

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

1. CHEMICAL: Bronopol
2. TEST MATERIAL: Technical 99.7% active ingredient
3. STUDY TYPE: 96 hour LC<sub>50</sub> Flow-thru study on a marine/estuarine fish

Species Tested: Sheepshead minnow (Cyprinodon variegatus)

4. STUDY IDENTIFICATION: Hill, R. W. November 1984. Bronopol: Determination of acute toxicity to sheepshead minnow (Cyprinodon variegatus). Study No. M167/D. Prepared by Imperial Chemical Industries PLC, Brixham, Devon. Submitted to Boots Company, PLC, Nottingham, England, EPA Accession No. 255965.

5. REVIEW BY: Elizabeth E. Zucker  
Wildlife Biologist  
Ecological Effects Branch/HED
6. APPROVED BY: David Coppage  
Supervisory Biologist  
Ecological Effects Branch/HED

Signature: *Elizabeth E. Zucker*

Date: 2/4/84

Signature: *David Coppage*

Date:

7. CONCLUSIONS:

This study may be used to fulfill a guidelines requirement for an acute toxicity test on a marine/estuarine fish species. With a 96-hour LC<sub>50</sub> of 59.55 mg/l (95% CL 51.33 to 70.39 mg/l), this chemical is considered slightly toxic to sheepshead minnows.

8. RECOMMENDATIONS: N/A

## 9. Background

It is not clear why this study was submitted. It was not required to support registration of a manufacturing-use product.

### Materials and Methods

#### A. Test Procedures

Minnows were obtained from Sea Plantations, Inc. in Salem, Mass., and held in the laboratory for 8 weeks prior to testing. Fish were acclimated to the test vessels for at least 3 days before the study's initiation. Additional test specifics of note include:

Fish mean weight: 0.67 g  
mean length: 27.9 mm

Twenty fish per concentration.  
Stock solutions prepared daily.  
Continuous flow-thru system constructed of glass.

Twenty-litre glass vessels holding a nominal 20 litre volume of diluent with a 200 ml/min. renewal rate. A 95% exchange of test solution every 4.5 hrs.

Nominal concentrations of test materials were: 0, 18, 32, 56, 75, 100 and 180 mg/l.

Samples were taken daily from test vessels and toxicant measured by HPLC.

Diluent was local seawater of approximately 34.5 ppt. pH, DO and temperature were measured daily. Symptoms of toxicity were noted twice daily. Food was withheld during the test period (from protocol).

#### B. Statistical Analysis

Data were analyzed according to the method described by Finney (1971).

12. Reported Results

Mortality Data

Nominal Conc. (mg/l)	Mean Measured Conc. (mg/l)	% Mortality			
		24	48	72	96
180	123.2	85	100	100	100
100	67.2	0	25	35	40
75	47.5	0	25	45	45
56	34.0	0	0	0	10
32	16.9	0	0	10	10
18	8.5	0	0	0	0
Control	-	0	0	0	0

pH values ranged from 7.8 to 8.3 in the exposure vessels.  
 Salinity ranged from 34.80 to 34.96%.  
 DO ranged from 5.8 to 8.6 mg/l  
 Temperature was 21.0 to 21.9°C in exposure chambers.

Symptoms of toxicity included loss of equilibrium, quiescence, darkening in color, rapid respiration and surfacing. Surfacing was noted in at least one fish at all levels except the 18 mg/l concentration. Most of the other symptoms were noted in the 75 mg/l or greater levels.

13. Study Author's Conclusion/QA Measures

Time	LC <sub>50</sub>	95% CL
24 hrs.	101	(88-114)
48 hrs.	70.8	(34-142)
72 hrs.	61.5	(34-110)
96 hrs.	57.6	(31-164)

The study was inspected by ICI in accordance with GLP.

14. Reviewer's Discussion and Interpretation of the Study.

A. Test Procedures

This study was performed under conditions that generally complied with current testing standards with the following notable exceptions:

1. Temperature was not monitored continuously.
2. Fish should have been acclimated to study conditions for a longer time period.
3. Photoperiod was not reported.

B. Statistical Analysis

Stephan's computerized program was utilized to analyze the data. The mean measured concentrations were used in the calculations. The LC50 value differed slightly from those found by the study author (Results appended).

C. Results/Discussion

According to the label, this chemical is 25% soluble in water (w/v). However, chemical analysis of diluent showed that actual concentration of the toxicant ranged from 47.2 to 68.4% of nominal concentrations. Each tank was sampled daily with the exceptions that the 180 mg/l vessel was sampled only on Day 1 and 2 and the 32 mg/l tank was sampled daily but 3 times on Day 2 and 4. Results of the analyses indicate that residues were consistent.

D. Adequacy of Study

1. Classification: Core for technical product
2. Rationale: N/A
3. Repairability: N/A

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ZUCKER	BRONOPOL	96 HOUR	TECHNICAL	SHEEPSHEAD MINNOW	MEASURED CONC
CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)	
123.2	20	20	100	9.53674E-05	
67.2	20	8	40	25.1722	
47.5	20	9	45	41.1901	
34	20	2	10	.0201225	
16.9	20	0	0	9.53674E-05	
8.5	20	0	0	9.53674E-05	

THE BINOMIAL TEST SHOWS THAT 34 AND 123.2 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 72.462

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.0918382	59.4376	51.8132 69.104

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.127714	1	.153635

SLOPE = 5.17263  
 95 PERCENT CONFIDENCE LIMITS = 3.32408 AND 7.02117

LC50 = 59.5451  
 95 PERCENT CONFIDENCE LIMITS = 51.3271 AND 70.3929

LC10 = 33.8316  
 95 PERCENT CONFIDENCE LIMITS = 24.2983 AND 40.6778  
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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
  - Sales or other commercial/financial information.
  - A draft product label.
  - The product confidential statement of formula.
  - Information about a pending registration action.
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