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

1. **CHEMICAL:** 2Chloro-4,6-bis(isopropylamino)-s-trizine
   PC Code No.: 080808

2. **TEST MATERIAL:** Propazine Purity: 98%

3. **CITATION**
   Authors: R. L.Boero; P.L. Kowalski; T. J. Ward
   Title: Flow through shell deposition test with propazine and the eastern oyster
   Study Completion Date: 8/4/95
   Laboratory: T.R. Wilbury Laboratories, Inc./ABC Laboratories, Inc.
   Sponsor: Griffin Corporation
   Laboratory Report ID: ABC Laboratories #41956
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4. **REVIEWED BY:** Thomas M. Steeger, Ph.D., Fishery Biologist, EFED, ERB IV, U.S. EPA

   Signature: Thomas M Steeger **Date:** 10/14/97

5. **APPROVED BY:** Ann Stavola, Aquatic Biologist, EFED, ERB IV, U.S. EPA

   Signature: Ann Stavola **Date:** 10/15/98

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Crassostrea virginica*
Age or Size of Test Organism:	juvenile
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured/Nominal

7. **CONCLUSIONS:** This study was not scientifically sound and does not fulfill guideline requirements. Precipitant was observed in the two highest treatment groups, 3.0 and 5.0 mg a.i./L. Recovery of Propazine ranged between 74 - 79% and underscores that solubility may have been problematic in all treatment groups. Thus the conclusion that the EC_{50} was greater than 3.72 ppm is not warranted as the oysters may not have been exposed to these levels due to the reduced solubility of Propazine in salt water.

Results Synopsis

EC_{50}: >3.72 ppm ai 95% C.I.: ___-___ ppm ai
NOEL: >3.72 ppm ai Probit Slope: ___

8. ADEQUACY OF THE STUDY

A. Classification: INVALID

B. Rationale: Conventional centrifugation is required for all test media where undissolved test material or precipitate is observed in the test chambers or where the solubility and hence bioavailability are in question. Filtration may be used instead of centrifugation if the analytical method is validated over a range of acceptable concentrations. For aquatic toxicity tests, the Environmental Fate and Effects Division defines solubility as the extractable chemical in the centrifuged or filtered supernate (EPA 1994).

Additionally, unfiltered sea water was used however data on complete water analysis is not provided. Total organic carbon is not reported.

C. Repairability: none

9. BACKGROUND

10. GUIDELINE DEVIATIONS

1. Salinity of dilution on Day 0 of acclimation was lower than the test salinity.

2. Samples at 0 hrs were stored in refrigerator until end of Definitive test rather than being shipped immediately

11. SUBMISSION PURPOSE: To determine the effect of Propazine to oysters by determining the median effective concentration of Propazine causing a 50% decrease in shell deposition (compared to a control) after a specified exposure period and to examine the sublethal/behavioral responses during the course of the study.

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*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	25 - 50 mm
Supplier	P. Cummins Oyster Co.
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	10 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
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	3-5 mm

Guideline Criteria	Reported Information
Feeding during the acclimation Must be fed to avoid stress.	Yes
Pretest Mortality <3% mortality 48 hours prior to testing	< 3% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Unfiltered natural sea water
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰	33‰
Water Temperature 15°-30° C, consistent in all test vessels	18.1 - 19.3°C
pH	7.7 - 8.5
Dissolved Oxygen ≥ 60% throughout	Range: 7.2 - 8.0 mg/L
Total Organic Carbon	not reported
Test Aquaria Should be constructed of glass or stainless steel.	Glass
Type of Dilution System Must provide reproducible supply of toxicant	proportional diluter delivering 0.3 ml stock into 3,000 ml of dilution water
Flow rate Consistent flow rate	8.3 vol/24 hours

Guideline Criteria	Reported Information
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: Dimethylformamide Maximum conc.: 0.1 ml/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, then no definitive test is required.	Not performed; relied on historical data
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	0, 0.75, 1.3, 2.0, 3.0, 5.0 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?	Yes
Biological observations made every 24 hours?	Yes

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	1. Temperature recorded daily 2. DO and pH recorded daily
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

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.	2.7 mm
Recovery of Chemical	88 - 104%
Raw data included?	Yes
Signs of toxicity (if any) were described?	N/A

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	<0.167	20	0	2.7 ± 0.8	--
Solvent Control	<0.167	20	0	2.9 ± 1.0	--
0.75	0.584	20	0	2.9 ± 0.7	ns
1.33	1.00	20	0	2.6 ± 0.6	ns
2.0	1.58	20	0	2.9 ± 0.9	ns
3.0	2.33	20	0	2.4 ± 0.8	ns
5.0	3.72	20	0	2.7 ± 0.9	ns

B. Statistical Results

Method:

96-hr EC_{50}: >3.72 ppm ai 95% C.I.: __ - __ ppm ai

Probit Slope: ___ NOEC: 3.72 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50}	computerized EC_{50} program (Stephan 1983)
EC_{50} (95% C.I.)	ppm ai
Probit Slope	N/A
Statistical Method for NOEC	
NOEC	ppm ai

15. REVIEWER'S COMMENTS:

Measured concentrations of propazine ranged from 74 - 79% of nominal. It is not clear whether the differences between test and nominal concentrations was a result of solubility. Propazine solubility reported to be 8.6 mg/L; in two of the treatments, i.e., 3.0 and 5.0 mg/L, there was a precipitate. It is interesting to note that the difference between nominal and test

concentrations was roughly 25% lower than in treatments involving bluegill.

Conventional centrifugation is required for all test media where undissolved test material or precipitate is observed in the test chambers or where the solubility and hence bioavailability are in question. Filtration may be used instead of centrifugation if the analytical method is validated over a range of acceptable concentrations. For aquatic toxicity tests, the Environmental Fate and Effects Division defines solubility as the extractable chemical in the centrifuged or filtered supernate (EPA 1994).

Unfiltered sea water was used for the exposure system and Table 1 provides a characterization of the dilution water; however, total organic carbon analysis is not included. Propazine is capable of adsorbing to organic matter thus rendering the herbicide less bioavailable; however, the compound is more likely to adsorb in its protonated form. At the pH of the test system (~ 8.0), Propazine would be predominately in its unionized form and less like to adsorb. The information on total organic carbon should be provided though.

References

EPA 1994. Pesticide reregistration rejection rate analysis: ecological effects. Office of Prevention, Pesticides, and Toxic Substances Publication EPA 738-R-94-035, Washington D. C.