



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 Rainbow Trout (Salmo gairdneri). Prepared by Springborn Life Sciences, Inc. Wareham, Massachusetts. Submitted by CIBA-GEIGY Corporation, Greensboro, North Carolina. Report #87-12-2570. EPA Accession No. 405098-01.

CONCLUSIONS: This study is scientifically sound and meets the requirements for a freshwater fish acute toxicity test. The 96-hour LC_{50} of Diazinon AG 500 to rainbow trout is 1.8 mg a.i./L. Diazinon AG 500 is classified as moderately toxic to rainbow trout. The NOEC was determined to be 0.23 mg a.i./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 Fish Static Acute Test.
Species Tested: Salmo gairdneri.
4. CITATION: Surprenant, D.C. 1987. Static Acute Toxicity of Diazinon AG 500 to Rainbow Trout (Salmo gairdneri). Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. Submitted by CIBA-GEIGY Corporation, Greensboro, North Carolina. Report #87-12-2570. Accession #405098-01. 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. HOUSEKNEHT

Clyde R. Housekneht 1-23-90

7. CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for a freshwater fish acute test. With the LC50 value of 1.8 mg A.I./L, Diazinon AG 500 is considered moderately toxic to Salmo gairdneri. The NOEC was determined to be 0.23 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 rainbow trout (*Salmo gairdneri*) were obtained from a commercial supplier in California and held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. The well water which flowed into this tank had total hardness and alkalinity ranges as calcium carbonate of 30-34 mg/L and 26-30 mg/L, respectively. Other parameters monitored in the holding tank were a specific conductivity range of 90-140 umhos/cm, pH range of 6.7-7.0, dissolved oxygen concentration range of 59-70% of saturation, and a flow rate of 8.8-15.0 tank volume replacements/day.

Test fish were maintained under these conditions for a minimum of 14 days. The temperature range in the holding tank was 12-13°C during this period. The fish were fed a dry commercial pelleted food, ad libitum, daily except the 48 hours prior to testing. There was no mortality of the test fish population during this 48-hour period. The mean wet weight of the test fish population was 0.67 g (range of 0.29-1.77 g) and the mean total length was 41 mm (range of 34-56 mm).

B. Test System: The test system consisted of 18.9-L glass aquaria which contained 15 L of test solution. The test solution depth was 18.4 cm with a surface area of 816 cm². The dilution water used was soft water reconstituted from deionized water with a total hardness and alkalinity as CaCO₃ of 46-50 mg/L and 35 mg/L, respectively. The pH of the water was 7.5 and specific conductivity was 120-160 umhos/cm. A cloudy white stock solution of 24 mg A.I./mL was prepared using distilled water.

All test solution temperatures were controlled by a system designed to maintain temperatures at 13 ± 1°C. Test solutions were not aerated. The photoperiod during testing was the same as that provided in the fish culture area. Light at an intensity of 25 foot-candles was provided at the solution surface.

C. Dosage: 96-hour acute static test.

D. Design: Ten rainbow trout selected impartially from the holding tank were placed in each aquarium (2 replicate aquaria per test concentration and the control) within thirty minutes after the test solutions had been prepared. The resulting test organism loading factor was 0.45 grams of biomass per liter of test solution. Fish were not fed during the exposure. The nominal

test concentrations were 0.11, 0.19, 0.31, 0.53, 0.86, 1.4, and 2.4 mg A.I./L. The test concentrations were measured at test initiation and termination.

All aquaria were examined after 0, 24, 48, 72, and 96 hours of exposure as follows: mortalities were recorded, dead fish were removed, and observations of the fish and the physical characteristics of the test solutions were recorded. Dissolved oxygen concentrations, temperature, and pH were measured in the controls and all test concentrations at each 24 hour observation interval.

E. Statistics: The LC50 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 concentration dropped below 8.2 mg/L at 24 hours in both replicates of all test concentrations. Therefore, the test solutions were aerated from 24 hours until the end of the test. The pH ranged between 7.1 and 8.1, while the temperature stayed at 13°C during the 96-hour test period.

Analyses of the exposure solutions at 0 hour resulted in measured concentrations which averaged 104% of the nominal levels. Measured concentrations at 96 hours established that the concentration of Diazinon in the exposure solutions generally decreased by an average of 41% between 0 and 96 hours. The mean measured test concentrations, the corresponding cumulative percent mortalities, and the observations made during the definitive test are presented in Table 2.

Table 3 summarizes the 0, 24-, 48-, 72-, and 96-hour LC50's and corresponding 95% confidence intervals, and presents the no-observed-effect concentration (NOEC).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC50 and 95% confidence interval (based on measured concentrations) was 1.8 (1.4-2.9) mg A.I./L. The NOEC was 0.43 mg A.I./L. Based on EPA (1985) criteria, the test material would be classified as moderately toxic to Salmo gairdneri.

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 No inert control was included in the study.
- o The age of the test fish was not provided.
- o The test was conducted at 13°C and the temperature was measured every 24 hours during the test period. According to the guidelines, coldwater fish tests should be conducted at 12°C and the temperature should be measured continuously (hourly) in at least one test vessel during the entire study period.
- 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 96-hour LC50 value using EPA's Toxanal program and obtained the same result (see attached).

C. Discussion/Results: The author reported the NOEC as 0.43 mg A.I./L. According to the results observed at 96 hours (Table 2), one of the surviving fish exhibited a partial loss of equilibrium in the 0.43-mg/L treatment level. Therefore, the NOEC should be reported as 0.23 mg A.I./L. With an LC50 value of 1.8 mg A.I./L, Diazinon AG 500 is considered moderately toxic to Salmo gairdneri.

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, corresponding cumulative mortalities and observations made during the 96 hour static exposure of Diazinon AG 500 to rainbow trout (*Salmo gairdneri*).

Mean Measured Concentration (mg A.I./L)	Cumulative Mortality (%)			
	24-hour	48-hour	72-hour	96-hour
Control	0	0	0	0
0.09	0	0	0	0
0.16	0	0	0	0
0.23	0	0	0	0
0.43	0	0	0	0 ^a
0.88	0	0	0 ^b	10 ^{bc}
1.2	0 ^{de}	0 ^{cf}	0 ^g	25 ^{hi}
1.8	0 ^{jkl}	0 ^{gjk}	15 ^{jm}	50 ^{jm}

- ^aOne of the surviving fish exhibited a partial loss of equilibrium.
^bTwo of the surviving fish exhibited a darkened pigmentation.
^cTwo of the surviving fish exhibited a complete loss of equilibrium.
^dOne of the surviving fish exhibited a complete loss of equilibrium.
^e<20% of the surviving fish exhibited a partial loss of equilibrium.
^f20-50% of the surviving fish exhibited a partial loss of equilibrium.
^g20-50% of the surviving fish exhibited a complete loss of equilibrium.
^h>50% of the surviving fish exhibited a darkened pigmentation.
ⁱ>50% of the surviving fish exhibited a complete loss of equilibrium.
^jAll of the surviving fish exhibited darkened pigmentation.
^kAll of the surviving fish exhibited a partial loss of equilibrium.
^l<20% of the surviving fish exhibited a complete loss of equilibrium.
^mAll of the surviving fish exhibited a complete loss of equilibrium.

Table 3. The LC50 values, 95% confidence intervals and No Observed Effect Concentration for the 96-hour static exposure of Diazinon AG 500 rainbow trout (*Salmo gairdneri*). Values presented are based on mean measured concentrations of active ingredient (Diazinon).

	LC50 (mg A.I./L)	Confidence Interval	
		Lower (mg A.I./L)	Upper (mg A.I./L)
24-hour ^a	>1.8	---	---
48-hour ^a	>1.8	---	---
72-hour ^b	>1.8	1.8	---
96-hour ^c	1.8	1.4	2.9

NOEC through 96 hours : 0.43 mg A.I./L

^aLC50 value empirically estimated as greater than the highest concentration tested. 95% confidence interval could not be calculated.

^bLC50 value empirically estimated as greater than the highest concentration tested; lower 95% confidence interval calculated by binomial probability.

^cLC50 and 95% confidence interval calculated by probit analysis.

KOSALWAT DIAZINON AG500 SALMO GAIARDNERI 6-24-88

INC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.8	20	10	50	58.80985
1.2	20	5	25	2.069473
.88	20	2	10	2.012253E-02
.43	20	0	0	9.536742E-05
.23	20	0	0	9.536742E-05
.16	20	0	0	9.536742E-05
9.000001E-02		20	0	0

9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.8

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	1.51662	1.8	1.389064 +INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

RATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.3237824	1	.9996808

SLOPE = 4.331195

95 PERCENT CONFIDENCE LIMITS = 1.866664 AND 6.795726

LC50 = 1.764282

95 PERCENT CONFIDENCE LIMITS = 1.446541 AND 2.892719

LC10 = .8981447

95 PERCENT CONFIDENCE LIMITS = .5133912 AND 1.106492

No.	Chemical Name	Chemical Class	Page	1	2	3
Study/Species/Lab/ Succession	(Diazinon AG 500)		Results	Reviewer/ Date	Validator/ State	
1) ay Single Dose Oral LD50	LD50 =	mg/kg (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	14-Day Dose Level mg/kg/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			
14-Day Single Dose Oral LD50	LD50 =	mg/kg (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	14-Day Dose Level mg/kg/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			
8-Day Dietary LC50	LC50 =	ppm (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	8-Day Dose Level ppm/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			
8-Day Dietary LC50	LC50 =	ppm (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	8-Day Dose Level ppm/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			
8-Day Dietary LC50	LC50 =	ppm (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	8-Day Dose Level ppm/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			
8-Day Dietary LC50	LC50 =	ppm (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	96-Hour Dose Level ppm/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			
96-Hour LC50	LC50 =	1.8 ppm (95% C.L.)	Contr. Mort.(%) = 0			
Species <u>Salmo gairdneri</u>	Slope =	4.3 # Animals/Level = 20	Sol. Contr. Mort.(%) = N/A			
Lab <u>Springborn Life Sciences, Inc.</u>	96-Hour Dose Level ppm/(% Mortality)		Temp. = 13°C			
Acc. <u>405098-01</u>	* Mean measured concentration					
96-Hour LC50	LC50 =	ppm (95% C.L.)	Contr. Mort.(%) =			
Species	Slope =	# Animals/Level =	Age(Days) =			
Lab	96-Hour Dose Level ppm/(% Mortality)		Sex =			
Acc.	() , () , () , () , () , ()		Comments:			

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