2-2-94

MRID No. 421455-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin. Shaughnessey No. 083401.

2. **TEST MATERIAL:** Benefin (N-(n-butyl)-N-ethyl-2,6-dinitro-∝,∝,∝-trifluoro-p-toluidine); Lot No. 231EF4; 95.64% purity.

71-4(a)

3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Bobwhite quail (*Colinus virginianus*).

4. **CITATION:** Murray, A.G., J.L. Seacat, and D.W. Grothe. 1991. The Toxicity of Benefin to Bobwhite in a One-Generation Reproduction Study. Laboratory Project No. A00690. Prepared by Toxicology Research Laboratories, Lilly Research Laboratories, Greenfield, Indiana. Submitted by DowElanco, Indianapolis, Indiana. EPA MRID No. 421455-02.

5. **REVIEWED BY:**

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Signature: Rosemary Graham Mora
Date: 4/23/92

6. **APPROVED BY:**

Michael L. Whitten, M.S.
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Signature: Michael L. Whitten
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Supervisor, HED/EFED
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Signature: 2/2/94
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2-2-94

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study. The mean measured dietary concentrations of benefin (96 ppm, 295 ppm, and 990 ppm) had no effects upon mortality or behavior in bobwhite quail during the 23-week exposure period. A high percentage of eggs cracked in the control prevents adequate analysis of this parameter. The ratio of two-week survivors/eggs set was significantly affected at all test levels (96, 295, and 990 ppm) and a treatment-related trend was obvious for the ratios of hatchlings/eggs set, two-week survivors/hatchlings, two-week survivors/eggs set, and viable embryos/eggs set. Therefore, an NOEC for bobwhite quail exposed to benefin could not be determined by this study.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in the test were pen-reared bobwhite quail (*Colinus virginianus*) purchased from Barrett's Quail Farm, Houston, Texas. All birds were of the same hatch date. For this study, 230 birds were quarantined and acclimated to the facilities for 4 weeks prior to test initiation. One male and one female were assigned to each pen. At test initiation, all pairs of birds were compatible. The birds were 14 weeks of age at the beginning of the preproduction phase of the study.

B. **Dose/Diet Preparation/Food Consumption:** Test diets (20-kg) were prepared on a weekly basis for each test level. Acetone with the appropriate amount of test material was added slowly to 8-kg of diet and mixed for 15 minutes. This treated batch was combined with the remaining 12-kg batch of untreated diet and mixed for 10 minutes. The control diet and three test concentrations (100, 300, and 1,000 parts per million [ppm]) were presented *ad libitum* to the birds. Each of the four groups of adult birds was fed the appropriate diet from test initiation until terminal sacrifice. The potency of the test material was assumed 100% for the purpose of diet preparation. Acetone accounted for no more than 1% of the total diet.

The composition of the basal diets for adult birds and their offspring was presented in the report. The test substance was not mixed into the diet of the offspring. Food and water were supplied *ad libitum* during acclimation and during the test for adults and offspring.

Samples of all diets were collected on three occasions during the study for the analysis of benefin using gas chromatography. Prior to this study, samples at 100 and 1,000 ppm were collected to evaluate the homogeneity and the stability of the test material.

C. **Design:** The pens were assigned to treatment levels using a computer-generated table of random numbers. The test birds were distributed to the test pens using a

computer-generated table of random numbers. The four groups were comprised of the following:

Benefin Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	20	1	1
100 ppm	20	1	1
300 ppm	20	1	1
1,000 ppm	20	1	1

Treatment levels were based, in part, upon acute toxicity data. Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 4 weeks
2. Pre-production - 10 weeks
3. Production - 13 weeks
4. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 weeks.

D. **Pen Facilities:** Adult birds were housed indoors in pens constructed of stainless steel. Pens measured approximately 25 x 45 cm with a sloped floor (height of 18-22 cm). The average temperature in the adult study room was 21 ±2°C with an average relative humidity of 50-70%.

The photoperiod for acclimation and the preproduction period of the study was 8 hours of light per day at an intensity of 235 lux. The photoperiod was increased to 17 hours of light per day two weeks prior to the production period.

E. **Adult Observations/Gross Pathology:** The adult and juvenile birds were observed twice daily on the weekdays and once daily on the weekends and holidays throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. The pen mate of dead birds was sacrificed and necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at the beginning and end of the production phase and weekly during acclimation and the preproduction period. Food consumption per pen was determined weekly throughout the study.

F. **Eggs/Eggshell Thickness:** During the production phase, eggs were collected daily, marked (date, pen number, and treatment level), candled, and stored in a refrigerator at 15°F. Cracked eggs or soft-shelled eggs were recorded and discarded. All cracked eggs for Set 4 and 6 were measured for eggshell thickness and discarded. The remaining eggs were incubated in an incubator with a dry bulb temperature of 99-100°F and a wet bulb temperature of 85-95°F. The eggs were candled on days 11 and 18. On day 21 of incubation, live eggs were transferred to a hatcher with a dry bulb temperature of 100°F and a wet bulb temperature of 80-100°F. All unhatched eggs were euthanized on day 24 of incubation.

When possible, the 7th, 14th, and 21st egg from each pen was collected for eggshell thickness determination. These eggs were opened at the girth, the contents removed, and the shell washed thoroughly to remove the albumen and allowed to air dry at room temperature for at least 48 hours. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.0001 in.) two points around each half of the waist of the egg using a micrometer. The measurements were converted to millimeters for statistical analysis.

G. **Hatchlings:** On day 24, all normal hatchlings were transferred from the hatcher to brooder pens where they were observed for 14 days for signs of toxicity. Hatchlings were wingbanded for identification by parental pen and placed in brooding pens until 14 days of age. The body weight of individual hatchlings was determined at the time of hatch and at 14 days post-hatch. Each brooding pen measured 61 x 46 x 18 cm high with plastic-coated wire mesh floors. Cheesecloth was placed on each pen floor until the hatchlings were 5-7 days old. Each brooder was equipped with a temperature controller which maintained a temperature gradient of 33-37°C in the brooding pens during the 14-day survival period. Continuous light was provided. Relative humidity was maintained at 30-70%.

H. **Statistics:** Upon completion of the study, two-factor repeated measures analysis of variance (ANOVA) was used to assess the effects of benefin on adult body weight, hatchling body weight, and hatchling body weight gain. All other variables were analyzed using a one-way ANOVA. Proportions data (e.g., EC/EL and VE/ES) were subjected to arcsine transformation prior to analysis. "F-statistics were used to test the statistical significance of all main effect and interactions terms.

Additionally, the statistical significance of linear trends across the concentration levels of benefin were examined to determine the concentration level below which no significant trend could be detected. All references to statistical significance represent $p \leq 0.05$."

Each of the following parameters was analyzed statistically:

Adult Body Weight	Offspring Body Weight
Adult Feed Consumption	Offspring Food Consumption
Egg Production	14-Day Old Survivors
Eggs Laid per Hen	14-Day Survivors per Hen
Number of Eggs Laid	14-Day Old Survivors of Eggs Laid
Eggs Cracked of Eggs Laid	14-Day Old Survivors of of Hatchlings
Viable Embryos of Eggs Set	Egg Shell Thickness
Live 3-Week Embryos of Viable Embryos	
Hatchlings of 3-Week Embryos	

12. REPORTED RESULTS

A. Diet Analysis: The results of the diet analyses are presented in Table 2 (attached). Nominal and mean measured concentrations of freshly prepared diets were as follows:

Benefin (ppm)		
Nominal Concentration	Mean Measured Concentration	Percent of Nominal
0	<2	NA
100	96	96%
300	295	98.3%
1,000	990	99%

Analysis conducted prior to this study showed that homogeneity and stability of the test material in the diet were within acceptable limits.

Subsequent discussions refer to treatment levels using their mean measured concentrations.

B. Mortality and Behavioral Reactions: "No signs of toxicity were observed at any treatment level. Nine bobwhite died during the study. One female at the 295-ppm treatment level was sacrificed due to pen-related

injuries. None of the deaths appeared to be treatment related" (Table 3, attached).

Necropsy results of all mortalities and sacrificed birds were included in the report. "No compound-related gross or histopathologic lesions were detected."

C. **Adult Body Weight and Food Consumption:** During the preproduction period, there were significant but slight reductions in food consumption between the control and all treatment levels (Table 8, attached). A significant decrease in food consumption during the production period was noted at the two higher test concentrations (Table 9, attached).

During the preproduction phase, body weight gains among male and female birds were lower at 990 ppm when compared to the control (Tables 6 and 7, attached).

D. **Reproduction:** When compared to the control group, there was a significant reduction for the 990-ppm group in eggs laid, viable embryos of eggs set, normal hatchlings of live 3-week embryos, and 14-day-old survivors of normal hatchlings. In addition, the percentage of eggs cracked was significantly higher at the higher test level (16.2%) when compared to the control (8.5%). At 295 ppm, there was a decrease of viable embryos of eggs set (Tables 10 and 11, attached).

E. **Egg Shell Thickness:** When compared to the control group, there were no significant differences in mean egg shell thickness for both normal and cracked eggs between the control and any test concentration (Table 13, attached).

F. **Offspring Survival and Body Weight:** When compared to the control, there was a significant reduction at 295 and 990 ppm in mean number of 14-day-old survivors (Table 11, attached). Mean body weight and body weight gain on day 14 for the 990-ppm group were significantly reduced when compared to the control (Tables 16 and 17, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based upon the results of this study, and maximum use rate patterns, benefin is not expected to have any adverse effect to bobwhite."

The report stated that the study was conducted in compliance with EPA (FIFRA 40 CFR, Part 160), OECD and Japanese MAFF

GLP standards. Quality assurance audits were conducted during the study and the final report was signed by the Quality Assurance Representative and the Study Director.

14. **Reviewer's Discussion and Interpretation of the Study:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, ASTM, and SEP guidelines except for the following deviations:

A withdrawal study period (using basal diet only) was not added to the test phase.

The physical properties of the test material (i.e., powder, liquid) were not reported.

For this study, the light intensity provided to the test birds was 235 lux (22 footcandles). The light intensity recommended by the guidelines is 65 lux (6 footcandles).

During the preproduction phase of this study, the period of light provided to adult birds was 8 hours. The SEP recommends 7 hours of light.

For this study, the brooder temperature gradient was 33-37°C. ASTM guidelines recommend a temperature gradient from the heat source down to about 21°C in order to allow the birds to seek a proper temperature.

The report did not indicate whether the bobwhite quail used in this study were phenotypically indistinguishable from wild bobwhite quail as recommended.

Behavioral observations of offspring were not reported.

Observations on food palatability were not reported.

B. **Statistical Analysis:** Statistical analyses of study parameters were performed by the reviewer using analysis of variance (ANOVA) following square-root transformation of the count data and arcsine square-root transformation of the ratio data. The comparison between control data and data from each treatment level was made using multiple comparison tests. The computer program used is based on the EEB Bigbird program, with an exception that the count data were square-root transformed before the ANOVA. The significance level was $p \leq 0.05$.

The reviewer's analyses showed a significant reduction in the number of eggs set, viable embryos, live 3-week embryos, hatchlings, and two-week survivors at 990 ppm when compared to the control. In addition, the number of viable embryos, live 3-week embryos, hatchlings, and two-week survivors was significantly reduced at 295 ppm when compared to the control. Analyses of other reproductive parameters matched those reported by the author, with exceptions (Table A, attached).

C. **Discussion/Results:** The percentages of cracked eggs in the control group (8.7%) and in all treatment groups (7.5-15.6%) are unusually high (Table 10, attached). Typically, 0.5% to 2.0% may be expected for the bobwhite quail (Technical Support Document to Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms). The authors provided no explanation for these high values. The high percentage of eggs cracked in the control prevents adequate analysis of this parameter.

This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study. The mean measured dietary concentrations of benefin (96 ppm, 295 ppm, and 990 ppm) had no effects upon mortality or behavior in bobwhite quail during the 23-week exposure period. However, the reviewer's analysis of two-week survivors/eggs set and 14-day survivor weight showed a significant reduction at all test levels. The reviewer's calculation of percentages reveal a treatment-related trend at all test levels (Table 10, attached). Although some differences from the control were not significantly different, the ratios of hatchlings/eggs set, two-week survivors/hatchlings, two-week survivors/eggs set, and viable embryos/eggs set, indicate a treatment-related effect (Table 10, attached). Therefore, an NOEC can not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** 1) An NOEC could not be determined. 2) A high percentage of eggs cracked in the control prevent adequate analysis of this parameter.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes; April 17, 1992.