MRID No. 423908-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Benfluralin.
Shaughnessey No. 084301.

2. **TEST MATERIAL:** Balan® EC (FN 0270); N-(n-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-p-toluidine; Lot No. ACD13701; an emulsifiable concentrate formulation containing 20.1% active ingredient (benefin).

3. **STUDY TYPE:** 72-1(b). 72-1. Freshwater Fish Acute Static-Renewal Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).

4. **CITATION:** Brock, D.E. 1992. The Acute Toxicity of Balan® EC (FN 0270), a Formulation Containing Benefin (EL-110, Compound 054521), to Bluegill (*Lepomis macrochirus*) in a Static-Renewal Test System. Laboratory Project ID: F00692. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 423908-01.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 10/21/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 10/21/92

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

Signature: 2/2/94 92 2-2-92
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a static acute toxicity test using freshwater fish. The 96-hour LC_{50} of 0.42 mg a.i./l mean measured concentration classifies Balan® EC as highly toxic to ~~rainbow trout~~ the Bluegill sunfish 2/17/94. The NOEC was 0.008 mg a.i./l mean measured concentration.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile bluegill sunfish (*Lepomis macrochirus*) were obtained from a commercial supplier in Osage Beach, MO, and were held in conditioned well water for approximately 50 days prior to test initiation. The fish were fed a dry pelletted food daily. During the 14-day period prior to test initiation, the average temperature was 21.2 (15.1-22.2)°C. The weight and length of the fish from the control group at test termination averaged 0.46 (±0.09) g and 35.7 (32-42) mm, respectively.

B. **Test System:** The test chambers were 18.9-l glass jars containing 15 l of test solution. A 16-hour light/8-hour dark photoperiod was used during acclimation and testing. Light intensity was reduced to help maintain benefin concentrations.

The test stock solution was prepared by dissolving 9.95 g of Balan® EC in acetone to a final volume of 100 ml. Appropriate volumes of the stock and acetone were diluted with conditioned well water to produce individual test solutions. Fresh solutions were prepared daily from the main stock. The final concentration of acetone in each solution was 0.5 ml/l.

The dilution water was well water obtained on-site. The water was treated to remove iron, 50% of the mineral content (using electrodialysis), and excess CO_2 (to adjust pH). The water was stored in underground tanks and warmed or cooled to test temperature before delivery to the diluter system.

The solutions were not aerated during the test.

C. **Dosage:** Ninety-six-hour static-renewal test. Based on a preliminary test, six nominal concentrations (0.010, 0.050, 0.10, 0.50, 1.0, and 5.0 mg a.i./l), an acetone control, and a dilution water control were used.

D. **Design:** Ten fish were randomly distributed to each vessel, one vessel per concentration. The biomass loading in the dilution water control was approximately 0.3 g/l.

Survival and sublethal responses were monitored daily. The physical condition/behavior pattern of the fish was

rated on a scale of 1.0 to 4.0, with 1.0 being normal and 4.0 being dead. On days 1, 2, and 3, the fish were transferred to clean vessels containing freshly-prepared solutions. Dead fish were removed each day. The fish were not fed for 48 hours prior to and during the test.

The dissolved oxygen concentration (DO), pH, and temperature were measured in the "new" and "old" solutions daily. The temperature of the water bath was monitored continuously. The hardness, alkalinity, and conductivity of the dilution water control were determined at test initiation. Ammonia concentrations were measured in the dilution water control and selected test solutions.

The concentration of benefin in the freshly prepared solutions (0 and 72 hours) and discarded solutions after renewal (24 and 96 hours) was determined using gas chromatography.

E. Statistics: Due to the nature of the mortality data, the median lethal concentration (LC_{50}) could not be definitely determined.

12. REPORTED RESULTS: The mean measured concentrations were 0.008, 0.037, 0.077, 0.37, 0.77, and 3.34 mg a.i./l (Table 1, attached). Concentrations of benefin in the "old" test solutions ranged from 31 to 62% of the "new" test solutions.

Mortality during the test was reported in Table 8 (attached). Fish exposed to test concentrations of ≥0.037 µg a.i./l showed signs of toxicity, such as hypoactivity and prostration. The 96-hour LC_{50} was >0.077 and <0.37 mg a.i./l. The no-observed-effect concentration (NOEC) was 0.008 mg a.i./l.

During the test, the DO was ≥70% of saturation in all test solutions. The pH ranged from 7.7 to 8.2 and temperature was 20.7-23.2°C. Hardness, alkalinity, and conductivity of the dilution water were 120 mg/l as $CaCO_3$, 162 mg/l as $CaCO_3$, and 268 µmhos/cm, respectively. Un-ionized ammonia levels were ≤0.003 mg/l at test initiation and termination.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The nonmonotonicity of the mortality data is not surprising considering that the benefin levels were well above benefin solubility in water and benefin was probably in suspension. The 96-hour LC_{50} of benefin determined using the formulated product (as in this study) was less than that attained using

the technical material (as in a previous study). However, the NOEC of benefin for the formulation and technical material was approximately the same.

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in compliance with EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance inspections were also reported.

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 physical characteristics of the test material (i.e., physical state, appearance) was not described.

The system used to control test temperature was not described in the report. Solution temperature measurements should have been made more often (i.e., hourly or at least every six hours) depending on the system used.

Each nominal test concentration was approximately 20-50% of the next highest concentration. The SEP states that each nominal concentration should be at least 60% of the next highest.

A 15- to 30-minute transition period between light and dark is recommended in the SEP. Transition periods were not used in the study.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour LC_{50} value as 0.42 mg a.i./l with a 95% C.I. of 0.29-0.73 mg a.i./l using the moving average method (see attached printout).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a static acute toxicity test using freshwater fish. The 96-hour LC_{50} of 0.42 mg a.i./l mean measured concentration classifies Balan® EC as highly toxic to rainbow trout. The NOEC was 0.008 mg a.i./l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core for a formulated product.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 10-15-92.

Table 1. Analyzed Concentrations of Benefin in Test Solutions. Study F00692.

Nominal Balan® EC Concentration (mg/L)	Nominal Benefin Concentration (mg/L)	Analyzed Benefin Concentration (mg/L)				
		0 Hour	24 Hour (Aged)	72 Hour (Renewed)	96 Hour (Aged)	Mean ± SD
0.0 (Water Control)	0.0 (Water Control)	ND[a]	ND	ND	ND	--
0.0 (Acetone Control)	0.0 (Acetone Control)	ND	ND	ND	ND	--
0.05	0.010	0.009	0.006	0.013	0.007	0.008± 0.003
0.25	0.050	0.040	0.025	0.051	0.031	0.037± 0.011
0.50	0.10	0.091	0.053	0.102	0.061	0.077± 0.023
2.50	0.50	0.48	0.23	0.50	0.25	0.37 ± 0.14
5.0	1.0	0.92	0.41	1.23	0.52	0.77 ± 0.38
24.9	5.0	5.08	1.59	--	--	3.34 ± 2.47

[a]ND = None detected. The analytical limit of quantitation was 0.5 µg/L.

Table 8. Cumulative Mortality Frequencies for Bluegill (*Lepomis macrochirus*) Exposed for 96 Hours to Balan® EC. Study F00692.

Nominal Balan EC Concentration (mg/L)	Averaged Assayed Benefin Concentration (mg/L)	24 Hour	48 Hour	72 Hour	96 Hour
ND (Water Control)	ND (Water Control)	0	0	0	0
ND (Acetone Control	ND (Acetone Control)	0	0	0	0
0.05	0.008	0	0	0	0
0.25	0.037	0	0	0	0
0.50	0.077	0	0	0	0
2.50	0.37	1	1	9	9
5.0	0.77	0	2	2	3
24.9	3.34	10	10	10	10

RIFICI BENFLURALIN BLUEGILL SUNFISH 10-15-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.34	10	10	100	9.765625E-02
.77	10	3	30	17.1875
.37	10	9	90	1.074219
.077	10	0	0	9.765625E-02
.037	10	0	0	9.765625E-02
8.000001E-03	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .077 AND 3.34 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 .5071292

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1994616	.4191967	.2870378	.725763

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.775356	4.22942	.0020051

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.037474
95 PERCENT CONFIDENCE LIMITS =-.6773071 AND 4.752254

LC50 = .4369136
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .1040099
95 PERCENT CONFIDENCE LIMITS = 0 AND .4958022

DATABASE ENTRY FORM
FOR ACUTE OR CHRONIC TOXICITY STUDIES

1. Chemical Benfluralin Shaughnessy 084301

2. Common Name Of Organism Tested Bluegill Sunfish

3. Scientific Name Lepomis macrochirus

4. Age Of Organisms > 40 days (juveniles)

5. Guideline No. 72-1

6. Type Of Dosing Method (Circle One) Or Study

1. Oral 2. Dietary 3. Reproduction 4. Static
(5. Static Renewal) 6. Flowthrough 7. Acute Contact
8. Other _____

7. % AI Of Test Substance 20.1

8. Study Duration (Hrs Or Days) 96 h

9. Dose Type (Circle One) A. LD50 (B. LC50) C. EC50 D. MATC

10. Toxicity Level A. mg/kg B. ppm (C. mg/l) D. µg/l E. ng/l
F. µg/bee G. Other

11. 95% C.L.s LC_{50} = 0.42 (95% C.I. = 0.29 - 0.73)

12. Curve Slope N/A

13. NOEL 0.008

14. Study Date (YEAR) '92

15. Study Review Date (YEAR) '92-Oct-15

16. Category (Circle One) (CORE) SUPPLEMENTAL INVALID

17. MRID of Accession Number 423908-01

18. Laboratory Lilly Research Labs.

19. Reviewer L. Rifici

20. For Reproductive Studies (avian or aquatic) Indicate Which Parameter Affected At What Toxicity Level.

Eggs Layed _____ % Cracked _____ % Viable _____
% Live Embryos _____ % Egghatch _____ 14D Survivors _____
Growth Effected at _____ Other Effects _____

FORM FOR SENDING DATA TO CONTRACTOR

REVIEWER Ann Stavola

CHEMICAL Benfluralin

SHAUGH. NO. 084301

ACTION TYPE List B Phase IV

or

REVIEW CODE

DATE DUE 10/22/92 / 11/5/92

STUDIES		MRID. NO./ACC. NO.	
Acute Bluegill Tox	72-1b	42390701	6
Acute Daphnia Tox	72-2b	42390702	6
Acute Rainbow Tox	72-1d	424192-01	6