

2-6-88  
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DATA EVALUATION RECORD

1. CHEMICAL: Diazinon MG-8
2. TEST MATERIAL: Diazinon MG-8; Code FL800945 (89.2%).  
Assigned Wildlife International i.d. No. WI-715.
3. STUDY TYPE: Avian Acute Oral LD<sub>50</sub>  
Species tested: Mallard Duck  
(Anas platyrhynchos)
4. CITATION: (No Author). 19081. Acute Oral LD<sub>50</sub> - Mallard Duck: Diazinon MG-8. Final Report. Prepared by Wildlife International Ltd., Solitude Creek Farm, St. Michaels, MD. Submitted by Ciba-Geigy Corp., Greensboro, NC. Project No: 108-197.

5. REVIEWED BY:

Jeffrey L. Lincer, Ph.D.  
Eco-Analysts, Inc.  
Sarasota, FL

Signature:  
Date: January 31, 1988

6. APPROVED BY:

James R. Newman, Ph.D.  
Proj. Mgr., KBN Engineering  
and Applied Sciences, Inc.

Signature:  
Date:

*James R. Newman*  
*2/6/88*

Henry T. Craven  
Chief EEB/HED  
USEPA

Signature:  
Date:

7. CONCLUSIONS:

This study is basically scientifically sound.

The approximate acute oral LD<sub>50</sub> of Diazinon MG-8 in the mallard duck is 8.7 mg/kg, with confidence limits (95%) of zero to infinity. Some regurgitation was observed, the significance of which may be clearer upon review of applicant's response to issues raised in 14D(2). If the above calculated LD<sub>50</sub> approximates the real LD<sub>50</sub> (see e.i) then Diazinon MG-8 is very highly toxic to mallards when gavaged with this chemical.

This study does not fulfill the guideline requirements but may upon submission of information requested in 14D(2).

8. RECOMMENDATIONS: Respond to questions in 14D(2).
9. BACKGROUND: N/A
10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: N/A
11. MATERIALS AND METHODS (PROTOCOLS):
  - A. Test Animals: Mallard ducks, six months old at the beginning of the study, were used. Source: Wildlife International Ltd.'s production flock, Easton, MD.
  - B. Dosage:

<u>Treatment</u>	<u>Pens</u>	<u>Birds/Pen</u>	<u>Dosage Level (mg/kg)</u>
Controls	1	10	Corn Oil Only
Experimental	5	10	1.59, 2.51, 3.98, 6.31 & 10.00

Feed was withheld from the control and test birds for 15 hours prior to oral administration of the experimental material.

The experimental material was dissolved in corn oil and intubated directly into the crop via a stainless steel catheter. Each bird was individually weighed and dosed on the basis of milligrams of material per kilograms of body weight. The control birds received a corresponding volume of corn oil only. The ratio of experimental material to diluent was adjusted so that each bird received an approximately constant volume to body weight does. "For the purposes of dosage administration and LD<sub>50</sub> calculations, the experimental material was assumed to be 100 percent active material and the LD<sub>50</sub>, as reported, is therefore of the experimental material as received."

C. Design:

Two weeks prior to initiation of the study, the birds were placed in pens identical to those to be utilized for testing. The birds were allowed to acclimate themselves to the environment under which testing would occur. Any group of birds which exhibited abnormal behavioral patterns or deviated from the norm were not utilized for testing purposes.

At the end of the acclimation period, the birds were randomly assigned to the above treatment groups, utilizing five birds of each sex per group.

D. Statistics: Mortality data were analyzed by probit analysis developed for Wildlife International.

12. REPORTED RESULTS:

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MORTALITY OF MALLARDS GAVAGED  
WITH DIAZINON MG-8 (89.2%)

<u>Dosage (mg/kg)</u>	<u>Mortality</u>
Controls	0
1.59	0
2.51	20
3.98	20
6.31	0
10.00	80

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"Controls - There were no mortalities in the negative control group during the course of the study. All birds were normal in both appearance and behavior throughout the test period.

"Experimental Material - There was a 20% mortality rate at both the 2.51 mg/kg and 3.98 mg/kg dosage levels, and an 80% mortality rate at the 10.0 mg/kg dosage level." There were no mortalities in the 1.59 or 6.31 mg/kg dosage levels.

"At the 1.59 mg/kg dosage level all birds appeared normal throughout the test period. At the 2.51 mg/kg dosage level no overt symptoms of toxicity were noted immediately after dosing. Symptoms of toxicity displayed by some birds at this dosage level during Day 1 included lethargy, loss of coordination and lower limb weakness. In addition, the two drakes which were found dead at this dosage level on Day 1 also exhibited lower limb rigidity and prolapse of the penis. A few birds at this dosage level remained asymptomatic during Day 1. All birds at this, and all other dosage level appeared normal by Day 2, and remained so until the termination of the study. At the 3.98 mg/kg dosage level symptoms of toxicity observed during Day 1 included lethargy progressing to depression, reduced reaction to external stimuli (sound and movement), with some wing droop, loss of coordination, and lower limb weakness, with a few birds also exhibiting prostrate posture and loss of righting reflex. The two drakes found dead at this dosage level also exhibited lower limb rigidity and prolapse of the penis.

"At the 6.31 mg/kg dosage level lower limb weakness was noted immediately after dosing. Regurgitation was also noted, and a few birds remained asymptomatic during Day 1. Symptoms of toxicity exhibited by most birds at this dosage level included lethargy progressing to depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, and lower limb weakness, with a few birds also exhibiting prostrate posture, loss of righting reflex and lower limb rigidity.

"At the 10.00 mg/kg dosage level depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, salivation, lacrimation and prolapse of the penis in drakes was observed following dosing, and, along with prostrate posture, loss of righting reflex and lower limb rigidity, continued to be exhibited through Day 1. Both surviving birds at this dosage level were asymptomatic by Day 2, but showed a very slight loss of body weight and a reduction in feed consumption for the test period.

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

"The acute oral LD<sub>50</sub> of Diazinon MG8 in the Mallard duck is 8.5 mg/kg, confidence limits (95%), 5.3 mg/kg to 13.7 mg/kg."

"The Quality Assurance Unit certifies the following with regard to 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(s):

This study was performed prior to the availability of both the Subdivision E Hazard Evaluation (Oct., 1982) and the current SEP for LD<sub>50</sub>'s (June, 1985). However, the test procedures used in this study are, basically, in compliance with those guidelines.

The following are exceptions:

(1) Reporting Requirements

- (a) SEP (pg. 5) requires that the slope of the dose-response line be calculated and reported. It was not reported.
- (b) SEP (pg. 5) indicates that gross necropsies are preferred; but none were reported.

(2) Materials and Methods

- (a) Regurgitation was reported for the 6.31 mg/kg group. SEP (pg. 7) indicates that if regurgitation is a problem, the test may have to be rerun.
- (b) Raw mortality data are consistent with the text.

- B. Statistical Analysis: The authors indicate that, "For purposes of dosage administration and LD<sub>50</sub> calculations, the experimental material was assumed to be 100 percent active material...." Although not clear from the text, for purposes of this review, it was assumed that all dosages were adjusted to 100% a.i.

Confirmation of statistical analysis indicated that because of the mortality pattern, neither the probit nor the moving average method could be used in calculating an LD<sub>50</sub>. An approximate LD<sub>50</sub> of 8.65 mg/kg with a confidence limit of zero to infinity was estimated using TOXANAL.

C. Discussion/Results:

(1) Behavioral Observations

- (a) Sublethal effects (i.e. depression and/or lethargy, reduced reaction to external stimuli, loss of coordination, lower limb weakness and/or loss of righting reflex) at even the next to the lowest dosage administered (i.e. 2.51 mg/kg) have negative implications with respect to survival in the wild.
- (b) In that regurgitation occurred in the 6.31 mg/kg group (but not higher groups), it would be desirable to confirm that it didn't occur in other groups. If it did not, applicant should comment on why regurgitation was so widespread during a similarly-carried-out study on Diazinon MG8, with the mallard (Project #108-281), but not this one.

(2) Implications of Dose-Mortality Response

- (a) The dose-response relationship is represented by a steep line, over a fairly narrow range.

(b) The absence of any mortality in the 6.31 mg/kg group is consistent with the observed regurgitation in this group.

(c) Since the lowest dosage (i.e. 1.59 mg/kg) resulted in no mortality or overt signs of toxicity, 1.59 mg/kg can be considered a NOEL.

(3) Gross Necropsies

(a) No comments - gross necropsies were not reported.

(4) Descriptive Categorization of Results

(a) With a calculated LD<sub>50</sub> (irrespective of some regurgitation) of 8.5 mg/kg, Diazinon MG-8 is very highly toxic to mallards when gavaged with this chemical.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: Study is scientifically sound and results may be useful in a risk assessment. However, the following issues need to be addressed:

(a) What is the slope of the dose-response curve?

(b) Confirm that gross necropsies were not performed or, if they were, provide the findings.

(c) Confirm that regurgitation only occurred in the 6.31 mg/kg group or provide details of additional observations.

(d) If regurgitation was limited to the 6.31 mg/kg group, why was this behavior so widespread under a similarly-conducted study with Diazinon MG-8 and the mallard (i.e. Project #108-281)?

(e) Were diets and/or birds analyzed for diazinon? If so, what were the results?

(3) Reparability: Depending on the applicant's response to the above questions, it is possible that this study could be upgraded to Core.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, on January 31, 1988.

16. CBI APPENDIX: N/A



J Newman Diazinon M68 MALLARD DUCK 02-08-88

PROJ. No. 108-197

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10	10	8	80	5.46875
6.31	10	0	0	9.765625E-02
3.98	10	2	20	5.46875
2.51	10	2	20	5.46875
1.59	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LD50 FOR THIS SET OF DATA IS 8.650862

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	3.747254	3.418193	1.652151E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.705944  
95 PERCENT CONFIDENCE LIMITS = -2.532176 AND 7.944064

LD50 = 8.537963  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LD10 = 2.897458  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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ONE LINER SHEET

Shaughnessey No. \_\_\_\_\_ Chemical Name Diazinon MG-8 Chemical Class \_\_\_\_\_ Page 1 of 1

Study/Species/Lab/ Accession # \_\_\_\_\_ Chemical % a.i. \_\_\_\_\_ Results \_\_\_\_\_ Reviewer/ Date \_\_\_\_\_ Validation Status \_\_\_\_\_

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14-Day Single Dose Oral LD<sub>50</sub> 95% C.L.  
 LD<sub>50</sub> = 8.65 mg/kg (0 - infinity) Contr. Mort. (%) = 0

Slope = (not given) # Animals/Level = 10 Age (Months) = 6 Lincer/ 1-31-88  
 Sex = 5 males + Supplemental

Species: Mallard duck 5 females/group

Lab: Wildlife Int. Ltd. 89.2

Project No: 108-197

AC #: 14-Day Dose Level mg/kg (% Mortality)  
 1.59 (0), 2.51 (20), 3.98 (20), 6.31 (0), 10.00 (80)

Comments: \* Information requested on occurrence of some regurgitation and missing information, required by guidelines.

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