

11/24/2003

DATA EVALUATION RECORD

Reviewed by: Anthony Q. Armstrong 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*

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| STUDY TYPE: | Nontarget Insect Testing, Tier I (885.4340) |
| MRID NO: | 458084-09 |
| DP BARCODE: | D290936 |
| TEST MATERIAL: | Mycogen Brand CryIF (synpro)/CryI Ac (synpro) Construct 281/3006 Cotton |
| PROJECT NO: | Springborn Smithers Study No. 12550.6257 Sponsor Protocol/Project No. 021123 |
| SPONSOR: | The Dow Chemical Company, Midland MI 48640 |
| TESTING FACILITY: | Springborn Smithers Laboratories, 790 Main St., Wareham, MA 02571-1075 |
| TITLE OF REPORT: | Assessment of Chronic Toxicity of Diets Containing CryIF and CryI Ac Microbial Protein, Lyophilized CryI Ac Cotton Leaf Tissue or PSC355 Control Cotton Leaf Tissue to Collembola (<i>Folsomia candida</i>) |
| AUTHOR: | D. Teixeira |
| STUDY COMPLETED: | November 7, 2002 |
| GOOD LABORATORY PRACTICE: | GLP Compliant, with exceptions |
| CLASSIFICATION: | Acceptable |

TEST MATERIAL:

- CryIF microbial protein with a purity of 15% CryIF/mg dry weight (TSN Number 101811; Lot Number 1650-85)
- Full length CryI Ac with a purity of 14% CryI Ac/mg dry weight. (TSN Number 102591; Lot Number 1757-66)
- CryI Ac event 3006-210-23 lyophilized cotton leaf (TSN Number 103879; Lot Number MXB-7)
- Positive Control: PSC355 lyophilized cotton leaf (TSN Number 103880; Lot Number PSC355)
- Positive Control: Thiodicarb (Lot Number 259-23A; CAS Number 59669-26-0)

- Negative Control: Brewer's yeast

METHODS: Juvenile (9-11 day old) Collembola (*Folsomia candida*) were placed in four-ounce glass jars containing a substrate (plaster of paris:activated charcoal, 8:1) and provided a treated diet of Brewer's yeast with nominal concentrations of 709 mg Cry1F alone, 22.6 mg Cry1Ac alone, or 702 mg Cry1F + 22.6 mgCry1Ac per kilogram of diet. These concentrations represented approximately 20X the concentration of these proteins in mature (pre-harvest) cotton plants. The control diet was Brewer's yeast only. In a separate assay, Collembola were fed Brewer's yeast containing nominal concentrations of 5% or 50% lyophilized Cry1Ac cotton leaves, representing about 1X and 10X the exposure from leaf tissue in soil, respectively, or 5% or 50% lyophilized PSC355 control cotton leaves. Samples of cotton leaf tissue were returned to the Study Sponsor at test termination to evaluate the stability of the Cry protein under test conditions. A reference test using Brewer's yeast and nominal concentrations of 1, 10, and 100 mg Thiodicarb per kg of diet was also conducted.

All tests consisted of four replicates of ten Collembola each for a total of 40 Collembola tested per treatment. The test chambers were placed in an incubator with controlled temperature of 18-23°C, humidity of 70-100%, and photoperiod of 16 hours of light and 8 hours of dark. Diets were stored in the freezer between feedings, and were available with water *ad libitum*. Survival, number of offspring and observations of sublethal effects such as lethargy were recorded at test initiation and at test termination on day 28. An analysis of variance (ANOVA) was conducted to compare reproduction between collembola fed test substances to the assay control. Significant differences were determined from a two-sample t-Test.

RESULTS: Mean adult survival was 98% for the control, 93% for Cry1F, 95% for Cry1Ac, and 98% for Cry1F + Cry1Ac (Table 1). Mean number of offspring per vessel was 440 for the control, 431 for Cry1F, 243 for Cry1Ac, and 410 for Cry1F + Cry1Ac (Table 1). The number of offspring in the Cry1Ac group was significantly reduced compared to the control. However, the study author believed this resulted from impurities in the test substance, and the Cry1Ac treatment was retested. In the retest, adult survival was 100% in the control and Cry1Ac groups, while the number of offspring was 387 in the control and 305 in the Cry1Ac treatment (Table 2). The number of offspring in the Cry1Ac group was again significantly lower than that in the control. To determine whether the decrease was due to Cry1Ac or to impurities associated with it, a third assay was conducted using diet containing either 5% or 50% lyophilized cotton leaves containing Cry1Ac or lyophilized leaves of an isoline control. In the third assay, mean adult survival was 100% in all groups (Table 3). Mean number of offspring per vessel was 397 in the control, 402/403 in the 5%/50% Cry1Ac cotton leaf groups, and 406/396 in the 5%/50% isoline cotton leaf groups (Table 3). There was no significant difference in the number of offspring between any of the cotton leaf groups and the control. Surviving Collembola exposed to the treated and control diets were observed to be healthy throughout all the tests. Survival/number of offspring in the 1, 10, and 100 mg/kg thiodicarb reference groups were 78%/288, 38%/50, and 0%/5, respectively (Table 3).

Table 1. Percent survival of parental organisms and number of offspring produced after 28 days of feeding collembola diet containing Cry1Ac and Cry1F microbial protein

| Nominal Diet Concentration (mg/kg) | Percent Survival (SD) | Cumulative No of Offspring (SD) |
|------------------------------------|-----------------------|---------------------------------|
| Assay Control (Brewers yeast) | 98 (5) | 440 (21.6) |
| 22.6 Cry1Ac | 95 (5.8) | 243 (38.0) |
| 709 Cry1F | 93 (9.6) | 431 (14.5) |
| 724.6 Cry1Ac + Cry1F | 98 (5.0) | 410 (12.6) |
| 1.0 mg/kg thiodicarb | 88 (5.0) | 284 (9.2) |
| 10 mg/kg thiodicarb | 33 (9.6) | 38 (7.9) |
| 100 mg/kg thiodicarb | 0 (0.0) | 5 (3.0) |

SD = Standard deviation

Table 2. Percent survival of parental organisms and number of offspring produced after 28 days of feeding collembola diet containing Cry1Ac microbial protein

| Nominal Diet Concentration (mg/kg) | Percent Survival (SD) | Cumulative No of Offspring (SD) |
|------------------------------------|-----------------------|---------------------------------|
| Assay Control (Brewers yeast) | 100 (0) | 387 (12) |
| 22.6 Cry1Ac | 100 (0) | 305 (29) |

SD = Standard deviation

Table 3. Percent survival of parental organisms and number of offspring produced after 28 days of feeding collembola diet containing Cry1Ac cotton leaf and lyophilized PSC355 control cotton leaf

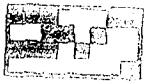
| Nominal Diet Concentration (mg/kg) | Percent Survival (SD) | Cumulative No of Offspring (SD) |
|------------------------------------|-----------------------|---------------------------------|
| Assay Control (Brewers yeast) | 100 (0.0) | 397 (13) |
| 5.0% PSC355 | 100 (0.0) | 406 (19) |
| 50% PSC355 | 100 (0.0) | 396 (13) |
| 5.0% Cry1Ac | 100 (0.0) | 403 (9.0) |
| 50% Cry1Ac | 100 (0.0) | 402 (9.0) |
| 1.0 mg/kg thiodicarb | 78 (5.0) | 288 (11) |
| 10 mg/kg thiodicarb | 38 (13) | 50 (11) |
| 100 mg/kg thiodicarb | 0 (0.0) | 5 (1.7) |

SD = Standard deviation

STUDY AUTHOR'S CONCLUSIONS: The study author concluded that exposure to 709 mg Cry1F/kg of diet, 702 mg Cry1F + 22.6 mg Cry1Ac/kg of diet, or lyophilized Cry1Ac cotton leaf at 5% or 50% of the diet did not adversely affect reproduction of Collembola, but that 22.6 mg Cry1Ac/kg in the diet did adversely affect reproduction of Collembola.

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REVIEWER'S COMMENTS: In 28-day dietary toxicity tests, 709 mg Cry1F/kg of diet or 702 mg Cry1F + 22.6 mg Cry1Ac per kg of diet did not adversely affect mortality or reproduction of *Collembola (Folsomia candida)*. Diet containing 22.6 mg Cry1Ac/kg alone did not affect mortality but decreased reproduction by up to 45%; however, the toxicity may have been due to impurities in the Cry1Ac test material. Lyophilized Cry1Ac cotton leaf at 5% or 50% of the diet had no adverse effect on mortality or reproduction.



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

HED Records Reference Center
6/28/2007

