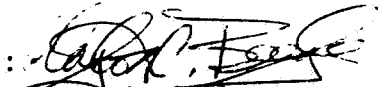
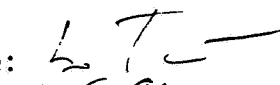


DATA EVALUATION REPORT

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: Kirkland, R. L. 1991. The effect of *Bacillus thuringiensis*, ABG-6305 technical powder, on the insect egg parasitoid, (*Trichogramma pretiosum* Riley) (Project ID. CAR 104L-91). Submitted by Abbott Laboratories. North Chicago, IL. EPA
✓ Access.No. 419748-07.
5. Reviewed By: Clayton C. Beegle
Entomologist
EFED/EEB
Signature: 
Date: 4-14-92

Les W. Touart
Head, Section 1
EFED/EEB
Signature: 
Date: 7-15-92
6. Conclusions: This study is invalid due to excessive untreated control mortality (6, 25, 32, 37, 46, and 50%, at 1, 2, 3, 4, 5, and 6 days after treatment, respectively) in Test I. Test I investigated the effects of ABG-6305 on adult *T. pretiosum*.
7. Recommendations: EEB recommends that the registrant repeat Test I using *T. pretiosum* which have developed in *Heliothis zea* eggs. Since the study states that poor quality *T. pretiosum* stock was often received when the supplying insectary refrigerated the eggs, it would be preferable if *T. pretiosum* were produced at the testing site. For example the study states that "two attempts to perform Test II using sterilized *Heliothis zea* eggs provided by the USDA failed, due to little or no female production". However, in Test III where *H. zea* eggs were parasitized at the test site by *T. pretiosum*, thousands of females were produced with a F:M sex ratio of nearly 2 (Table III). Site produced *T. pretiosum* would also be more vigorous, which should prevent the excessive untreated control seen in Table I.
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: *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)
Age/stage of maturity: One day old adults
Sex: Both sexes.

Source: Test I: Kunafin *Trichogramma* Insectaries, Quemado, TX
Test II and III: USDA-ARS Insect Biology Labs, Tifton, GA

B. Dosage Form:

Solvents/vehicles: Test I: Water and honey. Test III: Water.

Route of administration: Test I: Test containers were coated with treatments in water. After air drying, containers were streaked on inside with treatment-containing honey. Treatment-containing water was provided via saturated dental wicks. Test III: *H. zea* eggs which had been exposed to *T. pretiosum* females were sprayed with treatment solutions.

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: Test I: 50 (10/replicate, 5 replicates) adults/treatment. Test III: Number of *H. zea* eggs that 10 ♀ *T. pretiosum* will parasitize in 4 hrs (17-30 host eggs/♀)/cage, 5 cages/treatment.

Holding/acclimation: 24 hrs.

Pen/cage facilities: Test I: Plastic tubes (1.2 cm dia. x 8 cm length) with cork in one end with screened hole, and cork at other end with hole with dental cotton glued on inside cork surface. Test III: Disposable Petri dish (50 x 9 mm) used as holding cage for parasitized eggs. Emerged adults were collected in a glass test tube (75 x 13 mm).

Feeding: Water, streaked honey.

Physical condition: *T. pretiosum* used in Test I were reared in *Sitotroga cerealella* eggs and were very small, difficult to handle and often became stuck in the honey streaks and on the water wicks. During the winter months the stock was often of poor quality because the insectary would store the eggs under refrigeration. *T. pretiosum* used in Test III were reared in *Heliothis* eggs and were more robust.

Test conditions:

Temperature: 23-29 °C.

Relative humidity: 40-87%.

Photoperiod: 16L:8D.

Controls: Water treated controls.

Observation period: Test I: Adult mortality recorded at 1, 2, 3, 4, 5 and 6 days. Test III: Percent emergence recorded at 8, 9, and 10 days. Sex ratio of emerged adult *T. pretiosum* was determined at the conclusion of the emergence period.

Statistical methods: Means were compared by Duncan's Multiple Range Test ($p=0.05$).

10. Reported Results:

- A. **Effect of *B. thuringiensis* on adult *T. pretiosum*:** At day 3 the mean cumulative percent mortalities of adults treated with 0, 0.1X, 1X, and 10X the field dosage were 32, 17, 22, and 28, respectively. At day 6 the mean cumulative percent mortalities of adults treated with 0, 0.1X, 1X, and 10X the field dosage were 50, 41, 54, and 50, respectively. There were no significant differences between the means within either group.
- B. **Effect of *B. thuringiensis* on percentage emergence of adult *T. pretiosum*:** The percentage of *H. zea* eggs, treated with 0, 0.1X, 1X, and 10X the field *B. thuringiensis* dosage, yielding adult *T. pretiosum* were 59, 60, 52, and 48, respectively. There was no significant difference between any of the means.
- C. **Effect of *B. thuringiensis* on the sex ratio of adult *T. pretiosum*:** Parasitized *H. zea* eggs treated with 0, 0.1X, 1X, and 10X the field dosage, yielded adult *T. pretiosum* with sex ratios (F/M) of 2.0, 1.5, 1.7, and 2.0, respectively. There was no significant difference between any of the means.
- D. **NOEL:** Since there were no significant differences between the means of any of the treatments the NOELs were 10X the field rate. However, Test I, which determined adult mortalities, was invalid because of excessive control mortality.

11. Study Author's Conclusions/Quality Assurance Measures: "The results of this study indicated that *Bacillus thuringiensis* ABG-6305 had no significant adverse effects on *Trichogramma pretiosum* Riley at 10X, 1X, and 0.1X the field rate for the following tests:

Test I: Adults exposed to ABG-6305 through direct contact and ingestion.

Test III: Emergence rates and sex ratio of adult *T. pretiosum* emerging from host eggs treated with ABG-6305.

At the time of this writing, Test II had not been completed."

"The data submitted to the sponsor study monitor were collected and documented in accordance with 40 CFR Part 160, Good Laboratory Practice Standard, and accurately reflect the results of the study. Any deviations from the protocol and Standard Operating Procedure (SOP) have

been documented and reported to the Study Director". Signed by Reed L. Kirkland, Study Director, California Agricultural Research, Inc., 4141 N. Vineland, Kerman, CA 93630.

12. Reviewer's Discussion and Interpretation of:

- A. **Test Procedures:** The root cause of failures of Test I and II is the poor quality of *T. pretiosum* adults used in the experiments. The study report states "Our experience in these tests was that *T. pretiosum* reared on *S. cerealella* were extremely small, difficult to handle, and kept sticking in the honey or on the water wick. During the winter months the insectaries generally refrigerate the eggs and therefore the stock is often of poor quality. Those *T. pretiosum* reared on *H. zea* eggs were more robust. However, two attempts to perform Test II using sterilized *Heliothis zea* eggs provided by the USDA failed, due to little or no female production." The study protocol (1-CAR-NTO-TR-91) specifies that "The host eggs used will be from the cotton bollworm, *Heliothis zea*." Yet *S. cerealella* eggs were used to produce the adult *T. pretiosum* used in Test I, even though the performing laboratory was aware that such adults were generally unsatisfactory. This deviation from the study protocol was not noted in Appendix B: Alterations to the Study Protocol, even though it appears to be the critical factor in the failure of Test I. Test II failed because "*T. pretiosum* stock received from insectary sources were of inadequate vigor and produced produced (sic) insufficient numbers of females to complete the test in time to be included in this study." Test III, which was conducted with *H. zea* eggs parasitized by *T. pretiosum* at the performing laboratory, yielded nearly 3000 *T. pretiosum* females. The obvious answer to the problems encountered in Tests I and II is to produce *T. pretiosum* adults on *H. zea* eggs at the performing laboratory, and use those adults to conduct Tests I and II. The fact that poor quality insects were received from commercial and public insectaries does not negate the need for valid information. The conduction of quality research very often involves some methods development before the actual data generating research is done. It is obvious that such was not done in this case.
- B. **Statistical Analysis:** A mean separation test (Duncan's Multiple Range Test) was used to determine if there were significant differences between the means in Tables I-III. This is improper use of a mean separation test. A mean separation test is only used when an Analysis of Variance (ANOVA) of the data indicates that significant differences exist amongst the means, then a mean separation test is used to determine which means are significantly different. The study protocol states "If appropriate, data will be subjected to Analysis of Variance (ANOVA) ($P \leq 0.05$) using a randomized (sic) block design. If significant differences exist, Duncan's Multiple Range Test (DMRT) will be used to separate means."
- C. **Discussion/Results:** There were no Discussion or Results sections in the study report.

D. Adequacy of the Study:

1. Validation Category: Invalid
2. Rationale: Excessive control mortality in Test I due to use of improper host eggs and use of poor quality test insects. Test II was not conducted because of poor quality test insects.

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