2-6-88 X 070

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon MG8

2. TEST MATERIAL: Diazinon MG8; Fl. No. 861103: Reported purity of 86.6% (Assigned Wildlife International Ltd. i.d. No. WIL-1148).

3. STUDY TYPE: Avian Acute Oral LD_{50}
Species tested: Mallard Duck
(Anas platyrhynchos)

4. CITATION: Grimes, J. and M. Jaber. 1987. Diazinon MG8: An Acute Oral Toxicity Study with the Mallard. Project No. 108-281. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corp., Greensboro, NC. Project #: 108-281

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: James R. Newman
Date: 2/6/88

Henry T. Craven
Chief EEB/HED
USEPA

Signature:
Date:

7. CONCLUSIONS:

This study is basically scientifically sound but regurgitation makes any calculated LD_{50}, c.i. or dose-response line of questionable value.

A precise mallard acute oral LD_{50} value for Diazinon MG8 could not be determined due to regurgitation. Test data indicate the LD_{50} value might be greater than 6.0 mg/kg but less than 24.6 mg/kg. The calculated LD_{50}, irrespective of regurgitation, was 14 mg/kg with a 95% confidence interval of 11 mg/kg to 18 mg/kg. The no-observed-effect dosage could not be identified but it was less than 6 mg/kg, the lowest dosage tested, based upon mortality and overt signs of toxicity at the 6.0 mg/kg dosage.

Study does not fulfill the guideline requirements but may be useful for risk assessment purposes.

8. RECOMMENDATIONS: If the regurgitation problem cannot be corrected with mallards, Diazinon MG8 should be pilot-tested on quail and/or other species. Once a species is identified that can be used, range-finding studies should be carried out, followed by a formal LD_{50} study, using the appropriate dosages.

9. BACKGROUND: N/A

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: N/A

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: All mallards were 19 weeks of age and appeared to be in good health at initiation of the study. Mallards ranged in weight from 814 grams to 1286 grams at study initiation. The birds were obtained from Whistling Wings, Hanover, Illinois. All birds were pen-reared and phenotypically indistinguishable from wild birds.

B. Dosage: The test material was dispersed in corn oil. The concentration of the test material in the diluent was adjusted to provide a constant volume to body weight dosage for all treatment birds. All dosages were adjusted to 100% active ingredient. Therefore, all dosages and the LD_{50} value are reported as milligrams of active ingredient per kilogram of body weight. Nominal dosages used in this study were 6.0, 9.6, 15.4, 24.6 and 39.3 milligrams a.i. of Diazinon MG8 per kilogram of body weight.

C. Design: Groups of ten mallards (five males and five females) were assigned to each of the treatment groups and the control group by random draw. The test consisted of a geometric series of five dosage groups and a control group. Nominal dosages used in this study were 67.0, 9.6, 15.4, 24.6 and 39.3 milligrams of active ingredient (a.i.) Diazinon MG8 per kilogram of body weight. The dosages were established based upon known toxicity data. The control group was dosed with diluent only.

Birds were acclimated for twenty days prior to test initiation. The birds were fasted for at least 15 hours prior to dosing. At initiation of the test, a single dose of the test material in diluent was orally intubated directly into the crop or proventriculus of each bird using a stainless steel catheter. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received a corresponding volume of diluent only. All treatment and control birds received a constant dosage volume of 4 milliliters per kilogram of body weight.

The primary phases of this study and their durations were:

Acclimation - 20 days.

Fasting - At least 15 hours prior to dosing.

Dosing - The date of study initiation - March 10, 1987.

Post-dosing observation - 14 days.

D. Statistics: An LD_{50} value, along with 95% confidence limits, was calculated using the computer program of C. E. Stephan (U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota, 1978. Personal Communication.). In this study, probit analysis was used.

12. REPORTED RESULTS:

CUMULATIVE MORTALITY OF MALLARDS
GAVAGED WITH DIAZINON MG8

Dosage mg (a.i.)/kg	Percent Dead
Control	0
6.0	10
9.6	10
15.4	40
24.6	100
39.3	100

"CONTROLS

"There were no mortalities in the control group. All birds were normal in appearance and behavior throughout the test period...."

"DIAZINON MG8

"There was 10% mortality (1 out of 10) at the 6.0 and 9.6 mg/kg dosages, 40% mortality (4 out of 10) at the 15.4 mg/kg dosage and 100% mortality (10 out of 10) at the 24.6 and 39.3 mg/kg dosages....

"At the 6.0 mg/kg dosage, overt signs of toxicity were first observed approximately 1 3/4 hours after dosing. One bird was observed regurgitating at approximately 2 1/2 hours after dosing. The single mortality was noted on the morning of Day 1. All survivors were normal in appearance and behavior from the morning of Day 1 until study termination.

"At the 9.6 mg/kg dosage, overt signs of toxicity were first observed approximately 1 1/4 hours after dosing. Two males also were observed regurgitating at that time. A total of five birds were observed regurgitating on the day of dosing. The single mortality was noted approximately 2 1/2 hours after dosing. By the morning of Day 1, all survivors were normal in appearance and behavior until study termination.

"At the 15.4 mg/kg dosage, overt signs of toxicity were first observed approximately 1 hour after dosing. Five birds also were observed regurgitating at that time. A total of seven birds were observed regurgitating on the day of dosing. Mortalities were noted on Day 0 (approximately 2 hours after dosing) and on the morning of Day 1. All survivors were normal in appearance and behavior from the morning of Day 1 until study termination.

"At the 24.6 mg/kg dosage, overt signs of toxicity were first observed approximately 50 minutes after dosing. Four birds also were observed regurgitating at that time. A total of seven birds were observed regurgitating on the day of dosing. By the morning of Day 1, all birds had died.

"At the 39.3 mg/kg dosage, overt signs of toxicity were first observed approximately 30 minutes after dosing. One male was observed regurgitating and three birds were found dead approximately 55 minutes after dosing. Within approximately 1 3/4 hours of dosing, all birds had died.

"Overt signs of toxicity characteristic of intoxication with Diazinon MG8 included depression and/or lethargy, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, lacrimation, prolapsed penis, convulsions and coma.

"Body weight and feed consumption measurements were variable and may have been confounded at least in part by regurgitation of the test substance by some individuals. When compared to the controls, there was a reduction in body weight gain or body weight loss among males at the 9.6 mg/kg dosage and 15.4 mg/kg dosage from Day 0 to Day 3. In addition, there may have been a reduction in weight gain among females at 6.0 mg/kg. A corresponding reduction in feed consumption was noted among males at the 15.4 mg/kg dosage. Due to total mortality, effects on body weight and feed consumption could not be determined at the 24.6 and 39.3 mg/kg dosages."

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

Authors indicated, "... a precise LD_{50} value for Diazinon MG8 in this study could not be determined due to regurgitation. Although regurgitation was observed at all dosages tested, overt signs of toxicity and mortalities also were noted indicating that a significant amount of test material had been absorbed. When regurgitation was considered, test results indicated the LD_{50} value to be greater than 6 mg/kg and less than 24.6 mg/kg. Those results correspond closely with the calculated LD_{50} value for Diazinon MG8, irrespective of regurgitation, which was determined to be 14 mg/kg with a 95% confidence interval of 11 mg/kg to 18 mg/kg. The no-observed-effect dosage was less than 6.0 mg/kg, based upon mortality and overt signs of toxicity at the 6.0 mg/kg dosage.

"This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs (Federal Register, Volume 48, No. 230, November 29, 1983, pages 53946-53969). The final report was determined to be an accurate reflection of the data obtained. The dates of all audits and the ... results of those audits were reported to the Study Director/Laboratory Management ...." A total of 4 audits were carried out by QA personnel during the experimental and reporting phases of the study.

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

A. Test Procedure(s): Authors adjusted dosages to 100% a.i. for dosage administration and for the purpose of calculating LD_{50} and c.i. Raw mortality, weight change and feed intake data were consistent with written report.

Test procedures were, basically, in compliance with SEP protocol, with the following exceptions:

(1) Test Conditions

(a) SEP (pg. 4) suggests a photoperiod of 10-hours light/14-hours dark. Study provided 8-hours light/day.

(2) Reporting Requirements

(a) SEP (pg. 5) requires that the slope of the dose-response line should be calculated and reported. This was not done.

(b) SEP (pg. 5) indicates that gross necropsies are preferred. Apparently, these were not done.

(3) Regurgitation

(a) Regurgitation was observed in all but the control group. SEP (pg. 7) indicates that, "If [regurgitation] is a problem, the test may need to be rerun."

B. Statistical Analysis: Authors adjusted dosage for percent a.i. for purposes of calculating LD_{50} and c.i. (see attached printouts for statistical confirmation 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, convulsions and/or coma) at even the lowest dosage administered (i.e. 6.0 mg/kg) have negative implications with respect to survival in the wild.

(b) In that regurgitation was observed in all but the control group, the calculated LD_{50} and c.i. is of questionable value. If this problem cannot be avoided with mallards, perhaps exploratory tests could be done on bobwhite quail, and/or an acceptable passerine, to determine if they would be more acceptable species for this test with Diazinon MG8.

(2) Implications of Dose-Mortality Response

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

(b) Since the lowest dosage (i.e. 6.0 mg/kg) resulted in mortality and overt signs of toxicity, a NOEL could not be identified.

(3) Gross Necropsies

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

(4) Descriptive Categorization of Results

(a) With a calculated LD_{50} (irrespective of regurgitation) of 14 mg/kg, Diazinon MG8 is highly toxic to mallards when gavaged with this chemical.

(b) In that regurgitation was widespread in all groups, except the control, the above toxicity classification is probably a conservative estimate of this chemical's toxicity to mallards.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The study, itself, was carried out in a scientifically sound manner; however, the observed regurgitation by all but the control group makes calculation of the LD_{50}, c.,i. and dose-response line questionable. To that extent, the results do not meet the guideline requirements but the information produced may be useful in a risk assessment.

(3) Reparability: Not reparable.

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

16. CBI APPENDIX: N/A

J Newman Diazinon NGB MALLARD DUCK 02-08-88 PROJ. No. 108-201

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
39.3	10	10	100	9.765625E-02
24.6	10	10	100	9.765625E-02
15.4	10	4	40	37.69531
9.600001	10	10	1	10
1.074219				
6	10	1	10	1.074219

THE BINOMIAL TEST SHOWS THAT 9.600001 AND 24.6 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 16.34234

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LD50	95 PERCENT CONFIDENCE LIMITS	
4	.1677542	13.80801	10.17151	17.95075

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2063515	1	.1250697

SLOPE = 5.377947

95 PERCENT CONFIDENCE LIMITS = 2.934965 AND 7.820929

LD50 = 14.258

95 PERCENT CONFIDENCE LIMITS = 11.27171 AND 17.97494

LD10 = 8.277688

95 PERCENT CONFIDENCE LIMITS = 4.648009 AND 10.61684

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ONE LINER SHEET

Shaughnessey No._____ Chemical Name Diazinon MG8 Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Accession #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD_{50} = 14* mg/kg (95% C.L. (11-18*)) Contr. Mort. (%) = 0		
Species: Mallard Duck		Slope = 5.378 # Animals/Level = 10 Age (Weeks) = 19 Sex = 5 males + 5 females/group	Lincer/ 1-31-88	Supplemental
Lab: Wildlife Int. Ltd.	86.6			
Project No: 108-221		14-Day Dose Level mg/kg (% Mortality)		
AC #:		6.0 (10), 9.6 (10), 15.4 (40), 24.6 (100), 39.3 (100)		

Comments: * Regurgitation did not allow an accurate estimate of a LD_{50}, c.i. or NOEL.

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