

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

1. CHEMICAL: Triclopyr triethylamine.
Shaughnessey Number: 116002.
2. TEST MATERIAL: DOWCO 233; 98.9% AGR; 134832 GHC 25-1-47.
A low density, fibrous material with a slight odor. Date Received February 23, 1979. Wildlife International Identification No. WI-597.
3. STUDY TYPE: Avian Reproduction Study.
Species Tested: Anas platyrhynchos.
4. CITATION: Beavers, J.B. and R. Fink; 1980; Final Report; One-Generation Reproduction Study - Mallard Duck; Prepared by Wildlife International Ltd., Easton, MD; Project No. 103-192; Submitted by Dow Chemical Company (address not given); MRID Number ~~92189006~~ 3125D.

5. REVIEWED BY:

Jeffrey L. Lincer, Ph.D.
President
Eco-Analysts, Inc.

Signature: *Michael L. Whitten*
Date: 1/22/91 For J. Lincer

6. APPROVED BY:

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

Henry T. Craven
3-6-91
Signature: *Michael L. Whitten*
Date: 1/22/91

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

Signature: *Henry T. Craven*
Date: 3/15/91

7. CONCLUSIONS: This study appears to be scientifically sound, but does not meet the requirements for an avian reproductive test, since the statistical analyses could not be verified due to illegible raw data, and since the results of the chemical analyses of the diets were not presented. It appears from the report that the NOEC of DOWCO 233 is 100 ppm; a reduction in the ratio of 14 day-old survivors/eggs set occurred at 200 ppm and 500 ppm.
8. RECOMMENDATIONS: N/A
9. BACKGROUND: N/A
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Pen-reared mallard ducks (Anas platyrhynchos) were obtained from production flock, Wildlife International Ltd., Easton, MD. They were disease-free, previously untreated, approaching their first breeding season, phenotypically indistinguishable from wild birds and 6 months old at study initiation.
- B. Test System: The birds were housed indoors with two drakes and five hens per pen. Pens were constructed of wood supports, and were surrounded by one inch mesh, 20 gauge galvanized wire. Each pen was three feet in height and measured 60 square feet. Each pen contained a 12 kilogram gravity-fed feeder and a 17 liter water pan.

The temperature and humidity in the research facility were allowed to fluctuate in accordance with ambient temperature. Temperatures below 35°F and above 90°F were prevented through the use of cooling ventilators, exhaust fans, and ceiling insulation.

The photoperiod for the first eight weeks of the study was nine hours of light per day. The photoperiod was then increased to 17 hours of light per day for weeks 9 through 15 of the study, and to 22 hours of light per day for weeks 16 through 20 of the study. The birds received ten footcandles of illumination throughout the study, provided by standard incandescent lighting.

Eggs were collected daily and stored at 65°F and 65 percent relative humidity. Each egg collected was marked according to the pen from which it was taken. At weekly intervals the eggs were placed in a Kuhl eggwasher (Model No. F4220) and soaked for fifteen seconds, then washed for three minutes at 115°F in a bactericide (Kuhl "Super" "C" "D" chlorine base detergent) to reduce E. coli contamination. The clean eggs were then placed in a Petersime Incubator (Model S20) for incubation. On Day 22 or 23 of incubation, the eggs were placed in a Petersime Hatcher (Model S20) and allowed to hatch.

Throughout incubation the incubator temperature was maintained at 99.5°F ± 0.1°F with a wet bulb humidity index of 90.0°F ± 1.0°F. The incubator was equipped with a pulsator fan and blades that produce a mild breathing air movement that eliminated intracabinet

temperature and humidity variation during incubation.

The incubator was equipped with automatic egg rotation, assuring that each egg was rotated from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation was 100°) each hour through Day 22 or 23 of incubation. When the eggs were transferred to the hatcher, rotation was discontinued, the temperature was lowered to 99.0°F ± 1.0°F, and the wet bulb humidity index was raised to 92.0°F ± 1.0°F.

Hatchlings were housed according to appropriate parental grouping in Beach (Model B755) battery brooders until 14 days of age. Battery brooder temperature was maintained at 100°F from hatching to Day 7 of brooding and then lowered to 75°F for the remainder of the brooding period. During brooding the basal diet was Wildlife International Ltd.'s game bird starter ration. Starter ration and water were available ad libitum. At 14 days of age the ducklings were removed from the brooding units and the average body weight determined.

- C. Dosage: DOWCO 233 and corn oil were incorporated into aliquots of basal diet utilizing a Hobart mixer. The concentrates were frozen until utilized for the weekly preparation of fresh diets. The basal diet was Wildlife International Ltd.'s gamebird breeder ration containing 19.4% protein, 6.7% fat, and 3.8% fiber. Incorporation of the appropriate DOWCO 233 concentrate into bulk basal diet provided the experimental concentrations listed. Samples of the control diet and each of the DOWCO 233 diets were shipped to the sponsor for DOWCO 233 analysis during the study. The adult mallard ducks in both the control and experimental groups received the appropriate diets ad libitum for the duration of the study. They were fed the below dietary levels for a period of 20 weeks.
- D. Design: One hundred and forty mallard ducks (40 drakes and 100 hens) were randomly distributed into the following groups:

<u>Group</u>	<u>Dosage</u> (ppm)	<u># Pens</u>	<u>Birds Per Pen</u>	
			<u>Drakes</u>	<u>Hens</u>
1 - Controls	0	5	2	5
2 - DOWCO 233	100	5	2	5
3 - DOWCO 233	200	5	2	5
4 - DOWCO 233	500	5	2	5

Body weights were recorded at initiation, on weeks 2, 4, 6, 8 and at termination of the study. Body weights were not recorded during egg laying because of the adverse effect handling has on egg production. Feed consumption was recorded bi-weekly throughout the study.

Weekly throughout egg laying, one egg from each pen in each experimental group and the control was randomly selected for egg weight and eggshell thickness measurement. The eggs were first weighed to the nearest one-tenth of a gram, then they were opened at the waist. The contents were thoroughly washed out, and the shells were dried for one week at room temperature. The average thickness of the dried shell plus the membrane at the waist was determined by measuring at three to five points around the circumference of the egg using an eggshell thickness micrometer calibrated in 0.01 mm units.

E. Statistics: Cochran's Analysis, the Two-Way Analysis of Variance, the Student's T-Test, and the Chi-Square test were utilized, "where appropriate", to evaluate the differences between each of the following reproductive parameters:

Eggs Laid	Eggs Set
Eggs Cracked	Eggshell Thickness
Egg Weight	Viable Embryos (9-14 days)
Live 3-week Embryo	Hatchlings (No. Hatched)
Hatchlings' Body Weight	14-Day Old Survivors
14-Day Old Survivor Body Weight	

12. **REPORTED RESULTS:** "Mature mallard ducks receiving DOWCO 233 at dietary concentrations of 100 ppm, 200 ppm, and 500 ppm showed no symptoms of toxicity or behavioral abnormalities

for the duration of the study. Mortalities occurred as follows: Control group; one hen - week 12. 100 ppm group; one hen - week 14. 200 ppm group; no mortalities. 500 ppm group; one hen - week 14. The single mortality at the 500 ppm group occurred during the stress of egg production, and no gross compound-related abnormalities were noted upon necropsy...therefore, this death was considered to be incidental and not compound related. All other birds were normal in both appearance and behavior for the duration of the study.

"There was a slight, but statistically significant ($p < .01$) reduction in feed consumption by adult birds at the 100 ppm dosage level, and a statistically significant ($p < .01$) difference in the body weight of adult birds at the 500 ppm dosage level.

"Evaluation of the reproductive data..., and statistical analysis of the reproductive parameters: eggs laid, eggs cracked, viable embryos, live three-week embryos, normal hatchlings, hatchlings' body weight, 14 day-old survivors, 14 day-old survivors' body weight, egg weight, and eggshell thickness demonstrate that DOWCO 233 caused no statistically significant reproductive impairment at the dosage levels tested. However, statistical examination of the number of 14 day-old survivors as a percentage of the number of eggs set for each dosage level showed a statistically significant ($p < .01$) depression at the 500 ppm dosage level, and a depression approaching statistical significance ($p < .02$) at the 200 ppm dosage level."

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

"DOWCO 233 was fed to mature mallard ducks throughout a one-generation reproduction study at dietary concentrations of 100 ppm, 200 ppm, and 500 ppm. Moderate reproductive impairment was noted at the 500 ppm dosage level. Based on the results of this study, environmental concentrations of up to 200 ppm of DOWCO 233 do not represent a reproductive hazard to the mallard duck."

"With respect to the quality assurance, the QA Officer, Joy Faulcon, certified the following for this study:

- "1. It was conducted according to Standard Operating Procedures developed by Wildlife International Ltd.

2. It was conducted in accordance with the standards specified by Good Laboratory Practices as described in the Federal Register, Vol. 43, No. 247 - Friday, December 22, 1978.
3. It was inspected during its operational phase to insure compliance with items 1 and 2 above.
4. The information presented in this report accurately reflects the raw data generated during the course of conducting the study."

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

- A. Test Procedure: This study was carried out in 1979, before the current SEP and Subdivision E Guidelines were published. For the record, however, the below discrepancies were noted.
- (1) Temperature and humidity in the research facilities were allowed to fluctuate with ambient conditions (vs meeting SEP requirements; pg. 3).
 - (2) Pens were constructed of wood supports (vs. recommended stainless steel, etc.; SEP pg. 3).
 - (3) No bath was provided (SEP pg. 3).
 - (4) Photoperiod for first eight weeks was maintained at 9 hours light/day (vs. 8 hours; SEP pg. 4).
 - (5) Ten footcandles of light (vs. 6; SEP pg. 4) was received by the birds.
 - (6) Provisions for minimizing food spillage (SEP pg. 4) were not reported.
 - (7) The percent of the final diet, represented by corn oil, was not reported (SEP pg. 4).
 - (8) Pesticide levels in test diets were not reported (SEP pg. 5).
 - (9) Continuous and discrete variables should be analyzed in specifically different ways (SEP pg. 9). The report made reference to several statistical treatments but did not assign them to particular variables. Attachment X, which addressed the details of the statistical methods,

was unreadable because of the quality of copying.

- (10) Because of poor copy, raw data could not be totally checked to insure consistency with the written report.

As long as the statistical approach (item 9, above) and raw data (item 10) check out, none of the above are likely to be serious enough to invalidate the study.

- B. Statistical Analysis: Discussed above.
- C. Discussion/Results: The text of the report refers to reproductive data summarized in Tables 1A and 1B; these tables are missing from the report.

The results of chemical analyses of the diets were not presented with the report. Without these results, it is impossible to determine the concentrations to which the birds were exposed. These data should be submitted by the registrant, so that a useful risk assessment can be accomplished.

The significance level used in the statistical analyses was apparently $p = 0.01$ as noted in the author's reference of "...a depression approaching statistical significance ($p < .02$) at the 200 ppm dosage level" when discussing the number of 14 day-old survivors of eggs set. Since the significance level associated with most tests is $p = 0.05$, the reviewer considers the ratio of 14 day-old survivors/eggs set to be significantly lower in the 200 ppm group when compared to the controls. The NOEC, therefore, was 100 ppm.

Based on what was readable, and relevant to eggshell thickness, feed consumption of adults, and the weights of eggs, hatchlings, and 14 day survivors, levels of DOWCO 233 up to 500 ppm appear not to have a negative effect. Discussion of other parameters will await analysis of readable raw data and associated independent statistical treatment.

- D. Adequacy of the Study:
- (1) Classification: Supplemental.
- (2) Rationale: This study appears scientifically sound and to have been carried out in a way that meets the intent of the Guidelines. It also appears that the text properly reflects that raw data

which are legible. However, many of the attachments are not legible, and a proper review and analysis of the report is impossible. Additionally, the results of chemical analyses of the diets were not presented with the report.

- (3) **Repairability:** Reparable if readable attachments for raw data and subsequent independent statistical analyses meet normal review standards, and if the registrant can submit the results of chemical analyses of the diets.

15. COMPLETION OF ONE-LINER: Yes; January 21, 1991.

REFERENCES

1. HASEMAN, J.K. and KUPPER, L.L. Analysis of Dichotomous Response Data From Certain Toxicological Experiments Bionatics, 35, 281-283, March 1979.
2. KUPPER, L.L. Statistical Models For The Analysis of Certain Toxicological Experiments, University of North Carolina Institute of Statistics Mimeo Sciences, No. 1169, March 1978.
3. KLEINMAN, J.C. Proportions With Extraneous Variance. Single and Independent Samples, JASA, March 1973, Vol. 68, No. 341, pp. 45-54.
4. GLADEU, B. The Use of the Jackknife to Estimate Proportions From Toxicological Data in the Presence of Litter Effects, JASA, June 1979, Vol. 74, No. 366, pp. 278-283.
5. COCHRAN, W.G. Analysis of Variance for Percentages Based on Unequal Numbers, JASA, 1943, Vol. 38, pp. 287-301.
6. ARMITAGE, P. Statistical Methods in Medical Research, John Wiley and Sons, 1971.

Shaughnessy No. 116002

Study/Species/Lab/
Succession

Chemical
Active

Chemical Name Triclopyr + triethylamine
(Dowco 233)

Chemical Class

Page 1 of 1

Avian Reproduction,

Species: Anas platyrhynchos

98.9%

Lab: Wildlife International

Acc
NAID 92189006

Group	Dose (ppm)	Effectuated/Parameters*	Mort. (X)	100% Inh.	Reviewer/	Validat.
					Date	Status
Control	0		3%	N/A		
Treatment I	100		0			
Treatment II	200	# 14 day-olds/eggs set	0			
Treatment III	500	" " " "	3%			

M. Whitten
1-21-91
Supplemented

Study Duration: 20 weeks

Comments: *statistics not verified due to illegible raw data

Field Study (Simulated/Actual)
Species:

Group	Rate (ai/a)	Treatment Interval	Total # Treatments	Mort. (X)
Control				
Treatment I				
Treatment II				
Treatment III				

Lab:

Acc.

Crop/Site: _____
Study Duration: _____
Comments: _____

Chronic fish,

Species

Lab:

Acc.

Concentrations Tested (ppm) = _____
MAIC = > _____ < _____ ppm.
Effectuated Parameter = _____
Concr. Mort. (X) = _____ Sol. Contr. Mort. (X) = _____
Comments: _____

Chronic invertebrate

Species

Lab

Acc.

Concentrations Tested (ppm) = _____
MAIC = > _____ < _____ ppm.
Effectuated Parameter(s) = _____
Concr. Mort. (X) = _____ Sol. Contr. Mort. (X) = _____
Comments: _____