DATA EVALUATION REPORT

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

2. Test Material: Technical, primary powder

3. Study/Action Type: 154A-24. Honey bee toxicity/pathogenicity test: Tier I.

4. Study Identification: Kirkland, R. L. 1991. The effect of *Bacillus thuringiensis*, ABG-6305 technical powder, on the honey bee, (*Apis mellifera L.*). California Agricultural Research, Inc. Project CAR 196-90. Submitted by Abbott Laboratories. North Chicago, IL. EPA Access. No. 419748-08.

5. Reviewed By: Clayton C. Beegle
Entomologist
EFED/EEB

Signature: Clayton C. Beegle
Date: 3-24-92

Les W. Touart
Head, Section 1
EFED/EEB

Signature:
Date:

6. Conclusions: This study is scientifically sound and meets the EPA requirements for a core study. The study indicates that ABG-6305 is highly toxic *per os* to worker honey bees with an LE_{50} of 15 ppm (95% CL 10-21), and an LT_{50} of 6.7 days (95% CL 6.5-7.1) at the highest dose tested (1000 ppm).

7. Recommendations: EEB recommends that the registrant determine the source of the high toxicity of ABG-6305 to worker honey bees, 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 future tests the food solutions containing *B. thuringiensis* preparations should be agitated to ensure that they remain in suspension, and the bees should have access to the test suspensions through holes rather than through cotton plugs. Also probit or logit analysis programs should be used instead of linear regression analysis programs to calculate lethal effect or concentration levels.

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 organism:** *Apis mellifera* L.
Age/stage of maturity: Less than two weeks old
Form: Worker bees from three hives
Sex: Female
Source: Professional beekeeper

B. **Dosage Form:**
Solvents/vehicles: 1:1 v/v honey-water solution
Route of administration: *ad lib* oral from 1 dram vial with loosely fitted cotton plug. Continuously available.

C. **Referenced Protocol:**
Test levels: 1, 10, 100, and 1000 ppm
Dose spacing factor: 10X
Number per level: 225 (3 replicates of 25 each repeated 3 times over days)
Holding/acclimation: NA
Pen/cage facilities: Cages approximately 12.7 x 12.7 x 12.7 cm of 3.2 mm mesh with detachable lids held in place with rubber bands. Holes were made in lids in which vials were inserted.
Feeding: Continuously available 1:1 v/v honey-water solution (in which test substance was suspended where appropriate). Replacement vials containing newly prepared mixtures were provided daily.
Physical condition: Apparently healthy worker bees under one week of age at beginning of test.
Test conditions:
Temperature: 21-28 °C
Relative humidity: 40-87%
Photoperiod: Total darkness except for examination periods.
Controls: 1:1 v/v honey-water solution feeding control, and a 1:1 v/v honey-water solution evaporation control.
Observation period: Until untreated control mortality reached 20% (11 days in trial 1, 12 days in trial 2, and 9 days in trial 3).
Statistical methods: All treatment mortalities were corrected for control mortalities using Abbott's (1925) formula. To determine amount of material consumed per bee, evaporative losses were corrected for by amount lost in evaporative controls. LE_{50} values were calculated by linear regression analysis. *B. thuringiensis* dosage effects on net food consumption data were analyzed by Duncan's Multiple Range Test.

10. Reported Results:

A. **Effect of *B. thuringiensis* on food consumption of *A. mellifera*:**
There was no significant difference in the net amount of honey-water solution consumed by bees in the different treatment groups. The net amounts consumed at 0, 1, 10, 100, and 1000 ppm *B. thuringiensis* preparation were 51.4, 51.0, 48.7, 48.0, and 53.9 mg/bee/day, respectively.

B. **Dosage mortality response to *B. thuringiensis*:**
The calculated (by linear regression analysis) LE_{50} after 8 days of continuous feeding was 739 ppm. The LE_{50} calculated from data at the termination of each trial (9-12 days) was 326 ppm.

C. **NOEL:** No NOEL was determined since there was 17% corrected mortality at the conclusion of the test at the lowest dosage (1 ppm).

11. Study Author's Conclusions/Quality Assurance Measures:

"This study indicated that B. t. ABG-6305 was chronically pathogenetic /toxic to honey bees when continuously fed at high dosages over 9-12 days. It should, however, be realized that these high levels (i.e. 1000 and 100 ppm) of test substance which was fed to the honey bees represented a level of exposure which likely far exceeds the actual contact the insect would have under field conditions."

"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 Reed L. Kirkland, Study Director.

12. Reviewer's Discussion and Interpretation of:

A. **Test Procedures:** Since *B. thuringiensis* spores and crystals are particulate (1-3 m) and will fall out of suspension, the honey-water mixtures containing *B. thuringiensis* preparations should have been agitated throughout the test period. The viscosity of the 1:1 honey-water solution may have been high enough to retard settling out of the spore-crystal suspension, but that should have been established. If the spore-crystal suspension did settle out during the 24 hours between changing of the vials it would have resulted in a higher than intended dosage delivered to the test bees.

In addition, lids containing holes of the appropriate size (ca. three-penny nail) should have been used on the vials instead of cotton plugs as the cotton plugs may have filtered out some of the *B. thuringiensis* spore-crystal preparation.

B. **Statistical Analysis:** Linear regression analysis is not a proper technique for determining effect levels of pathogens and/or toxins. Dosage mortality responses follow an S-shaped curve and the most accurate part of the curve is around the 50% response point. For those reasons probit or logit analysis programs should be used to estimate effect levels. Using probit analysis the LE_{50} for 8 days of continuous feeding (using data in Table VI) is estimated to be 522 ppm (95% CL 378-720) compared to 739 ppm which was calculated by the study author using linear regression analysis. The probit analysis calculated LE_{50} value for the 9-12 days data in Table VII is 75 ppm (95% CL 55-104) compared to 326 ppm calculated by linear regression analysis. The LE_{50} calculated by probit analysis for day 12, the final day of the study, is 15 ppm (95% CL 10-21).

The use of a multiple range test on the data in Tables VIII and IX is inappropriate. Multiple range tests should only be used if an analysis of variance indicates that significant differences exists among the group of means. The F value for the data in Table VIII is 0.28, the required F_{05} value is 3.11.

C. **Discussion/Results:** An LE_{50} of 15 ppm indicates that the ABG-6305 preparation of *B. thuringiensis* subsp. *aizawai* is highly toxic to adult worker honey bees when fed continuously for 12 days. The LT_{50} (lethal time 50%) of the highest dose level of 1000 ppm) is 6.7 days (95% CL 6.5-7.1). However, there are two caveats. The first is that the ABG-6305 preparations may have settled out in the honey-water solutions which could have resulted in the test bees being exposed to higher than intended dosages. The second is that in susceptible Lepidoptera larvae, where *B. thuringiensis* spore-crystal complexes act primarily as stomach poisons, affected larvae exhibit greatly reduced food consumption and growth rates. This is the case with larvae exposed to sublethal as well as lethal doses. The data in Tables VIII and IX show that there are no differences in the amount of food consumed by bees in the check and any of the treatment groups, nor are there any trends. This suggests that the bees were not digesting the crystals and activating the toxin(s). To determine what is causing the mortality, the registrant should test well washed preparations, autoclaved preparations, and the first wash supernatant against bees. The results of these tests would show whether the activity is coming from the spore-crystal complex, exotoxins, or spent or unutilized growth medium components.

A typical *B. thuringiensis* preparation field application would be 1 lb. of formulated product in 20 gal. of water per acre. The spray droplets would contain about 6,000 ppm product. Most formulated *B. thuringiensis* products contain 25-50% technical powder, so the droplets would contain 1500-3000 ppm technical powder. The top rate (1000 ppm) used in this study is in that ball park. However, in the field the bees would only be directly exposed *per os* to the *B. thuringiensis* material that ends up in the nectar of flowers sprayed with *B. thuringiensis*. Only larval bees in the home hives would be exposed *per os* to *B. thuringiensis* preparations on pollen of flowers sprayed with *B. thuringiensis*. The activity half-life of *B. thuringiensis* on field crops is 2-3 days, and crops are normally not sprayed more often than once a week, so worker bees in a field situation would not be exposed to either the level or duration of exposure as they were in the study. Thus, it is not expected that honey bees would be killed by agricultural uses of formulated ABG-6305 product. However, the registrant should determine what material in the ABG-6305 technical powder is responsible for the honey bee activity, and take steps to alleviate it if possible.

D. **Adequacy of the Study:**

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements.