DATA EVALUATION RECORD

1. Chemical: Lindane

2. Test Material: Technical, 99.5%

3. Study/Action Type: Oyster Embryolarvae Acute Toxicity (Crassostrea virginica)

4. Study Identification: Acute Toxicity of Lindane to Embryos and Larvae of the Eastern Oyster (Crassostrea virginica). Prepared by Environmental Science and Engineering, Inc., Gainesville, Florida. May 19, 1986. Submitted by Rhone-Poulenc, Inc. for CIEL. EPA Accession No. 264036.

5. Reviewed by: Ann Stavola
Aquatic Biologist
EEB/HED
Signature: Ann Stavola
Date: 9 Dec 86

6. Approved by: Doug Urban
Supervisory Biologist
EEB/HED
Signature: Douglas J. Urban
Date: 12/31/86

7. Conclusions:

The study is scientifically sound but does not meet our Guidelines requirements for an oyster embryolarvae test. With an EC_{50} of 2.8 (2.6-3.0) mg/L, Technical Lindane is moderately toxic to oysters.

8. Recommendations:

The raw data for the larvae counts for each replicate beaker are needed to verify the statistical analysis.

9. Background:

The study was submitted in response to the data requirements of the Lindane Registration Standard.

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10. Materials and Methods:

a. Test Animals: Eastern oyster (Crassostrea virginica) embryolarvae. The embryos were obtained by induced spawning of sexually-mature oysters.

b. Dosage: Technical Lindane, 99.5% ai. The stock solution was prepared by adding 6.0136 g of Technical Lindane to acetone in a 50 mL volumetric flask. The dilution water was filtered natural seawater collected from Marineland, Florida, diluted to a salinity of 20 ppt with deionized water and refiltered using 0.45 um membrane filter.

c. Study Design: The test was conducted in glass beakers containing 900 mL of test solution. There were three beakers per control and test concentration. The nominal concentrations were 1.0, 1.7, 2.9, 4.8, and 8.0 mg/L. There were also solvent and seawater controls. The concentrations were analyzed according to EPA's Method Number 608 (40 CFR Part 136, October 1984). The measured concentrations were 1.06, 1.60, 2.56, 3.68, and 5.24 mg/L. Each beaker was inoculated with 26,700 embryos within 1 hour after fertilization and they were kept at 22 °C with a photoperiod of 14L:10D. After a 48-hour exposure period, 10 mL samples of larvae were collected from each beaker, preserved with buffer formalin and counted with a Sedgewick-Rafter slide.

d. Statistics:

$$\text{Percent reduction of normal larvae} = \frac{\text{Mean no. of normal larvae per conc.} - \text{Mean no. of normal larvae per solvent control}}{\text{Mean no. of normal larvae per solvent control}} \times 100$$

The EC_{50} and 95% C.I. were calculated by the computer program of Stephans. The One-Way Analysis of Variance and Williams Multiple Comparison test were used to identify those concentrations producing effects greater than the solvent control at a confidence level of 95%.

11. Report Results:

Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Mean No. of Normal Larvae Mean	SD	Reduction of Normal 48-hr Larvae (%)
Control	-	24,480	1514	-
Solvent Control	-	24,450	3273	-
1.0	1.06	19,740	406	-19
1.7	1.60	19,080	4445	-22
2.9	2.56	17,700	6954	-28
4.8	3.68	11,340	8574	-54
8.0	5.24	630	868	-97

48-hour EC_{50} and 95% C.I. = 2.8 (2.6-3.0) mg/L. There was a statistically significant reduction in normally developed larvae compared to the solvent control at the 3.68 and 5.24 mg/L concentrations.

Temperature ranged from 17 °C to 24 °C with most of the variation occurring on day 1 during a 2-hour period due to a system malfunction. The remainder of the time the temperature stayed within the range of 21 °C to 22 °C, and pH values were 8.0 at 0 hour and 8.1 at 48 hours. D.O. was 8.9 mg/L at 0 hour and 7.3 mg/L at 48 hour.

12. Study Author's Conclusions/QA Measures:

The 48-hour EC_{50} of Technical Lindane 99.5% to oyster embryolarvae was 2.8 (2.6-3.0) mg/L.

13. Reviewer's Evaluation:

a. Test Procedures: The test procedures generally follow the protocol recommended by ASTM, vol. 11.04, 1986.

b. Statistics: The method used to calculate percent reduction of normal larvae is acceptable. The EC_{50} was calculated with EEB's Toxanal program which is based on Stephan's program. The EC_{50} was computed to be 2.82 (2.76-2.90) mg/L. Because the raw data for each treatment replicate were not submitted the statistical significance of the reduction of normal larvae could not verified.

c. Discussion/Results: With an EC_{50} value of 2.8 (2.6-3.0) mg/L Technical Lindane is moderately toxic to oyster embryo-larvae. The raw data are needed to verify which test concentrations cause statistically significant reductions in normal larvae as compared to the controls.

d. Conclusions:

1. Category: Supplemental.

2. Rationale: Failure to include raw data on larvae counts for each replication beaker.

3. Repairability: If the raw data on the larvae counts are submitted and are determined to be valid, the study can be upgraded.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
5.24	1000	970	97	0
3.68	1000	540	54	0
2.56	1000	280	28	0
1.6	1000	220	22	0
1.06	1000	190	19	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 3.48565

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	2.154714E-03	2.826887	2.756616	2.900785

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.193057	148.908	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.076714
95 PERCENT CONFIDENCE LIMITS =-.2838934 AND 6.437322

LC50 = 2.778581
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.074101
95 PERCENT CONFIDENCE LIMITS = 0 AND 2.020506

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
5.24	100	97	97	0
3.68	100	54	54	0
2.56	100	28	28	0
1.6	100	22	22	0
1.06	100	19	19	0

THE BINOMIAL TEST SHOWS THAT 2.56 AND 3.68 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 3.485549

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS		
4	2.213963E-02		2.832452	2.616485	3.086947

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.193049	14.89072	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.076718

95 PERCENT CONFIDENCE LIMITS =-.2838819 AND 6.437318

LC50 = 2.778581

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.074102

95 PERCENT CONFIDENCE LIMITS = 0 AND 2.020505

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