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 French 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:	110046
DP Barcode:	D278110
MRID No.:	45422433

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: 4 days

7. **CONCLUSIONS:** This field study determined the residue levels of TI-435 (clothianidin) at 0.0017 mg/kg in pollen taken from the bees foraging on the seed-treated rape plants. 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 3/19/98 (plant date). This treatment related exposure level to the seed-treated rape came from samples taken between June 15-18, 1998, over 3 months after the seed treatment application of TI-435. Although there were no honey bees hived on the untreated (control) rape plot, thus lessening the scientific value of this study, this study is still considered to be scientifically sound but 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) A beehive was not installed on the control plot. The study authors stated that this was because the prepared beehive was not in a condition which permitted transport (p. 7). As a result, there was no comparison to residue levels found in honeybees from the treatment plot.

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

3) 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. Michel Riollett (French commercial beekeeper), Les Fourneaux, F27190 Faverolles la Campagne
All bees from the same source?	Yes. Before use, beehives stood at a forested area about 10 km from the trial site. Bees were transported to the test site on 6/15/98 and removed from test site on 6/18/98.

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 with rain on 6/18/98
Temperature:	3-26°C (37.4 - 78.8°F low to high range from 6/16 thru 6/18/98)
Relative humidity:	Not reported

Guideline Criteria	Reported Information
Precipitation:	A total of 4 mm (on 6/16/98) of rain was recorded over the 3 day study period (p. 9).
Site Characterization:	- The trial site was located in the vicinity of Conches between la Neuve Lyre and la Vieille Lyre in Northern France. - 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.

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).
Study Plots:	• Two rows of four rape-planted plots each were planted on the trial site (Figure 1, p. 11). • Each plot was 4 x 20 m, with a 1 m space between adjacent plots and an 8 m space between the two plot rows. • Of the 8 plots planted, only one was designated a control plot and one was a treatment plot.

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 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 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 (40 g/80 m^2 plot) (0.046 lb ai/acre) on March 19, 1998. • At the time of full rape blossom, tents of 4 x 4 x 2 m (see Fig. 1, p. 11) were installed on the treatment and control plots. • In the treatment plot (there was no beehive installed in the control plot) the hive entrance was 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.

Guideline Criteria	Reported Information
Definitive Test Sufficient number of time periods to yield statistically sound data.	No. Colonies were monitored from June 15-18, 1998 during field test. No hive for control was established on the untreated rape seed plot. Only one concentration (seed treatment application rate was used). Only one replicate for treatment group was used.
Controls: Negative control and/or diluent/solvent control	Negative control (untreated rape seed plot) with no hive control of bees placed on this untreated plot.
Number of colonies per group:	One colony (small beehive~5000 bees) in the treatment group. No beehive was installed in the control plot because the prepared beehive was not in a condition which permitted transport (p. 7).
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 reported
Observation (sampling) period and methods:	• Sampling of nectar, flowers and honeybees, and behavioral observations were performed between June 15 and 18, 1998. • Before placing beehives on the plots, approximately 100-200 honeybees were sampled for background residue levels in honeybees and honeybulbs.

Guideline Criteria	Reported Information
Sampling Procedures: Bees: Nectar form honey bulbs, pollen form pollen pockets	• For first three days (6/15-6/17/98) 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 the treatment group were placed in Eppendorf cap. • Pollen pockets were removed from prepared bees (not sufficient amount for analysis in blank 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 a 1.5 mL Eppendorf tube. • 10 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). Samples of bees (bees, nectar, & pollen) foraging from untreated rape plot were not taken because hive was not placed on untreated rape plot.
Raw data included?	Replicate data were not provided for residue analysis. There was no mortality or observation of behavioral anomalies.
Signs of toxicity (if any) were described?	During and after the test, study plots were examined for suspicious honeybee mortalities. Behavioral anomalies (exaggerated movements, discoordinated movements, apathy, and flight incapability) were also monitored.

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
Control	<0.0003	Not Sampled	Not determined	--[c]	<0.0003	--[c]

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
1.67 L TI 435 FS 600/ 100kg oilseed rape[d]	<0.0003	<0.0003	< LOQ	< LOQ	< LOQ	0

[a] Limit of quantitation (LOQ): 0.001 mg/kg; Limits of detection: 0.0003 mg/kg for TI-435
[b] HB = Honey Bee
[c] Amount insufficient for residue analysis.
[d] 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. Statistical analyses were not required and could not be conducted because a beehive was only tented on the treatment plot.

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical analysis were not required.

14. REVIEWER'S COMMENTS:

The study authors reported that during and after the test, study plots were examined for suspicious honeybee mortalities. Behavioral anomalies (exaggerated movements, discoordinated movements, apathy, and flight incapability) were also monitored. The results from these observations were not reported by the authors' and it is assumed by this reviewer that no anomalies in bee behavior or mortality were noted by the authors'.

On two (2) days (6/17 and 6/18/98) the temperature lows were 3°C and 7°C (37.4°F and 44.6°F), respectively. These temperatures would be considered by this reviewer to be unusually low temperatures in France during the month of June but no comment by the authors' was made

concerning these temperatures. It should be noted that normal honey bee flight and forage activity would not begin until the daily temperature reached approximately 55°F (Pacific Northwest Extension.1993). The temperatures on 6/17 and 6/18/98 did reach highs in the 70s°F.

Although there were no bees (control) exposed to the untreated rape plots (lessening the comparative value of this study) and there were insufficient sampling material to perform residue analysis of the nectar samples from the untreated rape plants, the sample of the rape flowers from the untreated rape plots did not produce any detectable levels of TI-435 (clothinianidin). In the treated rape plot, the only positive sample from the bees exposed or treated plants came from the pollen taken from the foraging bees which provided clothianidin levels of 0.0017 mg/kg. All other samples taken were either below levels of quanititation (0.001 mg/kg) or levels of detection (0.0003 mg/kg). The level of clothianidin in the pollen collected by the bees on the seed-treated rape was from a seed treatment made to the rape plants approximately 3 months (3/19/98) before the samples were taken. The application rate of the seed treatment made to the rape seeds was 10.4 lb ai/1000 lb seed or 0.046 lb ai/acre.

This toxicity study is scientifically sound, in that it determined the residue levels of TI-435 in pollen taken from the bees foraging on 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.

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 12, 2002

PMRA Submission Number: 2001-1293

Study Type: Residues of TI-435 in Nectar Blossoms, Pollen and Honey Bees Sampled from a French Summer Rape Field and Effects of These Residues on Foraging Honey Bees; PMRA DATA CODE 9.2.8, EPA MRID Number 45422433, 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. Bees were not placed on the control plot. The trial site was located in the vicinity of Conches between la Neuve Lyre and la Vieille Lyre in Northern France. 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 3/19/98 (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 rape seed. Residues were detected and quantified in rape pollen collected by bees at 0.0017 mg TI-435/kg. No detectable residues of TI-435 (<0.0003 mg TI-435/kg) were found in nectar samples from bees or nectar and blossom samples from plants exposed to TI-435 as treated seed. This treatment related exposure level to the seed-treated rape came from samples taken between June 15-18, 1998, over 3 months after the seed treatment application of TI-435.

No behavioral impacts (e.g., apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed in bees from the treated plot. This could not be compared to a control.

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 3/19/98 (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

Conches between la Neuve Lyre and la Vieille Lyre in Northern France.

One control plot and one plot seed-treated with TI-435 (4 x 20 m area) were planted with rape on the trial site. At the time of full rape blossom, one colony of honey bees (small beehive with ~5000 bees) was placed in a gauze tent (4 m x 4 m x 2 m) covering the treated plot. The hive to be used for the control plot could not be installed and, therefore, bee samples could not be taken from that plot. Honey bees were allowed to forage within the tent and were monitored from June 15-18, 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) 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:

Control samples (and quality control samples) contained non-detectable residues of TI-435 (<0.0003 mg TI-435/kg). There was insufficient sample for residue analysis of the nectar collected from the flowers in the control plot. No detectable residues of TI-435 (<0.0003 mg TI-435/kg) were found in nectar samples from bees or nectar and blossom samples from plants exposed to TI-435 as treated seed. Residues were detected and quantified in rape pollen collected by bees at 0.0017 mg TI-435/kg. These treatment related exposure levels to the seed-treated rape came from samples taken between June 15-18, 1998, over 3 months after the seed treatment application of TI-435.

No behavioral impacts (e.g., apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on bees collected for rape nectar and rape pollen. Behaviour could not be compared to a control as no bees were present in the control plot. 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, storage stability studies were not submitted, the exposure/observation period was short, raw data were not provided for mortality or behavioural effects, and no bees were present in tents on the control plot.

EAD Conclusion:

Due to the deficiencies mentioned above, this study provides limited information. TI-435 was only detected in bee-collected pollen from plants grown from treated seeds (0.0017 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 13, 2002
Secondary Reviewer:	Hemendra Mulye	Date