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 FS 600 Purity: 620g/L (62.0%)

3. **CITATION:**

Author:	R. Schmuck and R. Schöning
Title:	Residues of TI-435 in Nectar Blossoms, Pollen and Honey Bees Sampled from a Summer Rape Field in Sweden and Effects of These Residues on Foraging Honey Bees
Study Completion Date:	October 31, 2000
Laboratory:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Sponsor:	Bayer AG, GB Plant Protection, Marketing-Seed Treatment (Dr. Krohn/Altmann), D-40789 Monheim
Laboratory Report ID:	110282
DP Barcode:	D278110
MRID No.:	45422431
PMRA Submission#:	2000-1293

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation.

Signature: Rebecca Bryan **Date:** 6/28/02

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: [signature] **Date:** 6/28/02

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

Signature: Gabe Patrick **Date:** 7/1/03

APPROVED BY: Mike Rexrode, Ph.D., Senior Scientist, EPA/OPPTS/OPP/EFED/ERB5

Signature: M. Rexrode **Date:** 7/1/03

REVIEWED BY: Hemendra Mulye, Ph.D, Senior Evaluation Officer

Signature: [signature] **Date:** 7/16/03

PMRA/ Environmental Assessment Division **Date:**

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: 5 days

7. CONCLUSIONS: This field study determined the residue levels of TI-435 in various parts of seed-treated summer rape plants. The TI-435 treated rape seeds were treated at an application rate of 8.6 g a.i./kg seed (8.62 lb ai/1000 lb seed or 0.038 lb ai/acre) on 4/28/98 (plant date). The treatment exposure levels from the samples, indicated below, were a result of levels found in samples taken during the first week of July, 1998, over 2 months after the seed treatment application of TI-435.

forage bees: 0.0014 mg/kg
nectar in bees: 0.0086 mg/kg
nectar from rape flowers: 0.0012 mg/kg and 0.0072 mg/kg (sampled 7/3/98 and 7/2/98, respectively)
rape flowers: 0.0041 mg/kg

There were no levels of detection in the control bees (nectar or bees) hived on untreated rape or the control plants (nectar or flowers from untreated rape plants). The residue levels in the nectar taken from the bees (0.0086 mg/kg) is exceeding the acute oral NOAEL for honey bees (< 0.007 mg/kg from MRID No.: 45422426) and this nectar residue is only part of the exposure that the bees could be expected to incur while foraging on the seed-treated rape plants. However, as a result of this study, there did not appear to be any adverse effects to the foraging activity or any perceived increase in the mortality to the exposed bees.

The study is scientifically sound and 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, but it provides useful information for risk

assessment purposes.

C. Repairability: None

9. GUIDELINE DEVIATIONS:

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

2) The samples for residues were stored for approximately 8 months at -20°C before analysis was performed and storage stability information was not provided.

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* (assumed by reviewer since no scientific name was provided in study)
Age at beginning of test:	Commercial colonies with all life-stages present
Supplier	Mr. Krister Nilsson (Swedish commercial beekeeper), Smidarevagen 16, S-24196 Stockamollan
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Yes. Small beehives (~5000 honeybees) were caged on flowering rape plots using 4 x 4 x 2 m tents. Tents consisted of an aluminum frame covered by gauze material (2 x 2 mm mesh size).
Field study dates:	7/2 through 7/6/98
Lighting:	N/A
Temperature in field:	12-24°C (53-75°F)
Relative humidity:	Not reported.
Precipitation:	A total of 5 mm of rain was recorded over the 6 day study period (p. 9) with rain occurring from 7/4-7/6/98 .
Site Characterization:	• The trial site was located in the vicinity of Borlunda-Skelinge, South of Eslov in Sweden. • The field was previously cultivated with sugar beets in 1997. • Soil samples were analyzed from the study field. The soil was characterized as a "sandy loam". The organic carbon content was 1.8%, the water holding capacity was 63.1 g H_2O/100 g dry soil, and the pH was 6.0.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No

Guideline Criteria	Reported Information
Reference toxicant tested?	No, a reference compound was not specified for this type of material and use pattern (p. 6).
Study Plots:	• Two plots (1 untreated and 1 treated) were planted (drill seeded) with summer rape seed on 4/28/98. • Each plot was 8 x 16 m, with a crop-row spacing of 12.5 cm (~ 5 inches). • Plots were separated by 2 m-wide buffer strips.

Guideline Criteria	Reported Information
Method of administration:	• Bees were exposed to summer rape plants grown from seeds coated with 8.6 g a.i.(TI-435)/kg seed (analytically verified) (8.62 lb ai/1000 lb seed) . • The control plot was drilled with untreated rape seed (variety: "Maskot", summer rape) and the treatment plot was drilled with seeds dressed with test substance at a rate of 1.67 L TI-435 FS 600 per 100 kg oilseed rape at a drilling rate of 5 kg seed/ha (68 g/136 m^2 plot) (0.038 lb ai/acre) on April 28, 1998. • The delivery rate during drilling (verified prior to sowing) was between 4.2 and 4.3 g rape seed/application pipe over a 17 m drilling distance. The total seed delivery rate per plot was 16 x 4.2/4.3 g = 67.2/68.8 g per plot. • At the time of full rape blossom, tents of 4 x 4 x 2 m (see Fig. 1, p. 11) were installed on the control and treatment plots (one beehive/tent/plot). • The day after installment, hive entrances were disclosed and honeybees were allowed to forage on the study plots within the tent area.
Analytical determination of test substance on dressed seeds:	The seed coating rate was analytically determined to be 8.6 g a.i. TI-435/kg seed (8.62 lb ai/1000 lb seed) and the findings are reported in study number RA-2051/98 from July 20, 2000.

Guideline Criteria	Reported Information
Definitive Test Sufficient number of time periods to yield statistically sound data.	No. Colonies were monitored from July 2-6, 1998 with one control exposure and one treatment exposure at one concentration level or rate of application.
Controls: Negative control and/or diluent/solvent control	There was a negative control plot.
Number of colonies per group:	One colony per treatment and control group
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	No carrier with TI-435 FS 600 was mentioned in the study. Reviewer assumed treated rape seed was treated with TI-435 FS 600 alone.
Feeding:	No supplemental feeding of bees reported.
Observation (sampling) period and methods:	• Sampling of nectar, flowers and honeybees, and behavioral observations were performed between July 3 and 6, 1998. • Before placing beehives on the plots, approximately 100-200 honeybees were sampled for background residue levels in honeybees and honeybulbs (bee honey stomachs).

Guideline Criteria	Reported Information
Sampling Procedures: Bees: Nectar form honey bulbs, pollen form pollen pockets	• For first three days after hive installment, about 200 bees total were sampled after watching them feed on rape flowers for 10-30 seconds; killed by freezing (dry ice) • Honeybulbs removed from bees (halved between abdomen and thorax) with tweezers. All honeybulbs pooled from one treatment group and placed in Eppendorf cap.
Plants: Nectar from flower, Flowers	• Pollen pockets removed from prepared bees (not sufficient amount for analysis). • Plants outside of caged area (10-20 flowering plants protected from foraging insects with plastic bags): nectar from the rape flowers was directly sampled using 5 µL micropipettes, then emptied into 1.5 mL Eppendorf tube.
Storage conditions:	• 10 g of rape flowers were sampled by hand from plants outside tent area.
Same storage 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:	There was no control mortality and none of the control samples (or quality control samples) contained detectable residues of TI-435 (<0.0003 mg/kg TI-435) (Limits of Detection: 0.0003 mg/kg for TI-435).
Raw data included?	Raw data were not provided for residue analysis. There was no mortality, but data for flight and foraging activity were provided (Table 2, p. 13).
Signs of toxicity (if any) were described?	Flight and foraging intensity, behavioral anomalies, and mortality were observed (p. 9). Flight intensity and foraging observed once per day. Behavioral anomalies (exaggerated movements and discoordinated movements) were recorded with the date and time of observation.

Flight Activity [# of bees leaving (returning to) hive during 5 minutes]

Group	Days After Hive Installment				
	1	2	3*	4*	5*
Control	84 (65)	74 (91)	N/A	4 (13)	N/A
8.6 g a.i.(TI-435)/kg of seed**	88 (112)	71 (49)	N/A	7 (8)	N/A

* Rain occurred.
** Equivalent to 8.62 lb ai/1000 lb seed or 0.038 lb ai/acre

Foraging Activity (# of bees foraging on flowering rape during the check)

Group	Days After Hive Installment				
	1	2	3*	4*	5*
Control	12	6	N/A	1	N/A
8.6 g a.i.(TI-435)/kg of seed**	6	5	N/A	0	N/A

* Rain occurred.
** Equivalent to 8.62 lb ai/1000 lb seed or 0.038 lb ai/acre

Flight intensity: Once per day, over a period of 5 minutes, the number of bees leaving the hive and returning to the hive was recorded.
Forage intensity: Once per day the number of bees foraging within a haphazardly assigned area of 1 m^2 of flowering rape within the tent was recorded.
Behavioral anomalies: Whenever observed, the following behavioral anomalies were recorded with the date and daytime of observation:
- exaggerated motility
- discoordinated motility (trembling, shaking, apathy)

Mortality: Any suspicious numbers of dead bees in comparison to the controls during and after the test were recorded but no formal counts were made.

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

Group	Type of Sample					
	HB[b] before exposure	HB after exposure	Rape nectar sampled by bees	Rape nectar from flowers	Rape blossoms	Pollen sampled by bees [c]
Control	<0.0003	<0.0003	<0.0003	<0.0003	<0.0003	--
8.6 g a.i. (TI-435)/kg of seed [d]	<0.0003	0.0014	0.0086	0.0012-0.0072	0.0041	--

[a] Limit of quantitation: 0.001 mg/kg for TI-435; Limits of detection: 0.0003 mg/kg for TI-435
[b] HB = Honey Bee
[c] Amount insufficient for residue analysis.
[d] Equivalent to 8.62 lb ai/1000 lb seed or 0.038 lb ai/acre

Reported Statistical Results: No behavioral impacts (e.g., apathy, exaggerated motility, discoordinated movements) and no increased mortality was observed on bees collected for rape nectar and rape pollen. Due to poor weather conditions during the sampling period, flight and foraging intensity of honeybees was low. The study authors reported that this precluded the ability to make detailed conclusions other than there were no marked differences between the control and treatment groups. Statistical analyses were not required.

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical analysis could not be performed, as there was only one replicate in the control and treatment condition.

14. REVIEWER'S COMMENTS:

This toxicity study is scientifically sound, in that it determined the residue levels of TI-435 in various parts of seed-treated summer rape plants; however, it does not fulfill the requirements for a pollinator field test because a protocol was not approved by EPA for this insect field study prior to conducting the field study. A prior approved protocol would of required such things: conducting the study in the US, providing storage stability information on the test substance, a longer duration of honeybee activity observations, analysis of hive nectar/pollen/bees, etc.

Foraging activity and mortality did not appear to be affected by the bees' exposure (from 7/2 through 7/6/98) to the rape plants that received a seed treatment (on 4/28/98) of clothinianidin (TI-435) at a measured (analyzed) application rate of 8.62 lb ai/1000 lb seed or 0.038 lb ai/acre. The exposure period (5 days with 3 of these days receiving rain) was extremely limited for a small (< 5,000 bees) colony[1] that was moved to the site on 7/1/98 and then removed from the site on 7/6/98. However, the laboratory analysis of the bees exposed compared to the unexposed control bees (placed in a field receiving no TI-435 rape seed treatment) provides the following residue levels of TI-435 in the exposed bees and treated plants with no levels of detection in the control bees and plants:

forage bees: 0.0014 mg/kg
nectar in bees: 0.0086 mg/kg
nectar from rape flowers: 0.0012 mg/kg and 0.0072 mg/kg (sampled 7/3/98 and 7/2/98, respectively)
rape flowers: 0.0041 mg/kg

The above residue levels of TI-435 were detected in the samples collected more than 2 months after a TI-435 rape seed treatment application which occurred on 4/28/98. From a supplemental study (MRID No.: 45422426) the TI-435 honey bee acute oral LD50 is 0.0037 µg a.i./bee with a NOAEL of < 0.0009 µg a.i./bee. This would be equivalent to an LD50 of 0.0289 mg/kg and a NOAEL of < 0.007 mg/kg based upon an average fresh weight per honey bee of 128 milligrams(Mayer &. Johansen.1990).

The bees sampled were dissected to separate the honey stomachs (honeybulbs) from the bees. As a result the TI-435 residues found in nectar from the bees' honey stomachs (0.0086 mg/kg) is not counted in the residues found on the bees (0.0014 mg/kg). Presumably, the residue levels found on the forage bees (0.0014 mg/kg) was due mostly to exposure from the rape plants' pollen (in the insects' hairs) and not from the rape plants' nectar. Although pollen pockets (pollen baskets on bees legs) were dissected from bees there was not enough pollen to perform a residue analysis. The point, at issue, is that the residue levels in the nectar taken from the bees (0.0086 mg/kg) is exceeding the acute oral NOAEL for honey bees (< 0.007 mg/kg) and this nectar residue is only part of the exposure that the bees could be expected to incur while foraging on the seed treated rape plants. However, as a result of this study, there did not appear to be any adverse effects to the foraging activity or any perceived increase in the mortality to the exposed bees.

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

15. REFERENCES:

Mayer, D. & C. Johansen.1990. Pollinator Protection A Bee & Pesticide Handbook. Wicwas Press. Cheshire, Conn. p. 161

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/

US EPA. Code of Federal Regulations (CFR) Title 40 - Pesticide Programs Subchapter E - Pesticide Programs. Part 158 - Data Requirements for Registration.
URL: http://www.access.gpo.gov/nara/cfr/waisidx_00/40cfr158_00.html

DP Barcode: D278110
PMRA Submission #: 2000-1293 MRID No. 45422431

EAD Assessment of USEPA DER

Reviewer: Hemendra Mulye, PhD Date: January 14, 2003

PMRA Submission Number: 2000-1293

Study Type: Residues of TI-435 in nectar blossoms, pollen and honey bees sampled from a summer rape field in Sweden and effects of these Residues on foraging honey bees.

Reviewing Agency: US EPA

Executive Summary:

The objective of this field study was to determine residue levels of TI-435 in various parts of seed-treated summer rape plants. The TI-435 treated rape seeds were treated at an application rate of 8.6 g a.i./kg seed (8.62 lb ai/1000 lb seed or 0.038 lb ai/acre) on 4/28/98 (plant date). The treatment exposure levels from the samples were a result of levels found in samples taken during the first week of July 1998, over 2 months after the seed treatment application of TI-435, as follows: forage bees, 0.0014 mg/kg; nectar in bees, 0.0086 mg/kg; nectar from rape flowers, 0.0012 mg/kg and 0.0072 mg/kg (sampled 7/3/98 and 7/2/98, respectively); and rape flowers, 0.0041 mg/kg.

There were no levels of detection in the control bees (nectar or bees) hived on untreated rape or the control plants (nectar or flowers from untreated rape plants). The residue levels in the nectar taken from the bees (0.0086 mg/kg) is higher than the acute oral NOAEL for honey bees (< 0.007 mg/kg from MRID No.: 45422426) and this nectar residue is only part of the exposure that the bees could be expected to incur while foraging on the seed-treated rape plants.

However, the results of this study indicate that there did not appear to be any adverse effects to the foraging activity or a significant increase in the mortality of the exposed bees.

Material and Methods:

This study was conducted under field conditions near Borlunda-Skelinge, Eslov, Sweden. Two plots (1 untreated control and 1 treatment) were planted with summer rape on April 28, 1998. The plot dimensions were 8 X 16 m, with a row spacing of 12.5 cm. The control plot was drilled with untreated rape seed and the treatment plot was drilled with seeds that had been treated with clothianidin at a rate of 1.67 L/100 kg seed (8.6 g a.i./kg seed). Seeding rate was 5 kg/ha, equivalent to 68 g/136 m^2 (= 0.038 lb a.i./acre). At the time of full blossom, tents (dimennsions: 4 X 4 X 2 m) were installed on each of the plots. There was one bee hive/tent/plot. Then, honey bees were allowed

to forage on the study plots within each tent area. There was no supplemental feeding of the bees reported. For the first 3 days after hive installment, about 200 bees were sampled and killed by freezing and honey bulbs (stomachs) were removed. It was reported that pocket pollen was removed from the bees but there was insufficient quantity for analysis. Nectar from flowers as well as flowers were also sampled. All samples were stored frozen for periods up to 8 months. Hives were also observed for flight and foraging instensity, behavioural anomalies and mortality. Information on the stability of residues during storage, however, was not provided.

Results:

There were no behavioural impacts (i.e. apathy, exaggerated motility, lack of coordination) and no increased mortality reported. The treatment exposure levels from the samples were a result of levels found in samples taken during the first week of July 1998, as follows: forage bees, 0.0014 mg/kg; nectar in bees, 0.0086 mg/kg; nectar from rape flowers, 0.0012 mg/kg and 0.0072 mg/kg (sampled 7/3/98 and 7/2/98, respectively); and rape flowers, 0.0041 mg/kg. There were no detectable levels of residues in the control bees (nectar or bees) hived on untreated rape or the control plants (nectar or flowers from untreated rape plants).

Deviations:

This study was conducted by the registrant without prior agreed upon protocol between the registrant and US EPA.

Also, the samples for residue analysis were stored frozen (- 20 °C) for approximately 8 months. Information on the storage stability of residues, however, was not provided.

EAD comments:

The study is scientifically sound and is classified as **Supplemental** because this study was conducted without a prior agreed upon protocol between the registrant and the US EPA.

The residue levels in the nectar taken from the bees (0.0086 mg/kg) is higher than the acute oral NOAEL for honey bees (< 0.007 mg/kg) and this nectar residue is only part of the exposure that the bees could be expected to incur while foraging on the seed-treated rape plants. However, the results of this study indicate that there did not appear to be any adverse effects to the foraging activity or an increase in the mortality of the exposed bees.

The PMRA-EAD reviewer is in agreement with the conclusions reached by the US EPA.

Signatures:

Primary Reviewer: Hemendra Mulye, PhD Date: January 14, 2003

Secondary Reviewer: Linda Toy, MSc Date: January 24, 2003