238553
RECORD NO.

081901
SHAUGHNESSEY NO

REVIEW NO.

EEB REVIEW

DATE: IN 1 -25-89 OUT 8-31-89

FILE OR REG. NO. 50534-7

PETITION OR EXP. NO.

DATE OF SUBMISSION 1-12-89

DATE RECEIVED BY EFED 1-25-89

RD REQUESTED COMPLETION DATE 4-19-89

EEB ESTIMATED COMPLETION DATE 4-19-89

RD ACTION CODE/TYPE OF REVIEW 660

TYPE PRODUCT(S) Fungicide

DATA ACCESSION NO(S) 409641-01 thru 05

PRODUCT MANAGER, NO. L. Rossi (21)

PRODUCT NAME(S) Chlorothalonil

COMPANY NAME Fermenta Plant Protection

SUBMISSION PURPOSE Submission of avian reproduction studies with technical chlorothalonil and a Japanese quail acute oral LD50 study

SHAUGHNESSEY NO.	CHEMICAL	% A.I.
081901	Chlorothalonil	

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM August 31, 1989

SUBJECT: Submission of Avian Studies for Review
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 three avian studies for review. They are presented below:

1. STUDY TYPE: Avian Single-Dose Oral LD50 Test.
Species Tested: Japanese quail (Coturnix japonica).

CITATION: Shults, S.K., N. Wilson, and J. Killeen. 1987. Acute Oral Toxicity (LD50) Study in Japanese Quail With Technical Chlorothalonil. Laboratory Study No. 87-0041. Report prepared by Ricerca, Inc., Painesville, Ohio. Study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-107. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 409641-05.

CONCLUSIONS: With an LD50 value of greater than approximately 2000 mg/kg, Technical Chlorothalonil is considered practically non-toxic to Japanese quail. The test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical in Japanese quail, but the species is not a generally accepted one used in support of pesticide registration. It therefore does not meet the guideline requirements for an avian oral LD50 test.

2. STUDY TYPE: Avian Reproduction Study.
Species Tested: Mallard duck
(Anas platyrhynchos)

CITATION: Shults, S.K., N. Wilson, J. Killeen. 1988. Reproduction Study in Mallard Ducks with Technical Chlorothalonil. Laboratory Project No. 87-0004. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-109. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 409641-02.

CONCLUSIONS: This study is scientifically sound and meets the requirements for an avian reproductive test. Technical chlorothalonil, at nominal dietary concentrations of 1000, 5000, or 10,000 ppm did not result in treatment-related mortality or significant effects upon reproduction of mallards during the 18 week exposure period. Based upon unexplained discoloration (yellowing) of the skin on approximately one-half of the birds in the 5000 and 10,000 ppm groups, the NOEC was 1000 ppm.

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

CITATION: Shults, S.K., N. Wilson, J. Killeen. 1988. Reproduction Study in Bobwhite Quail with Technical Chlorothalonil. Laboratory Project No. 87-0006. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-108. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 409641-04.

CONCLUSIONS: This study is scientifically sound and meets the requirements for an avian reproductive test. Technical chlorothalonil, at a nominal dietary concentration of 5000 ppm, resulted in overt signs of toxicity and reduced reproduction. At 10,000 ppm, there were even more pronounced effects including overt signs of toxicity, mortalities, and profound effects upon several reproductive parameters related to egg production, hatching success, and survival of hatchlings. Based upon unexplained discoloration (yellowing) of the skin on all surviving birds in all treatment groups, the NOEC was less than the lowest test level of 1000 ppm.

3

Summary of Study Results

Species	Test Results	Category
Japanese quail	LD50>2000 mg/kg	Supplemental (test species)
Mallard duck	Reproduction NOEL = 1000 ppm Effects occurring at 5000 and 10000 ppm included discoloration of skin, no treatment related mortality or reduction in reproduction were observed at any test level.	Core
Bobwhite quail	Reproduction NOEL < 1000 ppm Effects occurring at 1000 ppm included discoloration of the skin on the head and body. However, no other signs of toxicity or reduced reproduction were observed in the 1000 ppm test group.	Core

Discussion

The bobwhite quail study reported a yellow discoloration at all test levels (1000, 5000 and 10000 ppm). In the mallard study, the yellowing occurred at the 5000 and 10000 ppm test level, but not at the 1000 ppm level. According to the report, the yellow discoloration of the skin, particularly around the eyes but also on the head and body, appeared to be stains rather than the result of a tissue reaction or other physiological process. However, no explanation of this conclusion was provided. According to the February 24, 1988 Chlorothalonil FRSTR, at the highest use rate of 7.5 lbs ai per acre, typical concentrations ranged from 938 ppm on short grass to 248 on insects and forage, and 11 ppm on fruit. Since the previous reproduction studies only tested to 50 ppm with no observed effects[1], and this quail study observed yellowing of an unknown origin at the lowest test level (i.e. 1000 ppm), the EEB is still in the same position; i.e. a NOEL of 50 ppm and a LOEL of 1000 ppm for chlorothalonil. The registrant should obtain additional information on the cause or source of the yellowing that was observed to show the cause and whether it is of biological significance. This may involve further laboratory testing. If so, it is recommended that they contact Dan Rieder.

In an another review dated 8-24-89, EEB addressed the two avian reproduction tests conducted with the degradate of chlorothalonil, DS-3701. Based on those studies, field testing is required for the turf use. The investigation into the yellowing

[1] MRID Number 41440 and 41441 as reported in the February 24, 1988 science chapter, page 3.

4

that occurred in these tests should be completed before the field study for the turf use is designed and initiated. If this yellowing represents an ecologically significant effect, it may be that field testing would be required. If so, the turf field study could be designed to address that phenomenon also.

Conclusion

The EEB cannot yet complete its evaluation of potential chronic or reproductive effects of parent chlorothalonil to birds. Further information is needed on the yellowing that occurred in these reproduction studies. Field testing may be required. Please direct any questions to Dan Rieder.

Accession No. 409641-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.

2. **TEST MATERIAL:** Technical Chlorothalonil. (tetrachloroisophthalonitrile). Ricera Blinded Identification Code: T-117-12. 98.6% active ingredient.

3. **STUDY TYPE:** Avian Single-Dose Oral LD50 Test. Species Tested: Japanese quail (Coturnix japonica).

4. **CITATION:** Shults, S.K., N. Wilson, and J. Killeen. 1987. Acute Oral Toxicity (LD50) Study in Japanese Quail With Technical Chlorothalonil. Laboratory Study No. 87-0041. Report prepared by Ricerca, Inc., Painesville, Ohio. Study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-107. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 409641-05.

5. **REVIEWED BY:**

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

Signature: Michael L. Whitten
Date: 3-17-89

6. **APPROVED BY:**

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

Signature: James R. Newman
Date: 3/17/89

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

Signature: Daniel Rieder 8-17-89
Date: Henry T. Craven 8/21/89

7. **CONCLUSIONS:** With an LD50 value of greater than approximately 2000 mg/kg, Technical Chlorathalonil is considered practically non-toxic to Japanese quail. The test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical in Japanese quail, but the species is not a generally accepted one used

in support of pesticide registration. It therefore does not meet the guideline requirements for an avian oral LD50 test.

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 study were 20-week old Japanese quail (Coturnix japonica) received from The Quail Castle, Lodi, Missouri. Birds were acclimated to the laboratory conditions for 20 days prior to test initiation. Birds exhibiting abnormal behavior or physical injury were not used in the test. Each bird was identified by a colored, numbered legband.

B. **Test System:** All birds were housed indoors in 78 x 51 cm pens. Floors were sloped resulting a ceiling height of 20 to 25 cm. Fluorescent lights provided seven hours of light per day. Average temperature was 27°C with an average relative humidity of 74%.

C. **Dosage:** 14-day single dose oral LD50 test. Based on "known toxicity data" nominal dosages selected for the definitive study were 260, 430, 720, 1200, and 2000 milligrams of T-117-12 per kilogram of body weight.

D. **Design:** Groups of ten birds (five males and five females) were randomly assigned to each of the five treatment groups and the control group. Each group was assigned two pens. One pen contained five males and the other five females. The birds were fed a game bird ration formulated to Wildlife International Ltd.'s specifications. Food and water were supplied ad libitum except for a period of "at least 15 hours" prior to dosing when the birds were fasted, with water allowed. At test initiation, a single dose of test material in diluent (corn oil) was orally intubated into the crop or proventriculus of each bird using a stainless steel catheter. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received diluent only. All treatment and control birds received a constant dosage volume of 6 milliliters per kilogram of body weight. The birds were individually weighed at test initiation and by group on days 3, 7, and 14. Group food consumption was recorded on test days 3, 7,

and 14. Observations were conducted at least twice daily for potential clinical signs indicative of test material effect. Gross necropsies were performed on all birds at study termination, 14 days after dosing.

E. **Statistics:** No statistical analyses were reported since no mortality occurred.

12. **REPORTED RESULTS:** There were no mortalities in the control or any treatment group. Feather picking, a form of aggression, was noted in the control and all treatment groups. Aggressive behavior between males was observed in thr 720 mg/kg group from day 2 until study termination. Aside from this aggressive behavior and feather picking, neither of which was considered treatment related, all birds were normal in appearance and behavior throughout the study.

Necropsy revealed signs of feather picking in some birds in all groups. One female in the 1200 mg/kg group was noted with slight enlargement of the kidneys. This was not considered to be treatment related. Necropsy results on all other birds were unremarkable.

There were no apparent treatment related effects upon body weight or food consumption at any test concentration. Body weight measurements were highly variable. This variability was attributed primarily to aggressive behavior among some birds. Additionally, some of the hens were in egg production causing hen weight to fluctuate.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The acute oral LD50 of T-117-12 for Japanese quail was determined to be greater than 2000 mg/kg, the highest dosage tested.

The study was conducted in conformance with Good Laboratory Practice regulations with one exception: Samples of the dosing mixtures were not taken for confirmation of the test concentrations.

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

A. **Test Procedure:** The Japanese quail (Corturnix japonica) is not a generally accepted test species. The report did not state the rationale for using this species. Unless the registrant can show sufficient reason for its use in this test, the species should not be used to support registration of a pesticide.

Individual body weights were not measured at the end of the study as recommended by the SEP. Instead, group body weights were measured.

Some females were in egg production during the test. This is not believed to have affected the results, since no deaths occurred even at the highest level tested.

As noted in the quality assurance statement, samples of the dosing mixtures were not taken for confirmation of the test concentrations. All dosages are therefore considered to be approximations of the intended dosages.

The test procedures were otherwise in accordance with SEP guidelines.

B. **Statistical Analysis:** LD50 calculations were not applicable since no mortalities occurred. No statistical analyses on body weights or food consumption were reported. The author did not provide data that would have enabled such analyses. The high variability in body weights (Table 2, attached) probably was not treatment related.

C. **Discussion/Results:** With an LD50 value of greater than approximately 2000 mg/kg, Technical Chlorothalonil is considered practically non-toxic to Japanese quail. The test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical in Japanese quail, but the species is not a generally accepted one used in support of pesticide registration. It therefore does not meet the guideline requirements for an avian oral LD50 test.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** Unacceptable test species. With the above noted discrepancies, the test is scientifically sound, but the species is not a generally accepted one used in support of pesticide registration.

(3) **Repairability:** Repairability pending sufficient rationale by registrant for use of Japanese quail as a test species.

15. **COMPLETION OF ONE-LINER:** Yes; March 17, 1989.

Accession No. 409641-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil. Shaughnessey Number: 081901.

2. **TEST MATERIAL:** Technical Chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile). Ricera Blinded Identification Code: T-117-12. 98.3% active ingredient.

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 Technical Chlorothalonil. Laboratory Project No. 87-0004. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-109. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 409641-02.

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: Henry T. Craven
Date: 8/21/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an avian reproductive test. Technical chlorothalonil, at nominal dietary concentrations of 1000, 5000, or 10,000 ppm did not result in treatment-related mortality or significant effects upon reproduction of mallards during the 18 week exposure period. Based upon unexplained discoloration of the skin on approximately one-

half of the birds in the 5000 and 10,000 ppm groups, the NOEC was 1000 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 23-week old mallards received from Whistling Wings, Hanover, Illinois. All birds had been under observation for a 6-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-117-2 into a pre-mix which was used for preparation of the final diet. Control diet and three test concentrations (1000, 5000, and 10,000 ppm) were prepared weekly and presented to birds on Tuesday of each week. 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 four groups as follows:

Nominal Concentration	Number Of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
1000 ppm	16	1	1
5000 ppm	16	1	1
10,000 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 - 6 weeks.
2. Pre-photostimulation - 9 weeks.
3. Egg laying - 9 weeks.
4. 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 19.5°C ± 2.3°C (SD) with an average relative humidity of 38%.

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. At study termination, all 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 37.4°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 24.3°C ± 2.0°C. The photoperiod was maintained at 16 hours of light per day. All offspring were obserevd at least once daily for signs of toxicity or abnormal behavior. 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
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

13

12. **REPORTED RESULTS**

A. **Diet Analysis**: The mean assay values for test chemical in the diets were 100%, 99%, and 99% of nominal concentrations for the 1000 ppm, 5000 ppm, and 10,000 ppm groups, respectfully.

B. **Mortality and Behavioral Reactions**: There were no mortalities during the course of the study among birds in the control or any treatment group.

No overt signs of toxicity were observed in any treatment group. One male in the 10,000 ppm group was noted with a yellow discoloration of the skin around the eyes beginning during week 11 and continuing until study termination. The discoloration appeared to be stains rather than the result of tissue reaction or other physiological process. Except for this bird, and aside from incidental signs unrelated to treatment (such as slight hyperexcitability, high wing carriage, lameness, or bumblefoot) all birds at all concentrations appeared normal throughout the study.

Necropsy of all surviving adults was conducted at study termination. Yellow discoloration on the skin of the head and body was noted in approximately one-half (predominately males) of the birds in the 5000 and 10,000 ppm treatment groups. This appeared to be staining rather than the result of tissue reaction or other physiological process. "While this finding appeared to be treatment related in this study, it did not appear to be toxicologically significant." All other lesions observed in the treatment groups 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 treatment groups were noted throughout the investigation.

Food consumption varied between pens due to excessive wastage by some birds. There were no significant differences in food consumption between the control and treatment groups throughout the investigation.

D. **Reproduction**: When compared to controls, there were no significant differences in reproductive parameters for any test concentration. "There may have been a very slight reduction in egg production at 10,000 ppm, with

four hens laying less than 30 eggs during the 9 weeks of egg production. However, average egg production for this treatment group (38 ± 10) was comparable to historical control values (39 ± 9). When compared to the control group, there also appeared to be a slight reduction in the percentage of hatchlings set. However, this value (72 ± 32) also was comparable to the historical control value (71 ± 10) for this parameter". Reproductive data are summarized in Tables 3 and 3A (attached).

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 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-117-2 at 1000, 5000, or 10,000 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 18 week exposure period. No treatment related effects upon reproductive performance were noted at 1000 or 5000 ppm. Although there may have been a slight reduction in reproductive performance at 10,000 ppm, values were similar to historical controls. The no-observed-effect concentration for T-117-12 in this study was at least 5000 ppm and may have been 10,000 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.

15

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.

As discussed in Section 12D, the author stated that the mean value for hatchlings/eggs set in the 10,000 ppm group was 72%, and therefore similar to the historical value for controls of 71%. The value reported in Table 3A and Appendix VII (attached) for the 10,00 ppm group is 65%. While this value is lower than the historical value given, it did not differ significantly from the control value.

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

C. **Discussion/Results**: Yellow discoloration on the skin of the head and body was noted in approximately one-half (predominately males) of the birds in the 5000 and 10,000 ppm treatment groups. The author provided no explanation for this phenomenon, but admitted that it appeared to be treatment related, though not toxicologically significant, and "appeared to be staining rather than the result of tissue reaction or other physiological process". The reviewer notes that while it did not appear to be toxicologically significant in this reproduction test, the condition is perplexing, and should not be so easily dismissed. The condition may justify testing beyond the scope of a reproduction test. Because the staining was evident in the 10,000 and 5000 ppm groups, the no-observed-effect concentration in the study was 1000 ppm, rather than the NOEC of at least 5000 ppm reported by the author.

Nominal dietary concentrations of T-117-12 at 1000, 5000, or 10,000 ppm did not result in treatment-related mortality or significant effects upon reproduction of mallards during the 18 week exposure period. 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.

17

Accession No. 409641-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil. Shaughnessey Number: 081901.

2. **TEST MATERIAL:** Technical Chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile). Ricera Blinded Identification Code: T-117-12. 98.3% active ingredient.

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 Technical Chlorothalonil. Laboratory Project No. 87-0006. Report prepared by Ricerca, Inc., Painesville, Ohio. Reproductive study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 230-108. Submitted by Fermenta Plant Protection Company, Mentor, Ohio. EPA Accession No. 409641-04.

5. **REVIEWED BY:**

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

Signature: Michael L. Whitten
Date:

6. **APPROVED BY:**

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

Signature: James R Newman
Date: 3/15/89

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

Signature: Daniel Rieder 8/17/89
Date: Henry T Craven 8/21/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an avian reproductive test. Technical chlorothalonil, at a nominal dietary concentration of 5000 ppm, resulted in overt signs of toxicity and reduced reproduction. At 10,000 ppm, there were even more pronounced effects including overt signs of toxicity, mortalities, and profound effects upon several reproductive parameters related to egg production, hatching success, and survival of hatchlings. Based upon unexplained

18

discoloration of the skin on all surviving birds in all treatment groups, the NOEC was less than the lowest test level of 1000 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 24-week bobwhite quail received from Fritt's Quail Farm, Phillipsburg, New Jersey. 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-117-12 into a pre-mix which was used for preparation of the final diet. Control diet and three test concentrations (1000, 5000, and 10,000 ppm) were prepared weekly and presented to birds on Tuesday of each week. 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 week 4, samples were analyzed only for even numbered weeks through week 20. Samples were also analyzed for week 21.

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

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

Nominal Concentration	Number Of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
1000 ppm	16	1	1
5000 ppm	16	1	1
10,000 ppm	16	1	1

19

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 - 8 weeks.
3. Pre-egg laying (with photostimulation) - 3 weeks.
4. Egg laying - 10 weeks.
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 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 20.5°C ± 2.1°C (SD) with an average relative humidity of 36%.

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 11.5° 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 37.4°C and 56% 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 the hatcher 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. Offspring were observed for signs of toxicity or abnormal behavior during a period of 14 days. Hatchlings were fed untreated diet. At 14 days of age the average body weight by parental pen of all surviving chicks 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

12. **REPORTED RESULTS**

A. **Diet Analysis**: The mean assay values for test chemical in the diets were 100%, 99%, and 100% of nominal concentrations for the 1000 ppm, 5000 ppm, and 10,000 ppm groups, respectfully (Table 4, attached).

B. **Mortality and Behavioral Reactions**: There were no mortalities during the course of the study in the control or 1000 ppm groups.

Two mortalities, both apparently unrelated to treatment, occurred in the 5000 ppm group. One male was noted to have an injured toe and was displaying reduced activity, reduced reaction to external stimuli, and lower limb weakness the day prior to death during week 7. Necropsy revealed a torn nail on the second toe of the right foot and evidence of extensive hemorrhage. The bird had an empty upper gastrointestinal tract. The second mortality in the 5000 ppm group was a male in week 14. Prior to death, the bird displayed severe foot lesions, yellow staining of the skin around the eyes, lethargy, reduced activity, reduced reaction to external stimuli, and a ruffled appearance. Necropsy revealed the bird to be dehydrated and emaciated, with extensive foot lesions and yellow discoloration of the skin. Necropsy of the bird's pen mate revealed a regressed ovary and yellow discoloration of the skin.

There were three mortalities in the 10,000 ppm group, two of which appeared to be treatment related. The first treatment related mortality occurred during week 12. The hen had displayed conjunctivitis, yellow skin discoloration, lethargy, wing droop and a ruffled appearance. Necropsy revealed the hen to be emaciated, with feather loss on the head, yellow skin discoloration, and a regressed ovary.

The second treatment related mortality in the 10,000 ppm occurred during week 18. The hen had displayed slight conjunctivitis, lethargy, wing droop and a ruffled appearance during week 8, but the bird appeared normal by week 9. After week 13, the bird displayed lethargy, reduced reaction to external stimuli, wing droop, a ruffled appearance, and slight loss of coordination. Necropsy revealed the hen to be emaciated with yellow skin discoloration. The bird also showed a severely

distended intestinal tract filled with a yellow-brown fluid and an inactive ovary.

The third mortality in the 10,000 ppm group was a hen with extensive foot lesions that died during week 17. During week 9 the bird was noted to be lethargic with ruffled feathers. During the week prior to death, extensive foot lesions, reduced activity, reduced reaction to external stimuli, wing droop, and a ruffled appearance were noted. Necropsy revealed foot lesions, emaciation, yellow skin discoloration, a regressed ovary, and small pale kidneys.

Necropsy of all surviving adults was conducted at study termination (Appendix IV, attached). There appeared to be an increase in the number of birds with regressing gonads in the 10,000 ppm group. There also appeared to be an increase in the number of hens with lesions of old egg yolk peritonitis. Yellow discoloration on the skin of the head and body was noted on all surviving birds in all treatment groups, but on none of the control birds. This "appeared to be stains rather than the result of a tissue reaction or other physiological process. While this finding appeared to be treatment related in this study, it did not appear to be toxicologically significant." All other lesions observed were considered to be incidental and not related to treatment.

No overt clinical signs attributable to treatment were observed in the control or 1000 ppm groups. Several birds in the 5000 and 10,000 ppm groups displayed clinical signs attributable to treatment.

In the 5000 ppm group, two females and two males displayed symptoms including lethargy, ruffled appearance, wing droop, and reduced activity at various times after week 12. Sixteen birds in this group displayed yellow discoloration of the skin at study termination.

In the 10,000 ppm group, one male was noted with conjunctivitis which was first observed during week 5. Five females and two males displayed symptoms including lethargy, reduced activity, wing droop, and a ruffled appearance beginning during week 8. All birds in this group displayed yellow discoloration of the skin at study termination.

C. **Adult Body Weight and Food Consumption**: No significant differences in body weights between the control and 1000 ppm or 5000 ppm groups were noted throughout the investigation.

In the 10,000 ppm group, body weights of males and females were significantly lower than controls for weeks 2, 4, 6, 8, and at termination. See Table 1 (attached) for body weights and significance levels.

Food consumption varied between pens due to excessive wastage by some birds. When compared to the control group, there was a slight but significant ($p<0.05$) increase in food consumption in the 1000 ppm group during week 3, and in the 5000 ppm group during week 4. These differences were considered to be unrelated to treatment. The 10,000 ppm group showed significant increases in food consumption during weeks 6 and 7, and reductions in weeks 1, 13, and 14. See Table 2 (attached) for mean food consumption values and significance levels.

D. **Reproduction**: There were no significant effects upon reproduction in the 1000 ppm group. In the 5000 ppm group there were "significant reductions on a number of reproductive parameters related to egg laying, hatching success and 14 day old survivors". (Table 3 and 3A, attached). "In the 10,000 ppm group, there was a profound effect upon reproductive performance. All parameters except the percentages of cracked eggs and live 3-week embryos were statistically different from the control group at $p<0.01$. There was only one 14-day old survivor from the 10,000 ppm group." (Tables 3 and 3A, attached).

E. **Egg Shell Thickness**: No significant differences in egg shell thickness were noted between the control group and any treatment group.

F. **Offspring Observations and Body Weights**: No signs of toxicity were observed in the offspring of adults in any treatment group. There were no significant differences in body weights of hatchlings at 1000 ppm or upon 14-day old survivors at 1000 ppm and 5000 ppm. There was a slight but significant ($p<0.05$) reduction in body weight of hatchlings at 5000 ppm (Tables 5 and 5A, attached). At 10,000 ppm, only four birds hatched and only one survived to 14 days of age. Therefore, a statistical analysis of body weights from the 10,000 ppm group was not reported.

24

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
" A dietary concentration of T-117-12 at 1000 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 upon reproductive performance were noted at 1000 ppm. At 5000 ppm, there were overt signs of toxicity and marked effects upon reproductive performance. At 10,000 ppm, there were even more pronounced effects including overt signs of toxicity, mortalities, and profound effects upon reproductive performance. The no-observed-effect concentration for T-117-12 in this study was 1000 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 11.5°C and a relative humidity of approximately 81%; 16°C and 65% are recommended.

Eggs were candled on day 21 to determine embryo survival; day 18 is recommended.

Hatchlings were removed from the hatcher on day 25 or 26 of incubation; the SEP recommends day 24.

B. **Statistical Analysis:** Statistical procedures for analyses of eggs laid and 14-day old survivors differed from recommended methods. Specifically, there is no basis for transforming these values to percentiles of the maximum number of eggs laid or set in any test group, which were then used in statistical procedures. According to the SEP, the number of eggs laid per hen and the number of 14-day old survivors per hen are the parameters that should be analyzed.

25

Analyses of reproductive parameters were verified (attached) and generally matched those reported by the author. Differences from the author's analyses were as follows:

Values for 14-day survivors per hen and eggs laid per hen in the 5000 ppm and 10,000 ppm groups were significantly different ($p<0.001$) from controls. As discussed above, the author did not analyze these parameters.

The value for hatchlings of three-week embryos did not differ among groups ($p=0.0930$) using EPA's ANOVA program.

The value for 14-day survivors of normal hatchlings differed between groups ($p=0.0002$) but Duncan's multiple range test did not identify significant pairwise comparisons. The author used Dunnett's test, which compares each treatment group to the control group, while Duncan's test does all pairwise compairisons.

C. **Discussion/Results:** Yellow discoloration on the skin of the head and body was noted in all surviving birds in the 1000, 5000 and 10,000 ppm treatment groups, but in none of the control birds. The author provided no explanation for this phenomenon, but admitted that it appeared to be treatment related, though not toxicologically significant, and "appeared to be staining rather than the result of a tissue reaction or other physiological process". The reviewer notes that while it did not appear to be toxicologically significant in this reproduction test, the condition is perplexing, and should not be so easily dismissed. The condition may justify testing beyond the scope of a reproduction test. Because the staining was evident in all treatment groups, the no-observed-effect concentration in the study was less than the lowest test level of 1000 ppm, rather than the NOEC of 1000 ppm reported by the author.

A nominal dietary concentration of T-117-12 at 1000 ppm did not result in mortality, overt signs of toxicity, or altered reproduction during the 21 week exposure period. At 5000 ppm, there were overt signs of toxicity and marked effects upon reproductive performance. At 10,000 ppm, there were even more pronounced effects including overt signs of toxicity, mortalities, and profound effects upon several reproductive parameters.

D. **Adequacy of the Study:**

(1) **Classification:** Core

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

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

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

27