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 Technical Purity: 99.7%

3. **CITATION:**

Author:	Ch. Maus and R. Schöning
Title:	Effects of TI-435 Technical Residues in Pollen on the Development of Small Bee Colonies and on Behavior and Mortality of Honey Bees
Study Completion Date:	January 28, 2001
Laboratory:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Sponsor:	Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany
Laboratory Report ID:	110059
DP Barcode:	D278110
MRID No.:	45422440

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:** 03/06/03

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 20/03

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: 41 days

7. **CONCLUSIONS:** This study is scientifically sound, in that it examined the effect of TI-435 residues in pollen on the development of small bee colonies and on the behavior and mortality of honey bees. One small beehive (about 500 bees) per treatment and control was tented on oat plots in cages and fed treated maize pollen; two control beehives were used in the study. The negative control bees were fed untreated pollen and the treatment group bees were fed pollen containing nominal concentrations of either 5, 10, or 20 µg TI-435 /kg. The measured concentrations were 5.4, 10.7, and 19.7 µg TI-435/kg. Bees fed TI-435 treated pollen, when compared to control colonies, did not exhibit treatment-related effects in mortality, foraging activity (including honey and pollen collection), comb production, honey storage behavior, population growth (including egg, larvae, pupae, and adult growth stages) or behavioral anomalies. Pollen treated with TI-435 at a measured concentration level up to 19.7 µg TI-435/kg produced no significant adverse effects to the parameters measured in this study based upon the visual inspection of the data. There was only one replicate hive per each treatment level, so a statistical analyses could not be made of the data provided.

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 effect of TI-435 residues in pollen on the development of small bee colonies and on the behavior and mortality of honeybees.

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	Mr. Josef Gilli, Reinatzstrasse 25, 53925 Kall
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Site Characterization:	• The trial site was located in the vicinity of Euskirchen-Billig (Germany, Nordrhein-Westfalen). • The field was cropped with oat. The oat field was drilled on 5/6/2000 (plant date). • Five oat-planted plots on the test field were confined with tunnel cages of ca. 50 m^2. Tent (tunnel) cages were placed on oat field on 6/8 & 6/9/00. Five (5) bee colonies were placed in cages on 6/14/00 with final evaluations of these colonies made on 7/25/00. • Temperature and precipitation events were continuously recorded, and included air and soil temperature, precipitation, cloudiness (% sky coverage), and wind speed (estimates).

Guideline Criteria	Reported Information
Cage size adequate?	Tunnel tent cages (10 x 5 m) consisted of an aluminum frame covered by plastic gauze material (ca. 2 x 2 mm mesh size).
Number of Plots/Treatment:	Five (5) plots total - 2 control plots and 3 treatment plots. Each plot had one small (4-frame) colony of bees.
Food Pollen Preparation:	• Food pollen was purchased from a Spanish supplier (Polen Joan Pinol El Perello, Tarragona) and was a mixture of pollen from different plants, mainly rosemary, but designated as "maize" pollen by the authors. This pollen was ground and analyzed to determine possible background contamination. According to these results, the maize pollen was free of background TI-435 contamination. • 50 mg TI-435 was dissolved in 500 mL of tap water. This stock solution was further diluted with tap water (p. 8) to prepared the 20 mL solution which was sprayed onto 200 g of maize pollen. • Five, 1 g samples were taken from each treated preparation (two, 1 g samples from the control) and analyzed for verification of the target concentration; see Table 1, p. 24 and Appendix XIV, p. 42-52.
Lighting:	The sky was mostly cloudy during the assessments.
Temperature (within control tent):	Maximum temperatures: 15-39°C (59-102°F) Minimum temperatures: 4-16°C (39-61°F) (from Appendix I of study- p. 25)

Guideline Criteria	Reported Information
Wind speed:	Mostly calm to moderate with two stormy days on Day 2 (6/15/00) and Day 28 (7/10/00) during biological work performance period of study from 6/14/00 through 7/25/00
Precipitation:	0 - 31 mm (0 - 1.3 in)
Relative humidity (within control tent):	40-100% (from Appendix I of study- p. 25)

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).
Method of administration:	• Pollen was provided in ca. 10-30 g portions both inside and outside the hive. One portion was offered in a separate, sheltered container next to the honey feeder. A second portion was offered in an open glass bowl placed on the hive bottom. • Bees were offered pollen at concentrations of 5.4, 10.7, and 19.7 µg/kg TI-435 Technical.
Definitive Test Sufficient number of time periods to yield statistically sound data.	Colonies were observed between June 14-July 25, 2000.

Guideline Criteria	Reported Information
Controls: Negative control and/or diluent/solvent control	Negative control
Number of colonies per group:	• Each colony was filled into a multiple-comb-fertilization-cage which contained 4 native comb strips (13 x 2 cm). One queen in egg laying activity was added to each of the hive colonies within a separate, closed cage. The queen cages were opened one or two days later. • One colony (500 adult bees with fertile queen) was randomly allocated to one tunnel cage per treatment and control group. • The 4-frame hives with bees and queen used in the study were prepared on 6/13/00 and set-up in tents on 6/14/00.
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A

Guideline Criteria	Reported Information
Feeding:	• Bees were fed fortified pollen (subsamples replaced in hive feeders on days 6, 9, 12, 16, 20, 24, 28, 33, and 36 and in field feeders on days 9, 14, and 26) and supplemental sunflower honey as a carbohydrate source (fresh portions offered each 2nd and 9th day of the study and old portions were removed and reweighed). • Honey was analyzed and confirmed to be free of background TI-435 contamination. • Fortified pollen and honey subsamples were stored during the study within a refrigerator between +6 and +9°C.

Guideline Criteria	Reported Information
Observations and frequency:	• Mortality was assessed daily, except weekends. • Comb cell production was regularly assessed for wax gland activity. • Food consumption was determined upon reweighing of feeders. • Honey storage behavior was assessed regularly as the weight increase of the colonies and as the percentage of honey comb-filled cells. • Egg laying activity was assessed regularly by inspection of brood combs. • Breeding success was determined during each inspection as the percentage of comb cells with honeybee larvae or pupae. • Colony strength was determined during each inspection as the percentage of comb cell area covered by honeybees. • Foraging intensity was determined daily, except weekends, as the number of bees foraging during a 5 minute observation period on the honey and pollen feeder; the number of honeybees on the tunnel roof was also counted. • Behavioral anomalies (i.e., exaggerated motility, discoordinated movements, apathy, and lethargy) were recorded whenever observed with the date and daytime observation.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (certified laboratory)
Control performance:	Mortality data for the two controls similar to treatment. A total of 49 and 43 bees died during the study in the controls.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Developmental and behavior anomalies of exposed honeybee colonies were recorded with the date of observations.

Table 1: Mortality (# of dead bees per colony)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Location		
	Front of Hive	Tent Edges	Total
Control 1	2	47	49
Control 2	2	41	43
5 (5.4)	2	43	45
10 (10.7)	0	17	17
20 (19.7)	1	38	39

a. In front of colony hives, cotton sheets of 60 X 50 cm were spread on the ground. Dead bees were collected from these sheets daily except during weekends. Any conspicuous mortality within the oat strip or at the tunnel edges was also recorded but no formal count were made on these bees.

Table 2: Foraging Activity (Average # of bees observed during five minute observations during 36 day observation period from 6/14-7/25/00)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Location			
	Pollen Feeder	Honey Feeder	Tent Roof	Total
Control 1	6	83	13	102
Control 2	7	83	17	107
5 (5.4)	6	83	14	103
10 (10.7)	4	77	16	97
20 (19.7)	7	92	15	114

a. Daily, except weekends, the number of bees foraging during a 5 minute observation period on the honey and pollen feeder were recorded. In addition, the number of honeybees encountered on the tunnel roof were counted. This figure (roof count) may give an indication of possible disorientation or repellent/antifeedant phenomena.

Table 3: Quantity of Honey and Pollen Collected by Foraging Honeybees[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Honey Collected (g)	Pollen Collected (g)
Control 1	438	93
Control 2	422	89
5 (5.4)	420	91
10 (10.7)	413	96
20 (19.7)	425	99

a The amount of pollen and honey consumption was determined by reweighing the respective feeders.

Table 4: Comb Production (increase in comb area, cm^2)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Days After First Exposure to Treated Substrate				
	6	15	22	33	41
Control 1	368	508	556	624	656
Control 2	386	520	560	672	672
5 (5.4)	424	502	548	638	638
10 (10.7)	382	514	538	616	644
20 (19.7)	418	510	510	616	668

a. Area represents estimate of 4 combs (2-sides) per group (one honey bee colony per group).

Table 5: Size of Honey Stores (cm^2 combs with honey)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Days After First Exposure to Treated Substrate				
	6	15	22	33	41
Control 1	106	173	163	152	25
Control 2	76	98	203	148	115
5 (5.4)	92	103	140	172	112
10 (10.7)	54	86	190	221	99
20 (19.7)	47	97	180	152	55

a. The percentage of comb cells which were filled with honey was estimated and a value in square centimeters was assigned to this estimate based on the proportion of the comb area (4 combs/colony/group) where the stored honey was recorded during the evaluation.

Table 6: Weight Increase of the Beehives(g)

Group (µg/kg TI-435 Technical) Nominal (Measured)	Hive Weight			
	Study Initiation	Day 37	Total Weight Gain	Difference (%)
Control 1	960	1005	45	4.7
Control 2	970	1050	80	8.3
5 (5.4)	965	995	30	3.1
10 (10.7)	990	1055	65	6.6
20 (19.7)	990	1060	70	7.1

Table 7: Population Growth (cm^2 occupied comb area)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Days After First Exposure to Treated Substrate				
	6	15	22	33	41
Control 1	132	234	190	272	283
Control 2	159	236	184	297	294
5 (5.4)	155	226	201	298	273
10 (10.7)	168	219	292	311	302
20 (19.7)	186	244	193	376	378

a. This is an estimate of the proportion of comb area (4 combs/colony/group) in square centimeters which was occupied by adult honey bees during the evaluation.

Table 8: Egg Laying Activity (cm^2 combs with eggs)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Days After First Exposure to Treated Substrate				
	6	15	22	33	41
Control 1	130	85	111	92	179
Control 2	87	144	131	88	98
5 (5.4)	132	89	106	111	150
10 (10.7)	183	108	103	94	196
20 (19.7)	125	100	109	115	143

a. This is an estimate of the proportion of comb area (4 combs/colony/group) in square centimeters which was occupied by bee eggs during the evaluation.

Table 9: Abundance of Honeybee Larvae (cm^2 combs with larvae)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Days After First Exposure to Treated Substrate				
	6	15	22	33	41
Control 1	0	23	35	21	31
Control 2	0	27	34	25	44
5 (5.4)	0	22	22	29	50
10 (10.7)	0	10	10	21	61
20 (19.7)	0	13	22	25	102

a. This is an estimate of the proportion of comb area (4 combs/colony/group) in square centimeters which was occupied by bee larvae during the evaluation.

Table 10: Abundance of Honeybee Pupae (cm^2 combs with pupae)[a]

Group (µg/kg TI-435 Technical) Nominal (Measured)	Days After First Exposure to Treated Substrate				
	6	15	22	33	41
Control 1	0	54	47	117	115
Control 2	0	5	35	140	169
5 (5.4)	0	17	55	121	106
10 (10.7)	0	25	29	89	135
20 (19.7)	0	17	22	140	149

a. This is an estimate of the proportion of comb area (4 combs/colony/group) in square centimeters which was occupied by pupae (capped bee cells) during the evaluation.

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:

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/

EAD Assessment of USEPA DER

Reviewer: Hemendra Mulye, PhD Date: January 15, 2003

PMRA Submission Number: 2000-1293

Study Type: Effects of TI-435 Technical residues in pollen on the development of small bee colonies and on behavior and mortality of honey bees.

Reviewing Agency: US EPA

Executive Summary:

The objective of this study was to determine the effects of residues of clothianidin technical in pollen on the development of small bee colonies and on the behaviour and mortality of honey bees. One small beehive (about 500 bees) per treatment and controls were placed in tent enclosures on oat plots in cages and fed treated pollen. The negative control bees were fed untreated pollen and the treatment group bees were fed pollen containing nominal concentrations of either 5, 10, or 20 µg TI-435 /kg. The measured concentrations were 5.4, 10.7, and 19.7 µg TI-435/kg. Bees fed TI-435 treated pollen, when compared to control colonies, did not exhibit treatment-related effects in mortality, foraging activity (including honey and pollen collection), comb production, honey storage behavior, population growth (including egg, larvae, pupae, and adult growth stages) or behavioral anomalies.

Material and Methods:

Apis mellifera were obtained from a commercial supplier near Kall, Germany. The trial site was located at near Euskirchen-Billig, Nordrhein-Westfalen, Germany. The field was planted with oat. Five plots on the test field were isolated with tunnel cages of approximately 10 X 5 m^2. The tunnel tent cages consisted of aluminium frames covered by plastic mesh (2 X 2 mm). There were 3 treatment plots and 2 control plots. Food grade pollen was purchased from a commercial supplier and was found to be a mixture of pollen from different plant species (mainly rosemary) but was designated as "maize (corn) pollen" by the researchers. Test pollen was fortified with TI-435 at a rate of 0 (control), 5.4, 10.7, or 19.7 µg a.i./kg. Pollen was offered in 10-30 g portions both inside and outside the hive. Sunflower honey was offered as supplemental source of carbohydrate. The following parameters were monitored: mortality, comb cell production, food consumption, honey storage behaviour, egg laying activity, breeding success, colony strength, foraging intensity and behavioural anomalies (i.e. exaggerated motility, lack of coordination, apathy and lethargy). There was only one replicate hive per each treatment level,

so statistical analyses could not be made of the data provided.

Results:

It was reported that there were no treatment related effects on any of the parameters monitored.

Deviations:

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

EAD comments:

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

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

Signatures:

Primary Reviewer: Hemendra Mulye, PhD Date: January 15, 2003

Secondary Reviewer: Linda Toy, MSc Date: January 20, 2003