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12-20-02

Document Number

Reviewer

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MRID No. 429524-01

IV-a-045

DATA EVALUATION RECORD

1. **CHEMICAL:** Benzisothiazolin, Proxel Press Paste. Shaughnessey Number: 098901.

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

3. **STUDY TYPE:** 72-3. Mollusc 48-hour Embryo-Larval Study. Species Tested: Pacific Oyster (*Crassostrea gigas*).

4. **CITATION:** Roberts, G.C., J.E. Caunter, and P.A. Johnson. 1993. Proxel Press Paste: Acute Toxicity to Larvae of the Pacific Oyster (*Crassostrea gigas*). Laboratory Study ID No. X060/D. Study performed by Brixham Environmental Laboratory, ZENECA Limited, Brixham, Devon, UK. Submitted by ZENECA Inc., Wilmington, DE. EPA MRID No. 429524-01.

5. **REVIEWED BY:**

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Associate Scientist
KBN Engineering and
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Signature: Rosemary Graham Mora
Date: 1/4/94

6. **APPROVED BY:**

Mark A. Mossler, M.S.
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Project Officer, EEB/EFED
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Date: 12/20/02

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity study using mollusc embryos and larvae. Based on percentage reduction in normal development of larvae and nominal concentrations, the 48-hour EC_{50} was 62 µg/l which classifies Proxel press paste as very highly toxic to the embryos of *Crassostrea gigas*. The NOEC was 32 µg/l nominal concentration.

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

9. **BACKGROUND:**

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

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11. MATERIALS AND METHODS:

A. Test Animals: Embryos of the Pacific oyster (*Crassostrea gigas*) were obtained by inducing oysters to spawn. Adult oysters were obtained from Guernsey Sea Farms, Channel Islands. The adult oysters were placed in 2-l glass beakers containing filtered seawater (20°C; 32 parts per thousand [ppt]). The beakers were placed in a water bath and the oysters were induced to spawn by raising the water temperature to 28°C, followed by cooling to approximately 20°C. Heat deactivated sperm suspension was added to each beaker containing a female oyster. To provide the embryo suspension used in this study, the egg suspension from one female was fertilized with the sperm from the induced gonad of one male. The embryo suspension was maintained at 20 ±1°C and agitated gently on an orbital shaker until test initiation.

B. Test System: The study was conducted in 250-ml glass beakers with loose-fitting lids, each containing 200 ml of test solution. The test temperature was maintained at 20 ±1°C by controlling the room temperature. A photoperiod of 16 hours of light with 10-minute transition periods was provided.

The dilution water was natural saltwater collected from Tor Bay, Devon. The seawater was filtered (0.2 µm) and adjusted to a salinity of 32 ppt with distilled water prior to use as dilution water.

A primary stock solution (10 mg/l) was prepared by dissolving 0.0107 g of test material in 1 l of dilution water. This stock was clear and colorless after 3 minutes of ultrasonic treatment. One liter of each exposure solution was prepared by adding appropriate amounts of stock solution to dilution water.

C. Dosage: Forty-eight-hour, static test. Six nominal concentrations (10, 18, 32, 56, 100, and 180 µg of proxel press paste/l) were used in this study. In addition, a dilution water control was included.

D. Design: The test was initiated with embryos which were 2 hours post-fertilization. A 0.760-ml aliquot of embryo suspension was added to each vessel, inoculating the vessels in a random sequence. Four replicates of the control and two replicates of each exposure concentration were prepared. Three additional vessels containing control water were inoculated for the

determination of stocking density which was 20 embryos/ml.

At 24 and 48 hours, visual observations of the larvae in the test solutions were recorded. After 48 hours, each vessel was mixed with a perforated plunger, 20 ml of solution removed, and 1 ml subsamples observed for the enumeration of normal and abnormal oyster larvae using a Sedgewick-Rafter counting cell.

The salinity of the dilution water control and of the 180 µg/l excess test solution was determined at test initiation. At the start of the test, the pH and dissolved oxygen concentration (DO) were measured in the excess test solution of each treatment. At test termination, the pH and DO in one replicate of each solution were measured. The temperature was measured daily in one replicate of each treatment and hourly in one additional vessel containing dilution water only.

Each test solution and the stock solution were sampled at test initiation and termination for determination of the test substance concentrations using high performance liquid chromatography. The analytical limit of detection was 62-91 µg/l.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal oyster larvae of each exposure concentration when compared to the control data. The median effective concentration (EC_{50}) and 95% confidence limits were determined using a computer program (Stephan, 1977).

12. **REPORTED RESULTS:** Concentrations of Proxel press paste at test initiation and termination were determined (Table 1, attached). The analytical limit of detection was 62-91 µg/l, therefore, only measured concentrations at 100 and 180 µg/l were determined. The measured concentration of the stock solution (10 mg/l nominal) was 11.8 mg/l at test initiation and 11.9 mg/l at test termination.

Based on the number of normal larvae and nominal concentrations, the 48-hour EC_{50} was 62 µg/l with a 95% confidence interval of 58-67 µg/l. Since the reduction in normal development at concentrations $\leq$32 µg/l did not exceed 2% which is considered to be not significant, the NOEC was determined to be 32 µg/l (Table 3, attached). The percentage survival in the control was calculated as 106%.

During the test, the test solutions had a DO of 7.2-7.6 mg/l and a pH of 8.30-8.38. The solution temperature ranged from 19.4-19.8°C. The salinity of the control and the highest test concentration was 31.5 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions in the report.

A GLP compliance statement and a quality assurance statement were included in the report indicating that the study was conducted in accordance 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, but deviated as follows:

The authors did not report percentage of mortality or the EC_{50} based on mortality of oyster embryos and larvae.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program and nominal test concentrations to calculate the 48-hour EC_{50} (95% confidence interval) for the reduction in normal development of oyster larvae and obtained results similar to those of the authors (printout, attached). The NOEC was determined using William's test and was the same as that of the author (printout, attached).

C. **Discussion/Results:** Although four of the six nominal concentrations were below the level of detection, the two highest mean measured concentrations were at least 140% of nominal concentrations. The use of nominal concentrations to calculate the 48-hour EC_{50} is appropriate for this study.

This study is scientifically sound and meets the guideline requirements for an acute toxicity study using mollusc embryos and larvae. Based on percentage reduction in normal development of larvae and nominal concentrations, the 48-hour EC_{50} was 62 µg/l which classifies Proxel press paste as very highly toxic to *Crassostrea gigas*. The NOEC was 32 µg/l nominal concentration.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes; 15 December 1993.

TABLE 1

PROXEL PRESS PASTE: ACUTE TOXICITY TO PACIFIC OYSTER LARVAE

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

Sponsor:	ZENECA Biocides
Test substance:	Proxel press paste
Test organism:	Pacific oyster larvae (*Crassostrea gigas*)
Test water:	32‰ seawater

Nominal concn. of Proxel press paste (µg/l)	Measured concn. of Proxel press paste (µg/l)		Mean measured concn. of Proxel press paste	
	0 h	48 h	µg/l	% of nominal
Dilution water control	<96	<86	-	-
10	<91	<66	-	-
18	<91	<66	-	-
32	<86	<62	-	-
56	68†	<62	-	-
100	169*	111*	140	140
180	216	206	211	117

*Mean of triplicate injections:
0 h : 164, 177, 166 µg/l
48 h : 109, 106, 118 µg/l

The 10 mg/l stock solution was also analysed at the start and end of the test. The measured concentration at the start was 11.8 mg/l, and at the end of the test it was 11.9 mg/l.

† The peak height for this sample was below the limit of detection, however the analyst was able to estimate the peak height manually.

- Values were below the limit of detection, therefore mean measured and % of nominal could not be determined.

*** HARTLEY TEST IS ABORTED ***

Proxel Press Paste: No. of Normal Oyster Larvae in Concentrate
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Bartletts test for homogeneity of variance

Calculated B statistic = 6.69
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.33
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Proxel Press Paste: No. of Normal Oyster Larvae in Concentrate
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	18.313	18.313	18.625
2	10	2	18.875	18.875	18.625
3	18	2	19.000	19.000	18.625
4	32	2	17.875	17.875	17.875
5	56	2	13.125	13.125	13.125
6	100	2	0.375	0.375	0.375

Proxel Press Paste: No. of Normal Oyster Larvae
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	18.625				
10	18.625	0.218		1.86	k= 1, v= 8
18	18.625	0.218		1.96	k= 2, v= 8
32	17.875	0.305		2.00	k= 3, v= 8
56	13.125	3.619	*	2.01	k= 4, v= 8
100	0.375	12.512	*	2.02	k= 5, v= 8

s = 1.655
Note: df used for table values are approximate when v > 20.

Ecological Effects Branch One-Liner Data Entry Form

Chemical Benzisothiazolin Shaughnessy No. 098901 Pesticide Use _____

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. *Crassostrea gigas*	76.1 w/w	62 (58-67) µg/l NA	48 h static	32 µg/l	1993/1993	429524-01 Core	ZEN	RGM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC_{50}	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								

COMMENTS: Results based on nominal test concentrations. ZEN=ZENECA Limited, Brixham Environmental Laboratory

Rosemary Graham Mora Proxel Press Paste Pacific Oyster

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
180	100	100	100	0
100	100	98	98	0
56	100	28	28	0
32	100	2	2	0
18	100	0	0	0
10	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 65.26484

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	8.831122E-03	61.72417	57.46039	66.40857

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	3.869983E-02	1	.1705161

SLOPE = 8.763846
95 PERCENT CONFIDENCE LIMITS = 7.039798 AND 10.48789

LC50 = 62.79066
95 PERCENT CONFIDENCE LIMITS = 59.28699 AND 66.56021

LC10 = 44.97607
95 PERCENT CONFIDENCE LIMITS = 40.6652 AND 48.46815

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TITLE: Proxel Press Paste: No. of Normal Oyster Larvae in Concentrate
FILE: e:\aquadata\42952401.nor
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	19.5000	19.5000
1	Control	2	15.5000	15.5000
1	Control	3	17.2500	17.2500
1	Control	4	21.0000	21.0000
2	10	1	17.7500	17.7500
2	10	2	20.0000	20.0000
3	18	1	18.7500	18.7500
3	18	2	19.2500	19.2500
4	32	1	18.5000	18.5000
4	32	2	17.2500	17.2500
5	56	1	13.7500	13.7500
5	56	2	12.5000	12.5000
6	100	1	0.5000	0.5000
6	100	2	0.2500	0.2500

Proxel Press Paste: No. of Normal Oyster Larvae in Concentrate
File: e:\aquadata\42952401.nor Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

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TABLE 3

PROXEL PRESS PASTE: ACUTE TOXICITY TO PACIFIC OYSTER LARVAE

EFFECT ON NORMAL DEVELOPMENT

Sponsor:	ZENECA Biocides
Test substance:	Proxel press paste
Test organism:	Pacific oyster larvae (*Crassostrea gigas*)
Test water:	32‰ seawater

Nominal concn. of Proxel press paste (µg/l)	Replicate	Number of normal larvae (N_n)	% normal $\frac{N_n}{N_i} \times 100$	Mean % normal	% Reduction compared to the control	Mean Number of Normal Larvae
Dilution water control	A	9.75	94	88	NA	9.2
	B	7.75	75			
	C	8.63	83			
	D	10.50	101			
10	A	8.88	85	91	0	9.4
	B	10.00	96			
18	A	9.38	90	92	0	9.5
	B	9.63	93			
32	A	9.25	89	86	2	8.9
	B	8.63	83			
56	A	6.88	66	63	28	6.6
	B	6.25	60			
100	A	0.25	2.4	1.9	98	0.19
	B	0.13	1.3			
180	A	*	*	*	100*	
	B					

Mean count of the number of dividing embryos in the inoculum samples (N_i) used to calculate % normal development = 10.37.

The number of normal larvae (N_n) was calculated from Table 2 by dividing by a factor of 2 to correct for concentration by sedimentation.

* Samples were not assessed since 98% of the larvae were abnormal at 100 µg/l of Proxel press paste.

NA Not applicable.

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