

**July 11, 2002**

**MEMORANDUM**

**SUBJECT:** Review of laboratory pollen feeding studies on two species of lady beetles (*Coleomegilla maculata* and *Hippodamia convergens*) in support of Monsanto's request to register *Bacillus thuringiensis* (Bt) Cry3Bb1 protein (event MON 863) expressed in field corn pollen. EPA Reg.No: 524-LRA, DP Barcode No. 282664, PC Code No 006484, MRID Nos. 453613-01 and 453613-02.

**FROM:** Robyn I. Rose, Entomologist  
Biopesticides and Pollution Prevention Division (7511C)

**TO:** Mike Mendelsohn, Regulatory Action Leader  
Biopesticides and Pollution Prevention Division (7511C)

**CLASSIFICATION:** **Acceptable**

**BACKGROUND:**

Monsanto Co. has request a registration for *Bacillus thuringiensis* (Bt) Cry3Bb1 protein (event MON 863) expressed in field corn pollen. EPA routinely requests studies on the effects of Bt proteins on beneficial nontarget insects such as lady beetles (Coccinellidae), green lacewings (*Chrysoperla carnea*), parasitic Hymenoptera and honey bees (*Apis mellifera*). Since the Cry3Bb1 protein specifically targets coleopteran (beetle) insects, particular attention is warranted regarding potential effects of MON 863 on lady beetles. In a memorandum from Robyn Rose to Mike Mendelsohn dated March 10, 2000, the Agency requested a test demonstrating the effect of lady beetles feeding on corn pollen containing Cry3Bb1. Monsanto conducted two laboratory studies (MRID Nos 453613-01 and 453613-02) on two different lady beetle species (*Coleomegilla maculata* and *Hippodamia convergens*) in response to this request. In addition, Monsanto conducted a lady beetle larvae pollen feeding study (MRID No 455382-04) that was classified as acceptable by EPA. In this study, lady beetle larvae (*Coleomegilla maculata*) were fed MON 863 corn pollen expressing the Cry3Bb1 protein in the laboratory. According to results of this study, the NOEC for Cry3Bb1 expressed in pollen is #93 µg/g fresh pollen weight. It can be concluded from this study that lady beetle larvae will not be adversely affected by Cry3Bb1 field corn.

**CONCLUSIONS:**

No adverse effects were detected when *Coleomegilla maculata* and *Hippodamia convergens* were fed MON 863 pollen in diet in the laboratory. Pollen levels fed on by the lady beetles in this study exceeded concentrations that are expected to be encountered in the field. Therefore, it can be concluded the MON 863 will not pose a risk to lady beetle adults in the field.

Lady beetle adults were feed diets of transgenic corn pollen mixed with fruit fly eggs to determine potential effects of transgenic pollen to beetles. The corn (MON 863) test pollen contained the variant Cry3Bb1 protein at a concentration of 37.4 µg/g pollen while the control pollen was produced from corn (MON 846) which was not genetically modified. After 30 days of diet exposure, 83.3 and 80.0% of adult *C. maculata* survived in the test and control pollen groups, respectively. While these survival rates were significantly less than that in the assay control group (bee pollen which exhibited 100% survival), there were no significant differences between the test and control pollen groups. All adults in the positive control (arsenate treated corn pollen) died in less than 8 days. Results indicated that transgenic Bt corn pollen expressing the variant Cry3Bb1 protein has no negative effects on the survival of *Coleomegilla maculata* adults.

*Hippodamia convergens* adults were fed diets of transgenic corn pollen in honey to determine potential effects of transgenic pollen to beetles. The corn (MON 863) test pollen contained the variant Cry3Bb1 protein while the control pollen was produced from corn (MON 846) which was not genetically modified. After 15 days of diet exposure, 84% and 81% of adult *Hippodamia convergens* survived in the test pollen and control pollen groups, respectively. There were no significant differences in survival among the test pollen, control pollen and the assay control (honey only) treatment groups. Only 5% of beetles exposed to the positive control (arsenate treated corn pollen) survived. Results demonstrate that transgenic Bt corn pollen expressing the variant Cry3Bb1 protein had no negative effects on the survival of *Hippodamia convergens* adults from dietary exposure.

## **DATA EVALUATION RECORD**

Reviewed By: Anthony Q. Armstrong and Eric B. Lewis, Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

EPA Reviewer: Robyn I. Rose, Biopesticides and Pollution Prevention Division (7511C)

**STUDY TYPE:** NonTarget Insect Testing, Tier I (OPPTS 885.4340); Dietary effects of adult lady beetles feeding on Bt corn pollen.

**TEST MATERIAL:** *Bacillus thuringiensis* Cry3Bb1 protein (event MON 863) expressed in corn pollen

**CITATION:**

Authors: Jian J. Duan, Graham Head, Mike McKee, Thomas E. Nickson  
Title: Dietary Effects of Transgenic *Bacillus thuringiensis* (Bt) Corn Pollen Expressing a Variant of Cry3Bb1 Protein on Adults of Ladybird Beetle, *Coleomegilla maculata*  
Study Completion Date: March 13, 2001  
Testing Laboratory: Monsanto's Biotechnology Regulatory Sciences, 700 Chesterfield Parkway North, St. Louis, MO 63198 and Ecological Technology Center, 800 North Lindbergh, St. Louis, MO 63011  
Sponsor: Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198  
Laboratory Report ID: MSL-16936  
MRID No: 45613-01  
EPA Reg.No: 524-LRA

**CLASSIFICATION:** Acceptable

**QUALITY ASSURANCE STATEMENT:** Acceptable

**GLP COMPLIANCE STATEMENT:** Acceptable

**STUDY PARAMETERS:**

Test Organism: Lady beetle (*Coleomegilla maculata*)  
Age of Test Organisms: Adult  
Definitive Study Duration: August 26, 2000 - September 30, 2000

**STUDY SUMMARY:**

**Objective:** To evaluate the potential hazards of *Bacillus thuringiensis* endotoxins Cry3Bb1 to adult lady beetles (*Coleomegilla maculata*).

**Materials & Methods:** This study was based upon procedures outlined in Series 885 of US EPA's, Office of Prevention, Pesticides and Toxic Substances Microbial Pesticide Test Guidelines (OPPTS Number 885.4340, February 1996). Treatments included test pollen from Bt corn pollen, control pollen, bee pollen provided by a commercial source and served as an assay control, and potassium arsenate treated pollen which served as a positive control. The test substance was transgenic corn pollen which contained the Cry3Bb1 protein from *Bacillus thuringiensis* produced by corn event MON 863. The Cry3Bb1 protein has insecticidal activity against the corn rootworm (*Diabrotica* spp.). Non-transgenic corn pollen from event MON 846 was used as control pollen. An ELISA was conducted to verify the level of Cry3Bb1 protein in the test and control pollen. The ELISA indicated that there was 37.4 µg Cry3Bb1 protein/g fresh pollen weight and no Cry3Bb1 detected in the control pollen. An additional ELISA was conducted at test termination to evaluate stability of the variant Cry3Bb1 protein. The estimated level of Cry3Bb1 protein verified by ELISA at test termination was 49.3µg/g fresh weight pollen.

The test organisms, lady beetles (*Coleomegilla maculata*), were reared in test chambers maintained at 25-30°C and fed a diet consisting of equal parts of insect diet and bee pollen. An insect diet consisting of equal amounts of dried fruit fly eggs and pollen was used for testing. Diets contained 50% pollen since this is the potential level of field exposure and an equal amount of the tephritid fruit fly diet. The dried fruit fly egg and test diet mixtures were stored in the freezer at -20°C until use. Adults that were less than three days old were randomly placed in test chambers with diet and treatments. The test chambers were polystyrene petri dishes (10 cm in diameter, 1.5 cm depth) with filter paper (8.5 cm diameter) placed on the bottom and covered with a ventillated top. Each test chamber contained one randomly selected beetle. A total of ten adults were tested in each assay and the assay was replicated three times. Water and diet mixtures were presented *ad libitum* to the beetles on glass microscope slides. The test chambers were maintained in an incubator at 27°C with a photoperiod of 14 hours light and 10 hours of darkness.

Daily observations of the adult beetles for mortality and number of eggs laid continued for 30 days after test initiation. However, egg production data was not evaluated because the male:female ratio varied between treatments. Concentration of Cry3Bb1 protein in the test and control substances was verified at test termination by counting pollen grains in sub-samples of the diet mixes. However, this data was rejected because of a missing data entry on the amount of the test pollen diet weighed for pollen counting. Statistical analysis on the percentage of surviving larvae in the test was conducted using Analysis of variance (ANOVA) and Tukey's studentized range (HSD).

**Reported Results:** Survival of beetles exposed to transgenic corn pollen, containing the Cry3Bb1 protein, in dietary form was 83.3% after 30 days of exposure and was numerically greater than the 80% survival exhibited by beetles exposed to the control corn pollen diet. There was no statistical difference between survival of *C. maculata* fed MON 863 pollen and non-transgenic pollen. Beetles exposed to bee pollen had a survival rate of 100% while beetles exposed to arsenate treated corn pollen did not survive longer than 8 days (0% survival verifies

lady beetles are ingesting pollen in this study). There were significantly more *C. maculata* surviving on bee pollen than on corn pollen (Bt and nonBt) suggesting that a corn pollen diet is probably not as optimal as a bee pollen diet.

**Study Authors Conclusions:** Results from this study demonstrated that when offered as a dietary component, transgenic Bt corn pollen containing the Cry3Bb1 protein had no effect on survival of adult lady beetles (*Coleomegilla maculata*).

**Reviewer's Comments:** This study was performed according to established protocols and guidelines making it acceptable for fulfilling FIFRA Guideline 153A-23. Both summarized and raw data were included in the study report (Table 1, pp.19-20). No deviations were noted in the study report or identified in this data evaluation record. Results demonstrate that transgenic Bt corn pollen expressing the variant Cry3Bb1 protein had no negative effects on the survival of *C. maculata* adults. Based on results of this study, no adverse effects are expected when *Coleomegilla maculata* feed on level of MON 863 pollen they will potentially encounter in the field.

Table 1. Number of adult lady beetles surviving after dietary exposures to test corn pollen (Cry3Bb1 protein), control corn pollen, arsenate treated corn pollen and bee pollen.

<i>Treatment Group</i>	<i>Day</i>														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Test pollen (MON 863 containing Cry3Bb1)	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
Control pollen (MON 846)	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
Bee pollen	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
Arsenate treated corn pollen	30/30	30/30	30/30	29/30	24/30	17/30	2/30	1/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30

<i>Treatment Group</i>	<i>Day</i>												
	15	16	17	18	19	20	21	22	23	24	25	26	27
Test pollen (MON 863 containing Cry3Bb1)	29/30	29/30	29/30	29/30	29/30	29/30	29/30	29/30	29/30	29/30	29/30	28/30	27/30
Control pollen (MON 846)	29/30	28/30	28/30	28/30	27/30	27/30	26/30	25/30	25/30	25/30	25/30	25/30	24/30
Bee pollen	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
Arsenate treated corn pollen	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30

Table 1. (Cont). Number of adult lady beetles surviving after dietary exposures to test corn pollen (Cry3Bb1 protein), control corn pollen, arsenate treated corn pollen and bee pollen.

<i>Treatment Group</i>	<i>Day</i>			
	28	29	30	Survival at Day 30 (%)
Test pollen (MON 863 containing Cry3Bb1)	27/30	26/30	25/30	83.3 <sup>a</sup>
Control pollen (MON 846)	24/30	24/30	24/30	80.0 <sup>a</sup>
Bee pollen	30/30	30/30	30/30	100
Arsenate treated corn pollen	0/30	0/30	0/30	0

<sup>a</sup> Means followed by the same letter are not significantly different at 0.05 error level as determined by ANOVA and Tukey's studentized range tests.

## DATA EVALUATION RECORD

Reviewed By: Anthony Q. Armstrong and Eric B. Lewis, Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

EPA Reviewer: Robyn I. Rose, Biopesticides and Pollution Prevention Division (7511C)

**STUDY TYPE:** NonTarget Insect Testing, Tier I (OPPTS 885.4340); Dietary effects of adult lady beetles feeding on Bt corn pollen.

**TEST MATERIAL:** *Bacillus thuringiensis* Cry3Bb1 protein (event MON 863) expressed in corn pollen

**CITATION:**

Authors: Rebecca L. Bryan, John R. Porch, Henry O. Krueger  
Title: Dietary Effects of Transgenic Bt Corn Pollen Expressing a Variant of Cry3Bb1 Protein on Adults of Ladybird Beetle, *Hippodamia convergens*  
Study Completion Date: March 15, 2001  
Testing Laboratory: Wildlife International, Ltd., 8598 Commerce Dr., Easton, MD 21601  
Sponsor: Monsanto Company, 700 Chesterfield Pkwy, North, Chesterfield, MO 63198  
Laboratory Report ID: 139-453  
MRID No: 45613-02  
EPA Reg.No: 524-LRA

**CLASSIFICATION:** Acceptable

**QUALITY ASSURANCE STATEMENT:** Acceptable

**GLP COMPLIANCE STATEMENT:** Acceptable

**STUDY PARAMETERS:**

Test Organism: Lady beetle (*Hippodamia convergens*)  
Age of Test Organisms: Adult  
Definitive Study Duration: September 25, 2000 - November 21, 2000

**STUDY SUMMARY:**

**Objective:** To evaluate the potential hazards of *Bacillus thuringiensis* endotoxins Cry3Bb1 to adult lady beetles (*Hippodamia convergens*).

**Materials & Methods:** This study was based upon procedures outlined in Series 885 of US EPA's, Office of Prevention, Pesticides and Toxic Substances Microbial Pesticide Test Guidelines (OPPTS Number 885.4340, February 1996). The test diets consisted of test pollen, control pollen and a positive control (potassium-arsenate treated pollen) were mixed with honey



in a 1:1 ratio. The test substance was transgenic corn pollen which contained the Cry3Bb1 protein from *Bacillus thuringiensis* produced by corn event MON 863. The Cry3Bb1 protein has insecticidal activity against the corn rootworm. Diets contained 50% pollen since this is the potential level of field exposure and an equal amount of honey. The assay control consisted of honey without pollen. The control pollen was a non-genetically modified corn MON 846. The test and control pollen were characterized by molecular analysis and ELISA as documented in Monsanto SOP BR-ME-0059-05. Concentrations of Cry3Bb1 protein determined by Monsanto in the transgenic corn pollen ranged from 55.86 to 72.45 µg/g pollen when supplied to Wildlife International for lady beetle testing. No Cry3Bb1 was detected in the control corn pollen. Stability analysis of CryBb1 indicated the presence of the protein in the corn pollen/honey diet supplied to the lady beetles; however, quantification of CryBb1 in the diet was not provided by Monsanto.

The test organisms, lady beetles (*Hippodamia convergens*), were adults obtained from A-Unique Insect Control (Citrus Heights, CA) and stored in a cold room for three months prior to test initiation. Beetles were placed in an incubator set at 26-28°C and 40% relative humidity and allowed *ad libitum* access to honey/water for four days prior to test initiation. Beetles were randomly selected and placed in test chambers consisting of disposable one-pint rolled paper containers approximately 9 cm in diameter and 9 cm high covered with a 10 cm in diameter petri dish and equipped with a 20-mL glass vial containing deionized water. Twenty-five beetles were placed in each chamber. A cotton swab containing the appropriate diet was inserted through the side of each chamber. Beetles were allowed *ad libitum* access to the diets and water. The chambers were maintained in an incubator where temperature averaged  $26.0 \pm 0.2^\circ\text{C}$  and relative humidity averaged  $75\% \pm 6\%$  for the test period. The photoperiod during the test was 12 hours of light and was controlled with an automatic timer.

The beetles were observed ½ hour and 1 ½ hours after test initiation and then daily to evaluate signs of clinical toxicity, abnormal behavior and mortality for the 14 day test period. Analysis of Variance (ANOVA) was used to analyze differences between treatments in the mean number of dead beetles.

**Reported Results:** Survival of beetles exposed to transgenic corn pollen, containing Cry3Bb1 protein, in a honey diet was 84.0% after 15 days of exposure (Table 1). Likewise, survival of the beetles exposed to control corn pollen diet was 81.3% and survival of the beetles exposed to honey was 78.6%. Survival in these three groups were not significantly different indicating no impact of the transgenic corn pollen on mortality of lady beetles. Beetles exposed to arsenate treated corn pollen had a low survival rate of 5% which verifies that the *H. convergens* were ingesting pollen.

**Study Authors Conclusions:** Results from this study demonstrated that when offered as a dietary component, transgenic Bt corn pollen containing the Cry3Bb1 protein had no effect on survival of adult lady beetles (*Hippodamia convergens*).

**Reviewer's Comments:** This study was performed according to established protocols and guidelines making it acceptable for fulfilling FIFRA Guideline 153A-23. Both summarized and raw data were included in the study report (Table 1, pp.14-17). No deviations were noted in the

study report or identified in this data evaluation record. Based on results of this study, no adverse effects are expected when *Hippodamia convergens* feed on level of MON 863 pollen they will potentially encounter in the field.

Table 1. Number of adult lady beetles surviving after dietary exposures to test corn pollen (Cry3Bb1 protein), control corn pollen, arsenate treated corn pollen and bee pollen.

<i>Treatment Group</i>	<i>Day</i>											
	1	2	3	4	5	6	7	8	9	10	11	12
Test pollen (MON 863 containing Cry3Bb1)	73/75	73/75	70/75	70/75	70/75	68/75	68/75	67/75	67/75	67/75	67/75	67/75
Control pollen (MON 846)	75/75	75/75	74/75	70/75	70/75	70/75	70/75	70/75	69/75	69/75	68/75	66/75
Honey only	74/75	73/75	73/75	72/75	71/75	69/75	69/75	66/75	64/75	64/75	63/75	62/75
Arsenate treated corn pollen	24/75	19/75	15/75	11/75	10/75	9/75	9/75	9/75	6/75	4/75	4/75	4/75

<i>Treatment Group</i>	<i>Day</i>		
	13	14	Survival at Day 15 (%)
Test pollen (MON 863 containing Cry3Bb1)	65/75	63/75	84.0 <sup>a</sup>
Control pollen (MON 846)	65/75	61/75	81.3 <sup>a</sup>
Honey only	61/75	59/75	78.6 <sup>a</sup>
Arsenate treated corn pollen	4/75	4/75	5.3

<sup>a</sup> Means followed by the same letter are not significantly different as determined by ANOVA( p=0.8039).

