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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 MNG Purity: 99.2%

3. **CITATION:**

Author: Wilkins, P.

Title: TI-435 Metabolite MNG: 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: GQ3203(Study Number); 110056(Report Number)

DP Barcode: D278110

MRID No.: 45422428

4. **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

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

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

2006172

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 MNG, a metabolite of Clothianidin (TI-435), for 48 hours. There was 0% mortality in the control and treatment groups. There were no sub-lethal effects observed at any observation period for any bees exposed to MNG ~~at 48 hours~~. **The LD_{50} was >153 µg a.i./bee** (highest dose tested) to the honey bee, *Apis mellifera*, on an acute oral toxicity basis. The NOAEL ≥ 153 µg a.i./bee. These toxicity values are based on analyzed content of MNG in the formulation [i.e., values were corrected for the actual purity of the test substance (99.2% ai) used in this study].

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

Results Synopsis:

Results - Oral Test (48 h):

LD_{50}: >153 µg a.i./bee (highest dose tested)	95% C.I.: N/A
NOAEL: ≥153 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: NA

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 MNG, 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 MNG at nominal concentrations of 0.109, 1.09, 10.9, and 109 µg ai/bee, with mortality rates of 3, 3, 0, and 0%, respectively. The definitive nominal concentrations were based upon these results.

Criteria	Reported Information
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:	0.181, 1.81, 18.1, and 181 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 MNG was removed and fresh aqueous sucrose (50% w/v) was provided *ad libitum*.
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. <u>REPORTED RESULTS:</u>

Guideline 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) bees were observed early on in the range-finding test. This sub-lethal effects disappeared by 24 hours. No signs of toxicity were present in the definitive test.

Mortality - 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.
(0.181)					
0.18	10.	1.	0.	0.	0.
0.18	10.	2.	0.	0.	0.
0.18	10.	3.	0.	0.	0.
(1.81)					
1.8	10.	1.	0.	0.	0.
1.8	10.	2.	0.	0.	0.
1.6	10.	3.	0.	0.	0.
(18.1)					
18	10.	1.	0.	0.	0.
18	10.	2.	0.	0.	0.
18	10.	3.	0.	0.	0.

Dosage[a] (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead		
			Hour of Study		
			4	24	48
(181)					
171	10.	1.	0.	0.	0.
158	10.	2.	0.	0.	0.
131	10.	3.	0.	0.	0.

Dosage[a] (µg ai/bee)	No. of bees	Rep.	Cumulative Number of Dead		
			Hour of Study		
			4	24	48
Reference Toxicant (Dimethoate)					
Negative Control	10. 10. 10.	1. 2. 3.	0. 0. 0.	0. 0. 0.	0. 0. 0.
(0.063) 0.061 0.063 0.057	 10. 10. 10.	 1. 2. 3.	 0. 0. 0.	 2. 1. 1.	 2. 1. 1.
(0.125) 0.12 0.11 0.12	 10. 10. 10.	 1. 2. 3.	 0. 0. 0.	 2. 1. 4.	 2. 1. 4.
(0.25) 0.23 0.19 0.21	 10. 10. 10.	 1. 2. 3.	 0. 0. 0.	 10. 3. 6.	 10. 3. 6.
(0.50) 0.43 0.35 0.41	 10. 10. 10.	 1. 2. 3.	 0. 0. 0.	 10. 9. 8.	 10. 10. 9.

[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: There was no mortality observed in the control or the MNG treatment groups. No bees were observed to be knocked down or stumbling at any of the observation times in the main oral dosing test ~~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 MNG LD_{50} value and the NOEL value were determined visually 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}: >153 µg a.i./bee 95% C.I.: N/A
NOEL: ≥153 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}.

Verified Results
LD_{50}: >153 µg a.i./bee 95% C.I.: N/A
NOAEL: ≥153 µg a.i./bee Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. The MNG LD_{50} was >153 µg a.i./bee to the honey bee, *Apis mellifera*, on an acute oral toxicity basis. The NOAEL ≥ 153 µg a.i./bee.

The nominally consumed 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 test substance (99.2% 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.

MNG (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. OPP does not have or require an acute oral LD_{50} study for honey bees.

15. REFERENCES:

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

1992. European and Mediterranean Plant Protection Organization. "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."

DP Barcode: D278110 MRID No.: 45422428

EAD Assessment of USEPA DER

Reviewer: Valerie Hodge Date: November 7, 2002

PMRA Submission Number: 2001-1293

Study Type: TI-435 Metabolite MNG: Acute Oral LD_{50} - Honeybee [*Apis mellifera*], PMRA DATA CODE 9.2.4.2, EPA MRID Number 45422428, 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 MNG, a metabolite of clothianidin (TI-435), for 48 hours (4 hour exposure followed by observation to 48 hours) at mean nominal doses of 0.18, 1.7, 18, and 153 µg a.i./bee. There was 0% mortality in the control and treatment groups. There were no sub-lethal effects observed at any observation period for any bees exposed to MNG. The acute oral LD_{50} was >153 µg a.i./bee (highest dose tested) to the honey bee, *Apis mellifera*. The NOEL was ≥ 153 µg a.i./bee (based on mortality and sublethal effects). These toxicity values were corrected for the actual purity of the test substance (99.2% ai) used in this study. MNG is "virtually non-toxic" to bees by ingestion.

Material and Methods:

Doses of MNG 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 MNG 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 0.181, 1.81, 18.1, and 181 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. Doses were also corrected for the purity of the test substance (99.2% ai). Actual mean nominaldoses were 0.18, 1.7, 18, and 153 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 and treatment groups at all observation times. There were no sub-lethal effects observed at any observation period for any bees exposed to MNG.

Statistical Results

LD_{50}: >153 µg a.i./bee	95% C.I.: N/A
NOEL: ≥153 µg a.i./bee	Probit Slope: N/A

Verified statistical results agreed with reported results.

Results for dimethoate toxicity were consistent with previous studies. Percent mean mortality in the dimethoate (actual) nominal 0.06, 0.12, 0.21, and 0.40 µg a.i./bee groups was 13, 23, 63 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), MNG 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: