

**State of New Jersey  
CHRISTINE TODD WHITMAN  
GOVERNOR**

**SEDIMENT TOXICITY TEST  
USING THE AMPHIPOD  
*Hyalella azteca*  
Watershed Management Area 9 and 10  
(South River and Millstone River)**



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

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New Jersey Department of Environmental Protection  
Division of Watershed Management  
P.O. Box 427, Trenton, NJ 08625-0427

## **WATER MONITORING MANAGEMENT**

James Mumman, Administrator

**January 1999**

# **SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca* Watershed Management Area 9 and 10 (South River and Millstone River)**

**Bureau of Freshwater and Biological Monitoring  
Biomonitoring Section**

**Assay Number(s): 98H006d, 98H006e, 98H006f**

**Report Prepared By:**

Victor Poretti

**Analysts:**

Thomas Miller  
Dean Bryson  
Victor Poretti

**Samplers:**

Thomas Miller  
Dean Bryson

**Supervisor:**

Paul Olsen

**Chief**

**Bureau of Freshwater & Biological Monitoring**  
Alfred Korndoerfer, Jr.

## EXECUTIVE SUMMARY

Toxicity tests using the amphipod *Hyalella azteca* were performed on sediments collected from three sites in the Raritan 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 Protection's Division of Watershed Management. One site tested is situated on the Millstone River within Watershed Management Area # 10, and two sites, one each on Manalapan Brook and McGellairds Brook, are within Watershed Management Area # 9, all within the Raritan Water Region. Suspected toxicity at two of the sites (Manalapan Brook and McGellairds Brook) was based on their severely impaired biological assessments (i.e. degraded quality of benthic macroinvertebrate communities) found in previous survey(s) of New Jersey's statewide Ambient Biomonitoring Network (AMNET). A reference site was selected at Millstone River because of its "non-impaired" AMNET assessment. 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 Bureau's 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, the test sites did not exhibit acute toxicity, as measured by survival, or chronic toxicity as measured by growth, of test organisms.

## 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 Jersey's 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 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 central New Jersey, which had exhibited varying degrees of impairment in previous AMNET sampling. The new initiative was designed to support management efforts in Watershed Management Areas (WMA) # 9 and # 10. Administratively, these include the Millstone and South river drainages, respectively, in the Raritan Water Region=

## METHODS

Sample sites were selected based on previous AMNET results(5) (see appendix A), proximity to urban and/or agricultural 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):

<u>AMNET STATION#</u>	<u>BIOLOGICAL ASSESSMENT</u>	<u>LOCATION</u> (see map)
AN0379	non-impaired	Millstone River @ Rt. 33 Bergan Mills
AN0439	severely impaired	Manalapan Bk. @ Federal Rd., Monroe Twp.
AN0447	severely impaired	McGellairds Bk. @ Rt. 527, Englishtown

Sediment samples were collected from all sites on December 4, 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:

98H006d = AN0379 (reference, nonimpaired site)  
98H006e = AN0439  
98H006f = AN0447

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  $35 \times 10^6$  cells  $\text{ml}^{-1}$  (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  $\geq 80\%$  survival (see Table 2) in the control test series (7). The survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxon Rank Sum Test was used when comparing test survival results. There was no significant difference in survival results between the reference test (98H006d), and the control. All test samples were then compared to the reference. The survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxon Rank Sum Test was used when comparing test survival results. The results for tests 98H006e and 98H006f both showed no significant difference in mortality from that of 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 the test samples showed no significant difference from the reference (see appendix B for statistical printout).

Although all 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 chambers 98H006d and 98H006e

contained several chironomid, or midge, larvae.

## DISCUSSION

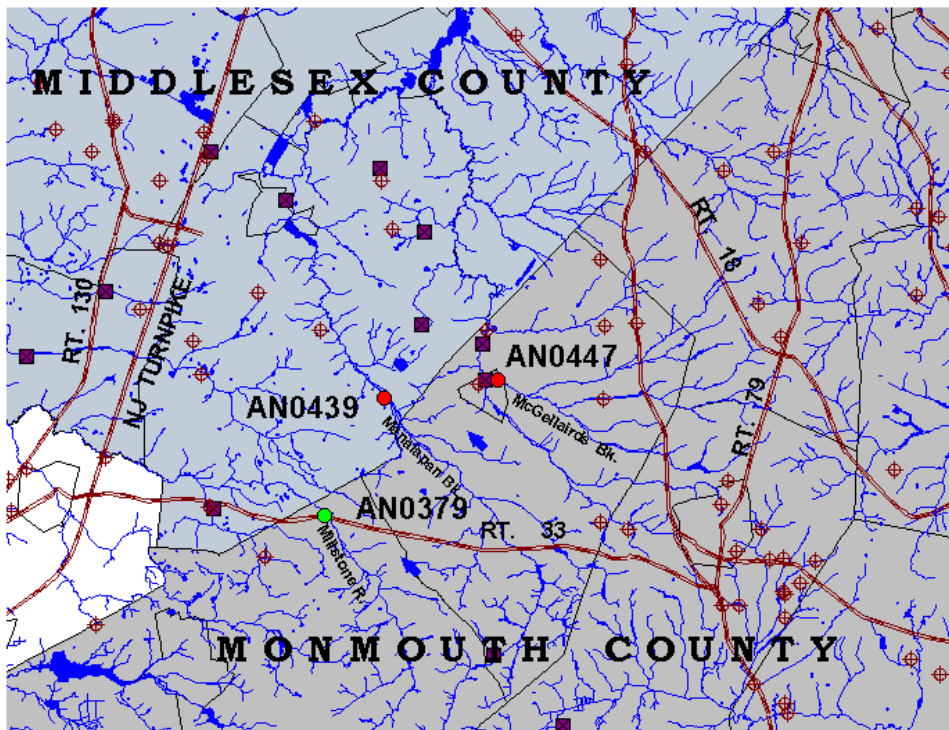
The test sites (one each on Manalapan Brook and McGellairds Brook) in WMA 9 were chosen based on the results of previous macroinvertebrate studies and the presence of likely sources of impairment; these included the proximity of NJPDES facilities, and of urbanization, or agriculture. The reference site at Millstone River in WMA 10, was chosen because of its prior *Nonimpaired* bioassessment in the AMNET survey(5), and because it is within the same Water Region (Raritan River Basin) 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 AN0439 (Manalapan Brook), and site AN0447 (McGellairds Brook), were chosen for testing based on *Severely impaired* bioassessment results, upstream presence of NJPDES facilities (on McGellairds Brook), and the high agricultural land use in the area.

Survival and dry weight results showed no significant differences between the reference and the test site treatments. Since the test 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. Therefore, it is advisable by these study results, that supplemental sampling be performed for target analytes, such as excessive nutrients (usually forms of nitrogen or phosphorus), and pesticides or other known toxic compounds.

## REFERENCES

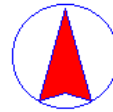
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**SEDIMENT  
TOXICITY TEST  
98H006d,  
98H006e, 98H006f  
Millstone and  
South Rivers**



- |  |   |
|--|---|
|  NONIMPAIRED AMHET SITE       |  MAJOR ROADS |
|  SEVERELY IMPAIRED AMHET SITE |  STREAMS     |
|  HJPDES SITE                  |  STREAM FLOW |
|  SOLID WASTE LANDFILL         |   |



NJDEP  
Division of Watershed Management  
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Biological Monitoring



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**Sediment Toxicity Tests  
Watershed Management Area # 9 and 10  
Millstone and South Rivers**

**Table 1  
Test Chamber Chemical/Physical Parameters**

<b>Control</b>	<b>HIGH</b>	<b>LOW</b>	<b>AVG.</b>	<b>STD. DEV.</b>	<b>% CV</b>
pH	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

<b>98H006d</b>	<b>HIGH</b>	<b>LOW</b>	<b>AVG.</b>	<b>STD. DEV.</b>	<b>% CV</b>
pH	7.5	6.8	7.2	0.26	3.60
cond. Fmhos	156	135	150	6.43	4.30
D.O. mg/L	8.7	5.9	7.3	1.01	13.96

<b>98H006e</b>	<b>HIGH</b>	<b>LOW</b>	<b>AVG.</b>	<b>STD. DEV.</b>	<b>% CV</b>
pH	7.3	6.8	7.1	0.23	3.29
cond. Fmhos	155	115	141	10.85	7.71
D.O. mg/L	8.7	5.8	7.5	1.03	13.66

<b>98H006f</b>	<b>HIGH</b>	<b>LOW</b>	<b>AVG.</b>	<b>STD. DEV.</b>	<b>% CV</b>
pH	7.4	6.8	7.3	0.19	2.61
cond. Fmhos	157	120	147	11.98	8.12
D.O. mg/L	8.7	6.8	8.0	0.63	7.84

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**Sediment Toxicity Tests  
Watershed Management Area # 9 and 10  
Millstone and South Rivers**

**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
98H006d	10	10	9	9	9	94
98H006e	9	10	10	10	10	98
98H006f	9	10	10	9	10	96

**Statistical Analysis**

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 98H006d: no significant difference from control  
98H006e: no significant difference from reference station  
98H006f: no significant difference from reference station

\*see appendix B for statistical printout

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**Sediment Toxicity Tests  
Watershed Management Area # 9 and 10  
Millstone and South Rivers**

**TABLE 3  
WEIGHT DETERMINATION**

Drying Oven Temperature: 105EC

Duration: 2 hours

Analyst: T. Miller

REPLICATE.	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 A	25.01	25.25	0.24	9	0.027	0.047
B	20.72	21.27	0.55	10	0.055	
C	23.34	23.94	0.60	10	0.060	
D	21.53	21.98	0.45	10	0.045	
E	26.55	27.03	0.48	10	0.048	
98H006d A	20.65	21.97	1.32	10	0.132	0.146
B	13.10	14.17	1.07	10	0.107	
C	18.14	19.73	1.59	9	0.177	
D	17.14	18.48	1.34	9	0.149	
E	17.63	19.12	1.49	9	0.166	
98H006e A	22.24	23.74	1.50	9	0.167	0.194
B	24.37	26.53	2.16	10	0.216	
C	20.23	22.05	1.82	10	0.182	
D	20.15	22.19	2.04	10	0.204	
E	16.57	18.60	2.03	10	0.203	
98H006f A	20.63	22.12	1.49	9	0.166	0.181
B	23.16	24.87	1.71	10	0.171	
C	14.56	16.54	1.98	10	0.198	
D	17.34	19.16	1.82	9	0.202	
E	18.62	20.31	1.69	10	0.169	

Statistical Analysis

Test Endpoint: Growth

Results:

F-test and T-test

98H006d:

no significant difference from control

F-test and T-test

98H006e:

no significant difference from reference station

F-test and T-test

98H006f:

no significant difference from reference station

\*see appendix B for statistical printout

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**APPENDIX A**

**AMNET DATA(5)**

**Sediment Toxicity Tests  
Watershed Management Area # 9 and 10  
Millstone and South Rivers**

## Appendix A

Raritan Basin - Jamesburg USGS Quadrangle  
Station AN0379  
Millstone River, Route 33, Bergen Mills  
July 25, 1990

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Family	Number of Individuals	Family Tolerance Value (FTV)
Hydropsychidae	25	4
Chironomidae	6	6
Heptageniidae	15	4
Tubificidae	7	10
Dytiscidae	4	4
Bryozoa	18	7
Cordulegastridae	1	3
Gastropoda	3	7
Nemertea	5	5
Ephemerellidae	1	1
Corydalidae	2	0
Tipulidae	4	3
BloodRedChironomidae	3	8
Sphaeriidae	2	8
Gomphidae	1	1
Aeshnidae	2	3
Elmidae	1	4

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### Statistical Analysis

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Number of Taxa: 17  
Total Number of Individuals: 100  
% Contribution of Dominant Family: 25.00  
Family Biotic Index: 5.21  
Scraper/Filterer Collector Ratio: 0.02  
Shredder/Total Ratio: 0.22  
E+P+T\*: 3 \*(Ephemeroptera, Plecoptera and Trichoptera)  
%EPT: 41.00  
EPT/C\*: 4.56 \*(Chironomidae)  
NJIS Score: 24  
Biological Condition: non-impaired  
Deficiency(s) noted: none

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### Observations

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Streamwater: slightly turbid...Flow: moderate...Width/Depth(ft): 15/<1...Substrate: gravel...Streambank Vegetation/Stability: good/good...Canopy: open...Other: brush-lined/commercial

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## Appendix A

Raritan Basin - Jamesburg USGS Quadrangle  
Station AN0439  
Manalapan Brook, Federal Road, Monroe Township  
September 29, 1993

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<b>Family</b>	<b>Number of Individuals</b>	<b>Family Tolerance Value (FTV)</b>
Hemiptera	45	8
Chironomidae	5	6
Aeshnidae	2	3
Simuliidae	1	6
Libellulidae	1	9
Sphaeriidae	1	8
Pyralidae	1	5
Nemertea	1	5
Gastropoda	1	7

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### Statistical Analysis

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Number of Taxa: 9  
Total Number of Individuals: 58  
% Contribution of Dominant Family: 77.59  
Family Biotic Index: 7.52  
Scraper/Filterer Collector Ratio: 0.00  
Shredder/Total Ratio: 0.00  
E+P+T\*: 0 \*(Ephemeroptera, Plecoptera and Trichoptera)  
%EPT: 0.00  
EPT/C\*: 0.00 \*(Chironomidae)  
NJIS Score: 3  
Biological Condition: severely impaired  
Deficiency(s) noted: Hemiptera overwhelmingly dominant, paucity of clean water organisms, significant organic pollution

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### Observations

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Streamwater: turbid...Flow: moderate...Width/Depth(ft): 20/1...Substrate: sand/gravel/concrete stones...Streambank  
Vegetation/Stability: good/good...Canopy: open...Other: wooded/grass fields

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## Appendix A

Raritan Basin - Freehold USGS Quadrangle  
Station AN0447  
McGellairds Brook, Route 527, Englishtown  
September 14, 1993

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Family	Number of Individuals	Family Tolerance Value (FTV)
Tubificidae	80	10
Sphaeriidae	1	8
Chironomidae	8	6
Gastropoda	2	7
Tipulidae	2	3
Nemertea	4	5
Sialidae	1	4
Hydropsychidae	1	4
Coenagrionidae	3	9
BloodRedChironomidae	1	8
Anthomyiidae	1	6
Elmidae	1	4

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### Statistical Analysis

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Number of Taxa: 12

Total Number of Individuals: 105

% Contribution of Dominant Family: 76.19

Family Biotic Index: 9.04

Scraper/Filterer Collector Ratio: 1.00

Shredder/Total Ratio: 0.07

E+P+T\*: 1 \*(Ephemeroptera, Plecoptera and Trichoptera)

%EPT: 0.95

EPT/C\*: 0.11 \*(Chironomidae)

NJIS Score: 6

Biological Condition: severely impaired

Deficiency(s) noted: paucity of clean water organisms, Tubificidae overwhelmingly dominant, significant organic pollution

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### Observations

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Streamwater: turbid...Flow: slow...Width/Depth(ft): 30/1...Substrate: sand/mud...Streambank Vegetation/Stability:  
fair/fair...Canopy: open...Other: tree-lined/suburban/residential; fish

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**APPENDIX B  
STATISTICAL DATA**

**Sediment Toxicity Tests  
Watershed Management Area # 9 and 10  
Millstone and South Rivers**



## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

#### SURVIVAL RESULTS

#### CONTROL VS. AN0379 (reference)

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

Blank	AN0379	Blank Trans	AN0379 Trans
0.9	1.0	1.249	1.4127
1.0	1.0	1.4127	1.4127
1.0	0.9	1.4127	1.249
1.0	0.9	1.4127	1.249
1.0	0.9	1.4127	1.249

#### Shapiro-Wilks Test for Normality

Blank Trans	AN0379 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.249	1.4127	1.249		-0.0982	-0.0982				
1.4127	1.4127	1.4127		0.0655	-0.0982				
1.4127	1.249	1.4127	1.3472	0.0655	-0.0982	0.0643	0.6403	0.842	Not Normal
1.4127	1.249	1.4127		0.0655	-0.0982				
1.4127	1.249	1.4127		0.0655	0.0655				
		1.4127		0.0655	0.0655				
Mean	Mean	1.4127		0.0655	0.0655				
1.38	1.3145	1.249		-0.0982	0.0655				
		1.249		-0.0982	0.0655				
		1.249		-0.0982	0.0655				

## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

**SURVIVAL RESULTS continued...**

**CONTROL VS. AN0379 (reference)**

**Wilcoxon Rank Sum Test**

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0379	Critical(from Table K=1)	Result
1.249	1.249	10	2.5	0	2.5	19	No Significant Difference
1.4127	1.249	9	2.5	0	2.5		
1.4127	1.249	8	2.5	0	2.5		
1.4127	1.249	1	2.5	2.5	0		
1.4127	1.4127	7	7.5	0	7.5		
1.4127	1.4127	6	7.5	0	7.5		
1.4127	1.4127	5	7.5	7.5	0		
1.249	1.4127	4	7.5	7.5	0		
1.249	1.4127	3	7.5	7.5	0		
1.249	1.4127	2	7.5	7.5	0		
				Sum	Sum		
				32.5	22.5		

## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

**SURVIVAL RESULTS continued...**

**AN0379 (reference) VS. AN0439**

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

AN0379	AN0439	AN0379 Trans	AN0439 Trans
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	1.4127
0.9	1.0	1.249	1.4127
0.9	1.0	1.249	1.4127

#### Shapiro-Wilks Test for Normality

AN0379 Trans	AN0439 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.4127	1.249	1.4127		0.0655	-0.0982				
1.4127	1.4127	1.4127		0.0655	-0.0982				
1.249	1.4127	1.249	1.3472	-0.0982	-0.0982	0.0643	0.6403	0.842	Not Normal
1.249	1.4127	1.249		-0.0982	-0.0982				
1.249	1.4127	1.249		-0.0982	0.0655				
		1.249		-0.0982	0.0655				
Mean	Mean	1.4127		0.0655	0.0655				
1.3145	1.38	1.4127		0.0655	0.0655				
		1.4127		0.0655	0.0655				
		1.4127		0.0655	0.0655				

**Appendix B**  
**Sediment Toxicity Tests**  
**Watershed Management Area # 9 and 10**

**SURVIVAL RESULTS continued...**

**AN0379 (reference) VS. AN0439**

**Wilcoxon Rank Sum Test**

Pooled	Sorted	Point	Wilcoxon Rank	AN0379	AN0439	Critical(from Table K=1)	Result
1.4127	1.249	6	2.5	0	2.5	19	No Significant Difference
1.4127	1.249	5	2.5	2.5	0		
1.249	1.249	4	2.5	2.5	0		
1.249	1.249	3	2.5	2.5	0		
1.249	1.4127	10	7.5	0	7.5		
1.249	1.4127	9	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	2	7.5	7.5	0		
1.4127	1.4127	1	7.5	7.5	0		
				Sum	Sum		
				22.5	32.5		

## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

#### SURVIVAL RESULTS continued...

AN0379 (reference) VS. AN0447

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

AN0379	AN0447	AN0379 Trans	AN0447 Trans
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	1.4127
0.9	0.9	1.249	1.249
0.9	1.0	1.249	1.4127

#### Shapiro-Wilks Test for Normality

AN0379 Trans	AN0447 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.4127	1.249	1.4127		0.0818	-0.0819				
1.4127	1.4127	1.4127		0.0818	-0.0819				
1.249	1.4127	1.249	1.3309	-0.0819	-0.0819	0.067	0.6547	0.842	Not Normal
1.249	1.249	1.249		-0.0819	-0.0819				
1.249	1.4127	1.249		-0.0819	-0.0819				
		1.249		-0.0819	0.0818				
Mean	Mean	1.4127		0.0818	0.0818				
1.3145	1.3472	1.4127		0.0818	0.0818				
		1.249		-0.0819	0.0818				
		1.4127		0.0818	0.0818				

## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

**SURVIVAL RESULTS continued...**

**AN0379 (reference) VS. AN0447**

#### Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0379	AN0447	Critical(from Table K=1)	Result
1.4127	1.249	9	3	0	3	19	No Significant Difference
1.4127	1.249	6	3	0	3		
1.249	1.249	5	3	3	0		
1.249	1.249	4	3	3	0		
1.249	1.249	3	3	3	0		
1.249	1.4127	10	8	0	8		
1.4127	1.4127	8	8	0	8		
1.4127	1.4127	7	8	0	8		
1.249	1.4127	2	8	8	0		
1.4127	1.4127	1	8	8	0		
				Sum	Sum		
				25	30		

## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

#### GROWTH

CONTROL VS. AN0379 (reference)

Average Dry Weight per Replicate (in mg)

BLANK	AN0379
0.027	0.132
0.055	0.107
0.060	0.177
0.045	0.149
0.048	0.166

Shapiro-Wilks Test for Normality

BLANK	AN0379	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.027	0.132	0.027		-0.0696	-0.0696				
0.055	0.107	0.055		-0.0416	-0.0516				
0.06	0.177	0.06	0.0966	-0.0366	-0.0486	0.0283	0.8914	0.842	Normal
0.045	0.149	0.045		-0.0516	-0.0416				
0.048	0.166	0.048		-0.0486	-0.0366				
		0.132		0.0354	0.0104				
Mean	Mean	0.107		0.0104	0.0354				
0.047	0.1462	0.177		0.0804	0.0524				
		0.149		0.0524	0.0694				
		0.166		0.0694	0.0804				

F-Test and T-Test

BLANK Var	AN0379 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0002	0.0008	4	6.3882	Equal	-7.0145	5	2.015	No Significant Difference

## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

**GROWTH** continued...

AN0379 (reference) VS. AN0439

Average Dry Weight per Replicate (in mg)

AN0379	AN0439
0.132	0.167
0.107	0.216
0.177	0.182
0.149	0.204
0.166	0.203

Shapiro-Wilks Test for Normality

AN0379	AN0439	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.132	0.167	0.132		-0.0383	-0.0633				
0.107	0.216	0.107		-0.0633	-0.0383				
0.177	0.182	0.177	0.1703	0.0067	-0.0213	0.0104	0.9648	0.842	Normal
0.149	0.204	0.149		-0.0213	-0.0043				
0.166	0.203	0.166		-0.0043	-0.0033				
		0.167		-0.0033	0.0067				
Mean	Mean	0.216		0.0457	0.0117				
0.1462	0.1944	0.182		0.0117	0.0327				
		0.204		0.0337	0.0337				
		0.203		0.0327	0.0457				

F-Test and T-Test

AN0379 Var	AN0439 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.0004	2	6.3882	Equal	-3.1113	7	1.8946	No Significant Difference



## Appendix B

### Sediment Toxicity Tests Watershed Management Area # 9 and 10

**GROWTH** continued...

AN0379 (reference) VS. AN0447

Average Dry Weight per Replicate (in mg)

AN0379	AN0447
0.132	0.166
0.107	0.171
0.177	0.198
0.149	0.202
0.166	0.169

Shapiro-Wilks Test for Normality

AN0379	AN0447	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.132	0.166	0.132		-0.0317	-0.0567				
0.107	0.171	0.107		-0.0567	-0.0317				
0.177	0.198	0.177	0.1637	0.0133	-0.0147	0.0073	0.943	0.842	Normal
0.149	0.202	0.149		-0.0147	0.0023				
0.166	0.169	0.166		0.0023	0.0023				
		0.166		0.0023	0.0053				
Mean	Mean	0.171		0.0073	0.0073				
0.1462	0.1812	0.198		0.0343	0.0133				
		0.202		0.0383	0.0343				
		0.169		0.0053	0.0383				

F-Test and T-Test

AN0379 Var	AN0447 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.0003	2.6667	6.3882	Equal	-2.3597	6	1.9432	No Significant Difference