X
2-25-88

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon MG8 Technical

2. TEST MATERIAL: Diazinon MG8 Technical, FL No. 861103, 86.6% a.i.

3. STUDY TYPE: 14-Day Acute Toxicity
   Species Tested: Canada Goose

4. CITATION: Fletcher, D. W. 1987. 14-Day Acute Oral Toxicity Study with Diazinon MG8 Technical in Canada Geese. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. BLAL 86 CD 1.

5. REVIEWED BY:

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

   Signature:
   Date: 2/17/88

6. APPROVED BY:

   James R. Newman, Ph.D.
   Proj. Mgr., KBN Engineering and Applied Sciences, Inc.

   Signature: James R Newman
   Date: 2/25/88

   Henry T. Craven
   Chief EEB/HED
   USEPA

   Signature:
   Date:

7. CONCLUSIONS:

   The study is scientifically sound. The LD_{50} of 6.16 mg a.i./kg (95% c.i. of 2.89 - 11.52 mg a.i./kg) make Diazinon MG8 Technical very highly toxic to Canada geese when given orally. Data requirements are met subject to applicant verifying test species with scientific name.

8. RECOMMENDATIONS: Use birds of more uniform age. Provide scientific name of test species. Use photoperiod 10 hours light per day (SEP, pg. 4).

9. BACKGROUND: N/A

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: The birds employed in the study were unmated (ranging in age from 6 months to 2> years old) Canada Geese received from Sherwood F. Krosch, Elmore, MN. The birds selected for the study had been under observation for a 63-day pre-test period to determine their suitability as test birds based on their general physical condition, and to acclimatize them to the laboratory conditions. The birds were identified by means of metal leg bands embossed with numbers unique within the study. Prior to the initiation of the project, all birds were examined and determined to be suitable for testing.

B. Dosage and Design: Birds were divided and gavaged according to the below table:

Group	Number of Birds		Dose Level (mg a.i./kg of body weight)
	Male	Female	
VC	3	3	0
T-I	3	3	2.15
T-II	3	3	4.64
T-III	3	3	6.81
T-IV	3	3	10.0
T-V	3	3	21.5

Note: The test material was diluted with corn oil such that 1 milliliter of the resulting solution equaled 10 mg Diazinon MG8 Technical (1 ml = 10 mg). The test solution was dispensed via disposable syringes. Each test bird received its respective dose of test material. The vehicle control birds each received an amount of corn oil equal to the largest volume dispensed in the T-V group (12.9 ml).

Dose levels were based on a geometric scale of 1.47. The geese were randomly assigned to test groups, and weighed individually at 0 hour on test day 1. Subsequent individual body weights were obtained on test days 3, 7, 14, and 21.

All birds were fasted (with water permitted) for approximately 19 hours prior to dosing at 0 hour on test day 1. Food and water were withheld for approximately 1 3/4 hours after dosing to reduce the possibility of regurgitation. The birds were permitted a standard laboratory diet of Purina Duck Grower w/o, plus water ad libitum, at all other times. Food consumption was recorded on test days 3, 7, 14, and 21. The doses for the individual test birds were volumetrically measured and administered via disposable syringes at 0 hour on test day 1. The vehicle control birds each received an amount of corn oil equal to the largest volume dispensed in the 21.5 mg/kg group (12.9 ml).

C. Statistics: The acute LD_{50} was calculated according to: Litchfield, J. T., Jr., and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments," The Journal of Pharmacology and Experimental Therapeutics, Vol. 96, No. 2, June 1949.

12. REPORTED RESULTS:

A. Reactions

At approximately two hours post-dosing, four 21.5 mg/kg birds were found dead, while the 6.81, 10.0 and surviving 21.5 mg/kg birds were lethargic. One hour later, three 6.81, four 10.0, and one additional 21.5 mg/kg bird were found dead while one 6.81 and the surviving 10.0 and 21.5 mg/kg birds remained lethargic. When the birds were checked one hour later, one 2.15, two 4.64, one 6.81 and the surviving 10.0 and 21.5 mg/kg appeared conscious but unresponsive to external stimuli with one 6.81 and both surviving 10.0 mg/kg birds also experiencing hypersalivation. One hour later, one 2.15 and one 10.0 mg/k bird were found dead. The conditions of the surviving birds remained unchanged, except for the 10.0 mg/kg bird, which was active.

At 21 hours post-dosing, one 4.64 and one 6.81 mg/kg bird were found dead. These were the last two mortalities recorded during the investigation. All birds

appeared to be active at this point in time, except for one 21.5 mg/kg bird which remained lethargic.

"Near the end of test day 2, the surviving 10.0 and 21.5 mg/kg birds were anorexic and lethargic, while all other birds appeared to be normal and active and remained so for the balance of the project. Near the close of test day 3 [, these] birds were becoming more active and were recorded as being normal and active near the end of test day 4. However, anorexia persisted through test day 7 in the [10.0 mg/kg] bird and through test day 15 in the [21.5 mg/kg] bird.

"Near the end of test day 7, it was noted that the surviving [21.5 mg/kg] bird had slight difficulty in walking (favored one leg over the other - presumed soreness or lameness in one leg) near the close of test day 14, this condition persisted and the bird appeared weak. Twenty-four hours later, it was observed that this bird was unable to walk. At the close of test day 18, this bird was immobile and its feces were bright green in color. This bird's condition remained unchanged at termination.

"The vehicle control birds were normal and active throughout the entire investigation."

B. Mortality and Post-Mortem Examinations

"No mortalities occurred in the [control] group. Mortality in dosed groups occurred as presented below:

Group	Dose Level (mg a.i./kg of body weight)	Number Dead Number Tested Male	Female	% Dead	Test Day Found Dead
VC	0	0/3	0/3	0	-
T-I	2.15	0/3	1/3	16.7	1
T-II	4.64	0/3	1/3	16.7	1
T-III	6.81	3/3	1/3	66.7	1,1,1,1
T-IV	10.0	2/3	3/3	83.3	1,1,1,1,1
T-V	21.5	3/3	2/3	83.3	1.1.1.1.1

C. Body Weight Data

"Statistical evaluation of the body weight data was conducted using Analysis of Variance. Statistical analysis of the body weights at each weighing interval revealed no statistically significant differences in the test groups' body weights when compared to the control group values."

D. Food Consumption Data

"Food consumption values in the vehicle control group ranged from 124 to 242 grams/bird/day during the investigation.

"Severe food avoidance was noted during the first three days of the project in all the test groups when compared in the vehicle control group. Also, severe food avoidance was noted through test day 14 in the [10.0 and 21.5 mg/kg] test groups. Food consumption values during test days 15 through 21 in the [same] test groups were within the normal control group range.

"All [2.15 through 6.81 mg/kg] test values during test days 4 through 21 were comparable to the vehicle control group's values."

E. Post Mortems

"Post-mortem examinations revealed no visible abnormal tissue alterations in the test birds found dead during the investigation.

"Gross pathological examinations on test day 21 of all birds sacrificed in each group revealed no abnormal tissue alterations."

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

"The results of the 14-Day Acute Oral Toxicity Study conducted with Diazinon MG8 Technical in Canada Geese showed the acute oral median lethal dose (LD_{50}) of the test material to be 6.81 mg/kg (5.90 mg a.i./kg) of body weight with 95% confidence limits of 3.72 to 12.46 mg/kg (3.22 to 10.79 mg a.i./kg) of body weight.

"In accordance with BLAL Laboratories' intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for Diazinon MG8 Technical was conducted and found to be in acceptable form by [the] Quality Assurance Officer. A final inspection of all data and records on February 19, 1987 indicates that the report submitted ... is an accurate reflection of the study as it was conducted by BLAL Laboratories."

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

A. Test Procedure(s):

Raw data, with respect to mortality, food consumption and weight, basically agreed with that reported in text. The one possible exception relates to food consumption. The text indicates that, "Food consumption values during test days 15 through 21 in [the 10.0 and 21.5 mg/kg] test groups were within the normal control group range." If compared with the controls, for the same time period, those values appear to be quite low.

To the extent that the SEP and subdivision E can be translated to the Canada goose, the study basically followed those guidelines. Possibly important exceptions are:

(1) Test Organisms. SEP (pg. 2) indicates a preference for the mallard duck, as a waterfowl species, at an age of at least 16 weeks old. Study used adult Canada geese, of widely-varying ages.

(2) Test Conditions. SEP (pg. 3) recommends 10 birds/treatment group. Study used 6 birds/treatment.

B. Statistical Analysis: The LD_{50} calculated by the probit method using Toxanal is slightly higher and has a wider c.i. than the author's values. 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 mostly adult birds may underestimate the risk to younger Canada geese.

(2) Repellency. Although the author doesn't dwell on it, geese gavaged with Diazinon consumed less feed than controls. These observations may relate to the issue of repellency with this species. Since the birds were not presented with food containing Diazinon, the birds weren't "repelled" by the chemical in their diet. Two possibilities are that (a) the intubated chemical resulted in some biochemical alteration, which caused lack of appetite, or (b) the treated birds were simply too sick to eat.

(3) Implications of Dose-Mortality Response. The response is rapid, with a relatively narrow range between 16.7% mortality (at 2.15 mg/kg) and 83.3% mortality (at 10.0 mg/kg). No NOEL was established due to mortality and significant reduction in food consumption at the lowest dose administered (i.e. 2.15 mg/kg).

(4) Descriptive Categorization of Results. The acute single oral LD_{50} of 6.16 mg a.i./kg (95% c.i. of 2.89 - 11.52 mg a.i./kg) makes Diazinon MG8 Technical very highly toxic to Canada geese, when given orally. Sublethal toxicity including lethargy, anorexia, weakness and paralysis have negative implications with respect to survival in the wild.

D. Adequacy of the Study:

(1) Classification: Supplemental, subject to applicant verifying species tested with scientific name.

(2) Rationale: This study is scientifically sound and does provide useful information for risk assessment. However, the use of Canada geese technically prevents this study from being Core. However, this species is, at the same time, one of the most appropriate to test, given the reported incidences of toxic effects with the species in areas of Diazinon use.

(3) Reparability: This study cannot be upgraded to Core.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, on February 17, 1988.

16. CBI APPENDIX: N/A

ONE LINER SHEET

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

Study/Species/Lab/ Accession #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD_{50} = 6.16 mg a.i./kg (95% C.L. (2.89 - 11.52)) Contr. Mort. (%) = 0		
Species: Canada Goose		Slope = 2.399 # Animals/Level = 6 Age (Months) = 6 - 30 Sex = both	Lincer/ 2-17-88	Supplemental
Lab: Bio-Life Assoc's.	86.6			
AC #: BLAL 86 CD 1		14-Day Dose Level mg/kg (% Mortality): 2.15 (16.7), 4.64 (16.7), 6.81 (66.7), 10.0 (83.3), 21.5 (83.3)		
		Comments:		

BLAL B6 CD 1

J. Newman Diazinon MG8 Technical Canada goose 02-24-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
21.5	6	5	83.33333	10.9375
10	6	5	83.33333	10.9375
6.81	6	4	66.66667	34.375
4.64	6	1	16.66667	10.9375
2.15	6	1	16.66667	10.9375

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 6.020227

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	2.652958	5.620936	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.5104779	1	.4723685

SLOPE = 2.399502
95 PERCENT CONFIDENCE LIMITS = .6851124 AND 4.113892

LC50 = 6.162584
95 PERCENT CONFIDENCE LIMITS = 2.894695 AND 11.51578

LC10 = 1.82174
95 PERCENT CONFIDENCE LIMITS = 6.537775E-02 AND 3.507885

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