State of New Jersey CHRISTINE TODD WHITMAN GOVERNOR

SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca* Watershed Management Area 5 (Hackensack Watershed)



New Jersey Department of Environmental Protection ROBERT C. SHINN, JR. COMMISSIONER

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# Bureau of Freshwater and Biological Monitoring Biomonitoring Section

Assay Number(s): 98H006a, 98H006b, 98H006c

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#### **EXECUTIVE SUMMARY**

Toxicity tests using the amphipod *Hyalella azteca* were performed on sediments collected from three sites in the Hackensack River system. This initiative was undertaken by the Bureau of Freshwater and Biological Monitoring in conjunction with the Office of Watershed Management, both administered under the New Jersey Department of Environmental Protections Division of Watershed Management. The sites tested, on Dwarskill, Tenakill, and Overpeck Creek, are situated within Watershed Management Area # 5, which encompasses the entire Hackensack drainage area. Suspected toxicity at two of the sites (on Tenakill and Overpeck Creek)was based on their Aseverely impaired@biological assessments (i.e. degraded quality of benthic macroinvertebrate communities) found in previous survey(s) of New Jerseys statewide Ambient Biomonitoring Network (AMNET). A reference site was selected at Dwarskill because of its "non-impaired" AMNET assessments. The sediment toxicity tests were conducted to provide further data, which could be related to the previous assessments. Tests were conducted in accordance with the Bureaus Standard Operating Procedures, which incorporate protocols recognized by the U.S. Environmental Protection Agency. When test results were statistically compared to that of the reference, acute toxicity, as measured by mortality of test organisms, was indicated for Overpeck Creek, but not for Tenakill. Chronic toxicity, as measured by growth of test organisms, was indicated for neither of the test sites.

## **INTRODUCTION**

The Ambient Biomonitoring Network (AMNET) program of the New Jersey Department of Environmental Protection (NJDEP), Bureau of Freshwater and Biological Monitoring (BFBM), is designed to establish a biological database for use in gauging stream quality throughout the state. This database, in turn, can be an invaluable aid to New Jerseys water quality and watershed planning and management efforts. Levels of impairment are shown through the use of Rapid Bioassessment Protocols (RBP) advised by the U.S. Environmental Protection Agency (EPA)(1). The RBP assesses impairment through the collection, identification, categorizing, and quantification of instream macroinvertebrate communities. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish whether impairment is due to water quality degradation or habitat destruction.

Sediment toxicity testing is an additional tool used to determine whether toxicity is the cause of impairment, before resorting to costly chemical monitoring. The test organism, *Hyalella azteca* is an epibenthic detritivore, reported to also digest bacteria and algae from ingested sediment particles (2). This amphipod crustacean inhabits lakes, ponds, and streams throughout North and South America, typically burrowing into the sediment surface (3,4). *H. azteca* is a sensitive *A*benchmark@species, which can be cultured in the laboratory with relative ease.

In December of 1998, the Bureau of Freshwater and Biological Monitoring conducted sediment toxicity tests on three stream sites, within an area of Hackensack watershed, which had exhibited varying degrees of impairment in previous AMNET sampling. The new initiative was designed to support management efforts in Watershed Management Area (WMA) # 5, which administratively encompasses the drainage from areas of the Hackensack River and its tributary, Pascack Brook.

#### METHODS

Sample sites were selected based on previous AMNET results(5) (see appendix A), proximity to urban areas, and proximity of point source discharges (i.e. effluents from facilities with New Jersey Pollutant Discharge Elimination System (NJPDES) permits). The sites selected are as follows (see map):

AMNET	BIOLOGICAL	
STATION#	ASSESSMENT	LOCATION(see map)
AN0208	non-impaired	Dwarskill @ Ruckman Road, Closter
AN0209	severely impaired	Tenakill @ Cedar Lane, Closter
AN0212	severely impaired	Overpeck Ck. @ Dean Drive, Englewood

Sediment samples were collected from all sites on December 3, 1998. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler and placed into one liter amber glass bottles, then stored at # 4EC until the start of the test (6).

Prior to test initiation the sample sites were assigned assay numbers, in accordance with our ongoing series of toxicity tests, as follows:

98H006a = AN0208(reference, nonimpaired site) 98H006b = AN0209 98H006c = AN0212

Testing methodology followed the BFBM Standard Operating Procedures(7). 24 hours prior to the start of the test, the sediment from each station was mixed to provide a homogeneous sample, and hand picked of any visible indigenous organisms. For each site, 100 ml of sediment was added to each of the five 300 ml replicate test vessels and topped with laboratory grade freshwater to the 250 ml mark. The test vessels were then held at the test temperature (23EC) for 24 hours to allow the sediment to settle(7). After this time period, the overlying water was siphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of overlying water only.

One to seven- day old *H. azteca* juveniles were collected, from our cultures, and held for one week prior to the start of the test (7).

Testing was initiated on December 8, 1998 at 10:00 hours, by adding ten 7 to 14- day old organisms from the holding chamber to each test replicate series. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of yeast, CEROPHYLL7, Trout chow(YCT)(8), and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35 X  $10^6$  cells ml<sup>-1</sup> (after centrifugation). Mortalities were noted if visible; pH, dissolved oxygen, and conductivity were measured from aliquots of each test series; measurements were made at the start of the test and after each 24 hour period (see Table 1).

The test was concluded after ten days (December 18, 1998). As a measure of acute toxicity, live organisms were counted against those dead or missing (see Table 2). As an indication of chronic toxicity, dry weights of surviving organisms were measured (see Table 3). Statistical analysis was performed following EPA guidelines (8). Results of the reference test were compared against the control, and, providing the reference and the control were statistically similar, the remaining tests were compared to the reference.

#### RESULTS

The tests were valid by virtue of meeting the acceptability requirements of \$ 80% survival (see Table 2) in the control test series (7). The survival data for the control was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxan Rank Sum Test was used when comparing test survival results. There was no significant difference in survival results between the reference test (98H006a), and the control. All test samples were then compared to the reference. For test 98H006b, the survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxan Rank Sum Test survival results. Test 98H006b, the survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxan Rank Sum Test was used when comparing test survival results. Test 98H006b showed no significant difference in mortality from the reference sample. For test 98H006c, the survival data was distributed normally as analyzed by the Shapiro-Wilks test for normality and therefore, an F-Test and T-Test were used when comparing test survival results. Test 98H006c showed a significant difference in mortality from the reference sample. Dry weights of both test samples were then compared to that of the reference (see Table 3). The dry weight data was distributed normally as analyzed by the Shapiro-Wilks test, and therefore, an F-Test and T-Test were used when comparing test dry weight results. In this comparison, both test samples showed no significant difference from the reference (see appendix B for statistical printout).

Although indigenous organisms observed in the samples before the start of the test were removed, some did remain; however, their presence did not invalidate test results. Test chamber 98H006a contained several caddisfly larva, while 98H006c contained several chironomid, or midge, larvae.

#### DISCUSSION

The test sites (one each on Tenakill and Overpeck Creek) in Watershed Management Area # 5 were chosen based on the results of previous macroinvertebrate studies and the presence of likely sources of impairment; these included the proximity of NJPDES facilities, and of urbanization. The reference site at Dwarskill in WMA # 5, was chosen because of its prior Anonimpaired@bioassessment in the AMNET survey(5), and because it is within the same Water Region (greater Passaic River) as the test sites. Also considered in choosing the reference site, were similarities in stream morphology and position within the New Jersey Ecomap or ecoregion scheme (1,9).

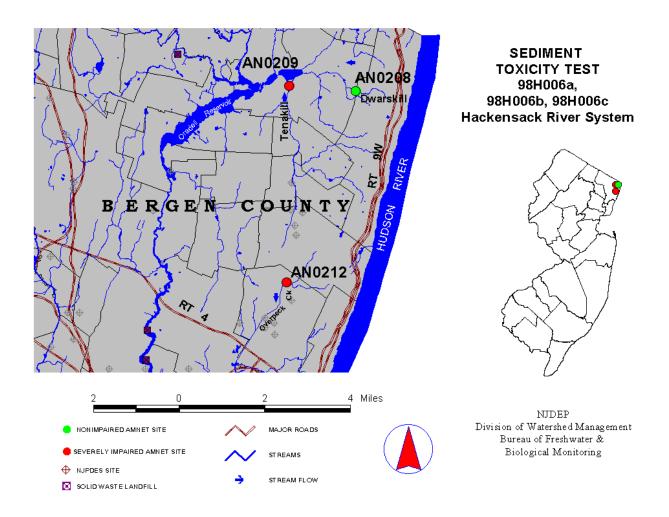
Site AN0209 (Tenakill), and site AN0212 (Overpeck Creek), were chosen for testing based on Aseverely impaired@bioassessment results, upstream presence of NJPDES facilities (on Tenakill), and to the high urban land use in the area.

Survival and dry weight results showed no significant differences between the reference and the test site treatments for the Tenakill. Since the Tenakill site results did not indicate acute toxicity, the severe impairment levels previously found may have been due to other factors including habitat degradation and/or various physiochemical parameters (e.g. temperature, turbidity, low dissolved oxygen, pH, etc...). Impairment may also have been caused by the presence of other toxic substances at chronically, but not acutely, toxic levels, which could be introduced episodically, rather than continuously, into the stream.

For Overpeck Creek dry weight results showed no significant differences between the reference and the test site treatments. Survival results for Overpeck Creek, however, exhibited acute toxicity by being significantly different, as determined by statistical analysis, in comparison to the reference site survival results. These results support the Aseverely impaired@bioassessment results demonstrated in the AMNET Survey. It is advisable, therefore, by these study results, for both Tenakill and Overpeck Creek, that supplemental sampling be performed for target analytes, such as excessive nutrients (usually in the form of nitrogen or phosphorus), and pesticides or other known toxic compounds, to determine the source of toxicity or impairment.

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#### Bureau of Freshwater and Biological Monitoring

#### Sediment Toxicity Tests Watershed Management Area # 5 Hackensack River System

			•	· · · · · ·	
Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pН	7.7	7.2	7.5	0.13	1.79
cond. Fmhos	154	136	146	6.05	4.14
D.O. mg/L	9.1	7.1	8.4	0.58	6.97
		1		1 1	
98H006a	HIGH	LOW	AVG.	STD. DEV.	% CV
pН	7.6	6.9	7.4	0.23	3.10
cond. Fmhos	176	126	160	13.89	8.70
D.O. mg/L	8.8	5.6	7.7	1.13	14.65
		1	1	· · · · · · ·	
98H006b	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.8	7.1	7.5	0.27	3.57
cond. Fmhos	193	161	172	9.14	5.31
D.O. mg/L	8.7	6.5	7.7	0.82	10.66
98H006c	HIGH	LOW	AVG.	STD. DEV.	% CV
pН	7.8	7.5	7.7	0.11	1.51
cond. Fmhos	185	220	197	10.01	5.09
D.O. mg/L	8.8	6.4	8.1	0.69	8.61

# Table 1Test Chamber Chemical/Physical Parameters

#### Bureau of Freshwater and Biological Monitoring Sediment Toxicity Tests Watershed Management Area # 5 Hackensack River System

## TABLE 2

#### MORTALITY DATA (number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	9	10	10	10	10	98
98H006a	9	8	9	10	10	92
98H006b	10	10	10	9	10	98
98H006c	7	6	5	1	3	44

## **Statistical Analysis**

Test Endpoint: Sur	vival	
Test Used:	Wilcoxan Ra	ank Sum Test
Results:	98H006a:	no significant difference from control
	98H006b:	no significant difference from reference station
Test Used:	T-test and F-	-test
Results:	98H006c:	significant difference from reference station

\*see appendix B for statistical printout

#### Bureau of Freshwater and Biological Monitoring Sediment Toxicity Tests Watershed Management Area # 5 Hackensack River System

Drying Oven Temperature:		<u>105EC</u> Duration: <u>2 hours</u>		Analyst: T. Miller			
REPLICATE	1.	WGT. OF BOAT (mg)	DRY WGT: BOAT + ORGANISMS (mg)	TOTAL WGT. OF ORGANISMS (mg)	NUMBER OF ORGANISMS	ORGANISM AVG. DRY WGT. (mg)	GROUP AVG. (mg)
CONTROL	А	25.01	25.25	0.24	9	0.027	
	В	20.72	21.27	0.55	10	0.055	
	С	23.34	23.94	0.60	10	0.060	0.047
	D	21.53	21.98	0.45	10	0.045	
	Е	26.55	27.03	0.48	10	0.048	
98H006a	A	20.13	21.48	1.35	9	0.150	
	В	19.87	21.43	1.56	8	0.195	
	С	18.28	20.46	2.18	9	0.242	0.185
	D	20.30	22.46	2.16	10	0.216	
	Е	23.67	24.87	1.20	10	0.120	
98H006b	А	16.33	17.79	1.46	10	0.146	
	В	16.29	18.24	1.95	10	0.195	
	С	19.32	20.90	1.58	10	0.158	0.168
	D	16.91	18.52	1.61	9	0.179	
	Е	20.47	22.10	1.63	10	0.163	
98H006c	А	21.75	22.49	0.74	7	0.106	
	В	17.49	18.75	1.261	6	0.210	
	С	14.29	14.72	0.43	5	0.086	0.140
	D	18.77	18.95	0.18	1	0.180	
	Е	17.47	17.83	0.36	3	0.120	

# **TABLE 3**WEIGHT DETERMINATION

Statistical Analysis Test Endpoint: Growth Results:

F- test andT-test	98H006a:	no significant difference from control
F- test andT-test	98H006b:	no significant difference from reference station
F- test andT-test	98H006c:	no significant difference from reference station

\*see appendix B for statistical printout

#### Bureau of Freshwater and Biological Monitoring

#### **APPENDIX A**

#### AMNET DATA(5)

Sediment Toxicity Tests Watershed Management Area # 5 Hackensack River System

#### Appendix A

Hackensack Basin - Yonkers USGS Quadrangle Station AN0208 Dwars Kill, Ruckman Road, Closter July 6, 1993

		NT 1	т. ч
	of	Number Tolerance	Family
Family	01	Individuals	Value (FTV)
Chironomidae		49	6
Ephemerellidae		3	1
Tipulidae		3	3
Baetidae		4	4
Empididae		3	6
Hydropsychidae		6	4
Elmidae		4	4
Leptophlebiidae		2	2
Ceratopogonidae		2	6
Glossosomatidae		1	0
Naididae		4	7
Lepidostomatidae		4	1
Philopotamidae		3	3
Nemertea		1	5
BloodRedChironomidae		2	8
Hemiptera		1	8
Rhyacophilidae		2	0
Choroperlidae		2	1
Dytiscidae		2	4
Peltoperlidae		1	1
Psephenidae		1	4

#### **Statistical Analysis**

Number of Taxa = 21 Total Number of Individuals = 100 % Contribution of Dominant Family = 49.00 Family Biotic Index = 4.81 Scraper/Filterer Collector Ratio = 0.78 Shredder/Total Ratio = 0.10 E+P+T\* = 9\*(Ephemeroptera, Plecoptera and Trichoptera)%EPT = 27.00EPT/C\* = 0.53\*(Chironomidae)NJIS Rating = 24 Biological Condition = non-impaired Deficiency(s) noted: none

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#### Observations

Streamwater: clear...Flow: slow...Width/Depth(ft): 5/<1... Substrate: rock/cobble/rubble/gravel/sand...Streambank Vegetation/ Stability: poor/poor...Canopy: mostly closed...Other: tree-lined

#### Appendix A

Hackensack Basin - Yonkers USGS Quadrangle Station AN0209 Tenakill, Cedar Lane, Closter July 6, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
BloodRedChironomidae	58	8
Tubificidae	3	10
Chironomidae	29	6
Asellidae	6	8
Gammaridae	1	4
Turbellaria	2	4
Hemiptera	1	8

#### **Statistical Analysis**

Number of Taxa = 7 Total Number of Individuals = 100 % Contribution of Dominant Family = 58.00 Family Biotic Index = 7.36 Scraper/Filterer Collector Ratio = 0.00 Shredder/Total Ratio = 0.44 E+P+T\* = 0 \*(Ephemeroptera, Plecoptera and Trichoptera) %EPT = 0.00 EPT/C\* = 0.00 \*(Chironomidae) NJIS Rating = 6 Biological Condition = severely impaired Deficiency(s) noted: paucity of clean water organisms significant organic pollution

#### Observations

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Streamwater: turbid...Flow: slow...Width/Depth(ft): 40/1+... Substrate: sand/cobble...Streambank Vegetation/Stability: poor/ /poor...Canopy: open...Other: tree-lined; siltation; fish; geese

#### Appendix A

Hackensack Basin - Yonkers USGS Quadrangle Station AN0212 Overpeck Creek, Dean Drive (near Brookside Cemetery), Englewood July 6, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Chironomidae	70	6
Gastropoda	2	7
BloodRedChironomidae	8	8
Turbellaria	9	4
Baetidae	3	4
Hydrophilidae	1	5
Aeshnidae	1	3
Tipulidae	1	3
Lumbricidae	3	10
Hydropsychidae	2	4

# Statistical Analysis

Number of Taxa = 10 Total Number of Individuals = 100 % Contribution of Dominant Family = 70.00 Family Biotic Index = 5.95 Scraper/Filterer Collector Ratio = 0.50 Shredder/Total Ratio = 0.19 E+P+T\* = 2 \*(Ephemeroptera, Plecoptera and Trichoptera) %EPT = 5.00 EPT/C\* = 0.06 \*(Chironomidae) NJIS Rating = 6 Biological Condition = severely impaired Deficiency(s) noted: paucity of clean water organisms Chironomidae overwhelmingly dominant

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#### **Observations**

Streamwater: clear...Flow: slow...Width/Depth(ft): 20/<1... Substrate: rubble/cobble/sand/gravel...Streambank Vegetation/ Stability: poor/fair...Canopy: mostly open...Other: tree-lined; right streambank is channelized; periphyton; several fish; from Palisades Drive, south, the creek is dead, black, and channelized

## Bureau of Freshwater and Biological Monitoring

#### APPENDIX B STATISTICAL DATA

Sediment Toxicity Tests Watershed Management Area # 5 Hackensack River System

## Sediment Toxicity Tests Watershed Management Area # 5

## SURVIVAL RESULTS

#### CONTROL VS. AN0208 (reference)

## Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0208	Blank Trans	AN0208 Trans
0.9	0.9	1.2	1.2
1.0	0.8	1.4	1.1
1.0	0.9	1.4	1.2
1.0	1.0	1.4	1.4
1.0	1.0	1.4	1.4

#### Shapiro-Wilks Test for Normality

Blank Trans	AN0208 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.249	1.249	1.249		-0.084	-0.2259				
1.4127	1.1071	1.4127		0.0797	-0.084				
1.4127	1.249	1.4127	1.333	0.0797	-0.084	0.1103	0.733	0.842	Not Normal
1.4127	1.4127	1.4127		0.0797	-0.084				
1.4127	1.4127	1.4127		0.0797	0.0797				
		1.249		-0.084	0.0797				
Mean	Mean	1.1071		-0.2259	0.0797				
1.38	1.2861	1.249		-0.084	0.0797				
		1.4127		0.0797	0.0797				
		1.4127		0.0797	0.0797				

# Sediment Toxicity Tests Watershed Management Area # 5

#### SURVIVAL RESULTS continued...

# CONTROL VS. AN0208 (reference)

#### Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0208	Critical(from Table K=1)	Result
1.249	1.1071	7	1	0	1	19	No Significant Difference
1.4127	1.249	8	3	0	3		
1.4127	1.249	6	3	0	3		
1.4127	1.249	1	3	3	0		
1.4127	1.4127	10	7.5	0	7.5		
1.249	1.4127	9	7.5	0	7.5		
1.1071	1.4127	5	7.5	7.5	0		
1.249	1.4127	4	7.5	7.5	0		
1.4127	1.4127	3	7.5	7.5	0		
1.4127	1.4127	2	7.5	7.5	0		
				Sum	Sum		
				33	22		

## Sediment Toxicity Tests Watershed Management Area # 5

#### SURVIVAL RESULTS continued...

## AN0208 (reference) VS. AN0209

#### Survival Proportions with Arc Sine Square Root Transformation

AN0208	AN0209	AN0208 Trans	AN0209 Trans	
0.9	1.0	1.249	1.4127	
0.8	1.0	1.1071	1.4127	
0.9	1.0	1.249	1.4127	
1.0	0.9	1.4127	1.249	
1.0	1.0	1.4127	1.4127	

#### Shapiro-Wilks Test for Normality

AN0208 Trans	AN0209 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.249	1.4127	1.249		-0.084	-0.2259				
1.1071	1.4127	1.1071		-0.2259	-0.084				
1.249	1.4127	1.249	1.333	-0.084	-0.084	0.1103	0.733	0.842	Not Normal
1.4127	1.249	1.4127		0.0797	-0.084				
1.4127	1.4127	1.4127		0.0797	0.0797				
		1.4127		0.0797	0.0797				
Mean	Mean	1.4127		0.0797	0.0797				
1.2861	1.38	1.4127		0.0797	0.0797				
		1.249		-0.084	0.0797				
		1.4127		0.0797	0.0797				

## Sediment Toxicity Tests Watershed Management Area # 5

#### SURVIVAL RESULTS continued...

## AN0208 (reference) VS. AN0209

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0208	AN0209	Critical(fro m Table K=1)	Result
1.249	1.1071	2	1	1	0	19	No Significant Difference
1.1071	1.249	9	3	0	3		
1.249	1.249	3	3	3	0		
1.4127	1.249	1	3	3	0		
1.4127	1.4127	10	7.5	0	7.5		
1.4127	1.4127	8	7.5	0	7.5		
1.4127	1.4127	7	7.5	0	7.5		
1.4127	1.4127	6	7.5	0	7.5		
1.249	1.4127	5	7.5	7.5	0		
1.4127	1.4127	4	7.5	7.5	0		
				Sum	Sum		
				22	33		

#### Sediment Toxicity Tests Watershed Management Area # 5

#### SURVIVAL RESULTS continued...

## AN0208 (reference) VS. AN0212

#### Survival Proportions with Arc Sine Square Root Transformation

AN0208	AN0212	AN0208 Trans	AN0212 Trans	
0.9	0.7	1.249	0.9912	
0.8	0.6	1.1071	0.8861	
0.9	0.5	1.249	0.7854	
1.0	0.1	1.4127	0.3218	
1.0	0.3	1.4127	0.5796	

#### Shapiro-Wilks Test for Normality

AN0208 Trans	AN0212 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.249	0.9912	1.249		0.2495	-0.6777				
1.1071	0.8861	1.1071		0.1076	-0.4199				
1.249	0.7854	1.249	0.9995	0.2495	-0.2141	1.1719	0.9382	0.842	Normal
1.4127	0.3218	1.4127		0.4132	-0.1134				
1.4127	0.5796	1.4127		0.4132	-0.0083				
		0.9912		-0.0083	0.1076				
Mean	Mean	0.8861		-0.1134	0.2495				
1.2861	0.7128	0.7854		-0.2141	0.2495				
		0.3218		-0.6777	0.4132				
		0.5796		-0.4199	0.4132				

AN0208 Var	AN0212 Var	F-Value	Critical-F (Two-Taile d 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0167	0.0709	4.2455	6.3882	Equal	4.3313	5	2.015	Significantly Different

## Sediment Toxicity Tests Watershed Management Area # 5

## GROWTH

## CONTROL VS. AN0208 (reference)

Average Dry Weight per Replicate (in mg)

BLANK	AN0208
0.027	0.150
0.055	0.195
0.060	0.242
0.045	0.216
0.048	0.120

#### Shapiro-Wilks Test for Normality

BLANK	AN0208	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
0.027	0.15	0.027		-0.0888	-0.0888				
0.055	0.195	0.055		-0.0608	-0.0708				
0.06	0.242	0.06	0.1158	-0.0558	-0.0678	0.0577	0.8786	0.842	Normal
0.045	0.216	0.045		-0.0708	-0.0608				
0.048	0.12	0.048		-0.0678	-0.0558				
		0.15		0.0342	0.0042				
Mean	Mean	0.195		0.0792	0.0342				
0.047	0.1846	0.242		0.1262	0.0792				
		0.216		0.1002	0.1002				
		0.12		0.0042	0.1262				

BLANK Var	AN0208 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-valu e	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0002	0.0024	12	6.3882	Unequal	-6.0342	4	2.1318	No Significant Difference

## Sediment Toxicity Tests Watershed Management Area # 5

## **GROWTH continued...**

## AN0208 (reference) VS. AN0209

Average Dry Weight per Replicate (in mg)

AN0208	AN0209
0.150	0.146
0.195	0.195
0.242	0.158
0.216	0.179
0.120	0.163

#### Shapiro-Wilks Test for Normality

AN0208	AN0209	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
0.15	0.146	0.15		-0.0264	-0.0564				
0.195	0.195	0.195		0.0186	-0.0304				
0.242	0.158	0.242	0.1764	0.0656	-0.0264	0.0119	0.9775	0.842	Normal
0.216	0.179	0.216		0.0396	-0.0184				
0.12	0.163	0.12		-0.0564	-0.0134				
		0.146		-0.0304	0.0026				
Mean	Mean	0.195		0.0186	0.0186				
0.1846	0.1682	0.158		-0.0184	0.0186				
		0.179		0.0026	0.0396				
		0.163		-0.0134	0.0656				

AN0208 Var	AN0209 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-valu e	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0024	0.0004	6	6.3882	Equal	0.693	5	2.015	No Significant Difference

## Sediment Toxicity Tests Watershed Management Area # 5

## **GROWTH continued...**

## AN0208 (reference) VS. AN0212

Average Dry Weight per Replicate (in mg)

AN0208	AN0212
0.150	0.106
0.195	0.210
0.242	0.086
0.216	0.180
0.120	0.120

#### Shapiro-Wilks Test for Normality

AN0208	AN0212	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
0.15	0.106	0.15		-0.0125	-0.0765				
0.195	0.21	0.195		0.0325	-0.0565				
0.242	0.086	0.242	0.1625	0.0795	-0.0425	0.0256	0.943	0.842	Normal
0.216	0.18	0.216		0.0535	-0.0425				
0.12	0.12	0.12		-0.0425	-0.0125				
		0.106		-0.0565	0.0175				
Mean	Mean	0.21		0.0475	0.0325				
0.1846	0.1404	0.086		-0.0765	0.0475				
		0.18		0.0175	0.0535				
		0.12		-0.0425	0.0795				

AN0208 Var	AN0212 Var	F-Value	Critical-F (Two-Taile d 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0024	0.0027	1.125	6.3882	Equal	1.384	7	1.8946	No Significant Difference