

Reviewer R. Lamb

③ 2-EEB-22
12-20-02
7/30/92
MRID No. 416871-01

Document Number



2001058

DATA EVALUATION RECORD

1. **CHEMICAL:** Proxel.
Shaughnessey No. 098901.
2. **TEST MATERIAL:** Proxel Press Paste; Code No. ADH 220040; a grey paste.
3. **STUDY TYPE:** Freshwater Fish Flow-Through Acute Toxicity Test. Species Tested: Rainbow Trout (*Salmo gairdneri*).
4. **CITATION:** Brown, D. 1979. "Proxel Press Paste": Determination of the Acute Toxicity to Rainbow Trout *Salmo gairdneri*. Project ID. BL/B/1986. Prepared by ICI Brixham Laboratory, Brixham, Devon, UK. Submitted by ICI Americas Inc., Wilmington, DE. EPA MRID No. 416871-01.
5. **REVIEWED BY:**

Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Louis M. Rifici</i> Date: 7/30/92
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6. **APPROVED BY:**

Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Rosemary Graham Mora</i> Date: 7/30/92
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature: <i>Henry T. Craven</i> Date: 12/20/02
7. **CONCLUSIONS:** This test is scientifically sound but does not meet the guideline requirements for an acute toxicity test using rainbow trout. The percentage active ingredient of the test material was not reported. Based on mean measured concentrations of whole product, the 96-hour LC₅₀ value was 1.6 mg/l. Therefore, Proxel Press Paste is classified as moderately toxic to rainbow trout. The NOEC was 0.82 mg/l mean measured concentration of whole product.
8. **RECOMMENDATIONS:** See Section 14.D.(3).
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

- A. Test Animals:** The rainbow trout (*Salmo gairdneri*) used in the test were obtained from a commercial supplier in Wiltshire, UK, and held in the laboratory for 24 days prior to testing. The fish were acclimated to the test vessels for 3 days and to the test temperature ($12 \pm 1^\circ\text{C}$) for 2 days. The mean length and weight of the test fish were 70 (55-77) mm and 4.41 (2.13-5.70) g, respectively.
- B. Test System:** A continuous flow-through system was used. Concentrated stock solutions and dilution water were fed to mixing chambers using peristaltic pumps. Mixed solutions were passed into the fish vessels. The test containers were 20-l glass vessels. The flow rate into the vessels was 20 ml/minute resulting in a 95% exchange in 4.5 hours.

The test dilution water was supplied from a 20,000 gallon reservoir. A stock solution of Proxel Press Paste was prepared by mixing 45 g of test material in 18 l of water at 50°C for 3 hours. The mixture was cooled overnight and filtered to remove a small amount of undissolved material. The filtrate (2.3 g/l of Proxel Press Paste) was used to prepare diluter stock solutions.

- C. Dosage:** Ninety-six-hour flow-through test. Five nominal concentrations (0.75, 1.0, 1.8, 3.2, and 4.2 mg/l) and a dilution water control were used. An additional level (5.6 mg/l) with a dilution water control was tested 5 days after the completion of the first test. The concentrations were expressed as mg/l of whole product.
- D. Design:** Twenty fish were used per concentration and control. The number of deaths was determined daily.

Dissolved oxygen concentration (DO), temperature, hardness, and pH were measured daily in vessels containing live fish.

Water samples were collected daily from all vessels containing live fish. The concentration of 1,2-benzisothiazolin-3-one (BIT) was determined using ultra-violet spectrophotometry. The measured concentrations were converted to mg/l of Proxel Press Paste.

E. **Statistics:** The median lethal concentrations (LC_{50}) and associated 95% confidence intervals (C.I.) were calculated using probit analysis.

12. **REPORTED RESULTS:** The mean measured concentrations for the initial test were 0.67, 0.82, 1.41, 2.84, and 3.64 mg/l and ranged from 78 to 89% of nominal concentrations (Table 2, attached).

Mortality was presented in Table 1 (attached). The 96-hour LC_{50} value was 1.6 mg/l (95% C.I. = 1.4-1.8 mg/l) as whole product.

During the first test, DO ranged from 7.7 to 10.6 mg/l. The pH values ranged from 7.3 to 7.8. The temperature was 11.9-12.8°C. The hardness of the dilution water ranged from 31.5 to 39.0 mg/l as $CaCO_3$.

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

The study was performed for non-regulatory purposes and the report was not subject to formal GLP requirements. No quality assurance statement was included.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures generally followed the SEP, except for the following:

The percentage active ingredient of the test material was not reported.

The system used to maintain solution temperature was not described in the report. Test solution temperature should be measured at least hourly in tests using air temperature to control solution temperature and at least every six hours in tests using a water bath.

The acclimation period (2-3 days) was shorter than recommended (2 weeks).

The hardness of the dilution water (31.5-39.0 mg/l as $CaCO_3$) was lower than recommended (40-200 mg/l as $CaCO_3$). In addition, the source of the dilution water was not described.

The photoperiod was not reported. A 16-hour light/8-hour dark photoperiod with 15- to 30-minute dawn and dusk simulations is recommended.

Given a mean fish weight of 4.41 g, a flow rate of 20 ml/minute (28.8 l/day), and a solution volume of 20 l, the biomass loading in the test chambers was approximately 3.1 g/l/day. The recommended loading for coldwater fish is 1 g/l/day.

The weight range of the test fish was 2.13-5.70 g. The SEP states that fish between 0.1 and 5 g should be used.

B. Statistical Analysis: Since data from the initial test were sufficient to produce an LC₅₀ value, data from the second test were not included in the reviewer's analysis. The reviewer used EPA's Toxanal program to determine the 96-hour LC₅₀ value and obtained similar results (see attached printout). The no-observed-effect concentration (NOEC) was 0.82 mg/l.

C. Discussion/Results: This test is scientifically sound but does not meet the guideline requirements for an acute toxicity test using rainbow trout. The percentage active ingredient of the test material was not reported. Based on mean measured concentrations of whole product, the 96-hour LC₅₀ value was 1.6 mg/l, therefore Proxel Press Paste is classified as moderately toxic to rainbow trout. The NOEC was 0.82 mg/l mean measured concentration of whole product.

D. Adequacy of the Study:

(1) **Classification:** Supplemental.

(2) **Rationale:** The percentage active ingredient of the test material was not reported.

(3) **Repairability:** The registrant must submit the percentage active ingredient of the test material (Code No. ADH 220040) used in this test. The test may be upgraded to "core" upon submission of the missing information.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 07-20-92.

TABLE 1

CONCENTRATIONS TESTED AND CORRESPONDING MORTALITIES

Material tested : "Proxel Press Paste"
 Species tested : Rainbow Trout

Nominal concn. (mg/l)	Mean measured concn. ¹ (mg/l)	Mortality observed ²			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
5.6	4.46	20	-	-	-
4.2	3.64	9	20	-	-
3.2	2.84	2	14	20	-
1.8	1.41	0	1	4	6
1.0	0.82	0	0	0	0
0.75	0.67	0	0	0	0
Control ³	-	0	-	-	-
Control ⁴	-	0	0	0	0

- NOTES :
1. See Table 2 for analytical results from which these mean concentrations were derived.
 2. 20 fish/test concentration
 3. Control for 5.6 mg/l level
 4. Control for all other levels.

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RIFICI PROXEL PRESS PASTE SALMO GAIRDNERI 7-20-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.64	20	20	100	9.536742E-05
2.84	20	20	100	9.536742E-05
1.41	20	6	30	5.765915
.82	20	0	0	9.536742E-05
.67	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .82 AND 2.84 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 1.650189

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.

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Study/Species/Lab/
MRID # _____ Chemical
% a.i. _____ Results _____ Reviewer/ Validation
Date _____ Status _____

48-Hour EC₅₀ _____
EC₅₀ - _____ PP (_____) 95% C.L. _____ Control Mortality (%) - _____
Slope - _____ # Animals/Level - _____ Solvent Control Mortality (%) - _____
Temperature - _____

Lab: _____
MRID # _____
48-Hour Dose Level pp / (% Effect)
(_____), (_____), (_____), (_____)

96-Hour LC₅₀ _____
LC₅₀ - 1.6 ^{*} 95% C.L. Probit
ppM (1.4 - 1.8) Control Mortality (%) - 0
Slope - not Reported # Animals/Level - 20 Solvent Control Mortality (%) - N/A
Temperature - 4°C

Species: Salmo gairdneri
Lab: 101 Bringham laboratory
MRID # 416 871-01
96-Hour Dose Level ppM / (% Mortality)
0.67 (0), 0.82 (0), 1.41 (30), 2.84 (100), 3.64 (100)
Comments: * mean measured concentration
LMR 7/20/98
Supplemental



TABLE 2

MEASURED LEVELS IN TEST VESSELS

Test material : "Proxel Press Paste"
 Species tested : Rainbow trout

Nominal concn. (mg/l)	Nominal exposure time (hrs.)	Concentration determined (mg/l)	Mean concn. (mg/l)	% Nominal
5.6	24	4.46	4.46	80%
	48	-		
	72	-		
	96	-		
4.2	24	3.65	3.64	87%
	48	3.62		
	72	-		
	96	-		
3.2	24	2.83	2.84	89%
	48	2.95		
	72	2.73		
	96	-		
1.8	24	1.43	1.41	78%
	48	1.47		
	72	1.36		
	96	1.39		
1.0	24	0.90*	0.82	82%
	48	0.81*		
	72	0.78		
	96	0.77		
0.75	24	0.71	0.67	89%
	48	0.66		
	72	0.67		
	96	0.65		

* Mean of 3 determinations : 0.83, 0.79, 0.81.