DATA EVALUATION REPORT

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

2. Test Material: Technical, primary powder

3. Study/Action Type: 154A-20. Freshwater aquatic invertebrate toxicity and pathogenicity testing: Tier I.

4. Study Identification: Ward, T. J. and Boeri, R. L. 1991. Chronic toxicity of ABG-6305 to daphnid *Daphnia magna*. EnviroSystems Division, Resource Analysts, Inc. Laboratory Project ID # 90162-A. Submitted by Abbott Laboratories. North Chicago, IL. EPA Access.No. 419748-07.

5. Reviewed By: Clayton C. Beegle
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Date: 5-6-92

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Date: 7-15-92

6. Conclusions: This study provides supplemental information. It does not satisfy 154A-20 core requirements because a sterile culture filtrate control [Section (b)(3)(ii) 154A-20 Subdivision M] was not tested, nor were a sufficient number of treatment concentrations tested to allow the determination of an accurate EC_{50} and 95% confidence limits [Sections (b)(7)(i) and (c)(1) 154A-20 Subdivision M]. Estimated EC_{50s}, based on the actual amounts of *B. thuringiensis* present in the tests (Appendix C) rather than the nominal concentrations, were 0.8 ppm when the daphnids were held individually and 2.7 ppm when they were held multiply (5). Thus, ABG-6305 is moderately to highly toxic to *D. magna*.

7. Recommendations: EEB recommends that the registrant repeat the toxicity tests using a graded series of dilutions (minimum of five, preferably seven) where about 0-5%, and 90-100% mortality, is expected at the lowest and highest dosages, respectively. LE_{50} values and corresponding 95% confidence limits should be calculated using the resulting dosage mortality data. Test materials should be ABG-6305 technical powder and well-washed purified spore-crystal complex of the *B. thuringiensis* isolate that ABG-6305 is based on. A sterile fermentation culture filtrate should be used as one of the controls. These tests would determine if the observed activity against *D. magna* is due to the spore-crystal complex, exotoxin(s), unutilized or waste fermentation materials, or materials added during recovery and stabilization. A better understanding of the observed toxic effects may alleviate the need for Tier II testing [Section (d) Tier progression, 154A-20 Subdivision M]. It would also be highly desirable for the test solutions to be gently agitated or circulated during the course of the test since it is apparent that the majority of the *B. thuringiensis* spores and crystals in the test solutions are falling out of suspension during the period of testing. The results of the statistical analyses should be included in the data tables in the Results section.

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:** *Daphnia magna*

Age/stage of maturity: Daphnids less than 24 hrs old (first instar) at beginning of study.

Sex: ♀

Source: In-house culture (lot # 01107912) maintained at EnviroSystems Division, Resource Analysts, Inc., One Lafayette Rd., Hampton, New Hampshire 03842.

B. **Dosage Form:**

Solvents/vehicles: Dilution water.

Route of administration: In suspension.

C. **Referenced Protocol:**

Test levels: Nominal concentrations of 0, 0.5, 5, and 74 mg/l. Actual concentrations (Appendix C) 0, 0.07, 0.97, and 20 mg/l.

Dose spacing factor: Approximately 10-20X.

Number per level: Seven replicates of individually-held, and four replicates of groups of five. Thus, 27/level.

Holding/acclimation: None.

Pen/cage facilities: 250 ml glass beakers containing 200 ml of test solution. Water depth approximately 6 cm.

Feeding: *Selenastrum capricornutum* alga and yeast-trout chow suspension at least twice daily.

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

Test conditions:

Temperature: 20 ± 2°C.

Dissolved oxygen: 8.7 ± 0.5 ml/l.

pH: 7.2-8.0.

Hardness: 160-180 mg/l.

Test conditions cont.:

Conductivity: 800 ± 100 umhos/cm.

Source of dilution water: Filtered well.

Static/renewal/flow-through: Static, replaced at 2, 4, 7, 9, 12, 14, 16, and 18 days.

Loading: 0.2 g/l.

Aeration: No.

Photoperiod: 16L:8D. Cool-white fluorescent with 25 uEs-1m-2 intensity.

Controls: Dilution water controls, seven individually held and four groups of five daphnids each.

Observation period: Initially and at 24 hr intervals for 21 days.

Statistical methods: In the Materials and Methods it is stated that "Shapiro-Wilk's test was used to determine if data were normally distributed, and Bartlett's test was used to determine if variances were homogeneous. If variances were homogeneous, a parametric one-way analysis of variance (ANOVA) and, if necessary, Dunnett's test was used to compare treatment and control means. If variances were heteroscedastic a nonparametric ANOVA (Steel's Many-One Rank test, Steel and Torrie, 1960) was used to compare control and treatment means. Dichotomous data were transformed (arc sin square root) prior to statistical analysis. All calculations were performed by the Study Director using nominal concentrations of test substance". However, no statistical test information was provided in any of the data tables.

10. Reported Results:

Table 2. Mean survival, reproduction, and dry weight of daphnids exposed to several concentrations of ABG-6305 after 21 days.

Nominal conc. (mg/l)	Actual conc.(mg/l)	% Survival 1 daph./rep	% Survival 5 daph./rep	# young produced	Dry wt. (mg) surv. adults
0 (control)		100	95	91	0.7
0.5	0.07	100	100	96	0.8
5.0	0.97	43	85	37	0.6
74	20.0	0	0	-	-

Table 5. No observed effect level (NOEL) and lowest observed effect level (LOEL) from toxicity test, in mg/l, with daphnids and ABG-6305.

Biological Endpoint	NOEL	LOEL
First generation survival (r1-7)	0.07[1] [0.5][2]	0.97 [5.0]
First generation survival (r8-11)	0.07 [0.5]	0.97 [5.0]
Time to first brood	0.97 [5.0]	>0.97 [5.0]
Number of young produced	0.07 [0.5]	0.97 [5.0]
Dry weight of survivors	0.07 [0.5]	0.97 [5.0]

[1] Actual concentration
[2] Nominal concentration

The mean actual (measured) concentrations of ABG-6305 in the test solutions were only 14.3, 19.4, and 27% of the intended nominal concentrations of 0.5, 5, and 74 mg/l, respectively. The calculated estimated (there were not enough data points to calculate valid values) EC_{50s} were 0.8 ppm for the singly held daphnids and 2.7 ppm for the multiply held.

11. Study Author's Conclusions/Quality Assurance Measures: "Survival of first generation daphnids exposed to 74 mg/L ABG-6305 was significantly different than the control and offspring production by daphnids exposed to 5.0 mg/L ABG-6305 was significantly different than the control. Survival of first generation daphnids, offspring production, weight, and occurance (sic) of sublethal effects was not significantly different in the 0.5 mg/L ABG-6305 treatment and the control. Production of young by the first generation daphnids was first observed on day 10 in test vessels containing 0.5 and 5.0 mg/L ABG-6305 and in the control exposure. Daphnids exposed to 74 mg/L were killed prior to sexual maturity.

The no observed effect level (NOEL) is 0.5 mg/L, and the lowest observed effect level (LOEL) is 5.0 mg/L ABG-6305 (Table 5). The maximum acceptable toxicant concentration (MATC), expressed as the geometric mean of the LOEL and NOEL is 1.6 mg/L ABG-6305."

12. Reviewer's Discussion and Interpretation of:

A. **Test Procedures:** An accurate determination of the level of toxicity of ABG-6305 to daphnids is not possible because an inadequate number of dosage levels were used. Because a sterile culture filtrate was not tested it is not possible to ascertain the source of the toxicity observed. Due to apparent settling out of the spores and crystals, the measured levels of material were much lower (14-27%) than the intended nominal levels.

B. **Statistical Analysis:** The results of the statistical analyses were not included in the tables in the Results section. If the statistical analyses described in the Materials and Methods section were actually conducted then the statistical analyses were satisfactory, with the exception that LE_{50} values and 95% confidence limits were not calculated for ABG-6305.

C. **Discussion/Results:** The NOEL and LOEL levels reported in the Results section are much too high as they were based on the nominal expected concentrations rather than the actual measured concentrations. In the Results and Discussion section of Appendix C (Bacteriological Analysis of Test Suspensions) it is stated that "There was generally no difference between old and new suspensions with respect to spore count." That statement is not correct. The data in the table of that appendix shows that the spore counts of 17 of the 18 new suspensions were higher than those of the old. Only one was lower.

D. **Adequacy of the Study:**

1. Validation Category: Supplemental.

2. Rationale: This study does not satisfy 154A-20 core requirements because a sterile culture filtrate control [Section (b)(3)(ii) 154A-20 Subdivision M] was not tested, nor were a sufficient number of treatment concentrations tested to allow the determination of an accurate EC_{50} and 95% confidence limits [Sections (b)(7)(i) and (c)(1) 154A-20 Subdivision M].