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Record No.

Review No.

057801

Shaughnessey No.

EEB REVIEW

JAN 23 1991

DATE: IN 12-21-88 OUT JAN 23 1991

FILE OR REG. NO. 100-524

PETITION OR EXP. NO.

DATE OF SUBMISSION 11-16-88

DATE RECEIVED BY EFED 12-24-88

RD REQUESTED COMPLETION DATA 03-20-89

EEB ESTIMATED COMPLETION DATE 01-20-91

RD ACTION CODE/TYPE OF REVIEW 660

TYPE PRODUCTS(S): I, D, H, F, N, R, S Insecticide

DATA ACCESSION NO(S). 408953-01, to -10

PRODUCT MANAGER NO. G. La Rocca (15)

PRODUCT NAME(S) Diazinon

COMPANY NAME CIBA-GEIGY Corporation

SUBMISSION PURPOSE Submission of toxicity data in response to registration standard

SHAUGHNESSEY NO.

CHEMICAL AND FORMULATION

% A.I.

	Diazinon	



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

TO: George LaRocca (PM15)
Insecticide-Rodenticide Branch
Registration Division

FROM: James A. Germain, Chief
Ecological Effects Branch
Environmental Fate and Effects Division

SUBJECT: Review of Diazinon studies

The Ecological Effects Branch has reviewed ten studies submitted by CIBA-GEIGY. The results of these reviews are summarized below.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 8-Day Acute Dietary LC50 Study in Mallard Ducklings. Laboratory Project No. 88 DC 105. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-02.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian dietary LC50 test. With an LC50 value of 32 ppm a.i., Diazinon MG8 Technical is considered very highly toxic to mallard ducklings (Anas platyrhynchos). The NOEL was determined to be 16 ppm a.i.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 14-Day Acute Oral LD50 Study in Mallard Ducks. Laboratory Study No. 88 DD 56. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-01.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 1.44 mg a.i./kg, Diazinon MG8 Technical is considered very highly toxic to mallard ducks (Anas platyrhynchos). The NOEL was 0.316 mg a.i./kg.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 8-Day Acute Dietary LC50 Study in Brown-headed

Cowbirds. Laboratory Project No. 88 SBLC 105. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-04.

CONCLUSIONS: With an LC50 value of 38 ppm a.i., Diazinon MG8 Technical is considered very highly toxic to brown-headed cowbirds (Molothrus ater). The NOEL was determined to be 8 ppm a.i. The test is scientifically sound with respect to procedures used to determine the LC50 of the test chemical in brown-headed cowbirds, but the species is not a generally accepted one used in support of pesticide registration.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon 14G: 14-Day Acute Oral LD50 Study in Mallard Ducks. BLAL No. 88-DD-54. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-05.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 2.34 mg a.i./kg body weight, Diazinon 14G is considered very highly toxic to mallard duck (Anas platyrhynchos). The NOEL was 0.681 mg a.i./L.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon 14G: 14-Day Acute Oral LD50 Study in Mallard Ducks. BLAL No. 88-DD-55. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-07.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 1.18 mg a.i./kg body weight, Diazinon AG500 is considered very highly toxic to mallard duck (Anas platyrhynchos). The NOEL was 0.316 mg a.i./L.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 14-Day Acute Oral LD50 Study in Brown-headed Cowbirds. Laboratory Project No. 88 SB 103. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-03.

CONCLUSIONS: With an LD50 value of approximately 69.0 mg a.i./kg of body weight, Diazinon MG8 Technical is considered moderately toxic to brown-headed cowbirds (Molothrus ater). The NOEL was determined to be less than 10.0 mg a.i./kg of body weight. A more precise NOEL could not be determined due to mortality and reduced food consumption during the first three days at the lowest test level of 10.0 mg a.i./kg. The test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical in brown-headed cowbirds, but the species is not a generally accepted one used in support of pesticide registration. It therefore does not meet the guideline requirements for an avian oral LD50 test.

3

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon 14G: 14-Day Acute Oral LD50 Study in Brown-Headed Cowbirds. BLAL No. 88-SB-101. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-06.

CONCLUSIONS: This study is scientifically sound but does not fulfill the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 6.85 mg a.i./kg body weight, Diazinon 14G is considered very highly toxic to brown-headed cowbirds (Molothrus ater). The NOEL was less than 2.15 mg a.i./L.

CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon AG500: 14-Day Acute Oral LD50 Study in Brown-Headed Cowbirds. BLAL No. 88-SB-102. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-09.

CONCLUSIONS: This study is scientifically sound but does not fulfill the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 46.4 mg a.i./kg body weight, Diazinon AG500 is considered highly toxic to brown-headed cowbirds (Molothrus ater). The NOEL was less than 10.0 mg a.i./L.

CITATION: Fletcher, D.W. and C.A. Pederson. 1988. Diazinon AG500: 8-Day Dietary LC50 Study in Mallard Ducklings. BLAL No. 88-DC-104. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-08.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian dietary LC50 test. With an LC50 value of 38 ppm a.i., Diazinon AG500 is considered very highly toxic to mallard duck (Anas platyrhynchos). The NOEC was determined to be 8 ppm a.i.

CITATION: Fletcher, D.W. and C.A. Pederson. 1988. Diazinon AG500: 8-Day Dietary LC50 Study in Brown-Headed Cowbirds. BLAL No. 88-SBLC-104. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-10.

CONCLUSIONS: This study is scientifically sound but does not meet the guideline requirements for an avian dietary LC50 test. With an eight-day LC50 value of 42 ppm a.i., Diazinon AG500 is considered very highly toxic to brown-headed cowbird (Molothrus ater). The NOEC was determined to be 16 ppm a.i.

If you have questions regarding these reviews, please contact Skip Houseknecht at 557-4372.

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon.
Shaughnessey No. 057801.
2. TEST MATERIAL: Diazinon MG8 Technical, FL # 880045, 88.2% active ingredient, CAS # 333-41-5.
3. STUDY TYPE: Avian Single-Dose Oral LD50 Test.
Species Tested: Anas platyrhynchos.
4. CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 14-Day Acute Oral LD50 Study in Mallard Ducks. Laboratory Study No. 88 DD 56. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-01.

5. REVIEWED BY:

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Michael L. Whitten*

Date: 2-3-89

6. APPROVED BY:

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *James R. Newman*

Date: 2/6/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*

Date: 9/6/89

- Clyde R. Houseknecht, Ph.D., M.P.H.*
7. CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 1.44 mg a.i./kg, Diazinon MG8 Technical is considered very highly toxic to mallard ducks (Anas platyrhynchos). The NOEL was 0.316 mg a.i./kg.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: The birds used in the study were 19-week old mallard ducks (Anas platyrhynchos), obtained from Whistling Wings, Inc., Hanover, IL. The birds had received P.A. Bactrin vaccinations at 7 and 14 days of age. Ducks selected for the study had been under observation for a 21-day quarantine period to determine their suitability as test subjects and to acclimate them to laboratory conditions. The animal room temperature during the quarantine period ranged from 52°F to 72°F with relative humidity between 83% and 94%.
- B. Test System: All birds were housed in 121.9 cm x 121.9 cm x 121.9 cm wire pens maintained over concrete in a thermostatically controlled indoor environment. Fluorescent lights provided eight hours of light per day. Maximum and minimum temperatures and relative humidity were monitored and recorded daily. Temperatures ranged from 52°F to 79°F with relative humidity between 57% and 88% during the test period.
- C. Dosage: 14-day single dose oral LD50 test. Based on two seven-day range finding tests, the dosages selected for the definitive study were 0.316, 0.681, 1.470, 3.160 and 6.810 milligrams of Diazinon MG8 Technical per kilogram of body weight.
- D. Design: Groups of ten ducks (five males and five females) were randomly assigned to each of the five treatment groups and the control group. The birds were fed Purina Duck Grower and supplied with water ad libitum except for a period of 21.5 to 22.25 hours prior to dosing when food was withheld from all birds. The doses for individual birds were volumetrically measured and administered via a single gelatin capsule at 0 hour on Test Day 1. Each control bird received one empty gelatin capsule. The birds were individually weighed at 0 hour on test day 1, and on test days 3, 7, and 14. Group food consumption was recorded on test days 3, 7, and 14. Daily observations were conducted for potential clinical signs indicative of test material effect. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds (two of each sex) from each of the

control, 0.316- and 0.681-mg/kg groups, as well as all survivors from the 1.47-, 3.16-, and 6.81-mg/kg groups, were subjected to gross pathological examinations on test day 14.

E. Statistics: The LD50 was calculated using the simplified method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949). Statistical evaluation of the body weight data was conducted using 1-Way Analysis of Variance. No other statistical analyses were reported.

12. REPORTED RESULTS: Mortalities occurred in each of the four highest treatment groups (Table 3, attached). All mortalities except one occurred during day 1. The first deaths occurred within one hour after dosing. Gross pathological examinations revealed no abnormalities in survivors and in 24 of 26 birds that died during the study. One mortality from the 3.16-mg/kg treatment group was emaciated, with loose brown fecal matter in the intestines, and stained feathers in the rectal area. One mortality from the 6.81 mg/kg treatment group showed penile protrusion.

No abnormal behavioral reactions or signs of toxicity were noted in the control or 0.316-mg/kg treatment groups. Signs of toxicity in the four highest treatment groups included lethargy, sitting, inability to walk, and regurgitation. Total remission of all signs was achieved by the end of test day 1 except for the bird from the 3.16-mg/kg group that died on day 8. This bird was lethargic on day 7 and had a weight loss of 122 grams from day 3 to day 7.

Statistical analysis of the body weights revealed no significant differences between groups at any of the weighing intervals. Food consumption was depressed during the first three test days only in the 3.16-mg/kg group. Severe food avoidance was noted during test days 4-7 only in the 6.81-mg/kg group. All other food consumption values were comparable to the control group (Table 4, attached).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 14-day acute oral LD50 of Diazinon MG8 Technical for mallard ducks was determined to be 1.44 mg a.i./kg of body weight with 95% confidence limits of 0.82 to 2.52 mg a.i./kg of body weight.

The study was designed and conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by the Quality Assurance Unit of Bio-Life Associates, Ltd.

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

- A. Test Procedure: The test procedures were in accordance with SEP guidelines. Although not mentioned, it was assumed that the birds were subjected to the same food and water regimen during the test as in the quarantine period.
- B. Statistical Analysis: The LD50 value calculated by using EPA's Toxanal computer program was similar to the value reported by the study author.
- C. Discussion/Results: With an LD50 value of 1.44 mg a.i./kg body weight, Diazinon MG8 Technical is considered very highly toxic to mallard ducks when administered as a single oral dose. The NOEL was considered to be 0.316 mg a.i./kg body weight. Abnormal behavioral reactions were noted only during day 1 post dosing, with the single exception of one bird that was lethargic on day 7 and died on day 8.
- D. Adequacy of the Study:
 - (1) Classification: Core
 - (2) Rationale: N/A
 - (3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, February 1, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 9 through 10 are not included in this copy.

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 - _____ Identity of product impurities.
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 - _____ Identity of the source of product ingredients.
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WHITTEN DIAZINON MGB ANAS PLATYRHYNCHOS 1-31-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
6.81	10	9	90	1.074219
3.16	10	9	90	1.074219
1.47	10	7	70	17.1875
.681	10	1	10	1.074219
.316	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .681 AND 3.16 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 1.156075

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.1677542	1.387058	.8685313 2.165249

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1988179	1	.184893

SLOPE = 2.918487
95 PERCENT CONFIDENCE LIMITS = 1.617163 AND 4.219811

LC50 = 1.417148
95 PERCENT CONFIDENCE LIMITS = .9437328 AND 2.121957

LC10 = .5203053
95 PERCENT CONFIDENCE LIMITS = .2028501 AND .8093253

No. _____

Chemical Name VIAZINON

Chemical Class _____

Page 1 of 1Study/Species/Lab/
Accession _____Chemical
X a.l.

Results

Reviewer/
DateValidat
Statu14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments:

14-Day Single Dose Oral LD₅₀LD₅₀ = 1.44 mg/kg. (0.82 - 2.52) Contr. Mort.(X) = 0Species Anas platyrhynchosSlope = 1.89 # Animals/Level = 10 Age(Days) = 133Lab Bio-Life Associates, Ltd.14-Day Dose Level mg/kg/(X Mortality)
0.316 (0.316), 0.631 (0), 1.26 (50), 2.52 (90), 5.04 (90)MLW
2-3-89

CORE

Acc. 408953-01

Comments:

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Lab _____

8-Day Dose Level ppm/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments:

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Lab _____

8-Day Dose Level ppm/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments:

48-Hour L₅₀LC₅₀ = (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Sol. Contr. Mort.(X) =

Lab _____

48-Hour Dose Level / (X Mortality) Temperature =
(), (), (), (), ()

Acc. _____

Comments:

96-Hour LC₅₀LC₅₀ = PP (95% C.L.) Con. Mor.(X) =

Species _____

Slope = # Animals/Level = Sol. Con. Mor.(X) =

Lab _____

96-Hour Dose Level pp / (X Mortality) Temp. =
(), (), (), (), ()

Acc. _____

Comments:

96-Hour LC₅₀LC₅₀ = PP (95% C.L.) Con. Mort.(X) =

Species _____

Slope = # Animals/Level = Sol. Con. Mort.(X) =

Lab _____

96-Hour Dose Level pp / (X Mortality) Temp. =
(), (), (), (), ()

Acc. _____

Comments:

12

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon.
Shaughnessey No. 057801.
2. TEST MATERIAL: Diazinon MG8 Technical, FL # 880045, 88.2% active ingredient, CAS # 333-41-5.
3. STUDY TYPE: Avian Dietary LC50 Test.
Species Tested: Anas platyrhynchos.
4. CITATION: Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 8-Day Acute Dietary LC50 Study in Mallard Ducklings. Laboratory Project No. 88 DC 105. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-02.

5. REVIEWED BY:

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Michael L. Whitten*

Date: 2-6-89

6. APPROVED BY:

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *James R. Newman*

Date: 2/6/89

[Signature] Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*

Date: 9/12/89

7. CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an avian dietary LC50 test. With an LC50 value of 32 ppm a.i., Diazinon MG8 Technical is considered very highly toxic to mallard ducklings (Anas platyrhynchos). The NOEL was determined to be 16 ppm a.i.

8. RECOMMENDATIONS: N/A

Aug 1989 ✓

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Two-day old mallard ducklings were received from Whistling Wings, Inc., Hanover, IL. The birds were acclimated to laboratory conditions for seven days. The animal room temperature during this period ranged from 64°F to 80°F with relative humidity between 40% and 87%. Following the quarantine period, birds were arbitrarily selected from the entire population and assigned to test groups. Birds were not sexed. Prior to the initiation of the test, all birds were examined and determined to be suitable for testing.
- B. Test System: The test system consisted of 45.7 cm x 61 cm x 45.7 cm wire pens maintained over concrete in a thermostatically controlled indoor environment. Fluorescent lights provided 24 hours of light per day. Maximum and minimum temperatures and relative humidity were monitored and recorded daily. Temperatures ranged from 73°F to 81°F with relative humidity between 74% and 82% during the 8-day study period.
- C. Dosage: Acute dietary LC50 test. Diazinon MG8 Technical was incorporated into a standard laboratory diet (Purina Game Bird Startena) via a premix with corn oil. Nominal dietary concentrations were 8, 16, 32, 64, and 128 ppm a.i.
- D. Design: Groups of ten birds were arbitrarily assigned to each of the five treatment and five control groups. Each group was fed the appropriate test diet or vehicle control diet for five consecutive days. The vehicle control birds received a standard laboratory diet mixed with corn oil. Following the five-day test period, all birds were removed from their respective treated diets and given plain feed during a three-day recovery period. Diet samples were collected immediately after preparation and on test day 3 from the vehicle control, 8-ppm and 128-ppm groups and were analyzed for confirmation of dietary levels. Observations were conducted daily for mortalities, potential clinical signs indicative of test material effect, abundance of food and water, and food spillage. Birds were weighed by groups at 0 hour on test day 1, and on test day 8. Food consumption was recorded for each group on test

days 3, 5, and 8. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds from the vehicle control groups and four from each of the 8-, 16-, and 32-ppm groups were subjected to gross pathological examinations at the termination of the project.

E. Statistics: The LC50 was calculated using the simplified method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949). No other statistical analyses were reported.

12. REPORTED RESULTS: Measured concentrations of Diazinon MG8 Technical in test diets averaged 92% of the nominal concentrations, with values ranging from 72% to 106% of nominal concentrations.

No mortalities occurred in the control groups or in the 8- and 16-ppm treatment groups. Five birds from the 32-ppm group and all birds from the 64- and 128-ppm groups died during the investigation (Table 3, attached). Deaths were noted on days 2, 3, 4, 5, and 6. Gross pathological examinations of selected survivors and of all birds that died during the investigation revealed no abnormalities. Within six hours after test diet administration, the 128-ppm birds were trembling and apparently confused. At the end of day 1, the 64- and 128-ppm birds were smaller in size than the control birds. Anorexia was noted at the end of day 2 in the 64- and 128-ppm birds. At the end of days 3 and 4, the 32-ppm and surviving 64-ppm birds were anorexic and small. At the end of day 6, the 32-ppm birds were slightly smaller in size than the control birds. On days 7 and 8, all birds appeared normal and active. No abnormal behavioral reactions or signs of toxicity were noted at any time in the control groups or in the 8- and 16-ppm groups.

Group body weights at the beginning and at the end of day 8 were essentially the same in the test and vehicle control groups (Table 4, attached).

Food consumption values are presented in Table 4, attached. Severe food avoidance was noted in the 64-ppm group during the first three test days, while food consumption in the 8-, 16-, and 32-ppm groups was normal or above normal during the same period. No birds from the 128-ppm group survived after the first three days.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 8-day dietary LC50 of Diazinon MG8 Technical for mallard ducklings was determined to be 32 ppm a.i. with 95%

confidence limits of 27 to 38 ppm a.i. The no observed effect level was determined to be 16 ppm a.i.

The study was designed and conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by the Quality Assurance Unit of Bio-Life Associates, Ltd.

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

- A. Test Procedure: The test procedures and the report were in accordance with SEP guidelines except for the following deviations:

Insig. The size of the test pens (127,398 cm³) was smaller than the recommended size of 168,000 cm³.

No Birds were weighed by groups. According to the SEP, individual body weights should be measured.

Prob. Insig. Food consumption was recorded on test days 3, 5, and 8. According to the SEP, food consumption should also be recorded at the beginning and end of the pretreatment period.

Formal The birds were not randomly assigned to groups. Instead, arbitrary selections were made from the entire population of ducklings.

Major Prob. The test dosages resulted in only one "partial kill". The statistical reliability of the calculated LC50 is therefore questionable. (See para. 14 B.) In this case, the "partial kill" (5 of 10 birds) at 32 ppm did in fact result in the death of 50% of the test animals.

Prob. Insig. Two birds were found dead approximately 3 hours after the beginning of test day 6. At the end of test day 8, therefore, 72 consecutive hours without mortality had not occurred.

- B. Statistical Analysis: The LC50 value calculated by using EPA's Toxanal computer program was the same as the value reported by the study author. Since there was only one concentration at which the percent dead was between 0 and 100, however, neither the moving average angle nor the probit method can give statistically sound results. The reported LC50 should therefore be regarded only as an approximation. A more precise value could be obtained by a dietary test with at least one dose level between 16 and 32 ppm and at least one dose level

between 32 and 64 ppm in order to produce "partial kills" surrounding the estimated LC50.

C. Discussion/Results: With an LC50 value of 32 ppm a.i., Diazinon MG8 Technical is considered very highly toxic to mallard ducklings. The NOEL was considered to be 16 ppm a.i. Abnormal behavioral reactions (confusion, weakness, anorexia) were noted in the 32-, 64-, and 128-ppm groups. All survivors appeared normal by the end of day 6.

D. Adequacy of the Study:

- (1) Classification: Core
- (2) Rationale: Although the test procedures deviated from the guidelines, the reviewer does not believe they affected the validity of the test results.
- (3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, February 3, 1989.

DIAZINON

Page _____ is not included in this copy.

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- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
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WHITTEN DIAZINON MGB ANAS PLATYRHYNCHOS 02-03-89

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CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED      DEAD        DEAD        PROB. (PERCENT)
128        10          10          100          9.765625E-02
64          10          10          100          9.765625E-02
32          10           5           50          62.30469
16          10           0           0           9.765625E-02
8           10           0           0           9.765625E-02
*****
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THE BINOMIAL TEST SHOWS THAT 16 AND 64 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 32

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.

No. _____

Chemical Name DIAZINON

Chemical Class _____

Page 1 of 1Study/Species/Lab/
Accession _____Chemical
X a.l. _____

Results

Reviewer/
Date _____Validat
Statu _____14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = # Animals/Level = _____ Age(Days) = _____
Sex = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg. (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = # Animals/Level = _____ Age(Days) = _____
Sex = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

8-Day Dietary LC₅₀LC₅₀ = 32 ppm (95% C.L.) Contr. Mort.(X) = 0Species Anas platyrhynchos

Slope = N.A. # Animals/Level = 10 Age(Days) = 9

Lab Bio-Life Associates, Ltd 88-2Sex = NOT DETERMINED MLW/2-3-89 COR
8 (0), 16 (0), 32 (50), 64 (100), 128 (100)Acc. 408953-02

Comments: _____

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = # Animals/Level = _____ Age(Days) = _____
Sex = _____

Lab _____

8-Day Dose Level ppm/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

48-Hour L₅₀LC₅₀ = (95% C.L.) Contr. Mort.(X) = _____
Sol. Contr. Mort.(X) = _____

Species _____

Slope = # Animals/Level = _____ Temperature = _____

Lab _____

48-Hour Dose Level / (X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = pp (95% C.L.) Con. Mor.(X) = _____
Sol. Con. Mor.(X) = _____

Species _____

Slope = # Animals/Level = _____ Temp. = _____

Lab _____

96-Hour Dose Level pp / (X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = pp (95% C.L.) Con. Mort.(X) = _____
Sol. Con. Mort.(X) = _____

Species _____

Slope = # Animals/Level = _____ Temp. = _____

Lab _____

96-Hour Dose Level pp / (X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

21

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon MG8 Technical, FL # 880045, 88.2% active ingredient, CAS # 333-41-5.
3. **STUDY TYPE:** Avian Oral LD50 Test.
Species Tested: Brown-headed cowbird
(Molothrus ater)
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988.
Diazinon MG8 Technical: 14-Day Acute Oral LD50 Study in Brown-headed Cowbirds. Laboratory Project No. 88 SB 103. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-03.

5. **REVIEWED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Michael L. Whitten*

Date: 2-23-89

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *James R. Newman*

Date: *3/1/89*

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:

Date: *Henry T. Craven 4/6/89*

- Clyde R. Houscknecht, Ph.D., M.P.H.*
7. **CONCLUSIONS:** With an LD50 value of approximately 85.0 mg a.i./kg of body weight, Diazinon MG8 Technical is considered moderately toxic to brown-headed cowbirds (Molothrus ater). The NOEL was determined to be less than 10.0 mg a.i./kg of body weight. A more precise NOEL could not be determined due to mortality and reduced food consumption during the first three days at the lowest test level of 10.0 mg a.i./kg. The test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon MG8 Technical, FL # 880045, 88.2% active ingredient, CAS # 333-41-5.
3. **STUDY TYPE:** Avian Oral LD50 Test.
Species Tested: Brown-headed cowbird
(Molothrus ater)
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988.
Diazinon MG8 Technical: 14-Day Acute Oral LD50 Study in Brown-headed Cowbirds. Laboratory Project No. 88 SB 103. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-03.
5. **REVIEWED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature:
Date: 2/23/89
6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:
Date:

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*
9/6/89
Date: *Clyde Hruschke* 9/6/89
7. **CONCLUSIONS:** With an LD50 value of approximately 69.0 mg a.i./kg of body weight, Diazinon MG8 Technical is considered moderately toxic to brown-headed cowbirds (Molothrus ater). The NOEL was determined to be less than 10.0 mg a.i./kg of body weight. A more precise NOEL could not be determined due to mortality and reduced food consumption during the first three days at the lowest test level of 10.0 mg a.i./kg. The test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical

in brown-headed cowbirds, but the species is not a generally accepted one used in support of pesticide registration. It therefore does not meet the guideline requirements for an avian oral LD50 test.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Brown-headed cowbirds were live-trapped in Michigan and Wisconsin by personnel from Bio-Life and the U.S. Fish and Wildlife Service. The birds from Michigan arrived at Bio-Life Associates on 4/29/88 and were immediately placed in quarantine for 52 days. The birds from Wisconsin were trapped from 5/02/88 until study initiation and taken to the laboratory at Bio-Life on a daily basis where they were immediately placed in quarantine. The quarantine periods were implemented in order to determine the birds' suitability as test units and for acclimation to laboratory conditions. All birds were fed Purina Duck Grower W/O during the quarantine period. The animal room temperature during this period ranged from 40°F to 96°F with relative humidity between 57% and 88%. Eighty-one out of 438 birds from Michigan and 44 out of 289 birds from Wisconsin died of natural causes during the quarantine period. Prior to initiation of the test, all birds were examined and their suitability for testing (based on general physical condition) was determined.
- B. Test System: The test system consisted of 61.0 cm x 45.7 cm x 45.7 cm steel wire pens maintained over metal pans in a thermostatically controlled indoor environment. Fluorescent lights provided 8 hours of light per day. Maximum and minimum temperatures and relative humidity were monitored and recorded daily. Temperatures ranged from 54°F to 96°F with relative humidity between 44% and 78% during the 14-day study period.
- C. Dosage: 14-day single dose oral LD50 test. Diazinon MG8 Technical was administered via gelatin capsule at levels of 10.0, 21.5, 31.6, 46.4, 68.1, 147.0, and 215.0 mg a.i./kg of body weight. The dosages were selected after four range-finding tests. Each test bird received its respective dose of test material by

administration of one capsule. Birds in the control group received one empty gelatin capsule only.

- D. **Design:** Five birds of like sex per pen (two pens per test group) were randomly assigned to each of the following test groups:

Group	Dose (mg a.i. kg/body weight)	# males	# females
Control	0	5	5
T-I	10.0	5	5
T-II	21.5	5	5
T-III	31.6	5	5
T-IV	46.4	5	5
T-V	68.1	5	5
T-VI	147.0	5	5
T-VII	215.0	5	5

Dose levels were based on a geometric scale of 1.47. The T-I through T-V and control birds were dosed on 6/21/88. The T-VI and T-VII birds were dosed on 6/22/88. Individual birds were identified by metal wing bands embossed with numbers unique within the study. Observations were conducted daily for mortalities, potential clinical signs indicative of test material effect, abundance of food and water, and food spillage. Birds were individually weighed at 0 hour on test day 1, and on test days 3, 7, and 14. Group food consumption values were recorded on test days 3, 7, and 14. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds from the vehicle control groups and four from each of the T-I, T-II, T-III, T-IV, and T-V groups, as well as the surviving T-VI and T-VII birds, were subjected to gross pathological examinations on test day 14.

- E. **Statistics:** The LD50 was calculated using the simplified method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949). Body weight data were analyzed using one-way analysis of variance.

12. **REPORTED RESULTS:** The following deaths were recorded during the investigation: two birds in the T-I group, two in the T-II group, one in the T-III group, one in the T-IV group, two in the T-V group, nine in the T-VI group, and eight in the T-VII group (Table 3, attached). No mortalities occurred in the control group. All deaths occurred within 24 hours of dosing. Gross pathological examinations of

24 hours of dosing. Gross pathological examinations of selected survivors and of all birds that died during the investigation revealed no abnormalities.

No abnormal behavioral reactions or systemic signs of toxicity were noted at any time in the control group. Signs of toxicity in the T-VI and T-VII groups included lethargy, tachypnea, yellow-colored diarrhea and inability to fly. With one exception, all birds that died in the T-I through T-V groups were asymptomatic prior to their deaths. The exception was a T-I male that exhibited tachypnea prior to death. One capsule was found under each of the T-VI male, T-VI female, and T-VII female pens shortly after dosing occurred. These three capsules were more pliable than they had been when dosing occurred. It is uncertain whether or not the three birds regurgitating these capsules actually received any of their dosages. Total remission of all signs was achieved by the end of test day 1.

Body weights in the T-VI and T-VII groups were lower ($p < 0.05$) than controls at test initiation. Significant decreases in the T-I, T-II, T-III, and T-IV test groups' body weights were noted on day 3 only (Table 4, attached). No other significant differences in body weights were noted throughout the investigation.

Food consumption in the control group ranged from 8 to 10 grams/bird/day throughout the investigation. All food consumption values in the test groups were comparable to the control group's range during the entire investigation (Table 4, attached).

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

The acute oral LD50 of Diazinon MG8 Technical for brown-headed cowbirds was determined to be 85.0 mg a.i./kg of body weight with 95% confidence limits of 34.6 to 209.1 mg a.i./kg of body weight (see EEB note below.) The no observed effect level was determined to be less than 10.0 mg a.i./kg of body weight.

The study was designed and conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by the Quality Assurance Unit of Bio-Life Associates, Ltd.

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

A. Test Procedure: The brown-headed cowbird (Molothrus ater) is not a generally accepted test species. The report states that the brown-headed cowbird was used "in

order to determine the toxicological effects of Diazinon MG8 Technical and to compare its toxicity to those of Diazinon 14G and Diazinon AG500." While the cowbird is certainly acceptable for those purposes, it should not be used as a species in a test to support registration of a pesticide.

- B. **Statistical Analysis:** The EEB reviewer recalculated the LC50 value (using EPA's Toxanal computer program) taking into consideration the capsules that were regurgitated by two birds in treatment group T-VI and one bird in treatment group V-II. Because nine and eight birds respectively of treatment groups V-I and V-II died, it is assumed that all birds in group V-I and nine birds in group V-II would have died had capsules not have been regurgitated. With that assumption, the LC50 value is 69.0 mg a.i./kg body weight.

- C. **Discussion/Results:** With an LC50 value of 69 mg a.i./kg, Diazinon MG8 Technical is considered moderately toxic to brown-headed cowbirds. The NOEL was considered to be less than the lowest test level of 10.0 mg a.i./kg of body weight. Abnormal behavioral reactions (lethargy, tachypnea, diarrhea, inability to fly) were noted in the T-VI and T-VII groups. The report did not clearly indicate how many birds in other groups showed abnormal behavioral reactions. The report stated, however, that all survivors appeared normal by the end of day 1.

Body weights in the T-VI and T-VII groups were lower ($p < 0.05$) than controls at test initiation. The author did not discuss the potential significance of this. Conceivably, the high mortality observed in these groups could have been due partially to the low weights at test initiation. For example, low body weights could indicate the birds were younger or of poor health compared to the other groups. If so, then these birds were not true replicates of the other groups.

- D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** Unacceptable test species. With the above noted discrepancies, the test is scientifically sound with respect to procedures used to determine the LD50 of the test chemical in brown-headed cowbirds, but the species is not a generally accepted one used in support of pesticide

Accession No. 408953-03

registration. The information provided by this test would be useful in comparisons between species or between chemicals.

(3) **Repairability:** Not applicable; see Section 14D.2.

15. **COMPLETION OF ONE-LINER:** Yes, September 5, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 30 through 31 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - _____ The document is a duplicate of page(s) _____.
 - _____ The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
215	10	9	90	1.074219
147	10	10	100	9.765625E-02
68.1	10	2	20	5.46875
46.4	10	1	10	1.074219
31.6	10	1	10	1.074219
21.5	10	2	20	5.46875
10	10	2	20	5.46875

THE BINOMIAL TEST SHOWS THAT 0 AND 147 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 86.76149

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD				
SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	.1649768	68.89733	46.49607	115.6752

RESULTS CALCULATED USING THE PROBIT METHOD			
ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.205727	3.540018	3.346801E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.026939
95 PERCENT CONFIDENCE LIMITS = -.1987534 AND 4.252631

LC50 = 73.42813
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 17.35009
95 PERCENT CONFIDENCE LIMITS = 0 AND 46.22371

Shaughnessey No. 057801Chemical Name DIAZINON

Chemical Class _____

Page 1 of 1

Study/Species/Lab/

Chemical

Access #

a.i

14-Day Single Dose Oral LD50,

Results

Reviewer/
DateValidation
StatusSpecies: MOLOTHRUS ATERLD50 = 69.8 mg/kg (46.5-115.7)Contr. Mort.(%) = 0

Slope =

Animals/Level =

Age(Days) = UNKNOWNLab.: BIO LIFE ASSOC-LTDSex = M/FORH9-5-89

Acc. #:

408953-03

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

0 (0), 10.0 (20), 21.5 (20), 31.6 (40), 46.4 (10), 68.1 (20), 147 (90)

Comments:

MOVING-AVERAGE METHOD, SLOPE N/A, 215 (80)

14-Day Single Dose Oral LD50,

LD50 = mg/kg (95% C.L.)

Contr. Mort.(%) =

Species:

Slope =

Animals/Level =

Age(Days) =

Lab.:

Sex =

Acc. #:

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

Comments:

8-Day Dietary LC50,

LC50 = ppm (95% C.L.)

Contr. Mort.(%) =

Species:

Slope =

Animals/Level =

Age(Days) =

Lab.:

Sex =

Acc. #:

8-Day Dose Level ppm/(% Mortality)

Comments:

8-Day Dietary LC50,

LC50 = ppm (95% C.L.)

Contr. Mort.(%) =

Species:

Slope =

Animals/Level =

Age(Days) =

Lab.:

Sex =

Acc. #:

8-Day Dose Level ppm/(% Mortality)

Comments:

96-hour LC50,

LC50 = PP (95% C.L.)

Contr. Mort.(%) =

Species:

Slope =

Animals/Level =

Sol. Contr. Mort.(%) =

Lab.:

Temperature =

Acc. #:

96-Hour Dose Level pp/(% Mortality)

Comments:

96-hour LC50,

LC50 = PP (95% C.L.)

Con. Mor(%) =

Species:

Slope =

Animals/Level =

Sol. Con. Mor.(%) =

Lab.:

Temp. =

Acc. #:

96-Hour Dose Level pp/(% Mortality)

Comments:

48-hour Invertebrate,

LC50 = PP (95% C.L.)

Con. Mort.(%) =

Species:

Slope =

Animals/Level =

Sol. Con. Mort.(%) =

Lab.:

Temp. =

Acc. #:

96-Hour Dose Level pp/(% Mortality)

Comments:

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon MG8 Technical, FL # 880045, 88.2% active ingredient, CAS # 333-41-5.
3. **STUDY TYPE:** Avian Dietary LC50 Test.
Species Tested: Brown-headed cowbird
(Molothrus ater)
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon MG8 Technical: 8-Day Acute Dietary LC50 Study in Brown-headed Cowbirds. Laboratory Project No. 88 SBLC 105. Prepared by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 408953-04.

5. **REVIEWED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Michael L. Whitten*

Date: 2-22-89

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *James R. Newman*

Date: 2/22/89

H Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*

Date: 9/12/89

Clyde R. Hornum 9/11/89

7. **CONCLUSIONS:** With an LC50 value of 38 ppm a.i., Diazinon MG8 Technical is considered very highly toxic to brown-headed cowbirds (Molothrus ater). The NOEL was determined to be 8 ppm a.i. The test is scientifically sound with respect to procedures used to determine the LC50 of the test chemical in brown-headed cowbirds, but the species is not a generally accepted one used in support of pesticide registration.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Brown-headed cowbirds were live-trapped in Michigan and Wisconsin by personnel from Bio-Life and the U.S. Fish and Wildlife Service. The birds from Michigan arrived at Bio-Life Associates on 4/29/88 and were immediately placed in quarantine for 19 days. These birds were fed Purina Game Bird Startena during the quarantine period. The birds from Wisconsin were trapped from 5/02/88 until 5/18/88 and taken to the laboratory at Bio-Life on a daily basis where they were immediately placed in quarantine. These birds were fed Purina Duck Grower W/O during the quarantine period until 5/16/88, when some birds were fed Purina Game Bird Startena for two days. The quarantine periods were implemented to determine the birds' suitability as test units and for acclimation to laboratory conditions. The animal room temperature during this period ranged from 40°F to 83°F with relative humidity between 57% and 88%. Seventy-eight out of 438 birds died of natural causes during the quarantine period. Prior to initiation of the test, all birds were examined and their suitability for testing (based on general physical condition) was determined.

B. Test System: The test system consisted of 45.7 cm x 61.0 cm x 45.7 cm steel wire pens maintained over metal pans in a thermostatically controlled indoor environment. Fluorescent lights provided 8 hours of light per day. Maximum and minimum temperatures and relative humidity were monitored and recorded daily. Temperatures ranged from 46°F to 86°F with relative humidity between 63% and 78% during the 8-day study period.

C. Dosage: Acute dietary LC50 test. Diazinon MG8 Technical was incorporated into Purina Game Bird Startena via a premix with corn oil. Nominal dietary concentrations were 8, 16, 32, 64, and 128 ppm a.i.

D. Design: Groups of ten birds of like sex were arbitrarily assigned to each of the five treatment and five control groups as follows:

Group	Nominal Concentration (ppm)	# males	# females
VC-I	0	10	0
VC-II	0	0	10
VC-III	0	10	0
VC-IV	0	0	10
VC-V	0	10	0
T-I	8	10	0
T-II	16	0	10
T-III	32	10	0
T-IV	64	0	10
T-V	128	10	0

Each group was fed the appropriate test diet or vehicle control diet for five consecutive days starting on 5/18/88 and ending on 5/23/88. The vehicle control birds received a standard laboratory diet mixed with corn oil. Following the five-day test period, all birds were removed from their respective treated diets and given plain feed during a three-day recovery period. Diet samples were collected immediately after preparation, at test initiation, and on test day 3 from the 8-ppm and 128-ppm diet, and from the vehicle control diet at test initiation and on test day 3. All samples were analyzed for confirmation of dietary levels. Observations were conducted daily for mortalities, potential clinical signs indicative of test material effect, abundance of food and water, and food spillage. Birds were weighed by groups at 0 hour on test day 1, and on test day 8. Food consumption was recorded for each group on test days 3, 5, and 8. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds from the vehicle control groups and four from each of the T-I, T-II, T-III groups, as well as the surviving birds from the T-IV group, were subjected to gross pathological examinations at the termination of the project.

E. **Statistics:** The LC50 was calculated using the simplified method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949). No other statistical analyses were reported.

12. **REPORTED RESULTS:** Measured concentrations of Diazinon MG8 Technical in test diets averaged 88% of the nominal

concentrations, with values ranging from 75% to 111% of nominal concentrations.

The following deaths were recorded during the investigation: One bird in the T-II group, four in the T-III group, eight in the T-IV group, and ten in the 128-ppm group (Table 3, attached). No mortalities occurred in the control groups or in the T-I group. Deaths were noted on days 1, 2, 3, 5, and 6. Gross pathological examinations of selected survivors and of all birds that died during the investigation revealed no abnormalities.

Signs of toxicity in the T-II, T-III, T-IV, and T-V groups included lethargy, anorexia, and decreased water consumption. On test days 7 and 8, all birds appeared normal and active. No abnormal behavioral reactions or signs of toxicity were noted at any time in the control groups or in the T-I group.

Group body weights at test initiation and at the end of day 8 were essentially the same in the test and vehicle control groups (Table 4, attached). The T-I, T-II, T-III, and T-IV test groups' average body weights on day 8 all fell within one gram of the range of the vehicle control groups.

Food consumption values are presented in Table 4, attached. Slight food consumption depression was noted in the T-III group during the test period. Severe food avoidance was noted in the T-IV group during the test period and in the T-V group during the first three days. Food consumption during the recovery period was normal or above normal for all surviving test groups.

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

The 8-day dietary LC50 of Diazinon MG8 Technical for brown-headed cowbirds was determined to be 38 ppm a.i. with 95% confidence limits of 26 to 57 ppm a.i. The no observed effect level was determined to be 8 ppm a.i.

The study was designed and conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by the Quality Assurance Unit of Bio-Life Associates, Ltd.

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

A. Test Procedure: The brown-headed cowbird (Molothrus ater) is not a generally accepted test species. The report states that the brown-headed cowbird was used "in order to determine the toxicological effects of Diazinon

MG8 Technical and to compare the toxicity to that of Diazinon AG500." While the cowbird is certainly acceptable for those purposes, it should not be used as a species in a test used to support registration of a pesticide.

Other discrepancies or deviations from recommended procedures are as follows:

Birds were weighed by groups. According to the SEP, individual body weights should be measured.

Food consumption was recorded on test days 3, 5, and 8. According to the SEP, food consumption should also be recorded at the beginning and end of the pretreatment period.

The birds were not randomly assigned to groups. Instead, arbitrary selections were made from the entire population of birds.

Two birds were found dead approximately 3 hours after the beginning of test day 6. At the end of test day 8, therefore, 72 consecutive hours without mortality had not occurred.

All birds in each pen were of the same sex. While this is not the usual procedure in LC50 tests, it did not appear to affect the results.

- B. Statistical Analysis: The LC50 value calculated by using EPA's Toxanal computer program was the same as the value reported by the study author.
- C. Discussion/Results: With an LC50 value of 38 ppm a.i., Diazinon MG8 Technical is considered very highly toxic to brown-headed cowbirds. The NOEL was considered to be 8 ppm a.i. Abnormal behavioral reactions (lethargy, anorexia, and decreased water consumption) were noted in the T-II, T-III, T-IV, and T-V groups. All survivors appeared normal by the end of day 6.
- D. Adequacy of the Study:
 - (1) Classification: Supplemental
 - (2) Rationale: Unacceptable test species. The test is scientifically sound with respect to procedures used to determine the LC50 of the test chemical in brown-headed cowbirds, but the species is not a

generally accepted one used in support of pesticide registration. The information provided by this test would be useful in comparisons between species or between chemicals.

(3) **Repairability:** Not applicable; see Section 14D.2.

15. **COMPLETION OF ONE-LINER:** Yes, February 20, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 40 through 41 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - _____ The document is a duplicate of page(s) _____.
 - _____ The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
128	10	10	100	9.765625E-02
64	10	8	80	5.46875
32	10	4	40	37.69531
16	10	1	10	1.074219
8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 16 AND 128 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 37.79736

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1144044	35.97114	26.1865	50.92806

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.2168915	1	.9496332

SLOPE = 3.967457
95 PERCENT CONFIDENCE LIMITS = 2.119749 AND 5.815166

LC50 = 36.57347
95 PERCENT CONFIDENCE LIMITS = 26.29892 AND 51.00929

LC10 = 17.50097
95 PERCENT CONFIDENCE LIMITS = 8.269921 AND 24.69243

No. _____

Chemical Name DIAZINON

Chemical Class _____

Page 1 of 1Study/Species/Lab/
Accession _____ Chemical
X a.l. _____

Results

Reviewer/
Date _____ Validat
Statu _____14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg. (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

8-Day Dietary LC₅₀LC₅₀ = 38 ppm (95% C.L.) Contr. Mort.(X) = 0Species Molothrus ater

Slope = 3.967 # Animals/Level = 10 Age(Days) = UNKNOWN

Lab Bio Life Associates88.2

Sex = M/F

MLW 2-20-898-day Dose Level ppm/(X Mortality)
8(0), 16(10), 32(40), 64(80), 128(100)Acc. 408953-04

Comments: _____

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____

Lab _____

8-Day Dose Level ppm/(X Mortality)
(), (), (), (), ()

Acc. _____

Comments: _____

48-Hour L₅₀LC₅₀ = (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Sol. Contr. Mort.(X) = _____

Lab _____

48-Hour Dose Level / (X Mortality)
(), (), (), (), () Temperature = _____

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = pp (95% C.L.) Con. Mor.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Sol. Con. Mor.(X) = _____

Lab _____

96-Hour Dose Level pp / (X Mortality)
(), (), (), (), () Temp. = _____

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = pp (95% C.L.) Con. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Sol. Con. Mort.(X) = _____

Lab _____

96-Hour Dose Level pp / (X Mortality)
(), (), (), (), () Temp. = _____

Acc. _____

Comments: _____

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DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon 14G, FL #880499, CAS #333-41-5,
14.7% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], brown-colored granules with a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Single-Dose Oral LD50 Test.
Species Tested: Mallard Duck
Anas platyrhynchos.
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988.
Diazinon 14G: 14-Day Acute Oral LD50 Study in Mallard Ducks.
BLAL No. 88-DD-54. Conducted by Bio-Life Associates, Ltd.,
Neillsville, WI. Submitted by Ciba-Geigy Corporation,
Greensboro, NC. Accession No. 408953-05.
5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: March 1, 1989
6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R. Newman
Date: 3/1/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA
Signature: Henry T. Craven
Date: 9/6/89

CLYDE R. HOUSEKNECHT, Ph.D., M.P.H. Clyde R. Houseknecht, 9/6/89
7. **CONCLUSIONS:** This study is scientifically sound and meets
the guideline requirements for an avian single-dose oral
LD50 test. With an LD50 value of 2.34 mg a.i./kg body
weight, Diazinon 14G is considered very highly toxic to
mallard duck (Anas platyrhynchos). The NOEL was 0.681 mg
a.i./L.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The birds employed in the study were 16-week-old mallard duck (Anas platyrhynchos) obtained from Whistling Wings, Inc., Hanover, IL. The ducks had received P.A. Bacterin vaccinations at 7 and 14 days of age. Upon receipt, they were placed on a 21-day quarantine period to determine their suitability as test units and to acclimate them to the laboratory conditions. All birds were fed Purina^R Duck Grower W/O and well water ad libitum. The animal room temperature ranged from 52 to 72°F with relative humidity between 83 and 94%. Lighting was provided by fluorescent lights which were left on eight hours per day.

Two birds died during the first 24 hours of the quarantine period. All other birds were normal and active throughout the entire quarantine period. Prior to the initiation of the study, all birds were examined and determined to be suitable for testing.

B. Test System: All birds were housed in 121.9 cm x 121.9 cm x 121.9 cm wire pens (10 birds per pen), maintained over concrete. Lighting condition in the room was the same as that used during the quarantine period. A thermostatically-controlled heated environment offered temperatures ranging from 52 to 79° with relative humidity between 57 and 88% during the 14-day study.

C. Dosage: 14-day single-dose oral LD50 test. Based on two seven-day, range-finding tests, the nominal dosages selected for the definitive study were 0.316, 0.681, 1.47, 3.16, and 6.81 mg a.i./kg body weight. Dosage levels were based on a geometric scale of 1.47.

D. Design: Groups of ten ducks, five males and five females, were randomly assigned to a control group and each of the five treatment groups. All birds were fasted (with water allowed) for approximately 20-22 hours prior to dosing. The dose for each individual test bird was gravimetrically measured and administered via a gelatin capsule at 0 hour on test day 1. Each control bird received one empty gelatin capsule.

The ducks were individually weighed at 0 hour on test day 1, and on test days 3, 7, and 14. Group food consumption values were recorded on test days 3, 7, and 14. Inspections were made daily for the presence (or absence) of clinical signs indicative of test material effects, mortalities, abundance of food and water, and food spillage. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds (two males and two females) from each group were also sacrificed on test Day 14 and subjected to complete gross pathological examinations.

E. **Statistics:** Statistical evaluation of the body weight data was conducted using One-Way Analysis of Variance. The acute oral median lethal dose (LD50) was calculated by employing the simplified method of Litchfield and Wilcoxon (1949).

12. **REPORTED RESULTS:** Table 3 (attached) presents daily mortality data of the test birds throughout the 14-day study period. The 14-day LD50 value was calculated to be 1.97 mg a.i./kg body weight (or 13.4 mg of test material/kg body weight or 59 granules/kg body weight) with 95% confidence limits of 1.47 to 2.64 mg a.i./kg body weight.

No abnormal behavioral reactions or systemic signs of toxicity were noted in the control, 0.316-, and 0.681 mg a.i./kg groups. Signs of toxicity in the 1.47-, 3.16-, and 6.81-mg a.i./kg groups included lethargy, stumbling, inability to walk, and regurgitation. Total remission of all signs was achieved by the end of test day 1.

Gross pathological examinations of all birds that died during the investigation revealed no abnormal pathological findings except for penile protrusion in 3 birds from the 3.16-mg a.i./kg group. No other abnormal pathological findings were observed in any bird examined.

Average body weight and estimated food consumption data are presented in Table 4 (attached). Statistical analysis of the body weights revealed no significant differences in the test groups' body weights at any of the weighing intervals. All food consumption values in the test groups were within or above the control group's range during the entire investigation.

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

The acute oral LD50 of Diazinon 14G for mallard duck was determined to be 1.97 mg a.i./kg body weight or 13.4 mg test material/kg body weight. The no-observed-effect level (NOEL) was determined to be 0.681 mg a.i./kg body weight.

A quality assurance statement was included, indicating that all studies conducted at Bio-Life Associates were designed and functioned in conformance with Good Laboratory Practice Regulations and the protocols for individual laboratory studies.

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

A. Test Procedure: The test procedures were in accordance with the SEP guidelines.

B. Statistical Analysis: Individual body weight data for the groups with no mortality were statistically compared with the control values using analysis of variance with Dunnett's test (see attached printouts). The reviewer recalculated the LD50 value using EPA's Toxanal computer program and obtained a similar result (attached).

C. Discussion/Results: The analysis of variance showed the same results as those analyzed by the authors, i.e., the body weights of the treatment birds were comparable to those of the control birds. With an LD50 value of 2.34 mg a.i./kg body weight (95% C.L. = 1.47-3.16 mg a.i./kg), Diazinon 14G is considered very highly toxic to mallard duck, when administered as oral single dose. Based on the signs of toxicity observed in dosages ≥ 1.47 mg a.i./kg, the NOEL was considered to be 0.681 mg a.i./kg body weight.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, February 24, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 48 through 49 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ _____ FIFRA registration data.
 - _____ The document is a duplicate of page(s) _____.
 - _____ The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

KOSALWAT DIAZINON 14G ANAS PLATYRHYNCHOS 02-24-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
6.81	10	10	100	9.765625E-02
3.16	10	9	90	1.074219
1.47	10	0	0	9.765625E-02
.681	10	0	0	9.765625E-02
.316	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 1.47 AND 3.16 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 2.336436

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.

50

Date: 02-24-1988

FILTER: None

Post-hoc tests for factor D (DOSE)

Level	Mean	<u>Dose</u> (mg a.i./kg body wt)
1	1123.175	Control
2	1111.750	0.316
3	1088.550	0.681
4	1107.325	1.470

Comparison	Dunnett
1 > 2	
1 > 3	
1 > 4	
2 > 3	N.A.
2 > 4	N.A.
3 < 4	N.A.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

Post-hoc tests for factor D (DAY)

Level	Mean	<u>Time</u> (days)
1	1061.825	1
2	1115.525	3
3	1114.800	7
4	1138.650	14

Comparison	Dunnett
1 < 2	0.0500
1 < 3	0.0500
1 < 4	0.0100
2 > 3	N.A.
2 < 4	N.A.
3 < 4	N.A.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

Sh. No. 057801Chemical Name Diazinon Chemical Class _____ Page 1 of 1
(Diazinon 14G)Study/Species/Lab/
Accession _____ Chemical
_____ a.i.14-Day Single Dose Oral LD₅₀

Species _____

Lab _____

Acc. _____

Results		Reviewer/ Date	Validati Status
LD ₅₀ =	mg/kg (<u>95% C.L.</u>)	Contr. Mort. (X) =	
Slope =	# Animals/Level =	Age (Days) =	
		Sex =	
14-Day Dose Level mg/kg/(X Mortality)			
() , () , () , () , ()			

Comments:

14-Day Single Dose Oral LD₅₀Species Anas 14.7
platyrhynchosLab Bio-Life AssociatesAcc. 408953-05

Results		Reviewer/ Date	Validati Status
LD ₅₀ =	2.3 mg/kg. (<u>95% C.L.</u>)	Contr. Mort. (X) =	
Slope =	N/A # Animals/Level =	Age (Days) =	
		Sex =	
14-Day Dose Level mg/kg/(X Mortality)			
0.316 (0) , 0.681 (0) , 1.47 (0) , 3.16 (90) , 6.81 (100)			

Comments: * based on active ingredient

3-Day Dietary LC₅₀

Species _____

Lab _____

Acc. _____

Results		Reviewer/ Date	Validati Status
LC ₅₀ =	ppm (<u>95% C.L.</u>)	Contr. Mort. (X) =	
Slope =	# Animals/Level =	Age (Days) =	
		Sex =	
3-Day Dose Level ppm/(X Mortality)			
() , () , () , () , ()			

Comments:

3-Day Dietary LC₅₀

Species _____

Lab _____

Acc. _____

Results		Reviewer/ Date	Validati Status
LC ₅₀ =	ppm (<u>95% C.L.</u>)	Contr. Mort. (X) =	
Slope =	# Animals/Level =	Age (Days) =	
		Sex =	
3-Day Dose Level ppm/(X Mortality)			
() , () , () , () , ()			

Comments:

48-Hour LC₅₀

Species _____

Lab _____

Acc. _____

Results		Reviewer/ Date	Validati Status
LC ₅₀ =	ppm (<u>95% C.L.</u>)	Contr. Mort. (X) =	
Slope =	# Animals/Level =	Sol. Contr. Mort. (X) =	
		Temperature =	
48-Hour Dose Level pp/(X Mortality)			
() , () , () , () , ()			

Comments:

96-Hour LC₅₀

Species _____

Lab _____

Acc. _____

Results		Reviewer/ Date	Validati Status
LC ₅₀ =	ppm (<u>95% C.L.</u>)	Con. Mort. (X) =	
Slope =	# Animals/Level =	Sol. Con. Mort. (X) =	
		Temp. =	
96-Hour Dose Level pp/(X Mortality)			
() , () , () , () , ()			

Comments:

96-Hour LC₅₀

Species _____

Lab _____

Acc. _____

Results		Reviewer/ Date	Validati Status
LC ₅₀ =	ppm (<u>95% C.L.</u>)	Con. Mort. (X) =	
Slope =	# Animals/Level =	Sol. Con. Mort. (X) =	
		Temp. =	
96-Hour Dose Level pp/(X Mortality)			
() , () , () , () , ()			

Comments:

53

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon 14G, FL #880499, CAS #333-41-5,
14.7% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], brown-colored granules with a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Single-Dose Oral LD50 Test.
Species Tested: Brown-Headed Cowbird
Molothrus ater.
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988.
Diazinon 14G: 14-Day Acute Oral LD50 Study in Brown-Headed Cowbirds. BLAL No. 88-SB-101. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-06.
5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: March 1, 1989
6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R. Newman
Date: 3/1/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA
Signature: Henry T. Craven
Date: 9/5/89

CLYDE R. HOUSEKNECHT, Ph.D., M.P.H.
Signature: Clyde R. Houseknecht, 09/05/89
7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 6.85 mg a.i./kg body weight, Diazinon 14G is considered very highly toxic to brown-headed cowbirds (Molothrus ater). The NOEL was less than 2.15 mg a.i./L.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Brown-headed cowbirds (Molothrus ater) used in the study were live-trapped in the state of Michigan. Upon receipt at Bio-Life Associates, Ltd., they were immediately placed on an approximate 45-day quarantine period to determine their suitability as test units and to acclimate them to the laboratory conditions. All birds were fed Purina^R Duck Grower W/O and well water ad libitum. The animal room temperature ranged from 40 to 92°F with relative humidity between 57 and 88%. Lighting was provided by fluorescent lights which were left on eight hours per day.

Approximately 18% (81 out of 438) of the birds died of natural causes during the quarantine period. Within the first 14 days, one bird was sacrificed in extremis because it was lethargic and anorexic, one was weak and found dead on the following day, two lethargic male birds had bloody heads due to fighting and four deaths were recorded within the next 24 hours. No deaths occurred during the last eleven days of the quarantine period. All other birds were normal and active throughout the entire quarantine period. Prior to the initiation of the study, all birds were examined and determined to be suitable for testing.

B. Test System: All birds were housed in 61.0 cm x 45.7 cm x 45.7 cm steel wire pens (5 birds of like sex per pen, 2 pens per test group), maintained over metal pans which were lined with an absorbent material. Lighting condition in the room was the same as that used during the quarantine period. An indoor environment offered temperatures ranging from 58 to 96°F with relative humidity between 44 and 82% during the 14-day study.

C. Dosage: 14-day single-dose oral LD50 test. Based on two seven-day, range-finding tests, the nominal dosages selected for the definitive study were 2.15, 4.64, 10.0, 21.5, and 31.6 mg a.i./kg body weight. Dosage levels were based on a geometric scale of 1.47.

D. Design: Groups of ten birds, five males and five females, were randomly assigned to a control group and each of the five treatment groups. All birds were

fasted (with water allowed) for approximately 4-5 hours prior to dosing. The dose for each individual test bird was gravimetrically measured and administered via a gelatin capsule at 0 hour on test day 1. Each control bird received one empty gelatin capsule.

The birds were individually weighed at 0 hour on test day 1, and on test days 3, 7, and 14. Group food consumption values were recorded on test days 3, 7, and 14. Inspections were made daily for the presence (or absence) of clinical signs indicative of test material effects, mortalities, abundance of food and water, and food spillage. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds (two males and two females) from each of the control, 2.15-, and 4.64-mg a.i./kg test groups, as well as the surviving birds of the 10.0-, 21.5-, and 31.6-mg a.i./kg test groups were also subjected to gross pathological examinations on day 14.

E. Statistics: Statistical evaluation of the body weight data was conducted using One-Way Analysis of Variance. The acute oral median lethal dose (LD50) was calculated by employing the simplified method of Litchfield and Wilcoxon (1949).

12. REPORTED RESULTS: Table 3 (attached) presents daily mortality data of the test birds throughout the 14-day study period. The 14-day LD50 value was calculated to be 5.65 mg a.i./kg body weight (or 38.44 mg of test material/kg body weight or 133 granules/kg body weight) with 95% confidence limits of 3.19 to 10.0 mg a.i./kg body weight.

No abnormal behavioral reactions or systemic signs of toxicity were noted in the control group. Signs of toxicity in the 4.64-, 10.0-, 21.5-, and 31.6-mg a.i./kg groups included quivering, laying in the pen, and yellow-colored diarrhea. The only sign of toxicity which was noted in the 2.15-mg a.i./kg group was yellow-colored diarrhea. Total remission of all signs was achieved by the end of test day 1. However, at the end of test day 8, two birds in the control group and one bird in the 2.15-mg a.i./kg group were noted to have bald heads. This condition prevailed at study termination.

All birds subjected to gross pathological examinations revealed no abnormal pathological findings. Average body weight and estimated food consumption data are presented in Table 4 (attached). Statistical analysis of the body

weights revealed no significant differences in the test groups' body weights at any of the weighing intervals. During the first three test days, food consumption in the 10.0-mg a.i./kg test group was slightly depressed while severe food avoidance was noted in the 21.5- and 31.6-mg a.i./kg test groups. All food consumption values in the test groups were within or above the control group's range during test days 4 through 14 of the investigation.

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

The acute oral LD50 of Diazinon 14G for brown-headed cowbirds was determined to be 5.65 mg a.i./kg body weight or 38.44 mg test material/kg body weight. The no-observed-effect level (NOEL) was determined to be less than 2.15 mg a.i./kg body weight.

A quality assurance statement was included, indicating that all studies conducted at Bio-Life Associates were designed and functioned in conformance with Good Laboratory Practice Regulations and the protocols for individual laboratory studies.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP guidelines, except for the following deviations:

- o Brown-headed cowbird (Molothrus ater) is not one of the SEP's recommended species.
- o The age of the test birds was unknown.
- o The SEP states that mortality of greater than 10% during an acclimation period is a sign that the lot should not be used. In this study, eighteen percent of the birds died during the quarantine period (45 days prior to the test). However, no mortalities occurred and all birds appeared normal and active during the last eleven days of the quarantine period. Therefore, the lot was probably suitable for the test.
- o The birds were fasted for approximately 4-5 hours prior to dosing. According to the SEP, feed should be withheld from all birds for at least 15 hours prior to oral dosing.

B. **Statistical Analysis:** The reviewer recalculated the LD50 value using EPA's Toxanal computer program and obtained a similar result (attached).

C. Discussion/Results: With an LD50 value of 6.85 mg a.i./kg body weight (95% C.L. = 4.32-10.13 mg a.i./kg), Diazinon 14G is considered very highly toxic to brown-headed cowbirds, when administered as oral single dose. Based on the signs of toxicity observed in all dosages tested, the NOEL was considered to be less than 2.15 mg a.i./kg body weight.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: Brown-headed cowbird (Molothrus ater) is not one of the SEP's recommended species.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, February 28, 1989.

KOSALWAT DIAZINON 14G MOLOTHRUS ATER 02-27-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
31.6	10	9	90	1.074219
21.5	10	9	90	1.074219
10	10	8	80	5.46875
4.64	10	4	40	37.69531
2.15	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 2.15 AND 21.5 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 5.579877

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.1783538	6.911339	3.934368 10.28867

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1991652	1	.3789305

SLOPE = 2.682348

95 PERCENT CONFIDENCE LIMITS = 1.485272 AND 3.879424

LC50 = 6.847861

95 PERCENT CONFIDENCE LIMITS = 4.319711 AND 10.13122

LC10 = 2.301914

95 PERCENT CONFIDENCE LIMITS = .7596518 AND 3.785044

DIAZINON

Page _____ is not included in this copy.

Pages 60 through 61 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ _____ FIFRA registration data.
 - _____ The document is a duplicate of page(s) _____.
 - _____ The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

No. _____

Chemical Name Diazinon Chemical Class _____Page 1 of 1Study/Species/Lab/
Accession _____ Chemical
a.i. _____

(Diazinon 14G)

Results

Reviewer/
Date _____ Validat/
Status _____14-Day Single Dose Oral LD₅₀LD₅₀ = _____ mg/kg (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____
Sex = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

14-Day Single Dose Oral LD₅₀
(Brown-headed cowbird)LD₅₀ = 6.85 mg/kg. (4.32 - 10.13) 95% C.L. Probit Analysis
Contr. Mort.(X) = 0Species Molothrus 14.7
aterSlope = 2.68 # Animals/Level = 10 Age(Days) = Unknown
Sex = M/FPK 2-28-89 SuppLab Bio-life Associates, Ltd.14-Day Dose Level mg/kg/(X Mortality)
2.15(0), 4.64(40), 10.0(80), 21.5(90), 31.6(90)Acc. 408953-06Comments: * active ingredient3-Day Dietary LC₅₀LC₅₀ = _____ ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____
Sex = _____

Lab _____

3-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

3-Day Dietary LC₅₀LC₅₀ = _____ ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____
Sex = _____

Lab _____

3-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

48-Hour LC₅₀LC₅₀ = _____ pp (95% C.L.) Contr. Mort.(X) = _____
Sol. Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Temperature = _____

Lab _____

48-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = _____ pp (95% C.L.) Con. Mort.(X) = _____
Sol. Con. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Temp. = _____

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = _____ pp (95% C.L.) Con. Mort.(X) = _____
Sol. Con. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Temp. = _____

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

62

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Formulation: Diazinon AG500, FL #861807, CAS #333-41-5, 48.1% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], an amber liquid a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Single-Dose Oral LD50 Test.
Species Tested: Mallard Duck
Anas platyrhynchos.
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988.
Diazinon 14G: 14-Day Acute Oral LD50 Study in Mallard Ducks. BLAL No. 88-DD-55. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-07.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: March 1, 1989

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R. Newman
Date: 3/1/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 9/6/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 1.18 mg a.i./kg body weight, Diazinon AG500 is considered very highly toxic to mallard duck (Anas platyrhynchos). The NOEL was 0.316 mg a.i./L.

8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The birds employed in the study were 16-week-old mallard duck (Anas platyrhynchos) obtained from Whistling Wings, Inc., Hanover, IL. The ducks had received P.A. Bacterin vaccinations at 7 and 14 days of age. Upon receipt, they were placed on a 21-day quarantine period to determine their suitability as test units and to acclimate them to the laboratory conditions. All birds were fed Purina^R Duck Grower W/O, and well water ad libitum. The animal room temperature ranged from 52 to 72°F with relative humidity between 83 and 94%. Lighting was provided by fluorescent lights which were left on eight hours per day.

Two birds died during the first 24 hours of the quarantine period. All other birds were normal and active throughout the entire quarantine period. Prior to the initiation of the study, all birds were examined and determined to be suitable for testing.

B. Test System: All birds were housed in 121.9 cm x 121.9 cm x 121.9 cm wire pens (10 birds per pen), maintained over concrete. Lighting condition in the room was the same as that used during the quarantine period. A thermostatically-controlled heated environment offered temperatures ranging from 52 to 79° with relative humidity between 57 and 88% during the 14-day study.

C. Dosage: 14-day single-dose oral LD50 test. Based on two seven-day, range-finding tests, the nominal dosages selected for the definitive study were 0.316, 0.681, 1.47, 3.16, and 6.81 mg a.i./kg body weight. Dosage levels were based on a geometric scale of 1.47.

D. Design: Groups of ten ducks, five males and five females, were randomly assigned to a control group and each of the five treatment groups. All birds were fasted (with water allowed) for approximately 21.5-22.3 hours prior to dosing. A stock solution was prepared with corn oil. The dose for each individual test bird was volumetrically measured and administered via a gelatin capsule at 0 hour on test day 1. Each control bird received one empty gelatin capsule.

The ducks were individually weighed at 0 hour on test day 1, and on test days 3, 7, and 14. Group food consumption values were recorded on test days 3, 7, and 14. Inspections were made daily for the presence (or absence) of clinical signs indicative of test material effects, mortalities, abundance of food and water, and food spillage. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds (two males and two females) from each of the control, 0.316-, and 0.681-mg a.i./kg groups, as well as the surviving birds from the 1.47- and 3.16-mg a.i./kg groups were also sacrificed on test Day 14 and subjected to gross pathological examinations.

E. **Statistics:** Statistical evaluation of the body weight, data was conducted using One-Way Analysis of Variance. The acute oral median lethal dose (LD50) was calculated by employing the simplified method of Litchfield and Wilcoxon (1949).

12. **REPORTED RESULTS:** Table 3 (attached) presents daily mortality data of the test birds throughout the 14-day study period. The 14-day LD50 value was calculated to be 1.10 mg a.i./kg body weight (or 2.29 mg of test material/kg body weight) with 95% confidence limits of 0.77 to 1.57 mg a.i./kg body weight.

No abnormal behavioral reactions or systemic signs of toxicity were noted in the control or the 0.316-mg a.i./kg groups. Signs of toxicity in the 0.681-, 1.47-, 3.16-, and 6.81-mg a.i./kg groups included lethargy, sitting, inability to walk, and regurgitation. Total remission of all signs was achieved by the end of test day 1.

Gross pathological examinations of all birds that died during the investigation revealed no abnormal pathological findings except for penile protrusion in 1 bird each from the 1.47- and 3.16-mg a.i./kg groups, and 2 birds from the 6.81-mg a.i./kg group. No other abnormal pathological findings were observed in any bird examined.

Average body weight and estimated food consumption data are presented in Table 4 (attached). Statistical analysis of the body weights revealed no significant differences in the test groups' body weights at any of the weighing intervals. Food consumption depression was noted during the first seven test days only in the 3.16-mg a.i./kg group. All other food consumption values in the test groups were within the

control group's range or within 7 grams/bird/day of the lowest control group value during the entire investigation.

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

The acute oral LD50 of Diazinon AG500 for mallard duck was determined to be 1.10 mg a.i./kg body weight or 2.29 mg test material/kg body weight. The no-observed-effect level (NOEL) was determined to be 0.316 mg a.i./kg body weight.

A quality assurance statement was included, indicating that all studies conducted at Bio-Life Associates were designed and functioned in conformance with Good Laboratory Practice Regulations and the protocols for individual laboratory studies.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP guidelines, except a vehicle control group (i.e., a group receiving the maximum amount of corn oil available to any treatment group) was not included in the test.

B. **Statistical Analysis:** Individual body weight data for birds in the group with no mortality (i.e., 0.316 mg a.i./kg) were statistically compared with the control values using analysis of variance (see attached printouts). The reviewer recalculated the LD50 value using EPA's Toxanal computer program and obtained a similar result (attached).

C. **Discussion/Results:** The analysis of variance showed no significant difference between body weights of the 0.316-mg a.i./kg birds and those of the control birds. Therefore, the NOEL in this study was considered to be 0.316 mg a.i./kg body weight. With an LD50 value of 1.18 mg a.i./kg body weight (95% C.L. = 0.82-1.70 mg a.i./kg), Diazinon AG500 is considered very highly toxic to mallard duck, when administered as oral single dose.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, February 27, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 67 through 68 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ FIFRA registration data.
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 - _____ The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

KOSALWAT DIAZINON AG500 ANAS PLATYRHYNCHOS 02-27-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
6.81	10	10	100	9.765625E-02
3.16	10	9	90	1.074219
1.47	10	7	70	17.1875
.681	10	2	20	5.46875
.316	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .316 AND 3.16 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 1.087462

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.1144044	1.216974	.8189337 1.723031

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.2216654	1	.8728044

SLOPE = 3.6675
95 PERCENT CONFIDENCE LIMITS = 1.940792 AND 5.394209

LC50 = 1.183127
95 PERCENT CONFIDENCE LIMITS = .8204738 AND 1.698191

LC10 = .5330237
95 PERCENT CONFIDENCE LIMITS = .2307115 AND .7783451

Date: 02-27-1988

Sh. No. 057801Chemical Name Diazinon Chemical Class _____Page 1 of 1Study/Species/Lab/
Accession _____Chemical
X a.i.

(Diazinon AG 500)

Results

Reviewer/
Date _____Validati
Status _____14-Day Single Dose Oral LD₅₀LD₅₀ = . mg/kg (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) =

Sex =

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments:

14-Day Single Dose Oral LD₅₀LD₅₀ = 1.18 mg/kg. (95% C.L.) Probit analysis
Contr. Mort. (X) = 0Species Anas 48.1

Slope = 3.67 # Animals/Level = 10 Age (Days) = 133

Sex = M/F

Lab platyrhynchos14-Day Dose Level mg/kg/(X Mortality)
0.316 (0) , 0.681 (20) , 1.47 (70) , 3.16 (90) , 6.81 (100)Lab Biolife Associates Ltd.Acc. 408953-07

Comments: * active ingredient

PK/2-27-89 Core8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) =

Sex =

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments:

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) =

Sex =

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments:

48-Hour LC₅₀LC₅₀ = PP (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Sol. Contr. Mort. (X) =

Temperature =

Lab _____

48-Hour Dose Level pp/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments:

96-Hour LC₅₀LC₅₀ = PP (95% C.L.) Con. Mort. (X) =

Species _____

Slope = # Animals/Level = Sol. Con. Mort. (X) =

Temp. =

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments:

96-Hour LC₅₀LC₅₀ = PP (95% C.L.) Con. Mort. (X) =

Species _____

Slope = # Animals/Level = Sol. Con. Mort. (X) =

Temp. =

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments:

71

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Formulation: Diazinon AG500, FL #861807, CAS # 333-41-5, 48.1% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], an amber liquid with a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Dietary LC50 Test.
Species Tested: Mallard duck
Anas platyrhynchos.

4. **CITATION:** Fletcher, D.W. and C.A. Pederson. 1988.
Diazinon AG500: 8-Day Dietary LC50 Study in Mallard Ducklings. BLAL No. 88-DC-104. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-08.

5. **REVIEWED BY:**


Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

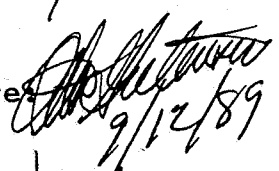
Signature: P. Kosalwat
Date: March 6, 1989

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R. Newman
Date: 3/8/89

 Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: 
Date: 9/12/89
Clyde R. Hume 9/14/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an avian dietary LC50 test. With an LC50 value of 38 ppm a.i., Diazinon AG500 is considered very highly toxic to mallard duck (Anas platyrhynchos). The NOEC was determined to be 8 ppm a.i.

8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Two-day old mallard ducklings, received from Whistling Wings Game Farm, Hanover, Illinois, were immediately placed under a seven-day quarantine period to determine their suitability as test units and to acclimate them to the laboratory conditions. During this period, the animal room temperature ranged from 64 to 80°F with relative humidity between 40 and 87%. Lighting was provided by fluorescent lights which were left on 24 hours per day. All birds were fed Purina^R Game Bird Startena^R and well water ad libitum.

Thirteen birds died during the quarantine period. Some weak, small birds were noted within the first 24 hours of the quarantine period. All other birds were normal and active throughout the quarantine period. Prior to initiation of the test, all birds were examined and their suitability for testing was determined.

- B. Test System: The test system consisted of 45.7 cm x 61 cm x 45.7 cm wire pens, maintained over a concrete slab. Lighting conditions in the room were the same as those employed during the quarantine period. During the 8-day study period, a thermostatically-controlled heated environment offered temperatures ranging from 73 to 81°F with relative humidity between 74 and 82%.
- C. Dosage: Eight-day acute dietary LC50 test. Stock diets of 128 ppm a.i. were prepared by incorporating Diazinon AG500 with corn oil into a basal diet (Purina^R Game Bird Startena^R). Nominal dietary concentrations were 128, 64, 32, 16, and 8 ppm a.i. The vehicle control diet was prepared by thoroughly mixing 13 kg basal diet with 100 ml corn oil. All diets were mixed for 10-15 minutes in Hobart mixer and were immediately frozen after preparation.
- D. Design: Groups of ten birds were assigned to each of the five treatment and five vehicle control groups. The birds were not formally randomized, but rather arbitrarily selected from the entire population of male and female birds. Each group was fed the appropriate

test diet or vehicle control diet for five consecutive days. After this five-day test period, treated diets were removed and were replaced with untreated feed for a three-day recovery period. The diet samples from the vehicle control, 8-, and 128-ppm groups, collected immediately after the preparation and on day 3 were analyzed for Diazinon.

Inspections were made daily for mortalities, the clinical signs indicative of test material effects, abundance of food and water, and food spillage. Birds were weighed by groups at 0 hour on day 1 and day 8. Food consumption was recorded for each group on days 3, 5, and 8. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds from the vehicle control groups and from each of the 8-, 16-, and 32-ppm groups, were also subjected to complete gross pathological examinations at test termination.

E. Statistics: The acute dietary median lethal concentration (LC50) was calculated employing the simplified method of Litchfield and Wilcoxon (1949).

12. REPORTED RESULTS: The measured dietary concentrations of the 8- and 128-ppm groups, collected immediately after the preparation and on day 3, ranged from 81.9 to 110.9% of the nominal concentrations.

Table 3 (attached) presents cumulative mortalities of the test birds throughout the 8-day study period. The LC50 value of the test material was determined to be 33 ppm a.i. (69 ppm of test material) with 95% confidence limits of 23 to 48 ppm a.i. Signs of toxicity noted at test concentrations ≥ 16 ppm a.i. included anorexia, lethargy, inability to walk, secretion of fluid from the nares, apparent weakness, and smallness in size. On test days 7 and 8, all birds appeared to be normal and active. No abnormal behavioral reactions or systemic signs of toxicity were noted among the five vehicle control groups or among the 8-ppm a.i. test group, throughout the investigation. Gross pathological examinations of all birds that died or were sacrificed on day 8 revealed no abnormal pathological findings.

Body weights of surviving birds in all test groups on test day 8 fell within the range of the vehicle control groups (Table 4, attached). All food consumption values in the test and vehicle control groups were comparable throughout

the study with one exception, i.e., severe food avoidance was noted in the 64-ppm a.i. group during the first three test days (Table 4, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 8-day acute dietary LC50 of Diazinon AG500 was determined to be 33 ppm a.i. or 69 ppm of test material for mallard ducklings. The no-observed-effect level was determined to be 8 ppm a.i.

A quality assurance statement was included, indicating that this toxicity test was conducted in compliance with U.S. EPA's Good Laboratory Practice Regulations (Pesticide Programs, 40 CFR 160).

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

- A. **Test Procedure:** The test procedures and the report were in accordance with the SEP guidelines, except for the following deviations:

- o The size of test pens ($127,398 \text{ cm}^3$) was smaller than the recommended size of $168,000 \text{ cm}^3$.
- o The birds were reported as not being formally randomized, but rather arbitrarily selected from the entire population. According to the SEP, the birds must be randomly assigned to the pens.
- o The temperature measured during the test was approximately $23\text{--}27^\circ\text{C}$ (reported as $73\text{--}81^\circ\text{F}$). According to the guidelines, the brooder temperature for the test should be about 35°C .
- o The birds were weighed by groups. According to the SEP, individual body weights should be measured.

- B. **Statistical Analysis:** The reviewer recalculated the LC50 value using EPA's Toxanal computer program and obtained a similar result (see attached printout).

- C. **Discussion/Results:** An LC50 value of 38 ppm a.i. (95% confidence limits of 28-50 ppm a.i.) classifies Diazinon AG500 as very highly toxic to mallard ducklings. The no-observed-effect level (NOEL) was determined to be 8 ppm a.i.

D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the validity of the test results.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, March 3, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 77 through 78 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

☒ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

KOSALWAT DIAZINON AG500 ANAS PLATYRHYNCHOS 02-27-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
128	10	10	100	9.765625E-02
64	10	10	100	9.765625E-02
32	10	1	10	1.074219
16	10	1	10	1.074219
8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 32 AND 64 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 42.06476

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.1144044	35.54655	25.85269 50.22071

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	.2935881	1	.0599466

SLOPE = 5.602214

95 PERCENT CONFIDENCE LIMITS = 2.566723 AND 8.637705

LC50 = 37.67671

95 PERCENT CONFIDENCE LIMITS = 28.09331 AND 50.46591

LC10 = 22.35511

95 PERCENT CONFIDENCE LIMITS = 10.94066 AND 29.56198

No. _____

Chemical Name DIAZINON
AG 500

Chemical Class _____

Page 1 of 1Study/Species/Lab/
Accession _____ Chemical
a.i. _____14-Day Single Dose Oral LD₅₀

Species _____

Lab _____

Acc. _____

Results _____ Reviewer/ _____ Validati
Date _____ Status _____LD₅₀ = mg/kg (95% C.L.) Contr. Mort. (X) = _____

Slope = # Animals/Level = _____ Age (Days) = _____

Sex = _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , ()

Comments: _____

14-Day Single Dose Oral LD₅₀

Species _____

Lab _____

Acc. _____

LD₅₀ = mg/kg (95% C.L.) Contr. Mort. (X) = _____

Slope = # Animals/Level = _____ Age (Days) = _____

Sex = _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , ()

Comments: _____

3-Day Dietary LC₅₀Species ANAS PLATYRHYNCHUSLab BIO-LIFE ASSOC. LTDAcc. 408953-08LD₅₀ = 38 ppm (95% C.L.) Contr. Mort. (X) = 0

Slope = N/A # Animals/Level = 10 Age (Days) = 9

Sex = NOT DETERMINED CORE

3-Day Dose Level ppm/(X Mortality)
8 (0) , 16 (10) , 32 (10) , 64 (100) , 128 (100) CRH/9/1/89

Comments: _____

3-Day Dietary LC₅₀

Species _____

Lab _____

Acc. _____

LC₅₀ = ppm (95% C.L.) Contr. Mort. (X) = _____

Slope = # Animals/Level = _____ Age (Days) = _____

Sex = _____

3-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , ()

Comments: _____

48-Hour LC₅₀

Species _____

Lab _____

Acc. _____

LC₅₀ = pp (95% C.L.) Contr. Mort. (X) = _____

Slope = # Animals/Level = _____ Sol. Contr. Mort. (X) = _____

Temperature = _____

48-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Comments: _____

96-Hour LC₅₀

Species _____

Lab _____

Acc. _____

LC₅₀ = pp (95% C.L.) Contr. Mort. (X) = _____

Slope = # Animals/Level = _____ Sol. Contr. Mort. (X) = _____

Temp. = _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Comments: _____

96-Hour LC₅₀

Species _____

Lab _____

Acc. _____

LC₅₀ = pp (95% C.L.) Contr. Mort. (X) = _____

Slope = # Animals/Level = _____ Sol. Contr. Mort. (X) = _____

Temp. = _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Comments: _____

80

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon AG500, FL #861807, CAS #333-41-5, 48.1% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], an amber liquid with a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Single-Dose Oral LD50 Test.
Species Tested: Brown-Headed Cowbird
Molothrus ater.
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988. Diazinon AG500: 14-Day Acute Oral LD50 Study in Brown-Headed Cowbirds. BLAL No. 88-SB-102. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-09.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date: — 3/1/89

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date:

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:

9/5/89

Date:

CLYDE R. HOUSEKNECHT, PH.D., MPH.

8/3/89 Clyde Houseknecht 9/5/89

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 46.4 mg a.i./kg body weight, Diazinon AG500 is considered highly toxic to brown-headed cowbirds (Molothrus ater). The NOEL was less than 10.0 mg a.i./L.

8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Brown-headed cowbirds (Molothrus ater) used in the study were live-trapped in the state of Michigan and the state of Wisconsin. Upon receipt at Bio-Life Associates, Ltd., they were immediately placed on an approximate 49- to 50-day quarantine period to determine their suitability as test units and to acclimate them to the laboratory conditions. All birds were fed Purina^R Duck Grower W/O and well water ad libitum. Ninety-one of the Michigan birds and fifty-nine of the Wisconsin birds were utilized in BLAL study numbers 88SB102 and 88SB103. The animal room temperature ranged from 40 to 96°F with relative humidity between 57 and 88%. Lighting was provided by fluorescent lights which were left on eight hours per day.

Approximately, 18% (81 out of 438) of the birds from Michigan died of natural causes during the quarantine period. Within the first 14 days, one bird was sacrificed in extremis because it was lethargic and anorexic, one was weak and found dead on the following day, two lethargic male birds had bloody heads due to fighting and four deaths were recorded within the next 24 hours. No deaths occurred during the last eighteen days of the quarantine period. Fifteen percent (44 out of 289) of the birds from Wisconsin died during the quarantine period. All other birds were normal and active throughout the entire quarantine period. Prior to the initiation of the study, all birds were examined and determined to be suitable for testing (based on general physical condition).

B. Test System: All birds were housed in 61.0 cm x 45.7 cm x 45.7 cm steel wire pens (5 birds of like sex per pen, 2 pens per test group), maintained over metal pans which were lined with an absorbent material. Lighting condition in the room was the same as that used during the quarantine period. An indoor environment offered temperatures ranging from 54 to 96°F with relative humidity between 44 and 78% during the 14-day study.

- C. **Dosage:** 14-day single-dose oral LD50 test. Based on four range-finding tests, the nominal dosages selected for the definitive study were 10.0, 21.5, 31.6, 46.4, 68.1, 147, and 215 mg a.i./kg body weight. Dosage levels were based on a geometric scale of 1.47.
- D. **Design:** Groups of ten birds, five males and five females, were randomly assigned to a control group and each of the seven treatment groups. All birds were fasted (with water allowed) for approximately 5-8 hours prior to dosing. A stock solution of Diazinon AG500 was prepared with corn oil. The dose for each individual test bird was volumetrically measured and administered via a gelatin capsule at 0 hour on test day 1. Each control bird received one empty gelatin capsule.

The birds were individually weighed at 0 hour on test day 1, and on test days 3, 7, and 14. Group food consumption values were recorded on test days 3, 7, and 14. Inspections were made daily for the presence (or absence) of clinical signs indicative of test material effects, mortalities, abundance of food and water, and food spillage. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds (two males and two females) from each of the control, 10-, 21.5-, 31.6-, 46.4-, and 68.1-mg a.i./kg test groups, as well as the surviving bird of the 215-mg a.i./kg test group were also subjected to gross pathological examinations on day 14.

- E. **Statistics:** Statistical evaluation of the body weight data was conducted using One-Way Analysis of Variance. The acute oral median lethal dose (LD50) was calculated by employing the simplified method of Litchfield and Wilcoxon (1949).

12. **REPORTED RESULTS:** Table 3 (attached) presents daily mortality data of the test birds throughout the 14-day study period. The 14-day LD50 value was calculated to be 60 mg a.i./kg body weight (or 124.7 mg of test material/kg body weight) with 95% confidence limits of 34.7 to 103.8 mg a.i./kg body weight.

No abnormal behavioral reactions or systemic signs of toxicity were noted in the control group. Signs of toxicity in the 147- and 215-mg a.i./kg groups included lethargy (sluggishness), tachypnea, and yellow-colored diarrhea. All birds that died in the 10- through 68.1-mg a.i./kg test

groups were asymptomatic prior to their deaths except for one bird in the 21.5-mg a.i./kg group which was noted as being near death 25 minutes prior to the discovery of its death. One capsule was found under the 215-mg a.i./kg female pen shortly after dosing occurred. The capsule was more pliable than it had been when dosing occurred. It was uncertain whether the bird regurgitating this capsule actually received any of its dosage. Total remission of all signs was achieved by the end of test day 1. One of the 46.4-mg a.i./kg bird, with its head severely pecked by its penmates, died on test day 14

Except for one bird from the 46.4-mg a.i./kg group which its head had been pecked by the penmates, all birds subjected to gross pathological examinations revealed no abnormal pathological findings. Average body weight and estimated food consumption data are presented in Table 4 (attached). Statistical analysis of the body weights revealed no significant differences in the test groups' body weights at any of the weighing intervals. Severe food avoidance was noted in the 215-mg a.i./kg group during the first three days of the test. All other food consumption values in the test groups were within or above the control group's range during the entire investigation.

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

The acute oral LD50 of Diazinon AG500 for brown-headed cowbirds was determined to be 60.0 mg a.i./kg body weight or 124.7 mg test material/kg body weight. The no-observed-effect level (NOEL) was determined to be less than 10.0 mg a.i./kg body weight.

A quality assurance statement was included, indicating that all studies conducted at Bio-Life Associates were designed and functioned in conformance with Good Laboratory Practice Regulations and the protocols for individual laboratory studies.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP guidelines, except for the following deviations:

- o Brown-headed cowbird (Molothrus ater) is not one of the SEP's recommended species.

o The age of the test birds was unknown. In addition, the test birds were from two sources, i.e., live-trapped from the state of Michigan and the state of Wisconsin. Within a given test all organisms must be from the same source.

o The SEP states that mortality of greater than 10% during an acclimation period is a sign that the lot should not be used. In this study, 18% of the birds from Michigan and 15% from Wisconsin died during the quarantine period (45 days prior to the test). However, no mortalities occurred and all birds appeared normal and active during the last eighteen days of the quarantine period. Therefore, the lots were probably suitable for the test.

o The birds were fasted for approximately 5-8 hours prior to dosing. According to the SEP, feed should be withheld from all birds for at least 15 hours prior to oral dosing.

o A vehicle control group (i.e., a group receiving the maximum amount of corn oil available to any treatment group) was not included in the test.

B. Statistical Analysis: The KBN reviewer recalculated the LD50 value using EPA's Toxanal computer program and obtained a similar result (attached). The EEB reviewer recalculated the LD50 value taking into consideration the capsule that was regurgitated by one bird in treatment group T-VII. Because all treated birds of treatment group T-VI died it is assumed that, had the capsule not been regurgitated, all members of group T-VII would have died. With that assumption, the LD50 value is 46.4 mg a.i./kg body weight.

C. Discussion/Results: Although one bird died on the last day (day 14), the cause of death was reported as being severely pecked on the head by its penmates which probably was not treatment-related. Therefore, the author did not extend the test period beyond 14 days.

With an LD50 value of 46.4 mg a.i./kg body weight (95% C.L. = 29.4-70.6 mg a.i./kg), Diazinon AG500 is considered highly toxic to brown-headed cowbirds, when administered as oral single dose. Based on mortalities observed in all dosages tested, the NOEL was considered to be less than 10.0 mg a.i./kg body weight.

D. Adequacy of the Study:

- (1) Classification: Supplemental.
- (2) Rationale: Brown-headed cowbird (Molothrus ater) is not one of the SEP's recommended species and not all test birds were obtained from the same source.
- (3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, March 1, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 87 through 88 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
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 - ☒ _____ FIFRA registration data.
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 - _____ The document is not responsive to the request.
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C. Houseknecht Diazinon Brown-headed Cowbird 08-31-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
215	10	10	100	9.765625E-02
147	10	10	100	9.765625E-02
68.1	10	2	20	5.46875
46.4	10	3	30	17.1875
31.6	10	3	30	17.1875
21.5	10	2	20	5.46875
10	10	3	30	17.1875

THE BINOMIAL TEST SHOWS THAT 0 AND 147 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 86.76149

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	.1693751	46.3633	29.44705	70.65498

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.19771	3.278012	5.814433E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.838285
95 PERCENT CONFIDENCE LIMITS = -.1735332 AND 3.850104

LC50 = 52.39567
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 10.67663
95 PERCENT CONFIDENCE LIMITS = 0 AND 31.77107

Shaughnessy No. 057801Chemical Name DIAZINON

Chemical Class _____

Page 1 of 1

Study/Species/Lab/

Chemical

(DIAZINON AG500)

Access # _____

a.i. _____

Results

Reviewer/
DateValidation
Status14-Day Single Dose Oral LD₅₀.LD₅₀ = mg/kg (95% C.L.)

Contr. Mort.(%)= _____

Species; _____

Slope= # Animals/Level= _____

Age(Days)= _____

Lab.; _____

Sex = _____

Acc. #; _____

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

Comments: _____

14-Day Single Dose Oral LD₅₀.(BROWN-HEADED COW BIRD)Species: MOLOTHRUS ATERLab.: BIO-LIFE ASSOC. LTD.Acc. #: 408953-09LD₅₀ = 46.4* mg/kg (95% C.L.)

MOVING AVERAGE METHOD

Contr. Mort.(%)= 0

Slope= # Animals/Level= _____

Age(Days)= UNKNOWNSex = M/F CB 18-2-89 SUPPL.

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

10(30), 21.5(20), 31.6(30), 46.4(30), 68.1(20), 147(100), 215(90)Comments: * ACTIVE INGREDIENT8-Day Dietary LC₅₀.LC₅₀ = ppm (95% C.L.)

Contr. Mort.(%)= _____

Species _____

Slope= # Animals/Level= _____

Age(Days)= _____

Lab.; _____

Sex = _____

Acc. # _____

8-Day Dose Level ppm/(% Mortality)

Comments: _____

8-Day Dietary LC₅₀.LC₅₀ = ppm (95% C.L.)

Contr. Mort.(%)= _____

Species: _____

Slope= # Animals/Level= _____

Age(Days)= _____

Lab: _____

Sex = _____

Acc. # _____

8-Day Dose Level ppm/(% Mortality)

Comments: _____

96-hour LC₅₀.LC₅₀ = PP (95% C.L.)

Contr. Mort.(%)= _____

Species: _____

Slope= # Animals/Level= _____

Sol. Contr. Mort.(%)= _____

Lab; _____

Temperature = _____

Acc. #; _____

96-Hour Dose Level pp/(% Mortality)

Comments: _____

96-hour LC₅₀.LC₅₀ = PP (95% C.L.)

Con. Mor(%)= _____

Species: _____

Slope= # Animals/Level= _____

Sol. Con. Mor.(%)= _____

Lab.; _____

Temp.= _____

Acc. #; _____

96-Hour Dose Level pp/(% Mortality)

Comments: _____

48-hour Invertebrate,

LC₅₀ = PP (95% C.L.)

Con. Mort.(%)= _____

Species: _____

Slope= # Animals/Level= _____

Sol. Con. Mort.(%)= _____

Lab.; _____

Temp.= _____

Acc. #; _____

96-Hour Dose Level pp/(% Mortality)

Comments: _____

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Diazinon AG500, FL #861807, CAS #333-41-5, 48.1% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], an amber liquid with a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Single-Dose Oral LD50 Test.
Species Tested: Brown-Headed Cowbird
Molothrus ater.
4. **CITATION:** Fletcher, D.W. and C.A. Pedersen. 1988.
Diazinon AG500: 14-Day Acute Oral LD50 Study in Brown-Headed Cowbirds. BLAL No. 88-SB-102. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-09.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: March 1, 1989

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R. Newman
Date: 3/1/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 3/6/89

Clyde Houscknecht, Ph.D., M.P.H.

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an avian single-dose oral LD50 test. With an LD50 value of 53.1 mg a.i./kg body weight, Diazinon AG500 is considered moderately toxic to brown-headed cowbirds (Molothrus ater). The NOEL was less than 10.0 mg a.i./L.

8. **RECOMMENDATIONS:** N/A.

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
Shaughnessey No. 057801.
2. **TEST MATERIAL:** Formulation: Diazinon AG500, FL #861807, CAS # 333-41-5, 48.1% active ingredient [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], an amber liquid with a slight sulfur-like odor.
3. **STUDY TYPE:** Avian Dietary LC50 Test.
Species Tested: Brown-Headed Cowbird
Molothrus ater.
4. **CITATION:** Fletcher, D.W. and C.A. Pederson. 1988.
Diazinon AG500: 8-Day Dietary LC50 Study in Brown-Headed Cowbirds. BLAL No. 88-SBLC-104. Conducted by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by Ciba-Geigy Corporation, Greensboro, NC. Accession No. 408953-10.

5. **REVIEWED BY:**


Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

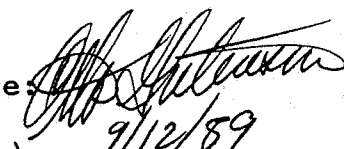
Signature: P. Kosalwat
Date: March 6, 1989

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R. Newman
Date: 3/6/89

 Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: 
Date: 9/12/89
Cyde R. Hruschman 9/11/89

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an avian dietary LC50 test. With an eight-day LC50 value of 42 ppm a.i., Diazinon AG500 is considered very highly toxic to brown-headed cowbird (Molothrus ater). The NOEC was determined to be 16 ppm a.i.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Brown-headed cowbirds were live-trapped in the states of Michigan and Wisconsin. Upon receipt, the birds were placed under quarantine for 16-19 days to determine their suitability as test units and to acclimate them to the laboratory conditions. The Michigan birds were fed Purina^R Game Bird Startena^R. The Wisconsin birds were offered Purina^R Duck Grower W/O until two days before the test on which they were switched to Purina^R Game Bird Startena^R. Well water was supplied ad libitum. During this period, the animal room temperature ranged from 40 to 83°F with relative humidity between 57 and 88%. Lighting was provided by fluorescent lights which were left on 8 hours per day.

Approximately 18% (78 out of 438) of the birds died during the quarantine period. Within the first 14 days of the quarantine period: one bird was sacrificed in extremis because it was lethargic and anorexic, one was weak and found dead on the following day, two lethargic male birds had bloody heads due to fighting and four deaths were recorded within the next 24 hours. All other birds were normal and active throughout the quarantine period. Prior to initiation of the test, all birds were examined and their suitability for testing was determined. Twenty birds from Wisconsin and 130 birds from Michigan were utilized in BLAL study numbers 88SBLCL104 and 88SBLCL105.

B. Test System: The test system consisted of 45.7 cm x 61.0 cm x 45.7 cm wire pens, maintained over metal pans which were lined with an absorbent material. Lighting conditions in the room were the same as those employed during the quarantine period. During the 8-day study period, a thermostatically-controlled heated environment offered temperatures ranging from 46 to 86°F with relative humidity between 63 and 78%.

C. Dosage: Eight-day acute dietary LC50 test. Stock diets of 128 ppm a.i. were prepared by incorporating Diazinon AG500 with corn oil into a basal diet (Purina^R Game Bird Startena^R). Based on an initial range test, nominal dietary concentrations selected for the definitive test

were 128, 64, 32, 16, and 8 ppm a.i. The vehicle control diet was prepared by thoroughly mixing 13 kg basal diet with 100 ml corn oil. All diets were mixed for 10-15 minutes in Hobart mixer and were immediately frozen after preparation.

- D. **Design:** Since it was desirable to separate male from female birds and also because the females weighed less than the males, the sex of the members of the vehicle control and test groups was alternated (male, female, male, female, etc.). The birds were not formally randomized, but rather arbitrarily selected from the entire population of male and female birds. Ten birds of like sex were assigned to each of the five treatment and five vehicle control groups. Each group was fed the appropriate test diet or vehicle control diet for five consecutive days. After this five-day test period, treated diets were removed and were replaced with untreated feed for a three-day recovery period. The diet samples from the vehicle control, 8-, and 128-ppm groups, collected immediately after the preparation and on day 3 were analyzed for Diazinon.

Inspections were made daily for mortalities, the clinical signs indicative of test material effects, abundance of food and water, and food spillage. Birds were weighed by groups at 0 hour on day 1 and day 8. Food consumption was recorded for each group on days 3, 5, and 8. All birds that died during the investigation were subjected to gross pathological examinations. Four arbitrarily selected birds from the vehicle control groups and from each of the 8-, 16-, and 32-ppm groups, as well as the surviving birds from the 64-ppm group were also subjected to complete gross pathological examinations at test termination.

- E. **Statistics:** The acute dietary median lethal concentration (LC50) was calculated employing the simplified method of Litchfield and Wilcoxon (1949).

12. **REPORTED RESULTS:** The mean measured dietary concentrations of the 8- and 128-ppm groups, collected immediately after the preparation were 83 and 103% of the nominal concentrations, respectively. The samples collected on day 3 contained slightly lower mean measured concentrations (78 and 96% of the nominals) than those collected immediately after the diet preparation.

Table 3 (attached) presents cumulative mortalities of the test birds throughout the 8-day study period. The LC50

value of the test material was determined to be 45 ppm a.i. (94 ppm of test material) with 95% confidence limits of 34 to 59 ppm a.i. Signs of toxicity noted at test concentrations ≥ 32 ppm a.i. included inactivity (lethargy), decreased water consumption, gasping, and anorexia. On test days 7 and 8, all birds appeared to be normal and active. No abnormal behavioral reactions or systemic signs of toxicity were noted among the five vehicle control groups or among the 8- and 16-ppm a.i. test groups, throughout the investigation. Gross pathological examinations of all birds that died or were sacrificed on day 8 revealed no abnormal pathological findings.

Body weights of surviving birds in all test groups on test day 8 fell within 2 grams of the range of the vehicle control groups (Table 4, attached). All food consumption values in the test and vehicle control groups were comparable throughout the study with one exception, i.e., severe food avoidance was noted in the 64- and 128-ppm a.i. groups during the first three test days (Table 4, attached).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 8-day acute dietary LC50 of Diazinon AG500 was determined to be 45 ppm a.i. or 94 ppm of test material for mallard ducklings. The no-observed-effect level was determined to be 16 ppm a.i.

A quality assurance statement was included, indicating that this toxicity test was conducted in compliance with U.S. EPA's Good Laboratory Practice Regulations (Pesticide Programs, 40 CFR 160).

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

A. Test Procedure: The test procedures and the report were in accordance with the SEP guidelines, except for the following deviations:

- o Molothrus ater is not one of the recommended species.
- o The age of the test birds was unknown. In addition, the birds were from two sources (i.e., the states of Michigan and Wisconsin). The SEP states that within a given test all organisms must be from the same source.
- o The birds were reported as not being formally randomized, but rather arbitrarily selected from the entire population. According to the SEP, the birds must be randomly assigned to the pens.

o The temperature measured during the test was approximately 8-30°C (reported as 46 to 86°F). According to the guidelines, the brooder temperature for the test should be about 35°C.

o The birds were weighed by groups. According to the SEP, individual body weights should be measured.

B. Statistical Analysis: The reviewer recalculated the LC50 value using EPA's Toxanal computer program and obtained a similar result (see attached printout).

C. Discussion/Results: An eight-day LC50 value of 42 ppm a.i. (95% confidence limits of 31-57 ppm a.i.) classifies Diazinon AG500 as very highly toxic to brown-headed cowbirds. The no-observed-effect level (NOEL) was determined to be 16 ppm a.i.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: Molothrus ater is not one of the recommended species.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, March 6, 1989.

DIAZINON

Page _____ is not included in this copy.

Pages 97 through 98 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ FIFRA registration data.
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 - _____ The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

KOSALWAT DIAZINON AB500 MOLOTHRUS ATER 02-27-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
128	10	10	100	9.765625E-02
64	10	8	80	5.46875
32	10	3	30	17.1875
16	10	0	0	9.765625E-02
8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 16 AND 128 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 41.98279

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.1144044	42.76147	31.35631 62.8506

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.284536	1	.9515336

SLOPE = 5.386989

95 PERCENT CONFIDENCE LIMITS = 2.513466 AND 8.260511

LC50 = 42.37151

95 PERCENT CONFIDENCE LIMITS = 31.48995 AND 57.001

LC10 = 24.62186

95 PERCENT CONFIDENCE LIMITS = 12.00897 AND 32.77382

Sh. No. 057801

Chemical Name Diazinon Chemical Class _____ Page 1 of 1Study/Species/Lab/
Accession _____ Chemical
a.i.

(Diazinon AG500)

Reviewer/
Date _____ Validati
Status _____14-Day Single Dose Oral LD₅₀LD₅₀ = _____ mg/kg (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments: _____

14-Day Single Dose Oral LD₅₀LD₅₀ = _____ mg/kg. (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments: _____

8-Day Dietary LC₅₀LC₅₀ = 4.2 ppm (95% C.L.) Contr. Mort.(X) = 0Species Molothrus ater

Slope = 5.4 # Animals/Level = 10 Age(Days) = Unknown

Lab Bio-life Associates
Ltd. 48.18-day Dose Level ppm/(X Mortality)
8 (0) , 16 (0) , 32 (30) , 64 (80) , 128 (100)

Acc. 408953-10

Comments: * active ingredient

PK/3-6-89 Sup

8-Day Dietary LC₅₀LC₅₀ = _____ ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Age(Days) = _____

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments: _____

48-Hour LC₅₀LC₅₀ = _____ pp (95% C.L.) Contr. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Sol. Contr. Mort.(X) = _____

Lab _____

48-Hour Dose Level pp/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = _____ PP (95% C.L.) Con. Mor.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Sol. Con. Mor.(X) = _____

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments: _____

96-Hour LC₅₀LC₅₀ = _____ PP (95% C.L.) Con. Mort.(X) = _____

Species _____

Slope = _____ # Animals/Level = _____ Sol. Con. Mort.(X) = _____

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , ()

Acc. _____

Comments: _____

100