3/30/04

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

OFFICE OF PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM **March 11, 2004**

Subject: EFED Review of Bayer CropScience's Draft Protocol of a Honeybee Field Study - Poncho 600 (264-789) [Clothianidin (044309), D295318]

To: Meredith F. Law, Chief Insecticide Branch
Dan Kenny, PM Team 4A
Stephanie Nguyen, Reviewer
Registration Division (RD) (7505C)

From: Gabe Patrick, Biologist Gabe Patrick
Environmental Risk Branch V
Environmental Fate and Effects Division (EFED) (7507C)

Through: Mah Shamim, Ph.D., Chief
Environmental Risk Branch V
Environmental Fate and Effects Division (7507C)

This memo provides EFED's changes to the Bayer CropScience's (BCS's) draft honeybee field study protocol. The protocol title is: An Investigation of the Potential Long-Term Impact of Clothianidin Seed Treated Canola on Honey Bees, *Apis mellifera* L. EFED recommends that RD assign an MRID No. to this protocol and later submissions. The table below reflects an update of the schedule for completion of this study. EFED proposed this schedule in a 4-7-03 addendum to EFED's Risk Assessment on clothianidin's use as a seed treatment on corn and canola.

Proposed Date	Action	Actual Date
Start (April/May 2003)	Conditional Registration of Clothianidin 600 FS	5/30/03

Proposed Date	Action	Actual Date
60 days (June/July 2003)	Registrant submission of draft protocol	11/28/03 (received by EFED)
120 days (August/September 2003)	EPA response with proposed changes to draft protocol	2/17/04 - 1st reviewer completed review 3/5/04 - 2nd reviewer completed review 3/8/04 - EFED's response completed
180 days (October/November 2003)	Registrant submission of final protocol for approval	
210 days (December 2003/January 2004)	EPA and registrant concur on study	
540 days (1.5 yrs.) (December 2004)	Final completed study submitted to EPA for review	May, 2005 (Bayer requested extension)

Overall we feel good about this protocol and believe it will yield valuable data about clothianidin's long-term effects on honeybee colonies. However, we do have some comments, questions, and changes that BCS needs to address before wrapping up this protocol. As pointed out in schedule, we are behind the proposed completion dates. By the design provided in this protocol, the clothianidin treated seed, plant dates would probably occur the last week of April, 2004 or the first week of May, 2004. The honeyflow and pollen production from theses sites would begin during the first week of July, 2004. BCS, in a 9/30/2003 memo, requested an extension until May, 2005 to complete the study. BCS is seeking this extension to allow time for samples analysis and writing the final report. EFED does not oppose this extension.

These dates mean that it is still possible to have a final agreement on the protocol before implementation which would allow this field study to occur during the 2004 crop growing year. However, should this not prove possible, EFED would recommend delaying this field study until the 2005 growing season. EFED wants usable data to decide the potential adverse effects to bees from clothianidin's seed treatment use and opposes rushing the study and having deficient information.

cc: Christine Cairns, Life Scientist
U. S. Environmental Protection Agency - Region 4

The specific comments, questions, and changes that need to be addressed by the registrant are as follows:

Trial Design

1. BCS recommends using canola as the representative seed treatment crop with test crops located in Ontario, Canada. Ontario is a major canola growing region in North America. Specifically, the test crops' locations are at the University of Guelph's Elora Research Station, Elora, Ontario. Normally EFED would prefer to have representative test crops in the United States (US). US field testing would account for variables caused by farming practices, meteorological conditions and soil conditions unique to the US. However, EFED feels the practices and conditions in Ontario are similar to those in US canola growing regions. The results from this study should provide satisfactory data to make an adverse effect determination for US concerns. EFED also agrees to the use of canola as the test crop since this crop is attractive to bee and will provide bee exposure from both pollen and nectar. An alternative crop, such as corn, which is less attractive to bees as a forage crop, would provide exposure from pollen, only.

2. BCS must include a map in this study which will show the locations of all the test fields and apiaries (including the fall apiary). This map will provide the distances between apiaries as well as the distances between apiaries and test canola fields. This map will also provide the locations of any other forage attractions available to the bees within a 10 km radius of each apiary. The map must provide the blooming or pollinating status of the plants during the test period and distances from the test apiaries. EFED assumes the 10 km distance is an unlikely flight distance for foragers but would be a possible flight range given a dearth in forage.

3. BCS states the canola seed treatment rate will be at the proposed US commercial rate. The clothianidin seed treatment rate used in study must be the maximum allowable labeled rate per area for clothianidin. BCS must provide this rate in the study as pounds active ingredient per acre (lb ai/A) or kilograms active ingredient per hectare (kg ai/ha) or both.

4. The protocol mentioned various other pesticide treatments (such as, fungicide, herbicides, and insecticides). When known, BCS must provide the application rates for any pesticides used for any treatment relevant to this study. BCS must provide these rates expressed as pounds active ingredient per acre (lb ai/A) or kilograms active ingredient per hectare (kg ai/ha) or both. At a minimum, BCS must provide the chemical's common name and percent active ingredient of the product used (for example, trifluralin, 44.5% for the product, Treflan®) for all pesticides mentioned. If the product is a multiple active ingredient product, then BCS must provide all active ingredients in the product with their respective active ingredient percentages. BCS must show these other treatments on the map filed with this study.

5. BCS proposes using 6, one hectare size fields of spring canola in this study. BCS will use

three of these fields as clothianidin seed treated fields and will use the remaining three fields as controls. EFED is strongly recommending BCS add an another test field and a control field to this study thus having 4 treatment fields and 4 control fields. Field studies are unpredictable and the use of the extra fields would provide a backup in case something goes wrong with one of the test plots. The extra fields would also increase the statistical significance of this study.

Planting Requirements

EFED has no comments, changes or questions.

Honey Bees

1. BCS intends to place the test hives at the edge of the test canola fields when 20% bloom occurs and remove the test hives when bloom has ended. The study must provide the method used for deciding when 20% canola bloom occurs and when the bloom has ended.

2. BCS will use "beekeeping techniques typical for southern Ontario." The study must provide a summary of these techniques. This summary should include bee management techniques used, the equipment (such as, Langstroth hives, queen excluders, or other) used, number of supers (that is, boxes) for each colony, and the number of frames in each super. BCS must also provide the estimated population sizes of all colonies at critical times during the study, and any extra feeding provided. EFED believes periodic population estimates of each colony is useful in assessing the health of the colonies including mortality rates. EFED assumes a normal colony has about 50,000 bees (Pacific Northwest Extension, 1993).

Colony Assessments

1. To measure bee mortality, BCS proposes counting the number of dead bees in front of each colony every 7 days beginning on Day 7 through the end of the study. BCS will perform this count by laying a linen sheet in front of each colony and counting the dead bees on this sheet. EFED believes mortality measurement to be an important value in this study. The proposed method will not account for dead bees lost to scavengers (for example, birds, mammals, and insects) and wind. EFED wants dead bees counted and collected twice daily at roughly 06:30 and 17:30 to better estimate mortality. These daily collections should begin on Day 1 (when BCS places bees next to the test fields) and continue to the end of the study. BCS needs to collect a sample of the bees and save the sample for possible laboratory analysis. As an alternative to these frequent collections from a sheet, EFED is recommending the use of dead bee traps for the collections. EFED is also recommending BCS use mortalities per day classifications from Todd and Reed (1969). Todd and Reed (1969) proposed the death of 100 bees/day near or inside the hive is "normal"; 200-400 bees/day represent "low mortality"; 500-900 "moderate" and more than 1,000 "high mortality".

Worker Assessments

1. To decide how long worker bees are living, BCS states "100 young (48 hr old), marked worker bees will be introduced to each of the colonies at the 6 field locations." BCS will survey these marked worker bees every 10 days until BCS finds no more marked worker bees. At that point, BCS will introduce a second group of marked worker bees to the hives and survey every 10 days until BCS finds none. It is not clear to EFED how BCS will introduce these marked worker bees to the hives or how BCS will conduct the survey. Does BCS intend to remove sealed brood from a hive, mark emerging workers, then reintroduce theses marked workers to the same hive? The marked worker bees origin is not clear to EFED. EFED feels this would be a useful measure of an adult bee's exposure to the clothianidin at the treated sites and after removal from the sites. BCS needs to elaborate on this method and reasoning behind this assessment technique.

2. BCS must supply a method for evaluating the potential disorientation of the forage bees. Are forage bees becoming disoriented and failing to return to the hive from their exposure to clothianidin because of clothianidin's seed treatment use on canola? EFED feels that some assessments in this protocol (such as, the worker longevity assessment, worker mortality assessment and overall colony health assessments) indirectly address this issue. However, EFED wants BCS to devise a more direct measurement for this possible disorientation effect on the bees and include an analysis in this study.

Queen Assessments

EFED has no comments, changes or questions.

Residue Assessment

1. This protocol shows collections of nectar, pollen and beeswax for residue analysis will begin on Day 1 (when BCS places the bees next to the test fields). EFED feels BCS must perform baseline sampling and analysis of nectar, pollen and beeswax before BCS places the bees next to the test fields. In other words, EFED must know the residue levels in the nectar, pollen and beeswax before testing exposure. EFED noted, under the section titled **Honey Bees**, the registant refers to Day 1 as the day the colonies are placed on the edge of test fields. However, under **Colony Assessments**, BCS refers to Day 1 as "24 hours prior to placement in canola." EFED assumes BCS will clarify this inconsistency in the final protocol and BCS intends to perform baseline sampling and analysis.

2. There is no mention of honey residue assessment in this protocol. Past field studies from clothianidin seed treatment uses have shown the presence of clothianidin in nectar from foraging bees' honey stomachs (MRID No. 45422431) and in the stored nectar samples from hives (MRID No. 45422435). The water content of nectar can be as high as 80% whereas the water content of honey is less than 20%. EFED needs to know what, if any,

the clothianidin concentration is in the honey as well as the nectar, pollen and beeswax. BCS must perform sampling and analysis of the honey according to the regimen used for the nectar, pollen and beeswax.

3. BCS must include sampling and analysis of the pollen from the canola, nectar from the canola blooms and nectar from the foraging bees as part of this study. Previous studies (MRID Nos. 45422431 and 45422433) confirmed the presence of clothianidin from canola seed treatment uses in these sample sources. EFED feels BCS must sample and analyze canola nectar and nectar from foraging bees to understand the bees' clothianidin exposure in these studies. EFED believes BCS's use of the Ontario Agricultural College (OAC) pollen trap should adequately assess the residue in the pollen on the test plots. Thus BCS would not need to sample pollen from the canola plants.

Added Inclusions

1. BCS must include weather during test plot planting and later during the honeybee foraging time period on the test plots. BCS must report daily temperatures ranges, daily wind speeds and directions, daily percent cloud cover estimates, and daily precipitation during the foraging time period. During planting BCS must report the daily temperature ranges, daily precipitation and the average soil moisture. BCS can use weather from acceptable local weather reporting stations as a source for this information.

2. BCS must include a characterization of the soil in the test plots which will include the soil type (for example, sandy loam), the percent organic matter and the soil pH.

3. BCS must provide Quality Assurance and Good Laboratory Practices (GLP) compliance statements for the laboratory analysis portion of study. BCS must provide a summary of methods taken to avoid contamination and degradation of the field samples taken during this study. This summary must include how and when BCS delivers the samples to the laboratory for analysis.

4. The study must include signed and dated reports of each of the individual scientists or other professionals involved in the study.

5. BCS must provide all raw data collected with the final report.

References

MRID No. 45422431. Schmuck R. and R. Schöning. 2000. Residues of TI-435 in Nectar Blossoms, Pollen and Honey Bees Sampled from a Summer Rape Field in Sweden and Effects of These Residues on Foraging Honey Bees. Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany. Bayer AG, GB Plant Protection, Marketing-Seed Treatment (Dr. Krohn/Altmann), D-40789 Monheim

MRID No. 45422433. Schmuck R. and R. Schöning. 2000. Residues of TI-435 in Nectar Blossoms, Pollen and Honey Bees Sampled from a French Summer Rape Field and Effects of These Residues on Foraging Honey Bees. Bayer AG, Crop Protection-Development, Leverkusen-Bayerwerk, Germany

MRID No. 45422435. Scott-Dupree C.; Spivak, M.; Bruns, G.; Blenkinsop, C.; & Nelson, S. 2001. The Impact of GAUCHO® and TI-435 Seed Treated Canola on Honey Bees, *Apis mellifera* L. University of Guelph, Guelph, Ontario, Canada; University of Minnesota, St. Paul, Minnesota, USA; Enviro-Test Laboratories, Edmonton, Alberta, Canada. Bayer Corporation, Agriculture Division, Stilwell, Kansas

Pacific Northwest Extension. Nov. 1993. PNW 245: Evaluating Honey Bee Colonies for Pollination: A Guide for Growers and Beekeepers. Pacific Northwest Extension Publication
URL: http://eesc.orst.edu/AgComWebFile/EdMat/PNW245.pdf

Todd, F.K. and C.B. Reed. 1969. Pollen Gathering of honey bees reduced by pesticide sprays. Journal of Economic Entomology. Vol 62 (4). Pp 865-867.

Memorandum

To: Gabe Patrick
From: Christine Cairns
Date: 2/5/04
Subject: Review of clothianidin field study protocol

I have had a chance to review the clothianidin field study protocol, and overall, I feel very good about this protocol as it is. I believe it will yield some very good data on the long term effects on honeybee colonies. However, I do have a few questions and comments about this study that I feel the researcher should address before the study protocol is finalized:

1. They should specify the location of the fall apiary with a brief description of landscape and food source(s). Saying they will use "beekeeping techniques typical for southern Ontario" isn't enough for me to know exactly how the bees will be managed at that point. However, this is mainly due to the fact that my own experience has been in the tropics. If others reviewing this protocol are familiar with what is typical for southern Ontario it may not be necessary for the researcher to expand on that, but for myself it would be.

2. They state that the colonies will be removed from the study fields when canola bloom has ended. I feel they should be a bit more specific because when the canola bloom ends may depend on various factors, and it could be slightly different for each field. It would be a better idea to simply remove all hives after a given number of days, so that at each site the hives will be placed there when 20% of blooms are open and removed *x* days later. Another possibility would be to remove the hives after a given percent of the blooms have dropped. The number of days chosen should give the bees a chance to exhaust a significant portion of the field but not exhaust it totally. If the resources are near exhausted, and you have four hives competing for the remaining nectar and pollen, then some bees will go off and forage elsewhere rather than compete. Although it is stated that no flowering crops will be planted in the vicinity, bees can forage as far as 10 km from the hive. This could pose a risk of cross-contamination in the study if bees start foraging in other fields. Not knowing a lot about the flowering phenology of canola, it is hard for me to suggest an exact number of days or to know how long it would take before the flowers are near tapped, but these are things the researcher should consider.

3. For statistical purposes it may be better to have two additional study fields for a total of N=8 (one more control and one more treated field) so that in case something goes wrong in one of the plots they would have backup and still be able to do a solid analysis on the remaining plots. Six is the absolute minimum for a study like this, so eight would increase statistical significance and decrease the problems that would occur if something did go wrong. Field studies are always a bit unpredictable and it is difficult to exact total control over everything that happens, and since they will only get one shot at this during a very narrow time frame of the canola flowering season, it would be better for them to hedge their bets and go with a higher number of reps. However, if six is the most they can afford to do with their existing resources and manpower than so be it.

4. For the mortality measure, they plan to count the number of dead bees which fall on a light colored linen sheet in front of each colony every seven days. I wondered if they have considered the role of natural predators - isn't it possible that something could eat the dead bees before they get to count them? This would effect the accuracy of the mortality assessment. In my experience you don't normally see lots of dead bees accumulating around a hive. Anything from ants to small reptiles and birds may carry them off. Again, my experience is in the tropics where there may be more of these types of predators and perhaps it is not so much of an issue in southern Ontario, but the researcher should address it and figure out a way to mitigate predation on dead bees if it is a problem. Perhaps using sticky pads instead of just a sheet is a possibility, or counting more frequently than once a week. They can review methodologies of other studies that have tried to keep a measure of hive mortality if they have not already done so.

5. In terms of the longevity assessment, they plan to introduce 100 young marked worker bees on day 4 to track their longevity, and another 100 after the hive has been removed form the canola field. If we are measuring longevity, shouldn't it be based on the bees native to that hive? The first set of bees could be introduced from another hive since at that time the hive will not have been exposed to the canola field for very long, but the second set I would think should be native to the hive, they should be young workers who were raised from the larval stage on honey and pollen extracted from the canola fields. I wasn't clear on where they are planning to get the 100 worker bees they are introducing. At the very least, this should be made clear.

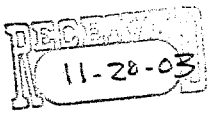
Again, I believe the study overall will yield good results. They are clearly looking at multiple dimensions of the effects of clothianidin including evaluations of bees at all stages, residue analysis, observing queen health, colony weight gain, honey yield, mortality etc. The primary researcher may have already considered some of these issues I just addressed and may have reasons for doing it they way she has chosen to, but if that is the case we should at least know what the reasoning is for choosing to do it this way. Feel free to contact me if you wish to discuss any of my comments or have any questions.

DP #: (295318)

Decision #: 334614

DATA PACKAGE BEAN SHEET

Date: 20-Nov-2003



* * * Registration Information * * *

Registration: 264-789 - PONCHO 600

Company: 264 - BAYER CROPSCIENCE LP

Risk Manager: RM 01 - Daniel - Kenny - (703) 305-7546 Room# CM-2 229

Risk Manager Reviewer: Stephanie Nguyen - SNGUYEN

Sent Date: 22-Oct-2003 Calculated Due Date: 20-Jan-2004 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: CONDITIONAL REGISTRATION FOLLOW-UP;DATA REQUIRED;REQUIRES SCIENCE REV

Ingredients:

* * * Data Package Information * * *

Expedite: ○ Yes ● No Date Sent: 23-Oct-2003 Due Back:

DP Ingredient: 044309, Guanidine, N-?(2-chloro-5-thiazolyl)methylU-N'-methyl-N''-nitro-,?C(E)U-

DP Title:

CSF Included: ○ Yes ● No Label Included: ○ Yes ● No Parent DP #:

	Assigned To	Date In	Date Out	
Organization:	EFED / ERB5	24-Oct-2003		Administrative Due Date: 01-Jan-2004
Team Name:				Negotiated Due Date:
Reviewer Name:	Patrick, Gabe	20-Nov-2003		Projected Completion Date:
Contractor Name:				

* * * Studies Sent for Review * * *

Printed on Page 2

* * * Additional Data Package for this Decision * * *

Printed on Page 3

* * * Data Package Instructions * * *

Mike Rexrode:

Please review attached draft protocol for the Honeybee Field Study to see whether it can be accepted and the meeting with the Registrant is needed.

Thanks, Stephanie

Bayer CropScience

September 30, 2003

Ms. Stephanie Nguyen
Document Processing Desk
Office of Pesticide Programs (7504C)
Registration Division
U.S. Environmental Protection Agency
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, Virginia 22202-4501

Re: Poncho 600 (EPA Reg. No. 264-789)

Submission of Draft Protocol of a Honeybee Field Study for Review

Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709
Phone: 919 549-2000

Dear Ms. Nguyen,

Attached please find five (5) copies of a draft protocol for a research study to investigate any possible chronic effects of clothianidin on bees, as requested in the memo from EFED on April 7, 2003 and in the May 30, 2003 Notice of Pesticide Registration for Poncho 600.

We have developed the protocol in collaboration with Dr. Cynthia Scott Dupree, a very experienced bee researcher from the University of Guelph. We believe that this protocol covers all of the specific requirements and endpoints detailed in the April, 2003 memo. We recommend carrying out the study in canola, as this represents the 'worst case' exposure scenario for bees. We also recommend carrying out the study in Ontario, Canada, as this represents a major canola region in North America. The only change we need to make concerns the timing of the experiments. The original request was for completion of the study at the end of 2004. However, we cannot start the 'field' portion of the study until the canola starts to bloom (around beginning of July). We then need to keep the colonies for two full life cycles (approximately 130 days), which means that the last samples will not be collected until November/December, 2004. To allow time for sample analysis and writing the report, we request an extension until May 2005.

My counterpart in Canada, Roy Lidstone, will also provide PMRA this protocol. We would be happy to meet with you and scientists in EFED and discuss this protocol in more detail. We could also have a joint meeting with PMRA to discuss this protocol. Please let us know how you would like to proceed.

Please contact me at jamin.huang@bayercropscience.com or at 919-549-2634 if you have any questions regarding this submission.

Sincerely,

Jamin Huang, Ph.D.
Product Registration Manager

Cc: Roy Lidstone (Bayer CropScience in Canada)

Attachment

PAGES 13-18 MAY BE OBTAINED UNDER FOIA SUBJECT TO THE ACCESS PROVISIONS OF FIFRA SECTION 10(g)