2-6-88

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 Mark Jaber. 1987. Diazinon MG8: A Comparison of Dietary LC_{50} Values with Mallards of Different Ages. Project No.: 108-278. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corp., Greensboro, N.C. Project No: 108-278.

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, Diazinon MG8 is very highly toxic to 10-day-old mallards. Data, resulting from tests with older mallards (31 and 87 days), are provided but the relevance is questioned because of the age of birds tested.

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 14A and C), thereby precluding the calculation of an LC_{50}. Although LC_{50} data were derived for 31 and 87-day-old mallard groups, these ages do not meet SEP guidelines and relevance of findings is questioned.

8. RECOMMENDATIONS: Repeat the LC_{50} study with 10-day-old mallards, using low enough dietary concentrations to meet the SEP guidelines regarding partial kills, a dose-response line and calculation of NOEL and LC_{50}. If diet is analyzed for residue, report findings.

9. BACKGROUND: N/A

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: Mallards for the three test groups were 10, 31 and 87 days of age, respectively, and appeared to be in good health at initiation of the study. The birds were obtained from Whistling Wings, Hanover, Illinois. The 10-day-old birds were hatched on December 29, 1986; the 31-day-old birds on December 8, 1986; and the 87-day-old birds on October 13, 1986. The 10-day-old birds were received at Wildlife International Ltd. on December 31, 1986, and the 31 and 87-day-old birds were received on December 30, 1986. All birds were pen-reared and phenotypically indistinguishable from wild birds.

B. Dosage: The test diets were prepared by making a pre-mix. The pre-mix prepared by mixing the test substance into a portion of the diet with corn oil. The concentration of corn oil in the treated and control diets was 2%. Mixing of the final test diet was done with a Patterson Kelley Twin Shell dry blender. Final diets were prepared on the day of study initiation. An amount of diet sufficient to last the five day exposure period was presented to the birds at initiation of the study. All dietary concentrations were adjusted to 100% active ingredient based upon the reported purity of the test substance. Therefore, all dietary concen-

trations 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, 750 and 1500 ppm a.i.

Diet samples were taken of the 47, 750 and 1500 ppm concentrations for residue analysis on the day of mixing. Samples were frozen after collection and shipped to Steve Blair, Ciba-Geigy Corporation, Greensboro, North Carolina.

C. Design: Groups of ten mallard ducks were assigned to each of the treatment and control groups by random draw. The 10-day-old and 31-day-old birds used in this study were too immature to sex. The 87-day-old birds were assigned to groups of 5 males and 5 females. Birds were acclimated from the day they were received until test initiation.

For the 10-day-old birds, the test consisted of a geometric series of five test concentrations and five control groups. Nominal dietary concentrations used in this study were 47, 94, 188, 375 and 750 parts per million (ppm, a.i.).

For the 31 and 87-day-old birds, test concentrations were 47, 94, 188, 375, 750 and 1500 ppm, a.i. One control group of 10 birds each was assigned to the 31 and 87-day-old age classes.

The dietary concentrations were established by Ciba-Geigy. Each group was fed the appropriate test or control diet for five days. During the exposure period, the control groups received an amount of the carrier in their diet equivalent to the greatest amount used in the treated diets (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:

1. Acclimation - 10-day-old birds - 8 days.
   31-day-old birds - 9 days.
   87-day-old birds - 9 days.
2. Exposure - 5 days.
3. Post-exposure observation - 3 days.

D. Statistics: For the 31 and 87-day-old age groups, an LC_{50} value and 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.). In this study, probit analysis was used. The mortality pattern for the 10-day-old age 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:

"CONTROLS

"There was 4% mortality (2 of 50) in the 10 day old age group.... One bird was noted as head-picked from the morning of Day 5 through the morning of Day 6. One bird 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. There were no mortalities in the 31 and 87 day old age control groups. All birds in those control age groups were normal in appearance and behavior throughout the test period.

"DIAZINON MG8

"Mortality

"There was 100% mortality (10 of 10) in the 10 day old age group 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.

"In the 31 day old age group, there were no mortalities at the 47, 94 and 188 ppm concentrations.... There was 30% mortality (3 of 10) at the 375 ppm concentration and 90% mortality (9 of 10) at the 750 and 1500 ppm concentrations. At the 375 ppm concentration, mortalities were noted on Days 0, 1 and 5. All mortalities at the 750 and 1500 ppm concentrations occurred on Day 0.

"In the 87 day old age group, there were no mortalities at the 47, 94, 188 and 375 ppm concentrations.... There was 20% mortality (2 of 10) at the 750 ppm concentration and 30% mortality (3 of 10) at the 1500 ppm concentration. All mortalities occurred on Day 0 of the study.

"Clinical Signs

"Overt signs of toxicity in the 10 day old age group were first noted 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.

"In the 31 day old age group, signs of toxicity were noted in one bird on Day 4 at the 47 ppm concentration, and in one bird on Day 1 at the 94 ppm concentration. All other birds at the above concentrations were normal in appearance and behavior throughout the test period. At the 188 ppm concentration, signs of toxicity were noted on Day 0 through Day 1 and on Day 3 through Day 5. At the 375, 750 and 1500 ppm concentration, signs of toxicity were first observed on Day 0. Overt signs of toxicity continued through Day 6 at 375 ppm, Day 5 at 750 ppm and Day 1 at 1500 ppm. Upon recovery, surviving birds remained normal in appearance and behavior until study termination.

"In the 87 day old age group, there were no overt signs of toxicity at the 47 and 94 ppm concentrations. All birds were normal in appearance and behavior throughout the test period. At the 188 ppm concentrations, signs of toxicity were first observed on Day 1 and continued intermittently through Day 6. At the 375, 750 and 1500 ppm concentrations, signs of toxicity were first observed on Day 0. By the afternoon of Day 6, all surviving birds had recovered and were normal in appearance and behavior until study termination.

"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, lower limb rigidity and coma.

"Body Weight and Feed Consumption

"When compared to the controls, there was a marked loss of body weight at all concentrations in the 31 day old and 87 day old age groups during the exposure phase (Day 0-5).... When comparing body weight change for the 8 day study

period, the 31 day old age group showed a loss at the 375, 750 and 1500 ppm concentrations and the 87 day old age group showed a weight loss at all concentrations. Due to total mortality in the 10 day old age group, effects on body weight could not be determined.... When compared to the controls, feed consumption was reduced in all age groups at all concentrations during the exposure phase."

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

"The mallard dietary LC_{50} value of DIAZINON MG8 for the 10 day old age group was determined to be less than 47 ppm a.i., the lowest concentration tested. The no-observed-effect level was less than 47 ppm based on total mortality at this concentration.

"The mallard dietary LC_{50} value of DIAZINON MG8 for the 31 day old age group was determined to be 510 ppm a.i. with a 95% confidence interval of 369 to 711 ppm. The no-observed-effect level was less than 47 ppm, the lowest concentration tested, based on loss of body weight and overt signs of toxicity at 47 ppm.

"The mallard dietary LC_{50} value of DIAZINON MG8 for the 87 day old age group was determined to be 2108 ppm a.i. with a 95% confidence interval of 1212 ppm to 8,284,993 ppm. The no-observed-effect level was less than 47 ppm based on loss of body weight at 47 ppm. The results indicate that the age of test birds has a significant effect on the LC_{50} value as determined by standard FIFRA LC_{50} methods."

According to the author, "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 took place six times during the study and report writing phases.

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

A. Test Procedure(s):

(1) Raw mortality data were consistent with written report.

(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 the 10-day-old mallard group (SEP, pg. 3).

(b) Three partial kills, surrounding the estimated LC_{50}, were not achieved in the 10-day-old group (SEP, pg. 3).

(c) The LC_{50} reported for the 10-day-old group was ... determined to be less than 47 ppm a.i. ...." The SEP requires that "Studies should be designed to establish an actual LC_{50} and 95% c.i." The choice of exposure levels, and resulting total mortality, at all levels of exposure prevented the authors from meeting the above criteria.

(d) A dose-response line for the 10-day-old group was not provided (SEP, pg. 6).

(e) The SEP (pg. 2) indicates that the age of mallard ducks, used for LC_{50} tests, "should be from 5 to 10 days old at the beginning of the test; the 5 day old birds being preferred." The study used birds that were 10, 31 and 87 days old at the beginning of the test. According to the authors, "Ciba-Geigy Corporation requested this evaluation to determine the extent to which risk assessment based on the standard FIFRA LC_{50} protocol (using 5-10 day old mallards) might overestimate effects for situations where typically older birds were exposed." (pg. 6 of study).

(f) The study (pg. 10) implied that some diet samples were analyzed for residues but no data were reported. The SEP (pg. 5) indicates that, "If the concentration of the test material was measured, the results should be reported."

B. Statistical Analysis: Reported LC_{50} values were confirmed using Stephan's computer program (TOXANAL). See attached printouts.

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

(1) What are the NOEL, dose-response line and LC_{50} for 10-day-old mallards fed Diazinon MG8? The toxicity data for this age group don't allow calculation of any of these parameters because chosen exposure levels did not include low enough concentrations, which led to total mortality at all exposure levels.

(2) What is the relevance of data resulting from the 31 and 87-day-old age groups? It is widely known that LC_{50}s often increase with age of bird tested (Hill and Camardese, 1981). Therefore, the study results are not unexpected. The SEP guidelines, which require the use of 5-10 day-old mallards, do so for a number of reasons (Turner, 1981). The large data base for comparative work, and the value of using birds young enough such that they cannot survive for 5 days without eating, are important among those reasons.

(3) What were the results of the diet residue analyses? Although the absence of these data alone is not enough to invalidate the study, their presence could be of some interpretive value.

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: Study does not provide adequate data to determine the LC_{50}, NOEL or dose-response line for the 10-day-old group of mallards. LC_{50} (c.i.) values for two other age groups (31 and 87-day-olds, respectively) were provided but the relevance of these data is questioned because the age of test birds does not meet SEP guidelines (see 14C).

(3) Reparability: Not reparable.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, January 24, 1988.

16. CBI APPENDIX: N/A

LITERATURE CITED

Hill, E. F. and Camardese, M. B., "Subacute Toxicity Testing with Young Birds: Response in Relation to Age and Interest Variablility of LC_{50} Estimates," Avian and Mammalian Wildlife Toxicology: Second Conference, ASTM STP 757, D. W. Lamb and E. E. Kenaga, Eds., American Society for Testing and Materials, 1981, pp. 41-65.

Turner, L. W., "Development of an Avian Dietary LC_{50} Toxicity Test for Potential Use Under the Toxic Substances Control Act," Avian and Mammalian Wildlife Toxicology: Second Conference, ASTM STP 757, D. W. Lamb and E. E. Kenaga, Eds., American Society for Testing and Materials, 1981, pp. 98-104.

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 (not given) 95% C.L. Contr. Mort. (%) = 4

Species: Mallard Slope = (not given) # Animals/Level = 10 Age (Days) = 10* Sex = ? Lincer 1/24/88 invalid

Lab: Wildlife Int. Ltd. 86.6
Project No: 108-278
AC #:

8-Day Dose Level ppm (% Mortality)
47 (100), 94 (100), 188 (100), 375 (100), 750 (100)

Comments: * Two older age groups were also tested (i.e. 31 and 87 days) but the relevance of resulting data is questioned.

Proj. No 108278 (31 days)

J. Newman Diazinon MG8 Mallard duck 02-02-88

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1500	10	9	90	1.074219
750	10	9	90	1.074219
375	10	3	30	17.1875
188	10	0	0	9.765625E-02
94	10	0	0	9.765625E-02
47	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 188 AND 750 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 465.6032

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1677539	529.0221	363.4335	884.2871

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2266766	1	.4736749

SLOPE = 4.094938
95 PERCENT CONFIDENCE LIMITS = 2.145315 AND 6.044561

LC50 = 510.0522
95 PERCENT CONFIDENCE LIMITS = 368.63 AND 710.9505

LC10 = 249.7329
95 PERCENT CONFIDENCE LIMITS = 118.1813 AND 349.7738

****

Proj. No 108-278 (87 days)

J. Newman Diazinon MG8 Mallard duck 02-02-88

****

CONC.	NUMBER	NUMBER	PERCENT DEAD	BINOMIAL PROB. (PERCENT)

Proj No 108-278
(87 days)

J. Newman Diazinon M68 Mallard duck 02-02-88

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1500	10	3	30	17.1875
750	10	2	20	5.46875
375	10	0	0	9.765625E-02
188	10	0	0	9.765625E-02
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 1060.66

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
6	.829193	1	.910265

SLOPE = 2.614442

95 PERCENT CONFIDENCE LIMITS = .2337301 AND 4.995154

LC50 = 2107.803

95 PERCENT CONFIDENCE LIMITS = 1211.699 AND 8237112

LC10 = 688.7576

95 PERCENT CONFIDENCE LIMITS = 17.19667 AND 1190.871

****