10-14-87

DATA EVALUATION RECORD PAGE 1 OF

CASE: GS0088 DIMETHOATE

CONT-CAT: 01 GUIDELINES: 71-4

00159768 Munk, R. (1986) 1-Generation Reproduction Study with Dimethoate on the Mallard Duck (Anas platyrhynchos L.) by Administration in the Diet: Test Report: Project No. 72W326/82. Unpublished study prepared by BASF Toxikologie. 190 p.

REVIEW RESULTS:
VALID____ INVALID____ INCOMPLETE x

GUIDELINE: SATISFIED ___ PARTIALLY SATISFIED ____ NOT SATISFIED x

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DIRECT RVW TIME = 32 hours START DATE: 7/8/87 END DATE: 8/3/87

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DATA EVALUATION REPORT

1. Chemical: Dimethoate

2. Test Material: Dimethoate (= DIM; test compound 82/326)
Batch No. 611A - 97.3% purity

3. Study Type: Avian Reproduction on Mallard Duck

Species Tested: Anas platyrhynchos

4. Study ID: Munk, R. (1986) 1-Generation Reproduction Study with Dimethoate on the Mallard Duck (Anas platyrhynchos L.) by Administration in the Diet: Test Report: Project No. 72W326/82. Unpublished study prepared by BASF Toxikologie. 190 p. TRID No. 470201-057, MRID No. 00159768.

5. Reviewed By: Candy Brassard
EEB/HED
Signature: Candy Brassard
Date: 8/8/87

6. Approved By: Douglas J. Urban
Head, Review Section III
EEB/HED
Signature:
Date: 8/19/87

7. Conclusions:

It appears from the submitted data that the NOEL was $\leq$ 30 mg/kg of the diet (ppm) when mallards were exposed to dimethoate. This study appears to be scientifically sound; however, there are major discrepencies that detract from the study. Therefore, this study is classified as Supplemental. Since two of the six control pens did not produce eggs, this study cannot be upgraded.

8. Recommendations:

- EEB recommends that 6-foot candles (60 Lux) be used for lighting instead of 220 Lux. This increase may have caused reproduction impairment such as infertility.

- The study author should implement a study design using the number of pens necessary to determine significant differences between treated and control birds. Ideally, a test should be designed to detect a difference of 25 percent at $p < 0.05$, in 8 of 10 such experiments (power, that is $1-\beta = 0.8$) (ASTM 1986).

- The study author should report cause of mortalities for both adults and chicks. Gross necropsies are preferred (D. McLane 1986).

9. Background:

This study is submitted in response to data requirements in the Registration Standard.

10. Discussion of Individual Test: N/A.

11. Materials and Methods:

a. Test Animals - Ducks approaching their first breeding season (6 months old) were kept in groups of two males and five females per replicate. The birds were obtained from Englemoorhof, Ornamental Waterfowl Farm, J.J. Wiard Fischer, Am Grun 19, D-2930 Varel 1, FRG (a total of 70 males and 140 females for acclimation). The birds were randomly assigned to their test cages. The birds were fed commercial feed "pputt LMS Legemehl" and water ad libitum.

b. Test System - The adult birds were housed in pens made of galvanized steel wire with stainless steel wire floors. The floor area was 1.95 m x 1.3 m, and height = 1.3 m. The temperature was generally 22 ± 3 °C, and relative humidity ranged from 50 to 80 percent.

The light intensity consisted of 220 Lux at the level of the ducks; warm light fluorescent lamps. The lighting regime was as follows:

Week of Test		Hours of Light	Hours of Dark
-2 to 0	Settling-in	7	17
1 to 8	Feeding period	7	17
9	before egg-	9	15
10	laying	12	12
11	Egg-laying period	12	12
12		14	10
13 to 22		17	7

Chicks - The chicks were housed the first week after hatch at 40 to 50 °C and the second week at 25 to 30 °C. Relative humidity was generally 50 to 80 °C. The photoperiod consisted of 16 hours light, 8 hours dark. The chicks were fed "Club" turkey starter and water ad libitum. The cages were made of stainless steel and the floors were made of stainless steel bars.

The chicks were not identified individually after hatching. They were kept in groups comprised of the chicks of each hatch of each replicate. Thus, the replicate was the smallest unit for statistical evaluation of chick data.

The following additives were put into the chick diet:

Vitamin A, Vitamin D_3, Vitamin E, Avoparcine, Ronidazol, Monensin-sodium, and BHT (an antioxidant known as butylated hydroxytoluene).

c. Dose - The ducks were kept in groups of two males/five females with a total of six replicates per concentration or control.

An additional two replicates with spare birds were maintained on each of the three treatments under the same conditions for use as replacement birds if necessary during the pre-egg production period.

The treatment levels were 6 mg/kg and 30 mg/kg in the diet.

d. Study Design - A 2-week acclimation period, 10-week pre-egg production period, and a 13-egg production period was implemented.

The following parameters were measured:

- No. eggs laid--at beginning of egg production total/day/replicate and total/week/replicate recorded.
- No. eggs cracked (7-day intervals-candling,days 14 and 21).
- Mean egg weight (7-day intervals).
- Mean egg shell thickness (mm) (measured weeks, 1, 3, 5, 7, 9, 11, 13, 15).
- No. of eggs incubated (at weekly intervals).
- No. of fertile eggs.
- No. of infertile eggs.
- No. of early embryonic mortalities.
- No. of late embryonic mortalities.
- No. of total embryonic deaths.
- No. of "dead in shell."
- No. of chicks hatched.
- No. of chicks surviving to 14 days.
- Mean body weight of chicks at hatching (g).
- Mean body weight of chicks 14 days after hatching (g).
- Total feed consumption/pen/day; mean/pen/week was calculated and recorded; observations on palatability were made.

- Body weight of adult birds were measured during acclimation period, day 0, and after 2, 4, 6, and 8 weeks of pre-egg production period and at test termination.
- Postmortem examination was performed on all birds which died during the test or were sacrificed at the end.
- All eggs were candled on days 14 and 21 of incubation period for evaluation of infertilities, early and late embryonic deaths (see Attachment A for definitions).

e. Statistics - Statistical analysis was carried out in the following parameters:

- Adult food consumption.
- Adult body weight.
- Number of eggs laid and proportion damaged.
- Egg weight.
- Eggshell thickness.
- Numbers of infertilities, embryonic mortalities, and hatching.
- Number of 14-day-old surviving chicks.
- Chick body weight at hatching and 14 days later.

12. Reported Results:

See Attachment B for a summary of egg production and chick data.

According to the study author, egg production was abnormally low in all groups (including the control). According to a note by the breeder, this was a general observation for mallard ducks hatched in the spring of 1983 in central Europe and England. Egg production in groups 1 and 2 was not significantly different from that in the control group.

- Proportions of infertile eggs were very variable, probably due to the stage of sexual maturation of the males and its different span.

- The numbers of early embryonic deaths were slightly higher in groups 1 and 2 than in group 0 (not statistically significant or dose-related).

- Late embryonic deaths were lower for group 2 (30 mg/kg), but not statistically significant.

- A few mortalities in the group 7 chicks were reported. The chicks were reported to be in good health.

- Initial body weights of F_1 chicks for weeks 1 to 4 were statistically significant for both treatment groups when compared to control. However, for weeks 5 to 9, 10 to 13, and for the whole egg-laying period (weeks 1-13), the body weights were not statistically significant.

All other parameters that were measured or observed were found to be in the "normal limits" and not expected to be dose-related.

13. Study Authors' Conclusions/QA Measures (excerpted from submission):

> Under the conditions of this study, there was no evidence that the dietary administration of Dimethoate at levels of 6 and 30 mg/kg diet had any adverse effects on any of the parameters examined.
>
> The quality assurance unit inspected the study, audited the final report, and reported findings to the study director and to management.

Dates of inspection ranged from February 21, 1984 to April 16, 1986.

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

The following discrepancies were noted in the study:

a. Test Procedures - The study author (or company) should justify the use of the following additives in the chicks' diets: Avoparcine, Ronidazol, and Monensin-sodium.

- Only four of the six control pens produced eggs. Therefore, this study could not be considered acceptable. Table A lists the data for each pen for the control and the two treatment levels.

- With regard to pen facilities, it is desirable to offer mallards water in which to bathe.

- The study author reported using a light intensity of 220 Lux. The SEP Guidelines recommend 6 foot candles at the bird level--this is approximately 60 Lux. The 220 Lux far exceeds the recommended 60 Lux.

- The dosage of 6 mg/kg is lower than what is expected for the birds to be exposed to. The Dimethoate Registration Standard indicated residue levels as high as 1250 ppm (based on a maximum application rate of 10 lb ai/A).

- This study, if conducted correctly, should have indicated a no-observable-effect level (NOEL) and a lowest-observed-effect level (LOEL). The level at which waterfowl may be exposed to is much higher than 6 to 30 mg/kg.

- With regards to mortalities, gross necropsies are preferred. When performed, all dead birds should be examined, as well as a sufficient number of survivors in order to provide characterization of gross lesions. Inspections of the GI tract, liver, kidneys, heart, reproductive organs, and spleen should be made. Also subcutaneous fat and muscles should be examined for evidence of deterioration (McLane 1986). These data should be recorded and submitted to EPA for review. With regards to the reported necropsies(p. 38 of the report), how were these mortalities not associated with exposure to Dimethoate, specifically bird no. 79?

- The SEP Guidelines do not require measurement of the reproductive organs. For future reference, if measurements are to be done, then the most appropriate method to determine a difference is by weight (mg). The reference points used in this study such as "size of cucumber seed" or "thick as a string" are inappropriate.

- The study author should report what criteria was used in the number of eggs /week were used for egg shell thickness measurements. The percent varied between the treatment groups.

b. Statistical Results - EEB used an ANOVA program on the data from the pens that produced eggs. The data indicate that there was no significant difference between the two treatment levels and the control. Therefore, the NOEL $\leq$ 30 mg/kg.

See Table B for the Analysis of Reproductive Effects.

c. Discussion/Results - The eggs laid per hen was only 9.2 for the control group. It is very clear that the control did not include enough pens that laid eggs.

The percent of normal hatchlings of live 3-week embryos of 50 percent is in the low end.

It appears from the analysis of reproductive effects Table B, the percent eggs cracked increased with both treatment levels, and the percent of normal hatchlings of live 3-week embryos decreased with both treatment levels.

The mean eggshell thickness slightly decreased in both treatment levels when compared to the control. In particular, weeks 10 to 13 showed a decrease in thickness of both treatment levels (.36 mm) when compared to control (.41 mm). No statistical significance was evident between the control and the two treatment levels.

The average feed consumption did not significantly decrease when the treatment levels were compared to control, indicating palatability did not seem to be hindered.

d. Adequacy of Study

1) Classification - Supplemental for 97.3% technical dimethoate.

2) Rationale - The study appears to be scientifically sound; however, there are major discrepancies that detract from the study.

One major concern is that 33 percent of the control hens did not produce any eggs. Other concerns are as follows:

- Additives in the chicks diet; and

- The levels tested were inappropriate since the level of exposure is expected to be much higher.

3) Repairability - Since 33 percent of the control hens did not produce eggs, the study cannot be repaired.

The data requirement for 71-4 using the waterfowl is still unfulfilled.

Citations/References

ASTM Standard E 1062-86 (1986) Standard Practice for Conducting Reproductive Studies with Avian Species. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

McLane, D. Avian Reproduction Test (1986) Standard Evaluation Procedure, Hazard Evaluation Division, U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC 20460, EPA 540/9-86-139.

Table A. Summary of Reproductive Data

	Eggs Laid	Eggs Cracked	Eggs Set	Viable Embryos	Live Embryos	Normal Hatchlings
Control (A)	56	0	52	34	33	21
	118	7	100	68	65	40
	0	-	-	-	-	-
	0	-	-	-	-	-
	60	5	51	50	46	17
	43	1	38	26	24	6
Treatment Level (B) 6 mg/kg	112	6	97	46	36	5
	43	2	38	36	33	11
	144	10	119	110	93	53
	35	2	31	9	6	0
	0	-	-	-	-	-
	42	4	34	34	34	18
Treatment Level (C) 30 mg/kg	53	2	48	46	42	24
	47	1	43	42	41	16
	135	12	112	82	76	33
	28	1	26	8	7	2
	0	-	-	-	-	-
	8	1	6	1	-	-

Table B. Analysis of Reproductive Effects

	Control	6 mg/kg	30 mg/kg
Eggs Laid/Hen	9.2	12.5	9.0
Eggs Cracked/ (%) Eggs Laid	4.6	6.3	6.2
Viable Embryos of Eggs Set (%)	74	74	74
Live 3-Week Embryos Percent of Viable Embryos	94	86	95
Normal Hatchlings of Live 3-Week Embryos (%)	50	43	45
14-Day-Old Survivors per Hen*	2.7	2.8	2.4
per Hen**	4.1	3.3	2.9
14-Day-Old Survivors of Normal Hatchlings (%)	98	95	96
Mean Egg Shell Thickness	0.37	0.36	0.34
Average Hatch Weight	35.9	33.3	33.3
Average 14-Day-Old Survivors Weight	261	236	258
Adult Body Weight (g)/Bird			
Females	1003	1040	999
Males	1136	1114	1097
Adult Body Weight % Increase Compared to Day 0			
Female	+6.4	+5.2	7.2
Male	+5.8	+16.5	9.6
Total Male & Female	+6.4	+8.0	+7.9
Mean Egg Weight	58.6	53.7	56.0
Average Feed Consumption Pre-Egg Production Period	155	149	170
Egg Production Period Mean Total	163	162	181
Mortalities - Adult	2	3	3
Chicks	2	4	3

*Total hens used per study--including pens that did not produce eggs.
**Excluding pens that did not lay eggs.

Attachment A

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5.3. Fertility, embryonic development and hatching

5.3.1. Infertile eggs

Recording of infertile eggs; infertile eggs are defined as being "clear" on day 14 candling.

5.3.2. Early embryonic deaths

These are mortalities before full differentation of the embryo and are recorded on day 14 candling.

5.3.3. Late embryonic deaths

These are mortalities recorded at day 21 candling when the embryo is fully differentiated.

5.3.4. Chicks "dead in shell"

Recorded at hatching chicks "dead in shell" are considered to have been fully formed and viable.

5.3.5. Number of chicks hatched

The number of chicks hatched was recorded.

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5.3.6. Abnormalities

Any abnormalities in chicks hatched were recorded.

5.4. F_1 chicks

All chicks hatched alive were reared until they were 14 days old. The following parameters were recorded:

5.4.1. Mortalities and toxic signs

Daily (incomplete recording for chicks from egg-laying weeks 2 and 3)

5.4.2. Body weights

Individual body weights were recorded within a period of 24 hours after hatching and on day 14.

5.4.3. Number of 14-day survivors

The number of 14-day survivors was recorded.

5.4.4. Post-mortem examination

All chicks which died during the 14-day observation period were examined macroscopically for gross-pathological abnormalities.

No post-mortem examination was carried out at termination.

Attachment 5/11 B

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Mallard Reproduction data

Summary of egg production and chick data

Group	0	1	2
Treatment (mg/kg)	0 (control)	6	30
No. of eggs laid	277	376	271
No. of cracked and broken eggs	13	24	17
Mean egg weight (g)	58.4	53.7	56
Mean egg shell thickness (mm)	0.37	0.36	0.37
No. of eggs incubated	241	319	235
No. of fertile eggs	178	235	179
No. of infertile eggs	63	84	56
No. of early embryonic mortalities	5	23	11
No. of late embryonic mortalities	5	10	2
No. of total embryonic deaths	10	33	13
No. of "dead in shell"	84	115	91
No. of chicks hatched	84	87	75
No. of chicks surviving to 14 days	82	83	72
Mean body weight of chicks at hatching (g)	36	33	33
Mean body weight of chicks 14 days after hatching (g)	261	236	258

Summary of egg production and chick data expressed as percentages

Group	0	1	2
Treatment (ppm)	0 (control)	6	30
Cracked and broken eggs as a % of total laid	4.7	6.4	6.3
Fertile eggs as a % of total incubated	73.9	73.7	76.2
Infertile eggs as a % of total incubated	26.1	26.3	23.8
Early embryonic mortalities as a % of fertile eggs	2.8	9.8	6.1
Late embryonic mortalities as a % of fertile eggs	2.8	4.3	1.1
% of total embryonic deaths of fertile eggs	5.6	14.0	7.3
"Dead in shell" as a % of fertile eggs	47	49	51
Hatchability (chicks hatched as a % of fertile eggs)	47	37	42
Chicks surviving to 14 days as a % chicks hatched	98	95	96.0

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