5-15-92

DATA EVALUATION REPORT

1. Chemical: *Bacillus thuringiensis* subsp. *aizawai* (ABG-6305)

2. Test Material: Technical, primary powder

3. Study/Action Type: 154A-23. Nontarget arthropod testing for toxicity/pathogenicity to arthropod predators/parasites.

4. Study Identification: Nelson, R. D. 1991. The effect of *Bacillus thuringiensis* , ABG-6305 technical powder, on the common green lacewing, *Chrysoperla carnea* (Stephens) (Project ID. 91.043). Submitted by Abbott Laboratories. North Chicago, IL. EPA Access.No. 422453-01.

5. Reviewed By: Clayton C. Beegle
Entomologist
EFED/EEB
Signature:
Date: 4-6-92

Les W. Touart
Head, Section 1
EFED/EEB
Signature:
Date: 5-15-92

6. Conclusions: This study is scientifically sound and meets EPA requirements for a core study. ABG-6305 is toxic to larvae of *C. carnea* at the 10X field rate. ABG-6305 is slightly toxic to *C. carnea* larvae at the field rate of 2.4 g/l (1 lb/acre in 50 gal water).

7. Recommendations: EEB recommends that the registrant determine the source of toxicity of ABG-6305 to green lacewings, as it is unlikely that the activity is due to the spore-crystal complex unless the isolate of *B. thuringiensis* subsp. *aizawai* used has a new and unique crystal toxin(s). In addition, it is recommended that dose responses be established by bioassaying ABG-6305 using at least five dose levels, with at least two dose levels above and below the anticipated LC_{50}. It is strongly recommended that in the future the registrant conduct this type of study in facilities with adequate control of photoperiod, temperature, and relative humidity.

8. Background: This study was submitted to support the request for the registration of the Abbott Laboratories *B. thuringiensis* subsp. *aizawai* product Centari.

9. Materials and Methods:

A. **Test organisms:** *Chrysoperla carnea* (Stephens), the common green lacewing.

Age/stage of maturity: 2-4 day old larvae.

Sex: Both sexes.

Source: Rincon-Vitova Insectaries, Inc., Oak View, CA.

B. **Dosage Form:**

Solvents/vehicles: Water.

Route of administration: Sprayed on *C. carnea* larvae, 1-ounce plastic cups and lids (cage), and prey (lepidopterous eggs) with an airbrush sprayer. All surfaces were sprayed to near runoff.

C. **Referenced Protocol:**

Test levels: 0.24, 2.4, and 23.9 g/l. These rates are equivalent to the field rates of 0.10, 1.0, and 10 lbs./50 gal./acre, which are 0.1X, 1.0X, and 10X the field rate.

Dose spacing factor: 10X.

Number per level: 100 (25/replicate, 4 replicates) larvae treated/dose. For oviposition and egg hatch studies 20 pairs of adults (5 pairs/cage, 4 cages/treatment) surviving each treatment were used, with the exception of the highest dose (10X field rate). Only one replicate was possible at that dose because of high larval mortality.

Holding/acclimation: None mentioned.

Pen/cage facilities: 1-ounce plastic cups (one larva/cup) with tight fitting lids were used in determining effects on larvae. 8-ounce styrofoam cups placed in 90 mm Petri dishes were used in determining adult fecundity and percentage egg hatch of survivors of larval treatments.

Feeding: *C. carnea* larvae were fed lepidopterous eggs. Adults were provided wheast paste and water soaked cotton.

Physical condition: Not mentioned.

Test conditions:

Temperature: 19-33.5 °C

Relative humidity: 26-68%

Photoperiod: Not mentioned in report. Trial notes Fax states that photoperiod was approximately 16L:8D.

Controls: Water sprayed controls.

Observation period: Larvae were evaluated for mortality at 2, 4, and 7 days after treatment. Larvae surviving treatment were observed until pupation and adult emergence. In the fecundity test eggs were counted every 2-3 days. Observations were made daily in determining percentage egg hatch.

Statistical methods: All data were subjected to a 2-way Analysis of Variance (ANOVA) (P=0.05). Significantly different means were separated using Duncan's New Multiple Range Test (DNMRT) (P=0.05).

10. Reported Results:

A. **Effect of *B. thuringiensis* on larval *C. carnea:*** The mean percentage mortalities after 7 days for larvae treated with 0, 0.1X, 1X, and 10X the field dosage were 5, 5, 17, and 61, respectively. There was no significant difference between the first three means, the 10X mean was significantly higher than the other means.

B. **Effect of *B. thuringiensis* on percentage pupation of *C. carnea:*** Mean percentage pupations of larvae surviving treatments of 0, 0.1X, 1X, and 10X the field dosage were 84, 80, 69, and 18, respectively. The first two means were not significantly different, the second and third means were not significantly different, and the 10X mean was significantly lower than all the other means.

C. **Effect of *B. thuringiensis* on percentage emergence from pupation of *C. carnea:*** Mean percentage emergence of adults from treatments 0, 0.1X, 1X, and 10X the field dosage were 74, 73, 74, and 79, respectively. There was no significant difference between any of the means.

D. **Effect of *B. thuringiensis* on number laid and percentage hatch of eggs of *C. carnea:*** Mean number of eggs laid by ♀ *C. carnea* developed from larvae treated with 0, 0.1X and 1X the field dosage were 243, 550, and 423, respectively. The mean percentage hatch of those eggs were 56, 55, and 51, respectively. There were no significant differences between any of the means of either group.

C. **NOEL:** The NOELs were 1X the field rate for mortality of *C. carnea* larvae treated with *B. thuringiensis*, number of eggs laid, and egg hatch; 0.1X the field rate for pupation; and 10X the field rate for adult emergence.

11. Study Author's Conclusions/Quality Assurance Measures: "The results of this study indicate that *Bacillus thuringiensis* ABG-6305 technical material would have no significant adverse effects on *Chrysoperla carnea* at the 1.0 X field rate. Bt ABG-6305 at the 10.0 X field rate would have significant adverse effects on *Chrysoperla carnea* by significantly increasing larval mortality".

"To the best of my knowledge, the study reported in this notebook was conducted in accordance with the Good Laboratory Practice standards (40 CFR Part 160) established by the Environmental Protection Agency". Signed by Richard Karstrom, Principal Investigator, and Richard D. Nelson, Study Director, Plant Sciences, Inc., 342 Green Valley Rd., Watsonville, CA 95076.

12. Reviewer's Discussion and Interpretation of:

A. **Test Procedures:** The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M, with the exception of control of temperature and humidity. Recommended temperature and relative humidity extremes are 26-30°C and 40-80%. The extremes during the conducted tests were 19-33.5°C and 26-68% RH. While EEB agrees that the deviations probably did not adversely affect the results of the study, such wide swings of temperature and humidity are undesirable and indicate that the experiments were conducted in a facility with no control over temperature or humidity. One of the most basic requirements for an insect rearing or testing facility is controlled light, temperature, and relative humidity.

When significant mortality is observed when the test organisms are exposed to the test dosages, a dosage-mortality response should be established by bioassaying the material using at least five dosages, with at least two above and below the anticipated LC_{50}.

B. **Statistical Analysis:** The appropriate statistical tests were used to analyze the data.

C. **Discussion/Results:** ABG-6305 is toxic to *C. carnea* larvae at the 10X field dosage. ABG-6305 appears to be slightly toxic to *C. carnea* larvae at the field rate of 2.4 g/l (1 lb/acre in 50 gal water). The registrant should determine the source of the toxicity of ABG-6305 to *C. carnea*, as it is unlikely that the activity is due to the spore-crystal complex unless the isolate of *B. thuringiensis* subsp. *aizawai* used has a new and unique crystal toxin(s).

D. **Adequacy of the Study:**

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements.