

US EPA ARCHIVE DOCUMENT

12/13/82

CASE GS0199 TERBUFOS PM 100

CHEM 105001 Terbufos

BRANCH ~~XXX~~ ^{EEG} DISC TOPIC GUIDELINE

FORMULATION

FICHE/MASTER ID 00097892 CONTENT CAT

Fink, R.; Reno, F.E. (1973) Final Report: One-Generation Reproduction Study--
Mallard Ducks: Project No. 362-146. (Unpublished study) CDL:090808.

SUBST, CLASS

OTHER SUBJECT DESCRIPTORS

PRIM:
SECI

DIRECT RYM TIME 12 hr. (MM) START-DATE 10/14/82 END DATE 12/2/82

REVIEWED BY: James D. Felkel
TITLE: Wildlife Biologist
ORG: Ecological Effects Branch, Hazard Evaluation Division (TS-769)
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APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

9 pages

DATA EVALUATION RECORD

1. Chemical: Terbufos (Shaughnessy #105001)
2. Formulation: Technical AC92100 89.0%
3. Citation: Fink, R.; Reno, F.E. (1973) Final Report: One-Generation Reproduction Study--Mallard Ducks: Project No. 362-146. (Unpublished Study) CDL:090808. MRID#00097892
4. Reviewed by: James D. Felkel, Wildlife Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769)
5. Date Reviewed: October 21, 1982
6. Test Type: Avian Reproduction
 - A. Test Species: Mallard (Anas platyrhynchos)
7. Reported Results: Technical AC92100 does not appear to pose a reproductive hazard to mallards at 2-20 ppm, as no statistically significant differences, compared to controls, were seen at these levels. However, 20 ppm is approaching a level at which reproductive impairment should be expected.
8. Reviewer's Conclusions: This study is scientifically sound but pen-by-pen data are required for statistical evaluation of results. The study may fully meet the intent of proposed guidelines (7/10/78) following submission and evaluation of pen-by-pen data.

Methods

The test material, a clear liquid, was received on 3/29/73. Pen-reared mallard ducks, nine months old, were received from Frost Game Farm, Coloma, WI and quarantined for 10 days at the Truslow Farms research facility. Birds were examined for injury and flight feathers were dipped on the right wing to facilitate handling. Five males and five females were sent to the Maryland Animal Health Dept. Laboratory for analysis and diagnosed negative for abnormal lesions at necropsy, bacteriology, and serology. 105 birds (30 males, 75 females) were randomly distributed into the following groups:

	Dosage	#Pens	Birds/Pen	
			Male	Female
1 - Controls	0	5	2	5
2 - AC92100	2 ppm	5	2	5
3 - AC92100	20 ppm	5	2	5

Technical AC92100 was added to corn oil and pre-mixed with a basal diet (commercial game bird breeder ration). These concentrates were frozen and used for weekly preparation of fresh diets.

All birds received the appropriate diet ad libitum for the entire 18-week study. Indoor pens (4x15x8') had an automatic watering trough, gravity-fed feeder and a 2x4' nesting area. Each pen was washed 3x/week and the nesting material (straw) changed 2x/week during egg laying.

The photoperiod for the first 8 weeks (4/4/73-5/30/73) was 7 hours of light/day. It was then increased by 17 hours of light/day and further increased by 15 minutes/week for the next 10 weeks. Illumination was 5 footcandles.

Body weights were recorded at initiation of the study, prior to egg-laying, and at termination. Food consumption was recorded bi-weekly.

Eggs were collected daily, marked as to pen, and stored at 16°C and 55% relative humidity. Weekly, they were treated with a 2% formaldehyde solution for 30 seconds to reduce E. coli contamination and placed in a Chick Master (Model 52E) incubator.

Incubation temperature was 37.2-37.4°C. Eggs were candled on day 0 for cracks, on day 14 to measure embryonation and on day 21 to measure embryo survival. On day 25, eggs were placed in a Robins' Incubator (Model 17H) and allowed to hatch; on day 28, all eggs were removed and hatchlings housed by parental grouping and maintained on the control diet for 14 days.

Weekly, approximately 5% of the eggs with sound shells from each group were randomly selected to measure eggshell thickness. Eggs were opened at the waist, thoroughly washed out, and the shells dried for one week at 24°C. The thickness of the dried shell plus membrane at the waist was measured by micrometer.

Statistical Analysis

Individual pen data were analyzed by a single classification analysis of variance, or F-test. Variances were tested for heterogeneity. If homogeneous variances were found, the F-Test was completed. When a significant F value was obtained, significant group differences were determined by Scheffe's method of multiple group comparisons. Where variances were heterogeneous, data were log-transformed and analyzed as above if variances were homogeneous. If variances were still heterogeneous, comparisons of individual groups were made by a T-test for unequal variances. Null hypothesis rejections were made at $p \leq 0.05$.

Results

There were no mortalities, toxicity symptoms, or behavioral abnormalities during the study. Reproductive data, eggshell thickness data, and body weight/food consumption data are presented in Table 1-3. Statistical analysis of body weight, food consumption, eggs laid, eggs cracked, eggs embryonated, live 3-week embryos, normal hatchlings, 14-day old survivors, and eggshell thickness revealed no differences between controls and treatments. The reduction in eggs laid at 20 ppm was approaching significance and considered by the investigators to be "biologically meaningful" (i.e., slightly less within-group variance and it would have been significant).

Reviewer's Evaluation

Procedures and analysis were generally consistent with proposed guidelines (7/10/78). None of the inconsistencies (e.g., body weights recorded at initiation, before egg laying and at termination rather than at weeks 0, 2, 4, 6, 8, and termination; eggshell thickness measured weekly on 5% of eggs with sound shells rather than on all newly-laid eggs every two weeks) would prevent the study from meeting the intent of the guidelines. However, results cannot be critically reviewed until the pen-by-pen data is submitted to permit statistical evaluation.

An initial screen was performed on the summary data submitted, using the "SUPER" (chi-square) program available at EEB. Impairment ($p < 0.05$) overall at 20 ppm and in viable embryos of eggs set at 2 ppm were detected. However, these results must be confirmed or rejected by ARSIN analysis (requiring pen-by-pen data) before any conclusions can be drawn. ANOVA analyses (e.g., of eggs laid and of 14-day old survivors at 20 ppm, relative to controls, since ARSIN analysis does not address these) also require pen-by-pen data.

Conclusions

1. Category: Supplemental.
2. Rationale: Methods were generally consistent with proposed guidelines (7/10/78). However, pen-by-pen data are required for statistical evaluation of results.
3. Repairability: Yes. Study may be upgraded following submission and evaluation of pen-by-pen data.



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TABLE 1a
Reproductive Data - Mallard Ducks

	Controls	Technical AC 92100	
		2 ppm	20 ppm
Eggs Laid	479	494	310*
Eggs Cracked	16	8	11
Eggs Set**	425	447	261
Eggs Embryonated	381	340	237
Live Three-Week Embryos	374	333	233
Normal Hatchlings	284	269	198
Fourteen-Day-Old Survivors	257	250	182

* Difference from control approaching significance.

** Excludes those cracked and those removed for eggshell thickness analysis.

TABLE 1b
Reproductive Success - Mallard Ducks

	Controls	Technical AC 92100	
		2 ppm	20 ppm
Eggs Laid Per Hen In Eight Weeks	19.2	19.8	12.4*
Eggs Cracked Of Eggs Laid (%)	3.3	1.6	3.5
Eggs Embryonated Of Eggs Set (%)	89.6	76.1	90.8
Live Three-Week Embryos Of Embryonated Eggs (%)	98.2	97.9	98.3
Normal Hatchlings Of Live Three-Week Embryos (%)	75.9	80.8	85.0
Fourteen-Day Survivors Of Normal Hatchlings (%)	90.5	92.9	91.9
Fourteen-Day Survivors Per Hen	10.3	10.0	7.3

* Difference from control approaching significance.



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TABLE 2
Eggshell Thickness - Mallard Ducks

	Controls	Technical AC 92100	
		2 ppm	20 ppm
No. Of Eggs Analyzed	40	40	40
Mean Shell Thickness (mm)	0.345	0.343	0.346

The above differences were not significant at the 95 per cent level of confidence.

TABLE 3
Body Weight and Food Consumption - Mallard Ducks

WEEK	Controls		Technical AC 92100 2 ppm		Technical AC 92100 20 ppm	
	B.W.	F.C.	B.W.	F.C.	B.W.	F.C.
	9	9	9	9	9	9
0	1028	-	1076	-	1057	-
2		100		99.7		101.8
4		90.5		87.3		93.3
6		100.8		101.5		102.6
8	1050	113.7	1065	130.1	1039	126.8
10		131.6		145.7		145.6
12		152.7		147.5		150.9
14		146.9		142.9		148.4
16		138.2		143.8		162.5
18	1092	135.1	1065	141.5	1030	157.0

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

The above differences were not significant at the 95 per cent level of confidence.

B.W. = Body Weight
F.C. = Food Consumption

END OF EXECUTION
 CPU TIME: 1.14 ELAPSED TIME: 1:5 .34
 EXIT
 @EXECUTE SUPER FOR
 LINK: Loading
 [LNKXCT FOR execution]

ENTER IN ORDER:
 EGGS LAID, EGGS CRACKED, EGGS SET, VIABLE EMBRYOS
 3-WEEK LIVE EMBRYOS, NORMAL HATCHLINGS, AND
 14-DAY SURVIVORS FOR CONTROLS

Mallard

479 16 425 381 374 284 257

ENTER IN ORDER:
 EGGS LAID, EGGS CRACKED, EGGS SET, VIABLE EMBRYOS
 14-DAY SURVIVORS FOR A TREATMENT GROUP

2 ppm

494 8 447 340 333 269 250

TOTAL CHISQUARE= 2.5552780

CONTROLS

LOST	ALIVE	TOTAL
16.00	463.00	479.00
44.00	381.00	425.00
7.00	374.00	381.00
90.00	284.00	374.00
27.00	257.00	284.00

TREATMENT GROUP

LOST	ALIVE	TOTAL	CHI
8.00	486.00	494.00	2.32
107.00	340.00	447.00	29.00
7.00	333.00	340.00	0.24
54.00	269.00	333.00	2.15
19.00	250.00	269.00	0.78

CHI 1DF=3.84 (P<0.05)

ENTER IN ORDER:
 EGGS LAID,EGGS CRACKED,EGGS SET,VIABLE EMBRYOS
 3-WEEK LIVE EMBRYOS,NORMAL HATCHLINGS,AND
 14-DAY SURVIVORS FOR CONTROLS

479 16 425 381 374 284 257

ENTER IN ORDER:
 EGGS LAID,EGGS CRACKED,EGGS SET,VIABLE EMBRYOS
 14-DAY SURVIVORS FOR A TREATMENT GROUP

20 ppm

310 11 261 237 233 198 182

TOTAL CHISQUARE= 4.462144D

CONTROLS

LOST	ALIVE	TOTAL
16.00	463.00	479.00
44.00	381.00	425.00
7.00	374.00	381.00
90.00	284.00	374.00
27.00	257.00	284.00

TREATMENT GROUP

LOST	ALIVE	TOTAL	CHI
11.00	299.00	310.00	0.13
24.00	237.00	261.00	0.13
4.00	233.00	237.00	0.03
35.00	198.00	233.00	6.63
16.00	182.00	198.00	0.14

CHI 1DF=3.84 (P<0.05)