11/24/2003

## DATA EVALUATION RECORD

**Reviewed by:** Eric B. Lewis and Sylvia S. Talmage, Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725

**EPA Reviewer:** Robyn Rose, Biopesticides and Pollution Prevention Division (7511C) Robyn Rose 11/24/03

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| **STUDY TYPE:** | Nontarget Insect Testing, Tier I (885.4340) |
| **MRID NO:** | 45808411 |
| **DP BARCODE:** | D290936 |
| **TEST MATERIAL:** | Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton |
| **PROJECT NO:** | 379-125A |
| **SPONSOR:** | The Dow Chemical Company, Midland MI 48640 |
| **TESTING FACILITY:** | Wildlife International, Ltd., 8598 Commerce Drive, Easton MD 21601 |
| **TITLE OF REPORT:** | Cry1F (synpro) ICP and Cry1Ac (synpro) ICP: Dietary Toxicity to Parasitic Hymenoptera (*Nasonia vitripennis*) |
| **AUTHOR:** | Sindermann, A.B., J.R. Porch, and H.O Krueger |
| **STUDY COMPLETED:** | October 9, 2002 |
| **GOOD LABORATORY PRACTICE:** | GLP Compliant |
| **CLASSIFICATION:** | Acceptable |

**TEST MATERIAL:** Mycogen Brand Cry1F (TSN No 101811; Lot No 1650-85); Cry1Ac (TSN No 102591; Lot No. 1757-66). Cry1F (15% a.i.) and full-length Cry1Ac (14.% a.i.) used in this study were a heat treated powders.

**METHODS:** In a limit test, parasitic wasps (*Nasonia vitripennis*) were provided with a nominal concentration of 5.2 µg Cry1F, 46.8 µg Cry1Ac, or 5.2 µg Cry1F + 46.8 µg Cry1Ac per mL of sugar water. These concentrations were stated to represent approximately 58X the concentration of Cry1F and 32X the concentration of Cry1Ac in pollen. Test concentrations of 5.2 µg Cry1F/mL diet and 46.8 µg Cry1Ac/mL diet were based on expression levels of 0.09 µg/g Cry1F and 1.45 0.09 µg/g Cry1Ac in pollen. An ELISA was conducted to analyze diets on the day of preparation and on the last day of use.

Test chambers were one-pint rolled paper containers (9 cm in diameter and 9 cm high) covered with plastic petri dishes. Fresh diet was provided daily by inserting diet-coated cotton through the

side of the chamber, and access to diet and water was *ad libitum*. Control diet consisted of sugar water only. At test initiation, wasps were immobilized with nitrogen and impartially distributed to the test chambers. There were three replicates of 25 wasps each for a total of 75 wasps per treatment. The test was conducted in an incubator with an average temperature of 27°C, and average of 65.5±6.3% (range of 50 to 80%) relative humidity and 12 hours of light. The wasps were observed for mortality and clinical signs of toxicity approximately 30 and 90 minutes after test initiation, and daily thereafter for 10 days. An $LC_{50}$ and NOEC were determined from visual inspection of the mortality and clinical observation data. A ANOVA was run and data was compared with a t-test using Dunnett's Test to determine if there were differences among treatments and controls.

**RESULTS:** The study authors reported no significant ($p \leq 0.05$) differences in mean mortality between the treated and control groups. Mean cumulative mortality on day 9 was 20% in the controls, 27% in the Cry1F group, 29% in the Cry1Ac group, and 40% in the Cry1F + Cry1Ac group. Survivors in the Cry1F group appeared normal throughout the test. Some wasps in the Cry1Ac group were lethargic on days 7 and 8, but were otherwise normal. Some wasps in the Cry1F + Cry1Ac group were lethargic on day 7, but otherwise normal.

**STUDY AUTHORS' CONCLUSIONS:** The study authors concluded that the dietary $LC_{50}$s for parasitic hymenoptera were >5.2 µg Cry1F/mL, >46.8 µg Cry1Ac/mL, and >5.2 µg Cry1F + 46.8 µg Cry1Ac/mL of diet. The NOECs were 5.2 µg Cry1F/mL, 46.8 µg Cry1Ac/mL, and 5.2 µg Cry1F/mL + 46.8 µg Cry1Ac/mL of diet.

**REVIEWER'S CONCLUSION:**
This study was conducted according to EPA guidelines Nontarget Insect Testing, Tier I (885.4340). Since this was a limit test, the $LC_{50}$s could not be determined statistically, and were estimated from the cumulative mortality.

In a limit test, the study authors concluded that prepared diets containing 5.2 µg Cry1F/mL, 46.8 µg Cry1Ac/mL, or 5.2 µg Cry1F + 46.8 µg Cry1Ac/mL did not affect the mean mortality of the parasitic hymenopteran *Nasonia vitripennis* after 10 days of exposure. Surviving larvae in all groups were generally normal in appearance and behavior. Based on this study, the dietary $LC_{50}$s were: > 5.2 µg Cry1F/gram of diet, >46.8 µg Cry1Ac/gram of diet, >0.52 µg Cry1F + 4.68 µg Cry1Ac/gram of diet, >5.2 µg Cry1F + 46.8 µg Cry1Ac/gram of diet, and > 5.2 µg heated Cry1F Ac + 46.8 µg heated Cry1Ac per gram of diet. The 40% mortality observed in the Cry1F + Cry1Ac group is less than a $LC_{50}$ at 32x the possible field exposure (EEC). The EPA level of concern for terrestrial wildlife is a $LC_{50}$ at less than 5x the field exposure ($EEC/LC_{50}$ = RQ > 0.2). Therefore since the $LC_{50}$ in this study is greater than 32x the EEC, no hazard to parasitic Hymenoptera is expected at field exposures which are minimal to nonexistent.



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# DATA EVALUATION RECORD

**Reviewed by:** Anthony Q. Armstrong and Patricia H. Reno, Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725

**EPA Reviewer:** Robyn Rose, Biopesticides and Pollution Prevention Division (7511C) Robyn Rose 11/24/03

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| STUDY TYPE: | Non-Target Insects, Field Study |
| MRID NO: | 458084-19 |
| DP BARCODE: | D290936/68467-G |
| TEST MATERIAL: | Cry1F (synpro) and Cry1Ac (synpro) |
| PROJECT NO: | GH-C 5578 |
| SPONSOR: | Dow AgroSciences LLC<br>Indianapolis, Indiana 46268 |
| TESTING FACILITY: | Phytogen Seed Co., LLC<br>850 Plymouth Ave., P.O. Box 787<br>Corcoran, CA 93212-0787 |
| TITLE OF REPORT: | 2002 Field Survey to Evaluate Effects on Non-Target Beneficial Arthropods of Cry1F/Cry1Ac Bt Cotton MXB-13 |
| AUTHORS: | J.F. Mahill and N.P. Storer |
| STUDY COMPLETED: | November 11, 2002 |
| GOOD LABORATORY PRACTICE: | Non-GLP compliant |
| CLASSIFICATION: | Supplemental to submitting 2003 field survey data and conducting additional field surveys on large plots that have been planted with Cry1F/Cry1Ac cotton for at least three consecutive years. |

## I. Test Material

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| Test Substance: | MXB-13 transgenic cotton line containing stacked ICPs Cry1F(synpro)/Cry1Ac(synpro) (events 281-24-236 and 3006-210-23, respectively) developed from conventional breeding in parent cotton variety PSC355 with both transgenes in homozygous condition. |
| Control Substance: | Cotton variety PSC355, the recurrent parent for the Cry1F and Cry1Ac transgenic cotton events, was used as the control material in these experiments. |

## II. Methods

A field study was conducted at two locations in cotton growing regions during 2002. The first trial at the NE Research Station, Winnsboro, LA is in a region that routinely implements control strategies for tobacco budworm (*Heliothis virescens*; TBW) and cotton bollworm (*Helicoverpa zea*; CBW). The LA trial consisted of three blocks per treatment with plots 16 rows x 50 ft. row length based on 40" row spacing. Plots were separated by four rows (160") planted with a mix of mustard and pigweed as alternate host to attract insect populations. The second trial at the Maricopa Agricultural Center, Maricopa, AZ is in a region that routinely implements control strategies for pink bollworm (*Pectinophora gossypiella*; PBW). The AZ trial consisted of two blocks per treatment with plots 24 rows x 80 ft. row length based on 40" row spacing. Plots were separated by four buffer rows of non-planted bare ground with a minimum width of 20 ft.

Three treatments of cotton and insecticide application were used at both locations.

Treatment 1 - Transgenic Bt cotton MXB-13 untreated for Lepidoptera. Only non-lepidopteran insecticides were applied for other pests when required to protect plant health.

Treatment 2 - Control cotton PSC355 sprayed with insecticides to control all pests including Lepidoptera.

Treatment 3 - Control cotton PSC355 with insecticide applications identical to those in treatment #1 for MXB-13.

Sampling of insects in Winnsboro, LA, was conducted at several weekly intervals using sweep nets, shake sheets, and plant structure sampling methods to observe and count beneficial insect populations for comparisons among test plots. The focus of sampling was on the period of time when tobacco budworm (TBW) and/or cotton bollworm (CBW) was present at infestation levels of economic importance.

Sampling of insects at Maricopa, AZ, was conducted utilizing sweep nets in June and August as well as aerial sticky traps in July and August in each experimental plot. Sampling occurred weekly on experimental plots. Sweep nets sampling consisted of 100 sweeps (4 x 25) per plot while 6 sticky traps were placed in plots for 24-hour capture. Sampling was conducted within the center 16 rows and the center 60 feet of each experimental plot.

## III. Results

NE Research Station, Winnsboro, LA

In the preliminary analysis, data were combined across dates. Where sufficient insects were observed, analysis of variance was performed and least significant differences between means were determined for the most abundant morphotypes. Analyses of all seasonal field survey data resulted in no deleterious effects of the MXB-13 plants to non-target beneficial arthropods. Significantly higher seasonal survey counts were found in MXB-13 than the control PSC355 cotton even when both were treated with non-lepidopteran insecticides. MXB-13 showed higher counts than the control for combined Heteroptera genera *Geocoris*, *Orius*, *Nabis*, *Podisus*, the



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family Reduviidae (Table 1) and for lady beetle adults from leaf sampling (Table 2). Sweep, shake sheet, square, boll and white flower survey data showed that MXB-13 effectively controlled CBW larvae(Tables 1, 3, 4, 5, 6). Boll and white flower control samples contained significantly more *Orius* than the MXB-13 and the control sprayed with insecticides for all pests (Tables 5 and 6).

Maricopa Agricultural Center, University of Arizona, Maricopa, AZ

Small treatment effects on some species for limited periods were found from preliminary analyses of the arthropod data. The greatest effect is due to the negative impact on arthropods from additional insecticides sprayed in the control PSC355 cotton. MXB-13 did not cause unintended effects to non-target arthropods. Of the 194 morphotypes recorded from sweep samples taken on 16 separate sample dates, few significant effects or trends were observed in the data. Season-long accumulated counts indicate four morphotypes were effected by treatments on at least one sample date; cotton leafhoppers, pale-striped flea beetles, *Drapetis* sp. and green lacewing larvae which were higher in the MXB-13 than in the control PSC355 cotton and both were higher than the control PSC335 treated with insecticides (Table 7). One-hundred-forty-three morphotypes were recorded from the aerial traps over the course of 13 sample dates. Similar to the results from the sweep analysis, few significant effects were detected (Table 8). Likewise, conclusions from the aerial trap data indicate no major non-target effects from the MXB-13 line on the 143 arthropod morphotypes examined.

## IV. Study Author's Conclusions

Preliminary analysis of the first year of field data indicate MXB-13 will not have an adverse impact on non-target arthropods compared to non-Bt cotton and will likely have a beneficial effect compared with conventionally managed non-Bt cotton. A more extensive analysis of data will be conducted using principle component analysis to examine effects on functional groups at different times during the cropping season after data is collected during the second year of planned field research.

## V. Reviewers Comments

Field surveys using sweep net and sticky trap sample methods were conducted to evaluate potential effects on non-target beneficial arthropods of MXB-13 stacked cotton line (Cry1F/Cry1Ac) in 2002. at two locations. Analyses of all data collected at Winnsboro, LA revealed that there were no adverse effects of MXB-13 on non-target beneficial arthropods. The MXB-13 with no chemical insecticide treatment for Lepidoptera showed significantly higher seasonal survey counts for beneficial Heteroptera in sweeps and for lady beetle adults in leaf sampling while demonstrating effective control of bollworm larvae. Likewise, field studies conducted at the Maricopa Agricultural Research Center in Arizona showed no apparent major negative effects to non-target organisms from the MXB-13 cotton line to the nearly 200 arthropods examined from sweep net collections and the 143 arthropods examined from aerial traps. Several insect groups were significantly more numerous in the MXB-13 plots than in the control plots sprayed with chemical insecticides for Lepidoptera.

This study was only conducted for one year using two sample methods in small plots. The study author also indicated that this study will be repeated which implies that it was replicated during the 2003 growing season. Additional field studies are needed on larger plots that have been planted with Cry1F/Cry1Ac cotton for at least three consecutive years. Since large plots are typically only available after registration, this study should be conducted three years after registration. In addition to sweep net and sticky trap sampling, the soil-dwelling arthropod community should be evaluated with a method such as pitfall trap sampling.



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13544

# R145228

**Chemical:** Bacillus thuringiensis var. aizawai Cry1F (synpro) and the genetic material (from the insert of plasmid pGMA281) necessary for its production in cotton
Bacillus thuringiensis var. kurstaki Cry1Ac (synpro) and the genetic material (from the insert of plasmid pMYC3006) necessary for its production in cotton

PC Code:
006512
006513

**HED File Code:** 41300 BPPD Eco Effects
**Memo Date:** 11/24/2003
**File ID:** DPD290936
**Accession #:** 000-00-9002

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