DATA EVALUATION REPORT

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

2. Test Material: Technical, primary powder

3. Study/Action Type: 154A-16. Avian oral pathogenicity/toxicity test: Tier I.

4. Study Identification: Beavers, J. B. 1991. ABG-6305: An avian oral pathogenicity and toxicity in the bobwhite. Laboratory Project ID # 161-117. Submitted by Abbott Laboratories. North Chicago, IL. EPA Access.No. 419748-04.

5. Reviewed By: Clayton C. Beegle
Entomologist
EFED/EEB
Signature: Clayton C. Beegle
Date: 5-13-92

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

6. Conclusions: This study provides scientifically valid information and demonstrates an LC_{50} > 8570 mg ABG-6305/kg. This indicates that ABG-6305 is practically nontoxic to bobwhite quail. It satisfies EPA Guideline requirements for avian oral toxicity and pathogenicity testing (Tier I).

7. Recommendations: None.

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:** Northern bobwhite quail, *Colinus virginianus*.

Age/stage of maturity: 21 days old at test initiation.

Sex: ♂ and ♀.

Size: Mean weight of 46 ± 4 g at initiation of test.

Source: Production flock of Wildlife International Ltd., Easton, Maryland 21601.

B. **Dosage Form:**

Solvents/vehicles: Distilled water.

Route of administration: Suspension directly introduced into crop or proventriculus using a stainless steel cannula.

C. **Referenced Protocol:**

Test levels: Distilled water at 10 ml/kg/day for 5 days (control), and 1.71 g/kg ABG-6305 in 10 ml/kg water daily for 5 days (total of 8.57 g/kg).

Dose spacing factor: NA.

Number per level: 30 birds (six groups of five birds each) received ABG-6305. 10 birds (two groups of five birds each) received distilled water.

Holding/acclimation: 21 days.

Pen/cage facilities: Galvanized sheeting and wire mesh cages 72 x 90 x 23 cm. Five birds (one replication)/cage.

Feeding: Water from the Easton public water supply and nonmedicated game bird diet formulated to Wildlife International Ltd.'s specifications (formula supplied) were supplied *ad libitum* during the acclimation and test periods.

Physical condition: Apparently free of disease, injuries, and abnormalities at the beginning of the test. Not treated for disease.

Test conditions:

Temperature: 21.8 ± 1.0°C.

Relative humidity: 43 ± 11%.

Photoperiod: 16L:8D. Fluorescent lights which approximate noon-day sunlight. Lamps 5000° Kelvin, 250 lux.

Controls: Distilled water oral gavage controls, two replicates of five birds each.

Observation period: Birds observed daily during acclimation period. During test period birds were observed at least twice daily. Individual bird weights recorded on days 0, 1, 2, 3, 4, 11, 18, 25, and 30. Estimates of feed consumption made for days 0-4, 5-11, 12-18, 19-25, and 26-30.

Statistical methods: Means and standard deviations calculated for body weights and means calculated for feed consumption. There were no mortalities in the control or treatment groups.

10. Reported Results:

A. **Mortalities:** No mortalities occurred in either the control or treatment groups.

B. **Appearances:** One control bird was lame in its right leg on days 4 and 5. One ABG-6305 treated bird had an ankle lesion on days 5-8. All other control and treatment birds were normal in appearance and behavior throughout the test period.

C. **Body weight and feed consumption:** The study report stated that "When compared to the negative control groups, there were no apparent treatment related effects upon body weight gain in any of the treatment groups (Table 3)." Analysis of the information in Table 3 indicates that is not correct. Starting on day 11 the treated birds were heavier, and by the end of the test (30 days) the difference was significant at the F_{05} level. The difference is probably due to the additional forced intake of nutrients supplied by ABG-6305.

There was no difference in feed consumption between the control and ABG-6305 treated groups.

D. **Necropsy:** One bird from treatment group T-3 had an enlarged spleen. Because this was the only instance of this abnormality it was not considered to be treatment related. No other abnormalities were noted in either the control or treated groups.

11. Study Author's Conclusions/Quality Assurance Measures: "ABG-6305 showed no apparent pathogenicity, toxicity or effect upon the survival of young bobwhite when administered by oral gavage at 1714 mg test substance/kg or approximately 3.4 X 10^{11} cfu/kg of body weight per day for five days."

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with the following exception: Samples of the dosing solutions were not collected for confirmation of the test concentrations, homogeneity or stability." Signed by Joann B. Beavers, Study Director, on 2-19-91.

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 exceptions that a sterile culture filtrate control was not included and samples of the dosing solutions were not collected for confirmation of the test concentrations, homogeneity or stability. The study report stated that "The study deviated from this guideline" (Section 154A-16 of the Environmental Protection Agency Registration Guidelines Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents) "in that it did not employ a sterile filtrate control group. Per the Sponsor, this material was inappropriate since ABG-6305 a technical material is a solid; no products are formulated from an unprocessed liquid from production culture." This reasoning is not correct. *Bacillus thuringiensis*, depending on the isolate and the conditions under which it is grown, can produce both heat labile

and heat tolerant exotoxins that can end up in dry solid material. The reason for testing sterile culture filtrates is that in the event of toxicity to nontarget organisms which are not normally sensitive to the spore-crystal complex, the results of the sterile culture filtrate test can indicate what is responsible for that unexpected activity. The sterile culture filtrate test is not necessary in this case because ABG-6305 had no deleterious effects on bobwhite quail. But this is so because of no deleterious effects, not because the test material is a dry solid.

The lack of dosing solution samples is not a significant omission because fresh ABG-6305 suspensions were made up daily for direct oral gavages.

B. **Statistical Analysis:** No analysis needed as there were no observed mortality or sublethal ill effects due to the test material.

C. **Discussion/Results:** An LC_{50} > 8570 mg ABG-6305/kg indicates that ABG-6305 is practically nontoxic to bobwhite quail.

D. **Adequacy of the Study:**

1. Validation Category: Core.

2. Rationale: This study meets EPA Guideline requirements.