UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM **DATE: November 16, 2007**

SUBJECT: **Review of Data Package DP336888 for Clothianidin, PC Code 044309**

FROM: **Allen Vaughan, Biologist** Allen W. Vaughan 11/30/07
Environmental Risk Branch V
Environmental Fate and Effects Division (7507P)

THRU: **Mah Shamim, Branch Chief** 11/30/07
Environmental Risk Branch V
Environmental Fate and Effects Division (7507P)

TO: **Kable Davis, Risk Manager Reviewer**
John Hebert, Risk Manager (RM 07)
Insecticide/Rodenticide Branch
Registration Division (7505P)

EFED has reviewed the following study submitted for clothianidin (PC Code 044309). The completed DER for this study is attached:

Cutler, C. 2006. An Investigation of the Potential Long Term Impact of Clothianidin Seed Treated Canola on Honey Bees, *Apis mellifera* L. Laboratory Report ID: 2005-CSD-EBTIX064. MRID 46907801 (with addendum 46907802).

This study is scientifically sound and satisfies the guideline requirements for a field toxicity test with honeybees (OPP Gdln. No. 141-5; OPPTS 850.3040).

Overall, there was no difference between colonies from clothianidin-treated and control fields. Although sporadic treatment or site differences were found on various dates, essentially no differences in worker or drone mortality, worker longevity, or brood development occurred during the study. Colonies in treated fields had similar weight gains and honey yields as those in control fields. Qualitative assessments, made the following spring by experienced bee researchers, confirmed that colonies from clothianidin-treated fields were as strong and healthy as those from control fields.

It was concluded that honey bees that forage on clothianidin seed-treated canola will be exposed to clothianidin residues in pollen, nectar, and honey; however, exposure concentrations are below those required to elicit acute and sublethal effects.

DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS
§141-5 (OPPTS 850. 3040)

1. **CHEMICAL:** Clothianidin — PC Code No.: 044309

2. **TEST MATERIAL:** 1) Prosper FL — Purity: 1) 9.49%
   2) Poncho 600 FS — 2) 48.0%

3. **CITATION:**

Author: Cutler, C.

Title: An Investigation of the Potential Long-Term Impact of Clothianidin Seed Treated Canola on Honey Bees, *Apis mellifera* L.

Study Completion Date: August 1, 2006

Laboratory: Department of Environmental Biology
University of Guelph
Guelph, Ontario, N1G 2W1

Sponsor: Bayer CropScience
P.O. Box 12014, 2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Laboratory Report ID: 2005-CSD-EBTIX064

DP Barcode: D336888

MRID No.: 469078-01

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 9/18/07

APPROVED BY: Teri S. Myers, Ph.D., Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 9/26/07

5. **APPROVED BY:** Allen Vaughan, Biologist, ERB - V

Signature: Allen W. Vaughan **Date:** 11/16/07

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the long-term toxicity of a pesticide to honey bees following an actual-use field exposure. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	Queens in all colonies were of the same lineage and *ca.* the same age.
Definitive Study Duration:	130 days (approximately 2 complete life cycles); 21-day exposure period (during peak bloom) followed by a 109-day post-exposure period.

8. **CONCLUSIONS:**

In a 130-day study (21-day exposure followed by 109-day post-exposure period), the long-term toxicity of clothianidin-treated seed was examined in the honey bee, *Apis mellifera* L., under open field conditions at four test sites. Each site contained one 1-ha field planted with canola seed, *Brassica napus* var. Hyola 420, that had been treated with the end-use products Prosper ® FL at 1250 mL/100 kg seed and Poncho® 600 FS at 417 mL/100 kg seed, delivering clothianidin at 400 g ai/100 kg seed, the highest commercial rate for use in Canada. In addition, each site contained one 1-ha control field planted with canola seed that had been treated at the same rate with specially-prepared Blank Prosper FL and Poncho 600 FS formulations. Each treated and control field were separated by at least 250 m. Four honey bee colonies were placed in the middle of each field (n=32) during a 3-week bloom period (Day 1 = July 1, 2005), and thereafter moved to a fall apiary for the remainder of the study (Day 130 = November 7, 2005). Throughout the study, colonies were assessed for bee mortality, worker longevity, and brood development. In addition, samples of honey, beeswax, and worker-gathered pollen and nectar were regularly analyzed

for clothianidin residues. Colony weight gain while in the canola fields and honey yield per colony was also determined.

Overall, there was no difference between colonies from clothianidin-treated and control fields. Although sporadic treatment or site differences were found on various dates, essentially no differences in worker or drone mortality, worker longevity, or brood development occurred during the study. Colonies in treated fields had similar weight gains and honey yields as those in control fields. Qualitative assessments by experienced bee researchers confirmed that colonies from clothianidin-treated field were as strong and healthy as those from control fields.

The majority of samples collected (>75%) for residue analysis had no detectable levels of clothianidin residues (LOQ = 0.5 ng/g). The maximum concentrations of clothianidin detected in honey, nectar, pollen, and beeswax samples were 0.928, 2.24, 2.59, and <0.5 ng/g, respectively. These levels were approximately 8-fold below the reported field relevant NOAEC of 20 ppb.

It was concluded that honey bees that forage on clothianidin seed-treated canola will be exposed to clothianidin residues in the form of pollen, nectar, and honey; however, exposure concentrations are below those required to elicit acute and sublethal effects.

In a study addendum (MRID 469078-02), the status of 29 of the original 32 over-wintered colonies was assessed on April 19-20, 2006. Observations included the presence/absence of the queen, presence/absence of eggs and larvae, area of sealed brood, number of frames of workers, and overall health of the colony. The spring assessment found no significant difference in the health between the treated and control colonies. Two treated colonies and two control colonies did not survive the winter. Of the 25 colonies that did survive the winter, a healthy queen was found in 21, and the presence of eggs and larvae in the remaining four indicated that these colonies were queen-right. There was no statistical difference between treated and control colonies in the amount of sealed brood or in the A number of frames of workers. Collectively, 24 colonies were classified "healthy", one was classified "weak" (<4 frames of live bees), and four were "dead".

This study is scientifically sound and satisfies EFED concerning the guideline requirements for a field toxicity test with honeybees (OPP Gdln. No. 141-5; OPPTS 850.3040).

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: N/A

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: N/A

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of clothianidin to honeybees in a field test for the purpose of chemical registration (new use).

Specifically, the test was conducted in response to a request by the Canadian PMRA and the U.S. EPA; as a condition for Poncho® registration in these countries, Bayer CropScience was asked to investigate the long-term toxicity of clothianidin-treated canola to foraging honey bees.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: **Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)**	*Apis mellifera* L.
Colony description at beginning of test:	Each colony consisted of a single brood chamber (24 cm deep, 10 frames per super) below a shallow honey super (originally empty, 16.5 cm deep, 9 frames per super). Queens in all colonies were of the same lineage and approximately the same age. A queen excluder was placed between the brood chamber and honey super to retain the queen in the brood chamber. Colonies were adjusted for strength to establish similar quantities of food stores (pollen and nectar), brood in all stages of development, and adults in each.
Pre-test health:	Colonies were assessed for presence of Varroa

Guideline Criteria	Reported Information
	mite, tracheal mite, and infectious honey bee diseases (American Foulbrood, European Foulbrood, and Chalkbrood) prior to placement in canola and throughout the study. Colonies were also treated with Checkmite® prior to placement in canola.
Supplier	Prior to field testing, the honey bee colonies were held at a spring apiary near the Townsend House Bee Research Facility, University of Guelph, Ontario.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Exposure Site Location and Establishment:	The four test sites were located in Elora, Ontario, Canada, at the University of Guelph, Elora Research Station (sites E1 and E2), and two neighboring farms owned by Allan and Phillip Wallace (sites W3 and W4). East test site consisted of two 1-hectare fields, one planted with clothianidin-treated canola seed and the other planted with control seed, giving a total of eight fields. Fields at each site were separated by at least 250 m. Planting of the canola seed occurred on May 20-21, 2005. Seeds were sown to a depth of 4 cm at the highest recommended rate of 15-20 seeds/m (8.0 kg/ha).

Guideline Criteria	Reported Information
Site Preparation:	All fields received a pre-plant treatment with Treflan EC® (43% trifluralin) at 2.0 L/ha, and with fertilizer (ammonium nitrate, 34-0-0) at 100 kg N/ha according to Ontario canola production recommendations. Prior to introduction of the colonies, a 10 m x 10 m clearing was mowed in the middle of each canola field to accommodate four colonies.
Number of Replicates/Treatment:	Four colonies per field, with 1 treated and 1 control field per site, and four sites (32 total colonies)
Post-exposure Site Location:	The fall apiary was located at the former University of Guelph, Cambridge Research Station, Ontario, Canada.
Lighting:	Natural; not further described.
Precipitation:	Daily precipitation ranged from 0.0 to 18.8 mm during the exposure period (data obtained from the Canadian weather website cited in the study report; refer to Reviewer's Comments section). The maximum rainfall event occurred on July 16 and 17, when 18.8 and 10.4 mm precipitation occurred, respectively. Total precipitation during the exposure period was 35.4 mm.
Temperature:	Mean daily temperatures ranged from 15.2 to 26.7°C during the exposure period.
Relative humidity:	Not reported

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported

Guideline Criteria	Reported Information
Reference toxicant tested?	No
Duration of Exposure Period	21 days, during canola bloom period
Duration of Post-exposure Period	109 days in the fall apiary
Test Substance(s):	Prosper FL Formulation Type: flowable suspension Batch No.: 312065M Ai: 9.64% clothianidin + the fungicides thiram, carboxin, and metalaxyl at *ca.* 9, 4, and 0.3%, respectively (refer to Reviewer's Comments section) Source: Gustafson, McKinney, TX Poncho 600 FS Formulation Type: flowable suspension Batch No: 407483M Ai: 48.0% clothianidin Source: Bayer CropScience, Kansas City, MO
Control Substance(s):	Prosper FL Blank Lot No.: TAM113:70-1 Ai: thiram, carboxin, and metalaxyl Source: Gustafson, McKinney, TX Poncho 600 Blank Lot No.: TAM113:67-1A Source: Gustafson, McKinney, TX
Canola Seed:	Variety A: Hyola 420, supplied from Interstate Payco Seed Company, West Fargo, ND

Guideline Criteria	Reported Information
Application Rate:	Treatment 1: Prosper FL at 1250 mL/100 kg and Poncho 600 FS at 417 mL/100 kg, to deliver clothianidin at the rate of 400 g ai/100 kg seed. Treatment 2: Blank Prosper FL (containing thiram, carboxin, and metalaxyl) and Blank Poncho 600 FS at the same ratios as used for Treatment 1.
Verification of Application Rate:	Mean (n=3) of 4175 ppm or 417 g ai/100 kg seed
Method of Seed Coating:	For both treatments, slurries were prepared by combining the appropriate quantities of each formulation. The slurries were applied to the seed using the Gustafson CBT-50 seed treater. Due to the large quantity of seed (100 kg per treatment), each treatment was divided into four 25-kg batches for treatment.
Colony Introduction:	The colonies were moved to the canola fields over a two-night period (June 27/28 and June 29/30), when approximately one-quarter to two-thirds of canola blooms in the test fields had opened (determined by visual estimation). All colonies were positioned so that the entrances faced approximately south. June 30, 2005 was identified as Day 0 of the 3-week exposure period. Honey supers were removed from and added to colonies as needed throughout the study.

Guideline Criteria	Reported Information
Post-exposure:	Colonies were moved at night from the canola fields to the fall apiary on July 20/21, when approximately 20% bloom remained in each field. Colonies remained there until study termination (Day 130; November 7). Control colonies were separated from those from the clothianidin-treated fields by at least 30 m. No other colonies were present at the fall apiary.

D. Biological Assessments

Guideline Criteria	Reported Information
Canola:	- Seedling emergence rates (determined on June 3 and June 7/8) - Development rates - Crucifer flea beetle (*Phyllotreta cruciferae* Goeze) and striped flea beetle (*Phyllotreta striolata* (F.) damage
Weight Gain:	Colonies were weighed on Days -1 (the night of transport to the canola fields) and Day 21 (the night colonies were moved to the fall apiary).
Honey Yield:	Honey yield per colony by weight.
Adult Mortality:	Dead workers and drones were collected and counted *ca.* every 7 days from Days 0 to 130. Mortality was assessed using Gary Dead Bee Traps (DBT) or 1 x 2 m white sheets placed on the ground extending out from the hive entrance. As only eight DBT units were available, one randomly selected colony at each field was fitted with a DBT, while the entrance sheet method was used for the remaining three colonies.

Guideline Criteria	Reported Information
Brood:	The area of sealed brood was determined on Days -2, 1/2, 14/15, 33/34, and *ca.* every 14 days up to Day 98 (refer to Reviewer's Comments section). The area of sealed brood was estimated by placing an empty template brood frame that was divided into six quadrants over each test brood frame, and estimating the percent sealed. Estimates were performed on both sides of each frame, for all 10 frames of each colony.
Worker Longevity:	Tagged worker bees were counted on Days 5 and 9 (post-introduction assessments), 14 and 15, and thereafter at *ca.* 14-day intervals up to Day 98. On Day 4 (allowing for a 3-day colony acclimation to the canola fields), newly-emerged (<24 hours) worker bees (from spare colonies maintained at the Townsend House Bee Research Facility) were marked with Opalith® colored/numbered thoracic tags (Graze, Bienenzuchtgeräte), and 50 marked workers were then introduced to each colony. Assessments on Day 5 indicated unsuccessful introductions in six colonies (three control and three treatment), and therefore a re-introduction was performed on Day 8. On Day 70, a second set of tagged workers was added to all colonies. At that time, 25 colonies had no tagged workers left, four colonies had one tagged worker, one colony had five tagged workers, and one colony still had 12 tagged workers. Following each reintroduction of tagged workers, those from subsequent introductions were disregarded during data collection.

Guideline Criteria	Reported Information
Queen Assessments:	Queen assessments were conducted at the time of brood assessment, i.e., Days -2, 1/2, 14/15, 33/34, and *ca.* every 14 days up to Day 98. Queens were located and visually inspected to ensure normal physical health and behavior. When queens were not located, the presence of eggs confirmed the presence of a laying queen in the colony within the last 3 days. Inspections were also conducted at these times for queen supercedure cells (elongate cells in which a new queen is reared). Most often, these cells were opened to verify the presence of a larva, and then destroyed. If the queen was absent in a colony, however, in some cases supercedure cells were left to allow a new queen to be reared by the colony. In other cases, marked queens were collected from spare colonies and introduced to the experimental colonies (refer to Reviewer's Comments section for further detail).

E. Residue Analysis

Guideline Criteria	Reported Information
Nectar Collection:	A 5-g pooled sample of nectar (when available) was collected from colonies at each field on Days -3/-1, 7, 14/15, 42, and thereafter at *ca.* 21-day intervals up to Day 83. Nectar was extracted from cells using a disposable syringe, or removed by gently shaking a brood frame over a sheet of waxed paper.

Guideline Criteria	Reported Information
Honey Collection:	A 5-g pooled sample of honey was collected from colonies at each field using a small disposable spatula on Days -3/-1, 7, 13, 40, and thereafter at *ca.* 21-day intervals up to Day 102.
Pollen Collection:	A 10-g pooled sample of pollen was collected from colonies at each field on Days -3/-1, 7, 14/15, 42, and thereafter at *ca.* 21-day intervals up to Day 106. Pollen was collected over a 24-hour period using an OAC pollen trap. Approximately 5 g of each sample was analyzed under a light microscope to confirm the bees foraged on canola. The remainder was used for residue analysis.
Beeswax Collection:	A 3-cm^2 pooled sample of brood and food-free beeswax was collected from colonies at each field on Days -3/-1, 7, 13, 40, and thereafter at *ca.* 21-day intervals.

Guideline Criteria	Reported Information
Storage of Samples:	All samples collected for residue analysis were held in a freezer at -20°C until shipment to the laboratory, and were shipped on dry ice. Conditions of storage (samples and extracts) once at the laboratory were not reported. Intervals of storage (reviewer-determined): Honey: 157 days prior to extraction, and 49 days prior to analysis. Nectar: 283 days prior to extraction, and 26 days prior to analysis. Pollen: 212 days prior to extraction, and 44 days prior to analysis. Beeswax: 273 days prior to extraction, and 22 days prior to analysis. **Storage stability assessments were apparently not performed.**
Extraction/Analysis:	The residue method for clothianidin in pollen, honey, and nectar was based on Bayer Method 00554 with minor modifications. For wax, a short summary supplied by Ralf Schoning of Bayer for imidacloprid was used as the basis for extraction. For all matrices, concentrations of clothianidin were determined using LC/MS/MS, and the limit of quantitation was 0.5 ppb (ng/g).

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Reviewer's Comments section for details regarding non-GLP sections).
Raw data included?	No, raw data were generally not provided for any measured parameter. Summarized data tables (means) were provided for worker and drone mortality, and worker longevity.
Signs of toxicity (if any) were described?	Yes

Canola Emergence:

Canola emergence, development, and flea beetle damage were compared by treatment and site. There were significantly more emerged plants per meter in clothianidin seed-treated fields than in untreated fields on both June 3 and June 8 ($p<0.0001$). Comparison among treated sites indicated that emergence was greatest at site E2 and lowest at site W4 at both intervals, although the difference was only significant on June 3 ($p = 0.0029$). On both sampling days, however, a significant site-treatment interaction was found, with generally greater emergence in treated fields ($p = 0.026$ on June 3 and $p = 0.0025$ on June 8).

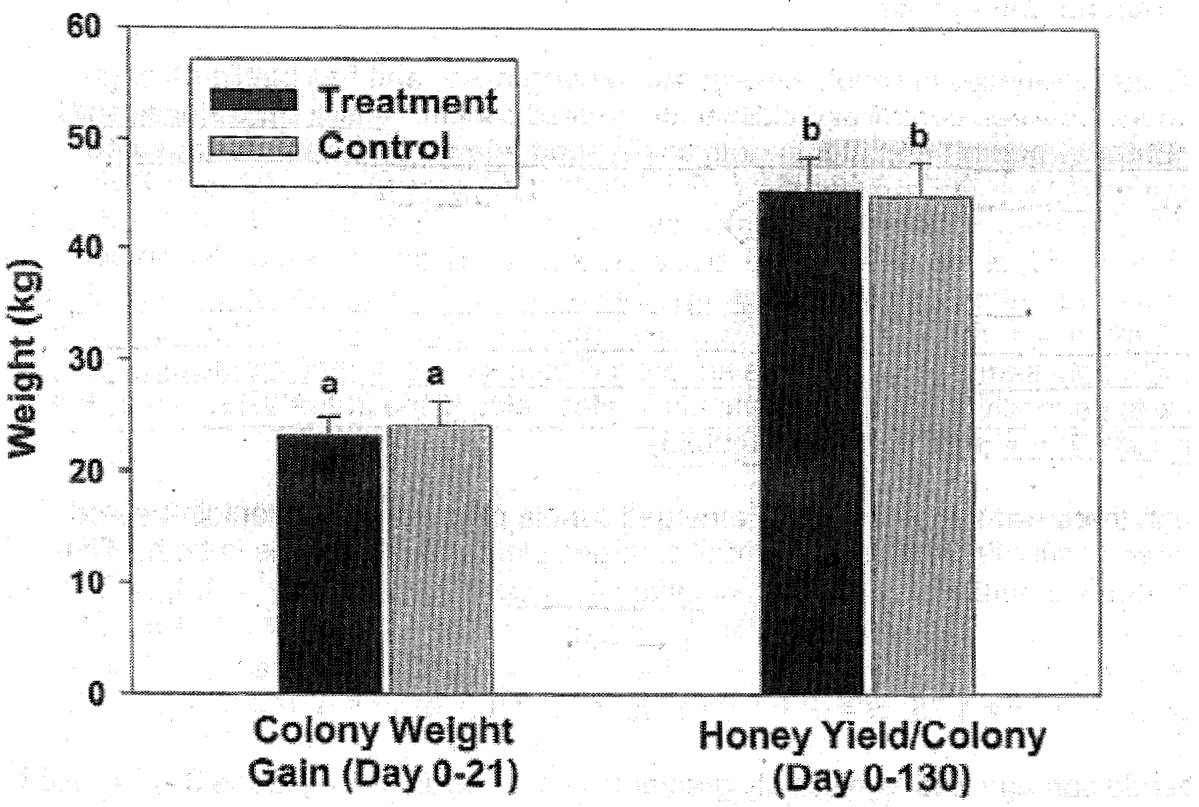
Although there were generally more emerged canola plants per meter in clothianidin-treated fields than control fields, development of emerged plants was the same in both. The growth stage of emerged plants did not differ with treatment or site, and there was no significant interaction of those effects.

Flea beetle damage was significantly greater in control fields on both June 3 and June 8 ($p<0.0001$). Although flea beetle damage did not vary with site on June 3, a difference between sites was found on June 8 ($p = 0.014$).

Weight Gain:

There was no significant difference in weight gain of colonies from control and clothianidin-treated fields (Figure 1). In both treatments, colony weights increased approximately 23-24 kg during the 3-week exposure period in canola fields. In addition, differences in colony weight gain were not significant among sites, and there was no significant treatment-site interaction.

Figure 1: *Mean (± SEM) honey bee colony weight gain and honey yield after exposure to clothianidin-treated (n=16) and control (n=15) canola. Colonies were in canola fields for 21 days during bloom (Days 0-21), and thereafter moved to a fall apiary, approximately 35 km away, where they were maintained for another 109 days.*



Honey Yield:

There was no significant difference in honey yield from colonies from control and clothianidin-treated fields (Figure 1). A mean of 45.3 kg and 44.7 kg of honey was harvested from treated and control fields, respectively, over the 130 days of the experiment. Values were comparable to the 2005 Ontario honey yield average of 46.6 kg. In addition, differences in honey yield were not significant among sites, and there was no significant treatment-site interaction for honey yield.

Adult Mortality:

In analyzing changes in the number of dead workers or drones over time, DBT (dead bee trap) and sheet data were analyzed separately, and the GLM incorporated effects of day, treatment, and the interaction of these terms. For analyses on individual days, the GLM in most cases was able to simultaneously incorporate analysis of effects of site, treatment, and method of dead bee collection. In some cases, however, inclusion of one of these parameters resulted in a significant Lack of Fit (LOF) in the model, while itself not contributing significantly to the model. In such cases, the parameter causing the LOF was removed, resulting in a simpler but more robust model (Table 1).

There were significant changes over time in the number of dead workers recovered from colonies with both the DBT (trap) and the sheet methods ($p<0.001$). However, regardless of the dead bee collection method used, there was no significant difference in dead workers due to treatment or the day-treatment interaction. Although the recovery of dead drones changed over time with the entrance sheet method ($p<0.001$), no change over time was found with DBT. As with workers, regardless of the dead bee collection method used, treatment and the day-treatment interaction had no significant effect on the number of dead drones found.

Table 1: *Honey bee mortality in colonies located in clothianidin-treated (n=16) and control (n=16) canola fields. At each of four sites (one treatment, one control field per site) were three colonies equipped with a white entrance sheet, and one colony fitted with a dead bee trap (DBT). Effects of site, treatment, dead bee assessment method, and their interaction were determined using a general linear model platform (SAS Institute 2003). Statistically-significant effects (α = 0.05) are in bold.*

Day	Dead Workers		Dead Drones	
	Effects Test	**Statistics**	**Effects Test**	**Statistics**
7[1]	Site	P = 0.45	Site	P = 0.11
	Treatment	P = 0.75	Treatment	P = 0.44
	Site*Trt	P = 0.51	Site*Trt	P = 0.45
13	Site[2]	P = 0.40	Site	P = 0.69
	Treatment	P = 0.13	Treatment	P = 0.61
	Site*Trt	P = 0.18	Method	P = 0.92
			Site*Trt	P = 0.39
			Site*Method	P = 0.56
			Trt*Method	P = 0.58

Day	Dead Workers		Dead Drones	
	Effects Test	**Statistics**	**Effects Test**	**Statistics**
18	Treatment[3]	P = 0.07	Treatment[3]	P = 0.18
	Method	**P < 0.001**	Method	P = 0.06
	Trt*Method	P = 0.13	Trt*Method	P = 0.12
21	Colonies moved from canola fields to fall apiary .			
28	Site	P = 0.77	Site	P = 0.97
	Treatment	P = 0.31	Treatment	P = 0.38
	Method	P = 0.34	Method	P = 0.32
	Site*Trt	P = 0.71	Site*Trt	P = 0.57
	Site*Method	P = 0.27	Site*Method	P = 0.31
	Trt*Method	**P = 0.03**	Trt*Method	P = 0.70
35[1]	Site	P = 0.51	Site	P = 0.41
	Treatment	P = 0.35	Treatment	P = 0.88
	Site*Trt	P = 0.06	Site*Trt	P = 0.45
42[1]	Site	P = 0.20	Site	P = 0.59
	Treatment	P = 0.46	Treatment	P = 0.35
	Site*Trt	P = 0.50	Site*Trt	P = 0.67
49	Treatment[3]	P = 0.15	Site	P = 0.93
	Method	P = 0.09	Treatment	P = 0.60
	Trt*Method	**P = 0.002**	Method	P = 0.32
			Site*Trt	P = 0.57
			Site*Method	P = 0.81
			Trt*Method	P = 0.71
64	Site	P = 0.96	Site	P = 0.87
	Treatment	P = 0.31	Treatment	P = 0.23
	Method	P = 0.32	Method	P = 0.97
	Site*Trt	P = 0.56	Site*Trt	P = 0.26
	Site*Method	P = 0.42	Site*Method	P = 0.37

Day	Dead Workers		Dead Drones	
	Effects Test	**Statistics**	**Effects Test**	**Statistics**
	Trt*Method	**P = 0.03**	Trt*Method	P = 0.90
71	Site	P = 0.39	Site	P = 0.67
	Treatment	P = 0.07	Treatment	P = 0.22
	Method	P = 0.52	Method	P = 0.84
	Site*Trt	P = 0.33	Site*Trt	P = 0.28
	Site*Method	P = 0.13	Site*Method	P = 0.95
	Trt*Method	P = 0.79	Trt*Method	P = 0.73
77 / 79	Site	**P = 0.002**	Site	P = 0.72
	Treatment	**P = 0.02**	Treatment	P = 0.97
	Method	**P < 0.001**	Method	P = 0.82
	Site*Trt	P = 0.98	Site*Trt	P = 0.54
	Site*Method	**P = 0.004**	Site*Method	P = 0.68
	Trt*Method	**P = 0.004**	Trt*Method	P = 0.47
89	Site	P = 0.50	Site	P = 0.99
	Treatment	P = 0.62	Treatment	P = 0.47
	Method	P = 0.46	Method	P = 0.29
	Site*Trt	P = 0.37	Site*Trt	P = 0.12
	Site*Method	P = 0.91	Site*Method	P = 0.88
	Trt*Method	P = 0.18	Trt*Method	P = 0.70
92	Site	**P = 0.03**	Site	P = 0.86
	Treatment	**P < 0.001**	Treatment	P = 0.57
	Method	**P < 0.001**	Method	P = 0.89
	Site*Trt	**P = 0.04**	Site*Trt	P = 0.99
	Site*Method	**P = 0.002**	Site*Method	P = 0.35
	Trt*Method	**P = 0.004**	Trt*Method	P = 0.87

Day	Dead Workers		Dead Drones	
	Effects Test	**Statistics**	**Effects Test**	**Statistics**
99	Site[4]	P = 0.19	Site	P = 0.16
	Method	**P < 0.001**	Treatment	P = 0.47
	Site*Method	P = 0.17	Method	**P = 0.04**
			Site*Trt	P = 0.06
			Site*Method	**P = 0.03**
			Trt*Method	P = 0.96
106	Site	P = 0.07	Treatment[3]	P = 0.07
	Treatment	P = 0.54	Method	P = 0.08
	Method	P = 0.38	Trt*Method	P = 0.10
	Site*Trt	P = 0.49		
	Site*Method	P = 0.08		
	Trt*Method	P = 0.89		
112	Site	**P < 0.001**	Treatment[3]	P = 0.07
	Treatment	P = 0.13	Method	**P = 0.05**
	Method	**P < 0.001**	Trt*Method	P = 0.07
	Site*Trt	P = 0.81		
	Site*Method	**P < 0.001**		
	Trt*Method	**P = 0.02**		
120	Treatment[3]	**P = 0.008**	Treatment[3]	P = 0.20
	Method	**P < 0.001**	Method	**P < 0.001**
	Trt*Method	**P = 0.009**	Trt*Method	P = 0.24

Day	Dead Workers		Dead Drones	
	Effects Test	**Statistics**	**Effects Test**	**Statistics**
127	Site	**P < 0.001**	Site	**P < 0.001**
	Treatment	P = 0.82	Treatment	P = 0.34
	Method	**P < 0.001**	Method	**P < 0.001**
	Site*Trt	P = 0.22	Site*Trt	P = 0.60
	Site*Method	**P < 0.001**	Site*Method	**P < 0.001**
	Trt*Method	P = 0.26	Trt*Method	P = 0.72

[1] DBT data unsuitable for analysis.
[2] Method parameter resulted in a significant Lack of Fit and therefore was omitted from the model.
[3] Site parameter resulted in a significant Lack of Fit and therefore was omitted from the model.
[4] Treatment parameter resulted in a significant Lack of Fit and therefore was omitted from the model.

On various collection dates, there were significant differences due to site, dead bee recovery method (DBT or sheet), treatment, and the interaction of these terms. However, there were no consistent trends in effect of these variables during the experiment. Recovery of dead bees using DBT was usually significantly greater than that with entrance sheets, although a significant method-site interaction was often found, indicating high variability in the number of dead bees recovered among the colonies fitted with a DBT. From a total of 18 sampling dates over 130 days, only one data set (Day 56) showed a statistically significant increase in worker mortality in treated colonies, whereas on three sampling dates (Days 77/79, 92, and 12) mortality from control colonies was statistically higher, Thus, there was overall no relevant difference between treatment and control colonies in worker mortality. In general, more dead workers than drones were recovered throughout the experiment, regardless of the collection method (Table 2). Effects on the number of dead drones recovered were minimal, but were occasionally found near the end of the experiment. As expected, the number of dead workers increased near the end of the experiment (e.g., Day 99) as colonies prepared for over-wintering.

Table 2: *Honey bee worker and drone mortality in colonies located in clothianidin-treated (n=16) and control (n=16) canola fields. At each of four sites (one treatment, one control field per site) were three colonies equipped with a white entrance sheet, and one colony fitted with a dead bee trap (DBT).*

Day	Mean No. Dead per Colony			
	Dead bee trap (DBT)		Entrance Sheet	
	Treated	Control	Treated	Control
Worker Mortality				
7	--	--	43.1	39.8
13	70.5	4.0	22.1	14.1
18	213.5	123.3	26.4	18.8
28	66.8	17.8	18.7	37.8
35	--	--	34.1	44.9
42	--	--	24.9	34.4
49	174.5	54.8	47.6	95.5
56	128.5	40.0	29.9	35.6
64	72.3	27.8	28.1	44.8
71	19.1	63.0	11.6	44.8
77	117.5	188.9	37.2	29.0
89	54.8	107.5	72.8	47.8
92	24.7	86.8	15.7	20.9
99	356.3	333.5	79.8	113.0
106	255.3	225.3	208.3	160.8
112	117.3	148.0	16.7	9.4
120	141.0	291.8	4.8	6.3
127	87.3	109.6	18.1	2.9
Drone Mortality				
7	--	--	4.2	5.4
13	10.8	3.0	7.1	7.3
18	56.8	14.3	6.0	8.8

Day	Mean No. Dead per Colony			
	Dead bee trap (DBT)		Entrance Sheet	
	Treated	Control	Treated	Control
28	0.2	5.8	6.3	8.5
35	--	--	8.5	7.8
42	--	--	2.7	5.0
49	7.3	9.3	15.5	27.3
56	6.8	12.0	14.8	19.0
64	4.5	14.5	5.8	13.8
71	1.8	4.0	1.4	5.3
77	6.0	13.7	11.6	3.2
89	6.9	36.3	45.2	54.1
92	3.4	4.8	3.3	5.8
99	42.0	38.0	29.7	25.0
106	9.5	143.8	6.2	11.9
112	4.0	93.8	0.3	0.3
120	4.3	2.3	0.3	0.2
127	37.8	36.0	2.0	1.2

There was a noticeable spike in the number of dead workers recovered in DBT on Day 18 (Table 2). In the week preceding collection of dead bees on Day 13, the mean maximum temperature was 30.9°C. Under these conditions, it is possible that DBT (essentially large metal boxes covering the colony entrance) caused poor ventilation and over-heating, resulting in an increased number of dead workers on this day.

Brood Assessment:

Although brood assessments were to be conducted up to Day 130, it was evident by Day 112 that there was no or minimal sealed brood in colonies in preparation for over-wintering. Therefore, the final brood assessment was conducted on Day 97/98.

The amount of sealed brood per colony changed significantly over time in colonies from control and clothianidin-treated canola fields ($p<0.0001$). However, on most days there was no effect of site, treatment, and/or sampler (the individual determining the amount of sealed brood), or the

interaction of these terms (Table 3). On Day 1/2, the amount of sealed brood per colony differed significantly across sites and on Day 33/34, the amount of sealed brood differed with the sampler. At no time during the experiment did the amount of sealed brood in colonies from clothianidin-treated field differ significantly from that found in colonies from control fields.

Table 3: *Mean area of sealed brood in honey bee colonies in clothianidin-treated (n=16) and control (n=16) canola fields. Colonies were placed in canola fields on July 1 (Day 1) and were moved to a fall apiary on July 21 (Day 21) for the remainder of the experiment (to Day 130). Effects of site, treatment, sampler (individual determining the amount of sealed brood), and their interaction were determined using a general linear model platform (SAS Institute 2003). Statistically-significant effects (α = 0.05) are in bold.*

Day	Mean Area (cm^2) Sealed Brood per Colony		Effect	Statistics
	Treated	Control		
1[1]	5304.2	5104.3	Site	**P = 0.007**
			Treatment	P = 0.06
			Site*Trt	P = 0.09
14	5126.14	4814.7	Site	P = 0.32
			Treatment	P = 0.61
			Sampler	P = 0.27
			Site*Trt	P = 0.48
			Site*Sampler	P = 0.39
			Trt*Sampler	P = 0.56
33/34[2]	4816.8	4762.0	Sampler	**P = 0.05**
			Treatment	P = 0.84
			Sampler*Trt	P = 0.88
48/49[2]	4975.0	4780.3	Sampler	P = 0.36
			Treatment	P = 0.69
			Sampler*Trt	P = 0.16
63/64[2]	4612.1	4238.6	Sampler	P = 0.17
			Treatment	P = 0.93
			Sampler*Trt	P = 0.14

Day	Mean Area (cm^2) Sealed Brood per Colony		Effect	Statistics
	Treated	Control		
76/84[1]	4597.4	4917.6	Site	P = 0.53
			Treatment	P = 0.49
			Site*Trt	P = 0.79
97/98[1]	1179.9	819.6	Site	P = 0.54
			Treatment	P = 0.15
			Site*Trt	P = 0.07

[1] Could not incorporate 'sampler' effect into model.
[2] Could not incorporate 'site' effect into model.

Worker Longevity:

The number of tagged workers decreased over time in colonies from both clothianidin-treated and control canola fields ($p<0.0001$). The GLM found no significant effect of site or treatment on longevity of tagged workers. There also was no significant date*site, date*treatment, or site*treatment interaction of the terms. Throughout the experiment, there was no significant difference in the number of tagged workers found in colonies from clothianidin-treated and control fields on any give day (Table 4). That is, workers lived as long in colonies in treated fields as in control fields.

Table 4: *Honey bee worker longevity in colonies in clothianidin-treated (n=16) and control (n=16) canola fields. Workers (n=50) tagged with colored/numbered thoracic tags were added to each colony on Day 4 (July 4); a second tagged worked introduction (n=50) was made on Day 70 (Sept. 8). Effects of site, treatment, and their interaction were determined using a general linear model platform (SAS Institute 2003).*

Day	Mean No. Tagged Workers		Effect	Statistics
	Treated	Control		
5/9	40.7	42.6	Site	P = 0.87
			Treatment	P = 0.30
			Site*Trt	P = 0.12
14/15	30.3	30.3	Site	P = 0.81
			Treatment	P = 0.98
			Site*Trt	P = 0.34

Day	Mean No. Tagged Workers		Effect	Statistics
	Treated	Control		
33/34	9.7	8.2	Site	P = 0.54
			Treatment	P = 0.48
			Site*Trt	P = 0.08
48/49	1.7	1.4	Site	P = 0.72
			Treatment	P = 0.70
			Site*Trt	P = 0.26
63/64	0.5	0.1	Site	P = 0.77
			Treatment	P = 0.22
			Site*Trt	P = 0.55
76/77	24.8	NA[1]	Site	--
			Treatment	--
			Site*Trt	--
84	NA[1]	12.4	Site	--
			Treatment	--
			Site*Trt	--
97/98	5.8	5.1	Site	P = 0.71
			Treatment	P = 0.62
			Site*Trt	P = 0.27

[1] Data not collected.

Disease:

Incidence of disease was low throughout the study. Colonies were treated with Checkmite® prior to placement in canola. As a result, Varroa and tracheal mite incidence was very low throughout the study; in the majority of colonies, no Varroa or tracheal mites were detected. American Foulbrood and European Foulbrood were not found in any colonies during the study. Chalk brood was sporadically detected at very low levels (i.e., 5-10 cells/colony throughout the study. However, as workers routinely remove chalkbrood mummies, the disease never affected the overall health of colonies.

Queen Losses and Overall Colony Health:

The presence of eggs and larvae were observed in colonies throughout the study. Due to losses of the queen, some colonies at some observations had low numbers of eggs and larvae. Loss of queens from colonies was expected given the intense amount of data collection, movement of colonies, and large number of colonies in the study. During the experiment, personnel replaced queens in eight colonies (Table 5). In five colonies (E2Cc, W3Cc, W3Cd, E2Tb, and E2Tc), original marked queens that died or were killed were naturally replaced by a virgin queen (i.e., the colony replaced the original queen on its own). Colonies W3Cc and E2Tc were both artificially and naturally re-queened. Therefore, a total of six colonies from clothianidin-treated fields and five colonies from control fields were naturally and/or artificially re-queened during the study. Colony W3Ta was problematic throughout the study. It was found to be queenless on July 8 (likely killed during the move of colonies to canola fields), and subsequently did not accept artificially re-introduced queens. However, the colony was found to be successfully naturally re-queened on Day 63 and thereafter.

Table 5: *Honey bee colonies to which new queens were introduced.*

	July 8	**August 4**	**August 18**
Treated Colonies			
E2Ta		X	
E2Tc		X	
W3Ta	X	X	X
W4Tc	X	X	
W4Td			X
Control Colonies			
W3Cc			X
W4Ca		X	
W4Cb	X		

Three colonies (W3Cc, W4Cb, and W4Td) were classified as "dead" part way through the study. These colonies were artificially or naturally re-queened during the experiment, but failed to successfully establish a queen. Although data from these colonies may have inadvertently been collected (prior to status was realized), these data were omitted from some statistical analyses, e.g., sealed brood analyses near the end of the experiment. Given that adequate data were collected from these colonies through much of the experiment, and that there were a large number of replicates in total, the loss of these colonies had no impact on the study overall.

As a general observation, experienced beekeepers/researchers qualitatively assessed colonies from clothianidin-treated and untreated canola fields throughout the study and found no differences in overall colony health and vigor.

Residue Analysis:

Clothianidin was detected in treated seed at the prescribed level at an average of 417 g ai/100 kg seed. In-phase recovery (± SD) of clothianidin residues from spiked samples of honey, nectar, pollen, and beeswax was 93 ± 13.0, 89 ± 13.3, 87 ± 13.8, and 104 ± 21.6%, respectively.

The majority of samples (>75%) collected had no detectable levels of clothianidin residues (LOQ = 0.5 ng/g), whether from colonies in treated or control fields (Table 6). The maximum concentration of clothianidin detected in honey, nectar, pollen, and beeswax samples was 0.928, 2.24, 2.59, and <0.5 ng/g, respectively. No clothianidin residues were detected in honey, pollen, or beeswax samples collected from control fields, although analyses conducted in January 2006 detected residues in three nectar samples from control colonies (field E1C, July 7; field W3C, July 7; and field W3C, August 11). Subsequent analyses of back-up nectar samples detected residues in two control colonies (field E1C, July 7; and field W3C, July 7), suggesting that workers in control colonies may have foraged on clothianidin-treated canola. This may have occurred because the separation between some pairs of control and treated fields was insufficient or because the forage in some control fields was of lower quality (due to insect damage and lower rates plant emergence), which may have lured workers from control fields to the treated fields. Clothianidin was also detected in two nectar samples when the colonies were not in canola fields (field W3T, June 27; field W3C, August 11).

Table 6: *Clothianidin residues in honey, nectar, pollen, and beeswax collected from honey bee colonies in clothianidin-treated and control canola fields. Pooled samples were collected at each site approximately every 21 days.*

Matrix	Treatment	Total No. Samples	Samples with Residues Detected	Residue Detected (ng ai/g)
Honey	Clothianidin	28	W4T, July 07 W3T, July 07 E2T, July 07 E1T, July 07 W3T, July 07	0.501 0.647 0.510 0.928 0.507
	Control	28	--	<0.5[1]

Matrix	Treatment	Total No. Samples	Samples with Residues Detected	Residue Detected (ng ai/g)
Nectar[2]	Clothianidin	15 (Jan.)	E1T, July 07 E2T, July 07 W3T, July 07 W4T, July 07	0.521 0.979 1.17 0.855
		23 (Mar.)	W3Td, July 27 E1T, July 07 E2T, July 07 W3T, July 07 W4T, July 07	0.693 2.24 0.717 1.74 1.14
	Control	15 (Jan.)	E1C, July 07 W3C, July 07 W3C, August 11	0.535 0.670 0.969
		23 (Mar.)	E1C, July 07 W3C, July 07	0.691 0.922
Pollen	Clothianidin	19	E1T, July 07 E2T, July 07 W3T, July 07 E1T, July 14	1.05 0.698 2.59 1.40
	Control	19	--	<0.5
Beeswax	Clothianidin	0	--	<0.5
	Control	0	--	<0.5

[1] 0.5 ng/g = LOQ

[2] Residue analyses conducted in January 2006 unexpectedly detected clothianidin residues in nectar samples collected from control colonies. Therefore, back-up nectar samples were sent to the laboratory in March 2006 for re-analysis.

Reported Statistical Results:

Plant emergence, development, and flea beetle damage were compared among treatments and sites using a general linear model (GLM) platform (SAS Institute, 2003). Colony weight gain during exposure, honey yield during exposure, worker and drone mortality, brood area per colony, and tagged worker longevity (based on the number of tagged workers recorded each collection day) were also compared over time using a GLM platform.

14. SUPPLEMENTAL ASSESSMENT OF OVERWINTERED COLONIES:

A supplemental report documenting further assessments of the colonies during over-wintering was concurrently-submitted [MRID 469078-02; Cutler, C., and C. Scott-Dupree. 2006. Spring

2006 Assessment of Overwintered Colonies Studied in an Investigation of the Potential Long-Term Impact of Clothianidin Seed Treated Canola on Honey Bees, *Apis mellifera* L. Unpublished report conducted by the University of Guelph, Ontario, Canada, and sponsored by Bayer CropScience, Research Triangle Park, NC. Report submitted July 12, 2006]. Data presented in the addendum report were not collected in accordance with GLP requirements, and raw data were not submitted.

As the colonies prepared for over-wintering beginning in late October, each colony was administered *ca.* 30 g of a mixture of oxytetracycline and icing sugar. Colonies were then provided access to 150 L of a sucrose:water (2:1) solution. In mid-November, colony entrances were reduced, an upper entrance was provided, and insulation was placed between the inner cover and the colony lid. On April 19-20, 2006, the status of over-wintered colonies was assessed for the presence/absence and health of queen, presence/absence of eggs and larvae, area of sealed brood, number of frames of workers, and overall health based on a collective assessment of all data per colony. Colonies were classified as "healthy" if they had ≥4 frames of live bees, and "weak" with <4 frames of live bees.

Overall, the spring assessment found no significant differences in the health of treated versus control colonies. Of the initial 32 colonies, three were classified as "dead" at the end of the fall 2005 data collection, and an additional four colonies (two from treated fields and two from control fields) did not survive the winter. It was reported that a loss of 10-15% of colonies in an apiary over winter is not uncommon in Canada. Of the 25 colonies that survived winter, a healthy queen was found in 21. The presence of eggs and larvae, however, confirmed that the remaining four colonies were queen-right. There was no difference between control and clothianidin-treated colonies in amount of sealed brood ($p = 0.56$) or in the number of frames of workers ($p = 0.95$). Collectively, 24 colonies were classified "healthy", one was classified "weak", and four were "dead".

15. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Replicate data were not provided to statistically verify the results of this study. The reviewer visually verified the reported results and agrees with the study author's assessments.

16. REVIEWER'S COMMENTS:

The reviewer=s conclusions agreed with the study author=s. Although sporadic differences between treatment and control colonies were found on various dates, essentially no differences in bee mortality, worker longevity, or brood development occurred throughout the study. In addition, colonies in clothianidin-treated field gained as much weight and yielded as much honey as those in control fields. No residues of clothianidin were detected (LOQ = 0.5 ppb) in the majority (>75%) of samples (honey, nectar, pollen, and beeswax) collected for residue analysis. The maximum concentration of clothianidin in any sample was 2.59 ng/g in pollen on Day 7. Based on the reported nectar/pollen oral NOAEC for honey bees of 20 ng/g (Schmuck and Keppler, 2003), the maximum concentration of clothianidin detected in any sample during this study was nearly 8-fold below the reported oral NOAEC, indicating a high margin of safety.

The flowable suspension Prosper FL nominally contains 9.49% clothianidin, 9.49% thiram, 4.43% carboxin, and 0.316% metalaxyl. The respective CAS Numbers are 210880-92-5, 137-26-8, 5234-68-4, and 57837-19-1. A Certificate of Analysis was not provided for this test substance, and only the actual percentage of the active ingredient of interest, i.e., clothianidin at 9.64%, was reported in the appendix (in "Appendix 4 – Seed Treatment Phase Report"). The actual percentages of the other active components were not reported.

To the knowledge of the study author, no other flowering crops or corn grown from seed treated with clothianidin were planted within a 1-km radius of the canola test plots. The availability to bees of alternative forage within 1 km of their colonies while situated in canola fields was also minimal. Although potential forage crops (e.g., soybean, corn, alfalfa) were within 1 km of some fields, none of these were in bloom while honey bee colonies were in the canola fields.

It was reported that brood assessments required opening the colony supers for *ca.* 60 minutes, sometimes under very hot, no-shade conditions, and that this procedure was very stressful for the bees. Coupled with the additional stress of moving the colonies to the fall apiary (which took several hours), it was decided not to conduct brood assessments on the day of colony removal from the canola fields. Furthermore, it was decided to wait a week after the move before continuing with the brood assessments, to allow the colonies to acclimatize to their new surroundings.

Originally, brood assessments were to include the presence/absence of eggs, unsealed larvae, and sealed brood for each colony. However, the study author reported it was apparent during the Day 1 assessment that it would not be possible to make all assessments for all 32 colonies [due to lack of time and/or adequate sunlight (fall apiary assessments only)]. Therefore, it was decided to determine the amount of sealed brood only, which would reflect development of egg and larval stages. It was reported that since normal, healthy, unsealed brood eventually are sealed by workers, effects of clothianidin on brood development would still be detected.

It was reported that queens were lost in some colonies during the experiment, e.g., they were accidentally killed during data collection, moving colonies, or rejected by the colony over time. In such cases, marked queens from the same/similar lineage were collected from spare colonies at the Townsend House Bee Research Facility and introduced to the experimental colonies. In other cases, a new queen was allowed to emerge from a supercedure cell to replace the old queen.

Swarm cells (queen cells usually found on the bottom of the combs before swarming) when found were destroyed to prevent swarming.

While the entrance sheet method of collecting dead bees resulted in no technical difficulties or inadequate data, the operation of the DBT (traps) was occasionally unreliable during the experiment. For example, on various collection dates, traps were left partially open, came loose from the colony, or became partially filled with water after heavy rainfall events. Another colony (E1Tc) was mistakenly thought to be "dead" on September 27-30. Therefore, adult mortality data from some colonies fitted with a DBT was not used on some data collection dates. Data were excluded if the number of dead bees in the DBT were unusually low (i.e., 0-1 dead bees) because, for example, the trap was not tightly fitted to the colony, or if the DBT was partially filled with water due to rain, which may have caused some live bees to drown. One dates where there were less than three treated and three control colonies with DBT from which data could be used, analysis comparing dead bee collection methods were not performed. In such cases, the "method" parameter (DBT vs. entrance sheet) was not used in the GLM. A total of n=18 collection dates incorporated entrance sheet dead bee collection data, while n=15 collection dates incorporated DBT collection data.

For the 3 years prior to the field studies, the sites had been planted with alfalfa, corn, soybean, barley, and wheat in 2002; soybean, barley, and corn in 2003; and soybean, corn white bean, and corn/barley in 2004.

The study timetable was as follows:

Seed Treatment Phase	Experiment Start: April 27, 2005 Test and Control Item Receipt: May 4, 2005 Report Completion: November 22, 2005
Field Study Phase	Seed planted May 20-21, 2005 Experiment Start (Day 1): July 1, 2005 Experiment Completion (Day 130): November 7, 2005 Final Report Completion: August 1, 2006
Residue Analysis Phase	Experiment Start (first samples received): August 30, 2005 Analytical Initiation Date: December 14, 2005 Analytical Completion: May 15, 2006 Report Completion: July 5, 2006

The GPS Coordinates for the canola fields were -80.4 X-coordinate and 43.6 Y-coordinate (combined and reduced to three significant figures), and for the fall apiary were -80.3 X-coordinate and 43.4 Y-coordinate.

Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted in compliance with the OECD and EPA Principles of Good Laboratory Practice. However, the following field study phase data were non-GLP compliant:

- Seed storage – before planting, seed was stored for *ca.* 2 weeks in a 10°C walk-in refrigerator at 39% relative humidity. Although no fluctuations in temperature or relative humidity were observed, the refrigerator maintenance and data logging was non-GLP compliant.
- Planting and maintenance (fertilizer and herbicide application) of fields.
- GPS coordinates of treatment (canola fields), pre-treatment (Airport apiary), and post-treatment (former University of Guelph – Cambridge Research Station) sites.
- Ground truthing.
- Weather data were obtained from Environment Canada weather stations in close proximity to sites at which colonies were maintained. Data are available on-line at http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html.
- Sample refrigeration temperatures.
- Statistical Analysis – the software used for statistical analysis (JMP Version 5.1, SAS Institute) was not GLP validated.

16. REFERENCES:

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