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

STUDY TYPE:	Nontarget Insect Testing, Tier I (885.4340)
MRID NO:	458084-10
DP BARCODE:	D290936
TEST MATERIAL:	Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton
PROJECT NO:	379-124A
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 Green Lacewing Larvae (*Chrysoperla carnea*)
AUTHOR:	Sindermann, A.B., J.R. Porch, and H.O Krueger
STUDY COMPLETED:	October 8, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant
CLASSIFICATION:	**Supplemental** to testing *Orius insidiosus*

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, newly-hatched green lacewing (*Chrysoperla carnea*) larvae were given nominal concentrations of either 5.2 µg Cry1F, 46.8 µg Cry1Ac, or a mixture of 5.2 µg Cry1F + 46.8 µg Cry1Ac per gram of moth egg (*Sitotroga* sp.) diet. These concentrations represent approximately 58X and 32X the concentrations of Cry1F and Cry1Ac, respectively, 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. A negative control group consisting of moth egg diet and deionized water was also included.

The test consisted of 30 replicates of each treatment group and the control, with one larva per

plastic test chamber. Test chambers consisted of a 1 oz semi-transparent plastic cup and lid. The test was conducted in an incubator with an average temperature of 21 to 22°C, 59 to 87% (75.5±7.4% average) relative humidity and 12 hours of light and dark. Fresh diet was provided on days 7 and 14 and larvae were allowed *ad libitum* access to food throughout the test. Larvae were observed about two hours after test initiation and daily thereafter for mortality and pupation. The test was terminated on day 16 because mortality exceeded 20% in the negative control group. An LC_{50} and NOEC were determined from visual inspection of the mortality and clinical observation data.

Additional treatment groups were given 0.52 µg Cry1F + 4.68 µg Cry1Ac, 5.2 µg Cry1F + 46.8 µgCry1Ac, or 5.2 µg heated Cry1F + 46.8 µg heated Cry1Ac per gram of diet and maintained as those in the limit test. The test with these additional groups was terminated after pupation in the control group exceeded 50% on day 18.

RESULTS: In the limit test, cumulative day 15 percent mortality/pupation were10%/50% for the control group. 20%/50% for the Cry1F group, 20%/63% for the Cry1Ac group, and 43%/53% for the Cry1F + Cry1Ac group. Surviving larvae were normal in appearance and behavior throughout the test period. There were no statistically significant differences in mortality between either of the Cry1F and Cry1Ac groups and the control group. Mortality in the Cry1F + Cry1Ac group was significantly increased ($p \leq 0.05$) over the control group.

In the additional tests, cumulative day 17 percent mortality/pupation were 10%/50% in the controls, 30%/47% in the 0.52 µg Cry1F + 4.68 µg Cry1Ac group, 33%/33% in the 5.2 µg Cry1F + 46.8 µgCry1Ac group, and 13%/53% in the 5.2 µg heated Cry1F + 46.8 µg heated Cry1Ac group. All surviving larvae in the additional test groups were normal in appearance and behavior throughout the test period. There was no indication of a dose response with the ten-fold increase in concentration of Cry1F or Cry1Ac, and there were no significant differences in mortality between any of the treated groups and the control group.

STUDY AUTHORS' CONCLUSIONS: Based on visual inspection of the mortality data, the study authors designated the following green lacewing larvae dietary LD_{50}s:

> 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 designated NOECs were 5.2 µg Cry1F, 46.8 µg Cry1Ac, 0.52 µg Cry1F + 4.68 µg Cry1Ac, 5.2 µg Cry1F + 46.8 µg Cry1Ac, and 5.2 µg heated Cry1F Ac + 46.8 µg heated Cry1Ac per gram of diet, based on visual inspection of the mortality and clinical observation data.

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

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determined statistically, and were estimated from the cumulative mortality. Statistical data were not included in the report. It is noted that in the additional tests, although the cumulative pupation percentages were roughly equal, the controls and heat-treated groups achieved pupation earlier than the Cry1F and Cry1Ac groups.

In tests of dietary toxicity of Cry1F, Cry1Ac, and Cry1F + Cry1Ac mixtures to green lacewing larvae (*Chrysoperla carnea*), mortality was statistically increased in larvae receiving 5.2 µg Cry1F + 46.8 µg Cry1Ac per gram of moth egg diet, but not in larvae receiving the same or lower concentrations of the individual proteins. Surviving larvae were 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.

Mortality was increased and pupation was affected in the Cry1F/Cry1Ac at 32x the concentration found in pollen. No effect is noted at Cry protein levels expressed in pollen that would be encountered by green lacewings in the field.

However, the appropriateness of the methodology for the green lacewing acute toxicity study is questionable. The August 2002 FIFRA Scientific Advisory Panel (SAP) noted concerns regarding the green lacewing study. The SAP recommended verifying levels of active protein throughout the test since the test protein was held for a week in diet and could have degraded considerably during this time. It was also noted that it would be more appropriate to conduct the green lacewing study through pupation rather than prematurely ceasing testing when 20% mortality is reached in the control group. The SAP also questioned the availability of Cry proteins to green lacewings when presented in a moth egg diet because the protein probably absorbs to the egg so only a small fraction of protein is contacted by larvae. Finally, the SAP questioned the appropriateness of testing green lacewing and recommended testing an alternate natural enemy such as the minute pirate bug (*Orius insidiosus*). Therefore, an additional Tier 1 nontarget insect test with the minute pirate bug should be conducted with the Cry1F and Cry1Ac proteins.

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R145227

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

HED Records Reference Center
6/28/2007