2-6-88 X
NTO

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon MG8

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

3. STUDY TYPE: Dietary LC_{50}
Species Tested: Mallard Duck
Anas platyrhynchos

4. CITATION: Grimes, J. and M. Jaber. 1987. A Comparison of Mallard Dietary LC_{50} Values with Constant and Declining Concentrations. Wildlife International Ltd. No.: 108-277. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corp., Greensboro, NC. Project #: 108-277

5. REVIEWED BY:

Jeffrey L. Lincer, Ph.D. Signature:
Eco-Analysts, Inc. Date: 1/26/88
Sarasota, FL

6. APPROVED BY:

James R. Newman, Ph.D. Signature: James R. Newman
Proj. Mgr., KBN Engineering Date: 2/6/88
and Applied Sciences, Inc.

Henry T. Craven Signature:
Chief EEB/HED Date:
USEPA

7. CONCLUSIONS:

The study is, basically, scientifically sound.

The mallard dietary LC_{50} value of DIAZINON MG8 at constant test concentrations was determined to be less than 47 ppm active ingredient (a.i.) At test concentrations simulating degradation of residues over five days, the mallard LC_{50} value was determined to be 59.6 ppm a.i.

These values are very rough estimates because of the lack of a proper range in mortalities. However, if they approximate the real LC_{50}s, Diazinon MG8 would be considered very highly toxic and highly toxic to the mallard, under the two above experimental exposures, respectively.

This study does not fulfill the intent of the Guidelines' requirements for an avian dietary LC_{50} determination, but is useful for risk assessment purposes.

8. RECOMMENDATIONS: Repeat constant concentration study, with appropriately lower concentrations, to determine LC_{50}, dose-response line, c.i. and other SEP requirements.

9. BACKGROUND: Although previously-reviewed Diazinon study (Project 108-276) was deemed invalid, which presented the same data as provided for the constant concentration diet herein, this study is considered supplemental because the LC_{50} derived from the "declining" concentration dietary test provides some confirmatory data.

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

11. MATERIALS AND METHODS (PROTOCOLS):

    A. Test Animals: All mallards were 10 days of age and appeared to be in good health at initiation of the study. The birds were obtained from Whistling Wings, Hanover, Illinois. The birds were hatched on December 29, 1986 and received at Wildlife International Ltd. on December 31, 1986. All birds were pen-reared and phenotypically indistinguishable from wild birds. Birds were assigned to pens by random draw with ten birds per pen. The ducklings were too immature to differentiate by sex. All birds were acclimated to the caging and facilities from the day of receipt until initiation of the study.

B. Dosage: The test diets were prepared by mixing the test substance into the diet with corn oil (2%). Diet premixes were prepared in a Hobart mixer. Final diets were mixed in a Patterson-Kelley Twin Shell blender. Diets were prepared on the day of study initiation. The declining concentration diets were stored frozen until presented to the birds. The constant concentration group received on Day 0 an amount of diet sufficient to last the five day exposure period. The declining concentration group received sufficient amounts of the appropriate diet on Days 0, 1, 2, 3 and 4. All dietary test concentrations were adjusted to 100% active ingredient based upon the reported purity of the test substance. Therefore, all dietary concentrations and the LC_{50} value are reported as parts per million of the active ingredient in the diet. Nominal dietary test concentrations used in this study were 47, 94, 188, 375 and 750 ppm a.i.

Diet samples were taken of the high and low concentrations for residue analysis on the day of mixing and on Day 4. Samples were frozen after collection and shipped to Ciba-Geigy Corporation, Greensboro, North Carolina.

C. Design: The test consisted of two treatment groups and a control group. One treatment group was exposed to DIAZINON MG8 at constant concentrations and the other was exposed to DIAZINON MG8 at declining concentrations. Five pens, each containing ten mallard ducklings, were assigned to each of the treatment and control groups. Birds were assigned to pens by random draw with ten birds per pen. The birds were acclimated from the day they were received until test initiation.

The constant concentration group consisted of a geometric series of five test concentrations with one pen of birds per concentration. The nominal concentrations used were 47, 94, 188, 375 and 750 parts per million (ppm) active ingredient (a.i.). Sufficient amounts of the appropriate test diet for the five day exposure period was presented to the birds on Day 0.

In the declining concentration group the test consisted of five test concentrations designed to simulate a degradation half-life of approximately 5 days. Nominal dietary concentrations used were as follows:

Days 0-1 ppm a.i.	Days 1-2 ppm a.i.	Days 2-3 ppm a.i.	Days 3-4 ppm a.i.	Days 4-5 ppm a.i.
47	41	36	31	27
94	82	71	62	54
188	164	143	124	108
375	327	284	248	215
750	653	569	495	431

The appropriate test diet was presented to the birds daily for the five day exposure period.

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. 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:

1. Acclimation - 8 days.

2. Exposure - 5 days.

3. Post-exposure observation - 3 days.

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.).

12. REPORTED RESULTS:

"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 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 at constant test concentrations, there was 100% (10 of 10) mortality 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 concentration. All birds had died by Day 1 at the 375 ppm concentrations and by the end of Day 2 at the 750 ppm concentration.

"When birds were exposed to DIAZINON MG8 at declining test concentrations, there was 20% mortality (2 of 10) at the 47-27 ppm concentration and 100% mortality (10 of 10) at the higher concentrations.... [see table below] At the 47-27 ppm concentration, the two mortalities were noted on the afternoon of Day 4 and on the morning of Day 6. At the 94-54 ppm concentration, mortalities were first noted on Day 2 and all birds had died by the afternoon of Day 4. At the 188-108 ppm concentration, mortalities were first noted on Day 1 and all birds had died by the morning of Day 4. At the 375-215 ppm concentration, mortalities were first noted on the afternoon of Day 0 and all birds had died by the morning of Day 2. At the 750-431 ppm concentration, all birds were found dead on the afternoon of Day 0."

CUMULATIVE MORTALITIES OF MALLARDS
EXPOSED TO DIAZINON MG8 FOR FIVE DAYS
WITH DECLINING CONCENTRATIONS

Concentration Range (ppm, a.i.)	Percent Dead (Day 5)
Control	4%
47- 27	20%
44- 54	100%
188-108	100%
375-215	100%
750-431	100%

"Clinical Signs

"Among the constant concentration group, overt signs of toxicity were first observed on Day 1 at the 47 and 94 ppm concentrations and on Day 0 at the 188, 375 and 750 ppm concentrations. Signs of toxicity continued to be displayed at all concentrations until all birds had died.

"Among the declining concentration group, overt signs of toxicity were first observed on the morning of Day 1 at the 47-27 ppm concentration. Most survivors appeared normal at observations from the morning of Day 7 until study termination; however, one bird was noted displaying overt signs of toxicity at each observation until study termination. At the 94-54, 188-108, 375-215 and 750-431 ppm concentrations, signs of toxicity were first noted on Day 0 and continued to be displayed until all birds had died.

"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 Feed Consumption

"In the declining concentration group, there was a decrease in body weight gain at the 47-27 ppm concentration from Day 0 to Day 5 when compared to the controls. Due to total mortality at the higher concentrations in the declining concentration group, and total mortality at all concentrations in the constant concentration group, no other effects on body weight could be determined....

"When compared to the controls, there was a decrease in daily feed consumption in the constant concentration group at 47 ppm, and in the declining concentration group at 47-27 ppm during the exposure period. However, feed consumption appeared to increase at 47-27 ppm in the declining concentration group from Day 3 to Day 5 as the concentration decreased from 36 to 27 ppm...."

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

"The mallard dietary LC_{50} value of DIAZINON MG8 at constant test concentrations for this study was determined to be less than 47 ppm a.i.. At test concentrations simulating degradation of residues over five days, the mallard LC_{50} value was determined to be approximately 58 ppm a.i. The

mortality responses in this test do not allow comparison of statistically derived LC_{50}'s for the two test groups. However, comparison of results between the 47 ppm constant concentration group and the 47 ppm declining concentration group indicate that mortality is reduced under conditions that simulate pesticide degradation."

According to the authors, "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 ...." Audits were performed six times during the experimental and reporting phases of this study.

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

A. Test Procedure(s):

(1) Raw data provided on mortality, weight gain and feed consumption was consistent with text.

(2) The protocol used for both the constant and declining concentrations were, for the most part, consistent with the SEP guidelines. Important exceptions were as follows:

(a) Constant Concentration. SEP requirements, for providing a statistically calculated LC_{50} with 95% confidence interval, a dose-rsponse line and a mortality ranging from 10-90% (and preferably three partial kills surrounding the LC_{50}), were not met. Feed samples were collected for residue analysis. Although it was not clear as to whether these samples were actually analyzed, no residue data were reported.

(b) Declining Concentration. In addition to not meeting the same objectives, discussed above for the constant concentration group, the declining concentration experimental procedures are questioned on other grounds:

(i) What was the basis for determining incremental decrease in dietary concentrations?

(ii) How different would the rate of degradation be in the diet of the "constant" concentration group than in food in the wild? What do the feed residue analyses reveal, if they were done?

The fact that the constant concentration resulted in higher mortality suggests that the diazinon in it did not degrade into something less toxic (if it degraded at all). However, the approach to preparing the declining concentration diets was done such that only the amount of the parent compound a.i. was decreased, giving no consideration to changing (and possibly increasing) levels of any degradates that might be produced under field conditions.

(iii) What concentration values were used as a basis for creating the dose-response curve and estimating the LC_{50}? Were the initial high values used? Were the final low values used? Was an average used for these calculations?

B. Statistical Analysis: The LC_{50} estimate is revised using TOXANAL. When there are less than two concentrations at which the percent dead is between zero and 100, neither the moving average nor probit method can give any statistically sound results. Because there was control mortatlity and none of the lower concentrations produced zero mortality, the data have been subjected to Abbott's correction (see attached sheet).

C. Discussion/Results:

(1) Post/Mortality Response. Neither test approach (constant or declining concentrations) provided a sound estimate of the dose-response line, a NOEL,

the LC_{50} or associated confidence interval. The constant concentration exposure resulted in 100% mortality in all groups. The declining concentration group test resulted in 100% mortality in all but the lowest exposure group.

Both tests, however, do provide some valuable information, in addition to a very rough approximation of the LC_{50}. The raw data (Tables 2 and 3) reveal that the mallard's response to this chemical is rapid over a narrow range. In addition, it is clear that, although the mallard's toxic response to diazinon is rapid at the medium to high concentrations tested, the response may be somewhat prolonged at the lower concentrations (even to the extent that additional mortality, in the declining 47 ppm group, occurred after the 5-day exposure phase).

(2) Protocol. The use of dietary concentrations which, purposefully, change over time creates some interpretive problems. How is the dose-response line established? What dietary concentration (for each exposure level) should be used as the basis for necessary calculations? In addition, there is little comparative value of resulting data since a data base from similar approaches is lacking.

(3) Descriptive Categorization of Results. With an estimated LC_{50} value of 47 ppm, Diazinon MG8 is very highly toxic to mallards under standard (constant concentration) dietary test conditions. Under declining dietary concentration conditions, but based on a weak estimate of the LC_{50} (at 59.6 ppm), this chemical would be considered highly toxic to the mallard.

D. Adequacy of the Study:
(1) Classification: Supplemental.
(2) Rationale: The studies were, for the most part, scientifically sound; however, the choice of high dietary concentrations resulted in all, or almost all, exposed birds dying. Although the results may provide some useful information in a risk assessment, they do not meet guideline requirements as discussed above.
(3) Reparability: Not reparable.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, January 26, 1988.

16. CBI APPENDIX: N/A

PROJ. No: 108-277

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

J Newman Diazinon MG8 mallard duck 02-08-88 (for declining concentrations)

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
750	9.600001	9.600001	100	9.765625E-02
375	9.600001	9.600001	100	9.765625E-02
188	9.600001	9.600001	100	9.765625E-02
94	9.600001	9.600001	100	9.765625E-02
47	9.600001	1.6	16.6667	5.46875

THE BINOMIAL TEST SHOWS THAT 0 AND 94 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 59.56565

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

ONE LINER SHEET

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

Study/Species/Lab/ Accession #4	Chemical % a.i.	Results	Reviewer/ Date	Validation Status

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Dietary LC_{50} LC_{50} = < 47 ppm (not given) 95% C.L. Contr. Mort. (%) = 4

Species: Mallard Slope = (not given) # Animals/Level = 10 Age (Days) = 10 Sex = ?

Lab: Wildlife Int. Ltd. 86.6

Project No: 108-277

AC #:

Lincer 1/26/88 supplemental

8-Day Dose Level ppm (% Mortality)

47 (100), 94 (100), 188 (100), 375 (100), 750 (100)

Comments: Complete mortality at all exposure levels prevents estimations of LC_{50}. A second study, using declining concentrations, estimated the LC_{50} to be 59.6 ppm.

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