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

1. **CHEMICAL(s):** Clothianidin (TI-435) & Imidacloprid (Gaucho) — PC Code No.: 044309 (Clothianidin) & 129099 (Imidacloprid)

2. **TEST MATERIAL:** TI-435 & Gaucho — Purity: TEP formulations not provided

3. **CITATION:**

Author:	Scott-Dupree C.; Spivak, M.; Bruns, G.; Blenkinsop, C.; & Nelson, S.
Title:	The Impact of GAUCHO® and TI-435 Seed Treated Canola on Honey Bees, *Apis mellifera* L.
Study Completion Date:	April 11, 2001
Laboratory:	University of Guelph, Guelph, Ontario, Canada; University of Minnesota, St. Paul, Minnesota, USA; Enviro-Test Laboratories, Edmonton, Alberta, Canada
Sponsor:	Bayer Corporation, Agriculture Division, Stilwell, Kansas
Laboratory Report ID:	110403
DP Barcode:	D278110
MRID No.:	45422435

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

Teri Myers 2/24/03

5. **Secondary Reviewer:** Gabe Patrick, Biologist, EPA/OPPTS/OPP/EFED/ERB5

Signature: Gabe Patrick **Date:** 3/5/03

Secondary Reviewer: Mike Rexrode, Ph.D., Senior Scientist, EPA/OPPTS/OPP/EFED/ERB5

Signature: M. Rexrode **Date:** 3/5/03

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* L.
Definitive Study Duration: ~100 days (includes initial application date through bee exposure)

7. **CONCLUSIONS:** This field study determined the residue levels of TI-435 (clothianidin) and imidacloprid in the pollen and nectar of seed-treated canola (rape) plants. The TI-435 treated rape seeds were treated at an application rate of 6 lb ai/1,000 lb seed or 0.04 lb ai /A and the imidacloprid treated rape seeds were treated at an application rate of 10 lb ai/1,000 lb seed or 0.06-0.07 lb ai/A. The treatment exposure levels from the samples, indicated below, were a result of levels found in samples taken during July, 2000.

Time Elapsed from Application to Sampling (Days)	TI-435 residue in pollen (ppb)	TI-435 residue in nectar (ppb)	Imidacloprid residue in pollen (ppb)	Imidacloprid residue in nectar (ppb)
61	3	3.7	not applicable	not applicable
68	1.6	0.9	not applicable	not applicable
50	2.3	1.1	7.6	0.81*
57	2.8	1	4.4	0.60*

* <Level of Quantification (LOQ) =1.0 ppb and >Level of Detection (LOD) = 0.3 ppb

While the study authors detected no significant treatment-related reductions in any parameters, the reviewer noted that several parameters (e.g., mortality, pollen foraging activity, mean # of foragers observed, and mean honey yield) appeared to be negatively impacted by treatment with either TI-435 or GAUCHO® (imidacloprid). The reviewer could not statistically verify these findings because replicate data were not provided. However, assuming there were no significant treatment related reductions, as indicated by the authors, the field exposure to the test substances and bee observation period were too brief (< 30 days) to fully evaluate the impact the exposure levels of clothianidin and imidacloprid would have on the bee colonies tested.

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:

- Replicate data for honeybee and hive parameters were not provided, so the study authors' conclusions could not be verified.
- Typical End-use Products (TEPs) formulations (percent active ingredient) used in study were not identified.
- Supplier(s) of bees for study was not identified.
- This study was conducted without a prior agreed upon protocol between the registrant and the Agency.

10. SUBMISSION PURPOSE:

This study was submitted to provide data on the residue levels of GAUCHO® and TI-435 in pollen and nectar collected from seed-treated canola blossoms and to determine their effects on the honey-producing ability and foraging and hive behavior of honeybees.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera*)	*Apis mellifera* L.
Age at beginning of test:	Commercial colonies with all life-stages present
Supplier	Townsend House Bee Lab (noted in acknowledgments and assumed to be supplier by reviewer)

Guideline Criteria	Reported Information
All bees from the same source?	Yes (assumed by reviewer)

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	N/A
Lighting:	N/A
Temperature:	**Ontario** May- average: 12.7°C, range: 0.2-27.7°C June- average: 16.4°C, range: 6.5-29.8°C July- average: 17.2°C, range: 7.3-26.9°C August- average: 17.5°C, range: 5.9-27.6°C **Minnesota** June - range: 5-34°C (41-94 °F) July - range: 10-32.8°C (50-91°F)
Relative humidity:	Not reported.
Precipitation:	**Ontario** May- 106.6 mm June- 95.4 mm July- 30.4 mm August- 46.0 mm **Minnesota** June - 109.0 mm (4.29 in.) July - 228.6 mm (9.0 in.)

Guideline Criteria	Reported Information
Wind speed:	**Ontario** May- average: 3.8 km/h June- average: 3.3 km/h July- average: 2.2 km/h August- 2.0 km/h **Minnesota** June - described as calm to windy July - described as calm to windy

Guideline Criteria	Reported Information
Site Characterization:	**Ontario** • The experiment was conducted at three locations in southern Ontario: • Site 1 (control) - Windy Acre Farms, Grand Valley, Ontario (GPS = 43°55'N, 80°15'W; • Site 2 (Gaucho) - Windy Acre Farms, Grand Valley, Ontario (GPS = 43°47'N, 80°13'W; • Site 3 (TI-435) - located 2 km south of Elora Research Station (GPS = 43°39'N, 80°25'W. • Sites 1 and 2 were located approximately 3.0 km from each other. Site 3 was located 47.0 and 44.0 km southwest of Sites 1 and 2, respectively. • The soil at the three sites was identified as "loam-textured" by the University of Guelph's Laboratory Services Department. • All sites were treated with the herbicide TREFLAN (trifluralin) EC® [2.3 L/ha (~32 fl.oz/A) (Dow AgroSciences Canada Inc.)] prior to planting. • Sites 1 and 2 were fertilized prior to planting with composted chicken manure (100 lbs N/acre). Site 3 was fertilized on May 2 with a mixed 20-10-10 fertilizer (300 kg/ha). • Soil moisture averaged 4.1% (July) at Site 1, 3.8% (July) at Site 2, and 5.7% (July and August) at Site 3. **Minnesota** • The experiment was conducted at the Rosemount Research and Outreach Center, Dakota County, Minnesota, 44°, 93°W: • Site 1 (control) - Section 28, range 19W, Township 155N; • Site 2 (Gaucho) - Section 35, range 19W, Township 115N; • Site 3 (TI-435) - Section 3, range 19W, Township 114N.

Guideline Criteria	Reported Information
Site Characterization (con't.):	**Minnesota (con't)** • Sites 1 and 2 were 3 km from each other. Site 3 was located 4.5 km from Site 2 and 7.5 km from Site 1. • There were no flowering plants in the area, which was predominantly planted with corn, soybeans, and peas. • The soil at the three sites was identified as Waukegan Silt Loam by the University of Minnesota Soils Department. • All sites were treated with the herbicide TREFLAN® (trifluralin) (PrePlant, Inc.) at 24 oz/acre prior to planting. • Site 2 was treated with HORNET® herbicide (clopyralid) in 1999 and the carryover in the soil negatively affected growth of canola at this site. Records of this herbicide treatment were not consulted prior to planting of canola in 2000 for this experiment.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No
Reference toxicant tested?	No
Study plots:	Each site (in Ontario and Minnesota) was planted with 1 ha of spring canola, *Brassica napus*, var. #46865. Seed for both the Ontario and Minnesota studies was provided by Pioneer Hi-Bred Production Ltd.-Plant Breeding Division (Georgetown, Ontario) and pesticide treated by the Gustafson Partnership (Guelph, Ontario).

Guideline Criteria	Reported Information
Method of Administration and Planting Information:	**Ontario** • Weather constraints and microclimate differences resulted in the sites being seeded on different dates. • Site 1 was seeded on May 16, 2000 using a Case IH Minimum till drill seeder with Vitavax RS Flowable (3.3% carbathiin, 6.6% thiram and 50% lindane (Uniroyal Chemical Co.), 2250 mL of formulated product/100 kg seed) treated seed at 6-7 lb/acre. • Site 2 was seeded on May 16, 2000 using a Case IH Minimum till drill seeder with GAUCHO® (1000 g AI./100 kg seed (Bayer Corp.)) plus RS Vitavax Fungicide canola seed at 6-7 lb/acre. • Site 3 (the most southerly exposed plot) was seeded on May 3, 2000 using a Hege Model 80 small plot planter with AMS 13945 (TI-435 (Bayer Corp.), 600 AI/100 kg seed)) plus Vitavax Fungicide (3.3% carbathiin + 6.6% thiram (Uniroyal Chemical Co.)), treated seed at 6 lbs/acre. • Seed treated with the three products were sent to Bayer AG-Monheim for analysis. Two separate analyses of two separate sub-samples indicated: Lindane content=1492 g a.i./100 kg (99.5%); Imidacloprid content=976 g a.i./100 kg (97.6%); TI-435 content=606 g a.i./100 kg (100.9%).

Guideline Criteria	Reported Information
Method of Administration and Planting Information (con't):	**Minnesota** • All sites were seeded on May 16, 2000 with a Tye No-Till Drill at 4.5 lb/acre (5 kg/ha), spaced 7 inches (17.5 cm) apart. The land was chisel-plowed in the fall and a field cultivator was used in the spring. Pesticide treatments to planted seed were identical to site listing and rates of application as listed for Ontario locations, above.
Measured Application Rates (seed treatments):	**Ontario & Minnesota** • Site 1 - Lindane content=1,492 g a.i./100 kg of seed at 6-7 lb treated seed/acre (15 lb ai lindane/1,000 lb seed or 0.09-0.11 lb ai lindane/A) • Site 2 - Lindane content=1,492 g a.i./100 kg of seed & Imidacloprid content=976 g a.i./100 kg of seed at 6-7 lb treated seed/acre (15 lb ai lindane/1,000 lb seed or 0.09-0.11 lb ai lindane/A) plus (10 lb ai imidacloprid/1,000 lb seed or 0.06-0.07 lb ai imidacloprid/A) • Site 3 - Lindane content=1,492 g a.i./100 kg of seed & TI-435 content=606 g a.i./100 kg of seed at 6 lb treated seed/acre (15 lb ai lindane/1,000 lb seed or 0.09 lb ai lindane/A) plus (6 lb ai TI-435/1,000 lb seed or 0.04 lb ai TI-435/A)

Guideline Criteria	Reported Information
Emergence Information:	**Ontario** • Planting emergence rates were determined approximately 18-30 days after planting by counting the number of canola seedlings per meter row in 10 random locations throughout each test field. An average emergence rate was computed for each of the three sites (Table 1, p. 5). • Emergence averaged 21.0% at Site 1, 28.7% at Site 2, and 64.0% at Site 3. The study authors attributed the low emergence rates at Sites 1 and 2 to unseasonably cool temperature and heavy rainfall following planting. • During the 2000 field season, flea beetle pressure was excessive on canola. Flea beetle damage was assessed at all sites by determining the percent damage to new leaves and the entire plant on 15 randomly selected plants in a marked 4-meter row from 5 random locations throughout each test field. **Minnesota** • Emergence rates were determined on June 26, 2000 after bloom initiation (June 22-24, 2000) at all sites by counting the number of canola seedlings per meter row in 10 random locations throughout each test field. For Site 2 (GAUCHO® treatment), an additional 10 locations (20 total) were counted because of anomalous growth of canola at this site. • Emergence was 15.6% at Site 1, 20.0% at Site 2 (average of 20 x 1 m rows), and 32.7% at Site 3. • The majority of plants at Site 2 did not develop normally, particularly in the center of the field. Many plants in the center of the field were either stunted or died, due to prior treatment of the site with HORNET® herbicide (clopyralid) which was carried over in the soil. When center plants did bloom, it was reported that bees visited the blossoms normally.

Guideline Criteria	Reported Information
Analytical determination of test substance:	TI-435 residues, Imidacloprid, Hydroxy Metabolite, and Olefin Metabolite in nectar and pollen were verified by HPLC-MS/MS (Appendix 4) to determine the limits of quanitfication and detection, and procedural recoveries.
Definitive Test Sufficient number of time periods to yield statistically sound data.	Ontario colonies at: Site 1 (Control) were observed between July 4-August 2, 2000 (29 days); Site 2 (Gaucho) were observed between July 4-August 1, 2000 (28 days); Site 3 (TI-435) were observed between June 26-July 20, 2000 (25 days). Minnesota colonies at all three sites were observed from June 28-July 28, 2000 (30 days).
Controls: Negative control and/or diluent/solvent control	Negative control-treated with VITAVAX RS (insecticide/fungicide) only, contains: Lindane (an organochlorine insecticide) acts by ingestion, contact and, to a lesser extent, by fumigant action against many soil-dwelling and insect pests. Thiram, a fungicide, controls seed-borne diseases. Carbathiin, a systemic fungicide, penetrates the seed coat to control diseases of the seed and seedling. Controls the diseases listed. Protects against flea beetles for a few days after crop emergence. http://www.canola-council.org/production/vitavax.html
Number of colonies per group:	When 20% of the canola blossoms were opened, 4 two-super colonies of honeybees containing sister queens of approximately the same age were placed at the easterly (Ontario) or southernly (Minnesota) edge of each of the sites.

Guideline Criteria	Reported Information
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A
Feeding:	No supplemental feeding of bees mentioned.

Guideline Criteria	Reported Information
Honeybee observation (sampling) period and methods:	• A 2 x 5 m^2 white sheet was secured on the ground in front of the hive entrances to collect and count dead bees. The number of dead bees found on the white sheet surrounding each colony was recorded and removed every other day. • To determine changes in colony strength over the course of the experiment, the total amount of sealed brood and frames of adult bees were estimated prior to colony removal at the end of the canola bloom period. • All hives were weighed weekly. Increases or decreases in honey super and/or brood super weights were assumed to be related to either nectar collection or consumption, respectively. Therefore, changes in colony weights were used to determine the total honey production per colony. • Foraging activity was determined by recording the number of bees entering the collection site (6 x 1 m^2) per 1 min interval, while noting the type of foraging activity they performed (i.e., landing on flowers no nectar or pollen collection; landing with pollen and/or nectar collection); because few bees were noted landing on flowers without collecting pollen, nectar, or both at the Minnesota site, this measure was not taken at this site. Foraging data collection alternated between morning and afternoon at each site. • Honeybees were monitored for abnormal behavior (e.g., convulsions, aggressiveness, or other erratic behavior) in the field at hive entrances for 2 minute intervals every other day during the bloom.

Guideline Criteria	Reported Information
Statistical Analysis:	All honeybee data were subject to ANOVA and Fisher's protected LSD test using Statistix® software (Ontario) or Tukey's studentized range test (HSD) using SAS statistical software (Minnesota).
Pollen and Nectar Collection:	**Ontario** • Pollen and nectar samples were collected twice (7 and 14 days after placement in the field) from each hive at all of the three test sites. • Prior to nectar collection (usually the day before), one empty hive frame was placed in each of the sampled colonies. Liquid nectar samples (5 g/hive) were collected using a pipette with a disposable tip. Nectar from each of the 4 colonies at a site was pooled. • Pollen collection devices were placed on colonies one day prior to collection and pollen samples (3 g/hive) were scooped out of the pollen tray. Pollen from each of the four colonies at a site was pooled. • All nectar and pollen samples were frozen at -24°C until residue analysis. **Minnesota** • Pollen and nectar samples were collected from all sites on July 6 and 12 (8 and 14 days after placement in the field). The method for pollen collection was identical to that described at the Ontario site above. • Because plants in the center portion of Sites 1 and 2 were stunted and bloomed about 1 week later than surrounding edge plants, an additional sample of nectar and pollen was collected on July 20 at the control and GAUCHO® sites. An extra sample was also collected from the VITAVAX RS FLOWABLE site, but the TI-435 site was done blooming at that time.

Guideline Criteria	Reported Information
Pollen and Nectar Collection (con't):	**Minnesota (con't)** • Pollen traps were placed on each colony one day prior to collecting samples and samples were scooped out of the pollen trays. Samples were frozen at -20°C until residue analysis. • Because colonies collected a variety of pollen from different plants, the pollen was later sorted by color (while still frozen) and compared to a representative color sample of pollen collected on bees specifically visiting canola. Samples of the same color were analyzed using standard pollen analysis techniques to identify the pollen to genus. Only pollen from the genus *Brassica* was analyzed.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	For most honeybee parameters, performance in the control group was similar to or less than that in the treatment groups. Replicate data were not provided for honeybee assessment parameters, so the control performance could not be statistically verified (against performance in the treatment groups) by the reviewer.
Raw data included?	No. Replicate data were not included for any of the honeybee data.
Signs of toxicity (if any) were described?	Behavioral anomalies (i.e., aggressive, convulsive, or erratic) were not observed in this study (p. 14).

Mortality (Mean # of dead bees per colony)

Treatment Group	Location	
	Ontario (mean no. of bees)	Minnesota (mean no. of bees)
Vitavax RS Flowable® (Control)	14.0a*	148.1a**
Gaucho®	20.6a	112.4a
TI-435	19.0a	101.5a

* Within each column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test.
** Within this column, means followed by the same letter are not significantly different [P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)]

Foraging Activity (mean number of bees visiting canola blossoms ($1m^2$ for 1 minute)

Treatment Group	Location					
	Ontario			Minnesota		
	Nectar	Pollen	Visit	Nectar	Pollen	Visit
Vitavax RS Flowable® (Control)	5.3b*	0.8b	1.1a	4.1a**	3.3a	N/A***
Gaucho®	8.4ab	0.9b	2.8a	6.8a	2.7a	N/A
TI-435	15.3a	3.0a	2.7a	4.0a	1.5a	N/A

* Within columns of Ontario data, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test.
** Within columns of Minnesota data, means followed by the same letter are not significantly different [P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)]
*** Data not available

Mean number of forager honey bees observed (1 m^2 for 1 min) during bloom period

Treatment Group	Location	
	Ontario	Minnesota
Vitavax RS Flowable® (Control)	2.4b*	3.7a**
Gaucho®	4.0ab	4.8a
TI-435	7.0a	2.7a

* Within each column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test.
** Within this column, means followed by the same letter are not significantly different [P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)]

Mean honey yields of colonies (kg)

Treatment Group	Location	
	Ontario	Minnesota
Vitavax RS Flowable® (Control)	42.5a*	9.2a**
Gaucho®	40.8a	11.1a
TI-435	38.2a	8.0a

* Within each column, means followed by the same letter are not significantly different (P>0.05 determined by ANOVA and Fisher's protected LSD test.
** Within this column, means followed by the same letter are not significantly different [P>0.05 determined by ANOVA and Tukey's Studentized range test (HSD)]

Flea beetle damage (%)

Treatment Group	36687		36696	
	Whole Plant	New Leaf	Whole Plant	New Leaf
Vitavax RS Flowable® (Control)	27	16	44	5.5
Gaucho®	57	14	64.4	13.5
TI-435	0.7	0	2.3	0.1

Residue of TI-435 in canola pollen and nectar (ppb) at locations in Ontario and Minnesota.

Site	Days After Hive Placement	TI-435 residue in pollen (ppb)	TI-435 residue in nectar (ppb)
Elora (Ontario)	7	3	3.7
Elora (Ontario)	14	1.6	0.9
Control (Ontario)	-	<0.5	<0.5
Rosemount (Minnesota)	7	2.3	1.1
Rosemount (Minnesota)	14	2.8	1
Control (Minnesota)	-	<0.5	<0.5

Residue of imidacloprid (I) and associated hydroxy (H) and olefin (O) metabolites in canola pollen and nectar (ppb) at locations in Ontario and Minnesota

Site	Days After Hive Placement	Analyte	Pollen Residue (ppb)	Nectar Residue (ppb)
GVF (Ontario)	7	O, H, I	<1.0	<1.0
GVF (Ontario)	14	O, H, I	<1.0	<1.0
Control (Ontario)	-	O, H, I	<1.0	<1.0
Rosemount (Minnesota)	7	O, H I	<1.0 7.6	<1.0 0.81*
Rosemount (Minnesota)	14	O, H I	<1.0 4.4	<1.0 0.60*
Control (Minnesota)	-	O, H, I	<1.0	<1.0

* <LOQ (1.0 ppb) and >LOD (0.3 ppb)

Reported Statistical Results: The study authors' statistical analysis detected no significant differences between the control and treatment groups for impacts on brood, foraging activity, bee mortality, honey yield, or bee behavior at either the Canadian or Minnesota sites. Furthermore, they reported that detection of residue levels that were substantially below the NOEC of 20 ppb (Schmidt and Schmuck. 2000) support the lack of a negative effect on bee behavior and hive variables in this study.

13. VERIFICATION OF STATISTICAL RESULTS:

The reviewer was unable to verify the study authors' conclusions via statistical analysis because replicate data for bee and hive parameters were not provided. Despite the lack of significant effects shown by the study authors, the reviewer notes that several parameters appeared to be negatively impacted by treatment with either GAUCHO® or TI-435. Mortality was 36% and 47% higher than the control group in the TI-435 and GAUCHO® treatment groups at the Ontario site. Pollen foraging activity was reduced 55% and 18% in the TI-435 and GAUCHO® treatment groups in Minnesota. The mean number of foragers observed was reduced 27% in the TI-435 treatment in Minnesota. Honey yield was reduced 10% and 4% in the TI-435 and GAUCHO® treatment groups in Ontario and 13% in the TI-435 treatment group in Minnesota. Incidentally, flea beetle damage was generally higher for the GAUCHO®-treated plants and substantially lower for the TI-435-treated plants.

14. REVIEWER'S COMMENTS:

While the study authors detected no significant treatment-related reductions in any parameters, the reviewer noted that several parameters (e.g., mortality, pollen foraging activity, mean # of foragers observed, and mean honey yield) appeared to be negatively impacted by treatment with either TI-435 or GAUCHO®; the reviewer could not statistically verify these findings because replicate data were not provided. However, assuming there were no significant treatment related reductions, as indicated by the authors, it should be noted that the field exposure time and monitoring the bees received was limited (≤30 days at all locations), that the complete life cycle for an individual worker bee during the time period tested would be approximately 63 days[1] and that monitoring of adverse effects to the colonies was not extended beyond the field exposure time.

The authors also indicated that all the residues detected in the nectar and pollen samples taken were substantially below the imidacloprid honey bee NOAEC of 20 ppb as determined by Schmidt and Schmuck. (2000) and cited this study as supporting their contention that "no negative impact on bee behavior and hive variables (ie. sealed brood, honey yield)" were noted in this study. The EFED has not received this study for review and an examination of the Canadian Honey Council's website (http://www.honeycouncil.ca/gaucho.html) provided the following listing of studies dealing with the imidacloprid honey bee NOAEC:

The [Imidacloprid] No Observed Effect Concentration (NOEC) on honeybees has been lowered as more research has been completed. The NOEC level accepted by Bayer scientists remains at 20 ppb.

Researcher	NOEC for honeybees
1997 Bayer	5,000 parts per billion
1998 Bayer	100 parts per billion
2000 Schmidt, Bayer	20 parts per billion
1998 Colin & Bonmatin, INRA	6 parts per billion
2000 Pham DeDelègue	4 parts per billion
2000 Colin & Bonmatin INRA	1-3 parts per billion

It should also be noted that the imidacloprid honey bee NOAEC cited by the authors provides no information concerning the TI-435 (clothianidin) NOAEC. To date, the EFED has not reviewed any of the imidacloprid studies cited in this Canadian Honey Council's table (above). The current listing of guideline 141 series (honey bee) studies received by EFED for imidacloprid are:

[1] Egg stage through pupae stage ≈ 21 days; adult house bee stage ≈21 days; and adult forager ≈ 21 days.

42273003 Cole, J. (1990) The Acute Oral and Contact Toxicity to Honey Bees of Compound NTN 33893 Technical: Lab Project Number: 101321. Unpublished study prepared by RCC, Research and Consulting Company AG. 13 p.

42480503 Mayer, D.; Lunden, J.; Husfloen, M. (1991) Integrated Pest and Pollinator Investigations 1991 (Including Honey Bee Toxicity of NTN 33893): Lab Project Number: 103815. Unpublished study prepared by Miles, Inc. 13 p.

42632901 Hancock, G.; Fischer, D.; Mayer, D.; et al. (1992) NTN 33893: Toxicity to Honey Bees on Alfalfa Treated Foliage: Lab Project Number: N3772902: 103938. Unpublished study prepared by Washington State University and Miles Residue Analysis Lab. 62 p.

As determined by this study (MRID No. 45422435) the following residues of TI-435 and imidacloprid were found in canola pollen and nectar:

Residue of TI-435 (Clothianidin) in canola pollen and nectar (ppb) at locations in Ontario and Minnesota.				
Site	Clothianidin Seed Treatment Application Rate (measured)	Days Elapsed from Application to Sampling (Days)	TI-435 residue in pollen (ppb)	TI-435 residue in nectar (ppb)
Elora (Ontario)	6 lb ai/1,000 lb seed or 0.04 lb ai /A	61	3	3.7
Elora (Ontario)	6 lb ai/1,000 lb seed or 0.04 lb ai /A	68	1.6	0.9
Control (Ontario)	n/a	-	<0.5*	<0.5*
Rosemount (Minnesota)	6 lb ai/1,000 lb seed or 0.04 lb ai /A	50	2.3	1.1
Rosemount (Minnesota)	6 lb ai/1,000 lb seed or 0.04 lb ai /A	57	2.8	1
Control (Minnesota)	n/a	-	<0.5*	<0.5*

* Residues not quantifiable

Residue of Imidacloprid in canola pollen and nectar (ppb) at locations in Ontario and Minnesota				
Site	Imidacloprid Seed Treatment Application Rate (measured)	Days Elapsed from Application to Sampling (Days)	Imidacloprid residue in pollen (ppb)	Imidacloprid residue in nectar (ppb)
GVF (Ontario)	10 lb ai/1,000 lb seed or 0.06-0.07 lb ai/A	56	<1.0	<1.0
GVF (Ontario)	10 lb ai/1,000 lb seed or 0.06-0.07 lb ai/A	63	<1.0	<1.0
Control (Ontario)	n/a	-	<1.0	<1.0
Rosemount (Minnesota)	10 lb ai/1,000 lb seed or 0.06-0.07 lb ai/A	50	7.6	0.81*

Rosemount (Minnesota)	10 lb ai/1,000 lb seed or 0.06-0.07 lb ai/A	57	4.4	0.60*
Control (Minnesota)	n/a	-	<1.0	<1.0

* <Level of Quantification (LOQ) =1.0 ppb and >Level of Detection (LOD) = 0.3 ppb

15. REFERENCES:

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EAD Assessment of USEPA DER

Reviewer: Valerie Hodge Date: November 18, 2002

PMRA Submission Number: 2001-1293

Study Type: The Impact of GAUCHO® and TI-435 Seed Treated Canola on Honey Bees, *Apis mellifera* L.; PMRA DATA CODE n/a, EPA MRID Number 45422435, OECD Data Point IIIA 10.4.5, EPA Guideline - none.

Reviewing Agency: U.S. EPA

EAD Summary:

This study is for supplemental information. The effects of the exposure of honeybees to canola blossoms produced from seed treated with TI-435 was studied at two sites, Ontario and Minnesota. Residue levels of TI-435 (clothianidin) in the pollen and nectar from seed-treated canola plants were also measured. The canola seeds were treated with TI-435 at 606 g ai/100 kg (100.9%), which is equivalent to an application rate of approximately 41 g ai/ha. Control and treated sites received approximately 100 g lindane/ha as they were also treated with Vitavax RS Flowable Fungicide (3.3% carbathin, 6.6% thiram, and 50% lindane). Honey bees were exposed to control and treatment sites when canola plants were in full bloom.

There were no significant treatment-related reductions ($p < 0.05$) in the parameters that were tested (amount of sealed honey bee brood, mortality, honey yields, foraging, pollen collection). Residues of TI-435 were detected in pollen and nectar at 7 and 14 days after hive placement.

Residues of TI-435 (ppb) in canola pollen and nectar at treated sites in Ontario and Minnesota (all controls < 0.5 ppb).

Treated Site	Time Elapsed from Application to Sampling (Days)	TI-435 residue	
		in pollen (ppb)	in nectar (ppb)
Ontario	61*	3.0	3.7
	68**	1.6	0.9
Minnesota	50*	2.3	1.1
	57**	2.8	1.0

Level of Quantification (LOQ) =1.0 ppb and Level of Detection (LOD) = 0.3 ppb.

* 7 days after hive placement

** 14 days after hive placement

Material and Methods:

This study was conducted at two locations, southern Ontario and Minnesota. Ontario sites were: Site 1 (control) - Windy Acre Farms, Grand Valley, Ontario (GPS = 43°55'N, 80°15'W; Site 3 (TI-435) - located 2 km south of Elora Research Station (GPS = 43°39'N, 80°25'W). Site 3 was located 47.0 km southwest of Site 1. Sites at the Rosemount Research and Outreach Center, Dakota County Minnesota (44°, 93°W) were: Site 1 (control) - Section 28, range 19W, Township 155N; Site 3 (TI-435) - Section 3, range 19W, Township 114N. (Site 2 for both locations was treated with GAUCHO®, imidacloprid. These results were not included in this summary for TI-435.)

Sites (in Ontario and Minnesota) were planted with 1 ha of spring canola on May 16, 2000 (Site 3 in Ontario was seeded on May 3). Seed was treated in Ontario and sent to Bayer AG-Monheim for analysis for TI-435 content (606 g a.i./100 kg; 100.9%). Sites were seeded at 6725 g treated seed/ha (6 lb treated seed/acre). This is equivalent to an application rate of approximately 41 g TI-435/ha. Control and treated sites received approximately 100 g lindane/ha as they were also treated with Vitavax RS Flowable Fungicide (3.3% carbathin, 6.6% thiram, and 50% lindane). TI-435 residues, Imidacloprid, Hydroxy Metabolite, and Olefin Metabolite in nectar and pollen were verified by HPLC-MS/MS to determine the limits of quanitfication and detection, and procedural recoveries.

When 20% of the canola blossoms were opened, 4 two-super colonies of honeybees

containing sister queens of approximately the same age were placed at the easterly (Ontario) or southernly (Minnesota) edge of each of the sites. Ontario colonies at Site 1 (control) were observed between July 4-August 2, 2000 (29 days) and at Site 3 (TI-435) were observed between June 26-July 20, 2000 (25 days). In Minnesota, colonies at all sites were observed from June 28-July 28, 2000 (30 days).

Honeybees were monitored for mortality (at hive entrance), total honey production (by hive weight), colony strength (numbers of sealed brood and adults), foraging activity, and abnormal behaviour. All parameters are measured during the entire bloom period except for numbers of sealed brood (Ontario, June 26 and July 27; Minnesota, July 24). Pollen and nectar, collected by foraging bees, was also collected from the hives and analysed for TI-435 residues at 7 and 14 days after hive placement. Analysis was conducted using HPLC with Electrospray MS/MS detection. All honeybee data were subject to ANOVA and Fisher's protected LSD test using Statistix® software (Ontario) or Tukey's studentized range test (HSD) using SAS statistical software (Minnesota).

Results:

Analytical recovery of TI-435 from pollen and nectar (honey) was 92 ± 9.5% and 86 ± 7.9%, respectively. The Limit of Quantitation for TI-435 is 0.5 ppb (ng/g).

There was no significant difference between the control group and honeybees that were exposed to TI-435 in Ontario and Minnesota ($p < 0.05$) for the following parameters: mean amount of sealed honey bee brood, mean number of dead honey bees, and mean honey yields (Table 1). Depending on the site, values for the control may be higher or lower than the treated group. A general trend was not evident as there were between-site differences. Mean number of forager bees observed during the entire canola bloom period was significantly higher for treated groups when compared to control groups in Ontario, but there was no significant difference for sites in Minnesota (TI-435 < control). The mean number of honey bees collecting nectar and pollen was significantly greater ($p < 0.05$) for bees exposed to TI-435 than controls at sites in Ontario. There was no significant difference between control and treated for this parameter at the Minnesota site, or for overall visits to canola blossoms in Ontario (not measured in Minnesota). Replicate data were not provided for honeybee assessment parameters, so the control performance could not be statistically verified (against performance in the treatment groups) by the US EPA reviewer. No abnormal behaviour was observed in honeybees.

Table 1. Results of observations of honey bees at canola test sites in Ontario and Minnesota.

Treatment	Ontario*			Minnesota**		
Mean amount of sealed honey bee brood (cm^2)						
Control (Vitavax RS Flowable)	3285a			5368a		
TI-435	2939a			5536a		
Mean number of honey bees visiting canola blossoms (1 m^2 for 1 min.) and collecting nectar and pollen during the entire bloom period						
	Nectar	Pollen	Visit	Nectar	Pollen	Visit
Control (Vitavax RS Flowable)	5.3b	0.8b	1.1a	4.1a	3.3a	n/a***
TI-435	15.3a	3.0a	2.7a	4.0a	1.5a	n/a
Mean number of forager honey bees observed (1 m^2 for 1 min.) during the entire canola bloom period						
Control (Vitavax RS Flowable)	2.4b			3.7a		
TI-435	7.0a			2.7a		
Mean number of dead honey bees collected outside colonies						
Control (Vitavax RS Flowable)	14.0a			148.1a		
TI-435	19.0a			101.5a		
Mean honey yields of colonies						
Control (Vitavax RS Flowable)	42.5a			9.2a		
TI-435	38.2a			8.0a		

* Within this column, means followed by the same letter are not significantly different ($p > 0.05$ determined by ANOVA and Fisher's protected LSD test.

** Within this column, means followed by the same letter are not significantly different ($p > 0.05$ determined by ANOVA and Tukey's Studentized range test, HSD).

*** Not measured.

Residues of TI-435 were detected in pollen and nectar samples from Ontario and Minnesota (Table 2). Controls had no quantifiable levels of TI-435 (< 0.5 ppb).

Table 2. Residues of TI-435 (ppb) in canola pollen and nectar at treated sites in Ontario and Minnesota (all controls < 0.5 ppb).

Treated Site	Time Elapsed from Application to Sampling (Days)	TI-435 residue	
		in pollen (ppb)	in nectar (ppb)
Ontario	61	3.0	3.7
	68	1.6	0.9
Minnesota	50	2.3	1.1
	57	2.8	1.0

Level of Quantification (LOQ) =1.0 ppb and Level of Detection (LOD) = 0.3 ppb.

* 7 days after hive placement

** 14 days after hive placement

EAD comments:

Results could not be validated as raw data were not provided. Variability of these data could, therefore, not be assessed. The US EPA evaluator concluded that the field exposure to the test substances and bee observation period were too brief (25-30 days) to fully evaluate the impact the exposure levels of clothianidin (and imidacloprid) would have on the bee colonies that were tested. The EAD evaluator agrees that longer-term effects could not be assessed due to the short time frame of the study (e.g., brood survival), but that the time may have been sufficient to provide information about other parameters (e.g., adult mortality). Residue levels were also measured which provides some information about exposure to bees. These measurements were, however, taken on only two days at 7 and 14 days after hive placement.

The EAD evaluator agrees that this study should be used as supplemental information.

EAD Conclusion:

This study is for supplemental information. Residues of TI-435 were detected in pollen and nectar. There did not appear to be any adverse effects on honeybees due to exposure to plants grown from seed treated with TI-435.

Signatures:

DP Barcode: D278110 MRID No. 45422435

Primary Reviewer: Valerie Hodge Date: November 18, 2002

Secondary Reviewer: Hemendra Mulye Date: