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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JAN 30 1990

MEMORANDUM

SUBJECT: Submission of Diazinon data in response to
DCI notice

FROM: James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division

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

Ecological Effects Branch has completed its review of ecotoxicity data for Diazinon AG 500 submitted by Ciba-Geigy Corporation. The following is a brief summary of the result of this review.

CITATION: Surprenant, D.C. 1987. Static Acute Toxicity of Diazinon AG 500 to Daphnids (Daphnia magna). Prepared by Springborn Life Sciences, Inc. Wareham, Massachusetts. Submitted by CIBA-GEIGY Corporation, Greensboro, North Carolina. Report #87-12-2572. EPA Accession No. 405098-03.

CONCLUSIONS: This study is scientifically sound and meets the requirements for a freshwater invertebrate acute toxicity test. The 48-hour EC₅₀ of Diazinon AG 500 to Daphnia magna is 1.1 ug a.i./L. Thus, Diazinon AG 500 is classified as very highly toxic to this species. The NOEC was determined to be < 0.89 ug ai./L.

If you have any any questions concerning this study, please contact Clyde Houseknecht at 557-4372.

DATA EVALUATION RECORD

1. **CHEMICAL:** Diazinon.
2. **TEST MATERIAL:** Formulation: Diazinon AG 500 (Lot #FL-861806); 48% active ingredient, a light yellow liquid.
3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Test.
Species Tested: Daphnia magna.
4. **CITATION:** Surprenant, D.C. 1987. Static Acute Toxicity of Diazinon AG 500 to Daphnids (Daphnia magna). Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. Submitted by CIBA-GEIGY Corporation, Greensboro, North Carolina. Report #87-12-2572. Accession #405098-03. Shaughnessey #057801.

5. **REVIEWED BY:**

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

Signature: P. Kosalwat
Date: 7-6-88

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: 7-11-88

Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 1/25/90

Clyde R. Hourcade

Signature: Clyde R. Hourcade
Date: 1-24-90

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a freshwater invertebrate acute test. With the EC50 value of 1.1 ug A.I./L, Diazinon AG 500 is considered very highly toxic to Daphnia magna. The NOEC was determined to be <0.89 ug A.I./L.

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

9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

A. Test Animals: The daphnids (Daphnia magna) were obtained from laboratory cultures maintained at Springborn Life Sciences, Inc. The culture water was prepared by fortifying well water based on the formula for hard water (ASTM 1980). The resulting water had total hardness and alkalinity ranges as calcium carbonate of 160-180 mg/L and 110-130 mg/L, respectively; a specific conductivity range of 400-600 umhos/cm; pH range of 7.9-8.3; and a dissolved oxygen concentration of greater than 60% of saturation.

The daphnid culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Light at the surface of the culture solution was at an intensity of 50-100 foot-candles. Daphnids were fed a solution of green algae and yeast suspension once daily. The ambient air temperature in the culture area was controlled in order to maintain the culture solution temperature at $20 \pm 1^{\circ}\text{C}$.

B. Test System: The test was conducted in 1000 mL glass beakers. The test solution depth was 10 cm with a surface area of 82 cm². Duplicate vessels were maintained for each concentration and the control. The dilution water used was from the same source as the culture water and had a pH of 7.9; a specific conductivity of 430 umhos/cm; a total hardness and alkalinity of 164 and 124 mg/L as CaCO₃, respectively. Primary and secondary stock solutions were prepared using distilled water.

The ambient air temperature in the laboratory was controlled in order to maintain test solution temperatures at $21 \pm 1^{\circ}\text{C}$. Test solutions were not aerated. The test area was illuminated at an intensity of 70 foot-candles at the surface of the solutions. The photoperiod during testing was the same as that provided in the culture area.

C. Dosage: 48-hour acute static test.

D. Design: Twenty daphnids, \leq 24 hours old, were impartially distributed to each concentration (ten daphnids per replicate) within twenty minutes after the test solutions had been prepared. Daphnids were not fed during the exposure. The nominal test concentrations were 0.91, 1.5, 2.5, 4.1, 6.7, 12.0, and 19.0 ug A.I./L. The test concentrations were measured at test initiation and termination.

The number of immobilized daphnids in each replicate test vessel was recorded at 24 and 48 hours of exposure. Biological observations and observations of the physical characteristics of each replicate test solutions were also made and recorded at 0, 24, and 48 hours. The pH, temperature, and dissolved oxygen concentrations were measured at 0, 24, and 48 hours in one replicate vessel of each treatment level and the control.

E. Statistics: The EC50 values and their corresponding 95% confidence intervals were calculated using a computer program modified from C. Stephan's program.

12. **REPORTED RESULTS:** The dissolved oxygen concentrations remained over 82% of saturation at the test termination. The pH and temperature ranges were 7.5-8.0 and 21-22°C, respectively.

Analyses of the exposure solutions at 0 hour resulted in measured concentrations which averaged 99% of the nominal levels. Measured concentrations at 48 hours established that the concentration of Diazinon in the exposure solutions generally decreased by an average of 11% between 0 and 48 hours. The mean measured test concentrations and corresponding cumulative number of immobilized organisms are presented in Table 2.

Table 3 summarizes the 24- and 48-hour EC50's and corresponding 95% confidence intervals, and presents the no-observed-effect concentration (NOEC) through 48 hours.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 48-hour EC50 and 95% confidence interval (based on measured concentrations) was 1.1 (1.0-1.3) ug A.I./L. The slope of the concentration-response curve was calculated by probit analysis to be 11.79. The NOEC was <0.89 ug A.I./L. Based on EPA (1985) criteria, the test material would be classified as very highly toxic to Daphnia magna.

The data and report were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice regulations except in the case of characterization and verification of the test substance identity. The report was signed by Springborn Life Sciences' Quality Assurance Unit.

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

A. Test Procedure: The test procedure is generally in accordance with the SEP guidelines, except for the following deviations:

o The dilution water used had total hardness of 164 mg/L as CaCO₃, which is much higher than the recommended hardness of 40-48 mg/L as CaCO₃.

o No inert control was included in the study.

o The test temperatures of 21-22°C deviated slightly from the recommended temperature of 20°C for daphnid test. Furthermore, the temperatures were measured every 24-hour interval instead of continuously (hourly) as recommended by the SEP.

o There was no report on a range-finding test.

B. Statistical Analysis: The statistical analyses performed by the author were appropriate. The reviewer recalculated the 48-hour EC50 value using EPA's Toxanal program and obtained the same result (see attached).

C. Discussion/Results: According to the raw data, the highest mean measured concentration was 18.3 ug/L. However, Tables 1 and 2 reported 18.0 ug/L as the highest concentration. With an EC50 value of 1.1 ug A.I./L, Diazinon AG 500 is considered very highly toxic to Daphnia magna. The NOEC was determined to be <0.89 ug A.I./L.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: Although the test procedure deviated from the SEP guidelines, the reviewer does not believe that it significantly affected the toxicity results.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, July 5, 1988.

Table 2. Concentrations tested and corresponding cumulative number of immobilized organisms and observations made during the 48-hour static exposure of daphnids (*Daphnia magna*) to Diazinon AG 500.

Mean Measured Concentration (µg A.I./L)	Cumulative Number of Immobilized Organisms (%)					
	24-hour			48-hour		
	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0
0.89	0ab	0a	0	20ab	0a	10
1.4	10abc	0ab	5	100a	70ab	85
2.5	40ad	50ad	45	100a	100a	100
4.0	100a	100a	100	100a	100a	100
5.7	100a	100a	100	100a	100a	100
9.8	100a	100a	100	100a	100a	100
18	100	100a	100	100	100	100

^aA film was present on the surface of the test solution.

^bSeveral of the surviving daphnids were caught on particulate matter.

^cSeveral of the surviving daphnids were at the surface of the test solution.

^dAll of the surviving daphnids were on the bottom of the test solution.

Table 3. The EC50 values, 95% confidence intervals and no observed effect concentration for daphnids (*Daphnia magna*) exposed to Diazinon AG 500. Values based on mean measured concentrations of active ingredient (Diazinon).

	EC50 ($\mu\text{g A.I./L}$)	Confidence Interval	
		Lower ($\mu\text{g A.I./L}$)	Upper ($\mu\text{g A.I./L}$)
24-hour ^a	2.2	1.9	2.6
48-hour ^b	1.1	1.0	1.3

NOEC through 48 hours: $<0.89 \mu\text{g A.I./L}^c$

^aEC50 and confidence limits calculated by Moving Average Angle Analysis.

^bEC50 and confidence limits calculated by Probit Analysis.

^cThe lowest concentration tested.

JALWAT DIAZINON AG500 DAPHNIA MAGNA 6-24-88

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CONC.  NUMBER  NUMBER  PERCENT  BINOMIAL
      EXPOSED  DEAD    DEAD    PROB. (PERCENT)
18     20      20      100     9.536742E-05
9.8    20      20      100     9.536742E-05
5.7    20      20      100     9.536742E-05
4       20      20      100     9.536742E-05
2.5    20      20      100     9.536742E-05
1.4    20      17      85      .1288414
.89    20      2       10      2.012253E-02
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THE BINOMIAL TEST SHOWS THAT .89 AND 1.4 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 1.138235

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	7.602794E-02	1.152628	.9787139	1.307858

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.1865899	1	1

SLOPE = 11.78812
95 PERCENT CONFIDENCE LIMITS = 6.69612 AND 16.88013

LC50 = 1.143265
95 PERCENT CONFIDENCE LIMITS = 1.025619 AND 1.273566

LC10 = .8920955
95 PERCENT CONFIDENCE LIMITS = .709978 AND .9996869

Study/Species/Lab/
Succession _____
Chemical
I.S.I.

(Diazinon AG 500)

Results

Reviewer/
Date _____
Valid:
Stat. _____

1 Day Single Dose Oral LD50

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

Species _____

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

Lab _____

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

Acc. _____

Comments:

14-Day Single Dose Oral LD50

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

Species _____

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

Lab _____

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

Acc. _____

Comments:

8-Day Dietary LC50

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

Species _____

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

Lab _____

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

Acc. _____

Comments:

8-Day Dietary LC50

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

Species _____

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

Lab _____

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

Acc. _____

Comments:

8-Day Dietary LC50

LC50 = PP (95% C.L.) Contr. Mort.(%) = _____
Sol. Contr. Mort.(%) = _____

Species _____

Slope = # Animals/Level = _____ Temperature = _____

Lab _____

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

Acc _____

Comments:

96-Hour EC50*

48

* EC50 = 1.1 ** 95% C.L.
PPb (1.0 - 1.3)

Species Daphnia magna

Slope = 11.79 # Animals/Level = 20

Con. Mort.(%) = 0
Sol. Con. Mort.(%) = N/A

Lab Springborn Life Sciences, Inc.

96-Hour Dose Level pp/(% Mortality)
0.89(10), 1.4(85), 2.5(100), 4.0(100), 5.7(100), 9.8(100), 18(100)

Temp. = 21-22°C PK Corr
7-5-88

Acc. 405098-03

Comments: * end point = immobilization, ** mean measured conc.

96-Hour LC50

LC50 = PP (95% C.L.) Contr. Mort.(%) = _____
Sol. Con. Mort.(%) = _____

Species _____

Slope = # Animals/Level = _____ Temperature = _____

Lab _____

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

Acc. _____

Comments: