DATA EVALUATION RECORD
HONEY BEE - ACUTE ORAL LD_{50} TEST
No OPP Guideline Applicable - Acute Oral

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

2. **TEST MATERIAL:** TI-435 Metabolite TZMU Purity: 98.8%

3. **CITATION:**

Author: Wilkins, P.

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

Study Completion Date: January 27, 2000

Laboratory: National Bee Unit
Central Science Laboratory
Sand Hutton, York Y041 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: GQ3202(Study Number); 110055(Report Number)

DP Barcode: D278110

MRID No.: 45422429

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, EPA/OPPTS/OPP/EFED/ERB5
Signature: Gabe Patrick **Date:** 3/5/03

Secondary Reviewer: Mike Rexrode, Ph.D., Senior Scientist, EPA/OPPTS/OPP/EFED/ERB5
Signature: M. Rexrode **Date:** 3/5/03

Secondary Reviewer: Valerie Hodge, MSc, Senior Evaluation Officer
Environmental Assessment Division, PMRA
Signature: Valerie Hodge **Date:** 3/20/03

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 orally to TZMU, a metabolite of TI-435, for 48 hours. Mean mortality was 10%, 0%, 0%, and 7% in the 0.16, 1.6, 16, and 113 µg a.i./bee treatment groups, respectively. Mortality in the control was 0%. Mortalities did not follow a dose-dependent pattern. There were no sub-lethal effects of TMZU observed for any bees at 48 hours. **The TZMU LD_{50} was >113 µg a.i./bee,** to the honey bee, *Apis mellifera*, on an acute oral toxicity basis. The NOAEL was ≥113 µg a.i./bee. These toxicity values are based on analyzed content of TZMU in the formulation [i.e., values were corrected for the actual purity of the test substance (98.8% ai) used in this study].

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

Results Synopsis:

LD_{50}: >113 µg a.i./bee	95% C.I.: N/A
NOAEL: ≥113 µg a.i./bee	Probit Slope: N/A

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 TZMU, a metabolite of TI-435 for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Criteria	Reported Information
Species: Species of concern (*Apis mellifera)*	*Apis mellifera*
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 TZMU at nominal concentrations of 0.109, 1.09, 10.9, and 109 µg ai/bee., with mortality rates of 7%, 13%, 3%, and 7%, respectively. The definitive nominal concentrations were based on 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:	0.163, 1.63, 16.3, and163 µg a.i./bee
Controls: Negative control and/or diluent/solvent control	Negative 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 first four-hour observations, the food solution containing TZMU was removed and fresh aqueous sucrose (50% w/v) was provided *ad libitum*.

Criteria	Reported Information
Observations period:	Mortality and behavior observations: 4 hours after test was initiated, then 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 co-ordinated manner) bees were observed early on, in both the range-finding and definitive tests. These sub-lethal effects disappeared by 24 hours in both tests.

Mortality - TZMU Oral Test

Dosage[a] (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead		
			Hour of Study		
			4	24	48
Negative Control	10.	1.	0.	0.	0.
	10.	2.	0.	0.	0.
	10.	3.	0.	0.	0.
(0.163)					
0.16	10.	1.	0.	2.	2.
0.16	10.	2.	0.	0.	0.
0.16	10.	3.	0.	1.	1.

Dosage[a] (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead		
			Hour of Study		
			4	24	48
(1.63)					
1.6	10.	1.	0.	0.	0.
1.6	10.	2.	0.	0.	0.
1.6	10.	3.	0.	0.	0.
(16.3)					
16	10.	1.	0.	0.	0.
16	10.	2.	0.	0.	0.
16	10.	3.	0.	0.	0.
(163)					
119	10.	1.	0.	1.	2.
104	10.	2.	0.	0.	0.
117	10.	3.	0.	0.	0.
Reference Toxicant (Dimethoate)					
Negative Control	10.	1.	0.	0.	0.
	10.	2.	0.	0.	0.
	10.	3.	0.	0.	0.
(0.063)					
0.061	10.	1.	0.	2.	2.
0.063	10.	2.	0.	1.	1.
0.057	10.	3.	0.	1.	1.
(0.125)					
0.12	10.	1.	0.	2.	2.
0.11	10.	2.	0.	1.	1.
0.12	10.	3.	0.	4.	4.
(0.25)					
0.23	10.	1.	0.	10.	10.
0.19	10.	2.	0.	3.	3.
0.21	10.	3.	0.	6.	6.

Dosage[a] (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead		
			Hour of Study		
			4	24	48
(0.50)					
0.43	10.	1.	0.	10.	10.
0.35	10.	2.	0.	9.	10.
0.41	10.	3.	0.	8.	9.

[a] Nominal dosages are listed in parentheses. Nominally consumed dosages, based on percent diet consumed, are listed for each 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 0.163 and 163 µg a.i./bee treatment groups, at rates of 10 and 7%, respectively. There was no mortality observed in the control or the 1.63 and 16.3 µg a.i./bee treatment groups. No bees were observed to be knockdown or stumbling by 48 hours.

Percent mortality in the Dimethoate nominal 0.063, 0.125, 0.25, and 0.50 µg a.i./bee groups was 13, 23, 63 and 97%, respectively, by 48 hours.

Statistical method: The LD_{50} value and the NOEL value were based on mortality data, since no treatment group exceeded 50% mortality. The study author determined these values based on the nominally consumed dosage concentrations.

Reported Statistical Results

LD_{50}: >113 µg a.i./bee 95% C.I.: N/A
NOEL: ≥113 µg a.i./bee Probit Slope: N/A

13. VERIFICATION OF STATISTICAL RESULTS:

Method: The mortality data were visually inspected to determine the NOAEL and LD_{50}. The LD_{50} could not be determined because mortality did not equal or exceed 50%..

Verified Results
LD_{50}: >113 µg a.i./bee 95% C.I.: N/A

NOAEL: ≥113 µg a.i./bee Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. The TZMU LD_{50} was >113 µg a.i./bee to the honey bee, *Apis mellifera*, on an acute oral toxicity basis. The TZMU NOAEL was ≥ 113 µg a.i./bee.

The study author reported that in both the TZMU range and main test when the stock solutions were made up, after sonication one or two very small crystals of test substance remained undissolved. These were so small that it was considered to have no effect on the results (p. 15). The reviewer also concluded that the presence of undissolved test material in stock solutions did not impact the acceptability of the study.

The nominally consumed TZMU 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 TZMU test substance (98.8% 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.

TZMU (a metabolite of clothianidin) was classified as "Virtually non-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."

EAD Assessment of USEPA DER

Reviewer: Valerie Hodge Date: November 7, 2002

PMRA Submission Number: 2001-1293

Study Type: TI-435 Metabolite TZMU: Acute Oral LD_{50} - Honeybee [*Apis mellifera*], PMRA DATA CODE 9.2.4.2, EPA MRID Number 45422429, 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 TZMU, a metabolite of clothianidin (TI-435), for 48 hours (4 hour exposure followed by observation to 48 hours) at nominal consumed doses of 0.16, 1.6, 16, and 113 µg a.i./bee treatment groups. Mean percent mortality was 10%, 0%, 0%, and 7%, respectively. Mortality in the control was 0%. Mortalities did not follow a dose-dependent pattern. There were no sub-lethal effects of TMZU observed for any bees at 48 hours. The acute oral LD_{50} was >113 µg a.i./bee. The NOEL was ≥113 µg a.i./bee (based on mortality and sublethal effects). TMZU is "virtually non-toxic" to bees by ingestion.

Material and Methods:

Doses of TZMU 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 TZMU 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 0.163, 1.63, 16.3, and 163 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 0.16, 1.6, 16, and 113.3 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 also exposed to dimethoate, as a reference toxicant, at nominal doses 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 TZMU

nominal 0.16, 1.6, 16, and 113.3 µg a.i./bee groups was 10, 0, 0 and 7%, respectively, by 48 hours. Mortalities did not follow a dose-dependent pattern. The NOEL was based on mortality and sublethal effects.

Only 5 bees in total, from various treatment groups, were observed to be knocked down at 4 hours. There were, however, no sub-lethal effects of TZMU observed for any bees at 24 or 48 hours.

Statistical Results

LD_{50}: >113 µg a.i./bee	95% C.I.: N/A
NOAEL: ≥113 µg a.i./bee	Probit Slope: N/A

Results for dimethoate toxicity were consistent with previous studies. Percent mean mortality in the dimethoate (actual) nominal 0.069, 0.12, 0.21, and 0.40 µg a.i./bee groups was 13, 23, 60 and 97%, respectively, by 48 hours (48 hour LD_{50}, 0.16 µg 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), TZMU is "virtually non-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 7, 2002

Secondary Reviewer: Hemendra Mulye Date: