

SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

JUN 26 1985

DATE: IN 4-23-85 OUT \_\_\_\_\_

FILE OR REG. NO 33753-3

PETITION OR EXP. NO. \_\_\_\_\_

DATE OF SUBMISSION 3-27-85

DATE RECEIVED BY HED 4-18-85

RD REQUESTED COMPLETION DATE 7-1-85

EEB ESTIMATED COMPLETION DATE 6-24-85

RD ACTION CODE/TYPE OF REVIEW 305/Amendment

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

DATA ACCESSION NO(S). 257569

PRODUCT MANAGER NO. J. Lee (31)

PRODUCT NAME(S) Bronopol

COMPANY NAME The Boots Company PLC

SUBMISSION PURPOSE Submission of mysid shrimp study for  
review

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

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EEB REVIEW

- 100.0 Pesticide Name: Bronopol
- 100.3 Submission Purpose: Submission of acute mysid shrimp study
- 101.0 Chemical: 2-Bromo-2-nitropropane-1,3-diol
- 103.0 Toxicological Properties: Mysid Shrimp  $LC_{50} = 5.9$  mg/l  
(5.1 - 7.5 mg/l)
- 105.0 Conclusions : This acute mysid shrimp study is scientifically sound and will fulfill registration requirements. An  $LC_{50} = 5.9$  mg/l suggest that Bronopol is moderately toxic to mysid shrimp.

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Michael Rexrode, Fishery Biologist  
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*Mike Klimak 6/26/85*  
Mike Klimak, Chief  
Ecological Effects Branch  
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1. Chemical: Bronopol 99.7% ai
2. Test Material: 2, Bromo-2-nitro-propate-1,3-diol
3. Study/Action Type: Flow-through Acute Mysid Shrimp Study
4. Study Identification: Thompson, R.S., Comber, M.H.I., 1985.  
"Bronopol: Determination of Acute Toxicity to Mysid Shrimp," Imperial Chemical Industries PLC, Brixham Laboratory, Brixham, Devon, England

5. Reviewed By:

Michael Rexrode  
Fishery Biologist  
EEB/HED

Signature: Michael Rexrode

Date: 6/25/85

6. Approved By:

Norman Cook  
Section Head  
EEB/HED

Signature: EQ Fite for

Date: 6/25/85

7. Conclusions: This test appears to be scientifically sound and will support registration. At 5.9 mg/l, Bronopol appears to be moderately toxic to mysid shrimp.

8. Materials and Methods

a. Test organisms:

The test organisms were mysid shrimp, Mysidopsis bahia, <24 hours old at the start of the test. These organisms were derived from a continuous laboratory culture. The original source of these cultures was stated as the US EPA Laboratory, Narragansett, Rhode Island and supplied by Sea Plantations Inc., Salem, MA.

b. Test material:

Bronopol concentration was 99.7% concentrated starch solution of the test material were made up daily, and kept in glass vessels.

c. Dilution water:

Test water was natural seawater obtained from Tor Bay, Devon. Salinity was adjusted to  $20 \pm 2\%$  (approximately 42% freshwater: 58% seawater).

d. Test Apparatus:

Test vessels were glass rectangular tanks of 14 liters. Each vessel contained 4 retention chambers, made from plastic beakers of 90 mm diameter and 400 ml vol, with a "window" cut in the side and covered by nylon mesh. Test vessels drained automatically to approximately one-third of working volume every 30 minutes, to ensure exchange of test solution between vessels and retention chambers. Dilution water flows of 500 ml/minute were obtained by use of fixed operative outlets from a constant head tank. Stock solutions of the test material were delivered by peristaltic pump to mix with the dilution water in chambers of about 0.4 liters volume, prior to delivery to test vessels.

e. Environmental Control:

Temperature was maintained at  $25 \pm 1^\circ$  C. Photoperiod was controlled at 14 hours light: 10 hours dark, with a gradual transition period of about 15 minutes.

f. Experimental Design:

Twenty mysid shrimp were randomly assigned to each concentration level and control (5 mysid in each of the 4 retention chambers within each vessel). Nominal concentration levels were as follows: control, 1.8, 3.2, 5.6, 10, 18, 32, 56 mg/liter. measured values; 1.48, 2.40, 3.61, 5.51, 12.6, 22.3, 25.3.

g. Test procedures:

During exposure, the mysid were fed nauplii of Artemia salina. Each chamber was fed twice per day with 1 ml from a liter suspension of Artemia (derived from 8 g of cysts).

h. Physical Parameter:

Dissolved oxygen; 6.1 to 6.9 mg/l; pH, 8.04 - 8.26;  
Salinity, 19.8 to 20.3‰

9. Results: Percent mortality is as follows:

MEASURED CONCENTRATION (MG/l)	PERCENTAGE MORTALITY			
	24 Hour	48 Hour	96 Hour	
25.3	100	100	100	20
22.3	70	100	100	20
12.6	5	55	100	20
5.51	10	10	35	7
3.61	5	10	10	2
2.40	0	0	0	0
1.48	0	0	0	0
control	0	0	0	0

The LC<sub>50</sub> values were calculated as follows:

24 hour LC<sub>50</sub> = 15.3 mg/l (6.1 - 39.9 mg/l)  
48 hour LC<sub>50</sub> = 9.6 mg/l (7.9 - 11.5 mg/l)  
96 hour LC<sub>50</sub> = 5.9 mg/l (5.1 - 7.3 mg/l)

Statistical analysis was calculated by Probit Analysis based on measured concentrations.

10. Reviewers Evaluation:

This study appears to be scientifically sound and will support registration. Test procedures were acceptable and measured concentrations were used. Toxicity values were verified by EEB and are within the range presented by the researchers. At 5.9 mg/l, Bronopol appears to be moderately toxic to mysid shrimp.

Category: Core

BRONOPAL ACUTE MYSID SHRIMP LC50 mg/l  
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
25.3	20	20	100	9.53674E-05
22.3	20	20	100	9.53674E-05
12.6	20	20	100	9.53674E-05
5.51	20	7	35	13.1588
3.61	20	2	10	.0201225
2.4	20	0	0	9.53674E-05
1.48	20	0	0	9.53674E-05

THE BINOMIAL TEST SHOWS THAT 3.61 AND 12.6 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 6.37768

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	.0231378	6.16747	5.26839	7.17238

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.180047	1	.963189

SLOPE = 7.02462  
 95 PERCENT CONFIDENCE LIMITS = 4.04394 AND 10.0053

LC50 = 5.94099  
 95 PERCENT CONFIDENCE LIMITS = 5.1428 AND 7.31548

LC10 = 3.91802  
 95 PERCENT CONFIDENCE LIMITS = 2.98577 AND 4.5646  
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