MRID No. 00133876

DATA EVALUATION RECORD

1. CHEMICAL: Proxel.
Shaughnessey No. 098901.

2. TEST MATERIAL: Proxel Press Paste; Batch No. 876, ADB 24276/76; 77.6% 1,2-benzisothiazolin-3-one (BIT), [redacted]

3. STUDY TYPE: Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Daphnia magna*.

4. CITATION: Thompson, R.S. and D.V. Smyth. 1977. Determination of the Acute Toxicity of Proxel Press Paste (Active Ingredient 1,2-Benzisothiazolin-3-one) to *Daphnia magna*. Report No. BL/B/1779. Prepared by Imperial Chemical Industries Limited, Brixham Laboratory, Brixham, Devon, UK. Submitted by ICI Americas Inc., Wilmington, DE. EPA MRID No. 00133876.

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6. APPROVED BY:

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

Signature: [signature] 1-16-92

Date: [signature] 7-20-92

7. CONCLUSIONS: This test is not scientifically sound. Since the test was performed in 1976, there were several significant differences in its design compared to the design recommended by the SEP, and the results are probably not comparable to tests meeting present-day standards. Based on nominal concentrations of whole product, the most conservative 48-hour EC_{50} value generated was 1.1 mg/l, therefore Proxel Press Paste is classified as moderately toxic to daphnids. The NOEC was 0.056 mg/l nominal concentration of whole product.

8. RECOMMENDATIONS: N/A.

Manufacturing process information is not included

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The *Daphnia magna* (12 ±12 hours old) used in the test were taken from in-house cultures maintained in glass beakers containing 1.5 l of reconstituted water at 19 ±1°C. The photoperiod was 16-hour light/8-hour dark and the cultures were fed *Chlorella vulgaris* daily. The daphnids used in the test were obtained by straining the cultures through nylon mesh.

B. Test System: The test vessels were graduated borosilicate glass test tubes. The tubes were randomly assigned to a test tube rack and maintained in total darkness at 19 ±0.5°C.

The test dilution water was soft reconstituted water prepared by dissolving $NaHCO_3$ (48 mg/l), $CaSO_4.2H_2O$ (30 mg/l), $MgSO_4$ (30 mg/l), and KCl (2 mg/l) in glass distilled water. A stock solution of Proxel Press Paste was prepared in distilled water within 24 hours of test initiation. Secondary stocks were prepared in dilution water by serial dilution and added to the test chambers. The final test concentrations were prepared by the addition of dilution water. The final test solution volume was 10 ml.

C. Dosage: Forty-eight-hour static tests. Three tests were performed. The concentrations were 1) control, 0.056, 0.18, 0.32, 0.56, and 1.33 mg/l; 2) control, 0.18, 0.75, 1.33, 2.4, and 3.2 mg/l; and 3) control, 0.18, 1.0, 1.33, 1.8, and 2.4 mg/l. The concentrations were expressed as mg/l of whole product.

D. Design: Five daphnids were added to each vessel, four vessels per concentration. The number of immobilized daphnids was determined after 24 and 48 hours. "*Daphnia* were recorded as immobile if they were unable to maintain active swimming for 5 seconds after agitation of the tube."

Dissolved oxygen concentration (DO) and pH were measured at 0 and 24 hours in solutions maintained concurrently but containing no daphnids. At termination, the individual replicate solutions were

pooled and the DO and pH measured. Temperature was monitored continuously using a recording thermograph.

Water samples from the two highest test concentrations of tests 2 and 3 were collected at the beginning (in the extra solutions) and end of each test (in the pooled solutions). The concentration of 1,2-benzisothiazolin-3-one (BIT) was determined using ultra-violet spectrophotometry.

E. Statistics: The median effective concentrations (EC_{50}) and associated 95% confidence intervals (C.I.) were calculated using the method of Litchfield and Wilcoxon (1949).

12. REPORTED RESULTS: The analytical measurements performed during test 2 and 3 were presented in Table 3 (attached).

The immobilization of the daphnids in the three tests were presented in Table 1 (attached). Based on the results of test 3, the 48-hour EC_{50} value was 1.35 mg/l (95% C.I. = 1.19-1.53 mg/l) as whole product. The 48-hour EC_{50} value based on the concentration of BIT was 1.05 mg/l (95% C.I. = 0.92-1.19 mg/l).

Dissolved oxygen levels were 94.5 to 100% of saturation in all tests. The pH values ranged from 7.0 to 7.65. The temperature was reported as 18.5-19.5°C.

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

The study was performed in 1976 before the adoption of formal GLP principles. No quality assurance statement was included in the report.

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

A. Test Procedure: The test procedures did not conform with the SEP in the following ways:

The test was performed in total darkness. A 16-hour light photoperiod and 15- to 30 minute dawn and dusk simulations are recommended.

The test vessels were glass test tubes containing 10 ml of test solution and 5 daphnids. The loading was therefore 1 daphnid/2 ml. The recommended test containers are 250-ml glass beakers containing 200 ml

of solution and 10 daphnids giving a loading of 1 daphnid/20 ml.

The test design for a formulated product study should include a control where organisms are exposed to just the carrier and/or inert ingredients. Such a control, specifically including [redacted] should have been included in the tests.

The length of time between test solution preparation and test initiation was not reported.

The report did not state whether the daphnids were randomly placed in the test vessels. Test daphnids must be randomly distributed to the test vessels.

Manufacturing process information is not included

B. Statistical Analysis: The reviewer used EPA's Toxanal program to determine the EC_{50} values for tests 2 and 3. The 48-hour EC_{50} for test 2 was 1.1 mg/l (95% C.I. = 0.91-1.3 mg/l) and for test 3 was 1.3 mg/l (95% C.I. = 1.1-1.5 mg/l). These values were nominal concentrations of whole product (see attached printouts). The no-observed-effect concentration (NOEC) was determined to be 0.056 mg/l.

C. Discussion/Results: This test is not scientifically sound. Since the test was performed in 1976, there were several significant differences in its design compared to the design recommended by the SEP. The small test vessels and lack of a photoperiod (tests were run in total darkness) suggest that the results are not comparable to tests meeting present-day standards. Based on nominal concentrations of whole product, the most conservative 48-hour EC_{50} value generated (reviewer calculated) was 1.1 mg/l, therefore Proxel Press Paste is classified as moderately toxic to daphnids. The NOEC was 0.056 mg/l nominal concentration of whole product.

D. Adequacy of the Study:

(1) **Classification:** Invalid.

(2) **Rationale:** The test design significantly deviated from the recommended design.

(3) **Repairability:** No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 06-03-92.

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TABLE 1

IMMOBILISATION OF DAPHNIA

Time (hrs)	Nominal concn of Proxel Press Paste (mg/l)	Series 1		Series 2		Series 3	
		No of Daphnia	% effect	No of Daphnia	% effect	No of Daphnia	% effect
24	0 (Control)	20	0	21	0	20	0
	0.056	23	0	–	–	–	–
	0.18	21	4.8	24	0	21	0
	0.32	21	0	–	–	–	–
	0.56	21	0	–	–	–	–
	0.75	–	–	20	0	–	–
	1.0	–	–	–	–	23	8.7
	1.33	21	28.6	21	9.5	20	35.0
	1.8	–	–	–	–	21	42.9
	2.4	–	–	21	90.5	23	60.9
	3.2	–	–	20	100	–	–
48	0 (Control)	20	15.0	21	0 0	20	0 0
	0.056	23	0 0	–	–	–	–
	0.18	21	14.8	24	28.3	21	0 0
	0.32	21	29.5	–	–	–	–
	0.56	21	14.8	–	–	–	–
	0.75	–	–	20	15.0	–	–
	1.0	–	–	–	–	23	28.7
	1.33	21	733.3	21	419.0	20	155.0
	1.8	–	–	–	–	21	571.4
	2.4	–	–	21	2100	23	2300
	3.2	–	–	20	2300		

NB : Only data enclosed by heavy line (Series 3) used for calculation of EC_{50}'s.

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TABLE 3

ANALYTICAL RESULTS

Series	Nominal concn Proxel Press Paste (mg/l)	Time (hrs)	Measured concn Prosel Press Paste (mg/l)
2	3.2	0 48	3.15 2.7
	2.4	0 48	2.55 2.55
3	1.8	0 48	1.75 1.75
	2.4	0 48	2.1 2.15

RIFICI PROXEL PRESS PASTE DAPHNIA MAGNA 6-3-92 Test 2

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.2	20	20	100	9.536742E-05
2.4	21	21	100	4.768372E-05
1.33	21	4	19.04762	.359869
.75	20	1	5	2.002716E-03
.18	24	2	8.333334	.0017941

THE BINOMIAL TEST SHOWS THAT 1.33 AND 2.4 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 1.604987

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	4.656381E-02	1.114432	.9120573 - 1.342328

~~1.342328~~

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RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	7.494907	20.71414	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 3.139604
95 PERCENT CONFIDENCE LIMITS =-5.455634 AND 11.73484

LC50 = 1.287523
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .5072771
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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RIFICI PROXEL PRESS PASTE DAPHNIA MAGNA 6-3-92 Test3

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2.4	23	23	100	1.192093E-05
1.8	21	15	71.42858	3.917694
1.33	20	11	55	41.19014
1	23	2	8.695652	3.302097E-03
.18	21	0	0	4.768372E-05

THE BINOMIAL TEST SHOWS THAT 1 AND 2.4 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 1.29469

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0743602	1.32642	1.142939	1.517006

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.116336	1	.318214

SLOPE = 8.271393

95 PERCENT CONFIDENCE LIMITS = 5.450181 AND 11.09261

LC50 = 1.395278

95 PERCENT CONFIDENCE LIMITS = 1.258922 AND 1.536584

LC10 = .9797594

95 PERCENT CONFIDENCE LIMITS = .7815065 AND 1.112019

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