2-6-91

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DATA EVALUATION RECORD

1. CHEMICAL: Octhilinone

2. TEST MATERIAL: Octhilinone technical, 98.5% active ingredient, yellow liquid.

3. STUDY TYPE: Mollusc 96-Hour Flow-Through Shell Deposition Study

4. CITATION: Dionne, Emily. 1990. (Octhilinone) - Acute Toxicity To Eastern Oysters (Crassostrea virginica) Under Flow-Through Conditions. Performing Laboratory; Springborn Laboratories, Inc. Wareham, MA. Study Sponsor; Rohm and Hass Company, Springhouse, PA. Accession # 417007- 01.

5. REVIEWED BY:

Greg Susanke, Biologist
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Greg Susanke 2/4/91

6. APPROVED BY:

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7. CONCLUSION: This study appears scientifically sound and fulfills the guideline requirement 72-3 for an estuarine/marine acute toxicity test with eastern oysters. The LC50 was not determined as no mortalities occurred at the concentrations tested. The EC50 for shell deposition is 0.013 ppm, and the 95% C.I. is 0.0064 - 0.025 ppm. The NOEC is < 0.003 ppm, the lowest concentration tested. Octhilinone is considered to be very highly toxic to mollusks.

8. MATERIALS AND METHODS:

A. Test Organisms:

Species- Eastern Oyster (Crassostrea virginica)

Supplier- Aquacultural Research Corporation, Dennis, MA.

Mean weight- not measured

Mean length- mean valve height 39 ± 4mm

Age- < 1 year, not sexually mature

Acclimation period- Test organisms were held for nine days at a temperature of 20 - 22° C, a salinity of 31 - 32 °/oo, a pH of 7.7 - 8.0, a DO concentration of 78 - 92% saturation. There was no mortality during this period. Oysters were fed a supplementary algal diet of Isochrysis galbana, clone T-ISO, and Tetraselmis maculata at 180 ml/aquarium/day.

Shell Preparation- 3 - 5 mm of new peripheral shell growth was removed from each oyster with the use of a fine-grit grinding wheel, 24 hours prior to test initiation. Immediately prior to test initiation, the outer shell edge was buffed with a metal file to remove any new shell deposition.

B. Test System:

Source of dilution water- Cape Cod Canal, Bourne, MA. Water quality was biologically monitored through the maintenance of continuous cultures of mysid shrimp.

Water temperature- 19 - 22 °C

pH- 7.4 - 8.0

Dissolved oxygen- 6.0 - 7.6 mg/L (82% - 100% saturation)

Salinity- 30 - 32 ppth

Total organic carbon- 2.7 mg/L (July sample)

Test aquaria- 60 x 30 x 30 glass aquaria containing 18 L of test solution or control seawater

Type of dilution system- serial diluter with a 50% dilution factor

Flow rate- 75 ml/minute or 6 volume additions per 24 hours

Biomass loading rate- not provided

Photoperiod- 16 hours light and 8 hours dark, fluorescent lighting was used and sudden transitions were avoided

C. Test Design:

Range finding test- Preliminary testing was conducted at nominal concentrations of .060, .024, .0096, .0038, and .0015 ppm. There were no mortalities but there was 33%, 30%, 7%, 15%, and 0% reduction in shell deposition, respectively.

Definitive test

Nominal concentrations- 0.1, 0.05, 0.025, 0.013, and .0063 ppm, each concentration is 50% of next higher level

Controls- seawater control and solvent control (30 ppm triethylene glycol)

Number of test organisms- 20 per replicate (40 per each treatment level and each control)

Biological observations- Observations for mortality and toxic effects were made at 24 hour intervals.

Water parameter measurements- Temperature, salinity, pH, and DO were measured in each treatment level and control at test initiation and at subsequent 24 hour intervals. Temperature was continuously monitored in one replicate of the control.

9. REPORTED RESULTS:

Mean measured concentrations- 0.057, 0.024, 0.011, 0.0062, and 0.003 ppm. Water samples were collected from both replicates of each treatment level and the controls on test days 0 and 4. The mean measured concentrations are 58%, 49%, 44%, 48% and 47% of the nominal concentrations, respectively.

Recovery of chemical- 97.2% $\pm$ 5% of Octhilinone was recovered from filtered seawater as determined by QC samples.

Mortality and observations- There were no mortalities in any treatment level or either control. Toxic effects such as reduced feeding and fecal production were observed at 0.057 ppm.

There was no difference in shell deposition between control groups. The solvent control group and water control group had a mean shell depositions of 2.0 mm and 1.9 mm, respectively. From high to low treatment concentrations, shell deposition was reduced by 79%, 68%, 42%, 37%, and 21%, respectively.

10. STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

The EC50 for Octhilinone to eastern oysters was estimated to be .013 ppm and the 95% C.I. is 0.0064 - 0.025 ppm. The NOEC is < 0.003 ppm.

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 set forth in 40 CFR Part 160.

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

A. Test Procedure: Test procedures were generally in accordance with protocols recommended by the Guidelines. The protocol deviations are listed below:

- Test solution temperature was not consistent as it ranged from 19 - 22° C.

- Each treatment groups concentration was only 50% of the next higher level. The recommended interval is 60%.

- The solvent control only used 81% (30 ppm) of the amount present in the highest treatment level (37 ppm). The amount used in the solvent control should be the same as that used in the treatment level. The amount of solvent used did not exceed the limit of 500 ppm.

- Minimal mean shell deposition in an acceptable control group is 2 mm. It should be noted that in this study the growth exhibited by the oysters was minimally acceptable (2.0 mm solvent control, 1.9 mm water control).

B. Statistical Analysis: The EC50 value was calculated by EEB's Toxanal Computer Program which used the Probit Method.

C. Discussion/Results: The study results appear to be scientifically valid. There were no mortalities from octhilinone up to 0.057 ppm, the highest concentration tested. The EC50 for shell deposition to Eastern oysters is .013 ppm, the 95% C.I. is 0.0064 - 0.025 ppm. The NOEC is < 0.003 ppm, the lowest concentration tested. Octhilinone is considered to be very highly to mollusks.

D. Adequacy of the Study: Classification - Core

Greg Susanke octhilinone EC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
57	40	32	80	0
24	40	27	67.5	0
11	40	17	42.5	0
6.2	40	15	37.5	0
3	40	8	20	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 13.86274 mg/l

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.127517	12.43378	8.800548	18.30455

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.1089968	1	.8768896

SLOPE = 1.320328

95 PERCENT CONFIDENCE LIMITS = .8844261 AND 1.75623

LC50 = 12.5008 mg/l

95 PERCENT CONFIDENCE LIMITS = 8.920352 AND 17.65131

LC10 = 1.364757

95 PERCENT CONFIDENCE LIMITS = .4341668 AND 2.514218

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