DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS, §141-5 or 850.3040

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

2. **TEST MATERIAL:** TI-435 Purity: 607.2 g/L (~ 5.2 lb ai/gal)(test substance) or 48.0% clothianidin (according to labeling provided separately from study)

3. **CITATION:**

Author:	Ch. Maus and R. Schöning
Title:	Residues Levels of TI-435 FS 600 and its Relevant Metabolites in Nectar, Blossoms, and Pollen of Summer Rape from Dressed Seeds and Effects of These Residues on Foraging Honeybees (Test Location: Farmland "Höfchen")
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:	110177
DP Barcode:	D278110
MRID No.:	45422437

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. **REVIEWED BY:** Gabe Patrick, Biologist, EPA/OPPTS/OPP/EFED/ERB5

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

APPROVED BY: Allen Vaughan, Entomologist, EPA/OPPTS/OPP/EFED/ERB5

Signature: Allen W. Vaughan **Date:** 03/04/03

6. **APPROVED BY:** Hemendra Mulye, PhD, Senior Evaluation Officer, Health Canada, Pest Management Regulatory Agency, Environmental Assessment Division, Environmental Fate and Effects
Signature: **Date:** March 19/03

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: 22 days (field sampling and observations)

7. **CONCLUSIONS:** This field study determined the residue levels of TI-435 in the nectar and pollen of seed-treated rape plants. The TI-435 treated rape seeds were treated at an application rate of 1056 g a.i./100 kg seed (1 lb ai/100 lb seed or 0.025 lb ai/A) on 4/28/00 (plant date). The treatment exposure levels from the samples, indicated below, were a result of levels found in samples taken from 6/30 through 7/18/00 over 2 months after the seed treatment application of TI-435 FS 600.

nectar from rape flowers: 5.4 µg ai/kg and 1.0 µg ai/kg (sampled 6/30/00 and 7/6/00, respectively)
pollen from rape flowers sampled from combs/forage bees: 1.9 to 2.5 µg ai/kg (combs sampled 7/12/00; forage bees sampled on 7/2 and 7/18/00)

There were no TI-435 levels of detection in the control. The metabolites of TI-435, TZMU and TZNG, were not detected in any of the nectar or pollen samples taken. Male and female blossoms were sampled from summer rape plants on Day 9 of the sampling period (7/5/00). However, these blossoms were not analyzed since nectar and pollen analysis was considered to be sufficient to detect residues of the test material. This was a deviation of the original study plan (p. 23 of the study).

Honey bee mortality in the controls was higher than in the treatment exposed honey bees however there was not a significant difference based on visual inspection of the data. Furthermore, there were no significant effects of TI-435 on the weight development of the beehives or foraging activity based on visual inspection of the data. It should be noted that with the exception of the residue samples found in the rape nectar and the residues detected in the pollen sampled, the results from other parameters measured (i.e., bee foraging behavior and the weight development of the beehives) are questionable due to the adverse weather conditions during the sampling period. There appeared to be an unusual amount of rainfall (6.2 inches in July, 2000) during most of the sampling period which would have restricted normal bee flight and foraging activity. It is also not clear whether or not the colonies used in this study were queen right. From the explanation provided on page 8 of this study, dealing with the hive preparation of the colonies used, it could readily be assumed that the colonies used in this study were queenless. The use of queenless, undersized colonies (2,000 - 3,000 workerbees)[1] would have provided additional factors

[1] Normal honey bee colony size is approximately 50,000 bees (Pacific Northwest Extension.1993).

that would make the results from the parameters measured questionable.

This toxicity study is scientifically sound, in that it determined the residue levels of TI-435 and its relevant metabolites in the pollen and nectar of seed-treated summer rape plants. The study is classified as Supplemental because this study was conducted without a prior agreed upon protocol between the registrant and the Agency as required by guideline 141-5. The information that it provides, however, may be useful for risk assessment purposes.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: These studies are only required on a case-by-case basis. A protocol was not approved by EPA for this insect field study as required by the guideline.

C. Repairability: None

9. **GUIDELINE DEVIATIONS:** N/A

10. **SUBMISSION PURPOSE:** This study was submitted to evaluate the exposure and residual toxicity of TI-435 to honey bees under field conditions.

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera*)	*Apis mellifera*
Age at beginning of test:	Commercial colonies with all life-stages present
Supplier	German beekeeper, Mr. Josef Gilli, Reinatzstrasse 25, 53925 Kall
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Small beehives (~2000-3000 honeybees) were caged on flowering rape plots using 10 x 5 x 3 m^3 tents. Tents consisted of an aluminum frame covered by gauze material (2 x 2 mm^2 mesh size).
Lighting:	Percent cloudiness ranged from 80-100% during the study period (6/27 - 7/18/00).
Temperature:	Month of June- minimum air temp: 5.0-19.0°C, maximum air temp: 16.2-34.0°C, soil temperature: 11.9-30.6°C Month of July- minimum air temp: 8.4-15.7°C, maximum air temp: 14.9-26.6°C, soil temperature: 12.0-21.1°C
Relative humidity:	Not reported
Precipitation:	A total of 64.5 mm (2.5 in.) of rain was recorded during the month of June and 157.6 mm (6.2 in.) during the month of July (p. 10). Average rainfall for April and May (prior to observation and sampling period) was 46.9 mm (1.8 in.)
Wind speed:	Ranged from calm to moderate (p. 11).
Site Characterization:	• The trial site was located at Bayer AG's experimental farmland "Höfchen", approximately 1 km from Burscheid (Bergisches Land Germany, 205 m above sea level) • The control and treatment plots were in field area "Auf dem Brachfeld", field number 502. The exact location is documented in the raw data (not included in the study report).

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. 7).
Study Plots:	• There was one treatment and one control plot planted on the trial site. • Each plot was 11.2 x 49 m^2 (5,907 sq.ft), with a between-row distance of 21.6 cm (8.5 in) and a 5.5 (2.2 in) cm in-row (seed to seed) drilling distance. • Test plots were adjacent to similar test plots which were cultivated with either maize or sunflower plants, and to a bean field. • Test plots were not treated with TI-435 before the study.
Additional Protection Treatments:	None

Guideline Criteria	Reported Information
Method of administration:	• Bees were exposed to summer rape plants grown from either TI-435-free or TI-435 FS 600-dressed summer rape seeds (607.2 g a.i.TI-435/L or 5.2 lb ai /gal). • Rape seeds (variety: "Lisonne", summer rape) were dressed at the Bayer Agricultural Research Centre at Monheim on 4/12/00 with TI-435 FS 600 at a rate of 1056 g a.i./100 kg seed (1 lb ai/100 lb seed) (nominal 1667 mL product/dt); treatment rate = 28.4 g a.i./ha.(0.025 lb ai/A). Seed from the treatment and control groups were also treated with a standard fungicide (thiram) (Tutan FS 500 @ 800 mL/100 kg seed). • The control plot was drilled with untreated rape seed and the treatment plot was drilled with seeds dressed with test substance at a drilling rate of 2.69 kg/ha on 4/28/00. • At the time of full rape blossom, bees were caged onto plots inside tents of 10 x 5 x 3 m^3 in the control and treatment plots (one beehive/tent/plot).
Analytical determination of test substance on dressed seeds:	The dressing rate was reportedly determined analytically to be 1056 g a.i./100 kg seed (1 lb ai/100 lb seed)(p. 7); however, no further information was provided.

Guideline Criteria	Reported Information
Definitive Test Sufficient number of time periods to yield statistically sound data.	Colonies were monitored 22 days between June 27-July 18, 2000.
Controls: Negative control and/or diluent/solvent control	There was a negative control plot.
Number of colonies per group:	One colony (small beehive~2000-3000 bees) per treatment and control group.
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A
Feeding:	None
Observation (sampling) period and methods:	• Sampling of nectar, flowers and honeybees, and behavioral observations were performed between 6/27-7/18/00. • Residue analysis was conducted from 9/13-9/25/00.

Guideline Criteria	Reported Information
Sampling Procedures: Bees: Nectar from honey bulbs, pollen from pollen pockets	• At Days 5 (7/2/00) and 21 (7/18/00) after hive installment, about 100 to 200 bees total were sampled with glass tubes while foraging on the summer rape plants; sampled honeybees were killed by freezing (dry ice). This method proved to be less efficient than other sampling methods, so no further processing of honeybees was conducted. • On Day 16 (7/12/00), combs were taken off beehives to cut out pollen stores and pollen pockets of sampled honeybees served as pollen sources for analysis. Combs were also removed on Day 16 (7/12/00) to take nectar samples of freshly collected nectar.
Plants: Nectar from flowers and flowers	• On Days 2 (6/28/00) and 4 (6/30/00) (control) and Days 4 (6/30/00) and 10 (7/6/00) (treatment), nectar from rape plants was sampled (0.5 mL minimum sample volume) directly from flowers with micro-capillaries. Sampled flowers were protected from other nectar-feeding insects by small gauze-covered tents (2 x 2 m^2).
Storage conditions:	• Male and female blossoms were sampled from summer rape plants on Day 9 (7/5/00).
Same procedure for all treatment groups:	Dry ice in the field, then refrigerated at -20°C until residue analysis Yes

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Control performance:	Control mortality exceeded treatment mortality. A total of 168 honeybees died in the control group and 132 bees in the treatment group.
Raw data included?	Raw data were provided for weight development of the beehives, foraging activity, and mortality.
Signs of toxicity (if any) were described?	Foraging intensity was measured on eleven days (Days 1, 2, 3, 6, 8, 9, 10, 15, 17, and 20 after 1st exposure). Behavioral anomalies(exaggerated motility, discoordinated movements, and apathy) were noted when observed. Mortality was recorded daily.

Mortality (# of dead bees per colony)[a]

Group	Days After 1st Exposure											Total Mortality
	0	1	2	3	6	8	9	10	15	17	20	
Control	n.r.	9	18	13	20	12	15	7	34	18	22	168
28.4 g a.i. TI-435/ha (0.025 lb ai/A)	n.r.	8	11	9	24	8	10	7	19	17	19	132

a. In front of the hive colonies, linen sheets approximately 60 X 50 cm were placed on the ground to trap the dead bees which were removed from the beehives during the time while colonies were confined within the tunnel cages. In addition, the number of dead honeybees around the tunnel edges were counted as an indicator whether a higher number of bees tried to leave the tent or failed to return to the hive. Also, any conspicuous numbers of dead bees in the study plots was recorded but no formal counts were made (p. 11).

Foraging Activity (total # of bees foraging during 1 min within 1 m^2 at two assessment areas)

Group	Days After 1st Exposure											Total
	0	1	2	3	6	8	9	10	15	17	20	
Control	21	21	19	13	24	4	11	17	3	10	1	144
28.4 g a.i. TI-435/ha (0.025 lb ai/A)	16	21	15	15	24	3	14	17	4	11	4	144

Weight Development of the Beehives(g)

Group	Hive Weight		
	Study Initiation	Study Termination	Difference (%)
Control	7920	7240	-8.6
28.4 g a.i. TI-435/ha (0.025 lb ai/A)	8310	7880	-5.2

Residue Analysis of Rape Nectar and Pollen(µg/kg)[a]

Group	Type of Residue		
	TI-435	TZMU	TZNG
Control			
Rape Nectar			
A (Capillary)	n.d.	n.d.	n.d.
B (Capillary)	n.d.	n.d.	n.d.
C (Honey Comb)	n.d.	n.d.	n.d.
Pollen A	n.d.	n.d.	n.d.

Residue Analysis of Rape Nectar and Pollen(µg/kg)[a]

Group	Type of Residue		
	TI-435	TZMU	TZNG
28.4 g a.i. TI-435/ha			
Rape Nectar			
A (Capillary)	5.4	<LOQ	n.d.
B (Capillary)	1.0	n.d.	n.d.
C (Honey Comb)	n.d.	n.d.	n.d.
Pollen A	1.9/2.5[b]	n.d.	n.d.

[a] Limit of quantitation (LOQ): 1 µg/kg; Limit of detection: 0.3 µg/kg
n.d.=Amount below limit of detection.
[b] Repetition of first analysis.

Reported Statistical Results: No behavioral impacts or increased mortality was observed on bees observed at hives. Statistical analysis was not necessary.

13. VERIFICATION OF STATISTICAL RESULTS:

It could be visually determined that there were no significant effects of TI-435 on bee behavior or mortality. Furthermore, statistical analyses could not be conducted, due to only one replicate hive per treatment level.

14. REVIEWER'S COMMENTS:

None.

15. REFERENCES:

Pacific Northwest Extension. Nov. 1993. PNW 245: Evaluating Honey Bee Colonies for Pollination: A Guide for Growers and Beekeepers. Pacific Northwest Extension Publication
URL: http://eesc.orst.edu/AgComWebFile/EdMat/PNW245.pdf

US EPA. Oct. 1982. Pesticide Assessment Guidelines Subdivision L Hazard Evaluation: Nontarget Insects. EPA-540/9-82-O19

US EPA. 1986. OPPTS 850.3040 - Field Testing for Pollinators. EPA 540/09-86-140
URL: http://www.epa.gov/docs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/