

State of New Jersey
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SEDIMENT TOXICITY TEST
USING THE AMPHIPOD

Hyalella azteca

Loantaka Brook, Black Brook,
Tributary to Dead River
(Passaic Basin)



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

APRIL 1998



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April 1998

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

Assay Number(s): 97H006b, 97H006c, 97H006d, 97H006e

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyalella azteca* was performed on sediments collected from selected sites in the Passaic Basin. One on Loantaka Brook, two sites on Black Brook, and a reference site on an unnamed tributary (UNT) to Dead River. Suspected toxicity at the Loantaka Brook and Black Brook sites 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). The reference site was selected on the UNT to Dead River because of its "non-impaired" biological assessment. Sediment toxicity testing provides further data which can be related to previous assessments. When statistically compared to the control, the reference provided inconclusive results for survival. The test sites were therefore compared to the control. The site on Loantaka Brook and one site on Black Brook was not significantly different from the control for survival results. The remaining Black Brook site was significantly different from the control for survival results. These results, however, are inconclusive because pooled percent survival results did not agree with the statistical analysis; these tests should be repeated to ensure more conclusive results. The test sites did not exhibit acute toxicity, as measured by the growth of test organisms.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program of the New Jersey Department of Environmental Protection (NJDEP) is designed to establish a biological database for use in gauging stream quality throughout the state. Levels of impairment are shown through the use of Rapid Bioassessment Protocol (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 if 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.

Hyalella azteca is an epibenthic detritivore reported to also digest bacteria and algae from ingested sediment particles (2). This amphipod inhabits lakes, ponds, and streams throughout North and South America, typically burrowing into the sediment surface (3,4). *H. azteca* is a sensitive benchmark, i.e. established, test species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data(5) (see appendix A) and proximity of 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(see map)</u>
AN0220	severely impaired	Loantaka Brook @ Blue Stone Terrace, Morristown Twp.
AN0222	severely impaired	Black Brook @ Southern Blvd., Harding Twp.
AN0223	severely impaired	Black Brook @ New Vernon Rd., Meyersville
AN0225	non-impaired	UNT to Dead River @ Somerville Rd., Bernards Twp.

Sediment samples were collected from sites AN0225, AN0223, AN0222, and AN0220 on December 2, 1997 at 10:00, 10:40, 11:10, and 11:40 hours respectively. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler, then placed into one liter amber glass bottles and 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:

97H006a = control
97H006b = AN0225
97H006c = AN0220
97H006d = AN0222
97H006e = AN0223

Testing methodology followed the Bureau of Water Monitoring 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. The overlying water for each test chamber and the control was aerated beginning at the time of the test set-up, and continued for the duration of the test.

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

The test was initiated on December 9, 1997 at 11:00 hours, by adding ten 7 to 14- day old organisms from the holding chamber to each test series replicates. 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 35×10^6 cells 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 19, 1997). Live organisms were counted (see Table 2) and the dry weights measured (see Table 3). Statistical analysis was performed following EPA guidelines (9). The reference test was compared against the control, and the remaining tests compared to the reference, providing the reference and the control were statistically similar.

RESULTS

The test was valid by virtue of meeting the acceptability requirements of \$ 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 to the blank control. Even though survival in the reference test was 86% (meeting the acceptability requirements of \$ 80% survival), there was a significant difference between the reference test, 97H006b, survival results and the control survival results. Since there was a significant difference demonstrated in the reference data, the tests 97H006c, 97H006d, and 97H006e were compared to the blank control rather than 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. For mortality, 97H006c and 97H006e showed no significant difference from the control. Test 97H006c did, however, have a low percent survival (62%). 97H006d showed a significant difference from the control for survival. The reference was not significantly different from the control when analyzed for growth. Test 97H006c, 97H006d, and 97H006e were then compared to the reference for dry weight (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. 97H006c, 97H006d, and 97H006e showed no significant difference from the reference for dry weight (see appendix B for statistical printout).

All indigenous macrofauna observed in the samples before the start of the test were removed. Some organisms, however, did remain although their presence did not invalidate test results. Several blood red chironomids* were found in test samples 97H006b, 97H006d and 97H006e; also, several nai worms and harpacticoid copepods, plus a snail were found in 97H006d; ostracods and a leech were present in 97H006e.

*pollution tolerant midges of several genera, which possess excess hemoglobin

DISCUSSION

The sample sites on Loantaka Brook and Black Brook were chosen based on the results of previous macroinvertebrate studies, the proximity of NJPDES facilities, and urbanization. Sites AN0220, AN0222, and AN0223 had severely impaired bioassessment results as analyzed in AMNET(4). The reference site at UNT to Dead River, AN0225, was chosen because of its nonimpaired bioassessment and proximity to the test sites. All sites are within the Passaic River drainage. Similar stream morphology and coinciding position in the New Jersey Ecomap or ecoregion scheme (based on geology, soil, natural vegetation, etc.) (9) was also considered in choosing the reference site.

Survival results for the reference site exhibited a significant difference from the control, even though the reference survival was 86% which is above the acceptable limit of 80% needed for test validity. This is probably due to the respective mortality ranking of individual test chambers, and how each compared to the control, using the required Wilcoxon Rank Sum Test, rather than comparability of pooled final results. Because the reference results were inconclusive, the test series results were compared to the blank control, rather than the reference, for survival. Test 97H006d showed a significant difference for survival when compared to the control. This result should be viewed as inconclusive because the pooled percent survival was relatively high (88%), even though it statistically differed from the control when individual test replicates were statistically ranked and compared. Some background mortality levels can be expected when performing toxicity testing, thus the 80% survival acceptability limit. The statistics, used for analysis, unfortunately can not take this cutoff into account and may sometimes give results that conflict with pooled survival percentages. Non-toxicity related mortalities can occur when testing ambient material. Indigenous organisms can effect mortality results through competition or predation, especially at reference sites where there is a large number of taxa present. False mortalities may be reported if organisms cannot be found in the sediment at the end of the test, or are damaged inadvertently during processing at test termination. Results were also inconclusive when comparing results between 96H006c and the control. Although this test had a low survival (62%), the test treatment did not show the expected significant difference from the control. Again, this is probably due to the statistical ranking and comparing of individual test chambers. In this case some test chambers had no mortalities while others had high mortalities; the statistics were able to factor out anomalous results (see table 2). These tests should be repeated to ensure more conclusive results. Test 96H006e showed no significant difference when compared to the control.

Growth results for the reference showed no significant difference from the control, and therefore, the reference was compared to the test treatments. No significant differences in growth were exhibited when comparing the reference to any of the test treatments.

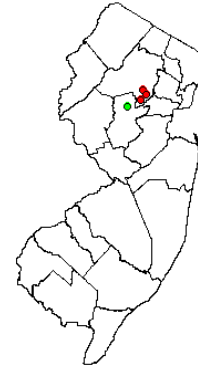
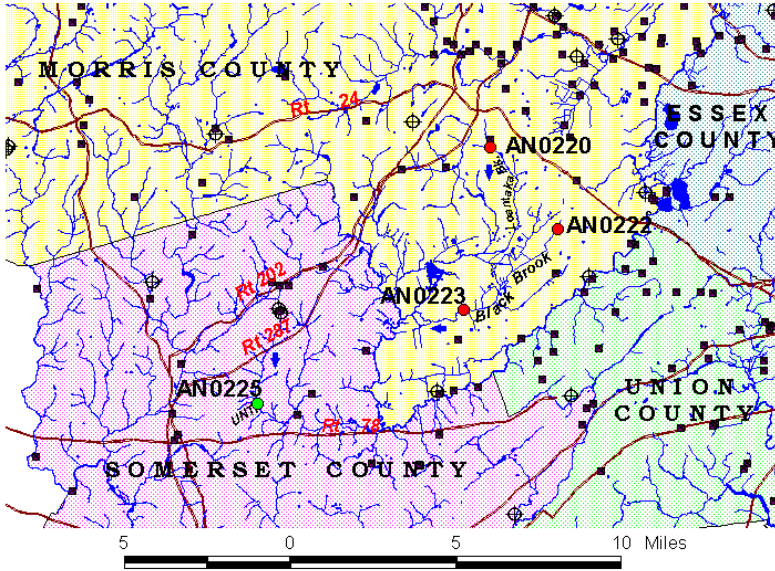
Since the test results are inconclusive, further sediment toxicity testing should be conducted at sites AN0225, AN0220, and AN0222, particularly site AN0220, which had a low percent survival. Since the test site results did not indicate acute toxicity, the severe impairment level previously found may have been due to elevated nutrient concentrations, or to the presence of other toxic substances at chronically, but not acutely, toxic

levels. These could have been 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 forms of nitrogen or phosphorus, and pesticides or other known toxic compounds.

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Map of Study Area
Loantaka Bk., Black Bk., Dead R. Tributary



- NONIMPAIRED AMHET SITE
- SEVERELY IMPAIRED AMHET SITE
- NJPDES SITE*
- ⊕ SOLID WASTE LANDFILL
- MAJOR ROADS
- STREAMS
- STREAM FLOW



NJDEP
Division of Science & Research
Water Monitoring Management
Bureau of Water Monitoring

*New Jersey Pollutant Discharge Elimination System, outfall site

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

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.8	7.3	7.6	0.16	2.12
cond. Fmhos	155	131	141	6.75	4.79
D.O. mg/L	7.9	5.6	7.1	0.65	9.16

97H006b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	8.0	7.6	7.8	0.13	1.65
cond. Fmhos	214	174	190	12.96	6.82
D.O. mg/L	8.1	6.2	7.3	0.52	7.12

97H006c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	8.1	7.7	7.9	0.14	1.74
cond. Fmhos	250	170	201	24.69	12.26
D.O. mg/L	8.1	6.5	7.3	0.49	6.63

97H006d	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.7	7.4	7.6	0.09	1.22
cond. Fmhos	237	184	196	15.59	7.94
D.O. mg/L	8.1	6.2	7.14	0.61	8.53

97H006e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.8	7.3	7.6	0.17	2.28
cond. Fmhos	177	151	162	7.03	4.34
D.O. mg/L	7.8	6.1	7.1	0.51	7.18

TABLE 2**MORTALITY DATA**
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	10	10	10	100
97H006b	8	7	9	10	9	86
97H006c	10	10	0	9	2	62
97H006d	8	9	9	9	9	88
97H006e	9	9	10	10	10	96

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 97H006b - significant difference from control

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 97H006c - no significant difference from control

97H006d - no significant difference from control

97H006e - no significant difference from control

*see appendix B for statistical printout

TABLE 3
WEIGHT DETERMINATION

Drying Oven Temperature: 105EC

Duration: 2 hours

Analyst: T. Miller

REPLICATE.	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	A	16.60	17.29	0.69	10	0.069	0.078
	B	19.87	20.74	0.87	10	0.087	
	C	15.97	16.75	0.78	10	0.078	
	D	16.54	17.41	0.87	10	0.087	
	E	12.75	13.43	0.68	10	0.068	
97H006b	A	11.95	14.21	2.26	8	0.283	0.255
	B	13.83	15.92	2.09	7	0.299	
	C	12.13	14.45	2.32	9	0.258	
	D	11.96	14.73	2.77	10	0.277	
	E	12.79	14.23	1.44	9	0.160	
97H006c	A	15.11	17.90	2.79	10	0.279	0.358
	B	14.73	18.09	3.36	10	0.336	
	C	----	----	----	----	----	
	D	11.10	13.41	2.31	9	0.257	
	E	13.58	14.70	1.12	2	0.560	
97H006d	A	11.93	13.64	1.71	8	0.214	0.215
	B	12.55	14.52	1.97	9	0.219	
	C	9.31	11.51	2.20	9	0.244	
	D	17.93	19.57	1.64	9	0.182	
	E	19.28	21.24	1.96	9	0.218	
97H006e	A	11.65	14.04	2.39	9	0.266	0.243
	B	13.47	15.65	2.18	9	0.242	
	C	14.04	16.49	2.45	10	0.245	
	D	11.99	14.88	2.89	10	0.289	
	E	12.25	14.00	1.75	10	0.175	

Statistical Analysis

Test Endpoint: Growth

Results: Rank Sum 97H006b - no significant difference from control
T-test, F-test 97H006c - no significant difference from reference station
T-test, F-test 97H006d - no significant difference from reference station
T-test, F-test 97H006e - no significant difference from reference station

*see appendix B for statistical printout

APPENDIX A

AMNET DATA(5)

AN0225 # Unnamed Tributary to Dead River
 Somerville Rd., Liberty Corner, Bernards Twp.
 USGS Quad +Bernardsville
 Latitude / Longitude: 40 39' 36.389"N / 74 35' 38.236"W

February 4, 1992

Taxon (Family Level)	FTV	NOI
SPHAERIIDAE	8	3
ODONTOCERIDAE	0	10
TUBIFICIDAE	10	17
ELMIDAE	4	4
CHIRONOMIDAE	6	24
HELICOSYCHIDAE	3	1
PSEPHENIDAE	4	2
LIMNEPHILIDAE	4	5
SIMULIIDAE	6	19
EPHEMERELLIDAE	1	2
HEPTAGENIIDAE	4	1
CAPNIIDAE	1	8
SIALIDAE	4	1
NEMOURIDAE	2	2
LEPTOPHLEBIIDAE	2	1

Number of Taxa + 15 # Number of Individuals+100

Dominant Family(s) +CHIRONOMIDAE = 24%
 Family Biotic Index + 5.23
 Scraper/Filterer Collector Ratio + 0.59
 Shredder/Total Ratio + 0.18
 E(phemeroptera)+P(lecoptera)+T(richoptera) +8
 %EPT + 30
 EPT/Chironomids + 1.25

NJIS/Rating + 24/non-impaired

OBSERVATIONS

Clarity + clear
 Flow + slow
 Width/Depth(ft) +5/<1
 Substrate + gravel/cobbles
 Streambank Vegetation/Stability + good/good
 Canopy +mostly open
 Other + woods, macrophyte, crayfish, small fish

FTV = Family Tolerance Value, NOI = Number Of Individuals(per 100 organism subsample)

AN0220 # Loantaka Bk, Bluestone Terrace, Morristown Twp
 USGS Quad +Morristown
 Latitude / Longitude: 40 46' 18.454"N / 74 27' 38.650"W

February 7, 1992

Taxon (Family Level)	FTV	NOI
BLOOD RED CHIRONOMIDAE* 8		85
TUBIFICIDAE	10	13
NAIDIDAE	7	1
CHIRONOMIDAE	6	1

Number of Taxa + 4 # Number of Individuals+ 100

Dominant Family(s) + BLOOD RED CHIRONOMIDAE = 85%

Family Biotic Index + 6.23

Scraper/Filterer Collector Ratio + 0.00

Shredder/Total Ratio + 0.01

E(phemeroptera)+P(lecoptera)+T(richoptera) +0

%EPT +0

EPT/Chironomids + 0

NJIS/Rating +0/severely impaired

Deficiency(s) noted + low diversity

BLOOD RED CHIRONOMIDAE overwhelmingly dominant

paucity of clean water organisms

Significant organic pollution

OBSERVATIONS

Clarity +turbid, grey

Flow + slow

Width/Depth(ft) +8/1

Substrate + sand/cobbles

Streambank Vegetation/Stability +fair/fair

Canopy +open

Other + woodlined, residential, sewage odor

*pollution tollerant midges of several genera which possess excess hemoglobin

FTV = Family Tolerance Value, NOI = Number Of Individuals(per 100 organism subsample)

AN0222 # Black Bk, Southern Blvd, Harding Twp.
 USGS Quad +Chatham
 Latitude / Longitude: 40 44' 12.731"N / 74 25' 21.871"W

February 7, 1992

Taxon (Family Level)	FTV	NOI
TUBIFICIDAE	10	80
SIMULIDAE	6	1
LUMBRICULIDAE	8	8
NEMATODA	6	2
BLOOD RED CHIRONOMIDAE* 8		2
TURBILARIA	4	2
PSYCODIDAE	10	3
CHIRONOMIDAE	6	2

Number of Taxa +8 # Number of Individuals+ 100

Dominant Family(s) + TUBIFICIDAE 80%
 Family Biotic Index + 9.48
 Scraper/Filterer Collector Ratio + 0.00
 Shredder/Total Ratio + 0.00
 E(phemeroptera)+P(lecoptera)+T(richoptera) +0
 %EPT + 0
 EPT/Chironomids + 0

NJIS/Rating + 3/severely impaired

Deficiency(s) noted + TUBIFICIDAE overwhelmingly dominant
 paucity of clean water organisms
 significant organic pollution

OBSERVATIONS

Clarity + clear
 Flow + slow
 Width/Depth(ft) +5/<1
 Substrate + sand/gravel
 Streambank Vegetation/Stability +poor/good
 Canopy +open
 Other +riprap on banks, golf course upstream

*pollution tollerant midges of several genera which possess excess hemoglobin

FTV = Family Tolerance Value, NOI = Number Of Individuals(per 100 organism subsample)

AN0223 # Black Bk., New Vernon Rd., Meyersville
 USGS Quad +Chatham
 Latitude / Longitude: 40 42' 04.382"N / 74 28' 33.316"W

February 7, 1992

Taxon (Family Level)	FTV	NOI
SPHAERIIDAE	8	13
CHIRONOMIDAE	6	62
TUBIFICIDAE	10	13
BRYOZOA	7	1
GAMMARIDAE	4	4
BLOOD RED CHIRONOMIDAE*	8	3
CERATOPOGONIDAE	6	1
NEMATODA	6	1
NAIDIDAE	7	1
ASELLIDAE	8	1

Number of Taxa +10 # Number of Individuals+ 100

Dominant Family(s) + CHIRONOMIDAE 62%
 Family Biotic Index + 6.80
 Scraper/Filterer Collector Ratio + 0.00
 Shredder/Total Ratio + 0.24
 E(phemeroptera)+P(lecoptera)+T(riehoptera) +0
 %EPT + 0
 EPT/Chironomids + 0

NJIS/Rating + 6/severely impaired

Deficiency(s) noted + CHIRONOMIDAE overwhelmingly dominant
 paucity of clean water organisms

OBSERVATIONS

Clarity + clear
 Flow +slow
 Width/Depth(ft) + 15/<1
 Substrate + sand/gravel/cobbles
 Streambank Vegetation/Stability + good/good
 Canopy + open
 Other +wooded, residential

*pollution tollerant midges of several genera which possess excess hemoglobin

FTV = Family Tolerance Value, NOI = Number Of Individuals(per 100 organism subsample)

APPENDIX B

STATISTICAL DATA

SURVIVAL RESULTS

BLANK CONTROL VS. AN0225(REFERENCE)

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0225	Blank Trans	AN0225 Trans
1.0	0.8	1.4127	1.1071
1.0	0.7	1.4127	0.9912
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
1.0	0.9	1.4127	1.249

Shapiro-Wilks Test for Normality

Blank Trans	AN0225 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.1071	1.4127		0.1054	-0.3161				
1.4127	0.9912	1.4127		0.1054	-0.2002				
1.4127	1.249	1.4127	1.3073	0.1054	-0.0583	0.2135	0.7403	0.842	Not Normal
1.4127	1.4127	1.4127		0.1054	-0.0583				
1.4127	1.249	1.4127		0.1054	0.1054				
		1.1071		-0.2002	0.1054				
Mean	Mean	0.9912		-0.3161	0.1054				
1.4127	1.2018	1.249		-0.0583	0.1054				
		1.4127		0.1054	0.1054				
		1.249		-0.0583	0.1054				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0225	Critical(from Table K=1)	Result
1.4127	0.9912	7	1	0	1	19	Significantly Different
1.4127	1.1071	6	2	0	2		
1.4127	1.249	10	3.5	0	3.5		
1.4127	1.249	8	3.5	0	3.5		
1.4127	1.4127	9	7.5	0	7.5		
1.1071	1.4127	5	7.5	7.5	0		
0.9912	1.4127	4	7.5	7.5	0		
1.249	1.4127	3	7.5	7.5	0		
1.4127	1.4127	2	7.5	7.5	0		
1.249	1.4127	1	7.5	7.5	0		
				Sum	Sum		
				37.5	17.5		

BLANK CONTROL VS. AN0220

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0220	Blank Trans	AN0220 Trans
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	0.0	1.4127	0.1581
1.0	0.9	1.4127	1.249
1.0	0.2	1.4127	0.4636

Shapiro-Wilks Test for Normality

Blank Trans	AN0220 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.2367	-1.0179				
1.4127	1.4127	1.4127		0.2367	-0.7124				
1.4127	0.1581	1.4127	1.176	0.2367	0.073	1.9412	0.5869	0.842	Not Normal
1.4127	1.249	1.4127		0.2367	0.2367				
1.4127	0.4636	1.4127		0.2367	0.2367				
		1.4127		0.2367	0.2367				
Mean	Mean	1.4127		0.2367	0.2367				
1.4127	0.9392	0.1581		-1.0179	0.2367				
		1.249		0.073	0.2367				
		0.4636		-0.7124	0.2367				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0220	Critical(from Table K=1)	Result
1.4127	0.1581	8	1	0	1	19	No Significant Difference
1.4127	0.4636	10	2	0	2		
1.4127	1.249	9	3	0	3		
1.4127	1.4127	7	7	0	7		
1.4127	1.4127	6	7	0	7		
1.4127	1.4127	5	7	7	0		
1.4127	1.4127	4	7	7	0		
0.1581	1.4127	3	7	7	0		
1.249	1.4127	2	7	7	0		
0.4636	1.4127	1	7	7	0		
				Sum	Sum		
				35	20		

BLANK CONTROL VS. AN0222

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0222	Blank Trans	AN0222 Trans
1.0	0.8	1.4127	1.1071
1.0	0.9	1.4127	1.249
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	AN0222 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.1071	1.4127		0.096	-0.2096				
1.4127	1.249	1.4127		0.096	-0.0677				
1.4127	1.249	1.4127	1.3167	0.096	-0.0677	0.1083	0.7812	0.842	Not Normal
1.4127	1.249	1.4127		0.096	-0.0677				
1.4127	1.249	1.4127		0.096	-0.0677				
		1.1071		-0.2096	0.096				
Mean	Mean	1.249		-0.0677	0.096				
1.4127	1.2206	1.249		-0.0677	0.096				
		1.249		-0.0677	0.096				
		1.249		-0.0677	0.096				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0222	Critical(from Table K=1)	Result
1.4127	1.1071	6	1	0	1	19	Significantly Different
1.4127	1.249	10	3.5	0	3.5		
1.4127	1.249	9	3.5	0	3.5		
1.4127	1.249	8	3.5	0	3.5		
1.4127	1.249	7	3.5	0	3.5		
1.1071	1.4127	5	8	8	0		
1.249	1.4127	4	8	8	0		
1.249	1.4127	3	8	8	0		
1.249	1.4127	2	8	8	0		
1.249	1.4127	1	8	8	0		
				Sum	Sum		
				40	15		

BLANK CONTROL VS. AN0223

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0223	Blank Trans	AN0223 Trans
1.0	0.9	1.4127	1.249
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0223 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.0327	-0.131				
1.4127	1.249	1.4127		0.0327	-0.131				
1.4127	1.4127	1.4127	1.38	0.0327	0.0327	0.0429	0.5093	0.842	Not Normal
1.4127	1.4127	1.4127		0.0327	0.0327				
1.4127	1.4127	1.4127		0.0327	0.0327				
		1.249		-0.131	0.0327				
Mean	Mean	1.249		-0.131	0.0327				
1.4127	1.3472	1.4127		0.0327	0.0327				
		1.4127		0.0327	0.0327				
		1.4127		0.0327	0.0327				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0223	Critical(from Table K=1)	Result
1.4127	1.249	7	1.5	0	1.5	19	No Significant Difference
1.4127	1.249	6	1.5	0	1.5		
1.4127	1.4127	10	6.5	0	6.5		
1.4127	1.4127	9	6.5	0	6.5		
1.4127	1.4127	8	6.5	0	6.5		
1.249	1.4127	5	6.5	6.5	0		
1.249	1.4127	4	6.5	6.5	0		
1.4127	1.4127	3	6.5	6.5	0		
1.4127	1.4127	2	6.5	6.5	0		
1.4127	1.4127	1	6.5	6.5	0		
				Sum	Sum		
				32.5	22.5		

GROWTH

BLANK VS. AN0225

Average Dry Weight per Replicate (in mg)

Blank	AN0225
0.069	0.283
0.087	0.299
0.078	0.258
0.087	0.277
0.068	0.160

Shapiro-Wilks Test for Normality

Blank	AN0225	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.069	0.283	0.069		-0.0976	-0.0986				
0.087	0.299	0.087		-0.0796	-0.0796				
0.078	0.258	0.078	0.167	-0.0886	-0.0886	0.0914	0.7943	0.842	Not Normal
0.087	0.277	0.087		-0.0796	-0.0796				
0.068	0.160	0.068		-0.0986	-0.0796				
		0.283		0.1164	-0.0066				
Mean	Mean	0.299		0.1324	0.0914				
0.078	0.255	0.258		0.0914	0.1104				
		0.277		0.1104	0.1164				
		0.160		-0.0066	0.1324				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0225	Critical(from Table K=1)	Result
0.069	0.068	5	1	1	0	19	No Significant Difference
0.087	0.069	1	2	2	0		
0.078	0.078	3	3	3	0		
0.087	0.087	4	4.5	4.5	0		
0.068	0.087	2	4.5	4.5	0		
0.283	0.160	10	6	0	6		
0.299	0.258	8	7	0	7		
0.258	0.277	9	8	0	8		
0.277	0.283	6	9	0	9		
0.160	0.299	7	10	0	10		
				Sum	Sum		
				15	40		

AN0225(reference) VS. AN0220

Average Dry Weight per Replicate (in mg)

AN0225	AN0220
0.283	0.279
0.299	0.336
0.258	0.000
0.277	0.257
0.160	0.560

Shapiro-Wilks Test for Normality

AN0225	AN0220	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.283	0.279	0.283		0.0121	-0.2709				
0.299	0.336	0.299		0.0281	-0.1109				
0.258	0.000	0.258	0.271	-0.0129	-0.0139	0.1749	0.8761	0.842	Normal
0.277	0.257	0.277		0.0061	-0.0129				
0.160	0.560	0.160		-0.1109	0.0061				
		0.279		0.0081	0.0081				
Mean	Mean	0.336		0.0651	0.0121				
0.255	0.286	0.000		-0.2709	0.0281				
		0.257		-0.0139	0.0651				
		0.560		0.2891	0.2891				

F-Test and T-Test

AN0225 Var	AN0220 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0031	0.0401	12.9355	6.3882	Unequal	-0.3335	4	2.1318	No Significant Difference

AN0225(reference) VS. AN0222

Average Dry Weight per Replicate (in mg)

AN0225	AN0222
0.283	0.214
0.299	0.219
0.258	0.244
0.277	0.182
0.160	0.218

Shapiro-Wilks Test for Normality

AN0225	AN0222	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.283	0.214	0.283		0.0476	-0.0754				
0.299	0.219	0.299		0.0636	-0.0534				
0.258	0.244	0.258	0.235	0.0226	-0.0214	0.0182	0.9631	0.842	Normal
0.277	0.182	0.277		0.0416	-0.0174				
0.160	0.218	0.160		-0.0754	-0.0164				
		0.214		-0.0214	0.0086				
Mean	Mean	0.219		-0.0164	0.0226				
0.255	0.215	0.244		0.0086	0.0416				
		0.182		-0.0534	0.0476				
		0.218		-0.0174	0.0636				

F-Test and T-Test

AN0225 Var	AN0222 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0031	0.0005	6.2	6.3882	Equal	1.4907	5	2.015	No Significant Difference

AN0225(reference) VS. AN0223

Average Dry Weight per Replicate (in mg)

AN0225	AN0223
0.283	0.266
0.299	0.242
0.258	0.245
0.277	0.289
0.160	0.175

Shapiro-Wilks Test for Normality

AN0225	AN0223	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.283	0.266	0.283		0.0336	-0.0894				
0.299	0.242	0.299		0.0496	-0.0744				
0.258	0.245	0.258	0.249	0.0086	-0.0074	0.0199	0.8532	0.842	Normal
0.277	0.289	0.277		0.0276	-0.0044				
0.160	0.175	0.160		-0.0894	0.0086				
		0.266		0.0166	0.0166				
Mean	Mean	0.242		-0.0074	0.0276				
0.255	0.243	0.245		-0.0044	0.0336				
		0.289		0.0396	0.0396				
		0.175		-0.0744	0.0496				

F-Test and T-Test

AN0225 Var	AN0223 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variiances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0031	0.0018	1.7222	6.3882	Equal	0.3833	7	1.8946	No Significant Difference