

1- EEB-11H
6/4/99

MRID No. 447620-02

DATA EVALUATION RECORD
S 72-3 - ACUTE EC₅₀ TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

1. **CHEMICAL:** Petroleum distillate **PC Code No.:** 063503

2. **TEST MATERIAL:** GB-1111 **Purity:** 98.75%

3. **CITATION:**

Authors: Kurt R. Drottar and Henry O. Krueger
Title: GB-1111: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)

Study Completion Date: February 11, 1999

Laboratory: Wildlife International Ltd., Easton, MD

Sponsor: Golden Bear Oil Specialties, Inc., Los Angeles, CA

Laboratory Report ID: 481A-106

MRID No.: 447620-02

DP Barcode: D255299

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

Signature:

Karl Bullock

Date: 6/1/99

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature:

P. Kosalwat

Date: 6/1/99

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: Mean valve height
32 mm

Definitive Test Duration: 96 hours
Study Method: Static

Type of Concentrations: Initial measured

7. **CONCLUSIONS:** The study is scientifically sound but does not fulfill the guideline requirements for a mollusk shell deposition study. Based on initial measured concentrations, the EC₅₀ was estimated to be 6.07 ppm ai, which classifies GB-1111 as moderately toxic to the Eastern oyster. The NOEC was determined to be 3.24 ppm ai.

OBS	CONC	LOG_CONC	REP	Y
1	0.00	.	1	1.85
2	0.00	.	2	2.45
3	0.00	.	3	3.00
4	0.00	.	4	1.25
5	0.00	.	5	2.70
6	0.00	.	6	2.15
7	0.00	.	7	1.90
8	0.00	.	8	2.35
9	0.00	.	9	2.10
10	0.00	.	10	2.75
11	0.00	.	11	0.95
12	0.00	.	12	1.90
13	0.00	.	13	2.30
14	0.00	.	14	2.15
15	0.00	.	15	2.80
16	0.00	.	16	1.70
17	0.00	.	17	3.05
18	0.00	.	18	1.90
19	0.00	.	19	2.20
20	0.00	.	20	2.05
21	2.52	0.40140	1	2.70
22	2.52	0.40140	2	3.15
23	2.52	0.40140	3	1.70
24	2.52	0.40140	4	2.60
25	2.52	0.40140	5	1.55
26	2.52	0.40140	6	2.50
27	2.52	0.40140	7	2.05
28	2.52	0.40140	8	2.00
29	2.52	0.40140	9	2.70
30	2.52	0.40140	10	0.65
31	2.52	0.40140	11	2.55
32	2.52	0.40140	12	2.20
33	2.52	0.40140	13	2.85
34	2.52	0.40140	14	2.10
35	2.52	0.40140	15	1.80
36	2.52	0.40140	16	2.20
37	2.52	0.40140	17	2.55
38	2.52	0.40140	18	1.65
39	2.52	0.40140	19	2.40
40	2.52	0.40140	20	2.85
41	3.24	0.51055	1	2.05
42	3.24	0.51055	2	1.60
43	3.24	0.51055	3	2.25
44	3.24	0.51055	4	1.30
45	3.24	0.51055	5	1.95
46	3.24	0.51055	6	2.20
47	3.24	0.51055	7	1.75
48	3.24	0.51055	8	2.55
49	3.24	0.51055	9	2.85
50	3.24	0.51055	10	1.60
51	3.24	0.51055	11	2.35
52	3.24	0.51055	12	0.95
53	3.24	0.51055	13	1.45
54	3.24	0.51055	14	1.90
55	3.24	0.51055	15	2.30
56	3.24	0.51055	16	2.00
57	3.24	0.51055	17	1.75
58	3.24	0.51055	18	1.90
59	3.24	0.51055	19	2.40
60	3.24	0.51055	20	2.65
61	4.67	0.66932	1	0.90
62	4.67	0.66932	2	1.45
63	4.67	0.66932	3	1.80
64	4.67	0.66932	4	2.10

MODEL: COUNT = CO * WEIGHTED REGRESSION 11:46 Thursday, May 13, 1999

GB-1111: SHELL DEPOSITION WITH EASTERN OYSTER
 PROBORN ((LOG EC50 - LOG CONC) / SIGMA)

Non-Linear Least Squares Iterative Phase
 Dependent Variable: COUNT Method: Gauss-Newton
 LOG EC50 SIGMA CO Weighted SS
 0 0.826586 0.27257 2.180000 34.092010

7	0.544278	2.330263	31.291680
8	0.544577	2.329754	31.292797
9	0.544452	2.329967	31.292529
10	0.544504	2.329878	31.292526
11	0.544482	2.329915	31.292443
12	0.544491	2.329900	31.292478
13	0.544487	2.329906	31.292463
14	0.544489	2.329903	31.292469
15	0.544488	2.329905	31.292467
16	0.544489	2.329904	31.292468
17	0.544488	2.329904	31.292468
18	0.544488	2.329904	31.292468

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Weighted MS	Dependent Variable	COUNT
Regression	3	179.15000000	59.71666667		
Residual	117	31.29246774	0.26745699		
Uncorrected Total	120	210.44246774			
(Corrected Total)	119	78.31922978			

Parameter	Estimate	Asymptotic Std. Error	Confidence Interval Lower	Confidence Interval Upper	Asymptotic 95 %
LOG_EC25	0.544488487	0.07411422948	0.3977080351	0.6912685384	CO
SIGMA	0.353458680	0.05052366777	0.2533984506	0.4535189086	CO
CO	2.329904152	0.16734094389	1.9984916890	2.6613166145	CO

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	CO
LOG_EC25	1		
SIGMA	-0.869906072	1	
CO	-0.81786456	0.6107587277	1

MODEL: YOUNG = CO * PROBNOEM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449) SUMMARY OF NONLINEAR REGRESSION

OBS	CONC	LOG_EC25	SIGMA	CO	RESID_SS	EC25
1	0	0.54449	0.35346	2.32990	31.2925	3.50339

MODEL: YOUNG = CO * PROBNOEM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449) Plot of COUNT*LOG_CONC. Symbol used is 'O'. Plot of PRED*LOG_CONC. Symbol used is 'O'.

COUNT 3.5

1	0.380933	2.396197	31.169492
2	0.342521	2.303591	31.340012
3	0.358183	2.341388	31.264737
4	0.351505	2.325106	31.302614
5	0.354283	2.331925	31.287968
6	0.353114	2.329057	31.294311
7	0.353604	2.330260	31.291687
8	0.353598	2.329794	31.292794
9	0.353484	2.329967	31.292331
10	0.353448	2.329878	31.292525
11	0.353463	2.329915	31.292444
12	0.353457	2.329906	31.292478
13	0.353458	2.329903	31.292469
14	0.353458	2.329905	31.292467
15	0.353459	2.329904	31.292468
16	0.353459	2.329904	31.292468
17	0.353459	2.329904	31.292468
18	0.353459	2.329904	31.292468

Convergence criterion met.

Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Weighted MS	Dependent Variable	COUNT
Regression	3	179.15000000	59.71666667		
Residual	117	31.29246774	0.26745699		
Uncorrected Total	120	210.44246774			
(Corrected Total)	119	78.31922978			

Parameter	Estimate	Asymptotic Std. Error	Confidence Interval Lower	Confidence Interval Upper	Asymptotic 95 %
LOG_EC50	0.782892831	0.04754013745	0.6887413724	0.8770425906	CO
SIGMA	0.353458680	0.05052366777	0.2533984507	0.4535189087	CO
CO	2.329904152	0.16734094391	1.9984916891	2.6613166147	CO

EC50 = 6.07 (4.88 - 7.53) Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.639348374	1	
CO	-0.837232406	0.6107587276	1

MODEL: COUNT = CO * PROBNOEM ((LOG_EC50 - LOG_CONC) / SIGMA - 0.67449) SUMMARY OF NONLINEAR REGRESSION

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
0	0	0.78289	0.35346	2.32990	31.2925	6.06587

MODEL: YOUNG = CO * PROBNOEM ((LOG_EC50 - LOG_CONC) / SIGMA - 0.67449) WEIGHTED REGRESSION - 11:46 Thursday, May 13, 1999

Non-Linear Least Squares Iterative Phase

Dependent Variable	COUNT	Method	Gauss-Newton	Weighted SS
LOG_EC25		CO	2.180000	35.958471
SIGMA		CO	0.643978	2.395845
CO		CO	0.506411	31.158937
		CO	0.560667	2.303456
		CO	0.342423	31.340845
		CO	0.537742	2.341485
		CO	0.558221	31.264494
		CO	0.547436	31.502695
		CO	0.543296	2.325067
		CO	0.354290	31.287931
		CO	0.353111	31.294326

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	55.13785417	11.02757083	36.43	0.0001
Error	114	34.50862500	0.30270724		
Corrected Total	119	89.64647917			
R-Square		C.V.	Root MSE	RESPONSE Mean	
0.615059		36.85325	0.550188	1.492917	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	55.13785417	11.02757083	36.43	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	55.13785417	11.02757083	36.43	0.0001

E: 47 obs had missing values. 132 obs hidden.
 GB-1111: SHELL DEPOSITION WITH EASTERN OYSTER
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 11:46 Thursday, May 13, 1999

General Linear Models Procedure
 Class Level Information

Class	Levels	Values
DOSE	6	0 12.5 2.52 3.24 4.67 7.58

Number of observations in data set = 120

GB-1111: SHELL DEPOSITION WITH EASTERN OYSTER
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 11:46 Thursday, May 13, 1999

General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	55.13785417	11.02757083	36.43	0.0001
Error	114	34.50862500	0.30270724		
Corrected Total	119	89.64647917			
R-Square		C.V.	Root MSE	RESPONSE Mean	
0.615059		36.85325	0.550188	1.492917	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	55.13785417	11.02757083	36.43	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	55.13785417	11.02757083	36.43	0.0001

GB-1111: SHELL DEPOSITION WITH EASTERN OYSTER
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 11:46 Thursday, May 13, 1999

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	20	2.17500000	0.53814985
12.5	20	0.51500000	0.42367440
2.52	20	2.23750000	0.57876343
3.24	20	1.98750000	0.47486148
4.67	20	1.23500000	0.62976604
7.58	20	0.80750000	0.62455774

GB-1111: SHELL DEPOSITION WITH EASTERN OYSTER
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure
 Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 114 MSE= 0.302707
 Critical Value of Dunnett's T= 2.260
 Minimum Significant Difference= 0.3932

Comparisons significant at the 0.05 level are indicated by ****.

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
2.52 - 0	-0.3307	0.0625	0.4557
3.24 - 0	-0.5807	-0.1875	0.2057
4.67 - 0	-1.3332	-0.9400	-0.5468
7.58 - 0	-1.7607	-1.3675	-0.9743
12.5 - 0	-2.0532	-1.6600	-1.2668

EPA PROBIT ANALYSIS PROGRAM
 USED FOR CALCULATING EC VALUES
 Version 1.4

B-1111: Toxicity to Eastern Oyster

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
2.5200	100	0	0.0000	0.0000	0.0594
3.2400	100	9	0.0900	0.0900	0.1231
4.6700	100	43	0.4300	0.4300	0.2820
7.5800	100	63	0.6300	0.6300	0.5772
12.5000	100	76	0.7600	0.7600	0.8394

Chi - Square Heterogeneity = 23.964

 WARNING *
 Significant heterogeneity exists. The results reported *
 for this data set may not be valid. The results should *
 be interpreted with appropriate caution. *

mu = 0.826589
 sigma = 0.272557

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	1.967276	0.644178	(-0.082497,	4.017049)
Slope	3.668963	0.816866	(1.069695,	6.268232)

Theoretical Spontaneous Response Rate = 0.0000

B-1111: Toxicity to Eastern Oyster

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence	Upper Limits
EC 1.00	1.5579	0.0623	2.8717
EC 5.00	2.3892	0.2611	3.8180
EC 10.00	3.0011	0.5513	4.5157
EC 15.00	3.5003	0.9007	5.1277
EC 50.00	6.7079	4.4346	14.1860
EC 85.00	12.8548	8.1611	104.9973
EC 90.00	14.9933	9.0836	174.9893
EC 95.00	18.8329	10.5672	375.7498
EC 99.00	28.8827	13.8556	1595.6814

$$\frac{4.33 - \text{intercept}}{\text{slope}} = \log_2 EC_{25}$$

$$= 0.643977$$

3-1111: Toxicity to Eastern Oyster

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE

