

# AMBIENT TOXICITY TEST REPORT FORM

GENERAL INFORMATION								
PROJECT NAME: <b>Manitowoc River Sediment Project</b>		LABORATORY NAME: Wisconsin State Laboratory of Hygiene		REPORT NUMBER: FZ000017-21				
REPORT TYPE: <input checked="" type="checkbox"/> Original <input type="checkbox"/> Amended		If amended, original report number: _____						
SAMPLE INFORMATION								
SAMPLE NO.	LAB NO.	FIELD NO.	SITE DESCRIPTION			STATION NO. (SWIMS, STORET or LAT/LONG)		
1	FZ000017	MT Tox 1	Reference Sediment Site 1					
2	FZ000018	MT Tox 2	Manitowoc River Sediment Site 2					
3	FZ000019	MT Tox 3	Manitowoc River Sediment Site 3					
4	FZ000020	MT Tox 4	Manitowoc River Sediment Site 4					
5	FZ000021	MT Tox 5	Manitowoc River Sediment Site 5					
6	FZ000022	MT Tox 3 Water	Manitowoc River Water from Site 3					
SAMPLE NO.	SAMPLE COLLECTION			SAMPLE TEMP. °C		HAND DELIVER? (If Yes, < 4 hr?)	HOLD TIME ≤ 36 HR?	SAMPLE ACCEP- TABLE?
	SAMPLE TYPE	SAMPLING DATE	DATE at LAB	COLLECTION	Temp Blanks			
1	Sediment	7/21/2014	7/21/2014	iced	13.2	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
2	Sediment	7/21/2014	7/21/2014	iced	2.0	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
3	Sediment	7/21/2014	7/21/2014	iced	2.0	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
4	Sediment	7/21/2014	7/21/2014	iced	13.2	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
5	Sediment	7/21/2014	7/21/2014	iced	11.2	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
6	Water	7/21/2014	7/21/2014	iced	10.9	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
<i>Describe any unusual conditions during sampling that may influence test results. (see Part 6.1.2 of the Methods Manual for examples.)</i> <b>COMMENTS:</b> Manitowoc River water was collected at Site 3 for use in toxicity tests. Sediment was collected at 5 sites via boat. Phase 1 tests were set within 72 hours of collection, other tests were set greater than 72 hours as test dates indicate.								
TEST INFORMATION								
<b>ACUTE</b>				<b>CHRONIC</b>				
Date Test Initiated:	Phase 1 DM and FHM 7/24/14			P. promelas 7/31/14, D. magna 7/31/14, P. Promelas Phase 3 8/28/14				
QA/QC CONDITIONS								
						ACUTE	CHRONIC	
Temperatures maintained during test? (25 ± 1°C)						<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
Dissolved oxygen ≥ 4.0 mg/l throughout tests?						<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
pH maintained within 6.0 - 9.0 s.u. throughout DM and FHM tests?						<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
Concurrent or monthly reference tests within acceptable limits?						<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
Tests conducted in a carbon dioxide atmosphere throughout test?						<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
Were samples modified prior to testing? (ex. filtration, aeration, chem addition)						<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
<b>COMMENTS:</b> Samples were modified in that elutriate samples were prepared from Manitowoc River water and sediment which was then filtered. Temperatures reached a high of 26.2°C in the acute P. promelas Trial 2 Test on days 1 - 3 and in the acute D. magna Trial 1								
WATER CHEMISTRY								
(All values reported in mg/L, except pH and Conductivity)								
SAMPLE TYPE	SAMPLE NO.	HARDNESS	ALKALINITY	TOTAL AMMONIA	DISSOLVED OXYGEN	pH (s.u.) After Warming	Conductivity (µS)	
Sites	Manitowoc River	244	235	0.06	9.7	9.02	511	
	Elutriate 3 Batch 2	272	255	NA - not enough volume	8.9	8.21	629	
	Elutriate 3 Batch 4	260	235	NA - not enough volume	9.9	8.21	572	
LAB WATER	Hard Water	180	135	NA	8.8	8.58	600	
	DC	196	335	NA	8.9	8.50	802	
<b>COMMENTS:</b> See narrative for other chemistry data notes pertaining to Daphnia magna and Pimephales promelas tests DC = Dechlorinated Madison tap water is used as the lab control for the fathead minnow test. Hard water is used for control in Daphnia magna tests. MR= Manitowoc River water Elut= elutriate DM= Daphnia magna								

## ACUTE PHASE 1 TRIAL 1 TEST CONTROL PERFORMANCE

<b>LAB WATER CONTROLS</b>		Daphnia magna Acute Trial 1 Set: 7/24/14
	<i>Daphnia magna</i>	
	Survival $\geq$ 90% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	

These are non-standard tests run using sediment and elutriate samples as described under each species test. This trial could not be done with fathead minnows due to limited volume of elutriate available.

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
Daphnia magna  Age of Organism: < 24 Hours Old	LC	LW Control	100	100	100	100	100.0	A
	1	MT Tox 1	100	90	100	100	97.5	A
	2	MT Tox 2	90	100	100	100	97.5	A
	3	MT Tox 3	20	30	30	30	27.5	B
	4	MT Tox 4	90	90	90	100	92.5	A
	5	MT Tox 5	90	100	90	100	95.0	A
	6							

Please describe any unusual behavior and/or appearance of organisms.(see Part 6.1.2 of the Methods Manual for ex.)

**COMMENTS:** \* Samples with the same letter are not statistically different from each other.  
**DM Trial 1** was done by making elutriate from sediment from each sample site mixed with Manitowoc River water. Each test treatment then had 30 ml sediment and 120 mL of elutriate in each beaker. Tests were static non renewal 48hours. Lab control associated with this test was 120 ml elutriate (made with lab water and lab control sediment) over 30 ml lab control sediment. 10 daphnia in each replicate were fed 1 mL YFC and 1 mL S.cap on Day 0. Some of the daphnia could not be found on shut down but were presumed dead. Additional hard water control is listed on DM Trial 3 and had 100% survival.

Please describe any unusual behavior and/or appearance of organisms.(see Part 6.1.2 of the Methods Manual for ex.)

**COMMENTS:** **FHM Trial 1** was not conducted due to limited elutriate volume.

Manitowoc River Sediment Project

Report # : FZ000017-21

Acute Test Date: Phase 1 DM and FHM 7/24/14

## ACUTE PHASE 1 TRIAL 2 TEST CONTROL PERFORMANCE

### LAB WATER CONTROLS

Fathead Minnow	<i>Daphnia magna</i>
Survival $\geq$ 90%	Survival $\geq$ 90%
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Daphnia magna Acute Trial 2 - Set: 7/24/14  
Fathead minnow Acute Trial 2 - Set: 7/24/14

COMMENTS: These are non-standard tests run using sediment and elutriate samples as described under each species test.

### ACUTE TEST DATA

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
Fathead Minnow  Age of Organism: 13 days	LC	LW Control	70	100	100	100	92.5	A
	1	MT Tox 1	100	100	100	100	100.0	A
	2	MT Tox 2	100	100	100	80	95.0	A
	3	MT Tox 3	80	100	100	100	95.0	A
	4	MT Tox 4	100	100	100	100	100.0	A
	5	MT Tox 5	100	100	100	100	100.0	A
	6	MR + Lab Sed Control	100	100	100	90	97.5	A

*Please describe any unusual behavior and/or appearance of organisms.(see Part 6.1.2 of the Methods Manual for ex.)*

COMMENTS: \* Samples with the same letter are not statistically different from each other.  
This trial involved sediment from each site with Manitowoc River water from site 3 as the overlying water. The first control is lab culture water - dechlorinated tap. An additional control is listed as treatment 6 which was lab sediment with Manitowoc River water overlying. Tests were run for 96 hour with partial water renewal daily. Beakers were filled with 1 part water: 4 parts sediment. Each beaker had of 40 mL of sediment and 160 mL of overlying water. FHM were fed 0.1 mL shrimp on Day 2.

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
<i>Daphnia magna</i>  Age of Organism: < 24 Hours Old	LC	LW Control	100	100	90	100	97.5	A
	1	MT Tox 1	100	100	100	90	97.5	A
	2	MT Tox 2	100	100	100	90	97.5	A
	3	MT Tox 3	90	80	100	100	92.5	A
	4	MT Tox 4	100	90	90	100	95.0	A
	5	MT Tox 5	100	80	100	100	95.0	A

*Please describe any unusual behavior and/or appearance of organisms.(see Part 6.1.2 of the Methods Manual for ex.)*

COMMENTS: \* Samples with the same letter are not statistically different from each other.  
This trial involved sediment from each site with Manitowoc River water from site 3 as the overlying water. The control is lab sediment with Manitowoc River water overlying. Daphnia that could not be found were presumed dead. Treatment 3 smelled like petroleum and had a film on top. Treatment 4 had a film on top of the water. Tests were nonrenewal and were fed 1 mL of *S.capricornutum* and YFC on Day 0.

Project Name : Manitowoc River Sediment Project

Report # : FZ000017-21

Acute Test Date : Phase 1 DM and FHM 7/24/14

## ACUTE PHASE 1 TRIAL 3 TEST CONTROL PERFORMANCE

### LAB WATER CONTROLS

Fathead Minnow	<i>Daphnia magna</i>
Survival $\geq$ 90% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Survival $\geq$ 90% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Daphnia magna Acute Trial 3 - Set 7/24/14  
Fathead Minnow Trial 3 - Set 7/24/14

**COMMENTS:**

These are non-standard tests run using sediment and elutriate samples as described under each species test.

### ACUTE TEST DATA

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
Fathead Minnow  Age of Organism: 13 Days	LC	LW Control	100	100	100	100	100.0	A
	1	MT Tox 1	100	100	100	100	100.0	A
	2	MT Tox 2	100	100	100	90	97.5	A
	3	MT Tox 3	90	100	90	100	95.0	A
	4	MT Tox 4	100	100	90	100	97.5	A
	5	MT Tox 5	100	100	100	100	100.0	A
	6	Lab Control Elutriate	90	90	80	100	90.0	A

*Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)*

**COMMENTS:**

\* Samples with the same letter are not statistically different from each other.  
FHM Trial 3 was done using elutriate made from sediment from each site mixed with Manitowoc River water. Only elutriate (no sediment) was used in each beaker. The lab control was dechlorinated tap water (non-elutriate), and treatment 6 was control elutriate, which was prepared using hard lab water and lab control sediment.  
Tests were run for 96 hour with partial water renewal daily. Beakers had 140 mL of elutriate, and were fed 0.1 mL shrimp on Day 2.

SPECIES	SITE DESCRIPTION		Percent Survival By Replicate				Mean Percent Survival	Statistical Significance*
			1	2	3	4		
<i>Daphnia magna</i>  Age of Organism: < 24 Hours Old	LC	Lab Control Elutriate	100	100	100	90	97.5	A
	1	MT Tox 1 Elutriate	100	100	100	100	100.0	A
	2	MT Tox 2 Elutriate	100	100	100	100	100.0	A
	3	MT Tox 3 Elutriate	90	100	100	100	97.5	A
	4	MT Tox 4 Elutriate	100	100	100	100	100.0	A
	5	MT Tox 5 Elutriate	100	100	100	100	100.0	A
	6	Hard Water Control	100	100	100	100	100.0	A

*Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)*

**COMMENTS:**

\* Samples with the same letter are not statistically different from each other.  
DM Trial 3 was done using elutriate made from sediment from each site mixed with Manitowoc River water. Only elutriate (no sediment) was used in each beaker. The lab control was control elutriate, which was prepared using hard lab water and lab control sediment. Treatment 6 was hard water, which was a non-elutriate lab control.

Project Name : Manitowoc River Sediment Project  
Report # : FZ000017-21  
Acute Test Date : Phase 1 DM and FHM 7/24/14

## CHRONIC PHASE 2 TEST CONTROL PERFORMANCE

### LAB WATER CONTROLS

<b>Fathead Minnow</b>	<b><i>Daphnia magna</i></b>
Survival > 80% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Survival > 80% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
> 0.25 mg/fish <input checked="" type="checkbox"/> Yes <input checked="" type="checkbox"/> No	> 15 neonates/female <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Survival Weight CV < 40% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Reproduction CV < 40% <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Survival Weight % CV = 4	Reproduction %CV= 8
	> 80% 3rd brood <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
	< 20% males <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

**Chronic Phase 2 *Pimephales promelas* and *Daphnia magna***

**COMMENTS:** 7/31/14 Set FHM and *Daphnia magna* Phase 2 Tests - focused on Site 3 due to toxicity seen in Phase 1. Site 1 is the reference site which did not show toxicity in Phase 1.

### CHRONIC TEST DATA

SPECIES	SITE DESCRIPTION		MEAN % SURVIVAL	MEAN DRY BIOMASS PER REPLICATE PAIR (mg)					MEAN BIOMASS (mg)	Growth Statistical Significance*
				1	2	3	4	5		
Fathead Minnow Growth & Survival Test	LC	Culture Water - DC	94	0.440	0.413	0.435	0.445	0.303	0.407	B
	<i>LW Survival Weight</i>			0.440	0.413	0.435	0.445	0.403		
	1	DC + Lab Sed Control	100	0.628	0.590	0.510	0.458	0.440	0.525	A
	2	DC + Site 1	100	0.463	0.438	0.453	0.720	0.403	0.495	AB
	3	MR + Site 3 Sediment	20	0.075	0.048	0.045	0.000	0.000	0.034	D
	4	S3 Elutriate	85	0.380	0.323	0.293	0.275	0.195	0.293	C
	5	Site 3 Elut +S3 Sediment	5	0.028	0.000	0.000	0.000	0.000	0.006	D

*Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)*

**COMMENTS:** \* Samples with the same letter are not statistically different from each other.

Phase 2 Test initiated 7/31/2014 - Chronic test was set with control treatments, site 1 as reference site, and multiple treatments involving Tox Site 3. All treatments are described in the site description above. Statistical significance is based on growth results.

SPECIES	SITE	NEONATE PRODUCTION BY REPLICATE										MEAN NEONATES	% ADULT SURVIVAL	Statistical Significance*
		1	2	3	4	5	6	7	8	9	10			
<i>D. magna</i> Reproduction & Survival Test	LC	47	51	57	56	52	52	60	52	57	60	54	100	A(Surv) A(Repro)
	1	30	30	29	29	30	22	23	23	35	31	28	90	A(Surv) B(Repro)
	2	51	48	27	65	65	62	71	44	59	52	54	90	A(Surv) A(Repro)
	3	56	56	58	59	51	52	56	49	60	59	56	100	A(Surv) A(Repro)
	4	39	44	57	29	26	67	52	40	56	51	46	50	B(Surv) A(Repro)
	5	50	66	72	64	56	57	0	58	74	0	50	80	A(Surv) A(Repro)

**Male Production ≤ 20% Over All Treatments? Yes**

*Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)*

**COMMENTS:** \* Samples with the same letter are not statistically different from each other.

Phase 2 Test initiated 7/31/14 - Chronic test was set with control treatment, site 1 as reference site, and multiple treatments involving Tox Site 3. Site descriptions are the same as the FHM treatments above, with the substitution of hard lab water for DC water in treatments LC, 1, and 2. Statistical significance was determined for both survival (Surv) and reproduction (Repro).

## CHRONIC PHASE 3 TEST CONTROL PERFORMANCE

RECEIVING WATER CONTROLS		LAB WATER CONTROLS	
<b>Fathead Minnow</b>		<b>Fathead Minnow</b>	
Survival $\geq$ 80%		Survival $\geq$ 80%	
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
$\geq$ 0.25 mg/fish		$\geq$ 0.25 mg/fish	
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
Survival Weight CV $\leq$ 40%		Survival Weight CV $\leq$ 40%	
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
%CV = 8		%CV = 9	

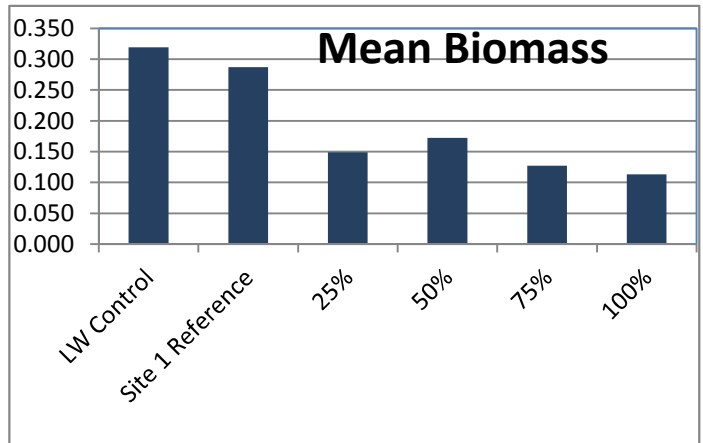
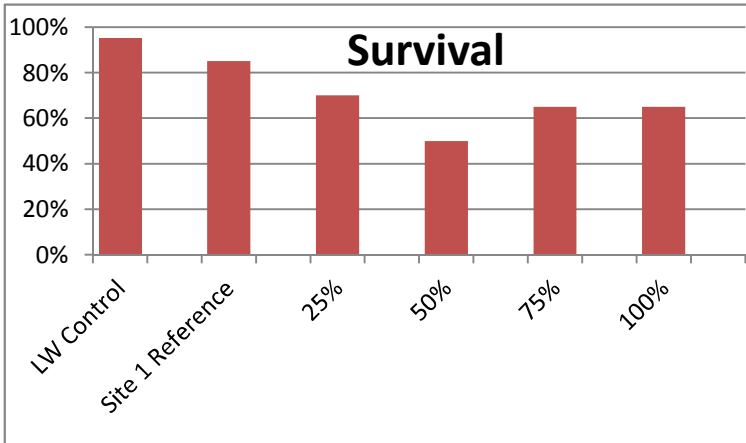
**COMMENTS:** Only the FHM test run with diluted sediments.

### CHRONIC TEST DATA

SPECIES	EFFLUENT TREATMENT	MEAN % SURVIVAL	MEAN DRY BIOMASS PER REPLICATE PAIR (mg)					MEAN BIOMASS (mg)	SURVIVAL WEIGHT %CV
			1	2	3	4	5		
Fathead Minnow Growth & Survival Test	LW Control	95%	0.338	0.325	0.315	0.293	0.325	0.319	
	<i>survival weight</i>		0.338	0.325	0.315	0.391	0.325		9
	Site 1 Reference	85%	0.230	0.317	0.255	0.265	0.368	0.287	
	<i>survival weight</i>		0.306	0.317	0.340	0.353	0.368		8
	25%	70%	0.250	0.075	0.160	0.165	0.095	0.149	
	50%	50%	0.258	0.133	0.083	0.210	0.180	0.173	
	75%	65%	0.233	0.210	0.073	0.120	0.000	0.127	
	100%	65%	0.120	0.130	0.098	0.088	0.130	0.113	

**FHM CHRONIC RESULTS:** IC<sub>25</sub> = 17%    C.I.% = 11-36%    rTUc = NA  
 Statistics Program:  EPA's ICp  Other (See Comments Below)

**COMMENTS:** Based on Phase 2 testing it was decided that a chronic P. promelas test should be run with a dilution series of sediments. Tox Site 3 sediments were diluted based on weight with the reference sediment from Tox Site 1. This was well mixed and then placed into cups with 1:4 ratio of sediment to Manitowoc River water. LW control is dechlorinated tap water only, and Site 1 reference is Tox Site 1 sediment with Manitowoc River water overlying.  
 Please describe any unusual behavior and/or appearance of organisms.(see Part 6.1.2 of the Methods Manual for ex.)



I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations. I also certify that these results relate only to these samples.

LAB REPRESENTATIVE:	Camille Turcotte	SIGNATURE:	
DATE:	10/31/2014		
PHONE:	(608) 224-6230	WDNR LAB CERT #:	113133790
LAB ADDRESS:	Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, Madison, WI 53718		
REVIEWED BY:	Mallory Ballard	DATE:	10/31/2014
PERMITTEE		SIGNATURE:	
PHONE:		DATE:	

Send **all pages** of this form (plus any attachments or additional information which you believe to be relevant to the test) to: **Biomonitoring Coordinator, Bureau of Watershed Management, Department of Natural Resources, 101 South Webster St., P.O. Box 7921, Madison, WI 53707-7921.**

Copies of the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual (Methods Manual) and the WET Guidance Document can be obtained from the WDNR Biomonitoring Coordinator at the address given above or at:  
<http://dnr.wi.gov/org/water/wm/ww/biomon/>

Yes  No

TO BE COMPLETED BY THE WISCONSIN DEPARTMENT OF NATURAL RESOURCES		
Results Entered Into Database?		
COMMENTS:		
REVIEWED BY:		DATE:
CC:		

Project Name : Manitowoc River Sediment Project  
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 Test Date : P. promelas 7/31/14,