

CORRESPONDENCE/MEMORANDUM

DATE: May 2, 1997

FILE REF: 3200

TO: Jim Reyburn - NOR/Green Bay

FROM: Tom Janisch - WT/2 *Tom Janisch*

SUBJECT: Toxicity Test Results From Surface Water and Wetland Soil Samples Collected In May 1996 From Kewaunee Marsh Arsenic Site.

Attached are three items related to the above samples and toxicity testing conducted on those samples. These items are:

- 1) Two tables that summarize the results of the statistical evaluation of the testing data for significance, i.e. are there identifiable differences between the results of the lab control and/or reference site and the results from the water and soils of the study sites. Table 1 summarizes the water sample testing and Table 2 summarizes the soil testing.
- 2) An April 21, 1997 memo from Linda Talbot who did the statistical interpretation of the data upon which the above summary tables are based.
- 3) Handout materials from our March 20, 1997 with STS and GeoTrans which provides information on the sampling sites, results for arsenic at each site, and raw data related to the toxicity testing.

Comments On Water Results

The Acute and Chronic Toxicity Criteria for arsenic in NR 105 for the protection of aquatic life are 339.8 and 148 ug/L, respectively. Based on the range of arsenic concentrations in the five site samples as shown in the following Table 1, toxicity is associated with the different tests and various test organisms when arsenic levels in water exceed 1,400 ug/L. Toxicity associated with test results are noted in Table 1 by the shading in the appropriate squares. The "Tox NS" designator in the square means toxicity was present in the replicates for the test at that site but the toxicity was not statistically significant. The designator "Toxicity" in a square indicates a statistically significant difference between the study site results and the lab control.

No toxicity was demonstrated at WT - 05 that had an arsenic concentration of 430 ug/L. Based on the above criteria levels, toxicity may have been predicted. However, criteria derivation methods, the chemical and physical characteristics of

surface waters in wetlands as opposed to those in stream conditions, and the form of arsenic present, may have ameliorated the toxicity at the 430 ug/L total arsenic level.

Comments On Wetland Soil Results

Toxicity related to exposure of the test organisms to the wetland soils is not as clearly grouped in a range of concentrations as it was for the surface water exposures. This is not unexpected given the number of factors related to controlling the availability of arsenic and exposure to organisms from the soil media. It is noted that in the chronic tests, *Daphnia magna* in the water column over all the study site soils had reduced survival. This includes site WT - 06 which had arsenic levels in the soil of 2.2 mg/kg which is comparable to a background level. *Chironomus tentans* also experienced reduced growth at this site and two other sites that had more elevated levels of arsenic.

If you have any questions or comments on the attached materials, give me a call. If you have any questions on the details of data interpretation of the toxicity testing, give Linda Talbot (608-266-8148) a call.

cc: Lee Liebenstein - WT/2
Linda Talbot - WT/2
Bob Strous - RR/3
Mike Berger - STS

Table 1. Acute and Chronic Toxicity Test Results Based On Exposures To Surface Water Samples Collected From Kewaunee Marsh In 1996.

Sample Station	Arsenic ug/L Water	Acute Toxicity Tests				Chronic Toxicity Tests		
		<i>C. dubia</i> 48 hr	<i>D. magna</i> 48 hr	Fathead Minnow 4 day	Microtox	<i>C. dubia</i> 7 day	Fathead Minnow	Algal Assay ⁵ 96 hr
WT - 01 Reference	1.0	100% Survival	100% Survival	NT ³	NT	NT	NT	NT
WT - 02	8,300	Tox NS ¹	100% Survival	Tox NS	Tox NS	Toxicity	Toxicity	Toxicity
WT - 03	1,400	100% ² Survival	100% Survival	Tox NS	NT	Toxicity	Toxicity	Toxicity
WT - 04	2,400	Tox NS	100% Survival	Toxicity ⁴	NT	Toxicity	NT	NT
WT - 05	430	100% Survival	100% Survival	NT	NT	NT	NT	NT
WT - 06	37	100% Survival	100% Survival	NT	NT	NT	NT	NT

Notes:

1. Tox NS = Toxicity was noted in the replicates from the site but compared to the control, the differences were not statistically significant.
2. 100 % Survival = The same number of viable test organisms were present at the end of the test period as were present at the start.
3. NT = No toxicity.
4. Toxicity = Impairments were present related to either survival or reproduction of young and the differences compared to the control results were statistically significant.
5. Algal Assay = Uses *Selenastrum capricornutum*.

Table 2. Acute and Chronic Toxicity Test Results Based On Exposures To Wetland Soil Samples Collected From Kewaunee Marsh In 1996.

Sample Station	Arsenic ug/kg Sediment	Acute Toxicity Tests			Chronic Toxicity Tests	
		<i>D. magna</i> 48 hr	<i>C. dubia</i> 48 hr	<i>Hyallela azteca</i> 10 day	<i>D. magna</i> 10 day	<i>C. tentans</i> 10 day
ST - 01	2.6	NT ¹	NT	NT	NT	NT
ST - 02	150	Tox NS ²	NT	NT	Toxicity	Toxicity ⁴
ST - 03	220	NT	NT	NT	Toxicity	NT
ST - 04	220	Tox NS	NT	NT	Toxicity	Toxicity ⁴
ST - 05	67	Toxicity ³	NT	NT	Toxicity	NT
ST - 06	2.2	NT	NT	NT	Toxicity	Toxicity ⁴

Notes:

1. NT = No toxicity
2. Tox NS = Toxicity was noted in the replicates from the site but compared to the control, the differences were not statistically significant.
3. Toxicity = Impairments were present related to either survival or reproduction of young and the differences compared to the control results were statistically significant.
4. *Chironomus tentans* survival in the lab control and all treatments was $\geq 70\%$, the test acceptability requirement. However, growth of *C. tentans* was significantly reduced in the field reference and at all the study sites compared to the lab control. If the growth results from the study sites are compared to the field reference site and not the lab control, sites ST - 02, ST - 04, and ST - 06 all show significantly reduced growth.

DATE: April 21, 1997

FILE REF:

TO: Tom Janisch - WT/2

FROM: Linda Talbot - WT/2 *Linda*

SUBJECT: Toxicity Test Results for C.D. Besadny Wildlife Area Waters and Sediments

I am reporting to you the results of the data analyses from the toxicity tests in which surface waters and sediments from the C.D. Besadny Wildlife Area were tested. All analyses on normally distributed data were conducted using the F-test for homogeneity of variance and the t-Test to detect differences at the $P=0.05$ level. Non-normal data was analyzed with Wilcoxon's Rank Sum Test.

Surface Waters

Acute Toxicity Test Results

Ceriodaphnia dubia were exposed for 48 hours. Two water samples from the marsh area reduced survival to 85% and 80%, from KMWT02 southwest of the capped area, and KMWT04, east of the capped area, respectively. However, neither reduction in survival was statistically significant in their difference from the lab control nor the field reference sample.

[I am reporting this because of a trend I've observed in the data from other exposures.]

There was 100% survival of *Daphnia magna* in all exposures for the acute 48 hour test.

The four-day survival of *Pimephales promelas* (fathead minnows) dropped to 40% in the KMWT02 (SW of capped area); 55% in KMWT03 (SE corner of capped area) and 70% in the KMWT04 (east of capped area) samples. Due to the data distribution, the differences in survival in the treatment samples KMWT02 and KMWT03 are not statistically significant; however, the KMWT04 difference is. All three treatments required aeration to maintain dissolved oxygen levels. Dropping DO and introducing aeration both contribute some level of stress on the organisms, which cannot be measured. Two replicate chambers from KMWT02 were excluded from the data analysis due to procedural difficulties with the aeration and trapping of some minnows in the air/water interface. Some treatment stress due to the water sample may have contributed to this situation, but it cannot be definitively determined.

Surface Waters

Chronic Toxicity Test Results

Survival of *C. dubia* over the seven-day chronic test dropped to 10% with no young produced in exposure KMWT02 and no organisms survived in the KMWT04 exposure with no young produced. Both results differ significantly from the lab control and field reference samples.

The statistical comparison of number of young produced in treatment KMWT03 and the lab control shows a statistically significant decline in the number of young produced. The lab control comparison was used in this analysis; the field reference treatment produced even more young than the lab control and would have produced a more significant difference..

Survival of *Pimephales promelas* (fathead minnows) was significantly affected in treatments KMWT02 and KMWT03. No other toxicity was exhibited in any of the remaining treatments, where survival and growth exceeded that of the lab control.

In the algal assay using *Selenastrum capricornutum*, a 96 hour chronic growth test, algal growth was significantly inhibited in exposures KMWT02 and KMWT03. The field reference, KMWT04, KMWT05 and KMWT06 all enhanced algal growth above the lab control.

No significant toxicity was seen in the Microtox test. The only sample that did not do better or as well as the control, was sample KMWT02.

Sediments

Acute Toxicity Test Results

No significant toxicity was observed in the exposure of *Ceriodaphnia dubia* over the 48 hour acute test.

Questionable results of mortality appeared in the *Daphnia magna* acute exposures of KMST02, KMST04 and KMST05. The consistent mortality evident in the replicates of treatment KMST05 is significantly different than the lab control and field reference. The percent survival for the exposure is 77%. This is respectively similar to and better than KMST02 at 77% and KMST04 at 57% where the mortality results within treatments (among replicates) are variable and a significant difference is not determined, unlike KMST05.

Sediments

Chronic Toxicity Test Results

Survival in the 10-day chronic *D. magna* test was significantly different than the control in treatments KMST02, KMST03, KMST04, KMST05 and KMST06.

[Just an aside note (more gray data): In treatments KMST02 and KMST05, where the t-statistic was within 1 unit of the t-table value, the number of young produced was not significantly different than the lab control. However, over time, the significant mortality difference would continue to result in many fewer young produced. Also, directions from EPA for data analysis say to do no further analysis of the data once survival has been determined to be significantly impaired. I was just curious.]

Though the percent survival in the field reference sample was only 53%, it was not significantly different from the lab control. [This is likely due to the large coefficient of variation in the replicates' survival within the field reference treatment - too variable to be statistically significant.] The number of young produced in the field reference treatment was significantly reduced relative to the control.

Survival of *Hyallela azteca* in the 10-day exposure was greater than 90% in all treatments and all treatments performed better than the lab control (66% survival).

Chironomus tentans survival in the lab control and all treatments was $\geq 70\%$, the test acceptability requirement. However, growth of *C. tentans* was significantly reduced in the field reference and all treatments compared to the lab control.

[An aside note: Just to look at effects above and beyond (excessive) the lab control comparison, I conducted a comparison of the growth results of the treatments to the field reference. KMST02, KMST04, and KMST06 resulted in significantly reduced growth relative to the field reference.]

Though it seems some of the results may be conflicting or confusing, there is something of a common thread of recurring problems to the various organisms with some of the samples. Those indicating problems that are not redundant between organisms should not be overlooked, due to differing sensitivities each has to various contaminants (i.e., the reason a suite of organisms is exposed). In the water treatments KMWT02, KMWT03 and KMWT 04 had fairly consistent detrimental impacts on the organisms. Sediment treatments KMST02, KMST04 and KMST05 seem to more consistently affect the organisms. However, the significant impacts of KMST03 and KMST06 should also be noted.

cc: Dale Patterson - WT/2
Lee Liebenstein - WT/2

Kewaunee Marsh

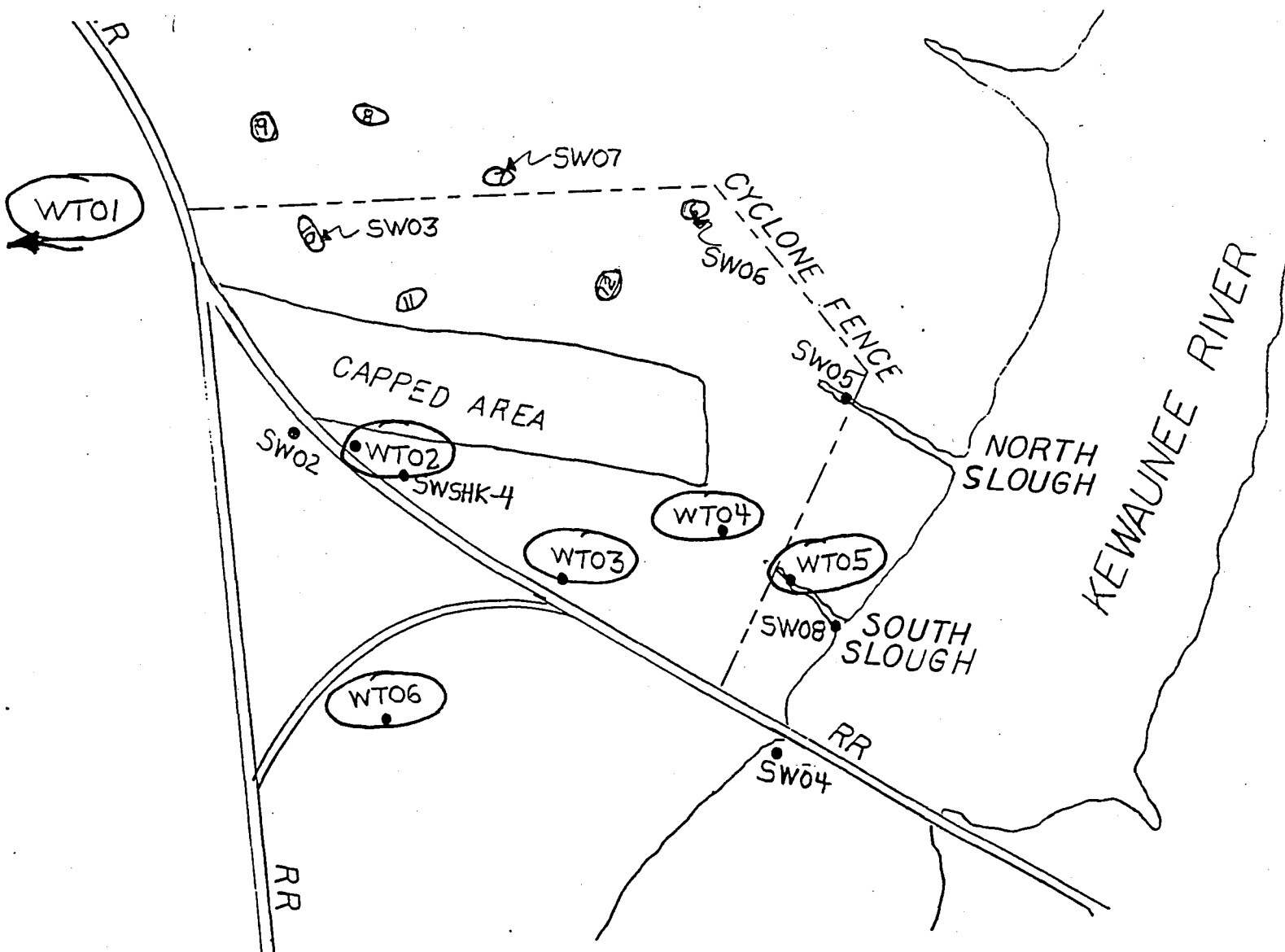
Preliminary Results From The Surface Water and Wetland Soil Toxicity Testing

Samples Collected May 1996

SURFACE WATER

Surface Water Sampling Locations and Analytical Results For Arsenic Related To Toxicity Testing

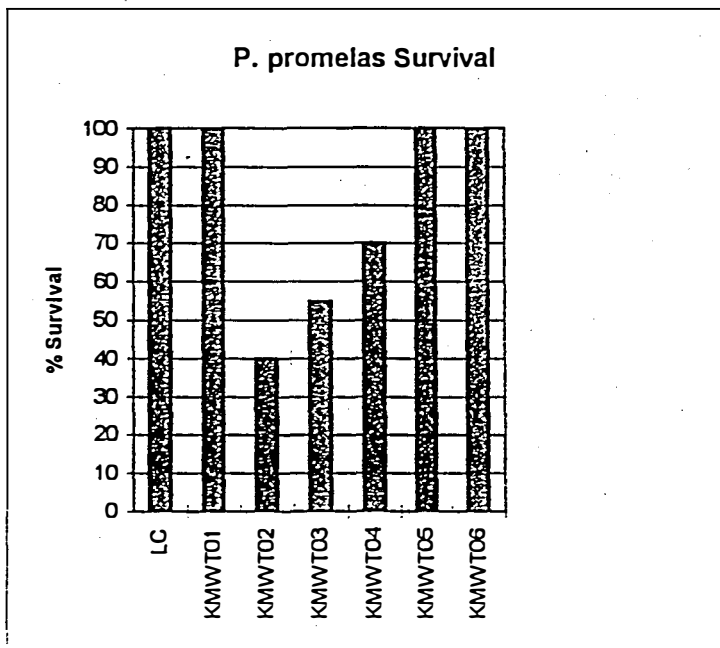
Surface Water Toxicity Testing Sample Site	Arsenic ug / L	General Location
WT01	1.0	Reference Site Off-Site Pond
WT02	8,300	SW of Cap
WT03	1,400	South of Cap
WT04	2,400	SE of Cap
WT05	430	South Slough to River
WT06	37	South of RR Tracks



C.D. Besadny Wildlife Area Surface Waters

Percent Survival (P. promelas Acute Test)

Site	% Survival
LC	100
KMWT01	100
KMWT02	40
KMWT03	55
KMWT04	70
KMWT05	100
KMWT06	100



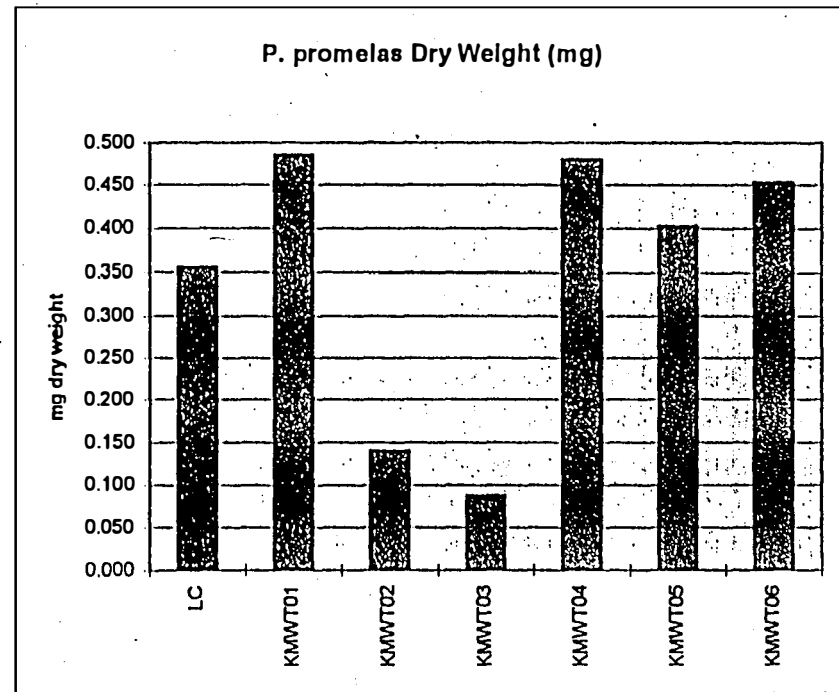
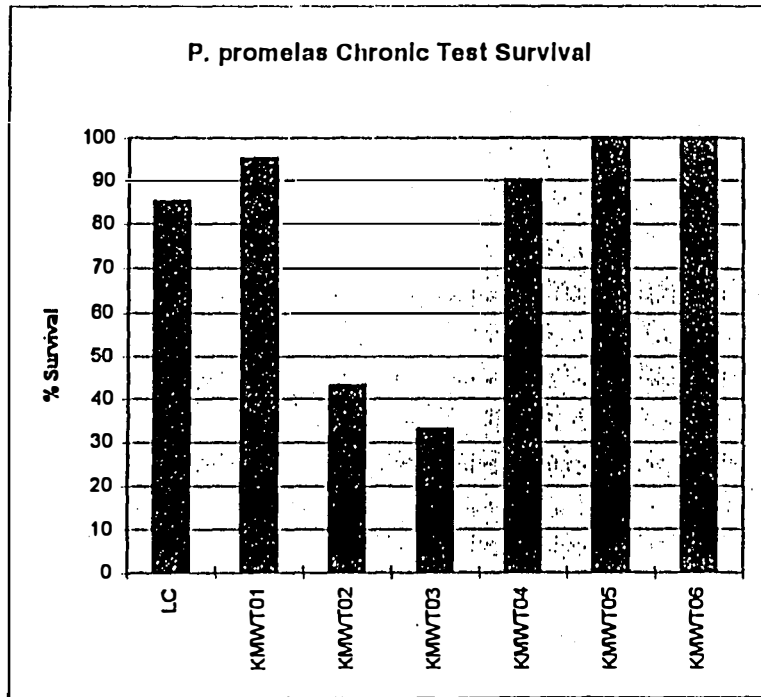
C.D. Besadny Wildlife Area Surface Waters

Percent Survival (*P. promelas* Chronic Test)

Site	% Survival
LC	85
KMWT01	95
KMWT02	43
KMWT03	33
KMWT04	90
KMWT05	100
KMWT06	100

Mean Dry Weight (*P. promelas* Chronic Test)

Site	Dry Weight (mg)
LC	0.355
KMWT01	0.485
KMWT02	0.140
KMWT03	0.087
KMWT04	0.480
KMWT05	0.402
KMWT06	0.452



WATER

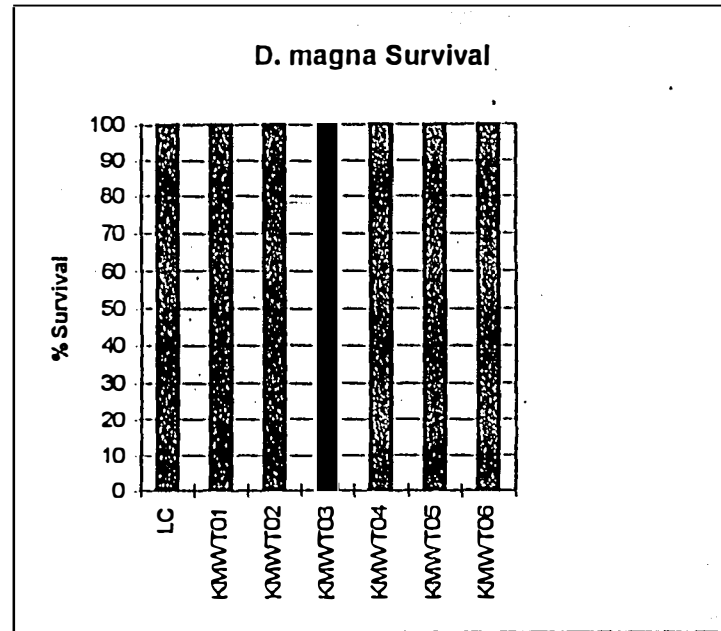
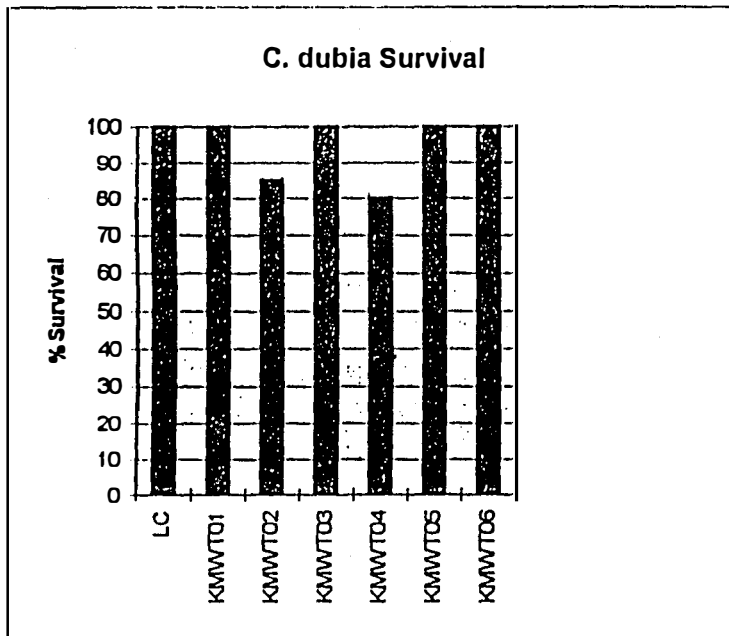
C.D. Besadny Wildlife Area Surface Waters

Percent Survival (*C. dubia* Acute Test)

Site	% Survival
LC	100
KMWT01	100
KMWT02	85
KMWT03	100
KMWT04	80
KMWT05	100
KMWT06	100

Percent Survival (*D. magna* Acute Test)

Site	% Survival
LC	100
KMWT01	100
KMWT02	100
KMWT03	100
KMWT04	100
KMWT05	100
KMWT06	100



WATER

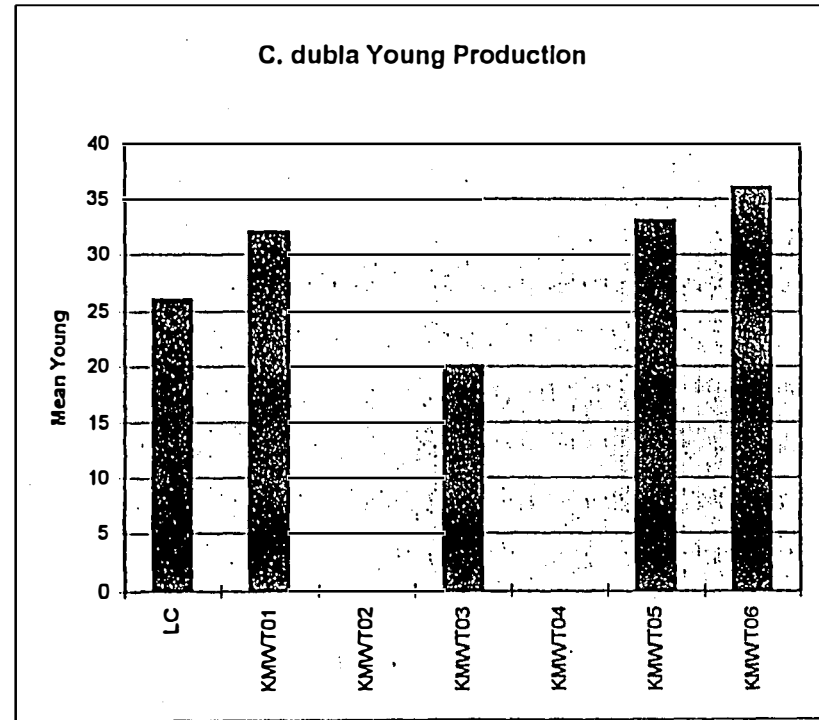
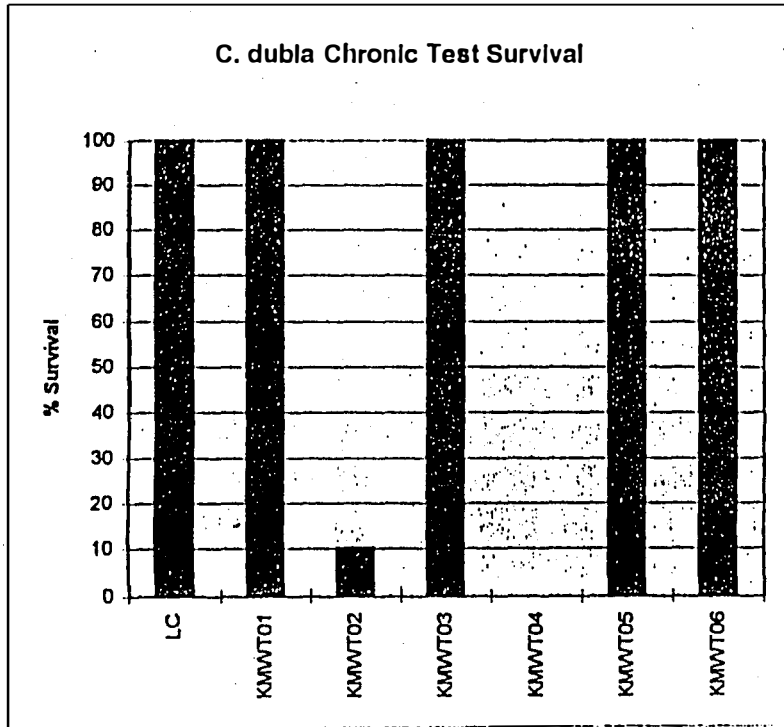
C.D. Besadny Wildlife Area Surface Waters

Percent Survival (C. dubia Chronic Test)

Site	% Survival
LC	100
KMWT01	100
KMWT02	10
KMWT03	100
KMWT04	0
KMWT05	100
KMWT06	100

Mean Young Produced (C. dubia Chronic Test)

Site	Mean Young
LC	26
KMWT01	32
KMWT02	0
KMWT03	20
KMWT04	0
KMWT05	33
KMWT06	36



WATER

Microtox Test Results

Test Date: 5/29/96
 Lab Numbers: 96069-96074

Site	Lab Number	Results after 5 minutes								Results after 15 minutes							
		1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	44	42	41	40	42	41	0.83	—	39	38	37	36	38	37	0.83	—
KMWT-01	96069	52	53	51	51	50	51	1.09	-24.24%	45	46	45	45	44	45	0.71	-20.81%
KMWT-02	96070	39	40	37	38	38	38	1.09	7.27%	37	36	35	34	34	35	0.83	6.71%

Site	Lab Number	Results after 5 minutes								Results after 15 minutes							
		1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	44	41	40	38	39	40	1.12	—	40	37	35	35	36	36	0.83	—
KMWT-03	96071	45	44	44	44	42	44	0.87	-10.13%	43	43	44	42	42	43	0.83	-19.58%
KMWT-04	96072	50	48	47	48	46	47	0.83	-19.62%	49	47	45	45	45	46	0.87	-27.27%

Site	Lab Number	Results after 5 minutes								Results after 15 minutes							
		1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	90	81	82	82	85	83	1.50	—	79	75	75	74	77	75	1.09	—
KMWT-05	96073	93	91	89	90	91	90	0.83	-9.39%	80	80	83	81	81	81	1.09	-7.97%
KMWT-06	96074	94	96	94	92	92	94	1.66	-13.33%	82	83	84	83	82	83	0.71	-10.30%

Site	Lab Number	Results after 5 minutes								Results after 15 minutes							
		1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC						ERR	ERR	—						ERR	ERR	—
							ERR	ERR	ERR						ERR	ERR	ERR
							ERR	ERR	ERR						ERR	ERR	ERR

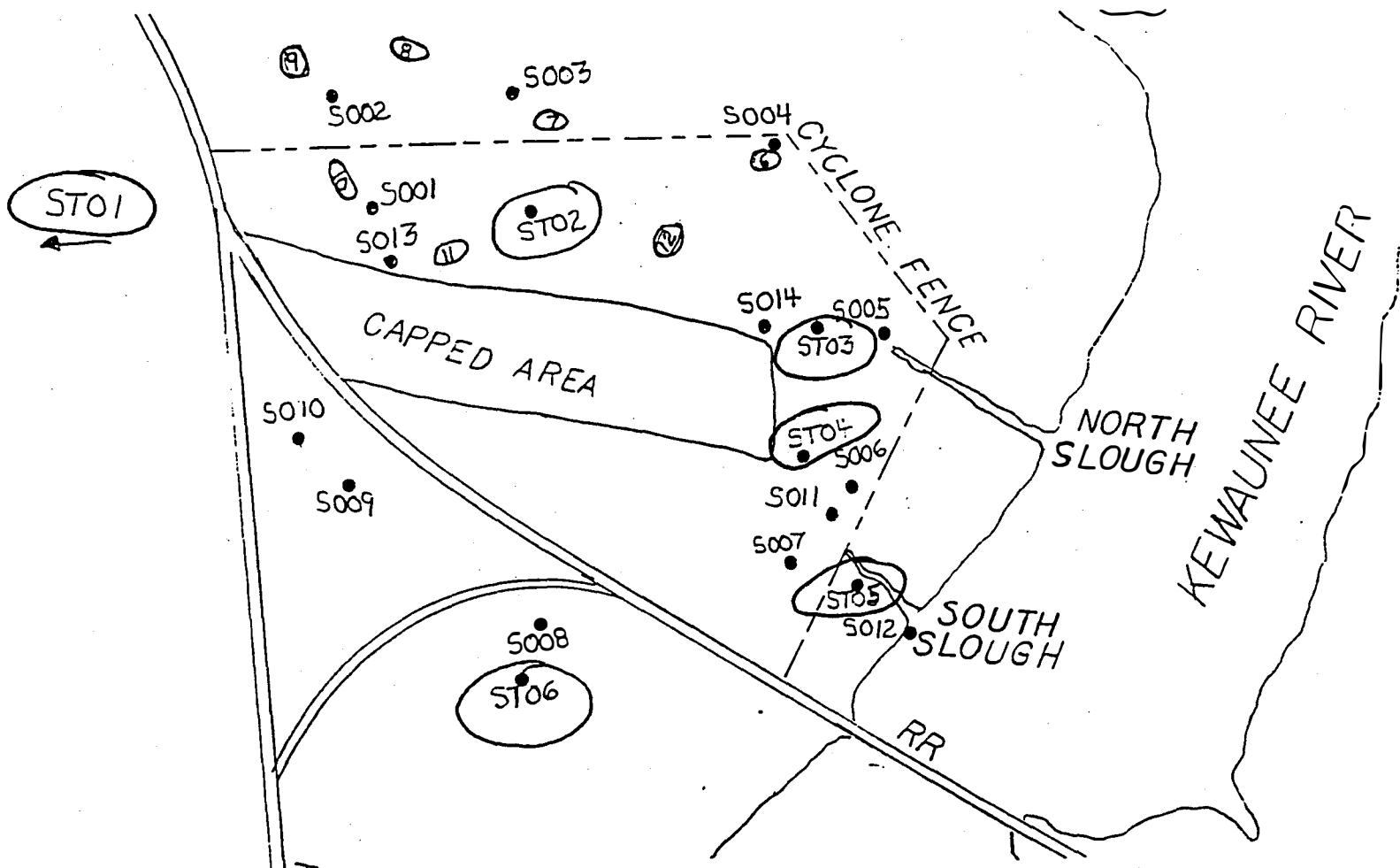
Comments: A negative percent effect means that the organisms did better in the sample than in the control.
 No significant toxicity was seen in the microtox.

WATER

SEDIMENT

Sediment Sampling Locations and Analytical Results for Arsenic Related to Sediment Toxicity Testing

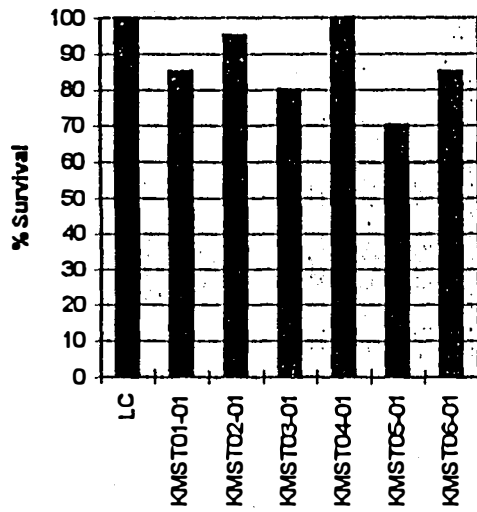
Sediment Toxicity Testing Sample Site	Arsenic ug / L	General Location
ST01	2.6	Reference Site Off-Site Wetland
ST02	150	North of Cap
ST03	220	NE of Cap
ST04	220	East of Cap
ST05	67	SE of Cap
ST06	2.2	South of RR Tracks



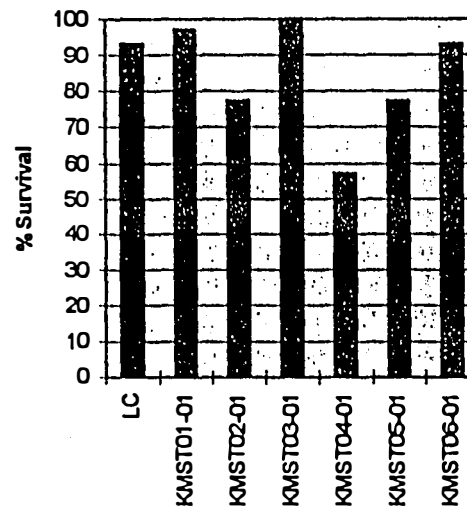
C.D. Besadny Wildlife Area Sediments

Percent Survival (<i>C. dubia</i> Acute Test)			Percent Survival (<i>D. magna</i> Acute Test)		
Site	% Survival		Site	% Survival	
LC	100		LC	93	
KMST01-01	85		KMST01-01	97	
KMST02-01	95		KMST02-01	77	
KMST03-01	80		KMST03-01	100	
KMST04-01	100		KMST04-01	57	
KMST05-01	70		KMST05-01	77	
KMST06-01	85		KMST06-01	93	

C. dubia Survival



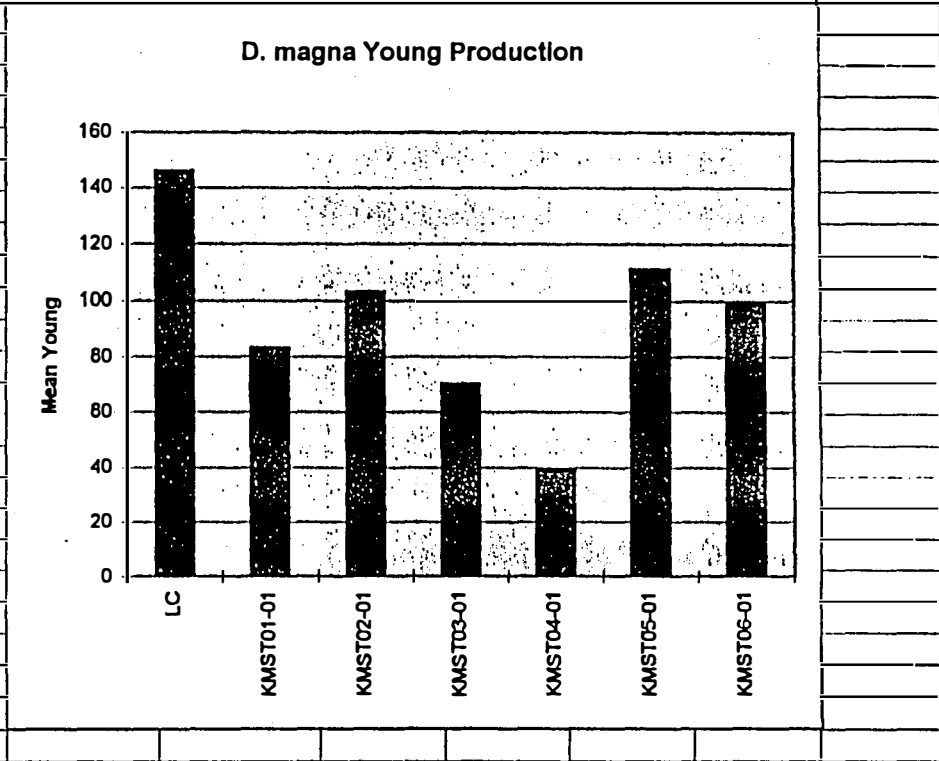
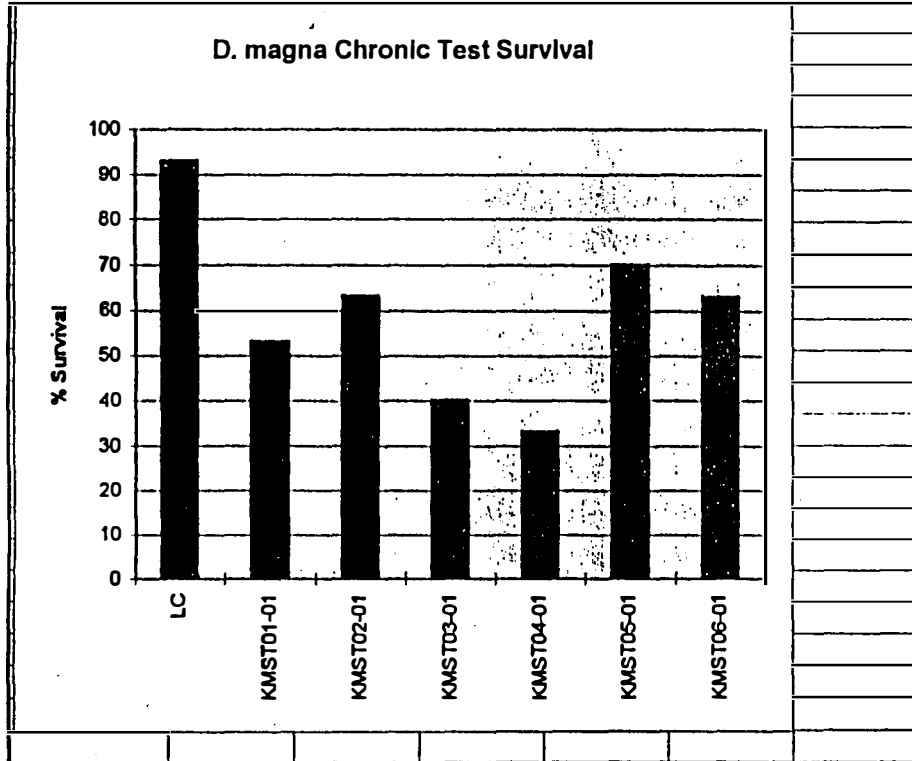
D. magna Survival



SEDIMENT

C.D. Besadny Wildlife Area Sediments

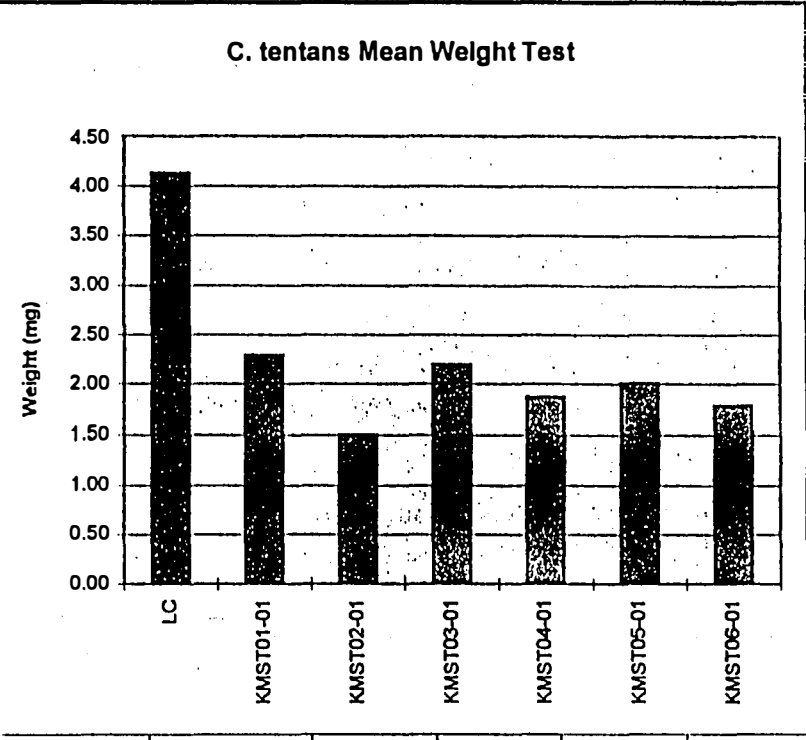
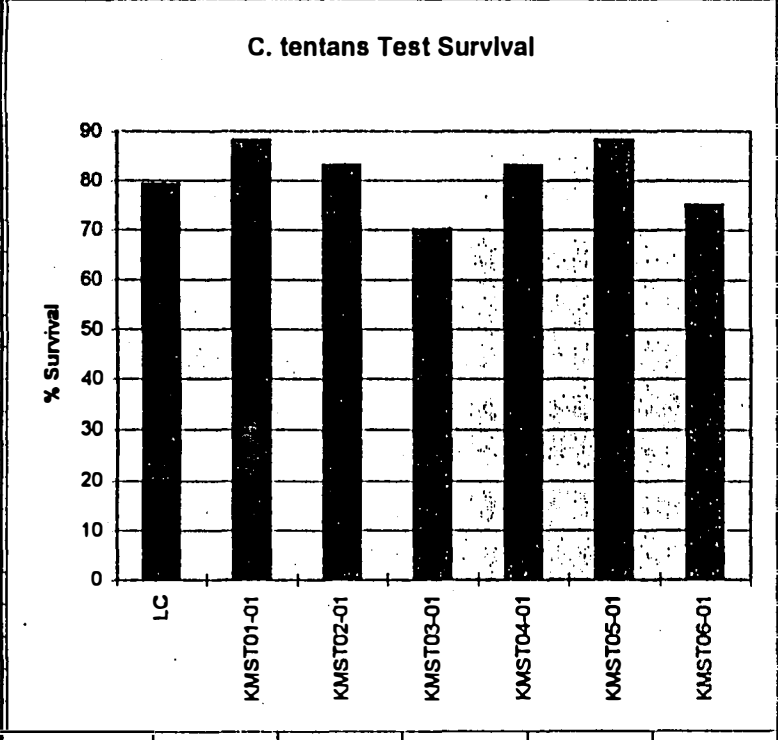
Percent Survival (D. magna Chronic Test)				Mean Young Produced (D. magna Chronic Test)			
Site	% Survival			Site	Mean Young		
LC	93			LC	146		
KMST01-01	53			KMST01-01	83		
KMST02-01	63			KMST02-01	103		
KMST03-01	40			KMST03-01	70		
KMST04-01	33			KMST04-01	39		
KMST05-01	70			KMST05-01	111		
KMST06-01	63			KMST06-01	99		



SEDIMENT

C.D. Besadny Wildlife Area Sediments

Percent Survival (<i>C. tentans</i> Test)			Mean Weight (<i>C. tentans</i> Test)		
Site	% Survival		Site	Weight (mg)	
LC	79		LC	4.12	
KMST01-01	88		KMST01-01	2.28	
KMST02-01	83		KMST02-01	1.50	
KMST03-01	70		KMST03-01	2.19	
KMST04-01	83		KMST04-01	1.87	
KMST05-01	88		KMST05-01	2.01	
KMST06-01	75		KMST06-01	1.79	



SEDIMENT