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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Diazinon Registration Standard Data Submissions; EPA Acc. Nos. 406255-01 and -02. EPA Reg. No. 100-524.

TO: George LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: Jim Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

CIBA-GEIGY Corporation has submitted the above referenced data in response to the Diazinon Registration Standard. EEB's review results are indicated below and the data evaluation records are attached.

Guide. Ref.No.	Test Species	% AI	Test Type	Test Results	Toxicity Category	Study Status
72-3	Mysid	87.7	96-hour LC50	4.2 ug/l	Very Highly Toxic	Core
72-3	Oyster	87.7	96-hour shell deposit.	0.88 mg/l	Very Highly Toxic	Core

John Noles 1/27/89
John Noles, Biologist
Ecological Effects Branch

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Accession No. 406255-02

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon Technical
Shaughnessey Number: 057801

2. TEST MATERIAL: Diazinon Technical; Lot No. FL-872049; CAS No. 333-41-5; 87.7% active ingredient; an amber liquid

3. STUDY TYPE: Acute Toxicity Test for Estuarine and Marine Organisms. Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster, Crassostrea virginica

4. CITATION: Surprenant, D.C. 1988. Acute Toxicity of Diazinon Technical to Eastern Oysters (Crassostrea virginica) under Flow-through Conditions. Laboratory Study #88-3-2656. Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. Submitted by Ciba Geigy Corporation, Greensboro, North Carolina. Accession Number 406255-02.

5. REVIEWED BY:

Kimberly Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences

Signature: Kimberly Rhodes
Date: May 5, 1989

6. APPROVED BY:

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

Signature: P. Kosalwat
Date: May 5, 1989

for Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: John Noles
Date: June 19, 1989

7. CONCLUSIONS: This study appears scientifically sound and fulfills the Guideline requirements for a flow-through acute oyster shell deposition study. The 96-hour EC50 value for eastern oysters exposed to Diazinon Technical was 0.88 mg a.i./L based on mean measusred concentration. Therefore, Diazinon Technical is classified as highly toxic to Eastern oysters (Crassostrea virginica). The NOEC was estimated to be 0.21 mg a.i./L.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Eastern oysters, Crassostrea virginica, were obtained from Aquacultural Research Corporation, Dennis, Massachusetts. Oysters were maintained at a salinity of 27 to 30°/oo, a temperature range of 18 to 20°C, and a dissolved oxygen concentration range of 90 - 98% of saturation for fourteen days prior to testing. Oysters were continuously fed a combination of marine algae which had an average density of 53 X 10^3 cells/mL during this period. During the 72-hours prior to testing, the temperature was maintained at 20 - 20.5°C, the salinity was 31 - 32°/oo, the pH ranged from 7.6 - 7.7, and the dissolved oxygen concentration ranged from 77 - 84 percent of saturation. No mortality occurred during the holding period. Oysters with a mean valve height of 43 ± 5 millimeters (mm) were tested.

B. Test System: The test was conducted in a continuous flow serial diluter with a dilution factor of 0.60. The system was a modification of the diluter described by Benoit et al. Each glass aquarium measured 60 x 30 x 30 centimeters (cm) and was equipped with a 10-cm high drain standpipe which maintained a test solution volume of approximately 18 liters (L). The flow rate provided 6-volume replacements per day. In addition, the contents of each aquarium were continuously circulated at a flow rate of 1.75 L per minute or about 5 L per oyster per hour. This circulation system aided in evenly distributing the algae fed to the oysters and in mixing the flow of fresh test solution throughout each aquarium. The temperature was maintained by a water bath at 20 ± 2°C. The photoperiod provided 16 hours of light and 8 hours of darkness per day.

The dilution water was natural unfiltered seawater collected from the Cape Cod Canal near Bourne, Massachusetts. The salinity of the dilution water was 31 to 32 °/oo with a pH of 7.4 to 8.1.

C. Dosage: 96-hour acute flow-through test.

D. Design: Twenty oysters were tested per duplicate test aquaria (40 per test and control solutions). A control, solvent control (62 microliter acetone/L), and nominal Diazinon Technical concentrations of 0.32, 0.54, 0.90, 1.5, and 2.5 mg/L were maintained. Twenty-four hours prior to testing, 2 - 5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge. Immediately prior to the test initiation, the outer shell was buffed with an emery board to remove any new shell deposition. Oysters were equally spaced from one another with their valve openings facing toward the flow from the circulator tube. During the holding period, 180 mL of a concentrated algal suspension of 10^7 cells/mL were added to each test aquarium three times daily. During the last 72 hours of acclimation and testing, oysters were fed a supplementary diet of Isochrysis galbana Parke, clone T-ISO and Tetraselmis maculata. Biological observations were made daily during the exposure in order to detect any mortality of oysters and to record any visible abnormalities. After 96 hours of exposure the oysters were removed and the new shell growth was measured microscopically to 0.1 mm using a calibrated micrometer. During the exposure, the pH, temperature, salinity and dissolved oxygen concentration were measured daily in each aquarium. In addition, temperature was monitored continuously in one test aquarium. Analytical determination of Diazinon Technical was performed from each replicate solution including the control at 0 and 96 hours.

E. Statistics: A computer program developed at the testing laboratory was utilized to compute four linear regression curves based on least squares. Percentage reduction in growth data were transformed to probits and concentrations to logs. Both untransformed and transformed data were regressed. The regression line which provided the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination. The regression equation was then applied to calculate the EC50 and its 95% confidence limits.

The no-observed-effect concentration (NOEC) was determined by subjecting the biological response (shell growth) data to analysis of variance and Williams' Test. The highest test concentration causing no significant reduction of shell growth was identified as the NOEC.

12. REPORTED RESULTS: The results of the oyster shell deposition test of Diazinon Technical are presented in Table 3 (attached). The shell growth of oysters exposed to Diazinon Technical concentrations ≥ 0.48 mg a.i./L was significantly reduced when compared to control growth. The 96-hour EC50 and 95 percent confidence interval calculated by linear regression was 0.88 (0.63 - 1.1) mg a.i./L. The mean shell growth of oysters exposed to a mean measured concentration of 0.21 mg a.i./L Diazinon Technical was not statistically different from the control oyster growth, which established 0.21 mg a.i./L as the no-observed-effect concentration (NOEC). Based on EPA (1985), the test material would be classified as highly toxic to oysters.

The mean measured concentrations of Diazinon Technical in the test solutions during the 96-hour definitive test were 0.21, 0.48, 0.65, 0.98, and 1.3 mg a.i./L. The mean measured concentrations of Diazinon Technical ranged from 52% to 89% of the nominal concentrations. No visible sign of undissolved material was observed in the dilution cells or the test aquaria. Water quality was unaffected by test concentrations of Diazinon Technical and was satisfactory for the survival and growth of the test organisms.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour EC50 and 95% confidence interval were calculated by linear regression analysis. The EC50 was 0.88 mg a.i./L with a 95% confidence interval between 0.63 and 1.1 mg a.i./L. The no-observed-effect concentration (NOEC) was 0.21 mg a.i./L. Based on EPA (1985) criteria, the test material would be classified as highly toxic to Eastern oysters.

The study was audited by the QA unit of Springborn Life Sciences, Inc. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o A flow rate of 75 mL/minute/test aquarium was utilized providing a flow of 225 mL/oyster/hour. This rate is only 4.5% of the rate set forth in test protocols recommended in the Guidelines (approximately 5 liter/oyster/hour). To offset the reduced flow, the

test facility supplemented the water with the algae, Isochrysis galbana Parke and Tetraselmis maculata at a density of 1 X 10^7 cells/mL three times daily, and utilized pumps to recirculate test solutions at a flow rate of 5 L/oyster/hour. It should be noted however, that a flow-through test, as defined by ASTM (1980), consists of a "test solution that flows through the test chamber on a once-through basis throughout the test" and not on a recirculated flow.

B. Statistical Analysis: The reviewer calculated the EC50 value using regression analysis (attached). This calculation provided a 96-hour EC50 value of 0.87 mg a.i./L which was similar to that estimated by the author (i.e., 0.88 mg a.i./L). The results of analysis of variance and Dunnett's test indicate that the shell growth in the two highest test concentrations were significantly different from that in the control which are the same as those analyzed by the author. However, Williams test (calculated by the author) gave a more conservative value of NOEC (0.21 mg a.i./L). Therefore, the no-observed-effect concentration was considered to be 0.21 mg a.i./L.

C. Discussion/Results: The 96-hour EC50 was estimated to be 0.88 mg a.i./L with a 95% confidence interval of 0.63 and 1.1 mg a.i./L mean measured concentration. Therefore, Diazinon Technical is classified as highly toxic to Eastern oysters (Crassostrea virginica). The NOEC was estimated by the author to be 0.21 mg a.i./L.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-24-89.

Table 3. Effects of DIAZINON Technical on shell deposition of Eastern oysters (Crassostrea virginica) after 96 hours of exposure.

Mean Measured Concentration (mg/L)	Mean (Standard Deviation) Shell Deposition[a] (mm)	Percentage Reduction[b]
1.3	0.6 (0.8)	70
0.98	0.8 (0.6)	60
0.65	1.3 (1.0)	35
0.48	1.4 (0.9)	30
0.21	1.6 (1.3)	20
Solvent Control	1.9 (1.4)	NA[c]
Control	2.0 (1.0)	NA
Pooled Control	2.0 (1.2)	NA

[a] The mean represents the measurements of 40 oysters per treatment level.

[b] % reduction compared to the pooled control value for shell growth.

[c] Not applicable.

Diazinon Technical
(EC50)

LISTING OF DATA

DATA POINT	X (Conc.)	Y % red. (probit)
1 0.21 mg/L	.21	4.16
2 0.48	.48	4.48
3 0.65	.65	4.61
4 0.98	.98	5.25
5 1.3	1.3	5.52

ACTUAL VERSUS ESTIMATED VALUES
X=CONCENTRATION Y=% REDUCTION

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	.21	4.16	4.131171	2.882862E-02
2	.48	4.48	4.484603	-4.602909E-03
3	.65	4.61	4.707134	-9.713364E-02
4	.98	5.25	5.139106	.1108942
5	1.3	5.52	5.557988	-3.798771E-02

REGRESSION EQUATION:
Y= 3.85628 + 1.309006 X

COEFFICIENT OF CORRELATION= .9904918

$Y = 3.85628 + 1.309006X$

where X = Concentration

Y = 5 (50% reduction converted into probit value)

Therefore, $X = \frac{1.14372}{1.309006} = 0.87$

$EC50 = 0.87$ mg/L

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Diazinon Technical
(NOEC)

Analysis of Variance File: diazoys Date: 04-25-1989

FILTER: None

N's, means and standard deviations based on dependent variable: REDUCT

* Indicates statistics are collapsed over this factor

Factors: C (mean measured Concentration (mg/L))		N	Mean	S.D.
*		14	1.3714	0.5622
1	Control & Solvent Cont.	4	1.9500	0.1000
2	0.21	2	1.7500	0.4950
3	0.48	2	1.3500	0.0707
4	0.65	2	1.2000	0.2828
5	0.98	2	0.7500	0.3536
6	1.3	2	0.6500	0.3536

Fmax for testing homogeneity of between subjects variances: 49.00
Number of variances= 6 df per variance= 1.

Analysis of Variance Dependent variable: REDUCT

Source	df	SS (H)	MSS	F	P
Between Subjects	13	4.1086			
(CONC)	5	3.4986	0.6997	9.177	0.0036
Subj w Groups	8	0.6100	0.0762		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.950	6	0.650
2	1.750		
3	1.350		
4	1.200		
5	0.750		

Comparison	Bon-ferroni	Dunnett
1 > 2		
1 > 3		
NOEC 1 > 4		
1 > 5	0.0162	0.0100 *
1 > 6	0.0099	0.0100 *
2 > 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
2 > 6		N.A.
3 > 4		N.A.
3 > 5		N.A.
3 > 6		N.A.
4 > 5		N.A.
4 > 6		N.A.
5 > 6		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).