2-6-88

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon MG8

2. TEST MATERIAL: Diazinon MG8, Fl. No. 861113. Reported purity of 86.6% (assigned Wildlife International Ltd. i.d. number WIL-1148).

3. STUDY TYPE: Avian Dietary LC_{50}
Species tested: Canada Goose
(Branta canadensis)

4. CITATION: Grimes, J. and M. Jaber. 1987. A Dietary LC_{50} Study with the Adult Canada Goose. Project No.: 108-279. Prepared by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corp., Greensboro, NC. Project No: 108-279

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: 2/4/88

Henry T. Craven
Chief EEB/HED
USEPA

Signature:
Date:

7. CONCLUSIONS:

This is a scientifically valid study but it falls short of meeting some critical protocol guidelines (see 14A and C). The findings, with this particular species, may be useful but because young birds were not used: (a) the resulting data are of limited comparative utility (i.e. to compare with a vast data base on young birds of various species); and (b) the toxicity estimates are likely to underestimate risk.

The dietary LC_{50} for Diazinon MG8, fed to adult Canada geese, was 3912 ppm (a.i.), with a 95% c.i. of 1167 - 1.7×10^{38} ppm. If the calculated LC_{50} approximates the real LC_{50} (which is questionable because of the large c.i. spread), this chemical would be considered slightly toxic to adult Canada geese when provided in the diet. Geese on Diazinon diets consumed less feed than controls and tended to lose more weight than controls.

Adequacy of data will depend on applicant's responses to issues raised and requests for additional information (see 14A and C).

8. RECOMMENDATIONS: Applicant should respond to issues raised and information requested in 14A and C. If EPA determines that study is to be repeated, it should be carried out with young Canadian geese and exposure levels should be chosen based on a screening study.

9. BACKGROUND: N/A

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: The birds were obtained from Carl Webb, Jr. of Betterton, Maryland. The precise age of the birds was not known by the supplier; however, the birds were in adult plumage and appeared to be in good health at initiation of the study. The birds were received at Wildlife International Ltd. on January 21, 1987. All birds were pen-reared and phenotypically indistinguishable from wild birds.

B. Dosage: The test diets were prepared by mixing the test substance into the diet with corn oil. The concentration of corn oil in the treated and control diets was 2%. Mixing of the test diet was done with a Hobart mixer. Diets were prepared on the day prior to study initiation and were stored in the freezer until presented to the birds. An amount of diet sufficient to last the five day exposure period was presented to the birds at initiation of the study. 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 188, 375, 750, 1500 and 3000 ppm a.i. Diet samples were

taken of the high and low concentrations for residue analysis on the day of mixing.

C. Design: Groups of six Canada Geese were assigned to each of the treatment and control groups by random draw. Birds were acclimated from the day they were received until test initiation. The test consisted of the above geometric series of five test concentrations and one control group. The dietary concentrations were established by Ciba-Geigy Corporation, based upon known toxicity data. Each group (three pens with two geese in each) was fed the appropriate test or control diet for five days. 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 - 7 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 (1978). In this study, probit analysis was used.

12. REPORTED RESULTS:

Dietary Concentration (ppm, a.i.)	Cumulative % Mortality (at Days 5 and 8)
0	0
188	0
375	0
750	33
1500	0
3000	50

"CONTROLS

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

"DIAZINON MG8

"There were no mortalities at the 188, 375 and 1500 ppm concentrations.... There were 33% mortality (2 of 6) at the 750 ppm concentration and 50% mortality (3 of 6) at the 3000 ppm concentration.

"There were no overt signs of toxicity at the 188 and 375 ppm concentrations. All birds were normal in appearance and behavior throughout the test period.

"At the 750 ppm concentration, the two mortalities were noted on the morning of Day 2 and on the afternoon of Day 3. No signs of toxicity were observed prior to death. All other birds at this concentration were normal in appearance and behavior until study termination.

"At the 1500 ppm concentration, one bird was observed with slight reduced reaction to external stimuli (sound and movement) on the morning of Day 3. All other birds were normal in appearance and behavior until study termination.

"At the 3000 ppm concentration, one mortality was noted on the afternoon of Day 0. Signs of toxicity observed prior to death included depression, reduced reaction to external stimuli (sound and movement), wing droop, prostrate posture, slight convulsions, lower limb rigidity and salivation. On the afternoon of Day 1, one bird was noted with wing droop, loss of coordination, lower limb weakness, salivation and lethargy. The bird was found dead approximately one and one-half hours later. The third mortality was noted on the morning of Day 5. No signs of toxicity were observed prior to death. All other birds at this concentration were normal in appearance and behavior until study termination.

"A decrease in body weight was noted at all concentrations and the control group during the exposure period (Days 0-5).... However, the loss in body weight among the treatment groups was greater than that observed in the control. In addition there was a marked reduction in feed consumption at all concentrations when compared with the control."

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

"...The Canada Goose dietary LC_{50} value of DIAZINON MG8 for this study was determined to be 3912 ppm a.i. with a 95% confidence interval of 1667 ppm to 1.7×10^{38} ppm. The no-observed-effect dosage was less than 188 ppm, the lowest dosage tested, based on body weight loss and a reduction in feed consumption at 188 ppm.

The authors indicated that, "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 five times during the experimental and report preparation phases of this study.

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

A. Test Procedure(s):

Raw mortality data were consistent with written report. Regarding reduced feed consumption, it wasn't clear whether the data in Table 4 were "average per bird" or "average per pen." If the latter, the data should be normalized by bird, so that they reflect the number of birds that died during the experiment.

To the extent that SEP, Subdivision E and ASTM Standards can be translated to the Canada goose, the study basically followed those guidelines. Important exceptions include:

(1) Test Organisms. SEP protocol (pg. 2) indicates a preference for the mallard duck, at an age of 5-10 days; 5 day-old birds being preferred. Study used Canada geese, in adult plumage, but of unknown age.

(2) Test Conditions. SEP protocol (pg. 3) recommends 10 birds per concentration. Study used 6 birds (2 per cage) for each concentration.

SEP (pg. 3) indicates that, in order to provide statistically-reliable results, the design should produce mortality ranging from 10 to 90 percent and three partial kills (i.e., between 0 and 100 percent) surrounding the estimated LC_{50}. Study did not accomplish either of these objectives.

SEP (pg. 4) provides guidelines for pen facilities for bobwhite quail and mallard ducks. There are, however, no guidelines for Canadian geese, making an assessment of study conditions difficult.

(3) Results of Residue Analyses. SEP (pg. 5) requires that if test material was analyzed, the results should be reported. Study (pg. 9) implied that samples were analyzed but no residue data were presented.

(4) Dose-Response Curve. SEP (pg. 5) requires that the slope of the dose-response line be calculated and reported. This was not done.

(5) Gross Necropsies. Gross necropsies are preferred by SEP (pg. 6). These were, apparently, not done.

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

C. Discussion/Results: Important issues and questions that are raised by this study are as follows:

(1) Test Organisms. The use of Canada geese is logical, given its exposure opportunity and documented problems in areas of diazinon use. The use of adult birds (especially of unknown sex), on the other hand, runs contrary to the intent of the SEP and other guidelines. It is widely known that LC_{50}s often increase with age of bird tested (Hill and Camardese, 1981). Therefore, the study results are likely to underestimate the risk to younger Canada geese. 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.

(2) Test Conditions. The choice of exposure levels and the resulting inconsistent mortality (i.e. 750 ppm resulting in 33% mortality, 1500 ppm - none, and 3000 ppm - 50%) creates some serious interpretive problems. The authors report an LC_{50} value of 3912 ppm but a 95% confidence interval of 1667 - 1.7×10^{38} ppm. Such a large c.i. doesn't give this reviewer much confidence in the LC_{50} estimate. If the above LC_{50} is an accurate reflection of toxicity (which could be questioned), Diazinon MG8 would be considered slightly toxic to adult Canada geese when provided in the diet.

(3) Residue Analyses. 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.

(4) Repellency. Although the authors don't dwell on it, geese on diazinon diets consumed less feed than controls and tended to lose more weight than the controls. These observations raise the issue of repellency with this species.

The way that data were presented in the two relevant tables, however, made interpretation difficult. To improve the value of these data: (a) body weight loss (Table 3) should be transformed into percentage of original weight; and, (b) feed consumption (Table 4) should represent amount per bird (vs. per pen) to take into account those birds that died during study (Note: This may already be the way that Table 4 data are presented but it is not clear to the reader).

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: This study is considered supplemental for the following reasons:

(a) Although the species is a good choice for reasons of risk opportunity, using adult birds does not meet the intent of the SEP, for reasons discussed in 14C(1).

(b) The inconsistent mortality, in response to the chosen exposure levels, and the resultingly large confidence interval (i.e. 1667 to 1.7×10^{38} ppm) raises the issue of confidence in the findings.

(3) Reparability: Possibly reparable to Core level, depending on applicant's response to issues raised in 14C(1 and 2).

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

16. CBI APPENDIX: N/A

REFERENCES CITED

Hill, E. F. and Camardese, M. B. "Subacute Toxicity Testing with Young Birds: Response in Relation to Age and Interest Variability 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.

Stephan, C. E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota; Personal Communication.

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, 11. 98-104.

Proj No. 108-279

J. Newman Diazinon MSB Canada geese 02-02-88

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3000	6	3	50	65.625
1500	6	0	0	1.5625
750	6	2	33.33334	34.375
375	6	0	0	1.5625
188	6	0	0	1.5625

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 3000

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
5	.9928161	1	.1524426

SLOPE = 1.681982

95 PERCENT CONFIDENCE LIMITS = 6.050945E-03 AND 3.357912

LC50 = 3911.784

95 PERCENT CONFIDENCE LIMITS = 1666.721 AND 1.701412E+38

LC10 = 687.5636

95 PERCENT CONFIDENCE LIMITS = 5.877472E-39 AND 1593.397

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} = 3912 ppm (95% C.L. (1667 - 1.7 x 10^{38})) Contr. Mort. (%) = 0		
Species: Canada Goose		Slope = (not given) # Animals/Level = 6 Age (Days) = ? Sex = ?	Lincer 1/24/88	Supplemental
Lab: Wildlife Int. Ltd.	86.6%			
Project No: 108-279		8-Day Dose Level ppm (% Mortality)		
AC #:		188 (0), 375 (0), 750 (33), 1500 (0), 3000 (50)		

Comments: Using adult birds and the above large confidence interval are important issues with respect to validity and completeness.

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