	Cumulative Number of Dead			% Mortality
				Hour of Study			
				4	24	48	
Negative Control	n/a	10. 10. 10.	1. 2. 3.	0. 0. 0.	0. 0. 0.	0. 0. 0.	0. 0. 0.
(1.25) 1.2 0.57 0.90	0.89	10. 10. 10.	1. 2. 3.	0. 0. 0.	0. 0. 0.	0. 0. 0.	0. 0. 0.
(2.5) 1.9 2.4 2.1	2.1	10. 10. 10.	1. 2. 3.	0. 0. 0.	2. 1. 3.	2. 1. 3.	20
(5) 2.9 3.0 3.4	3.1	10. 10. 10.	1. 2. 3.	0. 0. 0.	4. 2. 3.	4. 2. 4.	33
(10) 6.0 7.0 5.8	6.3	10. 10. 10.	1. 2. 3.	0. 0. 0.	10. 10. 8.	10. 10. 8.	93
(20) 16 17 15	16	10. 10. 10.	1. 2. 3.	0. 0. 0.	9. 10. 10.	9. 10. 10.	97
(40) 29 39 40	36	10. 10. 10.	1. 2. 3.	2. 7. 2.	9. 10. 9.	9. 10. 9.	93
Reference Toxicant (Dimethoate)							

Dosage ^a (μ g ai/bee)	Mean Nominally Consumed Dosage (μ g ai/bee)	No. of bees	Rep.	Cumulative Number of Dead			% Mortality
				Hour of Study			
				4	24	48	
Negative Control	n/a	10. 10. 10.	1. 2. 3.	0. 0. 0.	1. 0. 0.	1. 0. 0.	3
(0.063) 0.054 0.046 0.047	0.049	10. 10. 10.	1. 2. 3.	0. 0. 0.	0. 0. 0.	0. 0. 1.	3
(0.125) 0.067 0.10 0.096	0.088	10. 10. 10.	1. 2. 3.	0. 0. 0.	0. 5. 0.	0. 5. 0.	17
(0.25) 0.18 0.18 0.17	0.177	10. 10. 10.	1. 2. 3.	0. 0. 0.	9. 7. 5.	9. 7. 5.	70
(0.50) 0.26 0.32 0.38	0.32	10. 10. 10.	1. 2. 3.	0. 1. 0.	8. 10. 9.	8. 10. 9.	90

^a Nominal dosages are listed in parentheses. Nominally consumed dosages, based on percent diet consumed, are listed per replicate.

Note: There was a glitch in the above table whereby data numbers appear on a single line instead of separate lines. To prevent this I put a "." after each number.

Observations: Mortality was observed in the nominally consumed 2.1, 3.1, 6.3, 16, and 36 μ g a.i./bee treatment groups, at rates of 20, 33, 93, 97, and 93%, respectively. There was no mortality observed in the control group. No bees were observed to be in a state of knockdown or stumbling by 48 hours.

Percent mortality in the Dimethoate nominal 0.063, 0.125, 0.25, and 0.50 μ g a.i./bee groups was 3, 17, 70 and 90%, respectively, by 48 hours.

Statistical method: Mortality results were analyzed by the CSL probit programme (Probit 1, version 4). This program determined LD₅₀ values and corresponding 95% confidence intervals by probit analysis. The NOEL value was determined by mortality data. The study author determined these values based on the actual dosage concentrations.

Reported Statistical Results

LD ₅₀ : 3.9 µg a.i./bee	95% C.I.: 3.2-4.9 µg a.i./bee
NOEL: 0.89 µg a.i./bee	Probit Slope: 2.7

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Mortality data were visually inspected to determine the NOEC value, because there was no mortality in the control. The LD₅₀ was determined using the Probit method via ToxAnal software (see section 16). The data did not fit particularly well to the probit model, so 95% confidence intervals could not be estimated; however, the reviewer's LD₅₀ and probit slope estimates were identical to the study author's.

Verified Results

LD ₅₀ : 3.95 µg a.i./bee	95% C.I.: Could not be determined
NOAEL: 0.89 µg a.i./bee	Probit Slope: 2.72

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors. The TZNG LD₅₀ was 3.9 µg a.i./bee to the honey bee, *Apis mellifera*, on an acute oral basis. Significant mortality was shown at levels ≥ 2.1 µg a.i./bee, so the NOAEL was 0.89 µg a.i./bee.

The nominally consumed TZNG test concentration refers to the nominal dose consumed by the bees. The dose consumed was determined by the weight of dose remaining in the glass feeders after the 4 h treatment period and comparing this weight to the weight of the known volume of the test solutions at the beginning of the test. This is not a measured (analyzed) dose per se.

The toxicities values provided in this study by the author and reviewer were corrected for the actual purity of the TZNG test substance (98.6% ai) used in this study.

There was no data provided on the stability of the test substance in solution for this study. The test substance was assumed to be stable by the author and reviewer.

TZNG (a metabolite of clothianidin) was classified as "Moderately toxic" to honey bees on an acute oral basis by the author according to the following ICBB (1985) categorization:

> 100 µg a.i./bee	Virtually non-toxic
10-100 µg a.i./bee	Slightly toxic
1-10 µg a.i./bee	Moderately toxic
< 1.0 µg a.i./bee	Highly toxic

OPP does not have a categorization scheme for acute oral toxicity to honey bees.

This study was conducted in accordance with UK Good Laboratory Practice Regulations, USEPA Title 40 CFR 160, Japan Ministry of Agriculture, Forestry and Fisheries, and OECD Principles of Good Laboratory Practices.

15. REFERENCES:

1985. International Commission for Bee Botany Third Symposium on the "Harmonisation of methods for testing the toxicity of pesticides to bees".

1992. European and Mediterranean Plant Protection Organisation. "Guideline on test methods for evaluating the side-effects of plant protection products on honey bees" EPPO Bulletin 22, 203-215.

1996. Ministry of Agriculture, Fisheries and Food (UK), Pesticides Safety Directorate and the Health and Safety Executive, "The Registration Handbook Volumes 1 and 2, Pesticides, Biocides, Plant Protection Products, A guide to the policies, procedures and data requirements relating to their control within the United Kingdom".

1997. OECD (Draft April-Adopted 21/09/98). "OECD Guidelines for the testing of chemicals. Proposal for a new guideline 213. Honeybees, acute oral toxicity test."

16. Reviewer's Statistical Results

GP TZNG (CLOHIANIDIN METABOLITE) HONEYBEE ACUTE ORAL LD50 48h - TZNG

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
36	30	28	93.33334	4.339964E-05
16	30	29	96.66666	2.8871E-06
6.3	30	28	93.33334	4.339964E-05
3.1	30	10	33.33334	4.936858
2.1	30	6	20	7.154533E-02
.89	30	0	0	9.313227E-08

THE BINOMIAL TEST SHOWS THAT 2.1 AND 6.3 CAN BE
 USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
 CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
 ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.695539

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	2.838178E-02		4.159674	3.387419

5.074982

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	1.145468	8.78215

GOODNESS OF FIT PROBABILITY
 0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
 USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.722092
 95 PERCENT CONFIDENCE LIMITS = -.1912687 AND 5.635453

LC50 = 3.947835
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.348361
 95 PERCENT CONFIDENCE LIMITS = 0 AND 3.247872

EAD Assessment of USEPA DER

Reviewer: Valerie Hodge

Date: November 8, 2002

PMRA Submission Number: 2001-1293

Study Type: TI-435 Metabolite TZNG: Acute Oral LD₅₀ - Honeybee [*Apis mellifera*], PMRA DATA CODE 9.2.4.2, EPA MRID Number 45422430, OECD Data Point IIA 8.7.1, EPA Guideline - none.

Reviewing Agency: U.S. EPA**EAD Summary:**

The honey bee, *Apis mellifera*, was exposed to TZNG, a metabolite of clothianidin (TI-435), for 48 hours (4 hour exposure followed by observation to 48 hours) at nominal consumed doses of 0.89, 2.1, 3.1, 6.3, 16, and 36 µg a.i./bee. Mean percent mortality at these doses was 0, 20, 33, 93, 97, and 93%, respectively. Mortality in the control was 0%. Bee mortality from exposure to TZNG followed a dose-dependent pattern. No sub-lethal effects were observed by 48 hours. **The acute oral LD₅₀ was 3.95 µg a.i./bee for the honey bee, *Apis mellifera*. The NOEL, based on mortality, was 0.89 µg a.i./bee.** The LD₅₀ value was corrected for the actual purity of the test substance (98.6% ai) used in this study. TZNG is "moderately toxic" to bees by ingestion.

Material and Methods:

Doses of TZNG were prepared in 50% w/v aqueous sucrose. Adult worker bees (*Apis mellifera* L.), 3 replicates of 10 per treatment and control, were exposed in mesh cages to a given concentration (or control) of TZNG for 4 hours (200 µL per 10 bees in a glass feeder). After 4 hours, clean feeding solution replaced treatment solutions for the remainder of the study period. Nominal doses were 1.25, 2.5, 5, 10, 20, and 40 µg a.i./bee. Actual nominal doses were determined by weighing the feeding solution before and after exposure to bees, and determining a mean dose per bee. Actual mean nominal doses were 0.89, 2.1, 3.1, 6.3, 16, and 36 µg a.i./bee. Observations for mortality and behavior (knockdown or stumbling) were made at 4 hours after test was initiated, then 24 and 48 hours after treated diet was removed from cages.

Bees were exposed to dimethoate, as a reference toxicant, at nominal dose of 0.063, 0.125, 0.25, and 0.50 µg a.i./bee.

Results:

There was 0% mortality in the control groups. Percent mean mortality in the TZNG actual nominal 0.89, 2.1, 3.1, 6.3, 16, and 36 $\mu\text{g a.i./bee}$ treatment groups was 0, 20, 33, 93, 97, and 93%, respectively. Bee mortality from exposure to TZNG followed a dose-dependent pattern. The NOEL was based on mortality.

Significant knockdown was observed for bees exposed to doses of 16 and 36 $\mu\text{g ai/bee}$ at 4 hours. Most bees in these groups had died by 24 hours. No sub-lethal effects of TZNG were observed for any living bees in other dosing groups at 24 or 48 hours.

Reported Statistical Results

LD₅₀: 3.9 $\mu\text{g a.i./bee}$ 95% C.I.: 3.2-4.9 $\mu\text{g a.i./bee}$
NOEL: 0.89 $\mu\text{g a.i./bee}$ Probit Slope: 2.7

Verified Results

LD₅₀: 3.95 $\mu\text{g a.i./bee}$ 95% C.I.: Could not be determined
NOAEL: 0.89 $\mu\text{g a.i./bee}$ Probit Slope: 2.72

Verified results agreed with statistical results reported by the study author.

Results for dimethoate toxicity were consistent with previous studies. Percent mean mortality in the dimethoate (actual) nominal 0.049, 0.088, 0.18, and 0.32 $\mu\text{g a.i./bee}$ groups was 3, 17, 70, and 90%, respectively, by 48 hours (48 hour LD₅₀, 0.15 $\mu\text{g dimethoate/bee}$).

EAD comments:

The EAD evaluator agrees with the conclusions reached by the U.S. EPA evaluator.

EAD Conclusion:

Based on the results of this study, and the criteria of the International Commission for Bee Botany (1985), TZNG is "moderately toxic" to bees by ingestion.

Reference: 1985. International Commission for Bee Botany Third Symposium on the "Harmonization of methods for testing the toxicity of pesticides to bees".

Signatures:

Primary Reviewer: Valerie Hodge

Date: November 8, 2002

Secondary Reviewer: Hemendra Mulye

Date: