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: 620 g a.i.(TI-435)/L, (seed coating) or 62.0% ai

3. **CITATION:**

Author:	R. Schmuck and R. Schöning
Title:	Residues of TI-435 in Nectar Blossoms, Pollen and Honey Bees Sampled from a British Summer Rape Field and Effects of These Residues on Foraging Honey Bees
Study Completion Date:	October 17, 2000
Laboratory:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Sponsor:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Laboratory Report ID:	110024
DP Barcode:	D278110
MRID No.:	45422432

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

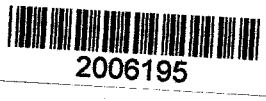
Signature: Teri Myers **Date:** 2/24/03

5. **Secondary Reviewer:** Gabe Patrick, Biologist, OPPTS/OPP/EFED/ERB 5

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

Secondary Reviewer: 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*
Definitive Study Duration: 3 days

7. **CONCLUSIONS:** This field study determined the residue levels of TI-435 in seed-treated summer rape flowers at a level of 0.0033 mg/kg. The TI-435 treated rape seeds were treated at a nominal application rate of 1.67 L TI-435 FS 600/100 kg oilseed rape (10.4 lb ai/1000 lb seed or 0.046 lb ai/acre) on 3/28/98 (plant date). The samples from the honey bees exposed to the treated rape provided no detectable levels of TI-435. There was insufficient sample for residue analysis of the pollen from the bees'pollen baskets (pockets) and nectar in the bees' honey stomachs (honeybulbs) in bees exposed to the treated rape plants. These treatment related exposure levels to the seed-treated rape came from samples taken between June 22-24, 1998, over 3 months after the seed treatment application of TI-435.

This toxicity study is scientifically sound, in that it determined the residue levels of TI-435 in blossoms 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. The study is classified as **Supplemental**.

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. Michael Graiptone (British commercial beekeeper), Troston, Suffolk
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	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).
Lighting:	Cloudy or overcast all 3 days of field study
Field study dates:	6/22 through 6/24/98
Temperature:	11-26°C
Relative humidity:	Not reported
Precipitation:	A total of 2.5 mm of rain (on 6/23/98) was recorded over the 3 day study period (p. 9).

Guideline Criteria	Reported Information
Site Characterization:	• The trial site was located in the vicinity of the Bayer UK experimental Elm Farm. • The field was previously grown with grass in 1997. • Soil samples were analyzed from the study field. The soil was characterized as a "sandy loam". The organic carbon content was 1.5% by weight, the water holding capacity was 55.3 g H_2O/100 g dry soil, and the pH was 5.4. At the time of flowering, the soil contained 17.4 g H_2O/100 g dry soil (=31% of the water holding capacity).

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).

Guideline Criteria	Reported Information
Study Plots:	• Two rows with three plots of rape plants each were planted on the trial site (Figure 1, p. 12). • Each plot was 4 x 15 m, with a between-row distance of 20 cm. • Rows were separated by a 0.5 m-wide buffer strip and plots in each row were separated by 1 m buffer strips. • The left plot in each row was planted with rape seeds treated with a developmental compound, while the right plot in each row was drilled with control seed. • The test substance was drilled in the middle plot of each row. • Sampling was done only in the lower row, while the upper row served as a reserve plot. • Treated and untreated plots were cultivated in the same way according to the practice of the region. Before initiation of sampling, no protection treatments other than the seed treatment was necessary.

Guideline Criteria	Reported Information
Method of administration:	• Bees were exposed to summer rape plants grown from seeds coated with 620 g a.i.(TI-435)/L. • Rape seeds (variety: "Lisonne", summer rape) were coated in a Centauer coating machine. TI-435 FS 600 (442.4 mL) was added to 26.5 kg rape seed together with 397.5 g (15 g/kg) Talcum blue and mixed over 35 seconds at 300 rpm. • The control plot was drilled with untreated rape seed and the treatment plot was drilled with seeds dressed with test substance at a nominal rate of 1.67 L TI-435 FS 600 per 100 kg oilseed rape (10.4 lb ai/1,000 lb seed) at a drilling rate of 5 kg seed/ha (30 g/60 m^2 plot) (0.046 lb ai/acre) on March 28, 1998. • At the time of full rape blossom, tents of 4 x 4 x 2 m 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:	Not conducted.
<u>Definitive Test</u> Sufficient number of time periods to yield statistically sound data.	No. Colonies were monitored from June 22-24, 1998.

Guideline Criteria	Reported Information
Controls: Negative control and/or diluent/solvent control	There was a negative control plot drilled at 5 kg seed/HA with untreated rape seed.
Number of colonies per group:	One colony (small beehive~5000 bees) per treatment and control group.
Solvent/Additives: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Talcum blue (15 g/kg of seed) added to seed treatment mixture
Feeding of bees:	No supplemental feeding
Observation (sampling) period and methods:	• Sampling of nectar, flowers and honeybees, and behavioral observations were performed between June 22 and 24, 1998. • Before placing beehives on the plots, approximately 100-200 honeybees were sampled for background residue levels in honeybees and honeybulbs (bee honey stomach).

Guideline Criteria	Reported Information
Sampling Procedures: Bees: Nectar from honey bulbs, pollen form pollen pockets	• For first two 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 were pooled from the treatment group and placed in an Eppendorf cap. • Pollen pockets were removed from prepared bees (not sufficient amount for analysis in treatment group).
Plants: Nectar from flower, Flowers	• Plants outside of caged area (10-20 flowering plants): nectar from the rape flowers were directly sampled using 5 µL micropipettes, then emptied into 1.5 mL Eppendorf tube. • 20 g of rape flowers were sampled by hand from plants outside tent area.
Storage conditions:	Dry ice in the field, then refrigerated at -20°C until residue analysis
Same procedure for all treatment groups:	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. 14).
Signs of toxicity (if any) were described?	Flight and foraging intensity, returning frequency, behavioral anomalies, and mortality were observed (p. 9). Flight intensity, foraging, and returning frequency were observed three times per day. Behavioral anomalies (exaggerated motility and discoordinated movements) were recorded with the date and time of observation.

Flight Activity [# of bees leaving (returning) to hive during 10 minutes]

Group	Days(hour)After Hive Installment					
	1 * (09:10-09:40)	1 * (12:00-12:38)	1 * (15:10-15:50)	2 (08:56-09:30)	2 (11:50-12:55)	2 (15:35-15:43)
Control	11 (0)	11 (7)	146 (210)	58 (9)	50 (22)	37 (45)
1.67 L TI-435 FS 600/100 kg oilseed rape**	6 (0)	4 (3)	43 (10)	84 (32)	64 (9)	N/A

* Rain occurred.
** Equivalent to 10.4 lb ai/1000 lb seed or 0.046 lb ai/acre

Foraging Activity (# of bees foraging per m^2 on flowering rape during 3 minute check)

Group	Days(hour)After Hive Installment					
	1* (09:10-09:40)	1* (12:00-12:38)	1* (15:10-15:50)	2 (08:56-09:30)	2 (11:50-12:55)	2 (15:35-15:43)
Control	0	2	22	18	8	7
1.67 L TI-435 FS 600/100 kg oilseed rape**	0	1	18	14	13	N/A

* Rain occurred.
** Equivalent to 10.4 lb ai/1000 lb seed or 0.046 lb ai/acre

Flight intensity: Three times per day, over a period of 10 minutes, the number of bees leaving the hive and returning to the hive was recorded.
Forage intensity: Three times per day the number of bees foraging within a haphazardly assigned area of 1 m^2 of flowering rape within the tent was recorded during a 3 minute period.
Returning frequency: Three times per day, over a period of 10 minutes, the number of bees arriving at the alighting board and returning to the hive is 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[c]	Rape blossoms	Pollen sampled by bees
Control	<0.0003	<0.0003	<0.0003	--	<0.0003	<0.0003
1.67 L TI-435 FS 600/100 kg oilseed rape[e]	<0.0003	<0.0003	—[d]	--	0.003	—[d]

[a] Limit of quantitation: 0.001 mg/kg; Limits of detection: 0.0003 mg/kg for TI-435
[b] HB = Honey Bee
[c] Sample lost due to a technical failure of the analyzer.
[d] Amount insufficient for residue analysis.
[e] Equivalent to 10.4 lb ai/1000 lb seed or 0.046 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. The study authors reported that flight and foraging intensity, as well as the returning frequency of honeybees was not different between bees foraging on control and treatment plots. Statistical analyses were not reported and probably could not be conducted because there was only one replicate plot in the treatment and control groups.

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical analysis could not be performed, as there was only one replicate in the control and treatment condition. The reviewer noted that there appeared to be greater flight and return activity in the control group for bees observed one day after hive installation (15:10-15:50); however, this difference did not appear to persist.

14. REVIEWER'S COMMENTS:

This toxicity study is scientifically sound, in that it determined the residue levels of TI-435 in blossoms 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 June 22 through June 24, 1998.) to the rape plants that received a seed treatment (on March 28, 1998) of clothinianidin (TI-435) at a nominal application rate of 10.4 lb ai/1000 lb seed or 0.046 lb ai/acre. The exposure period (3 overcast days with 1 of these days receiving rain) was extremely limited for a small (< 5,000 bees) colony[1] that was moved to the site on 6/22/98 and then removed from the site on 6/25/98. As indicated above, in the authors' reported results, TI-435 was only detected in seed treated rape flowers (0.0033 mg/kg) with no other samples in either the control samples or treatment samples providing any positive detection of TI-435. The samples from the honey bees exposed to the treated rape provided no detectable levels of TI-435. These samples were taken from bees exposed to seed treated rape plants approximately 3 months after the seed treatment to the rape plants. There was insufficient sample for residue analysis of the pollen from the bees'pollen baskets (pockets) and nectar in the bees' honey stomachs (honeybulbs) in bees exposed to the treated rape plants.

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

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/

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

EAD Assessment of USEPA DER

Reviewer: Valerie Hodge Date: November 8, 2002

PMRA Submission Number: 2001-1293

Study Type: Residues of TI-435 in Nectar, Blossoms, Pollen and Honey Bees Samples from a British Summer Rape Field and Effects of These Residues on Foraging Honeybees; PMRA DATA CODE 9.2.8, EPA MRID Number 45422432, OECD Data Point IIIA 10.4.4, EPA Guideline - none.

Reviewing Agency: U.S. EPA

EAD Summary:

This study should be considered as supplemental information only. Residue levels of TI-435 were determined in the nectar, blossoms, and pollen of summer rape flowers grown from seed which was treated with 10.4 g TI-435/kg seed. Honeybees foraging on these plants were also sampled for residues. The trial site was located in the vicinity of the Bayer UK experimental Elm Farm. Rape seeds were treated at a nominal application rate of 1.67 L of TI-435 FS 600 per 100 kg oilseed rape (10.4 g ai/kg seed) on 3/28/98 (plant date). At a drilling rate of 5 kg seed/ha, this is equivalent to an application rate of 52 g ai/ha. A control plot was seeded at the same rate with untreated seed. Residue levels of TI-435 in summer rape flowers from treated seed were determined to be 0.0033 mg ai/kg. Samples from honey bees exposed to the treated rape provided no detectable levels of TI-435. There was insufficient sample for residue analysis of the pollen from the bees' pollen baskets (pockets) and nectar in the bees' honey stomachs (honeybulbs) in bees exposed to the treated rape plants. These treatment related exposure levels to the seed-treated rape came from samples taken between June 22-24, 1998, over 3 months after the seed treatment application of TI-435. There were no effects on behaviour or mortality of bees.

Material and Methods:

Rape seeds were treated at a nominal application rate of 1.67 L of TI-435 FS 600 (620 g ai/L) per 100 kg oilseed rape (10.4 g ai/kg seed) on 3/28/98 (plant date). At a drilling rate of 5 kg seed/ha, this is equivalent to an application rate of 52 g ai/ha. A control plot was seeded at the same rate with untreated seed. The trial site was located in the vicinity of the Bayer UK experimental Elm Farm.

Two control plots and two plots seed-treated with TI-435 were planted to rape plants on the

trial site. Two other plots on the site were treated with an experimental compound and are, therefore, not relevant to the assessment of TI-435. Each plot was 4 x 15 m, with a between-row distance of 20 cm. Only one plot (treated and control) was sampled for analysis of plant material. At the time of full rape blossom, one colony of honey bees (small beehive with ~5000 bees) was placed on each treatment and control plot in a gauze tent (4 m x 4 m x 2 m). Honey bees were allowed to forage within the tents. Colonies were monitored from June 22-24, 1998. Flight intensity, foraging, and returning frequency were observed three times per day. Behavioral anomalies (exaggerated motility and discoordinated movements) were also recorded. Blank samples were obtained by sampling 100-200 honeybees from hives before they were placed in tents.

Plants (nectar; blossoms, 20 g) and bees (nectar from honey bulbs; pollen from pollen pockets) from control and treated plots were sampled and analysed for TI-435 by HPLC-MS/MS. The limit of detection of TI-435 was 0.0003 mg/kg. The limit of quantitation for TI-435 was 0.001 mg/kg.

Results:

There was no control mortality and none of the control samples (or quality control samples) contained detectable residues of TI-435 (<0.0003 mg TI-435/kg). No detectable residues of TI-435 (<0.0003 mg TI-435/kg) were found in pollen or nectar samples from bees and plants exposed to TI-435 as treated seed. Residues were detected and quantified in treated rape blossoms at 0.0033 mg TI-435/kg (wet weight). There was insufficient sample for residue analysis of the pollen from the bees' pollen baskets (pockets) and nectar in the bees' honey stomachs (honeybulbs) in bees exposed to the treated rape plants. These treatment related exposure levels to the seed-treated rape came from samples taken between June 22-24, 1998, over 3 months after the seed treatment application of TI-435.

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. The study authors reported that flight and foraging intensity, as well as the returning frequency of honeybees was not different between bees foraging on control and treatment plots. Raw data were not provided either for analysis of residues or bee mortality.

EAD comments:

The EAD evaluator agrees with the conclusions reached by the U.S. EPA evaluator. This study should be considered as supplemental information only. There was no replication of control or treated groups, raw data were not provided, and storage stability studies were not submitted. As indicated by the US EPA evaluator, the exposure period (3 overcast days with 1 of these days receiving rain) was extremely limited for a small (< 5,000 bees) colony

that was moved to the site on 6/22/98 and then removed from the site on 6/25/98.

EAD Conclusion:

This study provided limited information. TI-435 was only detected in rape flowers from treated seeds (0.0033 mg/kg) with no other samples in either the control or treatment plots providing any positive detection of TI-435.

Signatures:

Primary Reviewer:	Valerie Hodge	Date:	November 12, 2002
Secondary Reviewer:	Hemendra Mulye	Date:	