


DATA EVALUATION RECORD

1. **CHEMICAL:** Brodifacoum.
Shaughnessey No. 112701.
2. **TEST MATERIAL:** Brodifacoum bait (20 g blocks); 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one; CAS No. 56073-10-0; 0.006% w/w (50 mg brodifacoum/kg) blocks, containing heliogen blue, sugar, cornmeal, oat flour, and paraffin wax; blue waxy blocks.
3. **STUDY TYPE:** 72-1. Freshwater Fish Acute Toxicity Test.
Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).
4. **CITATION:** Sankey, S.A., J.E. Caunter, and R.D. Stanley. 1992. Brodifacoum: Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) of a 50 mg/kg Formulation. Report No. BL4614/B. Prepared by Imperial Chemical Industries PLC, Brixham, Devon, UK. Submitted by ICI Americas Inc. EPA MRID No. 426419-01.
5. **REVIEWED BY:**

Mark A. Mossler, M.S.
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Signature: 
Date: 8/9/93
6. **APPROVED BY:**

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Signature: P. Kosalwat
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Henry T. Craven, M.S.
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7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for an acute toxicity test. Extremely low DO and low pH resulting from the inert ingredients may have influenced the response of the fish to the active ingredient. Based on the conditions of the test, the 96-hour NOEC and LC₅₀ were 0.8 and 1.1 µg of brodifacoum/l, respectively (mean measured concentrations). Therefore, the test material is classified as very highly toxic to bluegill sunfish.

8. RECOMMENDATIONS: N/A.
9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

A. Test Animals: Bluegill sunfish (*Lepomis macrochirus*) were obtained from a commercial supplier in Salem, MA. The fish were acclimated to the test temperature ($22 \pm 1^\circ\text{C}$) for 31 days prior to testing. Twenty days before the test, the fish were treated with tetracycline for 5 days. Feeding was discontinued 24 hours before the test and <1% mortality occurred during the 7 days prior to testing. Terminal mean weight and length of the control fish were 0.86 g (range 0.60-1.21 g) and 34 mm (32-37 mm), respectively. Biomass loading rate in the control was 0.22 g/l.

B. Test System: The test vessels were glass aquaria measuring 610 x 305 x 385 mm. The test solution volume was 40 l. The test was performed in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) under a 16-hour light photoperiod with 10-minute low light transition periods.

The test dilution water was tap water which had been filtered and dechlorinated using sodium thiosulfate. At test initiation, the water had a hardness of 44 mg/l as CaCO_3 , an alkalinity of 22 mg/l as CaCO_3 , and a conductivity of 269 $\mu\text{S}/\text{cm}$. The pH of the water was 7.92.

C. Dosage: Ninety-six-hour static test. Five nominal concentrations (0.18, 0.32, 0.56, 1.0, and 1.8 mg brodifacoum/l) and a dilution water control were selected for the test. The solutions were prepared by adding blocks of the formulated bait to the water. Each block contained 1.2 mg of brodifacoum. Therefore, the number of blocks added to 40 l of dilution water was 6, 11, 19, 32, or 60 for the above five test concentrations, respectively.

D. Design: Ten bluegill were randomly placed in each vessel and one test vessel was used for each concentration. The fish were not fed during the test and the test solutions were gently aerated. Observations and mortality counts were made every 24 hours. Measurements were taken every 24 hours for pH,

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dissolved oxygen concentration (DO) and temperature within the test vessels.

Water samples were removed daily for chemical analysis. The concentration of brodifacoum was measured using high performance liquid chromatography.

- E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) and confidence interval were calculated using the moving average angle method.
12. **REPORTED RESULTS:** The mean measured concentrations were 0.31, 0.59, 0.77, 0.97, and 1.1 μg brodifacoum/l (Table 1, attached). These values were 0.06-0.18% of the nominal concentrations. The low levels of brodifacoum found in solution were probably due to the retention in the blocks of most of the brodifacoum. Although some cracks appeared in the blocks during the test, all were still complete at the end of the test.

There was no mortality in the control (Table 2, attached). Forty and fifty percent mortality was observed at the highest concentration level on days 3 and 4 of the study, respectively. Sublethal effects were observed at the two highest concentration levels and these consisted of gulping and surfacing (Table 3, attached). "It is likely that the dose and the time related reduction in dissolved oxygen and pH (Table 4) caused the mortalities and symptoms of toxicity. These changes in water quality probably resulted from microbiological activity associated with the sugar, flour, and cereal in the formulation. The test solutions became increasingly cloudy during the test and the blocks had a considerable growth on them by the end of the test. Bubbles of gas were also evolved from the growth on the blocks. The decrease in water clarity made observation of the fish more difficult.

As the previously reported 96 hour LC_{50} value for brodifacoum to bluegill sunfish was 0.165 mg/l it was considered unlikely that the measured concentrations of brodifacoum in the test solutions (2.4 to $<0.14 \mu\text{g/l}$) were having an acute toxic effect on the fish.

As a result of these observations the results have been quoted on the basis of the concentration of blocks in the test solutions. It is considered that the formulated blocks would not present an acute hazard to fish unless they were present at very high concentrations."

The 96-hour LC_{50} value was 1.5 blocks/l (95% C.I. = 1.2-3.2 blocks/l). The no-observed-effect concentration (NOEC) was 0.48 blocks/l.

During the test, the DO was 0.8-9.0 mg/l. The pH was 4.68-7.76 and the temperature ranged from 21.2 to 22.1°C (Table 4, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were presented.

Good laboratory practice and quality assurance statements were included in the report stating compliance with OECD principles and, indirectly, with 40 CFR Part 160.

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 material was a formulated product, a technical grade material is required.

Since the test material was a formulated product, control(s) containing the inert or carrier ingredients present in the formulation (i.e., blocks without brodifacoum) should have been included in the test design.

The test dilution water was dechlorinated tap water. Dechlorinated tap water is not recommended. However, free and combined residual chlorine were monitored in the dilution water and remained $<4 \mu\text{g Cl}_2/\text{l}$.

Continuous (hourly) temperature measurements were not recorded in at least one vessel.

B. **Statistical Analysis:** Statistical analysis using EPA's Toxanal program indicated that the 96-hour LC_{50} was 1.1 $\mu\text{g brodifacoum/l}$, based on mean measured concentrations.

C. **Discussion/Results:** The reviewer agrees with the authors that the mortality observed was due to decreasing DO and pH. The reviewer also believes that this study adequately represents what would happen to fish in a static system when this formulated bait is introduced. However, if the purpose of this study is to determine the acute toxic effects of the active ingredient in the formulation, the LC_{50} and NOEC

generated from this study may not represent the actual values due to the confounding effects from other factors (i.e., pH and DO).

This study is not scientifically sound and does not meet the guideline requirements for an acute toxicity test. Based on the conditions of the test, the 96-hour NOEC and LC₅₀ were 0.8 and 1.1 µg of brodifacoum/l, respectively (mean measured concentrations). Therefore, the test material is classified as very highly toxic to bluegill sunfish.

D. Adequacy of the Study:

- (1) Classification: Invalid.
- (2) Rationale: Extremely low DO and low pH resulting from the inert ingredients may have influenced the response of the fish to the active ingredient.
- (3) Repairability: No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 7-28-93.

Page _____ is not included in this copy.

Pages 6 through 9 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
- _____ Identity of product impurities.
- _____ Description of the product manufacturing process.
- _____ Description of quality control procedures.
- _____ Identity of the source of product ingredients.
- _____ Sales or other commercial/financial information.
- _____ A draft product label.
- _____ The product confidential statement of formula.
- _____ Information about a pending registration action.
- ☒ _____ FIFRA registration data.
- _____ The document is a duplicate of page(s) _____.
- _____ The document is not responsive to the request.
- _____ Internal deliberative information.
- _____ Attorney-Client work product.
- _____ Claimed Confidential by submitter upon submission to the Agency.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.1	10	5	50	62.30469
.97	10	0	0	9.765625E-02
.77	10	0	0	9.765625E-02
.59	10	0	0	9.765625E-02
.31	10	0	0	9.765625E-02

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 1.1

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

Ecological Effects Branch One-Liner Data Entry Form

Chemical Brodifacoum

Shaughnessy No. 112701

Pesticide Use Rodenticide

AQUATIC VERTEBRATE TOX.	% AI	LC ₅₀ (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. <u>Lepomis macrochirus</u>	0.000%	1.1mg/l [*] (4/24) ADD	96 hr static	0.81ug/l [*]	1992/1993	42649-01 Laval 110	JCE	MM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC ₅₀	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								

COMMENTS: * mean assessed concentration