

DATA EVALUATION RECORD

Mancozeb Avian Reproduction Bobwhite quail

1-30-92

1. CHEMICAL: Mancozeb.
2. TEST MATERIAL: Mancozeb (Dithane M-45 Technical fungicide
Percent ai = 81.4%
3. STUDY TYPE: 71-4
Avian Reproduction Study- Upland game
Bobwhite quail (*Colinus virginianus*).
4. CITATION:

Hakin, B. and D.O. Chanter. 1990. The Effect of Dietary Inclusion of Mancozeb on reproduction in the Bobwhite quail. HRC Report No.: R&H 60/89385. R&H Report No.: 88RC-0102. Huntingdon Research Centre Ltd., P.O. 2, Huntingdon, Cambridgeshire, PE18 6ES, UK. Submitted by: Rohm & Haas Co., Independence Mall West, Philadelphia, PA. 19105. MRID-415667-02.

5. REVIEWED BY:

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6. APPROVED BY:

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7. CONCLUSION:

This study does not meet the guideline criteria. See page 5 Table 1 for summary of results.

8. Background: Submitted as result of the Registration Standard.

9. MATERIALS AND METHODS:

A. Test Organisms:

1. Species Bobwhite Quail (*Colinus virginianus*).
 - a. Supplier: D.R. and R.E. Wise, Monkfield, Bourn, Cambridgeshire.

- b. Same hatch: Not reported.
 - c. Same source: Yes.
 - d. Approaching the first breeding season: Yes.
 - e. Indistinguishable from wild birds: Not reported.
 - f. Age: 10-11 months.
 - g. Pen reared: Not reported.
2. Health: Reported as healthy birds.
 - a. Weight loss: Not reported.
 - b. Sickness: Not reported.
 - c. Mortality: Not reported.
 - d. History: Not reported.
3. Acclimation period- 7 days.
- B. Test System:
1. No. per level: 20.
 - a. No. of dose levels: 3 (250, 500, 1000, ppm) + control.
 - b. No. of pens per level: 20.
 - c. Male to female ratio per pen: 1 to 1.
 2. Parents' Pen Facilities.
 - a. Temperature- maximum 23° C, minimum 21° C.
 - b. Relative humidity- 63%.
 - c. Minimize cross contamination: Not reported.
 - d. Pen materials: polytene coated steel wire pen; 30 x 40 x 25 cm.
 - e. Ventilation: Not reported.
 3. Chicks' Pen Facilities.
 - a. Temperature- maximum 25°C, minimum 22° C.
 - b. Relative Humidity- 46% ± 8.7.
 - c. Pen materials: wooden pens with concrete floors.
 - d. Ventilation: Continuous ventilation fans.

4. Photoperiod:

- a. Parents- Seven hours of light and 17 hours of darkness until the end of the 6th week. Then the light was increased to 16 hours a day until the end of the study.
- b. Chick- photoperiod: Continuous illumination.

5. Bodyweight.

Bird weights taken on the following days of the study -
-7, 0, 14, 28, 42, 56, 70, and 175.

6. Food consumption

Food consumption for adult birds was taken every week from -1 to 25.

7. Dose preparation-

- a. The rationale for the selection of the dosage levels was, "Known treatment levels in crops, and the OECD limit dose of 1000 ppm were to select dose levels."
- b. Dose level factor: 2.
- c. Vehicle 2% of the diet: no vehicle reported.
- d. Commercial game bird breeder ration: "The diet was known to contain no added antibiotics or other growth promoters..."
- e. Store to maintain stability: prepared once a week; stored at -20° C; concentration in the diet was measured every 4 weeks; after week-24 the change ranged from +13% to -17%.
- f. Amount of control diet vehicle: no vehicle.

7. Feeding and Husbandry.

- a. Appropriate diet: Quail layer diet.
- b. Water: domestic quality drinking water.
- c. Test diet for at least 10 weeks: egg production started on week-12.

8. Egg collection, Storage, and Incubation.

- a. Collected daily: yes.
- b. Stored at 16° C and 65% relative humidity: temp. 16° C, humidity not reported.
- c. Set at weekly intervals for incubation: yes.
- d. Canded day 0 cracks: yes.

- e. Day 11 fertility: yes.
- f. Day 18 early death of embryos: yes.
- g. Temp for hatching: 37.5%.
- h. Relative humidity: Not reported.

9. Observations of Chicks.

- a. Hatchability recorded: on day 22.
- b. Control diet for 14 days: HRC chick meal; length of time on this diet not reported.

10. Eggshell Thickness.

- a. Eggs were collected one day every two weeks starting the 2nd week of egg laying or the 15 week.
- b. Air dry for 48 hours: Yes.
- c. Measure 4 points in 0.01 mm: Yes.

11. Withdrawal.

Reduced reproduction was not evident.

10. REPORTED RESULTS:

A. Statistically significant parameters: (Excerpted from the citation).

TABLE 1.

Summary of reproductive data.

Observation	Control	Mancozeb		
		250 ppm	500 ppm	1000 ppm
Mean no. of eggs laid per pen	42.0	42.1	38.8	31.6
Mean egg weight (g)	10.5	10.0	10.1	9.9**
Egg shell thickness	0.2	0.2	0.2	0.2
Egg cracked or broken as % of eggs laid	11	16	16	16
Infertile eggs as % of eggs set	15	28	17	24
Early embryonic deaths as % of those set at Day 11	1	1	1	1
Late embryonic deaths as % of those set at Day 1	3	3	2	5
Hatchlings as % of fertile eggs	86	87	88	83
14-day survivors as % of hatchlings	92	90	91*	83*
Chick bodyweights (g)				
- Day 0	7.2	6.8	7.0	6.6**
- Day 14	26	23*	24*	23**

* Significantly different from the control ($P < 0.05$).

** Significantly different from the control ($P < 0.01$).

B. Statistical methods: (Excerpted from the citation).

"The factors for analysis of variance/covariance were (i) positional (batteries and rows by columns within batteries), (ii) treatment.

"Analysis of covariance was used when records spanned the start of treatment; this was appropriate for the analyses of adult food consumption and adult bodyweights. A covariate representing pre-dose data is used to adjust the post-treatment data for group differences that are evident before the treatments are applied and hence cannot be attributed the treatment.

"Data that were proportions were transformed before analysis of variance. Then the light was increased to 16 hours until the end of the study.

C. Chick-photoperiod- Continuous illumination.

D. Bodyweight.

Bird weights taken on the following days of the study -
-7, 0, 14, 28, 42, 56, 70, and 175

E. Food consumption.

Food consumption for adult birds was taken every week significance levels of the variance ratios from the analysis of variance.

"Comparisons between treatment means were made using Williams' test (2) for contrasting increasing dose levels of a compound with a zero dose control. Significant differences are indicated in the Tables using the conventional asterisk notation:

** $P \leq 0.01$

* $0.01 < P \leq 0.05$ "

Excerpted from study - See attached Addendum 2 for discussion of the statistical results.

F. Results of chemical analysis:

Measured concentrations of the test diet ranged from 9% over the nominal to 11% below the nominal with the exception of the 24 week sample which was minus 17% for the 1000 ppm level, 13% above the 500 ppm level and minus 14% for the 250 ppm level.

G. Mortality and observations:

Between weeks -1 to 13, 4 adult birds died.

Between weeks 14 and 25, the egg production period, 10 adult birds died.

H. Gross pathology- (Excerpted from the citation).

"Most of the post-mortem findings were of physical injuries and were considered to be related to treatment.

"All birds surviving until the study terminated at the end of week 25 were examined post-mortem. No abnormalities were detected bird."

11. STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

Signed GPL and quality control statements were attached.

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

A. Test Procedure- The several items do not meet the guideline criteria:

1. Acclimation period should have been a minimum of 2 weeks rather than 7 days.
2. During the acclimation period the temperature and humidity range from 23° C to 21° C and 63% ± 8.6. The recommended values are 21° C and 55%.
3. The photoperiod used was seven hours of light and 17 hours dark until the end of the 6th week. Then the light was increased to 16 hours until the end of the study. The recommended photoperiod is 7 hours of light during the 1st eight weeks and 16-17 hours during the following 12 weeks; both at 6 footcandles.
4. The light was reported in lux rather than footcandles.
5. Temperature and humidity at hatching should be 39° C and 70%, respectively, the temperature was reported as 37.5° C and the humidity was not reported.
6. Eggs stored at 16° C, but the humidity was not reported.
7. "Birds that died during the pre-laying phase were replaced and data from the replacements have been included in all the analyses." The replicates with new birds can not be compared to the original replicates.
8. Eggs shell cracking ranged from 11% for the control group and 16% for the treated groups. These values exceed normal parameters.
9. The lowest level 250 ppm was statistical significant for 14 day chick bodyweight. Therefore all dosages showed effects and the no-effect-level was not determined.
10. The highest dosage level, 1000 ppm, is below the expected field concentrations. The turf and fruit crops both can be expected to produce concentrations above 1000 ppm (see Appendix I for estimated concentrations on bird food items).
11. The cause of death of the birds (replicates 24B, 35B, 42C, and 46C) dying before the start of the study was not discussed.
12. The deaths during the study in replicates 59c, 41c, and 6a were discussed.
13. The description of the parent birds did not include whether the bird were from the same hatch, if they were

indistinguishable from wild birds, and if they were pen reared.

14. The description of the parental housing did not address ventilation.

B. Statistical Analysis:

The results both in the summarized form and in the Appendix II were provided on a weekly basis rather than the totals for each replicate, the basis for the reported statistical analysis.

The SAS program which addressed the following parameters agrees with the submitted results: Eggs laid, Eggs cracked, Eggs set, Viable embryos, Live embryos, Normal Hatchlings, and 14 day old survivors.

C. Discussion/Results:

The study does not meet the intent of the guidelines. Of the items listed in A. the following are the most significant:

1. The use of replacement pairs would jeopardize the integrity of the test population. This would require the pairs to be identical in every aspect. This is nearly impossible when items such as mate compatibility are considered.
2. The high number of eggs cracked may be an abnormal screen of the eggs, changing the egg depend variables.

The excessive percentage of cracked eggs in the control invalidates all of the variables. There are two lines of reasoning for rejecting all of the other variables: 1) underlying physical causes and 2) mathematical consequences.

The excessive cracking may have been caused by some factor(s) that also affected the other variables. Poor equipment or handling might directly cause cracking, disturbance might produce behavior in the parents that leads to cracking, or the strain of quail might be inbred and have a higher cracking rate. Any of these unknown causes might affect the other variables in an unpredictable manner.

The excessive cracking would change the number of specimens available for analysis. The mathematical effect of removing an unknown portion of fertilized eggs from the experimental population is not predictable. The excessive cracking would change the number of quail chicks produced, etc. in a manner that is not predictable.

HRC has had some problems with the egg cracking rate, but other laboratories have been able to meet this re-

quirement. EEB must conclude that the requirement is reasonable and should be applied to all registrants.

3. Cause of death of the birds dying before the start of the study was not discussed. This may reflect on the birds health and should be discussed.
4. The lack of a no-effect-level limits the usefulness of the study in risk assessment to determine minimal risk situations.
5. Highest dose did not consider the concentrations which may occur when the maximum application rates for fruits and turf are considered. Hence, the potential for reproductive impairment has not been addressed by this study for those uses.

Notice: There is a high probability that the last two items may not have occurred if the guideline requiring the use of a factor of 5 for the feed concentrations would have been used.

D. Adequacy of the Study:

1. Classification: Invalid.
2. Rationale: The excessive egg cracking invalidates this study by itself.
3. Repair: None.

13. COMPLETION OF ONE-LINER FOR STUDY- No.

APPENDIX I

Calculation of the potential mancozeb concentration of wild-life food.

Known Information:

1. Maximum turf application rate: 8 oz product/1000 ft²
 2. Percent active ingredient: 80%
 3. 43,560 ft²/A
 4. Assumption: 1 gal of mancozeb = 1 gal of water in weight.
 5. 1 gal of water weighs 8.3 lbs
- $8 \text{ oz product/1000 ft}^2 \times 0.8 \text{ (80\% ai)} = 6.4 \text{ oz/1000 ft}^2$
 $6.4 \text{ oz/1000 ft}^2 \times 43,560 \text{ ft}^2 / 1000 \text{ ft}^2 = 278.784 \text{ oz ai/A}$
 $278.784 \text{ oz ai/A} / 128 \text{ oz / gal} = 2.178 \text{ gals ai/A}$
 $2.178 \text{ gals ai/A} \times 8.3 \text{ lb} = 18 \text{ lbs/A}$

At an application rate of 18 lbs/A for turf and 10 lbs/A for fruit crops, basis on Hoerger and Kenaga (1972) the following concentrations can be expected on bird food items:

Plant Category	TURF		FRUITS	
	Upper Limit	Typical limit	Upper Limit	Typical Limit
Range Grass	4320	2250	2400	1250
Grass	1980	1656	1100	920
Leaves and Leafy Crops	2250	630	1250	350
Forage Crops	1044	594	580	330

APPENDIX II

	Control	Treatment		
		250	500	1000
Eggs laid	833	835	771	626
Eggs laid/hen/season	42	42.1	38.8	31.6
Eggs cracked*	95	132	126	98
Eggs cracked/20 hens/season	4.75	6.6	ons-	
Viable embryos (11 days)	575	465	494	363
Percent of eggs laid	69.0	55.7	64.1	58.0
Percent of eggs set	84.1	71.9	82.8	75.2
Live 18-day embryos	556	450	482	346
Percent of viable embryos	96.8	96.8	97.6	95.4
Hatchlings	506	407	436	303
Percent of eggs laid	60.7	48.7	56.6	48.4
Percent of eggs set	74.0	63.9	73.0	62.7
Percent of viable embryos	88.0	87.5	88.3	83.5
Percent of 18-day embryos	91.0	90.4	90.5	87.6
14-day survivors ***	456	364	390	260
Percent of normal hatchlings	90.1	89.4	89.4	85.8
Average hatch weight (g)	6.8	6.4	7.1	6.1
Average 14-day-old survivors' weight (g)	26	21	24	21
Adult body weight (g/bird) (at study termination)				
Females	212	208	208	203
Males	189	185	190	189
Adult bodyweight (g/bird) Increase compared with Day 0				
Females	-3.1%	-5.6%	3.1%	2.1%
Males	8.2%	6.1%	7.2%	5.2%
Mean eggshell thickness	.20	.198	.195	.198
Mean egg weight	10.5	10.0	10.1	9.9

LITERATURE CITATIONS

Hoerger, F.D. and E.E. Kenaga. 1972. Pesticide residues on plants correlation of representative data as a basis for estimation of their magnitude in the environment. in Environmental quality. Academic Press, New York, I:9-28.