2-EEB-30 b
(12-22-02)

Document Number

Reviewer:

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MRID No. 429450-01
AQ-a-SMN

DATA EVALUATION RECORD

1. **CHEMICAL:** Proxel Press Paste (Benzisothiazolin). Shaughnessey No. 098901.

2. **TEST MATERIAL:** Proxel press paste; 1,2-benzisothiazol-3(2H)-one; CAS No. 2634-33-5; purity of 76.1% w/w; a moist brown powder

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

4. **CITATION:** Roberts, G.C., J.E. Caunter, and A.J. Grinell. 1993. Proxel Press Paste: Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*). Project ID No. X060/A. Prepared by Brixham Environmental Laboratory, ZENECA Limited, Brixham, Devon, UK. Submitted by ZENECA Inc., Wilmington, DE. EPA MRID No. 429450-01.

5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is not scientifically sound ~~and~~ but does not meet the guideline requirements for an acute toxicity test using sheepshead minnows. The highest measured concentrations were more than twice the lowest measured concentrations. Based on mean measured concentrations, the 96-hour LC_{50} was 16.0 mg proxel press paste/l. Therefore, proxel press paste is classified as slightly toxic to sheepshead minnows. The NOEC was 4.4 mg proxel press paste/l.

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

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5.25

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Sheepshead minnows (*Cyprinodon variegatus*) were obtained from a commercial supplier in Salem, MA. The fish were acclimated to the test temperature (22 ±1°C) for 10 days prior to testing. Feeding was discontinued 48 hours before the test and no mortality occurred during the 3 months prior to testing. Mean weight and length of the fish were 0.57 g (range 0.33-0.79 g) and 27 mm (range 23-30 mm), respectively. Biomass loading rate in the control was 0.38 g/l.

B. **Test System:** The test was conducted under static conditions. The test containers were glass vessels measuring 360 x 230 x 230 mm. The test solution volume was 15 l. The treatment solutions were prepared by addition of the test material directly to dilution water. The solutions were thoroughly stirred. The test was performed in a temperature-controlled room (22 ±1°C) under a 16-hour light photoperiod with 10-minute transitions.

The test dilution water was filtered (10- and 25-µm) seawater which had been collected from Tor Bay, Devon, UK. At test initiation, the salinity and pH of the seawater were 35.1 parts per thousand (ppt) and 8.1, respectively.

C. **Dosage:** Ninety-six-hour static test. Five nominal concentrations (3.2, 5.6, 10, 18, and 32 mg proxel press paste/l) and a dilution water control were selected for testing.

D. **Design:** Ten minnows were randomly placed in each vessel and one test vessel was used for each treatment or control. The fish were not fed during the test. Test vessels were aerated during the third and fourth days of testing. Observations and mortality counts were made every 24 hours. Measurements of pH, dissolved oxygen concentration (DO), and temperature were taken daily. Temperature of the dilution water control was measured continuously.

Water samples were removed at test initiation, 48 hours after initiation, and at termination for analysis. The

samples were collected from the mid-point of the vessels. The concentration of 1,2-benzisothiazol-3(2H)-one was measured using high performance liquid chromatography.

E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) and confidence interval (C.I.) were calculated using the moving average angle method.

12. **REPORTED RESULTS:** Brown test material was present on the bottom of the treatment vessels and reported mean values are based on the mean of the 48- and 96-hr values, since the test material had not fully dissolved at test initiation. The mean measured concentrations were reported as 3.4, 5.7, 11, 19, and 33 mg proxel press paste/l (Table 1, attached). These values were 102-110% of nominal concentrations.

There was no mortality in the control or three lowest-concentration treatment groups (Table 2, attached). Sublethal effects and/or mortality were observed at the three highest-concentration levels (Table 3, attached). The 96-hour LC_{50} value was 22 mg proxel press paste/l (95% C.I.= 18-26 mg proxel press paste/l). The no-observed-effect concentration (NOEC) was 5.7 mg proxel press paste/l.

During the test, the DO was 4.2-7.0 mg/l. The pH was 7.7-8.1 and the temperature ranged from 21.2 to 22.3°C.

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 deviated as follows:
Precipitate noted on bottom of test vessels
The salinity of the dilution water was 35 ppt. The recommended salinity for euryhaline fish like the sheepshead minnow is 10-17 ppt.

The highest measured concentrations were more than twice the lowest measured concentrations at all treatment levels.

B. **Statistical Analysis:** The reviewer assumes that corrections were made to the analytical results so that

results are in terms of the total product (proxel press paste).

Mean (0, 48, and 96 hr) measured concentrations and EPA's Toxanal program were used to determine the 96-hour LC_{50}. A more conservative result was obtained using the binomial test (see attached printout). The 96-hour LC_{50} value was 16.0 mg proxel press paste/l (95% C.I.= 8.0-20.7 mg proxel press paste/l).

C. **Discussion/Results:** It is apparent that this test should have been initiated after the material had completely dissolved in the dilution water. Therefore, this study is not scientifically sound and does not meet the guideline requirements for an acute toxicity test using sheepshead minnows. Based on mean measured concentrations, the 96-hour LC_{50} was 16.0 mg proxel press paste/l. Therefore, proxel press paste is classified as slightly toxic to sheepshead minnows. The NOEC was 4.4 mg proxel press paste/l.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The highest measured concentrations were more than twice the lowest measured concentrations at all treatment levels.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 12-6-93.

TABLE 1

PROXEL PRESS PASTE: ACUTE TOXICITY TO SHEEPSHEAD MINNOW

CONCENTRATIONS OF PROXEL PRESS PASTE IN TEST VESSELS DETERMINED BY QUANTIFICATION OF 1-2-BENZISOTHIAZOL-3(2H)-ONE

Sponsor:	ZENECA Biocides
Test substance:	Proxel press paste
Test organism:	Sheepshead minnow (*Cyprinodon variegatus*)
Test water:	Seawater

Nominal concn. of Proxel press paste (mg/l)	Measured concentration of Proxel press paste mg/l			Mean measured concn. of Proxel press paste*		Mean Measured Conc. (0, 48, 96 h)
	0 h (5 April 1993)	48 h (7 April 1993)	96 h (9 April 1993)	mg/l	% of nominal	
3.2	1.0	3.3	3.5	3.4	106	2.6
5.6	1.9†	5.9†	5.5	5.7	102	4.4
10	2.9	11	10	11	110	8.0
18	4.7	19	19	19	106	14.2
32	8.4	33	TD	33	103	20.7
Dilution water control	<0.0099	<0.01	<0.01	<0.01	N/A	

†Mean of triplicate analyses: 0 h: 2.2, 1.7, 1.7 mg/l
48 h: 5.9, 5.9, 6.0 mg/l

*Values are the mean of 48 and 96 h values since at 0 h the test substance had not fully dissolved. The test substance was observed on the bottom of the tank. This was backed up by the analysis. The nominal 32 mg/l test was terminated before the end of the study, therefore no analysis was performed. The value at 48 h was used as the measured concentration of Proxel press paste.

N/A Not applicable.

TD Terminated due to complete mortality.

TABLE 2

PROXEL PRESS PASTE: ACUTE TOXICITY TO SHEEPSHEAD MINNOW

SUMMARY OF PERCENTAGE MORTALITIES DURING TEST

Sponsor:	ZENECA Biocides
Test substance:	Proxel press paste
Test organism:	Sheepshead minnow (*Cyprinodon variegatus*)
Test water:	Seawater

Nominal concn. of Proxel press paste (mg/l)	Mean measured concn. of Proxel press paste (mg/l)	Cumulative percentage mortality observed			
		24 h (6 April 1993)	48 h (7 April 1993)	72 h (8 April 1993)	96 h (9 April 1993)
3.2	3.4	0	0	0	0
5.6	5.7	0	0	0	0
10	11	0	0	0	0
18	19	0	0	10	20
32	33	20	100	100	100
Dilution water control	<0.01	0	0	0	0

Ten fish were exposed in each test concentration and in the dilution water control.

TABLE 3

PROXEL PRESS PASTE: ACUTE TOXICITY TO SHEEPSHEAD MINNOW

SYMPTOMS OF TOXICITY OBSERVED

Sponsor:	ZENECA Biocides
Test substance:	Proxel press paste
Test organism:	Sheepshead minnow (*Cyprinodon variegatus*)
Test water:	Seawater

Nominal concn. of Proxel press paste (mg/l)	Mean measured concn. of Proxel press paste (mg/l)	Exposure time in hours			
		24 h (6 April 93)	48 h (7 April 93)	72 h (8 April 93)	96 h (9 April 93)
3.2	3.4	A	A	A	A
5.6	5.7	A	A	A	A
10	11	C/a	C/ae	C/ce	B/e
18	19	C/abcde	C/abcdefghi	C/bcdehi	C/bcdehi
32	33	C/abcdefgh	C†	TD	TD

Definition of symbols

A	=	No significant effect. 10% or less of test population dead or exhibiting symptoms of toxicity.			
B	=	Partial effect. Between 11% and 30% of test population dead or exhibiting symptoms of toxicity.			
C	=	Significant effect. More than 30% of test population dead or exhibiting symptoms of toxicity.			
TD	=	Terminated	†	=	Mortalities only
a	=	Surfacing	e	=	Rapid respiration
b	=	Quiescent	f	=	Weak
c	=	Sounding	g	=	Ceased swimming
d	=	Dark discoloured	h	=	Loss of balance
			i	=	Laboured respiration

Ecological Effects Branch One-Liner Data Entry Form

Chemical Proxel Press Pask (Benzisothiazolin) **Shaughnessy No.** 098901 **Pesticide Use** Unknown

AQUATIC VERTEBRATE TOX.	% AI	LC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Cyprinodon variegatus	76.1	16.0 mg/l * (8.0-20.7) n/a	96 hr Static	4.4 mg/l *	1993/1993	429450-01 Invalid	ZEN **	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: * - based on mean measured concentration of proxel press pask. ** ZEN = ZENECA Inc.

MOSSLER PROXEL PRESS PASTE CYPRINODON VARIEGATUS 12-6-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
20.7	10	10	100	9.765625E-02
14.2	10	2	20	5.46875
8	10	0	0	9.765625E-02
4.4	10	0	0	9.765625E-02
2.6	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 8 AND 20.7 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 15.98847

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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