9-1-94

MRID No. 421449-01

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Chlorpyrifos. Shaughnessey No. 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: Mallard duck (*Anas platyrhynchos*).

4. **CITATION:** Hakin, B. 1990. The Effect of Dietary Inclusion of Chlorpyrifos on Reproduction in the Mallard Duck. Performed by Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, UK. Laboratory Project ID. MBS 28/881667. Submitted by Makhteshim-Agan (America) Inc. EPA MRID No. 421449-01.

5. **REVIEWED BY:**

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**Signature:** Michael L. Whitten
**Date:** 6/4/92

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6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
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7. **CONCLUSIONS:** Nominal dietary concentrations of chlorpyrifos at 10 ppm and 30 ppm had no effects upon behavior, food consumption, or reproduction in adult mallards during the 22-week exposure period. The diet in the highest treatment group (90 ppm) was changed to 60 ppm at the beginning of week 8 due to bodyweight loss and mortality at 90 ppm. After the diet change, bodyweight and mortality in this group appeared more normal. At the end of the study, the following parameters in this group were significantly lower than control values: male bodyweight, female bodyweight, and egg production. The NOEC was 30 ppm. This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Mallard ducks (*Anas platyrhynchos*) were purchased from the County Game Farms, Ashford, Kent. The birds were acclimated to the facilities for 14 days prior to initiation of the test. The birds were approximately 34 weeks of age at test initiation, and were identified by individual wing tags. At the beginning of acclimation, body weights ranged from 805 to 1255 g.

B. **Dose/Diet Preparation/Food Consumption:** A premix was created by dissolving the test substance in acetone and mixing. The acetone in the premix was evaporated by placing the premix in a rotary evaporator. The test diets were prepared by mixing aliquots of premix with basal diet. The control diet consisted of basal feed with an amount of acetone equal to that in the highest treatment group. After preparation, the diets were stored in paper bags at room temperature until fed to the birds.

The dietary concentrations were determined after a rangefinding test. The control diet and three test concentrations were prepared weekly. Initial treatment concentrations were 10, 30, and 90 ppm. The 90 ppm diet was changed to 60 ppm at the beginning of week 8 due to bodyweight loss and mortality at 90 ppm. In this report, this group is referred to as the 90/60-ppm group. Each of the four groups of adult birds was fed the appropriate diet for 22 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. No antibiotics or growth promoters were included in the diet. 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 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. 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) | 6 | 2 | 5 |
| 10 ppm | 6 | 2 | 5 |
| 30 ppm | 6 | 2 | 5 |
| 90/60 ppm | 6 | 2 | 5 |

In addition, 6 birds per group were maintained as replacements if needed prior to egg production.

D. **Pen Facilities:** Adult birds were housed indoors and randomly assigned to pens constructed of galvanized steel. Pens measured 1.4 x 0.7 m. The pens had solid sides and wire mesh floors. During egg production, the floors were covered with plastic "pillow" matting to minimize egg damage. The mean daily maximum and minimum temperatures in the adult study rooms were 20 and 17°C, respectively. The mean relative humidity ranged from 71% to 73%.

The photoperiod during acclimation and during the first 8 weeks of the study was 7 hours of light per day. At the end of week 8, the lighting was increased to 16 hours per day, and was maintained at that level throughout the remainder of the study.

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 and on all birds sacrificed at the termination of the study. Adult birds were individually weighed on the following days: -14, 0, 14, 28, 42, 56, 70, and 154.

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 and candled. Cracked eggs were recorded and discarded. All normal eggs (except those used for

eggshell thickness measurements) were placed in an incubator set to operate at 37.7°C and 55% relative humidity. Eggs were turned automatically every hour while in the incubator. Eggs were candled on day 14 to determine fertility and embryo viability and on day 21 to determine embryo survival. The eggs were placed in a hatcher at 37.5°C on incubation day 23. Eggs that did not hatch within 3 days were classified as "dead in shell" and the eggs were opened and ducklings examined for abnormalities.

All eggs collected the first day of even-numbered weeks were used for egg shell thickness measurements. The contents of each egg were washed out with tap water, and the shells were allowed to air dry for at least 48 hours at room temperature. 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, ducklings were individually weighed and identified by leg bands. The hatchlings were housed in pens measuring 1.5 m x 1.2 m which were constructed in the same manner as the adult pens. The mean daily maximum and minimum temperatures were 28°C and 26°C, respectively. The mean relative humidity was 64%. Hatchlings were fed untreated diet (HRC chick meal), and were observed daily. Food and water were available *ad libitum*. Individual body weights were measured within 24 hours of hatching and at 14 days after hatching. Gross pathological examinations were conducted on ducklings that died during the 14-day observation period.

H. **Statistics:** Analysis of variance 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/fertile eggs, eggs hatched/day 21 viable eggs, eggs hatched/fertile eggs, 14-day survivors/eggs hatched, and offspring body weight at hatching and 14 days later. Percentage data were subjected to angular transformation before analysis. Williams' test was used to compare individual treatment groups with the control.

12. **REPORTED RESULTS:**

A. **Diet Analysis:** All measured concentrations of chlorpyrifos taken from dietary samples were within 8%

of nominal values (Addendum 1, Table 1, 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:** One bird died during the acclimation period and was replaced. Adult mortality during the pre-egg laying period (weeks 1-10) was as follows: 1 control bird, 1 at 10 ppm, 2 at 30 ppm, and 11 at 90/60 ppm. Adult mortality during the egg laying (weeks 11-22) period was as follows: 1 control bird, 1 at 10 ppm, 5 at 30 ppm, and 4 at 90/60 ppm. Eight of the birds were replaced during the pre-egg laying period and no replacements were made during egg production. There was evidence of a treatment-related increase in mortality during the pre-egg production period for birds in the highest test group (90 ppm). Therefore, the dosage was reduced to 60 ppm at the end of week seven.

Most behavioral observations were of physical injuries resulting from "bullying" (pecking) by other birds (Appendix 4, attached).

Most abnormalities noted during gross pathological examinations were due to pecking (page 29, attached). Signs of emaciation were also evident.

C. **Adult Body Weight and Food Consumption:** There was a treatment-related reduction in body weight between days 0 and 42 in the highest treatment group (90 ppm). The dose was reduced to 60 ppm at the end of day 49. By day 56, the birds in this group showed an increase in body weight gain. After 56 days, no treatment related bodyweight changes were observed (Table 3, attached). When compared to the control group, there was a significant reduction in body weight in the highest treatment group during the pre-egg production period (days 1-70).

When compared to the control group, food consumption in the 30- and 90/60-ppm groups was significantly greater during the egg production period (Tables 4 & 5, attached). During week 1, food consumption in the 90-ppm group was depressed.

D. **Reproduction:** When compared to the control group, there was a significant reduction in the number of eggs

laid in the 90/60-ppm treatment group (Tables 6 & 7, attached).

When compared to the control group, there were no significant differences in cracked or broken eggs, egg weights, egg shell thickness, infertile eggs, or early or late embryonic deaths at any concentration (Tables 8-12, attached).

When compared to the control group, there was a significantly higher proportion of fertile eggs that hatched in the 90/60-ppm group (Table 13, attached).

When compared to the control group, there were no significant differences in the ratio of 14-day survivors/eggs hatched (Table 15, attached).

E. Offspring Body Weight: There were no significant differences in offspring bodyweight among groups for weight at hatch or at 14 days of age (Table 14, attached). No abnormalities were detected in post-mortem examinations of ducklings that died during the 14-day observation period.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "A treatment-related effect on adult bird mortalities and bodyweight occurred at a dose level of chlorpyrifos of 90 ppm and the dose level was reduced to 60 ppm at the end of week 7 of feeding. A reduction in egg production occurred at this dose level together with an increase in the proportion of fertile eggs that hatched. No other reproductive parameters were affected.

"At 30 and 90/60 ppm, birds ate more food than the control group during the egg production period.

"No treatment-related effects on reproductive parameters were observed at 10 or 30 ppm."

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 deviations:

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

The mean daily temperature in the adult study rooms ranged from 17°C to 20°C; the recommended temperature is 21°C.

The mean daily relative humidity ranged from 71% to 73%; the recommended value is 55%.

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 reproductive parameters supported the results reported by the authors. The reviewer's analyses showed significant differences from the control for some parameters that were not analyzed by the author. For example, at 90/60 ppm, the following parameters were significantly greater than control values: viable embryos/eggs set, live 21-day embryos/viable embryos, hatchlings/eggs set, and 14-day survivors/eggs set. These differences are not believed to be treatment-related.

The number of eggs set for incubation at 90/60 ppm was significantly lower than the control. This is attributed to reduced egg production, rather than being directly related to treatment. Since egg production was significantly reduced at 90/60 ppm, a reduction in the number of eggs set is expected.

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 8% 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 (20%) and in all treatment groups were unusually high (Table 8, attached). Typically, up to 6% may be expected for the mallard (Technical Support Document to Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms). The author 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 90 ppm diet was changed to 60 ppm at the beginning of week 8 due to bodyweight loss and mortality at 90 ppm. At the end of the study, the following parameters in this group were significantly lower than control values: male bodyweight, female bodyweight, and egg production. It is impossible to determine whether these parameters would have been affected if the group had been exposed only to a diet of 60 ppm. Therefore, the only valid concentrations tested were 10 ppm and 30 ppm. Since the expected field residue concentration is higher than 30 ppm, the study does not meet the guideline requirements regarding the highest concentration to be tested.

When compared to control values, food consumption in the 30- and 90/60-ppm groups was significantly increased during the study. However, the food consumption in the highest treatment group was lower than control values during weeks 1-5, and was especially depressed during week 1 (Tables 4 & 5, attached). The actual effect of the test material on food consumption could not be determined.

The NOEC was 30 ppm. The study is scientifically sound but does not fulfill the guideline 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. Additionally, the expected field residue concentration is higher than the highest valid concentration tested.

(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, and if the registrant can show that the EEC is lower than 30 ppm.

15. **COMPLETION OF ONE-LINER:** Yes; May 22, 1992.