079401

DATA EVALUATION RECORD

MRID 128636

1. CHEMICAL: Endosulfan

2. FORMULATION: Technical (95.9%) 72-3

3. CITATION: Boeri, R.H. and T. J. Ward. 1983. Acute toxicity of endosulfan to embryos of the eastern oyster (Crassostrea virginica) and the fiddler crab (Uca pugnax). Final Report. Submitted by American Hoechst Corp., Somerville, N.J.; perpared by Energy Resources Co., Inc., Cambridge, Mass. Reg. No. 8340-13. Acc. No. 250395.

4. REVIEWED BY: John J. Bascietto
Wildlife Biologist
EEB/HED

5. DATE REVIEWED: 7/11/83

6. TEST TYPE: Marine/Estuarine Organism - Acute Toxicity

7. REPORTED RESULTS: ("Nominal" values)

Eastern oyster 48-hr. EC_{50} = 0.45 ug/l (0.35-0.57 ug/l)
(Crassostrea virginica). NOEL = 0.10 ug/l

Fiddler Crab
(Uca pugnax). 96-hr. LC_{50} = 789.5 ug/l
NOEL = 100 ug/l

8. REVIEWER'S CONCLUSIONS: The study is scientifically sound. With an oyster embryo EC_{50} = 0.45 (0.35-0.57) ug/l and a fiddler crab LC_{50} = 789.5 ug/l, endosulfan is "very highly toxic" to these marine/estuarine species. The NOEL's were 0.1 ug/l for oyster and 100 ug/l for crabs. These results indicate that endosulfan can have a demonstrable effect at very low levels. The study partially fulfills the requirements for acute toxicity tests of marine/estuarine organisms (mollusc and crab) with technical endosulfan.

9. Materials/Methods

A. Procedures

Eastern Oyster Study - acute static bioassay procedures were based on: Am. Public Health Assoc. 1981. Standard Methods for the Examination of Water and Wastewater, 15th ed. Sec 809.

Embryos of oyster were obtained from laboratory stock cultures, in turn obtained by using adults from National Marine Fisheries Services Lab. Spawning was induced by elevation of H_2O temperature and addition of oyster sperm suspension. All embryos were combined and diluted to yield 1000 larvae per ml of seawater.

Exposure to endosulfan occurred under unaerated, static conditions in a 2-liter glass beaker containing 1 liter of solution. Temp. = 20 ± 0.5°C with a 14-hr light/10 hr dark photoperiod. 25,000 embryos per liter. Definitive test was conducted at nine (9) concentrations.

a) 0.0 ug/l (control)
b) 0.0 " + .1 ml/l acetone (solvent control)
c) 0.06 " endosulfan
d) 0.10 " "
e) 0.25 " "
f) 0.40 " "
g) 0.60 " "
h) 1.0 " "
i) 2.5 " "

After 48 hrs. oyster suspensions were concentrated 10X and subsamples were preserved in 10-ml vials in 3% formalin. Number of "normal" (fully shelled) and "abnormal" (not fully shelled) embryos were determined in 3 replicate samples using a Sedgwick-Rafter counting cell (microscopic). DO and pH were measured at 24-hr intervals; salinity was measured at 0 and 48 hrs; temperature of the environmental chamber used to house the experiment was continuously monitored.

Synthetic seawater used in the oyster and the fiddler crab study (see below) was "Instant Oceans" diluted with deionized water to a salinity of 30 ppt and had the following characteristics:

total suspended mater = 3-31 mg/l
total organic carbon = 1-13 mg/l
chem. 0_2 demand = 3-4.1 mg/l

Ar	<10 ug/l
Cd	<20 ug/l
Cr	<50 ug/l
Cu	<50 ug/l
Pb	<20 ug/l
Hg	<20 ug/l
Ni	<100 ug/l
Ag	<20 ug/l
Zn	<20 ug/l

Water was aerated prior to use.

Fiddler crab study - (based on ASTM E 729-80. 1980.)

Crabs were commercially obtained from Florida. They were acclimated to synthetic seawater (see above) at 30 ppt salinity and 20°C in the aquatic tox. laboratory for 7 days prior to test. Holding period mortality was 2%. Organisms appeared to be healthy. Crabs used on test were immature with carapace width <10 mm, weighing < 0.1 g.

Exposure of to endosulfan was under static conditions in 2-liter glass culture dishes containing 1 liter of solution. These were housed in an environmental chamber at 22 ± 0.5°C with a 14-hr light/10-hr dark photoperiod. Five (5) crabs were added to each dish; Four (4) replicates per concentration were run.

Solutions tested were:

a) 0.0 ug/l (control)
b) 0.0 " + 0.1 mg/l acetone (solvent control)
c) 100 " endosulfan
d) 250 " "
e) 400 " "
f) 600 " "
g) 1000 " "

Survival was recorded after 0, 2, 4, 24, 48, 72 and 96 hours. DO and pH were recorded at test initiation and at 24 hr intervals. Temperature was continuously monitored.

B. Statistical Analysis

Eastern Oyster Study -
Since control and solvent control data were identical, a determination of acceptability of pooling all control data was not done. One-way ANOVA was used to determine significant differences and Duncan's procedure used to identify significant defferences between control and treatment data.

EC_{50} was calculated by Stephan's computer program using the moving average and probit methods. Percent reduction of oysters exposed to each concentration was calculated by:

$$\% \text{ reduction} = \frac{\% \text{ control normal} - \% \text{ treatment normal}}{\% \text{ control normal}} \times 100$$

Fiddler Crab Study - LC_{50}'s were calculated by the moving average method on Stephan's computer program.

All concentrations and calculations for both studies are expressed as "nominal" concentrations of endosulfan.

10. Results

Oyster Study

Results ranged from 1.1% increase in "normal" oyster embryos to a 91.8% reduction, as compared to controls (see Tables), and was directly related to toxicant concentration.

EC_{50} (based on % reduction) = 0.45 ug/l endosulfan with a 95% confidence interval (c.i.) of 0.35 - 0.57 ug/l, moving average; or 0.37 - 0.51 ug/l, probit method. The NOEL = 0.10 ug/l with an observed effect level of 0.25 ug/l.

Fiddler crab survival measured at 24 and 48 hrs. showed LC_{50} >1000 ug/l; the 96-hr LC_{50} was 789.5 ug/l with a 95% c.i. of 641.7 - 1049.7 - (moving average method). After 2 hrs of exposure to endosulfan crabs were immobilized and rested on dorsal surfaces at 1000 ppb. As the exposure progressed this observation could be made at lower test concentrations. At 4 hrs it was seen at 600 ug/l; at 24 hrs at 400 ug/l; at 72 hrs at 250 ug/l at 96 hours only crabs exposed at 100 ug/l were unaffected.

11. Reviewer's Evaluation

A. Procedures - while no definitive protocols for either the oyster embryo survival or fiddler crab LC_{50} have been disseminated by EPA, the procedures used are sound and should provide meaningful test results.

B. Statistical Analysis - the authors used approved statistical procedures to establish LC_{50}'s (EC_{50}'s). The details of the statistical programs are attached. EEB did not validate the statistics since the authors used the same program (Stephan's) as EEB.

C. Results - the results are valid and show that endosulfan is very highly toxic to aquatic organisms at extremely low levels. The oyster embryo EC_{50} = 0.45 ppb is particularly low. Since the eastern oyster test was performed on embryos, the result may have reproductive implications as significant differences are noted between controls and embryos exposed at > 0.1 ppb. However reproductive impairment must be tested through chronic exposure studies where adults are placed in long-term exposure and reproductive success quantified and compared to controls.

D. Conclusions

1. Category: Core

2. Rationale: Guidelines

3. Repair: N/A

Table A.1.—Biological data from acute static bioassay with embryos of the eastern oyster[a,b,c]

Nominal Concentration of Endosulfan (ug/l)	Replicate	Number of Normal Oysters at 48-hr	Number of Abnormal Oysters at 48-hr	Percent Normal
0.0 (control)	1	100	11	90
	2	100	14	88
	3	100	13	88
	$\bar{x}$			88.7
0.0 (solvent control)[d]	1	100	16	86
	2	100	12	89
	3	100	10	91
	$\bar{x}$			88.7
0.06	1	100	13	88
	2	100	8	93
	3	100	12	89
	$\bar{x}$			90.0
0.10	1	100	32	76
	2	100	11	90
	3	100	16	86
	$\bar{x}$			84.0
0.25	1	100	65	61
	2	100	42	70
	3	100	40	71
	$\bar{x}$			67.3
0.40	1	100	76	57
	2	100	67	60
	3	92	100	48
	$\bar{x}$			55.0
0.60	1	75	100	43
	2	56	100	36
	3	63	100	39
	$\bar{x}$			39.3
1.0	1	40	100	29
	2	46	100	32
	3	65	100	39
	$\bar{x}$			33.3
2.5	1	8	100	7
	2	4	100	4
	3	12	100	11
	$\bar{x}$			7.3

[a]Toxicity test was conducted under unaerated conditions. Testing temperature was 20.0 ± 0.5°C and salinity was 30.0 ± 0.0 ppt throughout the test.

[b]The minimum and maximum pH values of each of the test solutions during the 48-hr test were: 7.9 and 8.0.

[c]The minimum and maximum dissolved oxygen concentrations (mg/l O_2) of test solutions during the 48-hr test were: 0.0 ug/l (control) - 7.5 and 7.8; 0.0 ug/l (solvent control) - 7.4 and 7.8; 0.06 ug/l - 7.5 and 7.8; 0.10 ug/l - 7.4 and 7.8; 0.25 ug/l - 7.4 and 7.8; 0.40 ug/l - 7.3 and 7.8; 0.60 ug/l - 7.4 and 7.8; 1.0 ug/l - 7.5 and 7.8; and 2.5 ug/l - 7.5 and 7.8.

[d]This control contained 0.1 ml/l acetone as a carrier. This concentration exceeds the highest concentration of carrier employed in the test.

Table A.2.—Biological data from acute static bioassay with fiddler crabs[a,b,c]

Nominal concentration of endosulfan (µg/l)	Replicate	Number of Live Crabs						
		0 hr	2 hr	4 hr	24 hr	48 hr	72 hr	96 hr
0.0 (control)	1	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5
	4	5	5	5	5	5	5	5
0.0 (solvent control)[d]	1	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5
	4	5	5	5	5	5	5	5
100	1	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5
	4	5	5	5	5	5	5	5
250	1	5	5	5	5	5	5[f]	5[f]
	2	5	5	5	5	5	5[f]	5[f]
	3	5	5	5	5	5	5[f]	5[f]
	4	5	5	5	5	4	4[f]	4[f]
400	1	5	5	5	5[f]	5[f]	5[f]	4[f]
	2	5	5	5	5[f]	5[f]	5[f]	5[f]
	3	5	5	5	5[f]	5[f]	5[f]	4[f]
	4	5	5	5	5[f]	5[f]	5[f]	5[f]
600	1	5	5	5[f]	5[f]	5[f]	5[f]	5[f]
	2	5	5	5[f]	5[f]	4[f]	3[f]	2[f]
	3	5	5	5[f]	5[f]	5[f]	5[f]	3[f]
	4	5	5	5[f]	5[f]	5[f]	5[f]	5[f]
1000	1	5	5[e]	5[f]	5[f]	5[f]	3[f]	1[f]
	2	5	5	5[f]	4[f]	4[f]	2[f]	1[f]
	3	5	5[e]	5[f]	4[f]	4[f]	2[f]	2[f]
	4	5	5	5[f]	3[f]	3[f]	3[f]	2[f]

[a]Toxicity test was conducted under unaerated conditions. Testing temperature was 22.0 ± 0.5° C and salinity was 30.0 ppt throughout the test.

[b]The minimum and maximum pH values of each of the test solutions during the 96-hr test were: 7.5 and 7.8.

[c]The minimum and maximum dissolved oxygen concentrations (mg/l O_2) of test solutions during the 96-hr test were: 0.0 µg/l (control) - 5.6 and 7.8; 0.0 µg/l (solvent control) - 5.5 and 7.8; 100 µg/l - 6.2 and 7.8; 250 µg/l - 6.1 and 7.8; 400 µg/l - 5.9 and 7.8; 600 µg/l - 5.8 and 7.8; and 1000 µg/l - 5.5 and 7.8.

[d]This control contained 0.1 ml/l acetone as a carrier. This concentration exceeds the highest concentration of carrier employed in the test.

[e]One crab immobilized and resting on dorsal surface.

[f]All crabs immobilized and resting on dorsal surface.

Table 1. Analysis of data from 48-hr acute static bioassay with embryos of the eastern oyster

Step 1. Percent normal oysters at 48 hr (from Appendix A.1)

Treatment (t):	Concentration of endosulfan (µg/l)								
	0.0 (control)	0.0 (solvent control)	0.06	0.10	0.25	0.40	0.60	1.0	2.5
Replicate (r)									
1	90	86	88	76	61	57	43	29	7
2	88	89	93	90	70	60	36	32	4
3	88	91	89	86	71	48	39	39	11
Mean ($\bar{x}$)	88.7	88.7	90.0	84.0	67.3	55.0	39.3	33.3	7.3

Step 2. Cochran's Test for Homogeneity of Control Variances

Treatment (t)	Percent normal oysters: Mean ($\bar{x}$)
0.0 µg/l endosulfan - Control	88.7
0.0 µg/l endosulfan - Solvent control	88.7

------- Further analysis not warranted. ($\bar{x}$ for control equal to $\bar{x}$ for solvent control, therefore, all control data can be pooled.) -------

Table 1. Continued

Step 3. Percent Reduction of Treatment Oysters at 48 hr (compared to pooled controls)

Treatment (t)	Percent Reduction
0.06 ug/l endosulfan	$\frac{88.7 - 89.7}{88.7} \times 100 = 1.1\%$ increase
0.10 ug/l endosulfan	$\frac{88.7 - 84.0}{88.7} \times 100 = 5.3\%$
0.25 ug/l endosulfan	$\frac{88.7 - 67.3}{88.7} \times 100 = 24.1\%$
0.40 ug/l endosulfan	$\frac{88.7 - 55.0}{88.7} \times 100 = 38.0\%$
0.60 ug/l endosulfan	$\frac{88.7 - 39.3}{88.7} \times 100 = 55.7\%$
1.0 ug/l endosulfan	$\frac{88.7 - 33.3}{88.7} \times 100 = 62.5\%$
2.5 ug/l endosulfan	$\frac{88.7 - 7.3}{88.7} \times 100 = 91.8\%$

Step 4. Cochran's Test for Homogeneity of Variances of Data

	Concentration of endosulfan (ug/l)								
Treatment (t):	0.0 (control)	0.0 (solvent control)	0.06	0.10	0.25	0.40	0.60	1.0	2.5
Mean percent normal ($\bar{x}$):	88.7	88.7	89.7	84.0	67.3	55.0	39.3	33.3	7.3
Variance (s^2):	1.14	0.82	4.3	52.0	30.3	39.0.	12.3	26.3	12.3

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$$C_{(cal.)} = \frac{s^2\ (max)}{\Sigma s^2} = \frac{52.0}{178.5} = 0.29\ ns,$$

as compared to: $C_{(tab.)} = 0.48$ for $\alpha = 0.05$, $k = 9$, and $v = 2$

Step 5. Parametric One-Way Analysis of Variance (ANOVA) of Data (Percent Normal)

Source of Variation	df	Sum of Squares	Mean Square	F(cal.)
Treatment (two controls and seven concentrations endosulfan)	8	21,210	2,651	126.2**,
Error	18	371	21	
Total	26	21,581		

as compared to: $F_{(tab.)} = 3.71$ for $\alpha = 0.01$, numerator df = 8, and denominator df = 18

Table 1.—Continued

Step 6. Duncan's Multiple Range Test for Identifying Cause of Significant Differences in Data

A. Ranking of Means ($\bar{x}$) from Lowest to Highest (excluding means > controls)

	Concentration of endosulfan (µg/l)						
	2.5	1.0	0.60	0.40	0.25	0.10	Pooled Controls
Mean percent normal ($\bar{x}$):	7.3	33.3	39.3	55.0	67.3	84.0	88.7

B. Comparison of Pooled Controls Mean with Treatment Means

Comparison of Means	Difference between Means	
Controls vs 2.5 µg/l endosulfan	88.7 - 7.3 = 81.4**,	as compared to LSR (least significant range) = 10.6 for $\alpha = 0.01$, $s_{\bar{x}} = 2.6$, and df = 18
Controls vs 1.0 µg/l endosulfan	88.7 - 33.3 = 55.4**,	as compared to LSR = 11.1 for $\alpha = 0.01$, $s_{\bar{x}} = 2.6$, and df = 18
Controls vs 0.60 µg/l endosulfan	88.7 - 39.3 = 49.4**,	as compared to LSR = 11.4 for $\alpha = 0.01$, $s_{\bar{x}} = 2.6$, and df = 18
Controls vs 0.40 µg/l endosulfan	88.7 - 55.0 = 33.7**,	as compared to LSR = 11.6 for $\alpha = 0.01$, $s_{\bar{x}} = 2.6$, and df = 18
Controls vs 0.25 µg/l endosulfan	88.7 - 67.3 = 21.4**,	as compared to LSR = 11.8 for $\alpha = 0.01$, $s_{\bar{x}} = 2.6$, and df = 18
Controls vs 0.10 µg/l endosulfan	88.7 - 84.0 = 4.7 ns,	as compared to LSR = 8.7 for $\alpha = 0.05$, $s_{\bar{x}} = 2.6$, and df = 18