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081901
SHAUGHNESSEY NO

REVIEW NO.

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TYPE PRODUCT(S) Fungicide

DATA ACCESSION NO(S) 407294-01 thru 04

PRODUCT MANAGER, NO. L. Rossi (21)

PRODUCT NAME(S) Chlorothalonil

COMPANY NAME Fermenta Plant Protection

SUBMISSION PURPOSE Submission of avian reproduction studies
with chlorothalonil metabolite, SDS-3701
in response to registration standard

SHAUGHNESSEY NO. CHEMICAL % A.I.

081901 Chlorothalonil



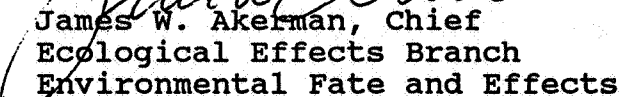
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

August 30, 1989

SUBJECT: Submission of Avian Reproduction Studies with DS-3701
Reg No: 50534-7

FROM:  James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division H7507C

TO: Lois Rossi PM 21
Insecticide/Rodenticide Branch
Registration Division H7505C

The registrant has submitted two avian reproduction studies for review. They are presented below:

1. STUDY TYPE: Avian Reproduction Study.
Species Tested: Mallard duck
(Anas platyrhynchos)
Test Material: DS-3701 (deg. of chloro.)

CITATION: Shults, S.K., N. Wilson, J. Killeen. 1988. Reproduction Study in Mallard Ducks with 4-hydroxy-2,5,6-trichloroisophthalonitrile. Laboratory Project No. 86-0064. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-106. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 407294-02.

CONCLUSIONS: This study is scientifically sound and meets the requirements for an avian reproductive test. 4-hydroxy-2,5,6-trichloroisophthalonitrile, at a nominal dietary concentration of 250 ppm, had a marked effect upon both the health of adult mallards (body weight, food consumption, and gonad development) and their reproductive performance (number

of eggs laid, embryonic development, egg shell thickness, hatchability, and hatchling survival). There was a slight reduction in egg shell thickness at 100 ppm. Nominal dietary concentrations of 10 ppm and 50 ppm did not appear to affect adult mallards or any reproductive parameter. The no-observed-effect concentration was 50 ppm.

2. STUDY TYPE: Avian Reproduction Study.
Species Tested: Bobwhite quail (Colinus virginianus).
Test Material: DS-3701 (deg. of chlorothalonil)

CITATION: Shults, S.K., N. Wilson, J. Killeen. 1988. Reproduction Study in Bobwhite Quail with 4-hydroxy-2,5,6-trichloroisophthalonitrile. Laboratory Project No. 86-0066. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-105. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 407294-04.

CONCLUSIONS: This study is scientifically sound and meets the requirements for an avian reproductive test. 4-hydroxy-2,5,6-trichloroisophthalonitrile, at nominal dietary concentrations of 10, 50, 100 and 250 ppm did not result in treatment related mortality, overt signs of toxicity, or significant reproductive effects during the 21 week exposure period. While not significantly different, a reduction in the number of eggs laid was observed at 250 ppm. A conservative no-observed-effect concentration was 100 ppm.

Summary of Study Results with DS-3701

<u>Species</u>	<u>Test Results</u>	<u>Category</u>
Mallard duck	Reproduction NOEL = 50 ppm	Core
Bobwhite quail	Reproduction NOEL= 100 ppm	Core

In a May 19, 1986 review EEB discussed expected exposure levels of the degradate, DS-3701. It assumed that the degradate will occur on vegetation at 7.5% of the applied parent. The highest use rate (turf, 7.5 lbs ai/acre) would result in residues of 52 to 70 ppm on long and short grass, respectively. Since DS-3701 is stable to photodegradation and hydrolysis, and chlorothalonil may be applied repeatedly to turf, the EEB expects this use to result in avian reproduction effects. Birds, including many waterfowl, which graze on grass in golf courses and other turf areas may be impacted. Field testing is required to address these effects.

The required field study must be designed to detect reductions in reproduction and other chronic effects to exposed avian species.

The EEB is also concerned with the yellowing that occurred to both bobwhite quail and mallard duck in the avian reproduction tests with technical chlorothalonil. It may be prudent to first determine exactly what caused the yellowing and what effect it has on birds before designing the field study for turf. If field work is necessary to address this yellowing phenomenon, the field study for turf should be designed to look at that effect.

If you have any questions, please contact Dan Rieder.

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil
Shaughnessey Number: 081901
2. **TEST MATERIAL:** 4-hydroxy-2,5,6-trichloroisophthalonitrile.
Test material identification number: SDS-3701-0301. Ricera
Blinded Identification Code: T-114-2. 99.6% active
ingredient. *Degradate*
3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Mallard duck
(Anas platyrhynchos)
4. **CITATION:** Shults, S.K., N. Wilson, J. Killeen. 1988.
Reproduction Study in Mallard Ducks with 4-hydroxy-2,5,6-
trichloroisophthalonitrile. Laboratory Project No. 86-0064.
Report prepared by Ricerca, Inc., Painesville, Ohio.
Reproductive study performed by Wildlife International Ltd.,
Easton, MD. Laboratory Project No. 230-106. Submitted by
Fermenta Plant Protection Company, Mentor, Ohio. EPA
Accession No. 407294-02.
5. **REVIEWED BY:**

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

Signature: *Michael L. Whitten*
Date: 3-13-89
6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *James R. Newman*
Date: 3/13/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*
Date: 8-21-89
7. **CONCLUSIONS:** This study is scientifically sound and meets
the requirements for an avian reproductive test. 4-hydroxy-
2,5,6-trichloroisophthalonitrile, at a nominal dietary
concentration of 250 ppm, had a marked effect upon both the
health of adult mallards (body weight, food consumption, and
gonad development) and their reproductive performance

(number of eggs laid, embryonic development, egg shell thickness, hatchability, and hatchling survival). There was a slight reduction in egg shell thickness at 100 ppm. Nominal dietary concentrations of 10 ppm and 50 ppm did not appear to affect adult mallards or any reproductive parameter. The no-observed-effect concentration was 50 ppm.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds employed in this study were unmated 20-week old mallards received from Whistling Wings, Hanover, Illinois. All birds had been under observation for a 7-week pre-test period for laboratory acclimation. Birds that did not appear healthy at test initiation were discarded.

B. **Dose/Diet Preparation/Food Consumption:** Test diets were prepared by mixing T-114-2 into a pre-mix which was used for preparation of the final diet. Control diet and four test concentrations (10, 50, 100, and 250 ppm) were prepared weekly and presented to birds on Monday of each week. The control diet contained an amount of the carrier (corn oil) and solvent (acetone) equal to that in the treated diets. Adults were fed a game bird ration formulated for breeding birds. All offspring received a game bird ration formulated for young growing birds. Water and feed were supplied ad libitum during acclimation and during the test.

Samples of the control and test diets were taken for analysis weekly after mixing and frozen immediately after collection. Diet samples were analyzed each week for the first four weeks. After the fourth week, samples were analyzed only for even numbered weeks.

Food consumption in each pen was determined weekly throughout the study.

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

Nominal Concentration	Number Of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
10 ppm	16	1	1
50 ppm	16	1	1
100 ppm	16	1	1
250 ppm	16	1	1

Treatment levels "were based upon data from a preliminary pilot study and consultation with the client." Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 7 weeks.
2. Pre-photostimulation - 8 weeks.
3. Pre-egg laying (with photostimulation) - 1 week.
4. Egg laying - 9 weeks.
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 5 weeks.

- D. **Pen Facilities:** Adult birds were housed indoors in 75 cm x 90 cm x 45 cm high galvanized wire pens. The average temperature in the adult study room was $21.6^{\circ}\text{C} \pm 3.0^{\circ}\text{C}$ (SD) with an average relative humidity of 59%.

During acclimation and upon initiation of the study, the birds were maintained under a photoperiod of eight hours of light per day. The photoperiod was increased to 17 hours of light per day during week 9, and was maintained at that length until terminal sacrifice. Birds received approximately 130 lux of illumination throughout the study.

- E. **Adult Observations/Gross Pathology:** All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. A record was maintained of all mortalities and observations. All birds that died during the study were necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination.

- F. **Eggs/Eggshell Thickness:** Eggs were collected daily, marked according to pen of origin, and washed to prevent pathogen contamination. The eggs were then stored at

11°C and 81% relative humidity until incubated. Eggs were removed from the storage room weekly and candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in an incubator at 37.5°C and 56% relative humidity. Eggs were candled again on day 14 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator and placed in hatching trays on incubation day 24. Temperature in the hatcher was 37°C with a relative humidity of 73%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were used for egg shell thickness measurements. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

G. Hatchlings: All hatchlings and unhatched eggs were removed from the hatcher on day 26 or 27 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were toe and web clipped for identification by pen of origin and then placed in galvanized wire mesh brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 24 cm high. Brooder temperatures were maintained at 38°C until the birds were 5 to 7 days of age and 26°C thereafter. Ambient room temperature was maintained at 21.1°C \pm 3.4°C. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age the average body weight by parental pen of all surviving ducklings was determined.

H. Statistics: Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data. Each of the following parameters was analyzed statistically:

Adult Feed Consumption

Offspring's Body Weight

Adult Body Weight
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of
Viable Embryos
Hatchlings of 3-Week
Embryos
Hatchlings of Eggs Set

Hatchlings of Maximum Set
14-Day Old Survivors of
Maximum Set
14-Day Old Survivors of
Eggs Set
14-Day Old Survivors of
of Hatchlings
Egg Shell Thickness

12. REPORTED RESULTS

- A. Diet Analysis: The mean assay values for test chemical in the diets were 101%, 101%, 103%, and 102% of nominal concentrations for groups of 10 ppm, 50 ppm, 100 ppm, and 250 ppm, respectfully (Table 6, attached).
- B. Mortality and Behavioral Reactions: There were no mortalities during the course of the study among birds in the control, 10, or 100 ppm groups.

There was one incidental mortality in the 50 ppm group. A male was found dead in week 13. Necropsy revealed slight bumblefoot (inflammation or abcess of the foot pads), slight petechial hemorrhage in the myocardium, and hyperemia of the upper intestinal tract.

Two mortalities that may have been treatment related occurred in the 250 ppm group. A male from this group was observed during week 15 exhibiting loss of coordination, lower limb weakness, and lacrimation prior to death in week 16. Necropsy revealed emaciation, minor feather loss around the eyes and slight bumblefoot. Internally, the gastrointestinal tract was empty, the ceca impacted, the kidneys were pale, and the spleen was pale and mottled. Necropsy of the pen mate showed slight feather loss on the breast and belly and slight bumblefoot. During weeks 7-9, a female from the 250 ppm group exhibited a slight ruffled appearance and slight emaciation. The bird was noted with bumblefoot and displaying lethargy during week 18, and died at the end of week 18. Necropsy revealed emaciation, bumblefoot, pasty green exudate around the vent, and extensive egg yolk peritonitis. Necropsy of the pen mate showed slight bumblefoot, an enlarged ceca, and regressed (dormant) testes.

No overt signs of toxicity were observed in the control, 10, 50, or 100 ppm groups.

Conditions which may have been related to treatment were observed in the 250 ppm group throughout the study. Four birds were noted to be slightly emaciated during week 2. One female exhibited slight ruffled appearance during weeks 6-16. Two females were noted standing on their toes; one during weeks 15-19 and another during week 19. The latter female also displayed reduced activity, reduced reaction to external stimuli, and a ruffled appearance during week 19. During week 17, one female was noted walking stiffly and another female displayed loss of coordination. Two males showed evidence of eye irritation; one beginning during week 18 and the second during week 19. Both males displayed evidence of eye irritation until study termination. "Except for the above mortalities and behavioral signs, and aside from incidental clinical signs, all birds at all concentrations appeared normal throughout the study".

Necropsy of all surviving adults was conducted at study termination. All lesions observed in the 10, 50, and 100 ppm treatment groups were considered to be incidental and not related to treatment.

There appeared to be an increased incidence of birds with regressed or regressing gonads (dormant or approaching dormant size) at 250 ppm (Appendix IV, attached).

- C. Adult Body Weight and Food Consumption: No significant differences in body weights between the control and 10, 50, or 100 ppm treatment groups were noted throughout the investigation. Body weights of males at 250 ppm were lower than controls for weeks 2, 4, and 6 ($P < 0.01$) and for week 8 and at termination ($P < 0.05$). Body weights of females at 250 ppm were lower than controls for weeks 2, 4, and at termination ($P < 0.01$) and for week 6 ($P < 0.05$).

Food consumption varied between pens due to excessive wastage by some birds. There were no significant differences in food consumption between the control and 10, 50, or 100 ppm treatment groups throughout the investigation. Food consumption in the 250 ppm group was lower than the control values during weeks 1, 2, 19 ($P < 0.01$) and 17 ($P < 0.05$). Food consumption in the

250 ppm group was higher than control values during weeks 4, 6, 7, 8, 9, and 12 ($P < 0.01$).

- D. **Reproduction:** When compared to controls, there were no significant differences in reproductive parameters for the 50 ppm and 100 ppm groups. At 10 ppm, there was a significant ($P < 0.05$) reduction in viable embryos of eggs set. "However, this reduction was principally the result of two pens in this group which failed to produce fertile eggs, and was not considered to be treatment related."

Several parameters of the 250 ppm group differed from the control group. Reductions that were significant (at $P < 0.05$ or $P < 0.01$) were observed in eggs laid, viable embryos, hatchlings, and 14-day old survivors (Tables 3 and 3A, attached). There were only five 14-day survivors at 250 ppm compared to 418 14-day survivors in the control group.

- E. **Egg Shell Thickness:** No significant differences in egg shell thickness were noted between the control group and the 10 ppm and 50 ppm groups. There was a slight but significant ($P < 0.05$) reduction in egg shell thickness in the 100 ppm group. There was a significant ($P < 0.01$) reduction in egg shell thickness at 250 ppm. There was also an increase in the percentage of cracked eggs at 250 ppm, but this difference was not significant.

- F. **Offspring Body Weights:** There were no significant differences in body weights of hatchlings or 14-day old survivors at concentrations of 10, 50, or 100 ppm. At 250 ppm, there was a decrease ($P < 0.01$) in the body weight of hatchlings and a slight, but not significant, decrease in the body weights of 14-day survivors (Tables 5 and 5A). No overt signs of toxicity were observed in the offspring of adults in any treatment group.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

"Dietary concentrations of T-114-2 at 10, 50, or 100 ppm did not result in treatment-related mortality, overt signs of toxicity, or effects upon body weight or feed consumption among adult mallards during the 19 week exposure period. No treatment related effects upon reproductive performance were noted at 10 or 50 ppm. There appeared to be a slight reduction in egg shell thickness at 100 ppm. However, there did not appear to be an effect on the percentage of cracked eggs at this concentration. T-114-2 at 250 ppm in the diet had a marked effect upon both the health of adult mallards

and their reproductive performance. The no-observed-effect concentration for T-114-2 in this study was 50 ppm."

A quality assurance audit was performed by the Quality Assurance Manager. The final report was determined to be an accurate reflection of the obtained results.

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

A. Test Procedures:

Deviations from required or recommended procedures were as follows:

Adult birds were exposed to 12 foot-candles of illumination (reported as 130 lux); 6 foot-candles is recommended.

Eggs were stored at a temperature of 11°C and a relative humidity of approximately 81%; 16°C and 65% are recommended.

- B. Statistical Analysis: Statistical procedures for analyses of eggs laid differed from recommended methods. Specifically, there is no basis for transforming this value to a percentile of the maximum number of eggs laid in any test group, which was then used in statistical procedures. According to the SEP, the number of eggs laid per hen is the parameter that should be analyzed.

Analyses of reproductive parameters were verified (attached) and found to generally match those reported by the author. Two parameters in which the author reported significant differences (number of hatchlings/live 3-week embryos and number of hatchlings/eggs set) were not found to be significantly different between groups using EPA's ANOVA program.

Several reproductive parameters were found to be reduced ($P < 0.0001$) in the 250 ppm group when compared to the control group: eggs set, viable embryos, live 3-week embryos, number hatched, eggs set/eggs laid, and 14-day survivors/number hatched.

The low number of viable embryos in the 10 ppm group is not believed to be due to a treatment effect. As the author reported, two pens in this group failed to produce fertile eggs.

C. Discussion/Results: Dietary concentrations of T-114-2 at 250 ppm had a marked effect upon both the health of adult mallards (body weight, food consumption, and gonad development) and their reproductive performance (number of eggs laid, embryonic development, egg shell thickness, hatchability, and hatchling survival. There was a slight reduction in egg shell thickness at 100 ppm. Dietary concentrations of T-114-2 at 10 ppm and 50 ppm did not appear to affect adult mallards or any reproductive parameter during the test. The no-observed-effect concentration for T-114-2 in this study was 50 ppm.

D. Adequacy of the Study:

- (1) Classification: Core
- (2) Rationale: N/A
- (3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, March 9, 1989.

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil. Shaughnessey Number: 081901
2. **TEST MATERIAL:** 4-hydroxy-2,5,6-trichloroisophthalonitrile. Ricerca Blinded Identification Code: T-114-2. 99.6% active ingredient. Degradate
3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Bobwhite quail (Colinus virginianus).
4. **CITATION:** Shults, S.K., N. Wilson, J. Killeen. 1988. Reproduction Study in Bobwhite Quail with 4-hydroxy-2,5,6-trichloroisophthalonitrile. Laboratory Project No. 86-0066. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-105. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 407294-04.
5. **REVIEWED BY:**

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

Signature: *Michael L. Whitten*
Date: 3-14-89
6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *James R. Newman*
Date: 3/14/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: *Daniel Rich*
8-21-89
Date: *Henry T. Craven*
8/21/89
7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an avian reproductive test. 4-hydroxy-2,5,6-trichloroisophthalonitrile, at nominal dietary concentrations of 10, 50, 100 and 250 ppm did not result in treatment related mortality, overt signs of toxicity, or significant reproductive effects during the 21 week exposure period. While not significantly different, a reduction in the number of eggs laid was observed at 250 ppm. A conservative no-observed-effect concentration was 100 ppm.

8. **RECOMMENDATIONS:** N/A
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds employed in this study were unmated 20-week old bobwhite quail received from Sand Prairie Quail Farm, Maquoketa, Iowa. All birds had been under observation for a 5-week pre-test period for laboratory acclimation. Birds that did not appear healthy at test initiation were discarded.

B. **Dose/Diet Preparation/Food Consumption:** Test diets were prepared by mixing T-114-2 into a pre-mix which was used for preparation of the final diet. Control diet and four test concentrations (10, 50, 100, and 250 ppm) were prepared weekly and presented to birds on Monday of each week. The control diet contained an amount of the carrier (corn oil) and solvent (acetone) equal to that in the treated diets. Adults were fed a game bird ration formulated for breeding birds. All offspring received a game bird ration formulated for young growing birds. The test substance was not mixed into the diet of the offspring. Water and feed were supplied ad libitum during acclimation and during the test.

Samples of the control and test diets were taken for analysis weekly after mixing and frozen immediately after collection. Diet samples were analyzed each week for the first four weeks. After the fourth week, samples were analyzed only for even numbered weeks.

Food consumption in each pen was determined weekly throughout the study.

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

Nominal Concentration	Number Of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
10 ppm	16	1	1
50 ppm	16	1	1
100 ppm	16	1	1
250 ppm	16	1	1

Treatment levels "were based upon data from a preliminary pilot study and consultation with the client." Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 5 weeks.
2. Pre-photostimulation - 7 weeks.
3. Pre-egg laying (with photostimulation) - 4 weeks.
4. Egg laying - 10 weeks.
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 5 weeks.

- D. **Pen Facilities:** Adult birds were housed indoors in 30 cm x 51 cm galvanized wire pens. The pens had sloping floors which resulted in a ceiling height ranging from 21 to 26 cm. The average temperature in the adult study room was $21.8^{\circ}\text{C} \pm 3.3^{\circ}\text{C}$ (SD) with an average relative humidity of 52%.

During acclimation and upon initiation of the study, the birds were maintained under a photoperiod of eight hours of light per day. The photoperiod was increased to 17 hours of light per day during week 8, and was maintained at that length until terminal sacrifice. Birds received approximately 130 lux of illumination throughout the study.

- E. **Adult Observations/Gross Pathology:** All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. A record was maintained of all mortalities and observations. All birds that died during the study were necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination.

- F. **Eggs/Eggshell Thickness:** Eggs were collected daily, marked according to pen of origin, and fumigated to prevent pathogen contamination. The eggs were then stored at 11°C and 75% relative humidity until incubated. Eggs were removed from the storage room weekly and candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in an incubator at 37.5°C and 54% relative humidity. Eggs were candled again on day 11 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in

the incubator and placed in hatching trays on incubation day 21. Temperature in the hatcher was 37°C with a relative humidity of 76%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were used for egg shell thickness measurements. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

- G. **Hatchlings:** All hatchlings and unhatched eggs were removed from the hatcher on day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg banded for identification by pen of origin and then placed in galvanized wire mesh brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 23 cm high. Brooder temperatures were maintained at 38°C. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age the average body weight by parental pen of all survivors was determined.

- H. **Statistics:** Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data. Each of the following parameters was analyzed statistically:

Adult Feed Consumption
Adult Body Weight
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of
Viable Embryos
Hatchlings of 3-Week
Embryos
Hatchlings of Eggs Set

Offspring's Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of
Maximum Set
14-Day Old Survivors of
Eggs Set
14-Day Old Survivors of
of Hatchlings
Egg Shell Thickness

17

12. REPORTED RESULTS

- A. **Diet Analysis:** The mean assay values for test chemical in the diets were 101%, 101%, 103%, and 102% of nominal concentrations for groups of 10 ppm, 50 ppm, 100 ppm, and 250 ppm, respectively).
- B. **Mortality and Behavioral Reactions:** There were no treatment related mortalities during the course of the study.

Incidental mortalities occurred in the control and all groups. One mortality occurred in the control group during week 11. Two mortalities occurred in the 10 ppm treatment group: one during week 11 and one during week 14. Three mortalities occurred in each of the other treatment groups as follows: 50 ppm - weeks 9, 11, 18; 100 ppm - weeks 12, 14, 19; 250 ppm - weeks 9, 15, 16.

All mortalities except one appeared to be related to either head or foot lesions apparently resulting from cannibalism or self-inflicted injury. The single exception was a female from the 100 ppm group observed with diarrhea during week 18 and found dead with a cloacal prolapse two days later. Prolapses are commonly the result of prolonged egg production or laying an overly large egg.

No overt signs of toxicity were observed in any group. Respiratory signs including coughing and sneezing were observed in 2-3 birds from each group. Signs were first observed during week 2 and were most noticeable during week 4. The signs had abated by week 6. One additional bird in the 250 ppm group was observed coughing and sneezing during week 8. Based on tissue cultures, the Animal Health Laboratory of the Maryland Department of Agriculture made a diagnosis of quail bronchitis. This viral disease is self limiting in adult birds and no attempts were made to treat the birds. All affected birds recovered uneventfully. The virus was not considered to have adversely impacted the results of the study.

"Other clinical signs not related to treatment, such as intermittent lethargy, depression (reduced activity, eyes partially closed), reduced reaction to external stimuli (sound and movement), ruffled appearance, wing droop or head curl, were observed in a few birds in all treatment groups during the course of the study. Except for the mortalities and clinical signs noted above, and

19

aside from any lesions or observations normally associated with pen wear and/or interaction among pen mates all other birds at all concentrations appeared normal throughout the study".

Necropsy of all surviving adults was conducted at study termination. All lesions observed were considered to be incidental and not related to treatment.

- C. **Adult Body Weight and Food Consumption:** No significant differences in body weights between the control and any treatment group were noted throughout the investigation.

Food consumption varied between pens due to excessive wastage by some birds. When compared to the control group, there was a slight but significant ($p < 0.05$) decrease in food consumption in the 50, 100, and 250 ppm groups during week 1. There was a slight but significant ($p < 0.05$) decrease in food consumption in the 100 ppm group during week 3 and an increase during week 21 (Table 2, attached). Based on the non-dose responsive nature of the occurrences and the lack of effects upon body weight, the differences were considered to be incidental to treatment.

- D. **Reproduction:** When compared to controls, there were no significant differences in reproductive parameters for any treatment group. While not statistically significant, there appeared to be a slight reduction in egg production at 250 ppm (Tables 3 and 3A attached). Four hens in this group each laid less than 20 eggs, while only one hen laid less than 20 eggs in the 0, 10, or 50 ppm groups and no hens laid less than 20 eggs in the 100 ppm group.
- E. **Egg Shell Thickness:** No significant differences in egg shell thickness were noted between the control group and any treatment group.
- F. **Offspring Body Weights:** There were no significant differences in body weights of hatchlings or 14-day old survivors at any concentration. No overt signs of toxicity were observed in the offspring of adults in any treatment group.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
 "Dietary concentrations of T-114-2 at 10, 50, 100 and 250 ppm did not result in treatment related mortality or overt signs of toxicity during the 21 week exposure period. When compared to the control group, no treatment related effects

19

upon reproductive performance were noted at 10, 50, or 100 ppm. While not significantly different, a slight reduction in the number of eggs laid was observed at 250 ppm. The no-observed-effect concentration for T-114-2 in this study was 100 ppm, and could have been 250 ppm."

A quality assurance audit was performed by the Quality Assurance Manager. The final report was determined to be an accurate reflection of the obtained results.

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

A. Test Procedures:

Deviations from required or recommended procedures were as follows:

Adult birds were exposed to 12 foot-candles of illumination (reported as 130 lux); 6 foot-candles is recommended.

Eggs were stored at a temperature of 11°C and a relative humidity of approximately 75%; 16°C and 65% are recommended.

Eggs were candled on day 21 of incubation to determine embryo survival; day 18 is recommended by the SEP.

- B. Statistical Analysis:** Statistical procedures for analyses of eggs laid differed from recommended methods. Specifically, there is no basis for transforming this value to a percentile of the maximum number of eggs laid in any test group, which was then used in statistical procedures. According to the SEP, the number of eggs laid per hen is the parameter that should be analyzed.

Analyses of reproductive parameters were verified (attached) and found to match those reported by the author.

- C. Discussion/Results:** Three hundred eighty two eggs were laid by the 250 ppm group, compared to 625 by the control group. This difference between groups ($p=0.0534$), while not significant at the 0.05 level, is nevertheless noted. As observed by the author, four hens in the 250 ppm group each produced less than 20 eggs (Appendix VII, p.3, attached). Additionally, the difference was partially the result of fewer hens available in the 250 ppm group compared to the control group. By the end of week 14 the 250 ppm group

consisted of 13 hens while the control group consisted of 15 hens. Though the difference between groups was not statistically significant, this reviewer concurs with the author's conservative estimate of the no-observed-effect concentration of 100 ppm.

The number of mortalities (one in the control group, two in the 10 ppm group, and three in each of the other treatment groups) was higher than normal, but the mortalities did not appear to be treatment related. The quail bronchitis that occurred in some birds is noted, but the condition did not appear to have affected the results of the study.

Dietary concentrations of T-114-2 at 10, 50, 100 and 250 ppm did not result in treatment related mortality, overt signs of toxicity, or significant reproductive effects during the 21 week exposure period. While not significantly different, a reduction in the number of eggs laid was observed at 250 ppm. A conservative no-observed-effect concentration for T-114-2 in this study was 100 ppm. This study is scientifically sound and meets the requirements for an avian reproductive test.

D. Adequacy of the Study:

- (1) **Classification:** Core
- (2) **Rationale:** N/A
- (3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, March 14, 1989.