2-2-94

MRID No. 416138-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin (or Benfluralin).
Shaughnessey Number: 084301.

2. **TEST MATERIAL:** Benefin; N-Butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine; Lot No. 231 EF4; CAS No. 1861-40-1; 96.6% active ingredient; a yellow-orange powder.

3. **STUDY TYPE:** 72-3(c) Estuarine Invertebrate Flow-Through Acute Toxicity Test. Species Tested: Mysid Shrimp (*Mysidopsis bahia*).

4. **CITATION:** Sousa, J.V. 1990. (Benefin) - Acute Toxicity To Mysid Shrimp (*Mysidopsis bahia*) Under Flow-Through Conditions. Prepared by Springborn Laboratories, Inc., Wareham, MA. SLI Report No. 90-06-3343. Submitted by DowElanco Products Company. EPA MRID No. 416138-04.

5. **REVIEWED BY:**

Kimberly Rhodes, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici for KR
Date: 6/25/91

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 6/25/91

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: 2/2/94 SR 2-2-94
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a flow-through acute toxicity study using estuarine shrimp. The 96-hour LC_{50} was 43 µg a.i./l mean measured concentration, which classifies benefin as very highly toxic to *Mysidopsis bahia*. The NOEC was 16 µg a.i./l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The mysid shrimp (*Mysidopsis bahia*) used in this test were obtained from in-house cultures. The culture water was from the same source as the dilution water used during the test. The mysid culture area received a photoperiod of 16 hours of light (intensity of 80-100 footcandles) and 8 hours of darkness. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 ±1°C. Mysids were fed brine shrimp nauplii twice daily.

B. **Test System:** The test system consisted of a constant-flow serial diluter, a temperature-controlled water bath and a set of 14 aquaria (29 x 15 x 19 cm). Each aquarium was equipped with a self-starting siphon attached to the drain. The solution volume in each aquarium to fluctuated between 3 and 6 1. The flow rate to each aquarium was approximately 17 volume replacements every 24 hours.

A photoperiod of 16 hours of light and 8 hours of darkness provided light with an intensity of 28-50 footcandles throughout the study period. The test solutions were not aerated.

The dilution water used during the study was filtered (20 and 5 µm) natural seawater collected from the Cape Cod Canal, Bourne, MA. The water had a salinity of 33 parts per thousand (ppt), a pH of 7.9, and a dissolved oxygen concentration of 7.1 mg/l at test initiation.

A 2.04 mg a.i./ml stock solution was prepared by dissolving 0.5281 g of benefin with acetone to volume in a 250-ml volumetric flask. The stock solution was mechanically injected into the mixing chamber of the diluter. A concentration equal to the highest test concentration (150 µg a.i./l) was produced in the mixing chamber and was subsequently diluted to produce the remaining test concentrations.

C. **Dosage:** Ninety-six-hour flow-through acute test. Based on the results of a preliminary test, five nominal concentrations (19, 32, 54, 90, and 150 µg a.i./l) were tested. A dilution water control and solvent control were also included in the test. The solvent control solution (74 µl/l) contained the maximum amount of acetone present in any concentration.

D. **Design:** Twenty mysids (≤24 hours old) were impartially selected and distributed to each concentration and the controls (ten mysids per replicate). Mysids were fed live brine shrimp nauplii twice daily during the test.

Biological observations and observations of physical characteristics of the test solutions were noted at test initiation and every 24 hours. Dead shrimp were removed at each observation interval.

The dissolved oxygen concentration, pH, salinity, and temperature were measured once daily in each replicate of the controls and each treatment level. The temperature was also continuously monitored in one replicate of the control throughout the study using a min/max thermometer.

Water samples were collected at test initiation and termination from both replicate test solutions of each treatment level and the controls. Quality control (QC) and test samples were analyzed for test material using gas chromatography.

E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval for each 24-hour interval were calculated using a computer program that employed multiple methods of analysis (i.e., probit analysis, moving average angle, and binomial probability). The no-observed-effect concentration (NOEC) was defined as the highest concentration tested at and below which there were no toxicant-related physical and behavioral abnormalities.

12. **REPORTED RESULTS:** Mean measured concentrations were 10, 16, 25, 45, and 66 µg a.i./l and ranged from 44 to 53% of nominal concentrations (Table 2, attached). Throughout the exposure period, test solutions were observed to be clear and colorless and contained no visible sign of insoluble test material.

The cumulative percent mortalities and observations made during the test are presented in Table 3 (attached). The 96-hour LC_{50} was determined to be 43 µg a.i./l with a 95% confidence interval of 36-52 µg a.i./l. The NOEC was 16 µg a.i./l.

During the study, pH ranged from 7.9 to 8.1, the dissolved oxygen concentration ranged from 6.2 to 7.2 mg/l (91 to 106% of saturation), salinity ranged from 31 to 33 ppt, and the daily temperature ranged from 25 to 27°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations (40 CFR Part 160). Maintenance of records on the test substance, including stability, characterization and verification of the test substance identity is the responsibility of the test sponsor.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following:

The test organisms were impartially selected and distributed to the test chambers; random assignment to the test vessels is required,

The number of volume replacements used was 17/day. The SEP recommends 5-10 volume replacements/day. The author used a higher number of replacements to aid the solubility of the test material.

Mortality of the test organisms prior to test initiation was not reported.

The report did not specify whether the photoperiod contained 15- to 30-minute transition periods between light and dark.

The continuously measured temperature ranged from 25 to 27°C. The SEP recommends a temperature of approximately 22°C and that it should not deviate more than 1°C during the test.

B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the LC_{50} value and obtained the same results as the author (see attached printout). The slope of the concentration-response curve was 4.5.

C. Discussion/Results: This study is scientifically sound and meets the requirements for a flow-through acute toxicity study using estuarine shrimp. The 96-hour LC_{50} was 43 µg a.i./l mean measured concentration, which classifies benefin as very highly toxic to *Mysidopsis bahia*. The NOEC was 16 µg a.i./l.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, May 20, 1992.

Table 2. **Measured concentrations of benefin in replicate (A,B) solutions during the 96-hour flow-through exposure of mysid shrimp (*Mysidopsis bahia*).**

Nominal Concentration (µg/L)		Measured Concentration (µg/L)			
		Day 0	Day 4	Mean (SD)[a]	% Nominal
150	A	64 (43)	49 33	66 (17)	44
	B	89 59	62 (41)		
90	A	57 63	35 39	45 (11)	50
	B	51 57	35		
54	A	35 (65)	18 33	25 (9.3)	46
	B	31 57	16 (30)		
32	A	20 63	11 34	16 (4.0)	50
	B	19 59	14 44		
19	A	11 58	8.2 43	10 (2.1)	53
	B	11 58	7.4 39		
Solvent Control	A	<1.6	<1.6		
	B	<1.6	<1.6		
Control	A	<1.6	<1.6		
	B	<1.6	<1.6		
QC#1[b]		171 (150)[c]	161 (150)	NA	
QC#2		53.9 (50.0)	77.5 (75.0)	NA	
QC#3		32.6 (30.0)	32.3 (30.0)	NA	

[a] Mean measured concentrations are presented with the corresponding standard deviation (S.D.) in parentheses. Mean values are calculated using the actual analytical results, rather than the rounded numbers (2 significant figures) reported in this table.

[b] QC = Quality Control sample

[c] Value in parentheses represents the nominal fortified concentration for each QC sample.

Table 3. **Concentrations tested, corresponding percent mortalities and observations made during the 96-hour flow-through toxicity test exposing mysid shrimp (*Mysidopsis bahia*) to benefin.**

Mean Measured Concentration (µg/L)		Cumulative Mortalities (%)			
		24-Hour	48-Hour	72-Hour	96-Hour
66	A	0	30	60	70
	B	10	50	70	70
	Mean	5[a]	40[a]	65[a]	70[a]
45	A	10	70	70	80
	B	10	20	40	60
	Mean	10[a]	45[a]	55[a]	70[a]
25	A	10	10	10	10
	B	10	20	20	20
	Mean	10	15[a]	15[a]	15[a]
16	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
10	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
Solvent Control	A	0	0	0	0
	B	0[b]	0[b]	0[c]	0
	Mean	0	0	0	0
Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0

[a] All of the surviving mysids were observed to be lethargic.
[b] All of the surviving mysids were observed to be lethargic and exhibited darkened pigmentation.
[c] Surviving mysids were observed to be lethargic.

benefin Mysidopsis bahia

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
66	20	14	70	5.765915
45	20	14	70	5.765915
25	20	3	15	.1288414
16	20	0	0	9.536742E-05
10	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND INFINITY 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 36.66428

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1136467	41.37666	34.51296	52.07589

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1279635	1	.2590439

SLOPE = 4.545838
95 PERCENT CONFIDENCE LIMITS = 2.919701 AND 6.171974

* LC50 = 42.59108
95 PERCENT CONFIDENCE LIMITS = (35.8392 AND 51.95668)

LC10 = 22.38422
95 PERCENT CONFIDENCE LIMITS = 15.4223 AND 27.6125

Shaughnessey # 128401 Chemical Name Pepe fin (or Bentluralin) Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} = pp (95% C.L.) Control Mortality (%) = Solvent Control Mortality (%) =		
Species:		Slope = # Animals/Level = Temperature =		
Lab:		48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )		
MRID #		Comments:		

96-Hour LC_{50}	96.6%	LC_{50} = 43 ppb* (95% C.L. 36 - 52) * probit Control Mortality (%) = 0 Solvent Control Mortality (%) = 0		
Species: Mysidopsis bahia		Slope = 4.5 # Animals/Level = 20 Continuous Temperature = 25-27 °C		
Lab: Springborn Laboratories, Inc.		96-Hour Dose Level pp b* /(% Mortality) 10 (0), 14 (0), 25 (15), 45 (70), 64 (70)	K.L 5/20/92	Core
MRID # 416138-04		Comments: NOEC = 14 ug a.i./l. * Based on mean measured concentrations		