MRID No. 416138-02

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-ρ-toluidine; Lot No. 231 EF4; CAS No. 1861-40-1; 96.6% active ingredient; a yellow-orange powder.

3. **STUDY TYPE:** 72-3(a) Estuarine Fish Flow-Through Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Sousa, J.V. 1990. (Benefin) - Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions. Prepared by Springborn Laboratories, Inc., Wareham, MA. SLI Report No. 90-08-3416. Study #1982.1289.6104.505. Submitted by DowElanco Products Company. EPA MRID No. 416138-02.

5. **REVIEWED BY:**

Alvaro A. Yamhure
Aquatic Biologist, EEB/EFED
USEPA

Signature:
Date: 2/2/94

6. **APPROVED BY:**

Daniel Rieder,
Head Section 3
EEB/EFED

Signature:
Date: 2-2-94

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the requirements for a flow-through acute toxicity study using estuarine fish. The initial test concentrations decreased considerably during the 96-hour exposure period. This decrease in solubility was probably enhanced by the sea water. This large decrease in test concentrations made it very difficult to determine the actual concentrations to which the test organisms were exposed. Under these testing conditions a precise 96-hour LC_{50} value could not be determined (the reported values were >0.79 mg a.i./l for the 96-hour LC_{50} and 0.16 mg a.i./l for the NOEC) . This study is rated to be **supplemental.**

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

9. **BACKGROUND:**

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-ρ-toluidine; Lot No. 231 EF4; CAS No. 1861-40-1; 96.6% active ingredient; a yellow-orange powder.

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5. **REVIEWED BY:**

Alvaro A. Yamhure **Signature:**
Aquatic Biologist, EEB/EFED
USEPA **Date:**

6. **APPROVED BY:**

Daniel Rieder, **Signature:**
Head Section 3
EEB/EFED **Date:**

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The sheepshead minnows (*Cyprinodon variegatus*) used in this test were obtained from a commercial fish supplier in Colorado. The fish were maintained in a 500-l fiberglass tank under recirculating conditions and a photoperiod of 16 hours of light and 8 hours of darkness. The water in the culture tank had a temperature of 20-23°C, a salinity of 30-34 parts per thousand (ppt), a pH of 7.3-7.4, and a dissolved oxygen concentration of 85-95% of saturation. The fish were maintained under these conditions for a minimum of 14 days prior to test initiation. The fish were fed a dry commercial flake food daily, except during the 48 hours prior to test initiation.

No mortality was observed during the 48-hour period prior to test initiation. A representative sample of the test fish population had a mean wet weight of 0.33 (0.12-0.83) g and a mean total length of 26 (21-36) mm.

B. **Test System:** The test system consisted of a constant-flow serial diluter, a temperature-controlled water bath and a set of 14 aquaria (29.25 x 14.5 x 19 cm). Each aquarium was equipped with a 14-cm high standpipe and an 8.0-cm high siphon drain which allowed the test solution volume to fluctuate between 5.9 and 3.4 l. The flow rate to each aquarium was approximately 17 volume replacements every 24 hours.

The aquaria were impartially positioned in a water bath which was designed to maintain the test temperature at 22 ±1°C. A photoperiod of 16 hours of light and 8 hours of darkness provided light with an intensity of 60-85 footcandles at the test solution surface throughout the study period. The test solutions were not aerated.

The dilution water used was filtered (20 and 5 µm) natural seawater collected from the Cape Cod Canal, Bourne, MA. The seawater had a salinity of 32 ppt and a pH of 7.9 at test initiation.
A 204 mg/ml stock solution was prepared by dissolving 42.2 g of benefin with acetone to volume in a 200-ml volumetric flask. The stock solution was delivered to the mixing chamber at a rate of 0.025 ml/min where it was diluted with seawater (0.340 l/min) to provide the

highest test concentration. The mixing chamber was partially submerged in an ultrasonicator water bath and the solution was constantly stirred. This solution was "proportionally" diluted (60% dilution factor) to provide the remaining four concentrations.

C. **Dosage:** Seven-day flow-through acute test. Based on the results of a preliminary test, five nominal concentrations (1.9, 3.2, 5.4, 9.0, and 15 mg/l) were tested. A dilution water control and solvent control were also included in the test. The solvent control (0.074 ml/l) contained the maximum amount of acetone present in any treatment level.

D. **Design:** Twenty fish were impartially selected and distributed to each concentration and the controls (ten fish per replicate). The organism loading rate was 0.034 g/l/day.

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

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

Water samples were collected on test days 0, 4, and 7 from both replicate test solutions of each treatment level and the controls. Quality control (QC) and test samples were analyzed for benefin 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:** The mean measured concentrations were 0.16, 0.21, 0.33, 0.85, and 1.1 mg a.i./l and ranged from 6 to 9% of nominal concentrations (Table 2, attached).

Undissolved material was observed in the diluter mixing chamber and in the three highest test solutions. "Variability between measured concentration was observed between sampling intervals. This variability was attributed to the presence of undissolved material in the system's mixing chamber, establishing that the nominal exposure levels exceeded the water solubility limit of benefin under the maintained test conditions."

The cumulative percent mortalities and observations made during the test are presented in Table 3 (attached). Based on the established exposure concentration range, the 96-hour LC_{50} was >1.1 mg a.i./l. The 168-hour LC_{50} was 1.7 mg a.i./l with a 95% confidence interval of 0.98-8.6 mg a.i./l. The 96- and 168-hour NOEC were 0.16 and <0.16 mg a.i./l, respectively.

During the study, the pH ranged from 7.7 to 8.0, the dissolved oxygen concentration ranged from 6.0 to 7.7 mg/l (83 to 107% of saturation), salinity ranged from 30 to 32 ppt, and the daily temperature ranged from 22-24°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 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.

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

The report does not indicate the age of the test organisms.

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

The pH of the holding water (7.3-7.4) during the acclimation period was not the same as the pH of the water (7.7-8.0) maintained during the study. The guidelines state that the organisms must be maintained under actual test conditions for at least 48 hours prior to test initiation. The salinity of the dilution water was 30-32 ppt. The recommended salinity for estuarine fish is 10-17 ppt.

B. **Statistical Analysis:** The 96-hour LC_{50} could not be calculated due to insufficient mortality.

C. **Discussion/Results:** This study is scientifically sound but does not meet the requirements for a flow-through acute toxicity study using estuarine fish. The measured concentrations decreased considerably during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown and much lower than intended. In addition, a precise 96-hour LC_{50} value could not be determined due to insufficient mortality.
The solubility of the test material may be increased by using other solvents (i.e., dimethylformamide). Since the concentrations attained during the 7-day test were sufficient to bring about responses from the fish (i.e., sublethal effects and mortality), further testing using different solvents or test conditions may result in a precise 96-hour LC_{50}. However, due to the low solubility of the test material, the analytical samples should filtered when taken (i.e., acrodisk and syringe) or prior to analysis to exclude any undissolved material. The author states that undissolved material was observed in the three highest test solutions and the variability in the analytical results may be explained by entrainment of undissolved particles into the unfiltered water samples.

Based on the conditions of this study, the 96-hour LC_{50} was >0.79 mg a.i./l mean measured concentration. The NOEC was 0.16 mg a.i./l.

D. **Adequacy of the Study:**

(1) Classification: Supplemental.

(2) **Rationale:** The measured test concentrations decreased greatly during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown and much lower than intended. A precise 96-hour LC_{50} could not be determined.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, May 21, 1992.

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-ρ-toluidine; Lot No. 231 EF4; CAS No. 1861-40-1; 96.6% active ingredient; a yellow-orange powder.

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4. **CITATION:** Sousa, J.V. 1990. (Benefin) - Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions. Prepared by Springborn Laboratories, Inc., Wareham, MA. SLI Report No. 90-08-3416. Study #1982.1289.6104.505. Submitted by DowElanco Products Company. EPA MRID No. 416138-02.

5. **REVIEWED BY:**

Alvaro A. Yamhure
Aquatic Biologist, EEB/EFED
USEPA

Signature:

Date:

6. **APPROVED BY:**

Daniel Rieder,
Head Section 3
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8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The sheepshead minnows (*Cyprinodon variegatus*) used in this test were obtained from a commercial fish supplier in Colorado. The fish were maintained in a 500-l fiberglass tank under recirculating conditions and a photoperiod of 16 hours of light and 8 hours of darkness. The water in the culture tank had a temperature of 20-23°C, a salinity of 30-34 parts per thousand (ppt), a pH of 7.3-7.4, and a dissolved oxygen concentration of 85-95% of saturation. The fish were maintained under these conditions for a minimum of 14 days prior to test initiation. The fish were fed a dry commercial flake food daily, except during the 48 hours prior to test initiation.

No mortality was observed during the 48-hour period prior to test initiation. A representative sample of the test fish population had a mean wet weight of 0.33 (0.12-0.83) g and a mean total length of 26 (21-36) mm.

B. **Test System:** The test system consisted of a constant-flow serial diluter, a temperature-controlled water bath and a set of 14 aquaria (29.25 x 14.5 x 19 cm). Each aquarium was equipped with a 14-cm high standpipe and an 8.0-cm high siphon drain which allowed the test solution volume to fluctuate between 5.9 and 3.4 l. The flow rate to each aquarium was approximately 17 volume replacements every 24 hours.

The aquaria were impartially positioned in a water bath which was designed to maintain the test temperature at 22 ±1°C. A photoperiod of 16 hours of light and 8 hours of darkness provided light with an intensity of 60-85 footcandles at the test solution surface throughout the study period. The test solutions were not aerated.

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highest test concentration. The mixing chamber was partially submerged in an ultrasonicator water bath and the solution was constantly stirred. This solution was "proportionally" diluted (60% dilution factor) to provide the remaining four concentrations.

C. **Dosage:** Seven-day flow-through acute test. Based on the results of a preliminary test, five nominal concentrations (1.9, 3.2, 5.4, 9.0, and 15 mg/l) were tested. A dilution water control and solvent control were also included in the test. The solvent control (0.074 ml/l) contained the maximum amount of acetone present in any treatment level.

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13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

No conclusions were made by the author.
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14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

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

The report does not indicate the age of the test organisms.

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

The pH of the holding water (7.3-7.4) during the acclimation period was not the same as the pH of the water (7.7-8.0) maintained during the study. The guidelines state that the organisms must be maintained under actual test conditions for at least 48 hours prior to test initiation. The salinity of the dilution water was 30-32 ppt. The recommended salinity for estuarine fish is 10-17 ppt.

B. **Statistical Analysis:** The 96-hour LC_{50} could not be calculated due to insufficient mortality.

C. **Discussion/Results:** This study is scientifically sound but does not meet the requirements for a flow-through acute toxicity study using estuarine fish. The measured concentrations decreased considerably during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown and much lower than intended. In addition, a precise 96-hour LC_{50} value could not be determined due to insufficient mortality.
The solubility of the test material may be increased by using other solvents (i.e., dimethylformamide). Since the concentrations attained during the 7-day test were sufficient to bring about responses from the fish (i.e., sublethal effects and mortality), further testing using different solvents or test conditions may result in a precise 96-hour LC_{50}. However, due to the low solubility of the test material, the analytical samples should filtered when taken (i.e., acrodisk and syringe) or prior to analysis to exclude any undissolved material. The author states that undissolved material was observed in the three highest test solutions and the variability in the analytical results may be explained by entrainment of undissolved particles into the unfiltered water samples.

Based on the conditions of this study, the 96-hour LC_{50} was >0.79 mg a.i./l mean measured concentration. The NOEC was 0.16 mg a.i./l.

D. **Adequacy of the Study:**

(1) Classification: Supplemental.

(2) **Rationale:** The measured test concentrations decreased greatly during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown and much lower than intended. A precise 96-hour LC_{50} could not be determined.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, May 21, 1992.

Sea Water

MRID No. 416138-02

DATA EVALUATION RECORD

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5. **REVIEWED BY:**

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

Signature: Louis M Rifici for KR
Date: 6/25/92

6. **APPROVED BY:**

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

Signature: Louis M Rifici
Date: 6/25/92

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

Signature:
Date:

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the requirements for a flow-through acute toxicity study using estuarine fish. The measured concentrations decreased considerably during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown. A precise 96-hour LC_{50} value (>0.79 mg a.i./l) could not be determined in this study due to insufficient mortality. The NOEC was 0.16 mg a.i./l.

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

9. **BACKGROUND:**

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

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

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C. **Discussion/Results:** This study is not scientifically sound and does not meet the requirements for a flow-through acute toxicity study using estuarine fish. The measured concentrations decreased considerably during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown. In addition, a precise 96-hour LC_{50} value could not be determined due to insufficient mortality.

The solubility of the test material may be increased by using other solvents (i.e., dimethylformamide). Since the concentrations attained during the 7-day test were sufficient to bring about responses from the fish (i.e., sublethal effects and mortality), further testing using different solvents or test conditions may result in a precise 96-hour LC_{50}. However, due to the low solubility of the test material, the analytical samples should filtered when taken (i.e., acrodisk and syringe) or prior to analysis to exclude any undissolved material. The author states that undissolved material was observed in the three highest test solutions and the variability in the analytical results may be explained by entrainment of undissolved particles into the unfiltered water samples.

Based on the conditions of this study, the 96-hour LC_{50} was >0.79 mg a.i./l mean measured concentration. The NOEC was 0.16 mg a.i./l.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The measured test concentrations decreased considerably during the 96-hour exposure period. Therefore, the actual concentrations to which the test organisms were exposed are unknown. A precise 96-hour LC_{50} could not be determined.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, May 21, 1992.

Shaughnessey # 084301 Chemical Name Benesin (Benfluralin) 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 (%) = Slope = # Animals/Level = Temperature =	_____	_____
Species:				
Lab:				
		48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )		
MRID #		Comments:		

96-Hour LC_{50}	96.6%	LC_{50} -> 0.79 ppm* (95% C.L. N/A) Control Mortality (%) = 0 Solvent Control Mortality (%) = 0		
Species: Cyprinodon variegatus		Slope = N/A # Animals/Level = 20 Continuous Temperature = 22-24°C		
Lab: Springborn Laboratories, Inc.			H.E. 5/21/92	Supplemental 11/24/92 Of amhuy Entered
		96-Hour Dose Level pp m*/(% Mortality) 0.16 (0), 0.23 (5), 0.57 (0), 0.5 (5), 0.79 (20)		
MRID # 416138-02		Comments:		

* Based on 0- and 96-hour mean measured concentrations.