DATA EVALUATION RECORD

§ 72-3(B) -- ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

1. CHEMICAL: Mepiquat Chloride PC Code No.: 109101

2. TEST MATERIAL: Mepiquat Chloride Purity: 54.6 %

3. CITATION

Authors: Drottar, Kurt R., James P. Swigler, and Catherine M. Holmes.
Title: Mepiquat chloride: A 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*).
Study Completion Date: January 16, 1995
Laboratory: Wildlife International Ldt.
Sponsor: BASF Corporation
Laboratory Report ID: 147A-120A
MRID No.: 435167-02
DP Barcode: D212401

4. REVIEWED BY: William S. Rabert, Biologist, EEB, EFED

Signature: William S. Rabert Date: Oct. 4, 1995

5. APPROVED BY: Harry Craven, Head of Section 4, EEB, EFED

Signature: Henry T. Craven Date: 10/12/95

6. STUDY PARAMETERS

Scientific Name of Test Organism: *Crassostrea virginica*
Age or Size of Test Organism: 28 mm (range 23 - 31 mm.)
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Mean measured

7. CONCLUSIONS: The 96-hour EC50 value for shell deposition for eastern oyster exposed to Mepiquat Chloride was reported as 14.6 mg a.i./L (ppm). The NOEC was reported as 12 mg a.i./L (statistically not significantly different. Calculation of oyster EC_{50} was 12.6 ppm. NOEC was < 4.0 mg a.i./L with 15 % inhibition.

Results Synopsis: Control shell growth was 2.6 mm ± 1.1 mm.

LC_{50}: 12.6 ppm ai 95% C.I.: 8.1 - 18 ppm
NOEL: < 4.0 ppm ai Probit Slope: 3.15

8. ADEQUACY OF THE STUDY

A. Classification: Core for 54.6 % formulation.

B. **Rationale:** N/A

C. **Repairability:** N/A

9. **BACKGROUND**

10. **GUIDELINE DEVIATIONS**

1.

2. (etc.)

11. **SUBMISSION PURPOSE:**

12. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	Eastern oyster *Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	28 mm (Range 23 - 31 mm)
Supplier	P. Cummins Oyster Co. Pasadena, Maryland 21122
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	10 days
Wild caught organisms were quarantined for 7 days?	Not reported
Were there signs of disease or injury?	None observed

Guideline Criteria	Reported Information
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	Not reported
Feeding during the acclimation Must be fed to avoid stress.	Supplied unfiltered seawater with supplemental algae
Pretest Mortality <3% mortality 48 hours prior to testing	0 % mortality prior to testing

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural, unfiltered seawater Water analysis identified no toxic chemicals present
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	20 (Range 18 - 20)
Water Temperature 15°-30° C, consistent in all test vessels	29.0 - 29.8 °C
pH	8.0 - 8.2
Dissolved Oxygen ≥ 60% throughout	Range: 4.5 - 6.1 mg/L 67 - 91 % (lowest % DO at 0 hour)
Total Organic Carbon	1.4 mg/L excluding addition of supplemental algal suspension
Test Aquaria Should be constructed of glass or stainless steel.	Teflon®-lined polyethylene aquaria; 56-L aquaria with 13 L of test water

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter with peristaltic pump; calibrated before test
Flow rate Consistent flow rate	370 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Unknown
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: None Maximum conc.: ml/L

D. **Test Design**

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 oyster then no definitive test is required.	Preliminary EC_{50} 16.7 ppm Shell growth insufficient (i.e., < 2 mm)
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	3.9, 6.5, 11, 18, 30 & 50 mg ai/L
Number of Test Organisms Minimum 20 individual per test level and in each control	20
Test organisms randomly or impartially assigned to test vessels?	Impartially divided
Biological observations made every 24 hours?	Not observed except at 96 hours

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	29.0 - 29.8 °C continuous measurement DO 4.4 - 6.1 mg/L lowest at 0 hour 4.4 / 6.71 = 66 % saturated pH 8.0 - 8.2
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Measured concentrations with ion chromatographic system

13. **REPORTED RESULTS**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0 %
Control Shell Deposition Must be at least 2 mm.	2.6 mm
Recovery of Chemical	66 - 112 %
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				

Control	--	18	0	2.6 ± 1.1	--
Solvent Control	--	N/A			--
3.9	4.0	20	0	2.2 ± 1.5	15
6.5	7.3	20	0	2.2 ± 1.4	15
11	12	20	0	1.6 ± 1.0	38
18	18	20	0	0.99 ± 0.7	62
30	30	20	0	0.20 ± 0.4	92
50	33	20	0	0.07 ± 0.2	97

B. Statistical Results

Method: TOXSTAT; method not specified, probably binomial

96-hr EC_{50}: 15 ppm ai 95% C.I.: 11 - 19 ppm ai

Probit Slope: not reported NOEC: 12 ppm ai (statistical)

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50}	(Bruce and Verteeg (1992), Probit, Binomial Test or Moving Average Angle)
EC_{50} (95% C.I.)	12.6 (8.1 - 18) ppm ai
Probit Slope	3.15
Statistical Method for NOEC	Arithmetic
NOEC	< 4.0 ppm ai

15. REVIEWER'S COMMENTS: Mepiquat is slightly toxic on oyster growth.

William Rabert Mepiquat Cloride 54.6 % a.i. Eastern Oyster Shell Growth

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
33	100	97	97	0
30	100	92	92	0
18	100	62	62	0
12	100	38	38	0
7.3	100	15	15	0
4	100	15	15	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 14.69693

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	1.099487E-02		12.42616	11.5037
13.38466				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.2805663	7.6414	
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 = 3.15092

95 PERCENT CONFIDENCE LIMITS = 1.481925 AND 4.819915

LC50 = 12.60381

95 PERCENT CONFIDENCE LIMITS = 8.072531 AND 18.28361

LC10 = 4.982474

95 PERCENT CONFIDENCE LIMITS = 1.424062 AND 7.853425

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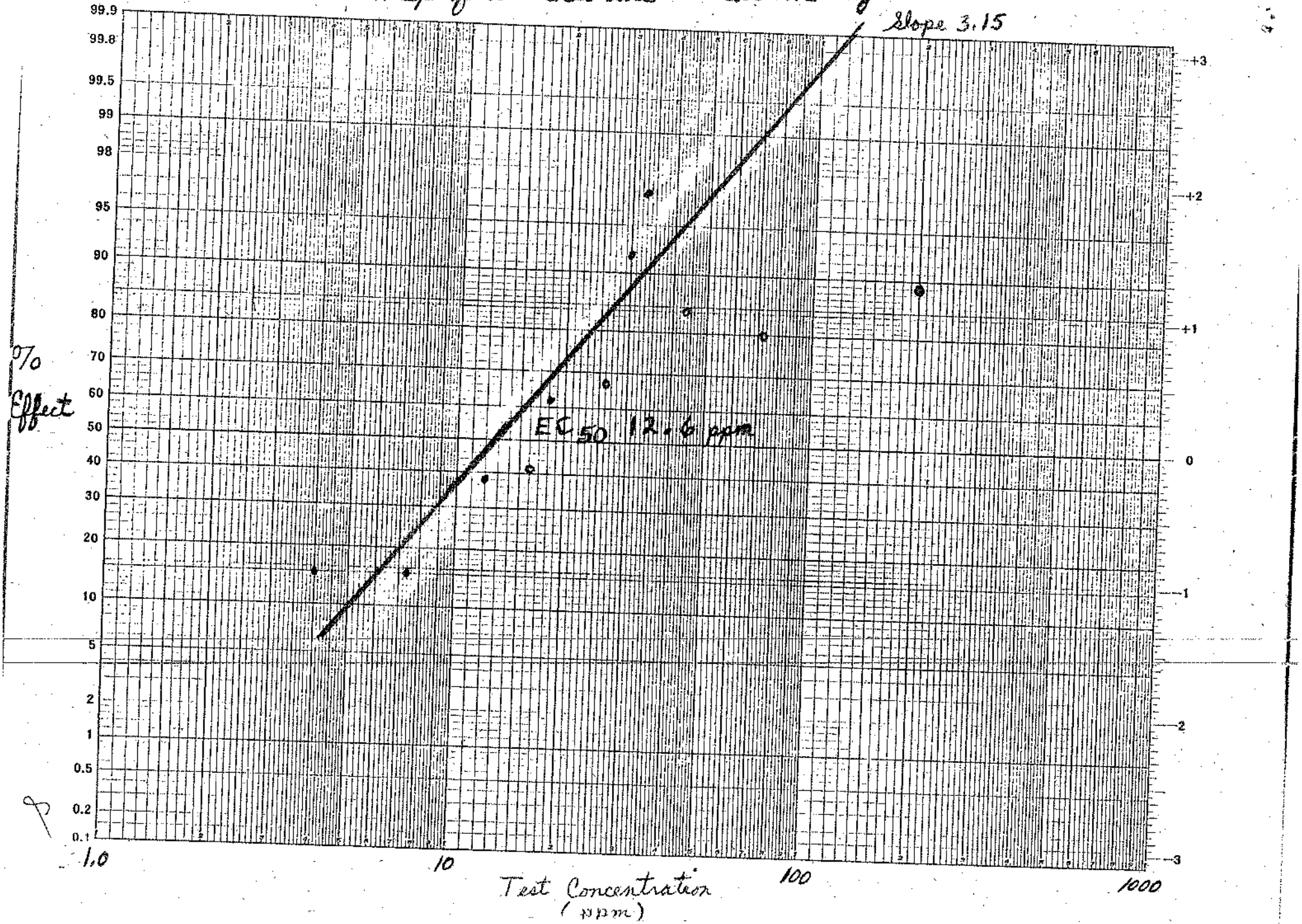
Mepiquat Chloride – Eastern Oyster
Slope 3.15
EC 50 12.6 ppm
% Effect
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
+3
+2
+1
0
-1
-2
-3
1.0
10
100
1000
Test Concentration (ppm)