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 predatory mite *Metaseiulus occidentalis* (Nesbit) and their host prey the twospotted spider mite *Tetranychus urticae* (Koch) (Project ID. 91.042). Submitted by Abbott Laboratories. North Chicago, IL. EPA Access.No. 419748-09.

5. Reviewed By: Clayton C. Beegle
Entomologist
EFED/EEB
Signature: Clayton C. Beegle
Date: 3-30-92

Les W. Touart
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Signature: L. T. T
Date: 7-15-92

6. Conclusions: This study is scientifically sound and meets the EPA requirements for a core study. ABG-6305 is somewhat toxic to the predacious mite *M. occidentalis*. The field dosage of 2.4 g/l killed about 24% (corrected mortality) of the treated gravid ♀s. The estimated LC_{50} was approximately 300 g/l. Adult prey mites, *T. urticae*, were adversely affected to about the same degree. *T. urticae* protonymphs were somewhat more susceptible, the estimated LC_{50} was approximately 200 g/l. The use of ABG-6305 on a crop where *T. urticae* was being controlled to some degree by *M. occidentalis*, would probably lessen the degree of control because ovipositing *M. occidentalis* ♀s eat about 11 immature *T. urticae* per day.

7. Recommendations: EEB recommends that the registrant determine the source of toxicity of ABG-6305 to mites, 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 for gravid *M. occidentalis* and *T. urticae* ♀s and *T. urticae* protonymphs 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}.

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:** Predatory mite *Metaseiulus occidentalis (Nesbit)* and the prey mite, twospotted spider mite *Tetranychus urticae* (Koch).

Age/stage of maturity: Gravid females and eggs of both species, and protonymphs of *T. urticae*.

Sex: Female adults, eggs and protonymphs of both sexes.

Source: *M. occidentalis* was obtained from the commercial insectary Biotactics Inc., Grand Torrance, CA. *T. urticae* was from a stock culture maintained by Plant Sciences, Inc., Watsonville, CA.

B. **Dosage Form:**

Solvents/vehicles: Water.

Route of administration: Sprayed on leaves with an airbrush sprayer.

C. **Referenced Protocol:**

Test levels: 0.24, 2.4, and 23.9 g/l. Each leaf sprayed 1/4 sec. 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 field rate.

Dose spacing factor: 10X

Number per level: Six gravid ♀ *M. occidentalis* per leaf, 3 *M. occidentalis* eggs per leaf, 3 gravid ♀ *T. urticae* per leaf, 3 *T. urticae* protonymphs per leaf, and as many *T. urticae* eggs per leaf that 4-5 ♀s lay in 24 hr. Each group replicated 12 times at each treatment level.

Holding/acclimation: 24 hrs.

Pen/cage facilities: Adult fecundity and mortality tests were conducted on leaf discs on absorbent moist cotton in 1 oz plastic cups. Larval mortality tests were conducted on whole, young leaves on moist cotton in 90 mm diameter Petri plates. All cups and plates were kept in a diurnal growth chamber.

Feeding: *T. urticae* fed on bush bean, *Phaseolus vulgaris* L., leaves on which they were confined. *M. occidentalis* fed on all stages of *T. urticae*.

Physical condition: Gravid ♀s and eggs of both *M. occidentalis* and *T. urticae*, and protonymphs of *T. urticae*. All stages were apparently healthy.

Test conditions:

Temperature: 22-25.5 °C

Relative humidity: 40-80%

Photoperiod: 16 hrs.

Controls: Water sprayed controls.

Observation period: Tests which assessed effects on gravid *M. occidentalis* ♀s and progeny of same were observed for seven days. Tests measuring the number of eggs laid, and their percentage hatch, by *M. occidentalis* were run for 5 days. Tests to determine sex ratios of *M. occidentalis* progeny were conducted until the mites reached maturity. All tests involving *T. urticae* were observed for eight days.

Statistical methods: "All data for Tests I, II, and III were subjected to a 3-way Analysis of Variance (ANOVA) (P=0.05). Significantly different means were separated using Duncan's New Multiple Range Test (DNMRT) for both observations and treatments".

10. Reported Results:

A. **Effect of *B. thuringiensis* on gravid ♀ *M. occidentalis*:** Average percent mortalities of mites treated with 0 X (check), 0.1 X (0.24 g/l), 1 X (2.4 g/l), and 10 X (23.9 g/l) the field dosage were 11.8, 25.7, 32.7, and 43.9, respectively. All values were significantly different at the P_{05} level.

B. **Number of eggs laid by *M. occidentalis* ♀s treated with *B. thuringiensis*:** Mean number of eggs laid by *M. occidentalis* treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 3.4, 2.2, 1.8, and 2.9, respectively. Females treated with 0.1 X and 1 X the field dosage laid significantly fewer eggs than untreated check ♀s.

C. **Hatchability of *M. occidentalis* eggs treated with *B. thuringiensis*:** Percentage hatch of eggs treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 81.5, 83.3, 92.6, and 82.4, respectively. There was no significant difference in average hatch between any of the treatment groups.

D. **Effect of *B. thuringiensis* on survivability of *M. occidentalis* hatched from eggs treated with *B. thuringiensis*:** The mean percentage mortalities from hatching to adulthood of *M. occidentalis* from eggs treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 6.9, 7.6, 10.3, and 6.2, respectively. There were no significant differences between treatment means.

E. **Effect of *B. thuringiensis* on the sex ratio of *M. occidentalis* hatched from eggs treated with *B. thuringiensis*:** The ♂:♀ ratios of *M. occidentalis* from eggs treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 1:1.7, 1:2.9, 1:2.5, and 1:3.3.

F. **Effect of *B. thuringiensis* on gravid ♀ *T. urticae*:** Average percent mortalities of mites treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 11.7, 20.1, 6.9, and 69.5, respectively. Mortality at the 10 X rate was significantly higher than mortalities at the other dosage rates.

G. **Effect of *B. thuringiensis* on protonymphs of *T. urticae*:** Average percent mortalities of protonymphs treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 5.5, 25.0, 26.9, and 82.7, respectively. The mortalities at 0.1 and 1 X were not significantly different from each other. The check mortality was significantly lower, and the 10 X mortality was significantly higher than mortalities at other rates.

H. **Hatchability of *T. urticae* eggs treated with *B. thuringiensis*:** Percentage hatch of eggs treated with 0 X, 0.1 X, 1 X, and 10 X the field dosage were 50.9, 48.4, 48.0, and 46.8, respectively. The percentage hatch of eggs in the control group were significantly higher than that in all the *B. thuringiensis* treated groups.

C. **NOEL:** The NOEL was 10 X the field rate for hatchability of *M. occidentalis* eggs treated with *B. thuringiensis*, and sex ratio and survival to the adult stage of mites hatched from such eggs. NOEL values for mortality of gravid ♀ *M. occidentalis*, number of eggs laid by ♀ *M. occidentalis*, mortality of *T. urticae* protonymphs, and percentage hatch of treated *T. urticae* eggs could not be determined because the lowest rate (0.1 X field rate) caused significant adverse effects. However, in the case of percentage hatch of treated *T. urticae* eggs, the effect was very slight. The NOEL for mortality of *B. thuringiensis* treated gravid ♀ *T. urticae* appears to be 1 X the field rate.

11. Study Author's Conclusions/Quality Assurance Measures: "The results of this study indicate there are some significant adverse effects of *Bacillus thuringiensis* ABG-6305 technical material to motile twospotted spider mites, *Tetranychus urticae* (Koch) (primarily at the 10X rate). There is a significant adverse effect of Bt ABG-6305 to the adult predatory mite *Metaseiulus occidentalis* at all concentrations but primarily at the 10X rate. Based on this data it is apparent that at the 1X field concentrations Bt ABG-6305 could have slight adverse effect on agricultural ecosystems that utilize this predatory mite for control of twospotted spider mites. This effect to the agricultural ecosystems would not be severe or long lasting due to the fact that Bt ABG-6305 has no adverse effect on the eggs of *Metaseiulus occidentalis*".

"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. However, when significant mortality is observed when the test mites are exposed to 0.1 X, 1.0 X, and/or 10 X the field dosage, 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}. LC_{50}s are calculated by analyzing the resulting data with a probit or logit analysis program.

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

C. **Discussion/Results:** ABG-6305 is somewhat toxic to the predacious mite *M. occidentalis*. The field dosage of 2.4 g/l killed about 24% (corrected mortality) of the treated gravid ♀s. The estimated LC_{50} was approximately 300 g/l. Adult prey mites, *T. urticae*, were adversely affected to about the same degree. *T. urticae* protonymphs were somewhat more susceptible, the estimated LC_{50} was approximately 200 g/l. The registrant should determine the source of the toxicity of ABG-6305 to mites, 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.