12-5-88

HTD

DATA EVALUATION RECORD

1. CHEMICAL: MIBK Process Diazinon

2. TEST MATERIAL: MIBK Process Diazinon, FL No. 871790, 97.0% a.i.

3. STUDY TYPE: 14-Day Acute Avian Oral
Species Tested: Mallard Duck

4. CITATION: Fletcher, D. W. 1987. 14-Day Acute Oral Toxicity Study with MIBK Process Diazinon in Mallard Ducks. Prepared by Bio-Life Associates, Ltd., Neillsville, WI for Ciba-Geigy Corporation, Greensboro, NC. BLAL 87 DD 49.

5. REVIEWED BY:

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

Signature:
Date: 2/16/88

6. APPROVED BY:

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

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

Henry T. Craven
Chief EEB/HED
USEPA

Signature: Henry T. Craven
Date: 12/5/88

7. CONCLUSIONS:

The study is scientifically sound. With an LD_{50} of 6.38 (95% c.i. of 4.90 - 8.50 mg/kg) MIBK Process Diazinon (97% a.il.) is very highly toxic to mallard ducks when given as an oral dose. Applicant should be requested to verify the test species by its scientific name and respond to 14C(4).

71-2

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8. RECOMMENDATIONS: When reporting results, use the actual dose (i.e. 3.75 mg/kg) rather than the group number (i.e. T-V). Identify the experimental species by its Latin name, in addition to its common name. Provide 10-hour light (14-hour dark lighting regime until guidelines are revised).

9. BACKGROUND: N/A

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: The birds employed in this study were unmated (16 weeks old) Mallard ducks received from Whistling Wings, Inc., Hanover, Ill. The birds selected for the study had been under observation for a 22-day quarantine period to determine their suitability as test birds based on their general physical condition, and to acclimatize them to laboratory conditions. The birds were identified by means of metal leg bands embossed with numbers unique within the study. Prior to the initiation of the project, all birds were examined and determined to be suitable for testing.

B. Dosage and Design: Dose levels were based on a geometric scale of 1.6. The ducks were randomly assigned to test groups, and were weighed individually at 0 hour on test day 1. Subsequent individual body weights were obtained on test days 3, 7, and 14.

All birds were fasted (with water allowed) for approximately 22> to 23> hours prior to dosing at 0 hour on test day 1. The birds were permitted a standard laboratory diet (Purina Duck Grower w/o) plus water ad libitum at all other times. Food consumption was recorded on test days 3, 7, and 14. The doses for the individual test birds were volumetrically measured and administered via disposable syringes at 0 hour on test day 1. All test and vehicle control birds received a constant dosage volume of approximately 4 ml/kg of body weight. The vehicle control birds each received corn oil only.

Dosage and experimental design were as follows:

Group	Number of Birds Male	Female	Dose Level (mg a.i./kg of body weight)
VC	5	5	0
T-I	5	5	0.57
T-II	5	5	0.91
T-III	5	5	1.46
T-IV	5	5	2.34
T-V	5	5	3.75
T-VI	5	5	6.00
T-VII	5	5	9.60
T-VIII	5	5	15.40

C. Statistics: LD_{50} was calculated using the method of Litchfield and Wilcoxon ("A simplified method of evaluating dose-effect experiments," The Journal of Pharmacology and Exp. Therapeutics, 96(2), June 1949).

12. REPORTED RESULTS:

"A. Reactions

"Treatment related signs of toxicity noted in birds receiving MIBK Process Diazinon included ataxia, regurgitation, lethargy, paralysis (legs stretched behind body), and penile protrusion.

"The vehicle control birds were dosed on June 26, 1987, from 1:30 p.m. to 1:35 p.m. The test group birds were dosed from 2:37 p.m. to 3:20 p.m. with the dosing order [following increasing concentration].

"At 8:30 a.m. on June 27, 1987 (17 hours post-dosing), the following deaths were recorded: [3 in the 3.75 mg/kg group; 4 - 6.00 mg/kg; 8 - 9.60 mg/kg; and 9 - 15.40 mg/kg. The remaining bird in the 15.40 mg/kg group] was aggressive and ataxic. Two [3.75 mg/kg] birds were aggressive and two [9.60 mg/kg] birds were lethargic at this time. Regurgitation had occurred in the [1.4 mg/kg] and [2.34 mg/kg] pens as evidenced by

examination under the pens and on the sides of the pens.

"At 8:20 a.m. on June 28, 1987, all birds appeared to be normal and active and remained so for the balance of the project.

"The vehicle control, [0.57 and 0.91 mg/kg] birds were normal and active throughout the entire investigation."

"B. Mortality and Post-Mortem Examinations

"No mortalities occurred in the [control through the 2.34 mg/kg] groups. Three mortalities were recorded in the [3.75 mg/kg] group, four in the [6.00 mg/kg] group, eight in the [9.60 mg/kg] group, and nine in the [15.40 mg/kg] group ... [see below]."

Group	Dose Level (mg a.i./kg of body weight)	Number Dead Number Tested Male	Female	% Dead	Test Day Found Dead
VC	0	0/5	0/5	0	-
T-I	0.57	0/5	0/5	0	-
T-II	0.91	0/5	0/5	0	-
T-III	1.46	0/5	0/5	0	-
T-IV	2.34	0/5	0/5	0	-
T-V	3.75	2/5	1/5	30	1,1,1
T-VI	6.0	2/5	2/5	40	1,1,1,1
T-VIII	9.60	5/5	3/5	80	1,1,1,1,1,1,1,1
T-VIII	15.40	4/5	5/5	90	1,1,1,1,1,1,1,1,1

- = No mortalities occurred.

"Post-mortem examinations revealed no visible abnormal tissue alterations in the test birds found dead during the investigation. However, all birds died with legs stretched behind their bodies.

"Gross pathological examinations on test day 14 of two male and two female birds sacrificed in the [control through the 6.00 mg/kg] groups as well as the surviving

[9.60 and 15.40 mg/kg] groups revealed no abnormal tissue alterations."

"C. Body Weight Data

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

"D. Food Consumption Data

"Food consumption values in the vehicle control group ranged from 113 to 126 grams/bird/day during the investigation.

"Severe food avoidance was noted during the first three days of the project in all of the test groups when compared to the vehicle control group. Food consumption remained depressed during test days 4 through 7 in the [0.57 and 9.60 mg/kg] test groups. Food consumption values were dose-correlatedly depressed during test days 8 through 14 in the [0.91, 3.75, 6.00, 9.60, and 15.40 mg/kg] test groups."

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

"The acute oral median lethal dose (LD_{50}) of MIBK Process Diazinon in Mallard ducks was determined to be 7.00 mg a.i./kg of body weight with 95% confidence limits of 5.11 to 9.59 mg a.i./kg of body weight.

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

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

A. Test Procedure(s):

(1) Raw data for mortality, body weight and food consumption was consistent with text.

(2) Study, basically, followed guidelines with the following exceptions:

(a) Test Organism. Test species was not verified by its scientific name (SEP, pg. 3).

(b) Body Weight and Food Consumption. Vomiting was reported for individuals in the 1.46 and 2.34 mg/kg groups. SEP (pg. 7) indicates that if vomiting is a problem, the test may need to be rerun.

B. Statistical Analysis: The calculated LD_{50} by the Probit method using Toxanal is slightly lower and has a narrower c.i. than the author's values. See attached printouts.

C. Discussion/Results:

(1) Mortality and Behavioral Observations. The distribution of mortality over dosage tracked the behavioral observations and no inconsistencies were obvious to the reviewer. The observed behavioral effects, such as ataxia, lethargy, paralysis and aggression (down to 3.75 mg/kg) have negative implications which could affect the bird's ability to develop and survive in the wild. Although vomiting was observed, it seems to have been limited to dosage groups, which would not have affected the LD_{50}.

(2) Implications of Dose-Mortality Response. The NOEL, for this study, was not established, since food consumption, for days 4 through 7, in the lowest exposure group (0.57 mg/kg), was depressed. The dose/mortality curve is steep, with a narrow range between no mortality (at 2.34 mg/kg) and 90% (at 15.40 mg/kg).

(3) Gross Necropsy. Gross necropsies were performed but then revealed no abnormal tissue alterations,

according to the author. All birds died with legs stretched out behind their bodies.

(4) Descriptive Categorization of Results. With an LD_{50} of 6.38 mg/kg (95% c.i. of 4.90 - 8.50 mg/kg), MIBK Process Diazinon is very highly toxic to mallard ducks.

D. Adequacy of the Study:

(1) Classification: This study is Core, subject to the verification of the test species by its scientific name.

(2) Rationale: N/A

(3) Reparability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, on February 16, 1988.

16. CBI APPENDIX: N/A

ONE LINER SHEET

Shaughnessey No._____ Chemical Name MIBK Process Diazinon Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Accession #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD_{50} = 6.38 mg/kg (95% C.L. (4.90 - 8.50)) Contr. Mort. (%) = 0		
Species: Mallard Duck		Slope = 3.89 # Animals/Level = 10 Age (Weeks) = 16 Sex = equal	Lincer/ 2-16-88	Core
Lab: Bio-Life, Ltd.	97.0			
AC #: 87 DD 49		14-Day Dose Level mg/kg (% Mortality): 0.57 (0), 0.91 (0), 1.46 (0), 2.34 (0), 3.75 (30), 6.00 (40), 9.60 (80), 15.40 (90)		

Comments: Test species needs to be confirmed by scientific name.

BLAL 87 00 49

J. Newman MIBK Process Diazinon Mallard duck 02-24-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
15.4	10	9	90	1.074219
9.600001		10	8	80
5.46875				
6	10	4	40	37.69531
3.75	10	3	30	17.1875
2.34	10	0	0	9.765625E-02
1.46	10	0	0	9.765625E-02
.91	10	0	0	9.765625E-02
.57	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 2.34 AND 15.4 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.717126

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1677539	6.273546	4.781142	8.380658

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1733351	1	.9345225

SLOPE = 3.892062
95 PERCENT CONFIDENCE LIMITS = 2.271659 AND 5.512465

LC50 = 6.376417
95 PERCENT CONFIDENCE LIMITS = 4.904668 AND 8.497744

LC10 = 3.007957
95 PERCENT CONFIDENCE LIMITS = 1.670586 AND 4.051303

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