DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS, §141-5

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

2. **TEST MATERIAL:** TI-435 Technical Purity: 100%

3. **CITATION:**

Author:	Ch. Maus and R. Schöning
Title:	Effects of Diet (Sugar Solution) Spiked with TI-435 Technical on Behavior and Mortality of Honey Bees (*Apis mellifera*)
Study Completion Date:	January 18, 2001
Laboratory:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Sponsor:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Laboratory Report ID:	110294
DP Barcode:	D278110
MRID No.:	45422434

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: Hemendra Mulye, PhD
Senior Evaluation Officer
Environmental Assessment Division, PMRA

Signature: **Date:**

Secondary Reviewer: Linda Toy, MSc
Environmental Assessment Division, PMRA

Signature: Linda Toy **Date:** March 20/03

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: 14 days

7. **CONCLUSIONS:** This study is not scientifically sound. The purpose of this study was to determine the effect of TI-435 residues in a diet (sugar solution) offered to bees on bee mortality and behavior and on the development of bee colonies. Measured concentrations were 11.8 µg/kg and 25.0 µg/kg and nominal concentrations were 10 µg/kg and 20 µg/kg, respectively. Beehives were placed in open (uncaged) fields and the distance between fields (e.g., control and treatment) was not provided. As a result, the reviewer is not convinced that bees foraged in the plots that they were intended to forage in. There were no apparent treatment-related effects on weight gain of the hives, syrup consumption, average number of marked and unmarked bees at the feeder, and average number of marked bees arriving at the hive. While mortality in the 25.0 µg/kg treatment group was notably higher than mortality in the control group, these data were reportedly unreliable because wasps were observed removing dead bees from the study plots.

The study is not scientifically sound because measures were not taken to ensure that bees remained in intended plots and mortality data were unreliable because wasps were observed removing dead bees from the study plots. The results of this study are unreliable and this study is classified as INVALID.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Invalid

B. Rationale: Beehives were placed in open (uncaged) fields and the distance between fields (e.g., control and treatment) was not provided. As a result, the reviewer is not convinced that bees foraged in the plots that they were intended to forage in. Furthermore, mortality data were unreliable because wasps were observed removing dead bees from the study plots.

C. Repairability: None. Additional evidence should have been provided to support the claim that bees allocated to a given plot remained foraging in that plot and measures should have been taken to prevent wasps from preying on dead bees.

9. GUIDELINE DEVIATIONS:

1) Bees were not caged onto study plots and the distance between study plots was not specified.

~~2) Aqueous sucrose solution is not a recommended solvent.~~ - gp

2) This study was conducted without a prior agreed upon protocol between the registrant and the Agency. - gp

10. SUBMISSION PURPOSE: This study was submitted to evaluate the effect of TI-435 residues in a diet (sugar solution) offered to bees on bee mortality and behavior and on the development of bee colonies under field conditions.

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:	Commercial colonies with all life-stages present
Supplier	Mr. Joseph Gilli, Reinartzstrasse 25, 53925 Kall
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	N/A. Beehives were placed on un-tented plots 165 m from a feeder containing spiked or untreated syrup.

Guideline Criteria	Reported Information
Lighting:	On the day after 1st exposure (Day 0), the sky was mostly cloudy. Days 1, 2, and 3, were sunny and Day 4 was cloudy (p.8).
Temperature: Control 10 µg/kg 20 µg/kg	 9.9-26.6°C 10.6-29.6°C 9.1-26.3°C
Relative humidity:	Not reported.
Precipitation:	A total of 8 mm 12 mm, 11 mm of rain was recorded in the control, 11.8, and 25.0 µg/kg treatment groups, respectively (pp. 33-35).
Site Characterization:	• The trial sites were located in the vicinity of Weilerswist, Germany. • The control group was placed on a beet field near Metternich (Field no. 1), the 11.8 µg/kg treatment group was placed on a reaped cereal field near Klein-Vernich (Field no. 2), and the 25.0 µg/kg treatment group was placed on a reaped cereal field near Lommersum (Field no. 3). • The study plots were located a "sufficient distance" from one another (and from other beehives) to make sure that no bees which did not belong to the respective test unit would feed on the respective feeder. • On study plots, there were no other food sources (e.g., flowers) which could be used by the bees.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No
Reference toxicant tested?	No, a reference compound was not specified for this type of material and use pattern (p. 6).
Preparation of the Food Substance:	• The test substance was presented to bees in a syrup solution which was prepared by dissolving 1 kg sugar in 1 L water (1:1). • A stock solution (1.04 mg TI-435/L) was prepared, which was used to produce the nominal 10 and 20 µg/kg treatment solutions. The control group received untreated syrup.

Guideline Criteria	Reported Information
Method of administration:	• Bee colonies were randomly assigned to one of the three study plots on 8/28/00. • Beehives were placed approximately 165 m from syrup feeders which contained either untreated or spiked food as a food source for bees. • Feeders consisted of a bottle filled with syrup and placed upside-down on a ground plate with three channels into which syrup was released from the bottle. • Feeders were replenished with syrup during the daily assessment period whenever syrup in a feeder was consumed by bees. • Every morning (9:00 a.m. or later), 50 of the first bees at the feeders were marked on the thorax with a day-specific color using water-unsoluble dye.
Analytical determination of test substance:	Syrup samples offered to the bees were analyzed by HPLC-MS/MS to verify test substance concentrations. Results are provided in Appendix IV.
Definitive Test Sufficient number of time periods to yield statistically sound data.	Colonies were monitored for 4 days.
Controls: Negative control and/or diluent/solvent control	Negative Control; the control group was fed untreated syrup.

Guideline Criteria	Reported Information
Number of colonies per group:	One colony (beehive with approx. 5000 - 10000 bees) per treatment and control group.
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	50% w/v aqueous sucrose
Feeding:	Prepared sucrose solutions was reportedly the only food source. Feeders were replenished daily during observations, if needed.

Guideline Criteria	Reported Information
Observation Periods:	• All assessments were made between 9:00 a.m. and 4:15 p.m.
Foraging activity:	• The number of marked bees and unmarked bees found close to the feeders was recorded every 15 minutes, daily. Where the total number of bees exceeded about 40, individual counting was not possible and the approximate number of bees was estimated. • Between the 15 minute assessments of bees foraging on or near the feeders, the number of bees approaching the hive was recorded for a 1 minute interval.
Syrup consumption:	• The syrup feeders were weighed before and after replenishing the syrup. The difference between these weights was assumed to be the quantity of consumed syrup. Additionally, the feeders were weighed at each assessment of the number of bees foraging at the syrup source. • Number of marked bees returning to hives recorded between feeder assessments (1 minute interval).
Mortality:	• Mortality was assessed in front of bee hives (on 1 x 1 m cotton sheets) daily; however, this number was not accurate because dead bees were preyed on by wasps. Dead bees found at the feeders were also recorded.
Colony Weight:	• Hives weighed daily to determine weight development of the colony.
Behavioral Anomalies:	• Exaggerated motility, discoordinated movements, and apathy were recorded

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	A total of 18 bees died from the control colony of 5,000-10,000 bees. However, counts were not reliable because wasps were observed removing dead bees.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Foraging activity and orientation around feeders and hives were reported. Behavioral anomalies (exaggerated motility, discoordinated movements, apathy, and lethargy) were noted when observed.

Mortality (# of dead bees found in front of hives)*

Group Nominal (Mean measured) concentrations (µg/kg)	Day After First Exposure					
	0	1	2	3	4	Total
Control	0	3	5	6	4	18
10 (11.8)	0	4	2	3	4	13
20 (25.0)	0	6	8	10	9	33

* Mortality data are not reliable because wasps were observed removing dead bees.

Weight Development of Bee Colonies(kg)

Group Nominal (Mean measured) concentrations (µg/kg)	Day After First Exposure						
	0	1	2	3	4	5	Total weight gain
Control	29.5	30.3	31.1	32.1	32.5	32.6	3.1
10 (11.8)	29.4	30.2	31.8	33.0	33.4	34.1	4.7
20 (25.0)	30.6	31.3	32.5	33.3	33.9	33.2	2.6

Syrup Consumption(g)

Group Nominal (Mean measured) concentrations (µg/kg)	Day After First Exposure					
	0	1	2	3	4	Total
Control	630	1235	1900	1874	543	6182
10 (11.8)	627	2308	2453	2479	1130	8996
20 (25.0)	639	2310	2419	2419	549	8336

Flight Activity (total # of marked bees returning to hives)

Group Nominal (Mean measured) concentrations (µg/kg)	Day After First Exposure					
	0	1	2	3	4	Total
Control	6	26	32	24	6	94
10 (11.8)	3	10	4	11	14	42
20 (25.0)	5	63	75	79	7	229

Foraging Activity (total # of bees at feeder during assessments)

Group Nominal (Mean measured) concentrations (µg/kg)	Day After First Exposure					
	0	1	2	3	4	Total
Control	17	274	640	626	285	1842
10 (11.8)	19	335	450	548	175	1527
20 (25.0)	28	267	513	422	186	1416

Diet Residue Analysis (µg/kg TI-435)[a]

Group	Residues		
	Measured	% Theoretical	Mean
Control	< 0.3	0	0
10 µg/kg	11.9 11.7	119 117	11.8

Group	Residues		
	Measured	% Theoretical	Mean
20 µg/kg	23.6 26.4	118 132	25.0

[a] Limit of quantitation (LOQ): 1 µg/kg
[b] Limit of Detection: 0.3 µg/kg

Reported Statistical Results: No behavioral anomalies were observed. The study authors reported that the increased mortality in the highest treatment group, 20 µg/kg, was likely to lie within the range of natural variation and that the values recorded were not reliable because wasps were observed removing dead bees from sheets in front of hives (p. 10). The study authors reported that weight gain and syrup consumption varied between the control and treatment groups and that dose-related effects were not observed. The average number of bees recorded at feeders was higher in the treatment groups than in the control and the average number of marked bees arriving at beehives or feeding on syrup did not exhibit a dose-dependent response. Statistical analyses were not conducted.

13. VERIFICATION OF STATISTICAL RESULTS:

The reviewer considered the results of this study to be unreliable. This and the experimental design (only one group per treatment) precluded the verification of results through statistical analysis.

14. REVIEWER'S COMMENTS:

The study is not scientifically sound because measures were not taken to ensure that bees remained in intended plots and mortality data were unreliable because wasps were observed removing dead bees from the study plots. The results of this study are unreliable and this study is classified as INVALID.