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12/24/90 128850

MRID No. 413961-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Glufosinate.
Shaughnessey No. 128850.

2. **TEST MATERIAL:** HOE 039866 Technical; Code #HOE 039866 OH ZC96 0002; 96.3% Active Ingredient; a liquid.

3. **STUDY TYPE:** Mollusc 48-hour Embryo-Larval Study.
Species Tested: Eastern Oyster (Crassostrea virginica).

4. **CITATION:** Ward, G.S. 1989. Acute Toxicity of HOE 039866 Technical Substance (Code: HOE 039866 OH ZC96 0002) to Embryos and Larvae of the Eastern Oyster (Crassostrea virginica). Prepared by Hunter/ESE, Gainesville, Florida. ESE Project No. 87341-0200-2130. Submitted by Hoechst Celanese Corporation, Somerville, New Jersey. MRID No. 413961-05.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Kimberly D. Rhodes
Date: June 1, 1990

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
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Signature: P. Kosalwat
Date: 6/1/90

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature: A.C. Petrus
Date: 12/20/90

M. Reynolds 12/24/90

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for an oyster embryo-larval test. The 48-hour EC50 based upon nominal concentrations of HOE 039866 to Eastern oysters (Crassostrea virginica) was 8.0 mg/L. Therefore, HOE 039866 is classified as moderately toxic to Eastern oysters. The NOEC was determined to be 4.9 mg/L after 48 hours of exposure.

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

1

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Adult Eastern oyster (Crassostrea virginica) were obtained from the Horn Point Environmental Laboratory of the University of Maryland. The oysters were maintained in natural seawater at a salinity of 20 parts per thousand (ppt) and a temperature of 20 to 22°C for 3 days prior to spawning.

Individual, sexually mature female oysters were induced to spawn by placing them in 1.6-liter (L) glass chambers containing 1 L of dilution water at approximately 23°C and increasing the water temperature to approximately 30°C in the presence of viable sperm stripped and/or released from a sexually mature male oyster. Fertilization occurred upon release of the eggs into the spawning chambers and was confirmed microscopically.

B. **Test System:** The test was performed in 1-L glass beakers containing 0.9 L of test solution. All test concentrations and the controls were triplicated. The test containers were maintained at 22 ± 1°C under fluorescent lighting on a photoperiod of 16 hours of light and 8 hours of darkness.

The dilution water was filtered natural seawater collected from the Atlantic Ocean near Marineland, Florida, and diluted to a salinity of approximately 19 ppt with well water. The water was filtered through a 0.45 µm filter membrane prior to addition to the test containers. The dilution water control was characterized as having a dissolved oxygen concentration of 7.4, a pH of 7.9, and a salinity of 19 ppt.

C. **Dosage:** Mollusc 48-hour embryo-larval static test.

D. **Design:** Based on the results of a range-finding test, a control and seven nominal HOE 039866 concentrations of 4.9, 8.1, 13.5, 22.8, 37.4, 62.3, and 104 mg/L were chosen for testing. The nominal concentrations were based on whole material. Each test container was inoculated with an estimated 27,000 embryos within 1 hour of fertilization. Initial embryo density was

determined by Sedgewick-Rafter counts of three 10-mL subsamples removed from the triplicate control flasks. Counts of embryos added to test containers at test initiation indicated the initial inoculum was actually only 20,370 on the average.

After 48 hours of exposure, 10-mL samples were collected by automatic pipet while agitating with a perforated plunger. The samples containing the larvae were preserved with 0.4 mL of buffered formalin. The number of normally developed 48-hour larvae was determined by a Sedgewick-Rafter counter from each triplicate test and control container.

The dissolved oxygen concentration and pH were measured and recorded at 0 and 48 hours in all replicates for all test concentrations and the control. The salinity was measured in one seawater control test container at test initiation. The temperature was measured and recorded hourly by a computerized temperature data logger.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal oyster larvae from each test concentration when compared to the control. The percentage reduction of normal 48-hour embryos was determined as follows:

$$\% \text{ Reduction} = \frac{\text{Mean number of normal larvae in each test concentration} - \text{Mean number of normal control larvae}}{\text{Mean number of normal control larvae}} \times 100$$

The numbers of normally developed larvae in the control were compared to the numbers of normally developed larvae in the test substance treatments to determine if exposure to any test concentration reduced the number of embryos developing normally. One-way analysis of variance (ANOVA) was conducted to determine if there was a significant difference among treatments. Dunnett's multiple comparison test was used to identify those test concentrations producing effects different from the control at a confidence level of 95 percent.

12. **REPORTED RESULTS:** Table 3-1 (attached) shows the number of normally developed larvae after 48 hours of exposure to HOE 039866 Technical and percentage reduction as compared to the control. HOE 039866 was acutely toxic to embryos and larvae at nominal test concentrations $\geq$8.1 mg/L. The percentage reduction of normal larvae, as compared to the control,

after 48 hours of exposure ranged form 66 percent in 8.1 mg/L to 100 percent in 104 mg/L. A 20 percent increase in the number of normal larvae was observed in the lowest test concentration. The 48-hour EC50 was 7.2 mg/L. The no-observed-effect concentration (NOEC) was 4.9 mg/L.

Test salinity was 19 ppt. The mean temperature was 22°C with a standard deviation of 1°C; temperature ranged from 20 to 24°C. The dissolved oxygen concentrations remained ≥6.0 mg/L (≥79% of saturation) in all test solutions throughout the test. The pH of all test solutions remained between 7.8 and 8.1 during the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA 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 The SEP states that natural or reconstituted seawater of 10 to 17 ppt salinity should be used when testing estuarine (euryhaline) mollusc species. The salinity of the seawater used in this toxicity test was 19 ppt.

o The SEP recommends a temperature of 20°C and states that the temperature should not vary more than 2°C during the test. During this toxicity test, the temperature ranged from 20 to 24°C.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether 15- to 30-minute transition periods between light and dark were maintained.

o There is a discrepancy in the report involving the percent saturation of the dissolved oxygen concentration. The author determined the percent saturation for the lowest dissolved oxygen concentration (6.0 mg/L) to be 79 percent. However, the reviewer determined the percent saturation for the

lowest dissolved oxygen concentration to be 70 percent at 19 ppt and 22°C.

B. **Statistical Analysis:** The reviewer recalculated the EC50 value using EMSL regression analysis. These calculations are attached. To determine the EC50 value, the log of concentration (X-axis) was plotted against percent reduction (Y-axis) expressed as probits. The lowest test concentration (4.9 mg/L) was not used for analysis since there were higher number of normal embryos than the control, indicating stimulation instead of inhibition. The regression equation formulated is as follows:

$$Y = 3.16711 + 2.030449\ X$$

where: X = Log concentration,
Y = 5 (50% reduction converted into probit value).

Therefore, X = 1.83289/2.030449
EC50 = 8.0 mg/L

Based on nominal concentrations of HOE 039866, regression analysis provides a 48-hour EC50 value of 8.0 mg/L, with a coefficient of correlation equal to 0.84. This regression analysis is similar to that reported by the author (i.e., 7.2 mg/L). The 48-hour NOEC was determined to be 4.9 mg/L based on nominal concentration.

C. **Discussion/Results:** The study results appear to be scientifically valid. The EC50, based on percentage reduction of normal oyster larvae after 48-hour of exposure to HOE 039866, was 8.0 mg/L nominal concentration. Therefore, HOE 039866 is classified as moderately toxic to Eastern oysters (Crassostrea virginica). The no-observed effect level (NOEL) was determined to be 4.9 mg/L nominal concentration.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 05-16-90.

Shaughnessey No. 128850 Chemical Name Glufosinate (HOE-039866 Technical) Chemical Class ____ Page ____ of ____

Study/Species/Lab/Accession	Chemical % a.i.	Results	Reviewer/Date	Valid/Stat
14-Day Single Dose Oral LD_{50}		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 LD_{50}		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 LC_{50}		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 LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
48-Hour EC_{50}		EC50 = 8.0 ppm (95% C.L. N/A) * Regression analysis Reduction Contr. Mort.(%)= N/A Sol. Contr. Mort.(%)= N/A		
Species Crassostrea virginica		Slope= N/A # Animals/Level= 20,370 Temperature = 20-24°C 5/16/90 Cone		
Lab Hunter/ESE	96.3%	48-Hour Dose Level ppt /(%Mortality) Reduction 4.9(+20), 8.1(-66), 13.5(-71), 22.8(-73), 37.4(-84) 62.3(-88) 104(-100)		
Acc. 413961-05		Comments: Based on nominal concentrations.		
96-Hour LC_{50}		LC50 = ppm (95% C.L.) Con. Mor(%)= Sol. Con. Mor.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level ppt /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = ppm (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level ppt /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		

HOE-039866
Oyster Embryo Larvae Toxicity Test

REGRESSION EQUATION:
Y= 3.16711 + 2.030449 X

COEFFICIENT OF CORRELATION= .8410612

PRESS ENTER TO CONTINUE.?

ACTUAL VERSUS ESTIMATED VALUES
X=conc (log) Y=% reduction (probit)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	.91	5.41	5.014819	.3951812
2	1.13	5.55	5.461517	8.848286E-02
3	1.36	5.61	5.92852	-.3185201
4	1.57	5.99	6.354915	-.3649149
5	1.79	6.18	6.801613	-.6216135
6	2.02	8.09	7.268617	.8213835

Regression Equation:

$Y = 3.16711 + 2.030449\ X_{log}$

$5.0 = 3.16711 + 2.030449\ X_{log}$

$$\frac{5.0 - 3.16711}{2.030449} = X_{log}$$

$0.9027 = X_{log}$

$7.99 = X$

EC50 = 8.0

Table 3-1. Number of Normally Developed Larvae After 48 Hours of Exposure to HOE 039866 Technical and Percentage Reduction as Compared to the Control

Nominal Concentrations (mg/L; ppm)	Mean Number of Normal Larvae		Percentage Reduction of Normal 48-Hour Larvae (%)
	Mean	SD*	
Control	16,650	1,838	---
4.9	20,131	5,324	+20
8.1	5,730	3,247	-66**
13.5	4,830	3,479	-71**
22.8	4,500	1,652	-73**
37.4	2,700	1,018	-84**
62.3	2,040	1,140	-88**
104.0	0	0	-100**

*SD = Standard deviation.
**Statistically different ($P \geq 0.95$) than the control.

Source: ESE, 1987.