2-6-88

X
Invalid

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. number WIL-1148).

3. STUDY TYPE: Avian Dietary LC_{50}
Species tested: Mallard Duck
(Anas platyrhynchos)

4. CITATION: Grimes, J. and M. Jaber. 1987. Diazinon MG8: A Comparison of Dietary LC_{50} Values Under Choice and No-Choice Feeding Conditions. Project No.: 108-276. Prepared by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corp., Greensboro, NC. Project No: 108-276.

5. REVIEWED BY:

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

Signature:
Date: 1/24/88

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 not scientifically valid.

With an LC_{50} of less than 47 ppm (under a no-choice situation), Diazinon MG8 is very highly toxic to mallard ducks. Reported conclusions, regarding repellency of this chemical, are challenged (see 14C).

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-71-2

This study does not fulfill the data requirements.

The primary reasons for rejecting the study are because choice of exposure levels did not include low enough concentrations to result in partial kills (see 14C), thereby precluding the calculation of an LC_{50}. Although repellency testing is not an SEP requirement, applicant has attempted to demonstrate this quality. Inconsistent results and an absence of replicates and feed residue data prevent concluding that this compound is repellant enough to be of biological significance.

8. RECOMMENDATIONS: Repeat the no-choice study, using low enough dietary concentrations to meet the SEP guidelines regarding partial kills and calculation of NOEL and LC_{50}. If a study is to be repeated to address the repellency question, the design should be such that confounding events are at least minimized to the greatest extent possible. Other considerations include: proper replicates, accuracy of diet intake measurements, dietary levels of environmental relevance, residue analyses of both feed and bird and, generally, an experimental design more sensitive to testing the null hypothesis.

9. BACKGROUND: N/A

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: Mallard duck (Anas platyrhynchos); 10 days old. Hatched on 12/29/86. Source: Whistling Wings, Hanover, Ill.

B. Dosage: Dietary exposure for 5 days. Test material was dispersed in corn oil and mixed with feed in a Hobart mixer. Corn oil concentration in treated and control diets - 2%. Final diets (for test duration) were prepared on the day of study initiation.

C. Design: The test consisted of two treatment groups: one treatment group was exposed to Diazinon MG8 in the diet for five days under test conditions that conform to FIFRA guidelines; the other group was treated similarly except that birds were exposed to a choice of treated or untreated diet. Nominal concentrations of Diazinon MG8 in treated diets in both treatment groups were 47, 94, 188, 375 and 750 parts per million (ppm) active ingredient (a.i.). The dietary

concentrations were established based upon known toxicity data. One pen of mallard ducklings (at 10 birds/pen) was assigned to each test concentration in each treatment group. Five pens of mallards were assigned to the control. Birds were randomly assigned to pens. The birds used in this study were too immature to differentiate by sex. During the exposure period, the control group received an amount of the carrier in their diet equivalent to the greatest amount used in the treated diets (i.e. 2%). Following the five-day exposure period, all groups were given untreated feed for three days. The primary phases of this study and their durations were: Acclimation - 8 days; Exposure - 5 days; and Post-exposure observation - 3 days.

Diet samples were taken of the high and low concentrations for residue analysis on the day of mixing. Samples were frozen after collection and shipped to Steve Blair, Ciba-Geigy Corporation, 410 Swing Road, Greensboro, North Carolina 27409.

D. Statistics: An LC_{50} value along with a 95% confidence interval was calculated using the computer program of C. E. Stephan (U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota, 1978; personal communication). For the diet choice group in this study binomial probability was used. The mortality pattern in the no-choice group was not conducive to calculating the LC_{50} value. Therefore, an estimation of the LC_{50} value was made by a visual inspection of the mortality data.

12. REPORTED RESULTS:

Dosage (ppm, a.i.)	Mortality	
Control	2/50	
	No Choice	Choice
47	10/10	0/10
94	10/10	0/10
188	10/10	1/10
375	10/10	6/10
750	10/10	1/10

"CONTROLS

There was 4% mortality (2 of 50) in the control group .... One bird was noted as head-picked on the morning of Day 5 and was found dead on the afternoon of Day 6. Another bird displayed loss of coordination and reduced reaction to external stimuli (sound and movement) on the afternoon of Day 7. The second mortality was

noted on the morning of Day 8. All other birds were normal in appearance and behavior throughout the test period.

"DIAZINON MG8

Mortality

When birds were exposed to DIAZINON MG8-treated diet for five days, there was 100% mortality (10 of 10) at all concentrations tested.... At the 47, 94 and 188 ppm concentrations, mortalities were first noted on Day 2. All birds had died by Day 5 at the 47 ppm concentration; by Day 4 at the 94 ppm concentration; and by the end of Day 3 at the 188 ppm concentration. Mortalities were first noted on Day 0 at the 375 and 750 ppm concentrations. All birds had died by Day 1 at the 375 ppm concentration and by the end of Day 2 at the 750 ppm concentration.

"When birds were given free choice of DIAZINON MG8-treated diet or untreated diet for five days, there were no mortalities at the 47 and 94 ppm concentrations.... There was 10% mortality (1 of 10) at the 188 and 750 ppm concentrations and 60% mortality (6 of 10) at the 375 ppm concentration. The single mortality at the 188 ppm concentration was noted on Day 0. Mortalities at the 375 ppm concentration were noted on Days 1, 2 and 3. At the 750 ppm concentration, the single mortality was noted on Day 1.

"Clinical Signs

Among the no-choice group, overt signs of toxicity were first observed on Day 1 at the 47 and 94 ppm concentrations and on Day at the 188, 375 and 750 ppm concentrations. Signs of toxicity continued to be displayed until all birds had died.

"In the diet choice group, overt signs of toxicity were observed in one bird at the 47 ppm concentration on the afternoon of Day 1. At the 94 ppm concentration, one bird was observed displaying signs of toxicity on the afternoon of Day 3 through the afternoon of Day 4. At the 188 ppm concentration, signs of toxicity were first observed on the afternoon of Day 0 and continued through the morning of Day 7. At the 375 and 750 ppm concentrations, signs of toxicity were first observed on the afternoon of Day 0 and continued through the afternoon of Day 3 at the 375 ppm concentration and through the morning of Day 4 at the 750 ppm concentration.

"Overt signs of toxicity typical 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 and coma.

"Body Weight and Fed Consumption

When compared to the controls, there was no effect on body weight among the diet choice group during the exposure period.... Due to total mortality among the no-choice group, effects on body weight could not be determined....

"In the diet choice group, feed consumption measurements indicated that there was a preference for the untreated diet at all concentrations during the exposure period.... Feed consumption data for the untreated diet was comparable with the control group during this period.... At the 47 ppm concentrations, total treated diet consumed by the diet choice group represented 55% of the total treated diet consumed by the no-choice group. The no-choice group showed a decrease in feed consumption at all concentrations during the exposure period when compared to the control...."

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

"The mallard dietary LC_{50} value of DIAZINON MG8 for mallards offered only treated feed in this study was determined to be less than 47 ppm a.i. When birds were given a choice of DIAZINON MG8 treated feed of untreated feed, the mallard dietary LC_{50} value was determined to be greater than 188 ppm a.i. Under the conditions of this test, young mallards demonstrated a concentration dependent increase in avoidance to DIAZINON MG8 treated feed in all test groups. The results indicate that birds offered a choice of DIAZINON MG8 treated and untreated diet showed significantly less mortality than birds offered DIAZINON MG8 treated diet without a choice.

"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 dates that results of those audits were reported to the Study Director/Laboratory Management...."

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

A. Test Procedure(s):

(1) Author adjusted dietary levels to 100% a.i. for purposes of calculating feed concentrations and LC_{50}. Raw mortality data were, for the most part, consistent with the written report. A possible exception is:

(a) Author reported (pg. 13) that, "When compared to the controls, there was no effect on body weight among the diet choice group during the exposure period (see Tables 4 and 6)." A comparison of the data in those tables (i.e. for the change in weight between Days 5 and 8) indicates that the diet choice group gained only 86% of that shown for the comparable control group.

(2) Test procedures were, basically, in accordance with protocols recommended by SEP. Important exceptions included:

(a) Mortality, ranging from 10% to 90%, was not achieved for either the experimental groups given a choice of feed or the group not given a choice (see SEP, pg. 3).

(b) Three partial kills, surrounding the estimated LC_{50}, were not achieved for the groups not given a choice (see SEP, pg. 3).

(c) Although three partial kills, surrounding the estimated LC_{50}, were achieved for the groups given a choice, the dose-response relationship was not consistent. That is, 10% died at 188 ppm (a.i.) and 60% died at 375 ppm, but only 10% died at the higher (and highest) dose of 750 ppm.

(d) The LC_{50} reported for the no choice mallards (i.e. offered only treated food) was "...determined to be less than 47 ppm a.i." That of the groups given a choice was reported to be "...greater than 188 ppm a.i." The SEP (pg. 3) requires that "Studies should be designed to establish an actual LC_{50} and 95% c.i." Neither reported LC_{50} meet these requirements.

(e) A dose-response line for neither test group was provided (SEP, pg. 6).

(f) The study (pg. 9, 2nd paragraph) implied that some diet samples were analyzed for residue but no data were provided.

B. Statistical Analysis: Lack of partial kills for birds under no-choice regime, and lack of consistent dose-response relationship for birds under choice regime, prevented author from providing required statistics on LC_{50} and C.I.

Statistical confirmation of the LC_{50} for the choice regime using Stephan's computer program (TOXANAL) gave a binomial LC_{50} estimate of 330 ppm versus the author's binomial estimate of 188 ppm.

C. Discussion/Results: The key issues that remain unanswered are:

(1) What are the NOEL and LC_{50} for mallards given diazinon under a no-choice test? The toxicity data for these mallards don't allow calculation of either value because chosen exposure levels did not include low enough concentrations.

(2) What is the LC_{50} for mallards given diazinon, but also given a choice of feed not containing the chemical? The dose-response relationship was not consistent, making interpretation difficult. The 375 ppm group did consume relatively more chemically-dosed feed/bird during Days 0-3, which is when the lower-dosed group (i.e. 375 ppm) exhibited greater mortality (see below table). However, even lower-dosed birds (45, 94 and 188 ppm) consumed comparable cumulative amounts (per bird) and didn't exhibit similar mortality. To understand the dose-response relationship, we have to know how much chemical is consumed by each bird (not "on the average") and relate that to observed mortality.

CUMULATIVE MORTALITIES AND CUMULATIVE DIAZINON MG8 INTAKE FOR MALLARDS EXPOSED TO DIAZINON MG8 FOR FIVE DAYS WITH A CHOICE OF DIET AVAILABLE

Concentration	Number Dead/Number Exposed (Cumulative Diazinon Intake*) Day of Study					
ppm a.i.	0	1	2	3	4	5
47	0/10	0/10 (705)	0/10 (1222)	0/10 (1833)	0/10 (2538)	0/10 (3196)
94	0/10	0/10 (564)	0/10 (1034)	0/10 (1410)	0/10 (1598)	0/10 (1974)
188	1/10	1/10 (376)	1/10 (752)	1/10 (1128)	1/10 (1504)	1/10 (1692)
375	0/10	3/10 (750)	4/10 (750)	6/10 (1875)	6/10 (3000)	6/10 (3750)
750	0/10	1/10 (750)	1/10 (1500)	1/10 (1500)	1/10 (2250)	1/10 (2250)

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*Micrograms; calculated based on the following: concentration in diet (ug/g, a.i.) x treated feed consumed (gm per bird per day). Feed consumption data taken from report is Table 9. Cumulative mortality data taken from report's Table 3.

(3) Does diazinon, at typical field application levels, have a repellant effect? The fact that fewer birds, given a choice, died when exposed to 750 ppm (vs. 375 ppm) could be explained if diazinon did provide a repellency. The reported percent treated feed consumed (Table 9 of study) suggests that the 750 ppm group consumed 1/3 the treated feed as did the 375 ppm group (1% vs. 3%). However:

(a) This table was based on intake estimates, the accuracy of which is not provided.

(b) The 375 and 750 ppm no-choice groups experienced almost complete mortality (i.e. 90%) on Day 0, indicating that, even at higher concentrations (when repellency should have been the greatest), it was not strong enough to keep these birds from feeding, which was an optional behavior.

(4) What were the results of the feed residue analyses? The SEP (pg. 5) indicates that, "If the concentration of test material was measured, the results should be reported." Although the absence of these data, alone, is not enough to invalidate the study, their presence might help interpret some of the non-linear mortality responses.

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: Study does not provide adequate data to determine an LC_{50} value for diazinon MG8 under either no-choice or choice feeding conditions. Nor does it allow the identification of a NOEL, under standard no-choice LC_{50} testing conditions.

(3) Reparability: Not reparable.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, January 23, 1988.

16. CBI APPENDIX: N/A

J. Newman Diazinon MGB Mallard duck 02-02-88

Proj. No 108-276
"Choice test"

108-

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
750	10	1	10	1.074219
375	10	6	60.00001	37.69531
188	10	1	10	1.074219
94	10	0	0	9.765625E-02
47	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 LC50 FOR THIS SET OF DATA IS 330.6611

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.7206816	330.6606	244.6661	1386.457

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	8.004182	4.007406	7.307351E-03

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

SLOPE = 1.395526
95 PERCENT CONFIDENCE LIMITS =-2.55265 AND 5.343702

LC50 = 1305.219
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 160.3381
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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ONE LINER SHEET

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

Study/Species/Lab/ Accession #4	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Dietary LC_{50}		LC_{50} = < 47 ppm (95% C.L. not given) Contr. Mort. (%) = 4		
Species: Mallard		Slope = (not given) # Animals/Level = 10 Age (Days) = 10 Sex = ?	Lincer 1/23/88	invalid
Lab: Wildlife Int. Ltd. Project No. 108-276 AC #:	86.6%	8-Day Dose Level ppm (% Mortality): 47 (100), 94 (100), 188 (100), 375 (100), 750 (100)		

Comments: Exposure levels were too high to provide partial kills and estimation of LC_{50} and NOEL. Authors' conclusions about repellency are challenged.

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