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WATER MONITORING MANAGEMENT
James Mumman, Administrator

March 1996

SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca* (S. BRANCH RAHWAY RIVER, ELIZABETH RIVER, & MIDDLE BROOK SEDIMENT) MARCH 1996

Assay Number(s): 96H002a, 96H002b, 96H002c

Report Prepared By:

Victor Poretti

Analysts:

Thomas Miller
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Samplers:

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Acting Chief
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STATE OF NEW JERSEY
DEPARTMENT OF ENVIRONMENTAL PROTECTION
DIVISION OF SCIENCE AND RESEARCH
BUREAU OF WATER MONITORING
BIOMONITORING OPERATIONS SECTION

REPORT

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INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program is designed to establish biologically impaired stream segments throughout the state using EPA's Rapid Bioassessment Protocol (RBP). The RBP assesses impairment through the collection, identification, and classification of macroinvertebrates. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish if impairment is due to water quality or habitat destruction. Sediment Toxicity Testing is an additional tool to narrow down the cause of impairment to an acute toxicity problem before resorting to costly chemical monitoring.

Hyalella azteca is an epibenthic detritovore reported to also digest bacteria and algae from ingested sediment particles (Hargrave, 1970). This amphipod burrows into the sediment surface and inhabits lakes, ponds, and streams throughout North and South America (de March, 1981; Pennak, 1989). *H. azteca* is a sensitive benchmark species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data (see appendix a) and proximity to NJPDES facilities.
The sites selected are as follows (see map):

AMNET STATION#	BIOLOGICAL ASSESSMENT	LOCATION(see map)
AN0201	severely impaired	S. Branch Rahway River @ Dover St. off Maplewood Terrace
AN0204	severely impaired	Elizabeth River @ Summer St.
AN0420	non-impaired	Middle Brook @ Rt. 28 (reference)

Sediment samples were collected from these sites on March 14, 1996 at 10:05, 11:05, and 13:13 hours respectively. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler and placed into two one liter amber glass bottles and stored at less than 4EC until the start of the test (NJDEP, 1992).

Prior to test initiation the sample sites were assigned assay numbers as follows:

96H002a = AN0420
96H002b = AN0201
96H002c = AN0204

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures (NJDEP, SM001.0795, 1995). 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 organism. 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(NJDEP, SM001.0795,1995). After this time period, the overlying water was syphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of overlying water only.

1 - 7 day *H. azteca* juveniles were collected and held for one week prior to the start of the test (NJDEP, 1995).

The test was initiated on March 19, 1996 at 11:00 hours, by adding ten 7 - 14 day old organisms from the holding chamber to each test series replicate. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of YCT and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35 X 10^6 cells/ml. 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 3).

The test was concluded after ten days (March 29, 1996). Live organisms were counted (see table 1) and the dry weights measured (see table 2). Statistical analysis was performed, following EPA guidelines (U.S.E.P.A., 1991). The reference test was compared against the control and the remaining tests compared to the reference, providing the reference and the control were statistically the same.

Salinity and conductivity analysis was performed, using a YSI 33 S-C-T meter, on April 24, 1996 on the surface water of the Elizabeth River at the toxicity test sample site, and on sites upstream and downstream. Analysis was performed at high tide to determine the maximum saltwater influence on the toxicity testing sample site. The sites are as follows:

Elizabeth River @ Atlantic Street (downstream)
Elizabeth River @ Summer Street
Elizabeth River @ South Street (upstream)

RESULTS

The test was valid by meeting the acceptability requirements of $ 80% survival (see table 1) in the control test series (NJDEP, SM001.0795,1995). The survival data of the tests was not normally distributed when 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 between the reference test, 96H002a, survival results and the control survival results. Test 96H002b and 96H002c survival data was then compared to the reference test. 96H002b had no significant differences from the reference test. 96H002c was significantly different from the reference test for mortality.

Growth data (see table 2) was normally distributed when comparing the control and reference station and also when comparing the reference test with 96H002b and 96H002c. Normality was analyzed using the Shapiro-Wilks test for normality, and an F-test and T-test was performed when comparing tests. Again there was no significant differences between the control and the reference test 96H002a. 96H002b and 96H002c exhibited a significant difference from the reference test for growth. 96H002b and 96H002c was also compared to the control treatment for growth endpoints. 96H002b data was not normally distributed and 96H002c data was normally distributed when compared to the control treatment. The Shapiro-Wilks test was used to analyze normality. F-test and T-test showed no significant differences between the control and test treatments.(see appendix b for statistical printout)

For salinity and conductivity results for the surface water of the Elizabeth River see Table 4.

DISCUSSION

The sample sites on the South Branch of the Rahway River and the Elizabeth River were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities. Sites AN0201 and AN0204 had severely impaired bioassessment results as analyzed in AMNET. The reference site, AN0420, was chosen because it had a nonimpaired bioassessment based on results from the AMNET and ECOREGION macroinvertebrate programs, and also because of similar stream morphology, and similar ecological region designation to the sample sites suspected of toxicity.

In the morning hours prior to sample collection, a residential fuel oil tank ruptured, spilling oil into storm drains which eventually deposited into the South Branch Rahway river, upstream of sample site AN0201(personal communication with DEP Central Emergency Response). At the time sediment samples were taken, a strong oil odor was present and a thick oil sheen was observed on the entire stream surface.

Site AN0204, on the Elizabeth River, had a very strong sewage odor at the time of collection which remained constant in intensity throughout the test and at test completion.

The reference site, AN0420 on Middle Brook is a highly productive stream as demonstrated in data obtained from the ECOREGION and AMNET Programs. Growth results for this site were greater than both test sites and the control treatment. When comparing growth results of the control treatment with the reference treatment, the analysis showed that there was not a significant difference. In calculating this result, only differences which show less growth than the control are considered. So, even though the reference growth averages were much higher than the control, the statistics did not consider this as significant, because growth was not shown to be diminished as compared to the control. Growth results were shown to be significantly less in tests 96H002b and 96H002c, when compared to the reference growth results. To confirm this difference, the control treatment growth results were also compared to test treatments 96H002b and 96H002c. Although the growth average for the control was higher than averages for 96H002b and 96H002c, a significant difference was not exhibited.

The control treatment had a 98 % survival, and Standard Reference Toxicant (SRT) tests, performed on a regular basis for quality control, were within expected limits. Therefore it is reasonable to assume that the growth values are valid for the control and subsequent statistical comparisons with the control are also valid. However the reference site also meets criteria for validity due to a 94 % survival, and the validity is substantiated with a non-impaired bioassessment score; comparisons between the reference site and other test treatments can be acceptably made. The control treatments are fed a standard diet during the test and it is assumed not to alter in any way during the test. Test treatments are fed the same diet, however additional food sources may occur naturally in the sediment. The reference station has been demonstrated to be a highly productive stream, therefore excellent food sources are available to the biological community. These additional food sources in the sediment of the reference test are the probable cause of the organisms high growth rate, as compared to the other test treatments including the control. Additional food sources may not be available in the sediments from 92H002b or 92H002c because of a depletion due to pollution. Test organism growth may also have been inhibited by stress due to contaminants in the sediment. These possibilities can be further substantiated given the severely impaired bioassessments displayed in the AMNET program. However, since the control treatment and reference treatment growth results are ambiguous relative to each other, growth inhibition due to toxicity can not be definitively demonstrated. Due to the equivocal growth results and the unknown impact of the fuel oil spill, site AN0201 on the South Branch Rahway River should be retested for toxicity , if macroinvertebrate data continues to show severe impairment when next sampled for the AMNET program. Chemical sampling of the S. Branch Rahway River would be premature, until further biological testing is performed.

Mortality results showed no significant differences between the control treatment and reference test, or between the reference test and 96H002b, the South Branch Rahway River.

Acute toxicity was exhibited, as a significant difference, when comparing the reference test mortality results with mortality results from 96H002c, the Elizabeth River. The Elizabeth River, upstream of the sampling location, is subject to numerous dischargers, and, being in an urban environment is likely impacted by nonpoint sources. This site also had a relatively high conductivity as compared to the reference site and also the S. Branch Rahway River site. This portion of the Elizabeth River is under tidal influence, however, tide charts indicate low tide occurred at the time of sampling(NJDEP, Tide Table, 1996). Conductivity in potable waters generally ranges from 50 to 1500 Fmhos (Standard Methods, 1992). Conductivity for the overlying water in the Elizabeth River test treatment was initially 2870 Fmhos and dropped to 416 Fmhos at the end of the test. A saltwater influence was suspected and an initial salinity was taken. The salinity was 2.4ppt, below the 5ppt criteria usually associated with conducting marine water toxicity tests. To assess the maximum impact of saltwater on the sample site and also to determine where the saltwater influence ends, subsequent salinity analysis was performed at high tide. Approximately 1.25 miles downstream of the sample site the salinity was 10.5ppt, the sample site was 7.8ppt (both over the 5ppt criteria usually associated with conducting marine water toxicity tests), and 0.8ppt approximately 1.25 miles upstream of the sample site. At high tide conditions the saltwater influence ends within 1.25 mile upstream of the sample site.

H. azteca can be used to evaluate the toxicity of estuarine sediments up to 15ppt salinity (Nebecker and Miller, 1988; Roach et al., 1992; Winger et al., 1993). It is unlikely that a salinity of 2.4ppt, as observed initially during the test, would have an adverse effect on the test organisms. Even at maximum saltwater influence, at high tide, the salinity only approaches 8ppt, well within the level that can be tolerated by *H. azteca.* Components in the sediment probably leached into the overlying water during the test, causing the high conductivity, which lessened after each 24 hour exchange, causing the decrease in conductivity over time. Daily conductivity variations in reservoir waters contrasts sharply with the daily fluctuations of some polluted river waters, and may contain significant trade waste(Standard Methods, 1992). The conductivity differed greatly from the reference site, but daily readings of the surface water of the Elizabeth River were not performed and daily variation cannot be properly assessed. However, the high conductivity as compared to the reference does indicate a contaminant in the river, and further study of the daily variability of the conductivity will reinforce the demonstration of point and/or nonpoint source contamination..

Impairment of site AN0204 as assessed in the AMNET program, was indicative of significant organic pollution. The severe impairment assessed in the AMNET program along with the acute toxicity demonstrated in the sediment, and high conductivity measurements, suggests an impact due to the additive effects of the numerous discharges and/or nonpoint sources which influence the Elizabeth River, and probably not the result of a single source. Chemical sampling should be performed to determine the sources and identities of the contamination present.

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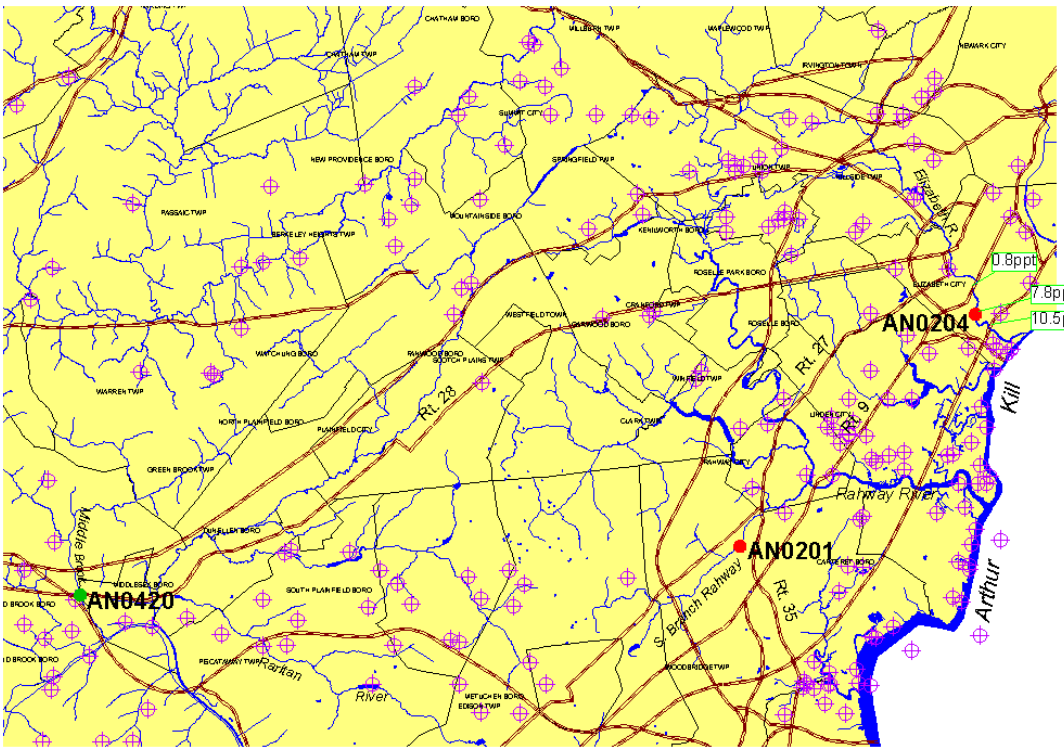
SEDIMENT TOXICITY TEST SITES

Assay #: 96H002a, 96H002b, 96H002c



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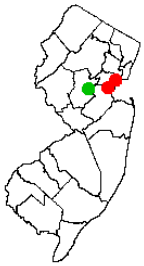

- Nonimpaired AMNET Site
- Severely Impaired AMNET Site
- NJPDES Sites
- Major Roads
- Municipal Boundaries
- Salinity at High Tide

1 0 1 2 3 4 5 Miles

Assay # Assignment

AN0420 = 96H002a
AN0201 = 96H002b
AN0204 = 96H002c

TABLE 1

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	9	10	10	98
96H002a	10	10	10	7	10	94
96H002b	10	8	8	9	10	90
96H002c	2	7	4	4	2	38

Statistical Analysis

Test Endpoint: Survival
Test Used: Wilcoxan Rank Sum Test
Results: 96H002a - no significant difference from control
96H002b - no significant difference from reference station
96H002c - significant difference from reference station

Test Endpoint: Growth
Test Used: F-test and T-test
Results: 96H002a - no significant difference from control
96H002b - significant difference from reference station
96H002c - significant difference from reference station

96H002b - no significant difference from control
96H002c - no significant difference from control

*see appendix b for statistical printout

TABLE 2

WEIGHT DETERMINATION

Drying Oven Temperature: 105EC Time/Date Start Drying: 1340 / 3-29-96
Time/Date End Drying: 1540 / 3-29-96
Analyst: T. Miller

REPLICATE / WEIGHING BOAT NO.	WGT. OF BOAT (mg)	DRY WGT: BOAT + LARVAE (mg)	TOTAL WGT. OF LARVAE (mg)	NUMBER OF LARVAE	LARVAE AVG. DRY WGT. (mg)	GROUP AVG. (mg)
CONTROL (A1)	15.42	16.77	1.35	10	0.135	
B(2)	11.63	12.78	1.15	10	0.115	
C(3)	8.43	9.38	0.95	9	0.106	0.126
D(4)	9.53	10.65	1.12	10	0.112	
E(5)	11.73	13.34	1.61	10	0.161	
95H012a A(6)	10.6	13.01	2.41	10	0.241	
B(7)	11.49	13.69	2.2	10	0.22	
C(8)	10.79	13.41	2.62	10	0.262	0.243
D(9)	14.78	16.69	1.91	7	0.273	
E(10)	7.92	10.11	2.19	10	0.219	
95H012b A(11)	8.99	10.23	1.24	10	0.124	
B(12)	9.86	10.8	0.94	8	0.118	
C(13)	7.4	8.33	0.93	8	0.116	0.14
D(14)	12.26	13.8	1.54	9	0.171	
E(15)	10.76	12.49	1.73	10	0.173	
95H012c A(16)	10.5	10.74	0.24	2	0.12	
B(17)	10.72	11.35	0.63	7	0.09	
C(18)	14.13	14.57	0.44	4	0.11	0.109
D(19)	8.02	8.38	0.36	4	0.09	
E(20)	13.95	14.22	0.27	2	0.135	

Table 3

Test Chamber Chemical/Physical Parameters

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.6	6.9	7.2	0.25	3.44
cond. Fmhos	152	134	142	5.97	4.22
D.O. mg/L	8.1	6.4	7.3	0.64	8.8

96H002a	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.3	6.6	6.8	0.2	2.89
cond. Fmhos	211	167	182	11.58	6.36
D.O. mg/L	6.8	4.5	5.6	0.79	14.27

96H002b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.3	6.3	6.7	0.27	4.05
cond. Fmhos	234	166	192	19.33	10.07
D.O. mg/L	6.9	3.6	5.2	1.05	20.07

95H012c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7	6.5	6.8	0.17	2.51
cond. Fmhos	2870*	416	921.5	707.79	76.79
D.O. mg/L	4.6	3.5	4	0.32	8

*High conductivity was the intial reading on the test vessel. A salinity reading was also taken due to the high conductivity. Salinity = 2.4 ppt.

TABLE 4

SALINITY AND CONDUCTIVITY RESULTS FOR ELIZABETH RIVER SURFACE WATER (At High Tide, April 24, 1996)

Elizabeth River @ Atlantic Street (downstream)

Salinity = 10.5ppt Conductivity = 13000Fmhos Time = 1300

Elizabeth River @ Summer Street (toxicity test site)

Salinity = 7.8ppt Conductivity = 10500Fmhos Time = 1250

Elizabeth River @ South Street (upstream)

Salinity = 0.8ppt Conductivity = 950Fmhos Time = 1310

APPENDIX A

AMNET DATA

Raritan Basin - Bound Brook USGS Quadrangle
Station AN0420
Middle Brook, Talmage Avenue, Bridgewater
September 14, 1993

Family	Number of Individual	Family Tolerance Value (FTV)
Pyralidae	2	5
Hydropsychidae	56	4
Chironomidae	6	6
Psephenidae	13	4
Elmidae	4	4
Nematoda	1	6
Baetidae	5	4
Turbellaria	7	4
Siphlonuridae	3	7
Gammaridae	2	4
Ephemerellidae	1	1

Statistical Analysis

Number of Taxa: 11
Total Number of Individuals: 100
% Contribution of Dominant Family: 56.00
Family Biotic Index: 4.22
Scraper/Filterer Collector Ratio: 0.25
Shredder/Total Ratio: 0.03
E+P+T*: 4 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT: 65.00
EPT/C*: 10.83 *(Chironomidae)
NJIS Score: 24
Biological Condition: non-impaired
Deficiency(s) noted: none

Observations

Streamwater: clear...Flow: fast...Width/Depth(ft): 30/1.5...Substrate: bedrock/cobble/rock...Streambank Vegetation/Stability: good/good...Canopy: mostly open...Other: tree-lined/residential; filamentous algae; minnows; darters

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Passaic Basin - Perth Amboy USGS Quadrangle
Station AN0201
South Branch Rahway River, Maplewood Ave (in Merrill Pk), Colonia
February 19, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
BloodRedChironomidae	2	8
Gammaridae	2	4
Tubificidae	42	10
Cambarinae	1	6
Turbellaria	2	4
Chironomidae	3	6

Statistical Analysis

Number of Taxa = 6
Total Number of Individuals = 52
% Contribution of Dominant Family = 80.77
Family Biotic Index = 9.15
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 Rating = 3
Biological Condition = severely impaired
Deficiency(s) noted: paucity of clean water organisms
significant organic pollution
Tubificidae overwhelmingly dominant

Observations

Streamwater: slightly turbid...Flow: slow...Width/Depth(ft): 50/1. ..Substrate: sand/gravel...Streambank Vegetation/Stability: poor/ poor...Canopy: open...Other: channelized in places; siltation; residential

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Elizabeth Basin - Elizabeth USGS Quadrangle
Station AN0204
Elizabeth River, Summer Street, Elizabeth
July 6, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Tubificidae	**21**	**10**

Statistical Analysis

Number of Taxa = 1
Total Number of Individuals = 21
% Contribution of Dominant Family = 100.00
Family Biotic Index = 10.00
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 Rating = 0
Biological Condition = severely impaired
Deficiency(s) noted: paucity of clean water organisms
low diversity
Tubificidae overwhelmingly dominant
significant organic pollution

Observations

Streamwater: turbid...Flow: slow...Width/Depth(ft): 50/10...
Substrate: rock/sand...Streambank Vegetation/Stability: poor/poor.
..Canopy: open...Other: fish

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APPENDIX B
STATISTICAL DATA

Survival Proportions with Arc-Sine Square Root Transformation

Blank	Blank Trans	96H002A	96H002A Trans
1	1.4127	1	1.4127
1	1.4127	1	1.4127
0.9	1.249	1	1.4127
1	1.4127	0.7	0.9912
1	1.4127	1	1.4127

Shapiro-Wilks Test for Normality

Blank	96H002A	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0585	-0.363				
1.4127	1.4127	1.4127		0.0585	-0.1052				
1.249	1.4127	1.249	1.3542	-0.1052	0.0585	0.1702	0.514	0.842	Not Normal
1.4127	0.9912	1.4127		0.0585	0.0585				
1.4127	1.4127	1.4127		0.0585	0.0585				
		1.4127		0.0585	0.0585				
Mean	Mean	1.4127		0.0585	0.0585				
1.38	1.3284	1.4127		0.0585	0.0585				
		0.9912		-0.363	0.0585				
		1.4127		0.0585	0.0585				

Wilcoxan Rank Sum Test

Pooled	Sorted	Wilcoxan Rank	Point	Blank	96H002A	Critical (from Table K=1)	Result
1.4127	0.9912	1	9	0	1	19	No Significant Difference
1.4127	1.249	2	3	2	0		
1.249	1.4127	6.5	1	6.5	0		
1.4127	1.4127	6.5	2	6.5	0		
1.4127	1.4127	6.5	4	6.5	0		
1.4127	1.4127	6.5	5	6.5	0		
1.4127	1.4127	6.5	6	0	6.5		
1.4127	1.4127	6.5	7	0	6.5		
0.9912	1.4127	6.5	8	0	6.5		
1.4127	1.4127	6.5	10	0	6.5		
				Sum	Sum		
				28	27		

Survival Proportions with Arc-Sine Square Root Transformation

96H002A	96H002A Trans	96H002B	96H002B Trans
1	1.4127	1	1.4127
1	1.4127	0.8	1.1071
1	1.4127	0.8	1.1071
0.7	0.9912	0.9	1.249
1	1.4127	1	1.4127

Shapiro-Wilks Test for Normality

96H002A	96H002B	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.1196	-0.3019				
1.4127	1.1071	1.4127		0.1196	-0.186				
1.4127	1.1071	1.4127	1.2931	0.1196	-0.186	0.2481	0.7381	0.842	Not Normal
0.9912	1.249	0.9912		-0.3019	-0.0441				
1.4127	1.4127	1.4127		0.1196	0.1196				
		1.4127		0.1196	0.1196				
Mean	Mean	1.1071		-0.186	0.1196				
1.3284	1.2577	1.1071		-0.186	0.1196				
		1.249		-0.0441	0.1196				
		1.4127		0.1196	0.1196				

Wilcoxan Rank Sum Test

Pooled	Sorted	Wilcoxan Rank	Point	96H002A	96H002B	Critical (from Table K=1)	Result
1.4127	0.9912	1	4	1	0	19	No Significant Difference
1.4127	1.1071	2.5	7	0	2.5		
1.4127	1.1071	2.5	8	0	2.5		
0.9912	1.249	4	9	0	4		
1.4127	1.4127	7.5	1	7.5	0		
1.4127	1.4127	7.5	2	7.5	0		
1.1071	1.4127	7.5	3	7.5	0		
1.1071	1.4127	7.5	5	7.5	0		
1.249	1.4127	7.5	6	0	7.5		
1.4127	1.4127	7.5	10	0	7.5		
				Sum	Sum		
				31	24		

Survival Proportions with Arc-Sine Square Root Transformation

96H002A	96H002A Trans	96H002C	96H002C Trans
1	1.4127	0.2	0.4636
1	1.4127	0.7	0.9912
1	1.4127	0.4	0.6847
0.7	0.9912	0.4	0.6847
1	1.4127	0.2	0.4636

Shapiro-Wilks Test for Normality

96H002A	96H002C	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	0.4636	1.4127		0.4197	-0.5294				
1.4127	0.9912	1.4127		0.4197	-0.5294				
1.4127	0.6847	1.4127	0.993	0.4197	-0.3083	1.4552	0.8345	0.842	Not Normal
0.9912	0.6847	0.9912		-0.0018	-0.3083				
1.4127	0.4636	1.4127		0.4197	-0.0018				
		0.4636		-0.5294	-0.0018				
Mean	Mean	0.9912		-0.0018	0.4197				
1.3284	0.6576	0.6847		-0.3083	0.4197				
		0.6847		-0.3083	0.4197				
		0.4636		-0.5294	0.4197				

Wilcoxan Rank Sum Test

Pooled	Sorted	Wilcoxan Rank	Point	96H002A	96H002C	Critical (from Table K=1)	Result
1.4127	0.4636	1.5	6	0	1.5	19	Significantly Different
1.4127	0.4636	1.5	10	0	1.5		
1.4127	0.6847	3.5	8	0	3.5		
0.9912	0.6847	3.5	9	0	3.5		
1.4127	0.9912	5.5	5	5.5	0		
0.4636	0.9912	5.5	7	0	5.5		
0.9912	1.4127	8.5	1	8.5	0		
0.6847	1.4127	8.5	2	8.5	0		
0.6847	1.4127	8.5	3	8.5	0		
0.4636	1.4127	8.5	5	8.5	0		
				Sum	Sum		
				39.5	15.5		

Shapiro-Wilks Test for Normality on Average Dry Weight per Replicate (in mg)

Blank	96H002A	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.135	0.241	0.135		-0.0494	-0.0784				
0.115	0.22	0.115		-0.0694	-0.0724				
0.106	0.262	0.106	0.1844	-0.0784	-0.0694	0.0387	0.8834	0.842	Normal
0.112	0.273	0.112		-0.0724	-0.0494				
0.161	0.219	0.161		-0.0234	-0.0234				
		0.241		0.0566	0.0346				
Mean	Mean	0.22		0.0356	0.0356				
0.1258	0.243	0.262		0.0776	0.0566				
		0.273		0.0886	0.0776				
		0.219		0.0346	0.0886				

F-Test

Blank	96H002A	Blank Var	96H002A Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances
0.135	0.241					
0.115	0.22					
0.106	0.262	0.0005	0.0006	1.2	6.3882	Equal
0.112	0.273					
0.161	0.219					

T-Test

Blank	96H002A	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.135	0.241				
0.115	0.22				
0.106	0.262	-7.9016	7	1.8946	No Significant Difference
0.112	0.273				
0.161	0.219				

Shapiro-Wilks Test for Normality on Average Dry Weight per Replicate (in mg)

Blank	96H002B	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.135	0.124	0.135		0.0019	-0.0271				
0.115	0.118	0.115		-0.0181	-0.0211				
0.106	0.116	0.106	0.1331	-0.0271	-0.0181	0.0059	0.8372	0.842	Not Normal
0.112	0.171	0.112		-0.0211	-0.0171				
0.161	0.173	0.161		0.0279	-0.0151				
		0.124		-0.0091	-0.0091				
Mean	Mean	0.118		-0.0151	0.0019				
0.1258	0.1404	0.116		-0.0171	0.0279				
		0.171		0.0379	0.0379				
		0.173		0.0399	0.0399				

Wilcoxan Rank Sum Test

Blank	96H002B	Pooled	Sorted	Wilcoxan Rank	Point	Blank	96H002B	Critical(from Table K=1)	Result
0.135	0.124	0.135	0.106	1	3	1	0	19	No Significant Difference
0.115	0.118	0.115	0.112	2	5	2	0		
0.106	0.116	0.106	0.115	3	2	3	0		
0.112	0.171	0.112	0.116	4	8	0	4		
0.161	0.173	0.161	0.118	5	7	0	5		
		0.124	0.124	6	6	0	6		
		0.118	0.135	7	1	7	0		
		0.116	0.161	8	5	8	0		
		0.171	0.171	9	9	0	9		
		0.173	0.173	10	10	0	10		
						Sum	Sum		
						21	34		

Shapiro-Wilks Test for Normality on Average Dry Weight per Replicate (in mg)

Blank	96H002C	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.135	0.12	0.135		0.0176	-0.0274				
0.115	0.09	0.115		-0.0024	-0.0274				
0.106	0.11	0.106	0.1174	-0.0114	-0.0114	0.0042	0.9483	0.842	Normal
0.112	0.09	0.112		-0.0054	-0.0074				
0.161	0.135	0.161		0.0436	-0.0054				
		0.12		0.0026	-0.0024				
Mean	Mean	0.09		-0.0274	0.0026				
0.1258	0.109	0.11		-0.0074	0.0176				
		0.09		-0.0274	0.0176				
		0.135		0.0176	0.0436				

F-Test

Blank	96H002C	Blank Var	96H002C Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances
0.135	0.12					
0.115	0.09					
0.106	0.11	0.0005	0.0004	1.25	6.3882	Equal
0.112	0.09					
0.161	0.135					

T-Test

Blank	96H002C	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.135	0.12				
0.115	0.09				
0.106	0.11	1.2522	7	1.8946	No Significant Difference
0.112	0.09				
0.161	0.135				

Shapiro-Wilks Test for Normality on Average Dry Weight per Replicate (in mg)

96H002A	96H002B	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.241	0.124	0.241		0.0493	-0.0757				
0.22	0.118	0.22		0.0283	-0.0737				
0.262	0.116	0.262	0.1917	0.0703	-0.0677	0.0321	0.9043	0.842	Normal
0.273	0.171	0.273		0.0813	-0.0207				
0.219	0.173	0.219		0.0273	-0.0187				
		0.124		-0.0677	0.0273				
Mean	Mean	0.118		-0.0737	0.0283				
0.243	0.1404	0.116		-0.0757	0.0493				
		0.171		-0.0207	0.0703				
		0.173		-0.0187	0.0813				

F-Test

96H002A	96H002B	96H002A Var	96H002B Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances
0.241	0.124					
0.22	0.118					
0.262	0.116	0.0006	0.0008	1.3333	6.3882	Equal
0.273	0.171					
0.219	0.173					

T-Test

96H002A	96H002B	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.241	0.124				
0.22	0.118				
0.262	0.116	6.1315	7	1.8946	Significantly Different
0.273	0.171				
0.219	0.173				

Shapiro-Wilks Test for Normality on Average Dry Weight per Replicate (in mg)

96H002A	96H002C	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.241	0.12	0.241		0.065	-0.086				
0.22	0.09	0.22		0.044	-0.086				
0.262	0.11	0.262	0.176	0.086	-0.066	0.0488	0.8634	0.842	Normal
0.273	0.09	0.273		0.097	-0.056				
0.219	0.135	0.219		0.043	-0.041				
		0.12		-0.056	0.043				
Mean	Mean	0.09		-0.086	0.044				
0.243	0.109	0.11		-0.066	0.065				
		0.09		-0.086	0.086				
		0.135		-0.041	0.097				

F-Test

96H002A	96H002C	96H002A Var	96H002C Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances
0.241	0.12					
0.22	0.09					
0.262	0.11	0.0006	0.0004	1.5	6.3882	Equal
0.273	0.09					
0.219	0.135					

T-Test

96H002A	96H002C	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.241	0.12				
0.22	0.09				
0.262	0.11	9.4752	7	1.8946	Significantly Different
0.273	0.09				
0.219	0.135				