DATA EVALUATION RECORD

12/2/1993

1. **CHEMICAL:** Oxyfluorfen.
   Shaughnessey number: 111601.

2. **TEST MATERIAL:** RH 2915 technical; Lot #7364; 74% active ingredient; a brown solid.

3. **STUDY TYPE:** Mollusc 48-Hour Embryo Larvae Study. Species tested: Eastern Oyster (Crassostrea virginica).
   72-3(b) Acute Est. | Marine Mollusk

4. **CITATION:** Vilkas, A.G. 1977. Acute Toxicity of RH 2915 Technical, Lot #7364 (74% Active Ingredient) To The Eastern Oyster (Crassostrea virginica). UCES Project Number 11506-33-02. Prepared by Union Carbide Environmental Services, Union Carbide Corporation, Tarrytown, New York. Submitted by Rohm and Haas Company, Research Laboratories, Spring House, Pennsylvania. EPA MRID Number 3097011-09.

5. **REVIEWED BY:**

   Jeffrey V. Wheat
   Aquatic Toxicologist
   Toxikon Environmental Sciences

   Signature: Jeffrey V. Wheat
   Date: 4/24/91

6. **APPROVED BY:**

   Pim Kosalwat, Ph.D.
   Senior Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: P. Kosalwat
   Date: 5/24/91

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

   John Notes 5/19/92

   Signature: Henry T. Craven
   Date: 12/2/93

7. **CONCLUSIONS:** This study is not scientifically valid and does not fulfill the guideline requirements for a mollusc 48-hour embryo larvae test in that the concentrations tested were not high enough to derive a precise EC_{50} and mortality was not used as an endpoint. The 48-hour EC_{50} based upon abnormal development and calculated using nominal concentrations of RH 2915 technical to the Eastern Oyster (Crassostrea virginica) was 53.6 µg/L which classifies this compound as highly toxic. The no-observed-effect concentration (NOEC) was 10 µg/L.

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

6 hrs

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Adult Eastern Oysters (Crassostrea virginica) used in this test were obtained from a commercial oyster hatchery on Long Island, New York. The eggs were spawned and handled as described by Loosanoff and Davis (1963). No other acclimation details were provided.

B. **Test System:** The test was conducted in 1-liter (L) Pyrex beakers containing 1 L of natural seawater (22 $^{o}/_{oo}$ salinity). Zero-hour measured control water parameters of this dilution water had a dissolved oxygen (DO) concentration of 7.2 mg/L and a pH of 8.26. The test was conducted in a temperature controlled incubator which maintained temperature at 24 $\pm$1°C.

C. **Dosage:** Forty-eight-hour static test. Five nominal concentrations of the test substance were tested: 3.2, 5.6, 10, 18, and 32 µg/L. A stock solution of RH 2915 technical was prepared at 0.1 mg/mL (100 mg/L). A solvent control was also maintained at a concentration of 320 µL/L acetone which was the amount present in the highest test concentration. All treatments containing test compound were dosed after addition of the fertilized eggs.

D. **Design:** Five concentrations, a solvent control, and a control were selected for the study. Treatments were duplicated. One liter of dilution water containing approximately 50 fertilized eggs per mL was added to each beaker and then dosed. At 48 hours, the contents of each vessel was filtered through a 37-µm sieve into a 100-mL beaker. The larvae were then subsampled and 100 embryos from each duplicate were observed under 60X magnification for abnormal development. The DO and pH were measured in both controls and the low, middle and high test concentrations at initiation and termination. Salinity was measured in the dilution water at test initiation. Temperature was reported to have been maintained at 24 $\pm$1°C. No confirmation of test concentrations was performed.

E. **Statistics:** The 48-hour EC_{50} value and associated 95% confidence limits were produced by extrapolation and then the method described by Litchfield and Wilcoxon

(1949). Only abnormal development was used as the endpoint.

12. **REPORTED RESULTS:** Results of the test are presented in Table 1 (attached).

The reported 48-hour EC_{50}, based upon abnormal development and using nominal concentrations, was extrapolated to be 95.0 µg/L. An NOEC was not reported.

During the course of the study, DO concentration ranged from 6.9 mg/L to 7.3 mg/L (94% - 100% of saturation). The pH ranged from 7.74 to 8.26. Temperature in the incubator was 24 ±1°C.

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

No conclusions were made by the authors.

A GLP compliance statement was not included in the report and no indication was given that the study was audited by a quality assurance unit.

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

A. **Test Procedure:** Test procedures deviated significantly from the SEP as follows:

- The SEP states that the embryos should be tested within one hour of spawning and after fertilization. The report states that the test was initiated within four hours of fertilization.
- The SEP recommends a salinity range of 10 to 17 $^{0}/_{00}$ and a pH of 7.7 to 8.0 for estuarine species of which the eastern oyster is a member. Salinity for this study was 22 $^{0}/_{00}$ and the pH was 8.26.
- The SEP states that each designated treatment group should be exposed to a concentration of toxicant that is at least 60% of the next highest concentration. Each designated treatment group for this test was only 56% of the next highest concentration.
- The SEP advocates the use of a 16-hour light : 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark cycles of the photoperiod. The report does not state whether any photoperiod was maintained.

- The SEP states that the study should be initiated with 20,000 to 30,000 fertilized eggs per liter. This study was initiated with 50,000 fertilized eggs per liter.

- The SEP states that if the test is not conducted in a water bath, then the temperature should be measured in one control replicate hourly during the study. The report does not state that this was done.

- The SEP states that there should be at a minimum of four control replicates for this study. Only two were used.

- The SEP states that mortality should be used in association with abnormal development to assess the toxicity of a compound. For this study, only abnormal development was used as an endpoint.

- It was not clearly stated in the report if the test concentrations were reported as active ingredient or whole material.

B. **Statistical Analysis:** The reviewer used the EPA's Probit Analysis computer program to calculate the 48-hour EC_{50}. The NOEC was calculated by ANOVA and the treatment means were compared using Dunnett's test (attached).

C. **Discussion/Results:** The study results do not meet the guideline requirements for a 48-hour mollusc embryo larvae study. Based upon the results as presented, the 48-hour EC_{50} based upon nominal concentrations of RH 2915 technical was 53.6 µg/L with 95% confidence limits of 43.1 and 86.5 µg/L. The NOEC was calculated to be 10 µg/L.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The test procedures deviated significantly from the guidelines. The concentrations tested were not high enough to derive a precise EC_{50} value and mortality was not utilized as an endpoint.

(3) **Repairability:** None.

15. **COMPLETION OF ONE-LINER:** Yes, April 24, 1991.

AUTHOR REFERENCES:

Davis, H. C. and V. L. Loosanoff. 1963. Rearing of bivalve mollusks. In: F. S. Russel (ed.). Advances in Marine Biology. Academic Press, Inc. London, 1:1-136.

Litchfield, J. T. and G. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharm. Exp. Ther. 96: 99-113.

RIN 0637-00

EFED Review - Oxyfluorfen

Page 6 is not included in this copy.

Pages _____ through _____ are not included.

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.

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

Oxyflourfen Oyster Embryo Larvae Study

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	200	1	0.0050	0.0000	0.0124
3.2000	200	2	0.0100	-.0024	0.0000
5.6000	200	5	0.0250	0.0128	0.0004
10.0000	200	3	0.0150	0.0026	0.0061
18.0000	200	13	0.0650	0.0533	0.0517
32.0000	200	46	0.2300	0.2203	0.2208

Chi - Square Heterogeneity = 2.603

Mu = 1.729084
Sigma = 0.290965

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	-0.942577	1.017114	(-2.936121,	1.050967)
Slope	3.436835	0.710562	(2.044134,	4.829536)
Spontaneous Response Rate	0.012408	0.004437	(0.003712,	0.021104)

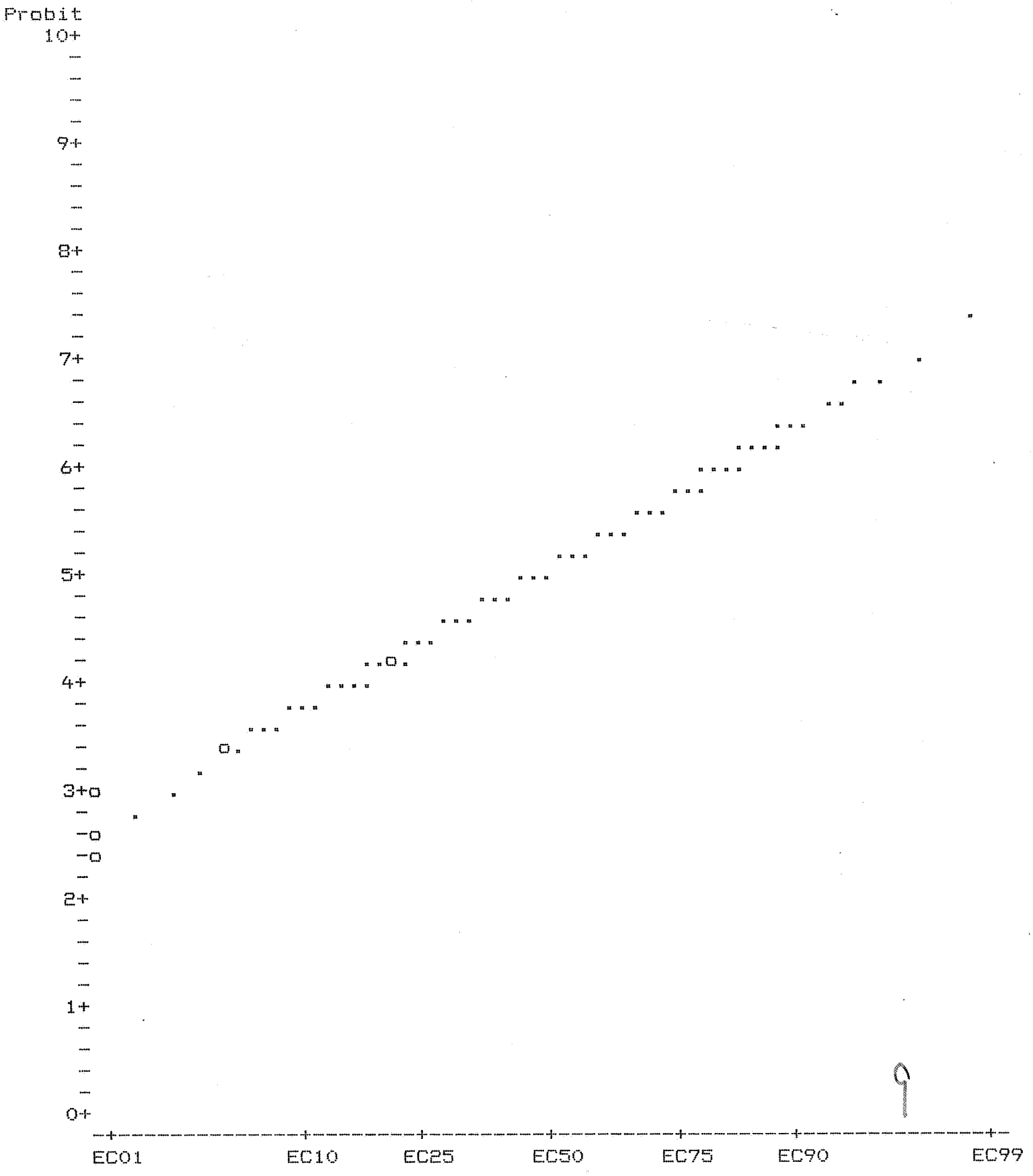
Oxyflourfen Oyster Embryo Larvae Study

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	11.2775	6.0342	14.8520
EC 5.00	17.8023	12.7791	20.9094
EC10.00	22.7082	18.6829	25.6064
EC15.00	26.7625	23.4188	30.2658
EC50.00	53.5900	43.1449	86.5359
EC85.00	107.3100	71.5725	274.7809
EC90.00	126.4693	80.5525	361.7245
EC95.00	161.3217	95.9286	543.8279
EC99.00	254.6577	133.0063	1169.4238

Oxyflourfen Oyster Embryo Larvae Study

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE



Oxyflourfen Oyster Embryo Larvae test

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	2	.5000	.7071	141.4
2	2	1.0000	1.4142	141.4
3	2	2.5000	2.1213	84.9
4	2	1.5000	2.1213	141.4
5*	2	6.5000	2.1213	32.6
6*	2	23.0000	1.4142	6.1

*) the mean for this group is significantly greater than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = 4.901704
This difference corresponds to 980.34 percent of control

Between groups sum of squares = 753.666667 with 5 degrees of freedom.

Error mean square = 3.000000 with 6 degrees of freedom.

Bartlett's test p-value for equality of variances = .963

ONE LINER

Shaughnessey Number	Chemical Name	Chemical Class	Chemical % A.I.	Accession Number
111601	Oxyfluorfen		74%	3097011-09

Study Type : 48-Hr. EC50

Test Species: Crassostrea virginica

Lab: Union Carbide Envir. Serv.

95% Confid. Limits

LC50 = 53.6 PP B (43.1 to 86.5)

Slope = 3.44 # Animals/Level = 50,000

Control Mortality: 0.5 %

Test Temperature: 24°C

Dose Level PP B / (% [illegible] Effect

3.2 (1)	5.6 (2.5)	10 (4.5)	18 (6.5)	32 (23)	( )

Comments: Concentrations based upon (mean) nominal / measured concentrations. (Circle one)

Reviewer: JW | Date: 4-24-91 | Validation Status: Invalid

Study Types : 48-Hour LC50
96-hour LC50

Comments should indicated whether test concentrations were based upon Nominal or Measured concentrations.

Validation Status codes to be used above: CORE, SUPPLEMENTAL, INVALID.

Accession Number = MRID Number.