9-1-94

MRID No. 421449-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorpyrifos.
Shaughnessey Number: ~~038011~~. 059101

2. **TEST MATERIAL:** Chlorpyrifos technical (Pyrinex); Batch No. 489205; 96.8% purity; an off-white semi-solid.

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

4. **CITATION:** Hakin, B. 1990. The Effect of Dietary Inclusion of Chlorpyrifos on Reproduction in the Bobwhite Quail. Performed by Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, UK. HRC Report No. MBS 27/881666. Submitted by Makhteshim-Agan (America), Inc. EPA MRID No. 421449-02.

5. **REVIEWED BY:**

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Date: 6/4/92

6. **APPROVED BY:**

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7. **CONCLUSIONS:** Nominal dietary concentrations of chlorpyrifos technical at 10 and 40 ppm had no effects upon behavior, food consumption, or reproduction in adult bobwhite quail during the 24-week exposure period. At 130 ppm, adult food consumption and egg production were significantly reduced. The NOEC was 40 ppm. This study is scientifically sound, but does not fulfill the guideline requirements for an avian reproduction study, due to high percentages of cracked eggs in all groups.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bobwhite quail (*Colinus virginianus*) were purchased from a supplier in Cambridgeshire, England. The pre-treatment period was 14 days. The birds were approximately 6 months of age at the start of the pre-treatment period, and were identified by individual wing tags. Individual bodyweights ranged from 155 to 245 g at the start of the pre-treatment period.

B. **Dose/Diet Preparation/Food Consumption:** Chlorpyrifos was dissolved in acetone. A premix (1500 ppm) was prepared using the stock solution. Acetone was evaporated using a rotary evaporator at 40°C. The premix was mixed thoroughly and portions removed to prepare the test diets. The diets were mixed in a double-cone blender for a minimum of 7 minutes. The control diet was prepared using an amount of acetone equal to that used for the highest treatment concentration. The control diet and three test concentrations (10, 40, and 130 ppm) were prepared weekly. After preparation, the diets were stored in closed paper sacks at room temperature until fed to the birds. Each of the four groups of adult birds was fed the appropriate diet for 24 weeks.

Basal diet for adult birds was quail layer diet manufactured by Special Diets Services, Witham, Essex. The composition of the diet was presented in the report. Food and water were supplied *ad libitum* during acclimation and during the test. Homogeneity and stability samples were taken from a trial mix of treatment diets (10 ppm and 130 ppm). Stability of the test chemical was determined in the trial mix by analyzing subsamples stored for 0, 4, 9, and 14 days at room temperature in the animal room. Samples were taken from the test diets during weeks 1, 12, and 22 for confirmation of dietary concentrations of chlorpyrifos. These samples were stored at -20°C until analyzed. Analyses were performed by Huntingdon Research Centre (HRC) Department of Analytical Chemistry. Group food consumption was determined weekly throughout the study.

C. **Design:** The birds were distributed into four groups using a randomized block design as follows:

Chlorpyrifos Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	20	1	1
10 ppm	20	1	1
40 ppm	20	1	1
130 ppm	20	1	1

In addition, 4 male and 4 female birds per group were maintained as replacements if needed prior to egg production.

D. **Pen Facilities:** Adult birds were housed indoors in pens constructed of polythene-coated steel wire. Pens measured approximately 30 cm x 40 cm x 25 cm and had sloping floors with 10-cm egg-catchers. The mean daily maximum and minimum temperatures in the adult study rooms were 23°C and 21°C, respectively. The mean relative humidity was 59%.

The photoperiod during acclimation and during the first 6 weeks of the study was 7 hours of light per day. At the beginning of week 7, the lighting was increased to 16 hours per day, and was maintained at that level throughout the remainder of the study. Light intensity ranged from 200 to 610 lux.

E. **Adult Observations/Gross Pathology:** Observations were made daily throughout the study for signs of toxicity or abnormal behavior. Gross pathological examinations were conducted on all birds that died during the study, as well as on all birds that survived until study termination. Adult birds were individually weighed on the following days: -14, 0, 14, 28, 42, 56, 70, and 168.

F. **Eggs/Eggshell Thickness:** Eggs were collected daily during the 12-week production period, and stored at 16°C. Following each 7-day collection period, the eggs were weighed, candled, and any cracked eggs were recorded and discarded. All remaining eggs (except those used for eggshell thickness measurements) were incubated at 37.7°C and 55% relative humidity. Eggs were turned automatically every hour while in the incubator. Eggs were candled on day 11 to determine early embryonic death and on day 18 to determine late

embryonic death. The eggs were placed in a hatcher at 37.5°C on incubation day 21. Eggs which did not hatch within 24 hours were classified as "dead in shell".

The first egg laid on the first day of weeks 14, 16, 18, 20, 22, and 24, in each pen was removed for shell thickness measurements. The thickness of the shells was measured at 4 points around the circumference using a micrometer calibrated to 0.01 mm.

G. **Hatchlings:** Upon removal from the hatcher, chicks were individually weighed and identified by leg bands. The hatchlings were housed in wooden pens with concrete floors. Each pen contained two drinking fountains and two food hoppers. Wood shavings were used as bedding. The mean daily minimum and maximum temperatures were 24°C and 28°C, respectively. Heat was supplied using infra-red lamps in each pen. The mean relative humidity was 58%. Hatchlings were fed untreated diet (HRC chick meal), and were observed daily. Food and water were available *ad libitum*. At 14 days of age, individual body weights were measured. Gross pathological examinations were conducted on chicks that died during the 14-day observation period.

H. **Statistics:** Analysis of variance (ANOVA) was used to evaluate adult food consumption, adult body weight, number of eggs laid, mean egg weight, % eggs damaged, egg shell thickness, infertile eggs/eggs set, early embryonic deaths/fertile eggs, late embryonic deaths/day 11 fertile eggs, eggs hatched/day 18 viable eggs, eggs hatched/fertile eggs, 14-day survivors/eggs hatched, and offspring body weight at hatching and 14 days later. Proportional data were angular transformed prior to ANOVA. Williams' test was used to compare individual treatment groups with the control.

12. **REPORTED RESULTS:**

A. **Diet Analysis:** All mean measured concentrations of chlorpyrifos taken from dietary samples were within 7% of nominal values (Addendum 1, Table 2, attached). Analyses of samples taken from the trial mix showed that chlorpyrifos was homogeneously blended and was stable throughout the 14-day storage period (Addendum 1, Tables 3 & 4, attached).

B. **Adult Mortality and Behavioral Reactions:** Prior to week 13, three birds (two at 10 ppm, one at 130 ppm) were sacrificed due to injury or poor condition. These

birds were replaced with spare birds fed the same diet. From week 13 until the end of the test, two mortalities occurred at 10 ppm and one at 40 ppm. These birds were not replaced since this was during the egg production phase of the test.

The results of gross pathological examinations conducted on birds that died or were sacrificed during the study were included in the report (Section 3.4., attached). The observations were not considered to be treatment related.

"In general, bird health was good throughout the study...The majority of the observations made were of physical injuries and were not considered to be related to treatment." Individual bird observations are given in Appendix 4 (attached).

C. **Adult Body Weight and Food Consumption:** Mean bodyweights were similar for all groups and there was no evidence of any difference between treatments (Table 3, attached). Analysis of food consumption over the entire test period indicated that values in the 130-ppm group were significantly lower than in the control group. When the pre-egg laying and egg laying periods were analyzed separately, no significant differences in consumption were observed.

D. **Reproduction:** When compared to the control group, significantly fewer eggs were laid at 130 ppm (Table 8, attached). There were no significant differences from the control for the following parameters at any concentration tested: egg weights, cracked or broken eggs, infertile eggs/eggs set, early embryonic deaths/fertile eggs, late embryonic deaths/fertile eggs, eggs hatched/fertile eggs, and 14-day survivors/eggs set (Tables 8, 9, 12, 13, & 15, attached).

E. **Egg Shell Thickness:** The presence of the test material in the feed had no significant effect on egg shell thickness (Table 10, attached).

F. **Offspring Body Weight:** Chick bodyweights in the treatment groups at hatch were not significantly different from the control (Table 14, attached). However, after 14 days of age, body weights in all three treatment groups were significantly higher than control weights. Post-mortem examinations of chicks that died during the 14-day observation period revealed

abnormalities in four chicks. At 10 ppm, one chick was unable to walk properly. At 40 ppm, one chick had three extra toes on each leg and another had curled toes. At 130 ppm, a chick was unable to straighten its leg.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Following treatment of adult Bobwhite quail with Chlorpyrifos in diet at 130 ppm, food consumption was reduced and egg production was reduced by 27% relative to control values ($P<0.01$). Chicks in all three treated groups (Chlorpyrifos at 10, 40, and 130 ppm) were between 5% and 10% heavier at Day 14 than those in the control group ($P<0.05$). There were no other treatment-related effects either on adult birds or on any reproductive parameters. The only treatment-related effect observed at 40 ppm was an increase in chick 14-day bodyweights; otherwise, no effects were observed at this level."

The report stated that study was conducted in conformance with USEPA Good Laboratory Practice regulations (40 CFR Part 160). The GLP statement was signed by the Study Director. Quality assurance audits were conducted during the study and the final report was signed by the Systems Compliance Auditor of Huntingdon Research Centre Ltd.

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 deviation:

A recovery period (exposure to basal diet only) was not added at the end of the treatment phase of the study.

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

Analyses of all parameters supported the results reported by the authors, with the exception that body

weights of 14-day old chicks at 130 ppm were not significantly different from control values (P=0.085), while the author reported otherwise. Egg weights were not subjected to statistical analysis by the reviewer.

C. **Discussion/Results:** Chemical analyses of food samples taken during weeks 1, 12, and 22 show that measured concentrations of chlorpyrifos were very similar to nominal concentrations; all measured values were within 7% of nominal values. Homogeneity and stability was measured on a trial mix of treatment diets. Therefore, homogeneity and stability of the actual treatment diets were not measured. However, judging from the data using the trial mix, chlorpyrifos was very stable in the diet, and the method of preparation achieved a homogeneous mix.

The percentages of cracked eggs in the control group (19%) and in all treatment groups were unusually high (Table 8, 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. Statistical analysis of this parameter showed no significant differences between groups. However, the high values in the control group are unusual, and may have confounded the analysis (all treatment groups had high values for this parameter). In view of this, conclusions regarding cracked eggs are not clear. The registrant should provide an explanation for the high percentages of cracked eggs. Additionally, the registrant should investigate the circumstances surrounding the collection, handling, and storage of eggs, so that procedures can be implemented to reduce the incidence of cracked eggs.

The number of eggs set for incubation at 130 ppm was lower than the control (P=0.053). This was due to reduced egg production, rather than being directly related to treatment. Since egg production was significantly reduced at 130 ppm, a reduction in the number of eggs set is expected.

The weights of chicks at hatch in all three treatment groups were slightly greater than control values (Table 14, attached), though the differences were not significant. The weights 14-day old chicks at 10 and 40 ppm were significantly greater than control values. The weights of 14-day old chicks at 130 ppm were also

greater than control values, and approached the level of significance (P=0.085). The proportion of hatchlings that survived to 14 days of age in all treatment groups was slightly higher than in the control group (Table 15, attached). These differences were probably not treatment-related. Instead, the control birds apparently exhibited a slightly lower fitness than treatment birds, with fewer birds surviving to 14 days of age, and slightly lower body weights than treatment birds.

The author's conclusion of reduced adult food consumption and egg production in the 130-ppm group is accepted. The NOEC, therefore, was 40 ppm.

Because of the high percentages of cracked eggs in all groups, the study (although scientifically sound), does not fulfill the requirements for an avian reproduction study.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** A high percentage of cracked eggs in the control group precludes a meaningful analysis of this parameter.

(3) **Repairability:** The study can be upgraded to "Core" if the registrant can successfully show that the analysis of cracked eggs was not confounded by a high percentage of cracked eggs in the control group.

15. **COMPLETION OF ONE-LINER:** Yes; 5-13-92.