MRID No. 31251 ~~92189005~~

DATA EVALUATION RECORD

1. **CHEMICAL:** Triclopyr triethylamine.
Shaughnessey Number: 116002.

2. **TEST MATERIAL:** DOWCO 233; 98.9% purity; AGR 134832; GHC 25-1-47; a low density, fibrous material with a slight odor.

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

4. **CITATION:** Beavers, J.B. 1979. DOWCO 233: A One-Generation Reproduction Study with the Bobwhite (Colinus virginianus). Laboratory Project No. 103-191. Prepared by Wildlife International Ltd., Easton, Maryland. Submitted by Dow Chemical Company. MRID No. 31251.

5. **REVIEWED BY:**

Wayne R. Marion, Ph.D.
Wildlife Ecologist
Wildlife Resources Management

Signature: Michael L. Whitten
Date: 1/22/91 For W. Marion

6. **APPROVED BY:**

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

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:** DOWCO 233 was administered to adult bobwhite quail at nominal dietary concentrations of 100 ppm, 200 ppm, and 500 ppm during a 19 week exposure period. At the dosage levels tested, DOWCO 233 appeared to have no effect on behavior or standard reproductive parameters. The study is scientifically sound, but does not meet the requirements for an avian reproductive test, since the results of the chemical analyses of the diets were not presented.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

-5 hrs

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in this study were unmated 5-month-old bobwhite quail from the production flock of Wildlife International Ltd., Easton, Maryland. A description of the acclimation period did not appear in the report. The study included 144 bobwhites (48 cocks, 96 hens) that were pen-reared, approaching their first breeding season, previously untreated, disease free, and phenotypically indistinguishable from wild birds. Immediately prior to the study, gross necropsy and serological examinations of representative specimens indicated that these birds were healthy.

B. **Test System:** The birds were housed in indoor pens (Georgia Quail Farm Breeding Pens Model 206) with 10 footcandles of light provided by Chroma 50 fluorescent lamps and temperatures maintained at between 65° and 85°F. Photoperiods of 9 hours of light per day were maintained for the first 7 weeks of the experiment and then 17 hours of light per day for the remainder of the test interval. Fresh air was provided 6 times per hour by time-clocked exhaust fans. A freezer, scales, and an incubator were available for use in the appropriate phases of the experiment.

C. **Dose/Diet Preparation/Food Consumption:** The basal diet used in these experiments was Wildlife International Ltd.'s game bird breeder ration containing 19.4% protein, 6.7% fat, and 3.8% fiber. The test chemical, DOWCO 233, and corn oil were incorporated into aliquots with a Hobart mixer and these concentrates were stored frozen until used for the weekly preparation of fresh diets. The experimental concentrations of 0 ppm (control), 100 ppm, 200 ppm, and 500 ppm were developed by mixing the appropriate DOWCO 233 concentrate with the bulk basal diet mix described above. Samples of the control diet and each of the treatment diets were shipped to the sponsor for DOWCO 233 analyses during the study. Both the control and experimental groups received the appropriate diets and water ad libitum for the duration of the study. Feed consumption was recorded bi-weekly throughout the study.

D. **Design:** The birds were randomly distributed into 4 groups as follows:

Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	12	1	2
100 ppm DOWCO 233	12	1	2
200 ppm DOWCO 233	12	1	2
500 ppm DOWCO 233	12	1	2

Adult bobwhites were housed indoors in pens described in the report as Georgia Quail Farm Breeding Pens (Model 206), but no other specific descriptions were provided.

E. **Adult Observations/Gross Pathology:** Adult birds were fed ad libitum the above diets for 11 weeks prior to egg laying and for 8 weeks during egg collection. Body weights for the adult birds were recorded at initiation of the experiment, on weeks 2, 4, 6, 8, and at the termination of the study. Body weights were not recorded during egg laying to avoid any possible adverse effects of handling during this period. Observations of adults for signs of toxicity and/or gross pathology apparently occurred regularly during this experiment, but these observations were not described in detail in the report.

F. **Eggs/Eggshell Thickness:** During egg laying, eggs were collected daily, marked, and stored at 65°F and 65% relative humidity. At weekly intervals, the stored eggs were fumigated and then placed in an incubator to be incubated at 99.5°F. Weekly throughout egg laying, one egg was randomly selected from every other pen in each experimental group and the controls to obtain egg weight and eggshell thickness measurements. Eggs were first weighed to the nearest 0.1 g. and then they were opened at the waist and prepared for the measurement of eggshell thickness. The average thickness of the dried shell plus the membrane was determined by measuring in 3-5 locations around the waist of the egg using a micrometer calibrated to 0.01 mm. All other eggs were candled on Day 1 of incubation for eggshell cracks; on Day 11 to measure embryo viability; and on Day 21 to measure embryo survival. On Day 21 of incubation, the eggs also were placed in a Humidaire Hatcher (Model No. 50) and allowed to hatch by Day 24 or 25.

G. **Hatchlings:** Average body weights of hatchlings were initially determined on Day 24 or 25 of incubation as the birds were removed from the incubator. Hatchlings were then housed according to the appropriate parental grouping in Beacon (Model B755) battery brooders maintained at 100°F until 14 days of age. During this time, the young birds were fed a basal diet of Wildlife International Ltd.'s game bird starter ration and water ad libitum. At age 14 days, the chicks were removed from the brooding units and the average body weights by parental pen of all survivors were determined.

H. **Statistics:** At the completion of the study, reproductive parameters were tested for statistically significant differences between the control group and each of the treatment groups using analysis of variance. Sample units were the individual pens within treatment groups. Percentage data were arcsine transformed prior to analysis. When a significant effect was found, t-tests were used for individual comparisons of doses versus the control. The following parameters were analyzed:

Eggs Laid
Eggs set
Eggs Cracked
Egg Weight
Eggshell Thickness
Viable Embryos
Live Three-Week Embryos
Hatchlings
Body-Weight of Hatchlings
14 Day-Old Survivors
Body Weight of 14 Day-Old Survivors

12. REPORTED RESULTS

A. **Diet Analysis:** No information was presented in the report regarding measured concentrations of DOWCO 233 in the diets.

B. **Mortality and Behavioral Reactions:** There were 10 mortalities reported during this study and all were among hens. Mortalities included 2, 1, 6, and 1 hens in the control, 100 ppm, 200 ppm, and 500 ppm groups, respectively. Necropsies of these birds indicated no evidence that these deaths were treatment-related, and they were considered incidental to treatment. Instances of abnormal physical conditions and behaviors

were reported, but these were rare and considered not related to treatment.

C. **Adult Body Weight and Food Consumption:** Neither adult body weights nor food consumption data were statistically analyzed. Adult body weight data appeared to be comparable between the control and treatment groups and there was no evidence of a treatment effect on this parameter. Similarly, food consumption did not appear to be dose responsive.

D. **Reproduction:** Statistical analysis revealed no significant reproductive impairment at the dosage levels tested.

E. **Eggshell Thickness:** The only statistically significant effect reported was a reduction in eggshell thickness ($p < 0.01$) at the 200 ppm dosage level, but there was no significant effect on the reproductive success of the birds at this level.

F. **Offspring Body Weight:** When compared to the control group, there were no significant differences in the body weights of hatchlings and at 14 days of age.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "DOWCO 233 was fed to mature bobwhite quail throughout a one-generation reproduction study at dietary concentrations of 100 ppm, 200 ppm, and 500 ppm. Based on the results of this study, environmental concentrations of up to 500 ppm of DOWCO 233 do not represent a reproductive hazard to bobwhite quail."

The study was conducted in conformance with Good Laboratory Practice Regulations. The data were inspected and the final report signed by the Quality Assurance Officer of Wildlife International, Ltd.

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

A. **Test Procedures:** The test procedures employed in this study were generally in compliance with the SEP, ASTM, and Subdivision E guidelines. Exceptions were:

No information was presented in the report regarding measured concentrations of DOWCO 233 in the diets.

The photoperiod during the first 7 weeks of the study was 9 hours of light per day; a 7-hour photoperiod is recommended during this period.

The recommended temperature in the adult pens is 21°C; the temperature in this study ranged from a low of 18°C to a high of 29°C (reported as 65°F and 85°F, respectively).

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

The report did not state whether the birds were acclimated to the facilities prior to the study.

The report did not state the schedule for making behavioral observations.

Behavioral observations of young birds were not reported.

B. **Statistical Analysis:** Statistical procedures used were generally in compliance with recommended methods. An exception was the author's use of t-tests for individual comparisons of treatment groups versus the control. Repeated applications of the t-test to test various treatments versus a control is discouraged, since this procedure increases the probability of committing a Type I error (saying two means are unequal when, in fact, they are equal). Multiple comparison procedures should have been used. In this case, however, the results were not affected, since the author found no differences between treatment and control values (except in eggshell thickness at 200 ppm, which was verified by the reviewer).

Analyses of reproductive parameters were verified (attached) and generally matched those reported by the author, except as discussed below.

Confirmation of the analyses revealed differences at 100 ppm for eggshell thickness and eggs cracked. At 200 ppm, differences were seen for eggs cracked, egg weight, eggshell thickness, and eggs hatched. However, the reproductive data in Tables 1-4 (attached) show that these differences do not appear to be dose related. Thus, the conclusion that reproductive success was not affected by treatments is supported.

C. **Discussion/Results:** The percentage of cracked eggs ranged from 5% to 9% (control = 5%). These values are of concern. Typically, for the bobwhite quail, 0.6 to 2.0% of the eggs may be expected to be cracked

(Technical Support Document to Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms). The authors provided no explanation for this unusually high percentage of cracked eggs. In this case, the values for cracked eggs do not appear to be treatment related, and the conclusion of no treatment effect for this parameter is accepted. Procedures should be implemented in future studies, however, to reduce the number of cracked eggs.

A discrepancy exists regarding the number of mortalities at 200 ppm; the text states (page 11) that 6 hens died in that group while the necropsy report included as Attachment I shows pathological observations for only 5 hens.

The mortality, behavioral data, and differences between groups in adult body weights and food consumption do not appear to be related to treatment. Similarly, the minor differences in various reproductive parameters do not appear to be dosage related and support the conclusion that there was no reproductive impairment.

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.

At nominal dietary concentrations of DOWCO 233 of up to 500 ppm, there was no evidence presented in this study that would indicate any toxicity to bobwhite quail in the areas of body weight, food consumption, behavior, and reproduction. The study is scientifically sound, but does not meet the requirements for an avian reproductive test, since the results of the chemical analyses of the diets were not presented with the report.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The results of chemical analyses of the diets were not presented with the report.

(3) **Repairability:** The report can be upgraded to core if the registrant can submit the results of chemical analyses of the diets.

15. **COMPLETION OF ONE-LINER:** Yes; January 7, 1991.

TABLE 1A

REPRODUCTIVE DATA - BOBWHITE QUAIL

	Controls	DOWCO 233 (ppm)		
		100	200	500
Eggs Laid	706	778	522	767
Eggs Cracked	33	71	49	46
Eggs Set	629	662	431	679
Viable Embryos	502	504	332	561
Live Three-Week Embryos	485	472	298	544
Normal Hatchlings	308	303	227	309
14 Day-Old Survivors	236	215	188	233
Eggs Laid/Hen in 8 Weeks*	29	32	22	32
14 Day-Old Survivors/Hen*	10	9	8	10

*Based on 24 hens.

TABLE 1B

REPRODUCTIVE SUCCESS - BOBWHITE QUAIL

	Controls	DOWCO 233 (ppm)		
		100	200	500
Eggs Laid of Theoretical Maximum (%)	53	58	39	57
Eggs Cracked of Eggs Laid (%)	5	9	9	6
Viable Embryos Of Eggs Set (%)	80	76	77	83
Live Three-Week Embryos Of Viable Embryos (%)	97	94	90	97
Normal Hatchlings Of Live Three-Week Embryos (%)	64	64	76	57
14 Day-Old Survivors Of Normal Hatchlings (%)	77	71	83	75
14 Day-Old Survivors Of Eggs Set (%)	38	32	44	34

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TABLE 25

BODY WEIGHT DATA - HATCHLINGS

BOBWHITE QUAIL

	Controls	DOWCO 233 (ppm)		
		100	200	500
No. Of Chicks Weighed	308	303	227	309
Mean Body Weight (g)	5.8	5.7	6.1	5.2

TABLE 26

BODY WEIGHT - 14 DAY-OLD SURVIVORS

BOBWHITE QUAIL

	Controls	DOWCO 233 (ppm)		
		100	200	500
No. Of Chicks Weighed	236	215	188	233
Mean Body Weight (g)	18	13	20	17

TABLE 27

EGG WEIGHT DATA - BOBWHITE QUAIL

	Controls	DOWCO 233 (ppm)		
		100	200	500
No. Of Eggs Weighed	44	44	41	42
Mean Egg Weight (g)	9.2	9.0	9.3	9.0

TABLE 3B

EGGSHELL THICKNESS DATA - BOBWHITE QUAIL

	Controls	DOWCO 233 (ppm)		
		100	200	500
No. Of Eggs Measured	44	41	41	42
Mean Shell Thickness (mm)	.221	.210	.206*	.217

*Statistically significant reduction ($p < .01$)

TABLE 4

BODY WEIGHT AND FEED CONSUMPTION DATA - ADULT BOBWHITE QUAIL

	Controls		DOWCO 233 (ppm)					
			100		200		500	
WEEK	B.W.	F.C.	B.W.	F.C.	B.W.	F.C.	B.W.	F.C.
0	169	-	171	-	181	-	165	-
2	192	19	195	20	198	18	190	19
4	201	18	199	20	200	17	198	20
6	200	14	202	18	204	15	200	17
8	205	15	206	16	206	14	204	16
10	-	15	-	18	-	14	-	16
12	-	17	-	18	-	14	-	17
14	-	18	-	20	-	19	-	18
16	-	19	-	22	-	19	-	20
18	-	20	-	23	-	19	-	22
Term.	220	23	225	25	215	21	213	24

The body weight data are presented as a group mean.
The feed consumption data are presented as the group mean feed consumption per bird per day.

B.W. - Body weight in grams
F.C. - Feed consumption in grams

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16

CONFIRMATION OF STATISTICS

```
DATA RAWDATA; INFILE DATA; INPUT EL EC EW ET ES VE LE NH NHW SV SVW;
DATA TRANFORM; SET RAWDATA;
 RESP=TOTAL/BASE; TRANRESP=ARSIN(SQRT(RESP));
PROC GLM; CLASS TRT;
 MODEL TRANRESP = TRT;  WEIGHT BASE;
 LSMEANS TRT / PDIFF; RUN;
```

General Linear Models Procedure

Class	Levels	Values
TRT	4	100 200 500 C (T1 T2 T3)

Number of observations in data set = 48

Dependent Variable	Weight	TRT Pr > F		Least Squares Means	100	200	500	C
EL	112	0.0383		LSMEAN	0.868	0.665	0.860	0.813
			C vs TRT	P > |T|	0.472	0.059	0.535	.
EC (Eggs cracked)	EL	0.0430		LSMEAN	0.297	0.285	0.240	0.185
			C vs TRT	P > |T|	**0.008**	**0.032**	0.183	.
EW (egg weight)		0.0013		LSMEAN	8.975	9.783	9.000	9.192
			C vs TRT	P > |T|	0.316	0.008 T2 > C	0.374	.
ET (eggshell thickness)		0.0095		LSMEAN	0.210	0.206	0.217	0.221
			C vs TRT	P > |T|	**0.023**	**0.002**	0.424	.
VE	ES	0.7773		LSMEAN	1.079	1.137	1.174	1.144
			C vs TRT	P > |T|	0.499	0.950	0.743	.
LE	VE	0.0829		LSMEAN	1.358	1.302	1.468	1.436
			C vs TRT	P > |T|	0.229	0.068	0.609	.
NH (number hatched)	LE	0.0177		LSMEAN	0.934	1.075	0.867	0.927
			C vs TRT	P > |T|	0.906	0.025	0.270	.

observations in data set = 32

NHW	NH	0.0167		LSMEAN	5.732	6.125	5.202	5.763
			C vs TRT	P > |T|	0.904	0.192	0.034	.
SV	NH	0.2442		LSMEAN	1.005	1.159	1.103	1.075
			C vs TRT	P > |T|	0.327	0.274	0.686	.
SV	ES	0.3512		LSMEAN	0.603	0.720	0.621	0.650
			C vs TRT	P > |T|	0.440	0.309	0.629	.
SVW	SV	0.0695		LSMEAN	18.73	19.80	16.96	18.25
			C vs TRT	P > |T|	0.636	0.143	0.196	.

Due to missing values, only 31 observations can be used in this analysis.

Shaughnessey No. 116002 Chemical Name Triclopyr triethlamine (DowCo 233) Page 1 of 1

Study/Species/Lab/Succession | Chemical % Active | Results | Reviewer/Date | Validat. Status

Avian Reproduction,

Species: Bobwhite quail (Colinus virginianus) 98.9%

Lab: Wildlife International Ltd

~~Acc~~ MRID No. 92189005

Group	Dose(ppm)	Effected/Parameters	Mort.(%)	1C% Inh.
Control	0	NONE	6%	N/A
Treatment I	100		3%	
Treatment II	200		17%	
Treatment III	500		3%	

Study Duration: 19 weeks

Comments:

M.L. Whitten 1-7-91 Supplemental

Field Study(Simulated/Actual)

Species:

Lab:

Acc.

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

Crop/Site: Study Duration:

Comments:

Chronic fish,

Species

Lab:

Acc.

Concentrations Tested (pp__)=

MATC = >____ <____ PP___. Effected Parameter =

Contr. Mort.(%)= Sol. Contr. Mort.(%)=

Comments:

Chronic invertebrate

Species

Lab

Acc.

Concentrations Tested (pp__)=

MATC =>____< ____PP___. Effected Parameter(s)

Contr. Mort.(%)= Sol. Contr. Mort.(%)=

Comments: