UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

4-7-94

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of Mallard Duck Reproduction Study (Guideline 71-4) Submitted by Cheminova Agro A/S in Support of Malathion Reregistration

From: for Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Douglas D. Urban 4/7/94

To: Peg Perreault, Chemical Review Manager, Team 73
Reregistration Division (7508W)

We reviewed the mallard reproduction study (MRID 42782101) submitted by Jellinek, Schwartz and Connolly, Inc., on behalf of Cheminova Agro A/S, in support of reregistration of malathion. A copy of the Data Evaluation Record for this study is enclosed.

The study was judged to be scientifically sound, and fulfills the guideline requirement for an avian reproduction study in a waterfowl species. A NOEC of 1200 ppm was established based on no observed effects upon behavior, food consumption, weight or reproduction at this dose level. At the highest dose level, 2400 ppm, treatment effects were observed in male body weight, eggshell thickness and embryo viability.

If you have any questions concerning this DER, please contact Joanne Edwards. She may be reached at (703) 305-6736.

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3/22/1994

MRID No. 427821-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Malathion (AC 6,601 technical). Shaughnessey No. 057701.

2. **TEST MATERIAL:** AC 6,601 technical; Lot No. AC 6015-136B; CAS No. 000121-75-5; 0, 0-dimethyl phosphorodithioate of diethyl mercaptosuccinate; $C_{10}H_{19}O_6PS_2$; 94.0% active ingredient; a clear, brown to colorless liquid with a characteristic mercaptan odor.

3. **STUDY TYPE:** 71-4. Avian Reproduction Study. Species Tested: Mallard duck (*Anas platyrhynchos*).

4. **CITATION:** Pedersen, C.A. and D.W. Fletcher. 1993. AC 6, 601 Technical: Toxicity and Reproduction Study in Mallard Ducks. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Laboratory Project ID No. BLAL No. 90 DR 39. Submitted by Cheminova Agro A/S, Lemvig, Denmark. EPA MRID No. 427821-01.

5. **REVIEWED BY:**

Charles G. Nace Jr., M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten for C.G. Nace
Date: 3-22-94

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 3-22-94

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study. There were no treatment-related effects observed in mallard ducks that were fed AC 6,601 for 20 weeks at a nominal concentrations of 240 and 1200 ppm a.i. At 2400 ppm a.i. treatment-effects were observed in male body weight, eggshell thickness, and embryo viability. The no-observed-effect concentration (NOEC) was 1200 ppm a.i (nominal concentration).

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in this study were mallard ducks (*Anas platyrhynchos*) purchased from a commercial supplier in Hanover, IL. The birds were 23 weeks of age at study initiation and were acclimated to the laboratory environment for 32 days. All birds were phenotypically indistinguishable from wild birds. At test initiation, all birds were examined and their suitability for testing (based on general physical condition) was determined.

B. **Dose/Diet Preparation/Food Consumption:** Diets were prepared by mixing a standard premix with stock diet. The standard premix was a mixture of the appropriate amount of test substance, acetone, and stock diet. Diets were prepared fresh weekly. The control diet consisted of stock diet and acetone in an amount equivalent to the test diets. Dietary concentrations were adjusted to reflect the purity of the test substance and are reported as parts per million (ppm) of active ingredient (a.i.). Each of the three treatment groups and the control group were fed the appropriate diet for 20 weeks.

Adult birds were fed Purina® Duck Grower® W/O for the first eight weeks. Basal diet for adult birds throughout the rest of the study was Purina® Game Bird Breeder Layena®. The compositions of these diets were presented in the report. The test substance was not mixed into the diet of the offspring. Food and water were supplied *ad libitum* throughout the study.

Homogeneity and stability of the test substance in avian feed was measured in diets used during a 28-day pilot study. Verification samples were collected from the control and treated diets during test weeks 1, 6, 12, and 18. All samples were immediately frozen after collection. Samples were shipped under dry ice to American Cyanamid for analysis using GLC/FPD method M-776.

C. **Design:** The birds were randomly distributed into four groups as follows:

AC 6,601 Technical Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm a.i.)	16	1	1
240 ppm a.i.	16	1	1
1200 ppm a.i.	16	1	1
2400 ppm a.i.	16	1	1

Treatment levels were based upon results of a 28-day dietary pilot study. Adult birds were identified by individual leg bands.

D. **Pen Facilities:** Adult birds were housed in wire mesh pens which measured 2 x 4 x 2 ft. The average daily temperature in the adult study room was 16°C with an average relative humidity of 88%.

The photoperiod during the first 8 weeks of the study was 7 hours of light per day. At the start of week 9, the photoperiod was increased to 17 hours of light per day and maintained at that level for the duration of the study. The light intensity was at least 6 footcandles at bird level.

E. **Adult Observations/Gross Pathology:** Adult birds were observed daily throughout the study for signs of toxicity. Mortalities occurring prior to terminal adult sacrifice were recorded and necropsied. Necropsies were also conducted on half of all surviving adult birds in each group at study termination. Adult body weights were measured at study initiation, biweekly through week 8, and at study termination. Feed consumption was calculated on a biweekly basis.

F. **Eggs/Eggshell Thickness:** Eggs were collected daily during the production period and were labeled according to pen of origin. Normal eggs were stored at an average temperature of 15°C with an average relative humidity of 65%. The eggs were turned once daily during each seven-day collection period. Eggs were removed from the egg cooler weekly and eggs not cracked or used for eggshell thickness measurements were placed in an incubator maintained at an average temperature of 37.7°C with an average relative humidity of 61%. All eggs were turned automatically every four hours while in the incubator. Eggs were candled on day 14 of incubation to determine fertility and again on day 21

to determine embryo survival. On incubation day 23, the eggs were placed in hatching trays.

Eggs collected on the first day of alternate weeks of the test period were used to determine eggshell thickness. The eggs were broken (if not already so), the contents removed, and the shells thoroughly washed. The shells were allowed to air dry for at least 48 hours at room temperature prior to measurement. Measurements were recorded to the nearest 0.01 mm. The eggshells and contents were frozen and retained for analysis, if requested by the sponsor.

G. **Hatchlings:** Hatchlings were housed according group and pen number in cages measuring 61 x 61 x 46 cm. All hatchlings were observed daily and received untreated diet during the 14-day observation period. The average minimum and maximum temperatures in the hatchling study room ranged from 22-27°C and relative humidity ranged from 43-87%. Hatchling body weights were measured and recorded at hatch and on day 14. Gross pathological examinations were performed on hatchlings that died and on selected hatchlings at termination of the growth period.

H. **Statistics:** Analysis of variance (ANOVA) was used to statistically analyze the following parameters:

Adult Body Weight	Hatchling Body Weight
Adult Feed Consumption	Eggshell Thickness

Contingency Table Analysis was used to statistically analyze the following parameters:

Eggs Set of Eggs Laid	Eggs Laid Per Hen
One Week Eggs of Fertile Eggs Set	Midterm Eggs of Fertile Eggs Set
Full Term Eggs of Fertile Eggs Set	Cracked Eggs of Eggs Laid
Infertile Eggs of Eggs Set	Live 3-Week Embryos of Fertile Eggs Set
Hatchlings of Fertile Eggs Set	14-Day Old Survivors of Hatchlings
Normal Eggs of Eggs Laid	Fertile Eggs of Eggs Set
Defective Eggs of Eggs Laid	

12. **REPORTED RESULTS:**

A. **Diet Analysis:** The diets used in the pilot study were homogeneously mixed and were stable in the diet for up

to seven days (Appendix 6, Pages 197 and 198, attached). The percent recoveries for the diets prepared during test weeks 1, 6, 12, and 18 of the definitive study averaged 106.1, 106.9, and 107.0% for the 240, 1200, and 2400 ppm a.i. test diets, respectively (Appendix 6, Page 217, attached).

B. **Mortality and Behavioral Reactions:** There were two male deaths (one during week 7 and one during week 19) at 2400 ppm a.i. Because of the differences noted in bird behavior prior to death, the time interval between the deaths, gross necropsy findings, and the number of mortalities, these mortalities are attributed to factors other than the test material. No other mortalities occurred during the study. There were no treatment-related behavioral signs observed at any concentration throughout the study. Gross pathological examinations of the two birds that died during the study and of one-half of the surviving adults in each group showed no treatment-related findings.

C. **Adult Body Weight and Food Consumption:** A significant difference (lower value) in body weights was noted only among the males at 2400 ppm a.i. at study termination. No significant differences were noted among the females at this level. Although not statistically significant except at termination, the males at 2400 ppm a.i. displayed weight loss at each successive interval throughout the study. This finding was considered to be treatment-related. No significant differences were found in the other test groups (Table 1, attached). There were no significant differences in feed consumption at any concentration tested when compared with the control (Table 2, attached).

D. **Reproduction:** There were significant differences (higher values) noted in the numbers of infertile eggs at 2400 ppm a.i. This was considered to be a treatment-related finding. A significant difference (lower value) was noted in the number of infertile eggs at 1200 ppm a.i. This was considered to be an incidental finding.

Significant differences (lower values) were noted in the numbers of one week eggs at 1200 and 2400 ppm a.i and in the number of full term eggs at 2400 ppm a.i. These were considered to be incidental findings.

Although not statistically significant, a slight reduction in the number of viable embryos was noted at 2400 ppm a.i.

There were no other significant findings in any other reproductive parameter (Tables 5A and 5B, attached).

E. **Eggshell Thickness:** When eggshell thicknesses were statistically analyzed from all of the intervals, a significant difference (lower value) was noted at 2400 ppm a.i. When values representing the average eggshell thickness per pen were statistically analyzed, a significant difference (lower value) was noted at 2400 ppm a.i. These were considered to be treatment-related findings. The slightly thinner eggshells noted at 2400 ppm a.i. may be correlated with the slightly higher percentage of cracked or broken eggs in this test group when compared to the control group (Tables 7A and 7B, attached).

F. **Offspring:** There were significant higher and lower differences in offspring bodyweight in various hatches (Table 8, attached) These differences were not consistent or dose correlated and were not considered to be treatment-related. There were no significant differences in hatchling survival when compared with the control (Table 9, attached). No treatment-related abnormal behavior or signs of toxicity were observed. Gross pathological examinations of selected ducklings on day 14 revealed no treatment-related findings.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "The ingestion of AC 6, 601 Technical at 2,400 ppm a.i. by the parental generation appeared to produce signs of toxicity (lower male body weights at termination, eggshell thinning, and greater numbers of infertile eggs). Based on parameters investigated (body weights, feed consumption, egg production, hatchability, viability, etc.), the no-observed-effect level was determined to be 1,200 ppm a.i."

The report stated that the study was conducted in conformance with Good Laboratory Practice (GLP) regulations (40 CFR Part 160), except the feed component analysis by Ralston Purina and the quarterly water contaminant analysis by Hazleton Laboratories were not performed under GLP regulations. These did not have an effect on the conduct or the interpretation of the results of the study. Quality assurance audits were conducted during the study and the final report was signed by a Quality Assurance Officer for Bio-Life Associates, Ltd. An additional statement of

conformance with GLP (40 CFR part 160) guidelines was included in the analytical report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, ASTM, and SEP guidelines except for the following deviation:

Average daily temperature and relative humidity in the adult mallard duck study room were 16°C and 88%, respectively; 21°C and 55% are recommended.

B. **Statistical Analysis:** Statistical analyses of reproductive parameters were performed by the reviewer using analysis of variance (ANOVA) following arcsine square-root transformation of the ratio data. The comparisons between control data and data from each treatment level were made using Dunnett's procedure and Bonferroni's procedure. The computer program is based on the EEB Birdall program. The significance level was $p \leq 0.05$.

The results of the statistical analyses were similar to those reported by the authors (see attached printouts) with the following exception: The number of hatchlings per live 21-day embryos at 2400 ppm a.i. was significantly higher than in the controls. This is not considered to be treatment-related.

C. **Discussion/Results:** This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study. There were no treatment-related effects observed in mallard ducks that were fed AC 6,601 for 20 weeks at nominal concentrations of 240 and 1200 ppm a.i. At 2400 ppm a.i. treatment-related reductions were observed in male body weight, eggshell thickness, and embryo viability. The NOEC was 1200 ppm a.i.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

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

15. **COMPLETION OF ONE-LINER:** Yes; 02/25/94.