DATA EVALUATION RECORD

§ 72-3(B) -- ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

1. **CHEMICAL:** Pirate (AC 303,630) PC Code No.: 129093

2. **TEST MATERIAL:** AC 303,630 Purity: 94.5%

3. **CITATION:**

Authors: G.S. Ward and J.D. Wisk
Title: Effect of AC 303,630 on New Shell Growth in the Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Test Conditions
Study Completion Date: July 7, 1993
Laboratory: Toxikon Environmental Sciences, Jupiter, FL
Sponsor: American Cyanamid Company, Princeton, NJ
Laboratory Report ID: J9203020d
MRID No.: 434928-17
DP Barcode: D210808 and D222690

4. **REVIEWED BY:** William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: William Evans **Date:** 10/9/96 added

5. **APPROVED BY:** Ann Stavola, Sectioon Chief, Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: Ann Stavola **Date:** 11/7/96

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: 28 ±2.8 mm
Definitive Test Duration: 96 hours
Study Method: Flow Through
Type of Concentrations: Mean Measured

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for an oyster shell deposition study. The average control shell growth was <2 mm which might have been caused by an inadequate acclimation. Test oysters were abruptly transferred from seawater with a salinity of 20‰ to the dilution water with a salinity of 30-31‰, and then acclimated for only two days prior to testing. In addition, the test temperature varied by 5°C during the test (range of 20.5-25.5°C). Results are not recorded for invalid studies. added

Results Synopsis

EC_{50}: 95% C.I.:
NOEC: Probit Slope:

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Invalid

B. **Rationale:** The average control shell growth was <2 mm which might have been caused by an inadequate acclimation (see the above conclusions). Also, the test temperature varied by 5°C during the test.

C. **Repairability:** No

9. **BACKGROUND:**

10. **GUIDELINE DEVIATIONS:**

1. The test temperature was variable during the test with a range of 20.5-25.5°C.

2. The test organisms were held in filtered seawater with a salinity of 20‰ for 7 days, then abruptly transferred to the unfiltered seawater with a salinity of 30-31‰ (i.e., dilution water) and acclimated for only two days prior to testing. The test organisms must be held for at least 10 days for acclimation to avoid unnecessary stress.

3. The new shell growth of oysters in the control was variable and averaged 1.51 and 1.39 mm for the dilution water control and solvent control, respectively (Tables 2 and 3, attached). The low growth might have been caused by an inadequate acclimation and variable temperature.

4. The ratio of the lowest to the highest mean measured concentration was 1.7, 1.5, 1.2, 1.5, and 1.4 for the measured concentrations of 1.81, 4.32, 5.46, 8.17, and 13.2 ppb ai, respectively.

11. **SUBMISSION PURPOSE:**

12. **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	28 ±2.8 mm (range of 22-33 mm)
Supplier	Shellfish Culture, Inc., Pass Christian, MS
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported.

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	Held in filtered seawater with a salinity of 20‰ for 7 days, then acclimated to dilution water (unfiltered seawater) with a salinity of 30-31‰ for 2 days.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
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	2-5 mm removed two days before the test, and additional new shell growth removed immediately before the test.

Guideline Criteria	Reported Information
Feeding during the acclimation Must be fed to avoid stress.	Fed a mix of marine algae (*Isochrysis galbana* and *Skeletonema costatum*) during acclimation. During testing, oysters received food through unfiltered seawater, and a supplement of 600 mL of *I. galbana*/day/vessel.
Pretest Mortality <3% mortality 48 hours prior to testing	No mortality occurred during holding or acclimation.

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater from the Jupiter River which was aerated prior to use.
Does water support test animals without observable signs of stress?	No
Salinity 30-34‰ salinity, weekly range < 6 ‰	30-33‰
Water Temperature 15-30°C, consistent in all test vessels	20.5-25.5°C
pH	7.1-8.0
Dissolved Oxygen ≥ 60% throughout	≥68% of saturation throughout the test
Total Organic Carbon	3.12 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	11.2-L glass tanks with 9.2 L of test solution

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	Constant-flow diluter
Flow rate Consistent flow rate	63 volume additions/24 hours
Was the loading of organisms such that each individual sits on the bottom with water flowing freely around it?	Not reported.
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L	Solvent: DMF Maximum conc.: Not reported.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 fish, then no definitive test is required.	One range-finding test showed 100% mortality at concentrations ≥0.05 ppm ai and no reduction in new shell growth at 0.01 ppm ai. A second range-finding test demonstrated 29% reduction in new shell growth at 5 ppb ai, 48% reduction at 10 ppb ai and 27% mortality and no new shell growth at 20 ppb ai.
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; solvent control; and 2.1, 3.2, 5.4, 9.2, and 15 µg ai/L.
Number of Test Organisms Minimum 20 individual per test level and in each control	20 oysters per test aquarium, one aquarium per treatment or control.

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
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	1. Temperature was measured daily in the dilution water control. As implied by the information provided in Table 4 (attached), temperature was also continuously measured using a min/max thermometer during the first and second 24-hour period of the test. 2. DO and pH were measured daily in all test chambers.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

13. 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 occurred in the control or solvent control.
Control Shell Deposition Must be at least 2 mm.	1.51 mm in the control 1.39 mm in the solvent control
Recovery of Chemical	86-135%
Raw data included?	Yes

Guideline Criteria	Reported Information
Signs of toxicity (if any) were described?	None reported.

Shell Growth

Concentration (ppb ai)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction Compared to Pooled Control
Nominal	Mean Measured				
Control	<0.5	20	0	1.51	-
Solvent Control	<0.5	20	0	1.39	-
2.1	1.81	20	0	1.63	-12*
3.2	4.32	20	0	1.27	12
5.4	5.46	20	0	1.25	14
9.2	8.17	20	1	0.96	34
15	13.2	20	12	0.16	89

* Negative value means increase.

B. Statistical Results

Method: Non-Linear Interpolation
96-hr EC_{50}: 9.29 ppb ai 95% C.I.: 8.17-13.2 ppb ai
Probit Slope: N/A NOEC: 5.46 ppb ai

14. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Statistical Method for EC_{50}	Moving Average
EC_{50} (95% C.I.)	8.96 (8.41-9.58) ppb ai
Probit Slope	N/A

Statistical Method for NOEC	ANOVA with Bonferroni's t-test and Williams' test (compared treatments to pooled control)
NOEC	5.46 ppb ai

Note: An LC_{50} of 12.4 ppb ai was also determined based on mortality observed during this study.

15. **REVIEWER'S COMMENTS:** This study is not scientifically sound, does not fulfill the guideline requirements for an oyster shell deposition study, and is classified as **Invalid**.

The authors state that the test organisms were held for seven days in filtered seawater (20‰ and 23.9-24.9°C). Two days prior to test initiation, 2-5 mm of shell growth were removed from the oysters. Oysters were then transferred to a control chamber for two days acclimation prior to test initiation. The salinity and temperature of the dilution water during acclimation was reported to be 30-31‰ and 21.6-22.4°C, respectively. An acclimation period of 10 days is required to avoid unnecessary stress to the test organisms. The reviewer believes that the acclimation period in this test was not adequate and, indeed, may have been stressful to the test organisms. The oysters were removed from seawater with a salinity of **20‰**, had their shell growth removed by a high speed grinder, and were transferred to the dilution water with a salinity of **30-31‰**. The abrupt change in salinity and short period of acclimation (2 days) do not appear to be conducive to healthy new shell which is reflected by the variable new shell growth of the control oysters (range of 0-2.4 mm and mean of 1.45 mm; Tables 2 and 3, attached).

In addition, the test temperature (20.5-25.5°C) was variable throughout the test according to temperatures reported in Table 4 (attached).

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Pages 9 through 11 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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RGMora Oyster Ac Shell Deposition

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
13.2	100	89	89	0
8.17	100	34	34	0
5.46	100	14	14	0
4.32	100	12	12	0
1.81	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 9.289005

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.720811E-02	8.960655	8.412929	9.583198

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.3282929	4.429774	4.050613E-03

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

SLOPE = 5.041061
95 PERCENT CONFIDENCE LIMITS = 2.152691 AND 7.92943

LC50 = 8.587604
95 PERCENT CONFIDENCE LIMITS = 6.777196 AND 12.29366

LC10 = 4.80774
95 PERCENT CONFIDENCE LIMITS = 2.387081 AND 6.205006

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RGMora Mortality

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
13.2	20	12	60.00001	25.17223
8.17	20	1	5	2.002716E-03
5.46	20	0	0	9.536742E-05
4.32	20	0	0	9.536742E-05
1.81	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 12.24741

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.2481507	12.24741	10.8468	15.41987

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.2909513	1	.9996418

SLOPE = 9.201482
95 PERCENT CONFIDENCE LIMITS = 4.238212 AND 14.16475

LC50 = 12.38084
95 PERCENT CONFIDENCE LIMITS = 10.94968 AND 14.79321

LC10 = 9.010086
95 PERCENT CONFIDENCE LIMITS = 6.411849 AND 10.30782

AC 303,630: Shell Deposition of Exposed Oysters
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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	9.380	33.880	53.480	33.880	9.380
OBSERVED	9	23	64	35	9

Calculated Chi-Square goodness of fit test statistic = 5.6311
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

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Hartley's test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 2.79
Closest, conservative, Table H statistic = 6.7 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 15
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 19.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, Hartley's test may still be used as an approximate test (average df are used).

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t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN	=	1.5050	CALCULATED t VALUE = 0.7150
GRP2 (BLANK CRTL) MEAN	=	1.3900	DEGREES OF FREEDOM = 38
DIFFERENCE IN MEANS	=	0.1150	

TABLE t VALUE (0.05 (2),40) = 2.021 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

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TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	2.0000	2.0000
1	GRPS 1&2 POOLED	2	2.4000	2.4000
1	GRPS 1&2 POOLED	3	1.7000	1.7000
1	GRPS 1&2 POOLED	4	1.1000	1.1000
1	GRPS 1&2 POOLED	5	2.0000	2.0000
1	GRPS 1&2 POOLED	6	1.3000	1.3000
1	GRPS 1&2 POOLED	7	1.0000	1.0000
1	GRPS 1&2 POOLED	8	1.9000	1.9000
1	GRPS 1&2 POOLED	9	1.7000	1.7000
1	GRPS 1&2 POOLED	10	2.2000	2.2000
1	GRPS 1&2 POOLED	11	2.0000	2.0000
1	GRPS 1&2 POOLED	12	1.9000	1.9000
1	GRPS 1&2 POOLED	13	1.0000	1.0000
1	GRPS 1&2 POOLED	14	0.9000	0.9000
1	GRPS 1&2 POOLED	15	1.8000	1.8000
1	GRPS 1&2 POOLED	16	1.8000	1.8000
1	GRPS 1&2 POOLED	17	1.0000	1.0000
1	GRPS 1&2 POOLED	18	1.0000	1.0000
1	GRPS 1&2 POOLED	19	1.4000	1.4000
1	GRPS 1&2 POOLED	20	0.0000	0.0000
1	GRPS 1&2 POOLED	21	1.4000	1.4000
1	GRPS 1&2 POOLED	22	1.6000	1.6000
1	GRPS 1&2 POOLED	23	1.1000	1.1000
1	GRPS 1&2 POOLED	24	1.2000	1.2000
1	GRPS 1&2 POOLED	25	1.2000	1.2000
1	GRPS 1&2 POOLED	26	1.0000	1.0000
1	GRPS 1&2 POOLED	27	1.4000	1.4000
1	GRPS 1&2 POOLED	28	1.4000	1.4000
1	GRPS 1&2 POOLED	29	0.0000	0.0000
1	GRPS 1&2 POOLED	30	1.6000	1.6000
1	GRPS 1&2 POOLED	31	1.4000	1.4000
1	GRPS 1&2 POOLED	32	1.7000	1.7000
1	GRPS 1&2 POOLED	33	2.1000	2.1000
1	GRPS 1&2 POOLED	34	1.7000	1.7000
1	GRPS 1&2 POOLED	35	2.1000	2.1000
1	GRPS 1&2 POOLED	36	1.2000	1.2000
1	GRPS 1&2 POOLED	37	1.2000	1.2000
1	GRPS 1&2 POOLED	38	1.9000	1.9000
1	GRPS 1&2 POOLED	39	1.2000	1.2000
1	GRPS 1&2 POOLED	40	1.4000	1.4000
2	1.81	1	1.9000	1.9000
2	1.81	2	1.8000	1.8000
2	1.81	3	2.2000	2.2000
2	1.81	4	1.7000	1.7000
2	1.81	5	1.6000	1.6000
2	1.81	6	1.3000	1.3000
2	1.81	7	1.8000	1.8000
2	1.81	8	1.7000	1.7000
2	1.81	9	2.1000	2.1000
2	1.81	10	1.7000	1.7000

2	1.81	11	1.4000	1.4000
2	1.81	12	2.0000	2.0000
2	1.81	13	1.9000	1.9000
2	1.81	14	1.9000	1.9000
2	1.81	15	0.0000	0.0000
2	1.81	16	1.6000	1.6000
2	1.81	17	1.8000	1.8000
2	1.81	18	1.3000	1.3000
2	1.81	19	1.5000	1.5000
2	1.81	20	1.4000	1.4000
3	4.32	1	1.9000	1.9000
3	4.32	2	1.7000	1.7000
3	4.32	3	0.9000	0.9000
3	4.32	4	0.9000	0.9000
3	4.32	5	0.0000	0.0000
3	4.32	6	1.7000	1.7000
3	4.32	7	1.0000	1.0000
3	4.32	8	1.0000	1.0000
3	4.32	9	2.3000	2.3000
3	4.32	10	1.4000	1.4000
3	4.32	11	1.9000	1.9000
3	4.32	12	0.9000	0.9000
3	4.32	13	1.3000	1.3000
3	4.32	14	1.6000	1.6000
3	4.32	15	1.1000	1.1000
3	4.32	16	1.7000	1.7000
3	4.32	17	1.3000	1.3000
3	4.32	18	0.0000	0.0000
3	4.32	19	1.5000	1.5000
3	4.32	20	1.3000	1.3000
4	5.46	1	1.2000	1.2000
4	5.46	2	1.0000	1.0000
4	5.46	3	1.6000	1.6000
4	5.46	4	1.1000	1.1000
4	5.46	5	1.9000	1.9000
4	5.46	6	1.0000	1.0000
4	5.46	7	0.8000	0.8000
4	5.46	8	1.2000	1.2000
4	5.46	9	1.8000	1.8000
4	5.46	10	1.0000	1.0000
4	5.46	11	1.5000	1.5000
4	5.46	12	1.5000	1.5000
4	5.46	13	1.0000	1.0000
4	5.46	14	0.8000	0.8000
4	5.46	15	0.8000	0.8000
4	5.46	16	1.2000	1.2000
4	5.46	17	1.7000	1.7000
4	5.46	18	1.0000	1.0000
4	5.46	19	1.8000	1.8000
4	5.46	20	1.1000	1.1000
5	8.17	1	1.0000	1.0000
5	8.17	2	1.2000	1.2000
5	8.17	3	1.5000	1.5000
5	8.17	4	1.4000	1.4000
5	8.17	5	0.0000	0.0000
5	8.17	6	1.7000	1.7000
5	8.17	7	0.0000	0.0000
5	8.17	8	1.2000	1.2000
5	8.17	9	1.3000	1.3000
5	8.17	10	1.1000	1.1000

5	8.17	11	1.2000	1.2000
5	8.17	12	1.4000	1.4000
5	8.17	13	0.0000	0.0000
5	8.17	14	0.8000	0.8000
5	8.17	15	1.1000	1.1000
5	8.17	16	1.1000	1.1000
5	8.17	17	1.1000	1.1000
5	8.17	18	0.8000	0.8000
5	8.17	19	1.3000	1.3000
5	8.17	20	0.0000	0.0000
6	13.2	1	1.0000	1.0000
6	13.2	2	0.0000	0.0000
6	13.2	3	0.0000	0.0000
6	13.2	4	0.0000	0.0000
6	13.2	5	0.0000	0.0000
6	13.2	6	1.1000	1.1000
6	13.2	7	0.6000	0.6000
6	13.2	8	0.5000	0.5000
6	13.2	9	0.0000	0.0000
6	13.2	10	0.0000	0.0000
6	13.2	11	0.0000	0.0000
6	13.2	12	0.0000	0.0000
6	13.2	13	0.0000	0.0000
6	13.2	14	0.0000	0.0000
6	13.2	15	0.0000	0.0000
6	13.2	16	0.0000	0.0000
6	13.2	17	0.0000	0.0000
6	13.2	18	0.0000	0.0000
6	13.2	19	0.0000	0.0000
6	13.2	20	0.0000	0.0000

AC 303,630: Shell Deposition of Exposed Oysters
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	40	0.000	2.400	1.448
2	1.81	20	0.000	2.200	1.630
3	4.32	20	0.000	2.300	1.270
4	5.46	20	0.800	1.900	1.250
5	8.17	20	0.000	1.700	0.960
6	13.2	20	0.000	1.100	0.160

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	GRPS 1&2 POOLED	0.269	0.518	0.082	35.81

2	1.81	0.211	0.459	0.103	28.16
3	4.32	0.337	0.580	0.130	45.71
4	5.46	0.129	0.359	0.080	28.73
5	8.17	0.287	0.535	0.120	55.78
6	13.2	0.121	0.349	0.078	217.83

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	28.922	5.784	24.932
Within (Error)	134	31.090	0.232	
Total	139	60.012		

Critical F value = 2.29 (0.05,5,120)
Since F > Critical F REJECT Ho: All equal

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BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.448	1.448		
2	1.81	1.630	1.630	-1.383	
3	4.32	1.270	1.270	1.346	
4	5.46	1.250	1.250	1.497	
5	8.17	0.960	0.960	3.696	*
6	13.2	0.160	0.160	9.760	*

Bonferroni t table value = 2.36 (1 Tailed Value, P=0.05, df=120,5)

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BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	40			
2	1.81	20	0.311	21.5	-0.182
3	4.32	20	0.311	21.5	0.178
4	5.46	20	0.311	21.5	0.198

5	8.17	20	0.311	21.5	0.488
6	13.2	20	0.311	21.5	1.288

AC 303,630: Shell Deposition of Exposed Oysters
File: C:\TOXSTAT\43492817.DEP Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	40	1.448	1.448	1.508
2	1.81	20	1.630	1.630	1.508
3	4.32	20	1.270	1.270	1.270
4	5.46	20	1.250	1.250	1.250
5	8.17	20	0.960	0.960	0.960
6	13.2	20	0.160	0.160	0.160

AC 303,630: Shell Deposition of Exposed Oysters
File: C:\TOXSTAT\43492817.DEP 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
GRPS 1&2 POOLED	1.508				
1.81	1.508	0.461		1.66	k= 1, v=134
4.32	1.270	1.346		1.73	k= 2, v=134
5.46	1.250	1.497		1.75	k= 3, v=134
8.17	0.960	3.696	*	1.77	k= 4, v=134
13.2	0.160	9.760	*	1.77	k= 5, v=134

s = 0.482
Note: df used for table values are approximate when v > 20.