

SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

JUL 8 1985

DATE: IN 5-22-85 OUT _____

FILE OR REG. NO 33753-3, 33753-1

PETITION OR EXP. NO. _____

DATE OF SUBMISSION 5-13-85

DATE RECEIVED BY HED 5-21-85

RD REQUESTED COMPLETION DATE 7-5-85

EEB ESTIMATED COMPLETION DATE 7-1-85

RD ACTION CODE/TYPE OF REVIEW 303/Amendment

TYPE PRODUCT(S) : I, D, H, F, N, R, S Microbiocide

DATA ACCESSION NO(S). _____

PRODUCT MANAGER NO. J. Lee (31)

PRODUCT NAME(S) Bronopol

COMPANY NAME The Boots Company PLC

SUBMISSION PURPOSE Submission of oyster study for review.

SHAUGHNESSEY NO. CHEMICAL, & FORMULATION 8 A.I.

EEB REVIEW

- 100.0 Pesticide Name: Bronopol
- 100.3 Submission Purpose: Submission of acute oyster larvae study
- 101.0 Chemical: 2-Bromo-2-nitropropane-1,3-diol
- 103.0 Toxicological Properties: Oyster larvae LC₅₀ = 0.77 mg/l
(.72 - 1.0 mg/l)
- 105.0 Conclusions: This acute oyster larvae study is scientifically sound and will fulfill registration requirements. An LC₅₀ = 0.77 mg/l suggests that Bronopol is very highly toxic to oyster larvae.

Michael Rexrode

Michael Rexrode, Fishery Biologist
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Michael Slimak 7/8/85
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Ecological Effects Branch
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1. Chemical: Bronopol 99.7%
2. Test Material: 2-Bromo-2-nitropropane-1,3-diol
3. Study/Action Type: Acute Oyster Larvae 48-hour Toxicity
4. Study Identification: Thompson, R.S. 1985. Bronopol: Determination of the acute toxicity to larvae of the Pacific oyster (Crassostrea gigas), Imperial Chemical Industries, PLC, Brixham Laboratories, Brixham, Devon, England.

5. Review By:

Miachel Rexrode
Fishery Bioloigist
EEB/HED

Signature: Miachel Rexrode
Date: 7/9/85

6. Approved By:

Norman Cook
Sectin Head
EEB/HED

Signature: Norman Cook
Date: 7.8.85

7. Conclustions: This study appears to be scientifically sound and will support Registration. A mean of measured concentrations was calculated, producing an $LC_{50} = 0.77$ mg/l. This study suggests that Bronopol is very highly toxic to oyster larvae.

8. Materials and Methods:

a. Test organisms:

The test organisms were 3-hour post-fertilization embryos of the Pacific Oyster (Crassostrea gigas). These were obtained by briefly raising the water temperature to 28-30° C, thus inducing female oysters (92-124 mm shell length) to spawn. Oysters were maintained in unfiltered seawater at 17-19°C.

b. Test material:

Bronopol concentration was 99.7% technical.

c. Dilution water:

Natural seawater from Tor Bay, Devon. Salinity was adjusted to $20 \pm 2\%$ (approximately 42% freshwater, 58% seawater).

d. Test apparatus:

Test vessels were 250 ml glass beakers. Two replicates of the control and each concentration were prepared, each containing 200 ml of test solution.

e. Tet procedures:

A nominal concentration of 35 embryos/ml (Coulter Counter) was added to each vessel at the start of the test. Test solutions prepared by the addition of stock solutions and distilled water to dilution water. The following nominal concentrations of bronopol were tested: Control, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, and 5.6 mg/l. After 48-hours each vessel was mixed with a perforator plunger and 10 ml of solution removed and fixed with 5% borax-buffered formalin. Normal and abnormal larvae were counted in each of four 1 ml subsamplers from each sample. D-veliger larvae were defined as normal if the bivalve shell was fully formed.

f. Chemical Parameters:

Temperature measurements were 20.4 to 20.8 °C; salinity of dilution water was 32.2‰; dissolved oxygen concentration was 7.05 to 7.40 mg/l; and pH was 7.9 to 8.0.

The measured concentrations at the start of the test ranged from 88 to 104% of the nominal values. The measured concentrations at the end of the test ranged from below the detection limit to 5% of the nominal values. Decline in concentration over the test period was considered to be due to hydrolysis of the test material (TABLE 1).

TABLE 1

Nominal and Measured Concentration of Bronopol

NOMINAL CONCENTRATION (MG/L)	TIME FROM START OF EXPOSURE PERIOD (hr)	MEASURED CONC/NTRATIN (MG/L) (x)	% NOMINAL
5.6	0	5.84	104
	48	(3.06) 0.28	5
3.2	0	3.32	104
	48	(1.7) 0.08	3
1.8	0	1.86	---
	48	(.95) < 0.03	100
1.0	0	1.0	---
	48	-(.52) < 0.03	88
0.56	0	0.49	---
	48	-(.26) < 0.03	91
0.32	0	0.29	---
	48	-(.16) < 0.03	100
0.18	0	0.18	---
	48	(.11) < 0.03	---
Control	0	< 0.03	---

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9. Results:

No significant effects on larval development or survival were observed at or below a nominal concentration of 0.32 mg/l. The 48-hour LC₅₀ was 1.7 mg/l (1.3 - 2.4 mg/l) and the 48hour EC₅₀ was 0.69 mg/l (0.56 - 1.0 mg/l). Survival data corresponding to nominal concentrations are listed in table 1.

TABLE 1

ACUTE TOXICITY OF BRONOPOL TO OYSTER LARVAE

NOMINAL CONCENTRATION (MG/L)	MEAN MEASURED CONCENTRATION (MG/L)	NUMBER SERVIVING/ML
5.6	3.06	1.5
3.2	1.7	12.5
1.8	.95	16.3
1.0	.52	22.2
0.56	.26	33.6
0.32	.16	37.7
0.18	.11	35.6
CONTROL	CONTROL	35.8

10. Reviewer's Evaluation:

This test appears to be scientifically sound and will support registration. However, a decline in Bronopol concentrations was noted during testing. EEB compensated for the change by calculating a mean value for measured concentrations at the the beginning and end of the test. These values are as follows: 3.06, 1.7, 0.95, 0.52, 0.26, 0.16, and 0.11 mg/l. Using these values, an LC₅₀ value was calculated at 0.78 mg/l and suggest that Bronopol is extremely toxic to oyster larvae.

Category: Core

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NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY. THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

BRONOPOL OYSTER LARVAE 48-HR TOXICITY *mg/l*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.06	36	35	97.2222	0
1.7	36	24	66.6667	0
.95	36	20	55.5556	0
.52	36	14	38.8889	0
.26	36	3	8.33333	0
.16	36	0	0	0
.11	36	0	0	0

THE BINOMIAL TEST SHOWS THAT .52 AND .95 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.

APPROXIMATE LC50 FOR THIS SET OF DATA IS .777735

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	.0323535	.835675	.714142	.988726

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.041491	1	.182186

SLOPE = 2.7097
 95 PERCENT CONFIDENCE LIMITS = 2.15775 AND 3.26165

LC50 = .859783
 PERCENT CONFIDENCE LIMITS = .719803 AND 1.03571

LC10 = .292215
 95 PERCENT CONFIDENCE LIMITS = .212515 AND .369075
