2-6-88

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon MG8

2. TEST MATERIAL: Diazinon MG8; Fl. Number 861103; reported purity of 86.6% (assigned Wildlife International i.d. number WIL-1148).

3. STUDY TYPE: Avian Single Dose Oral LD_{50}
Species tested: Canada Goose
(Branta canadensis)

4. CITATION: Grimes, J. and M. Jaber. 1987. Diazinon MG8: An Acute Oral Toxicity Study with the Canada Goose. WIL Project No.: 108-280. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corp., Greensboro, NC. Project No.: 108-280

5. REVIEWED BY:

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

Signature:
Date: 1/28/88

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 basically scientifically sound but the occurrence of regurgitation substantially weakens estimate of LD_{50}.

A precise Canada goose acute oral LD_{50} value for DIAZINON MG8 could not be determined due to regurgitation. Test data (ignoring regurgitation) indicate the LD_{50} value to be greater than 6 mg active ingredient (a.i.)/kg, and less than 39.3 mg a.i./kg. The calculated LD_{50} value, irrespective of regurgitation, was 25 mg a.i./kg with a 95% confidence interval of 18 mg/kg to 45 mg/kg. The no-observed-effect dosage was less than 6 mg a.i./kg. With the above, as a conservative estimate of the LD_{50}, Diazinon MG8 is at least highly toxic to adult Canada geese when intubated.

8. RECOMMENDATIONS: Study to determine standard LD_{50} should be redone in such a way as to avoid subject regurgitation. If it can be done with the Canada goose, use younger (16 week old) birds. If regurgitation can't be avoided with Canada geese, use mallard ducks or other species of the appropriate age.

9. BACKGROUND: N/A

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: All Canada geese appeared to be in good health at initiation of the study. Canada geese ranged in weight from 2997 grams to 5813 grams at study initiation. The birds were obtained from Carl Webb, Jr., Betterton, Maryland. The precise age of the birds was not known by the supplier; however, the birds were in adult plumage. All birds were pen-reared and phenotypically indistinguishable from wild birds. Birds were assigned to five test groups and one control group. Each treatment or control group contained six geese. All test birds were acclimated to the caging and facilities for 9 days prior to the initiation of the study.

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 six Canada geese were assigned to each of the treatment groups and the control group by random draw. Individuals could not be differentiated by sex. The test consisted of a geometric series of five dosage groups (see above) and a control group. The dosages were established by Ciba-Geigy based upon known toxicity data. The control group was dosed with diluent only.

Birds were acclimated for nine 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 1 milliliter per kilogram of body weight.

The primary phases of this study and their durations were:

1. Acclimation - 9 days.

2. Fasting - At least 15 hours prior to dosing.

3. Dosing - The date of study initiation - January 30, 1987.

4. 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 was used.

12. REPORTED RESULTS:

Cumulative Mortality of Canada Geese Gavaged with Diazinon MG8

Dosage (mg a.i./kg)	Percent Dead
Control	0
6.0	0
9.6	0
15.4	33
24.6	33
39.3	83

"CONTROLS

"There were no mortalities in the control group (see table above). All birds were normal in appearance and behavior throughout the test period.

"DIAZINON MG8

"There were no mortalities at the 6.0 and 9.6 mg/kg dosages.... There was 33% mortality (2 of 6) at the 15.4 and 24.6 mg/kg dosages and 83% mortality (5 of 6) at the 39.3 mg/kg dosage.

"At the 6.0 mg/kg dosage, signs of toxicity were first noted approximately 1 and 1/2 hours after dosing when two birds regurgitated. Approximately 4 hours after dosing, one bird was noted with slight loss of coordination and lethargy. One bird was noted as lethargic on Day 1 and feather-picked on Day 2. This condition continued for the length of the study with the bird noted as severely feather-picked by Day 3. All other birds at this dosage level were normal in appearance and behavior from the morning of Day 1 until study termination.

"At the 9.6 mg/kg dosage, signs of toxicity and regurgitation were first noted approximately one hour and fifteen minutes after dosing. A total of three birds were observed regurgitating. By the morning of Day 1, all birds were normal in appearance and behavior until study termination.

"At the 15.4 mg/kg dosage, signs of toxicity were first noted approximately forty minutes after dosing when one bird regurgitated. Approximately one hour and fifteen minutes after dosing, other overt signs of toxicity became apparent and two bird were observed regurgitating. The two mortalities were noted approximately three hours after dosing. By the morning of Day 1, all survivors were normal in appearance and behavior and remained so until study termination.

"At the 24.6 mg/kg dosage, one bird regurgitated approximately one half hour after dosing. Approximately fifty minutes after dosing other overt signs of toxicity became apparent, and within approximately two and one-half hours of dosing two mortalities had occurred. By the morning of Day 1, all survivors had recovered and were normal in appearance and behavior until study termination.

"At the 39.3 mg/kg dosage, signs of toxicity were first noted approximately fifteen minutes after dosing when one bird regurgitated. Approximately forty-five minutes after dosing, other overt signs of toxicity became apparent and three more birds were observed regurgitating. Within approximately two and one-half hours of dosing, three mortalities had occurred. Signs of toxicity continued through Day 1 with two mortalities noted during the afternoon observation. The sole survivor was normal in appearance and behavior from the morning of Day 2 until study termination.

"Overt signs of toxicity typical of Canada Geese dosed with DIAZINON MG8 included depression and/or lethargy, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, salivation and lower limb rigidity.

"When compared to the control group, there was a reduction in body weight gain or body weight loss at all dosage groups tested during the first 3 days of the study.... There was a corresponding reduction in feed consumption at the 24.6 and 39.3 mg/kg dosages for that same period.... A reduction in feed consumption continued to be noted at the 24.6 and 39.3 mg/kg dosages for the remainder of the study.

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

"... 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 a.i./kg, but was less than 39.3 mg a.i./kg. These results correspond closely with the calculated LD_{50} value for Diazinon MG8, irrespective of regurgitation, which was determined to be 25 mg a.i./kg with a 95% confidence interval of 18 mg/kg to 45 mg/kg. The no-observed-effect dosage was less than 6 mg a.i./kg."

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...." Five audits were performed during the experimental and reporting phases of this study.

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

A. Test Procedure(s): Author adjusted dosages to 100% a.i. for dosage and LD_{50} calculations.

Raw mortality, weight change and feed consumption data were consistent with written report.

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 16 weeks. Study used Canada geese, in adult plumage, but of unknown age and sex.

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

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

(3) Calculated LD_{50}. SEP (pg. 5) requires that an LD_{50}, 95% c.i. and the slope of the dose-response line be calculated and reported. This was not done.

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

(5) Vomiting. Regurgitation was noted in all groups except the controls. SEP (pg. 7) indicates that this may mean that the test has to be rerun.

B. Statistical Analysis: Author adjusted dosages for percent a.i. for purposes of calculating LD_{50}. The LD_{50} was confirmed using Stephan's Computer program (TOXANAL) See attached printout.

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, which might result in varying degrees of aggression), on the other hand, runs contrary to the intent of the SEP and other guidelines. It is widely known that toxicity often decreases with age of bird tested. Therefore, the study results are likely to underestimate the risk to younger Canada geese.

(2) Vomiting. Regurgitation was observed in all but the control groups, which made determination of an LD_{50} value questionable.

(3) Descriptive Categorization of Results. In that 83% of the birds died, when gavaged with 39.3 mg/kg Diazinon MG8 (and these birds were older and regurgitated), this chemical is at least highly

toxic to Canada geese under these conditions. Reduced food intake, depression and/or lethargy, reduced reaction to stimuli, and/or loss of co-ordination and lower limb weakness and rigidity, even at 6 mg/kg, have negative implications with respect to survival in the wild.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The study is, basically, scientifically sound and does provide some useful information for risk assessment. However, the occurrence of regurgitation in all but the control group, and the use of adult Canada geese, of unknown age and sex, prevents this study from being Core.

(3) Reparability: Not reparable to Core classification.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, on 1/28/88.

16. CBI APPENDIX: N/A

J Newman Diazinon MGB CANADA GOOSE 02-08-88 Proj. No 108-280

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
39.3	6	5	83.33333	10.9375
24.6	6	2	33.33334	34.375
15.4	6	2	33.33334	34.375
9.600001		6	0	0
1.5625				
6	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 LD50 FOR THIS SET OF DATA IS 28.59573

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LD50	95 PERCENT CONFIDENCE LIMITS	
3	.3638307	24.39012	17.67444	43.60154

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.4446959	1	.6193631

SLOPE = 4.268254

95 PERCENT CONFIDENCE LIMITS = 1.421946 AND 7.11456

LD50 = 25.1603

95 PERCENT CONFIDENCE LIMITS = 17.72881 AND 44.5288

LD10 = 12.68149

95 PERCENT CONFIDENCE LIMITS = 3.731001 AND 17.94099

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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

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14-Day Single Dose Oral LD_{50}

LD_{50} = 25* mg/kg

95% C.L. (18-45 mg/kg)

Contr. Mort. (%) = 0

Species: Canada goose

Slope = 4.268

Animals/Level = 6

Age (Days) = ?

Sex = ?

Lincer/ 1-28-88

Supplemental

Lab: Wildlife Int. Ltd. 86.6

Project No: 108-280

AC #:

14-Day Dose Level mg/kg (% Mortality)

6 (0), 9.6 (0), 15.4 (33), 24.6 (33), 39.3 (83)

Comments: * Birds regurgitated; LD_{50} calculation corrected for % a.i.
Adult Canada geese, of unknown sex and age were used.

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