DATA EVALUATION RECORD
§ 72-3 - ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSC SHELL DEPOSITION STUDY

1. **CHEMICAL:** Hydrogen cyanamide **PC Code No.:** 014002

2. **TEST MATERIAL:** Aqueous hydrogen cyanamide **Purity:** 50.8%

3. **CITATION:**

Authors: Gettmann, W., K.R. Drottar, and H.O. Krueger
Title: Hydrogen Cyanamide: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
Study Completion Date: November 24, 1998
Laboratory: Wildlife International Ltd., Easton, MD
Sponsor: SKW Trostberg AG, Trostberg, Germany
Laboratory Report ID: 248A-104
MRID No.: 448047-04
DP Barcode: D255592

4. **REVIEWED BY:** Mark Mossler, M.S., Environmental Scientist, Golder Associates Inc.

Signature: [signature] **Date:** 9/20/99

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

Signature: P. Kosalwat **Date:** 9/20/99

5. **APPROVED BY:**

Signature: [signature] Andrew Bryceland **Date:** 1/27/00

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: Mean valve height - 36.4 mm
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** The study is scientifically sound and fulfills the guideline requirements for a mollusc shell deposition study. A 96-hour EC_{50} of 2.3 ppm ai classifies hydrogen cyanamide as moderately toxic to the Eastern oyster.

Results Synopsis

EC_{50}: 2.3 ppm ai
NOEC: 0.56 ppm ai
95% C.I.: 1.8 - 3.0 ppm ai
Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

   A. Classification: Core

   B. Rationale: N/A

   C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:** No guideline deviations of consequence were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

   A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	Mean: 36.4 mm Range: 27.4-42.6 mm
Supplier	P. Cummins Oyster Co., Annapolis, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

   B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days after collection	10 days of acclimation
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No

Guideline Criteria	Reported Information
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	Only reported that peripheral shell was ground off
Feeding during the acclimation Must be fed to avoid stress.	Algae (*Skeletonema* sp., *Chaetoceros* sp., *Isochrysis* sp., and *Thalassiosira pseudonana*) were added as a supplement during acclimation and testing
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater pumped from Indian River Inlet, Delaware, aerated and adjusted to 20 ‰ with well water
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	20 ‰
Water Temperature 15°-30° C, consistent in all test vessels	22 - 23°C
pH	8.0 - 8.1
Dissolved Oxygen ≥ 60% throughout	≥82% of saturation throughout the test
Total Organic Carbon	<1.0 mg/L

Guideline Criteria	Reported Information
Test Aquaria Should be constructed of glass or stainless steel.	52-L stainless steel aquaria lined with Teflon® and a fill volume of 13 L
Type of Dilution System Must provide reproducible supply of toxicant	Continuous flow serial diluter with mixing boxes
Flow rate Consistent flow rate	37 volume additions per day
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: none Maximum conc.: N/A

D. **Test Design**

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 oysters, then no definitive test is required.	Concentrations were based on a exploratory range finding test and consultation with the study sponsor
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Control, 0.58, 0.97, 1.6, 2.7, 4.5, and 7.5 mg/L (0.29, 0.49, 0.81, 1.4, 2.3, and 3.8 mg active ingredient [ai]/L)
Number of Test Organisms Minimum 20 individual per test level and in each control	20 oysters per treatment or control
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	Temperature was measured at 0 and 96 h in all aquaria and continuously in one replicate of the dilution water control DO and pH were measured at 0, 48, and 96 h in each aquarium
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes, daily samples were analyzed by HPLC

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No mortality reported
Control Shell Deposition Must be at least 2 mm.	Negative control: 4.035 mm
Percent Recovery of Chemical: **1) % of nominal,** **2) procedural recovery,** **3) limit of quantitation (LOQ)**	1) 97-138% 2) 106% 3) 0.2 ppm ai
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Analytical Results

Nominal concentration (ppm ai)	Measured concentration (ppm ai)				
	Hour of Study				
	0	24	48	72	96
Negative control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.29	0.19	0.30	0.35	0.30	0.29
0.49	0.52	0.60	0.61	0.52	0.49
0.81	1.0	1.2	1.3	1.1	1.0
1.4	1.5	1.8	2.0	1.5	1.5
2.3	2.3	2.7	2.7	2.3	2.3
3.8	4.4	4.8	4.9	4.1	4.2

Shell Growth

Concentration (ppm ai)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Decrease
Nominal	Mean Measured*				
Control	<LOQ	20	0	4.035	N/A
0.29	0.28	20	0	4.140	-3
0.49	0.56	20	0	4.475	-11
0.81	1.1	20	0	2.760	32
1.4	1.7	20	0	2.825	30
2.3	2.5	20	0	1.893	53
3.8	4.5	20	0	1.160	71

*Measured concentrations were not corrected for a procedural recovery of 106%.

Other Significant Results: Oysters from all groups appeared healthy and normal throughout the testing period.

B. Statistical Results

Method: Linear interpolation was used to estimate the EC_{50}, and Dunnett's test was used to determine the NOEC.

96-hr EC_{50}: 2.3 ppm ai 95% C.I.: 1.6 - 3.0 ppm ai
Probit Slope: N/A NOEC: 0.56 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Statistical Method for EC_{50}	Non-linear regression
EC_{50} (95% C.I.)	2.3 (1.8 - 3.0) ppm ai
Probit Slope	N/A
Statistical Method for NOEC	Williams' Test
NOEC	0.56 ppm ai

14. REVIEWER'S COMMENTS: The study is scientifically sound and fulfills the guideline requirements. The EC_{50} was 2.3 ppm ai, which classifies hydrogen cyanamide as moderately toxic to Eastern oysters. The NOEC was determined to be 0.56 ppm ai. This study is classified as **Core.**

Oyster shell deposition
File: oys Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	20	4.035	4.035	4.217
2	0.28 ppm ai	20	4.140	4.140	4.217
3	0.56 ppm ai	20	4.475	4.475	4.217
4	1.1 ppm ai	20	2.760	2.760	2.793
5	1.7 ppm ai	20	2.825	2.825	2.793
6	2.5 ppm ai	20	1.893	1.893	1.893
7	4.5 ppm ai	20	1.160	1.160	1.160

Oyster shell deposition
File: oys Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	4.217				
0.28 ppm ai	4.217	0.445		1.66	k= 1, v=133
0.56 ppm ai	4.217	0.445		1.73	k= 2, v=133
1.1 ppm ai	2.793	3.045	*	1.75	k= 3, v=133
1.7 ppm ai	2.793	3.045	*	1.77	k= 4, v=133
2.5 ppm ai	1.893	5.250	*	1.77	k= 5, v=133
4.5 ppm ai	1.160	7.045	*	1.78	k= 6, v=133

s = 1.290
Note: df used for table values are approximate when v > 20.

hydrogen cyanamide - shell deposition

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OBS	CONC	LOG_CONC	REP	Y
1	0.00	.	1	6.20
2	0.00	.	2	7.00
3	0.00	.	3	5.35
4	0.00	.	4	3.85
5	0.00	.	5	4.80
6	0.00	.	6	3.10
7	0.00	.	7	1.85
8	0.00	.	8	5.55
9	0.00	.	9	6.05
10	0.00	.	10	1.90
11	0.00	.	11	2.35
12	0.00	.	12	5.05
13	0.00	.	13	2.55
14	0.00	.	14	2.60
15	0.00	.	15	3.25
16	0.00	.	16	3.40
17	0.00	.	17	3.00
18	0.00	.	18	4.25
19	0.00	.	19	2.30
20	0.00	.	20	6.30
21	0.28	-0.55284	1	5.45
22	0.28	-0.55284	2	4.00
23	0.28	-0.55284	3	2.85
24	0.28	-0.55284	4	2.10
25	0.28	-0.55284	5	4.70
26	0.28	-0.55284	6	5.20
27	0.28	-0.55284	7	4.45
28	0.28	-0.55284	8	3.15
29	0.28	-0.55284	9	4.10
30	0.28	-0.55284	10	3.85
31	0.28	-0.55284	11	4.35
32	0.28	-0.55284	12	3.20
33	0.28	-0.55284	13	6.65
34	0.28	-0.55284	14	5.45
35	0.28	-0.55284	15	5.65
36	0.28	-0.55284	16	3.10
37	0.28	-0.55284	17	4.35
38	0.28	-0.55284	18	4.20
39	0.28	-0.55284	19	4.05
40	0.28	-0.55284	20	1.95
41	0.56	-0.25181	1	5.05
42	0.56	-0.25181	2	3.05
43	0.56	-0.25181	3	5.55
44	0.56	-0.25181	4	3.60
45	0.56	-0.25181	5	3.45
46	0.56	-0.25181	6	7.20
47	0.56	-0.25181	7	4.00
48	0.56	-0.25181	8	5.45
49	0.56	-0.25181	9	3.35
50	0.56	-0.25181	10	4.20
51	0.56	-0.25181	11	3.50
52	0.56	-0.25181	12	5.70
53	0.56	-0.25181	13	4.60
54	0.56	-0.25181	14	3.45
55	0.56	-0.25181	15	4.90
56	0.56	-0.25181	16	6.90
57	0.56	-0.25181	17	3.95
58	0.56	-0.25181	18	4.70
59	0.56	-0.25181	19	3.50
60	0.56	-0.25181	20	3.40
61	1.10	0.04139	1	4.10
62	1.10	0.04139	2	3.45
63	1.10	0.04139	3	2.50
64	1.10	0.04139	4	2.05

65	1.10	0.04139	5	2.60
66	1.10	0.04139	6	4.20
67	1.10	0.04139	7	3.00

hydrogen cyanamide - shell deposition

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OBS	CONC	LOG_CONC	REP	Y
68	1.1	0.04139	8	5.50
69	1.1	0.04139	9	3.05
70	1.1	0.04139	10	3.90
71	1.1	0.04139	11	2.10
72	1.1	0.04139	12	2.05
73	1.1	0.04139	13	1.10
74	1.1	0.04139	14	1.25
75	1.1	0.04139	15	3.75
76	1.1	0.04139	16	1.70
77	1.1	0.04139	17	3.85
78	1.1	0.04139	18	2.10
79	1.1	0.04139	19	1.45
80	1.1	0.04139	20	1.50
81	1.7	0.23045	1	3.20
82	1.7	0.23045	2	6.55
83	1.7	0.23045	3	3.55
84	1.7	0.23045	4	2.80
85	1.7	0.23045	5	5.85
86	1.7	0.23045	6	3.80
87	1.7	0.23045	7	1.10
88	1.7	0.23045	8	1.95
89	1.7	0.23045	9	3.10
90	1.7	0.23045	10	4.20
91	1.7	0.23045	11	2.10
92	1.7	0.23045	12	2.95
93	1.7	0.23045	13	0.85
94	1.7	0.23045	14	1.05
95	1.7	0.23045	15	1.90
96	1.7	0.23045	16	3.15
97	1.7	0.23045	17	3.40
98	1.7	0.23045	18	1.00
99	1.7	0.23045	19	0.55
100	1.7	0.23045	20	3.45
101	2.5	0.39794	1	2.70
102	2.5	0.39794	2	2.05
103	2.5	0.39794	3	3.60
104	2.5	0.39794	4	2.10
105	2.5	0.39794	5	1.75
106	2.5	0.39794	6	1.55
107	2.5	0.39794	7	0.60
108	2.5	0.39794	8	0.00
109	2.5	0.39794	9	0.95
110	2.5	0.39794	10	2.40
111	2.5	0.39794	11	2.75
112	2.5	0.39794	12	0.00
113	2.5	0.39794	13	4.60
114	2.5	0.39794	14	0.80
115	2.5	0.39794	15	3.05
116	2.5	0.39794	16	2.10
117	2.5	0.39794	17	1.40
118	2.5	0.39794	18	1.20
119	2.5	0.39794	19	1.35
120	2.5	0.39794	20	2.90
121	4.5	0.65321	1	2.10
122	4.5	0.65321	2	1.10
123	4.5	0.65321	3	0.00
124	4.5	0.65321	4	0.50
125	4.5	0.65321	5	0.75
126	4.5	0.65321	6	1.10
127	4.5	0.65321	7	0.00
128	4.5	0.65321	8	0.00

129	4.5	0.65321	9	1.15
130	4.5	0.65321	10	1.30
131	4.5	0.65321	11	3.10
132	4.5	0.65321	12	0.60
133	4.5	0.65321	13	0.45
134	4.5	0.65321	14	1.40

hydrogen cyanamide - shell deposition
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OBS	CONC	LOG_CONC	REP	Y
135	4.5	0.65321	15	1.90
136	4.5	0.65321	16	1.95
137	4.5	0.65321	17	1.65
138	4.5	0.65321	18	0.00
139	4.5	0.65321	19	2.10
140	4.5	0.65321	20	2.05

hydrogen cyanamide - shell deposition
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
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Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	0.384000	0.385000	4.035000	86.242377
1	0.360181	0.462819	4.272632	82.464980
2	0.365180	0.448864	4.249892	82.467870
3	0.363297	0.452920	4.258751	82.461815
4	0.363811	0.451775	4.256286	82.464231
5	0.363662	0.452102	4.256997	82.463628
6	0.363704	0.452009	4.256795	82.463806
7	0.363692	0.452036	4.256852	82.463756
8	0.363695	0.452028	4.256836	82.463770
9	0.363694	0.452030	4.256841	82.463766
10	0.363695	0.452030	4.256839	82.463768
11	0.363694	0.452030	4.256840	82.463767

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	425.75000000	141.91666667
Residual	137	82.46376724	0.60192531
Uncorrected Total	140	508.21376724	
(Corrected Total)	139	159.81671272	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	0.363694440	0.05736888055	0.2502504166	0.4771384630
SIGMA	0.452029745	0.08451778348	0.2849001569	0.6191593335
C0	4.256839795	0.26989600134	3.7231342751	4.7905453153

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.526411971	-0.778029961
SIGMA	-0.526411971	1	0.5858133714
C0	-0.778029961	0.5858133714	1

hydrogen cyanamide - shell deposition
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION

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OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	0.36369	0.45203	4.25684	82.4638	2.31044

hydrogen cyanamide - shell deposition
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION
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Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC25	SIGMA	C0	Weighted SS
0	0.127000	0.385000	4.035000	85.877323
1	0.048099	0.463078	4.272161	82.448093
2	0.062485	0.448806	4.249788	82.468304
3	0.057789	0.452936	4.258784	82.461779
4	0.059098	0.451771	4.256277	82.464238
5	0.058722	0.452103	4.256999	82.463626
6	0.058829	0.452009	4.256794	82.463807
7	0.058798	0.452036	4.256853	82.463756
8	0.058807	0.452028	4.256836	82.463771
9	0.058804	0.452030	4.256841	82.463766
10	0.058805	0.452030	4.256839	82.463768
11	0.058805	0.452030	4.256840	82.463767

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	425.75000000	141.91666667
Residual	137	82.46376724	0.60192531
Uncorrected Total	140	508.21376724	
(Corrected Total)	139	159.81671269	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	0.058804896	0.09992030081	-0.1387823812	0.2563921737
SIGMA	0.452029746	0.08451778362	0.2849001573	0.6191593344
C0	4.256839797	0.26989600167	3.7231342759	4.7905453174

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	C0
LOG_EC25	1	-0.872756234	-0.78092058
SIGMA	-0.872756234	1	0.5858133718
C0	-0.78092058	0.5858133718	1

hydrogen cyanamide - shell deposition
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC25	SIGMA	C0	RESID_SS	EC25
1	0	0.058805	0.45203	4.25684	82.4638	1.14500

hydrogen cyanamide - shell deposition
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
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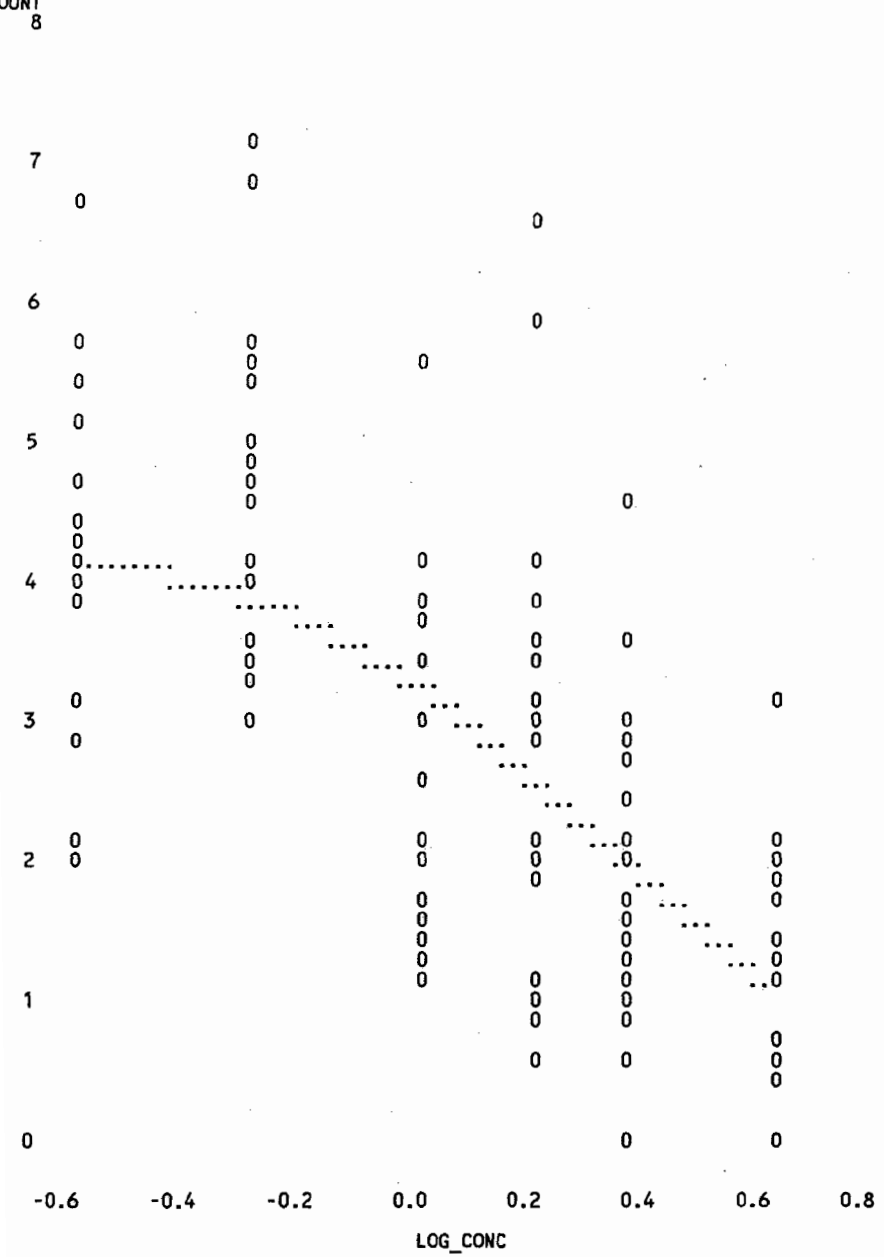
Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.



E: 1247 obs had missing values. 1279 obs hidden.

hydrogen cyanamide - shell deposition
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	7	0 1.1 1.7 2.5 4.5 0.28 0.56

Number of observations in data set = 140

hydrogen cyanamide - shell deposition
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	184.7004643	30.7834107	18.49	0.0001
Error	133	221.4858750	1.6653073		
Corrected Total	139	406.1863393			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.454719	42.43465	1.290468	3.041071

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	6	184.7004643	30.7834107	18.49	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	6	184.7004643	30.7834107	18.49	0.0001

hydrogen cyanamide - shell deposition
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	20	4.03500000	1.63514847
1.1	20	2.76000000	1.18982529
1.7	20	2.82500000	1.59163107
2.5	20	1.89250000	1.17934806
4.5	20	1.16000000	0.87728649
0.28	20	4.14000000	1.19907859
0.56	20	4.47500000	1.19840903

hydrogen cyanamide - shell deposition
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:48 Thursday, September 16, 1999

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= 133 MSE= 1.665307
Critical Value of Dunnett's T= 2.316
Minimum Significant Difference= 0.945

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.56 - 0	-0.5050	0.4400	1.3850	
0.28 - 0	-0.8400	0.1050	1.0500	
1.7 - 0	-2.1550	-1.2100	-0.2650	***
1.1 - 0	-2.2200	-1.2750	-0.3300	***
2.5 - 0	-3.0875	-2.1425	-1.1975	***
4.5 - 0	-3.8200	-2.8750	-1.9300	***