

MAR 1 1 2005

RESULTS OF TOXICITY TESTING WITH Leptocheirus pumulosus ON 15 DECEMBER 2005 SEDIMENT SAMPLES FROM THE DELAWARE RIVER

Prepared for:

Department of Natural Resources and Environmental Control Division of Air and Waste Management 391 Lukens Dr. New Castle, DE 19720

Prepared by:

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Results relate only to the items tested or to the samples as received by the laboratory.

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This report contains 10 pages plus 2 attachments.

Wayne L. McGulloch

Laboratory Director

11 June



1. INTRODUCTION

At the request of the State of Delaware Department of Natural Resources and Environmental Control (DNREC), EA Engineering, Science, and Technology performed 10-day solid phase survival toxicity tests with the amphipod *Leptocheirus plumulosus*. The purpose of this study was to evaluate the toxicity of three sediment samples collected from the Delaware River following an oil spill upstream. These three samples were collected on 15 December 2004, 16 days after sediment samples were collected to establish baseline toxicity profiles. The results of the *L. plumulosus* sediment toxicity testing performed on the 29 November 2004 baseline sediment samples is presented in EA Report #4677.

2. MATERIALS AND METHODS

2.1 SAMPLE RECEIPT AND PREPARATION

Three sediment samples were collected by DNREC personnel on 15 December 2004 and transported on ice to EA's Ecotoxicology Laboratory in Sparks, Maryland. Upon receipt at EA on 22 December 2004, the sediment samples were logged in and assigned EA laboratory accession numbers, and were stored in the dark in a secured walk-in cooler at ≤4°C until used for testing. Prior to use in testing, each sediment was homogenized, and large rocks and debris were manually removed and discarded from the sample. Table 1 summarizes the sample identifications, accession numbers, and collection and receipt information for the sediment samples. Chain-of-custody records are included in Attachment I.

2.2 CONTROL SEDIMENT

A sample of sediment from Codorus Creek, Pennsylvania, was used as the control sediment for the toxicity testing. The sediment was collected in an area designated as a state wild trout stream. Sediment collected from this location has historically been non-toxic and is routinely utilized as a control in EA's sediment toxicity tests.

2.3 OVERLYING WATER

Artificial sea water (Forty Fathoms sea salts) at 5 ppt salinity was used as the overlying water. Dechlorinated tap water was used to prepare the artificial sea water. The source of the tap water was the City of Baltimore municipal water system. Upon entering the laboratory, the water was passed through a high-capacity, activated-carbon filtration system to remove any possible contaminants such as chlorine, detergents, and other possible trace organic contaminants. This water source has proven safe for aquatic organism toxicity testing at EA as evidenced by maintenance of multigeneration *Daphnia* sp., *H. azteca* and fathead minnow cultures with no evident loss of fecundity.

2.4 TEST ORGANISMS

Whole sediment toxicity testing was conducted with the estuarine amphipod *Leptocheirus* plumulosus. The amphipods (2-4 mm) were acquired from Aquatic BioSystems. Organism lot number LP-026 was received on 6 January 2005 and used to initiate the toxicity test on 11 January 2005. During the holding period, the organisms were gradually acclimated to laboratory water at 20°C and the appropriate test salinity of 5 ppt. The organisms were fed finely ground Tetramin flake food during the acclimation period.

2.5 TOXICITY TEST METHODS

All toxicity testing was conducted following EA's standard operating procedures (EA 2003) which are in accordance with US EPA guidance (1994).

The whole sediment toxicity tests were conducted as static, non-renewal tests with ten days of exposure to the whole sediments and overlying water. Prior to initiation of the toxicity tests, the sediments and overlying water were added to the test chambers, and the suspended sediments were allowed to settle overnight. The addition of the test organisms to the exposure chambers on the following day marked the initiation of the toxicity tests.

The *L. plumulosus* tests utilized 1-L beakers as the exposure chambers, with each beaker containing 200 ml of sediment and 700 ml of overlying water. There were five replicate chambers for each sediment sample and control. Test organisms were randomly assigned to the test chambers, 20 organisms per replicate chamber for a total of 100 organisms per sample.

The tests were maintained at 20±1°C with a 16-hour light/8-hour dark photoperiod. The test chambers were visually inspected daily for abnormal organism behavior/lack of burrowing. Water quality measurements of temperature, pH, dissolved oxygen, and salinity were recorded daily on one replicate of each sample and control. The water quality parameters measured during the toxicity tests are summarized in Table 2. The test organisms were not fed during the 10-day exposure period. After ten days of exposure, the test organisms were retrieved from the samples and the number of live organisms per replicate was recorded.

Statistical analyses were performed on the whole sediment test data according to US EPA (1994) guidance and using the ToxCalc statistical software package (Version 5.0, Tidepool Scientific Software). Statistical analyses were performed to determine if exposure to either of the sediment samples resulted in significantly lower (p=0.05) survival of the test organisms as compared to the control sediment. A summary of the survival data for the *L. plumulosus* exposed to each sediment sample is provided in Table 3. Copies of the original data sheets are included as Attachment I.

2.6 REFERENCE TOXICANT TESTING

In conformance with EA's quality assurance/quality control program requirements, reference toxicant testing was performed on the acquired lot of *L. plumulosus*. The reference toxicant test consisted of a graded concentration series of cadmium chloride in water only tests, with no sediment present in the test chambers. The results of the reference toxicant test was compared to established control chart limits.

2.7 ARCHIVES

Original data sheets, records, memoranda, notes, and computer printouts are archived at EA's Baltimore Office in Sparks, Maryland. These data will be retained for a period of 5 years unless a longer period of time is requested by the State of Delaware Department of Natural Resources and Environmental Control.

3. RESULTS AND DISCUSSION

The results of the *Leptocheirus plumulosus* whole sediment toxicity tests met the current NELAC standards, where applicable.

Table 3 summarizes the results of the toxicity tests conducted on the 15 December 2004 Delaware River sediment samples. There was 91 percent survival of *L. plumulosus* in sample DRSED01, and 95 percent survival in sample DRSED02, after 10 days of exposure. The 10-day survival in sediment DRSED03 was only 32 percent. The DRSED03 sample gave off an oily sheen, and had a strong petroleum smell. Survival in the control sediment was 93 percent. Statistical analysis indicated that the DRSED03 sample was the only post oil spill sediment sample that was significantly (p=0.05) different from the control. There was no evidence of any oil in the DRSED01 and DRSED02 test treatments.

The 48-hour LC50 for the reference toxicant test conducted on Lot LP-026 was 10.9 mg/L Cd, which was within EA's established laboratory control chart limits of 1 - 15.0 mg/L Cd, indicating that the acquired organisms were of acceptable quality.

4. REFERENCES CITED

- EA. 2003. EA Ecotoxicology Laboratory Quality Assurance and Standard Operating Procedures Manual. EA Manual ATS-102. Internal document prepared by EA's Ecotoxicology Laboratory, EA Engineering, Science, and Technology, Inc., Sparks, Maryland.
- US EPA. 1994. Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and marine Amphipods. EPA 600/R-94/025. U.S. Environmental Protection Agency, Office of Research and Development, Narragansett, Rhode Island.

TABLE 1 SUMMARY OF COLLECTION AND RECEIPT INFORMATION FOR 15 DECEMBER 2004 SAMPLES FROM DELAWARE RIVER

Sample Identification	EA Accession Number	Collection Time and Date	Receipt Time and Date
CONTROL	AT5-021	10 January 2005	10 January 2005
DRSED 01	AT4-800	1345, 15 December 2004	1600, 22 December 2004
DRSED 02	AT4-798	1530, 15 December 2004	1600, 22 December 2004
DRSED 03	AT4-799	1130, 15 December 2004	1600, 22 December 2004

TABLE 2 SUMMARY OF WATER QUALITY PARAMETERS FROM 10-DAY WHOLE SEDIMENT TOXICITY TESTING WITH Leptocheirus plumulosus ON DELAWARE RIVER SEDIMENTS

Test Species:

Leptocheirus plumulosus

Test Number:

TN-05-013

		Water Quality Parameters – Range												
Sample ID	EA Accession Number	Temperature (°C)	рН	Dissolved Oxygen (mg/L)	Salinity (ppt)									
Lab Control	AT5-025	19.0-20.9	6.7-7.4	3.9-8.5	4.3-5.0									
DRSED 01	AT4-800	19.0-21.0	6.7-7.6	3.6-8.6	4.3-5.0									
DRSED 02	AT4-798	19.0-20.9	6.8-7.6	4.4-8.4	4.5-4.9									
DRSED 03	AT4-799	19.0-20.5	6.7-7.4	3.2-8.5	4.4-5.0									

TABLE 3 RESULTS OF 10-DAY WHOLE SEDIMENT TOXICITY TESTING WITH Leptocheirus plumulosus ON DELAWARE RIVER SEDIMENTS

Test Species:

Leptocheirus plumulosus

Test Number:

TN-05-013

Sample Date:

15 December 2004

Test Date:

11-21 January 2005

<u>Test Treatment</u>	10-Day <u>% Survival</u>
LAB CONTROL	93
DRSED 01	91
DRSED 02	95
DRSED 03	32 ^(a)

⁽a) Significantly different (p=0.05) from the laboratory control.

ATTACHMENT I

Data Sheets and Statistical Analyses (12 pages)

FIELD CHAIN OF CUSTODY

Environmental Laboratory Section - Division of Water Resources Department of Natural Resources and Environmental Control 89 Kings Highway, Dover, DE 19901 (302) 739-4771

Sample Disposal: Return to Client [] Disposal by ELS [] Authorized by:



PROJECT NAI SAMPLERS (P	Phone No.:	35/10 35/10	adle?	5 DX1 DE 19 2600	1720		No	18 To 18	winner e protes	Invoi Acco ELS	ce To unt Batch	#:_	KO Ro	Cord	t Schulle Schulte	
(ELS Use Only) Lab Log No.	Client Sample Description	Sample. Date	Sample Time	Matrix'	Comp	Grab	Of Con- taluers	Leptochinus Toc.	BOATHACNOCYDANA	The see also the second		TEN POPOLOGICA CONTRACTOR CONTRAC			REM.	ARKS
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	Please Coll Kissen Tord Identification: Non-Hazard				, -										SD - solid SE - sediment	water X – Other

		0 0000
Yes No Yes No Yes No	La tradition is a second	
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8. Custody Seal was Present/Unbroken. 9. Discrepancies between sample labels and COC record? 10. Any Exceptions (see Comm	ente)	
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or Picked up by ELS Yes No Yes No Yes No Yes No	rea	Yes No
	Yes No	Fig. X.
Shipped, Hand-delivered 2. Received broken/leaking 3. Cooler Temperature Bottle 4. Properly preserved 5. Holding times expired 6.	Bottles supplied by	ELS 7. Field Filtered
Sample Condition (circle response):		
ILS USE ONLY		
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SEDIMENT TOXICITY TEST SET-UP BENCH SHEET

Project Number:	70005.08		
Client: DNRE			
QC Test Number:	TN-05-01	3	
		TEST ORGANISM INFOR	RMATION
Common Name:	1. '	the state of the s	plated (Time, Date):
Scientific Name:		Neonates	Pulled (Time, Date):
Source: ABS			10 -
Source: 1100		Culture vv	/ater (T/S): 19.2 °C ppt
		TESTUNITIATIO	N
, <u>Date</u>	Time	<u>Initials</u>	Activity
1/10/05	1/30	CES/PG	Sediment Added to Chambers
1	1650	Ρ6	Overlying Water Added to Chambers
1/11/05	1015	P6	Organisms Transferred
		TEST SET-UP	475-021
Sample Number(s):	AT4-798	,799,800, 395	A75-021 025 www. 2/24/05
Overyling Water Nu	mber: $5pp$	ot PF	na de comina de vica da de vica de vic
<u>Treatment</u>	<u>V</u>	olume Test Sediment	Volume Overlying Water
(maha)		200nJ	700ml
Control		1	
AT4-798			
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AT4-799 AT4-800			
1 A74-000			
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A Take NH3 sample at Day & before renewal / loading and at Day 10. A Day 10.



SEDIMENT TOXICITY TEST OBSERVATION DATA SHEET

Project Number:	TEST ORGANISM	^	Beginning Date:	<u>Y11105</u>	_ Time: 1015
Client: DNREC	Common Nam	ne: <u>Amphipod</u>	Ending Date:	1/21/05	Time: 1115
QC Test Number: TN-05-013	Scientific Nam	ne: L. plumulosus			
Test Material(s): <u>Sediment</u>	AT5-021 Wilmy/05				
Accession Number(s): <u>ATY-798,797,8</u> 6	O, 395TEST TYPE:	Static / Flowthrough	Test Container:		
Overlying Water: 5ppt FF		Renewal / Non-renewal	Test Volume:	200ml Se	d. / 700ml Hzc
Accession Number:			Test Duration: _	10 day	
				•	

						Number o	of Surviving	Organisms				
Treatment	Rep	Day O Date Villy5	Day 10 Date 1/21	Pay	Day	Day	Day	Day	Day	Day	Day	Day
				Date	Date	Date	Date	Date .	Date	Date	Date	Date
Control	A	20	18									
AT5-021	<u>B</u>	20	19									
	C	20	19									
	D	20	19									
	ε	20	18									
AT4-798	A	20	19									
	В	20	18									
	C	20	19									
	D	20	19									
	E	20	20									
Time /	Initials	101261	1115 90									



SEDIMENT TOXICITY TEST OBSERVATION DATA SHEET

Project Number:	TEST ORGANISM	Beginning Date: 1105 Time: 1015
Client: DNREC	Common Name: Umphipad	Ending Date: 1/21/05 Time: 1115
QC Test Number: <u>TN-05-013</u>	Scientific Name: L. plumulosus	
Test Material(s): Sediment	375 AT5-04 WILL	•
Accession Number(s): <u>A74-798,79</u> °	7, 800 TEST TYPE: Static / Flowthrough	Test Container: 1 beaker
Overlying Water: 5pp+ FF	Renewal Non-renewal	Test Volume: 200ml / 700ml H20
Accession Number:		Test Duration: 10 day
		· ·

						Number (of Surviving	Organisms				
Tooland	D	Day O	Day 10	Ray	Day	Day	Day	Day	Day	Day	Day	Day
Treatment	Rep	Date '(1)	Date —	Date	Date	Date	Date	Date	Date	Date	Date	Date
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TOXICITY TEST WATER QUALITY DATA SHEET - OLD SOLUTIONS

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AT4-798	6,191	20.0	20.7	0 ⁰	19.8	19.6	19.2	<u>8 ما</u>	છ.છ	7.6	1.0	70	7.2	7.3	44	4.5	1:1	દુ.9ૄ	1.2	<u>8,0</u>	ક. <u>પ</u>	<u> </u>	47	4.7	4.6	4.60	4.5	<u>4,5</u>
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AT4-800	19.2	203	319.8	410	<u>19,5</u>	F1.2	19.0	6.8	し _ず :コ	7,60	7.2	7.0	7. 2.	7.2	4.1	3,6	1.7	7.7	8.18	<u>19</u>	8,2	47	4,6	4.7	4.6	4.3	45	4,4
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TOXICITY TEST WATER QUALITY DATA SHEET - OLD SOLUTIONS

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		Temperature (°C)						рН							Diss	olved	Охур	jen (m	g/L)		Conductivity (µS/cm) Salinity (ppt)								
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TOXICOLOGY LABORATORY BENCH SHEET

Project Number:	70005.08
Client: DNR	80
QC Test Number:	TN-05-018

Date/Ti	me/Initials		Comments/Activity
1/10/05	1115	P6/CES	ausamples had a lot of dead clams in the sample.
			Sample AT4-799 had a very
			Strong oil-like smell. (petroleum)
1/11/05	800	P6/LES	NH3 samples taken and all
			beakers renewed.
1/3/05	1340	P6	Dissolved oxygen fell below 40% Beakers were aerated
			lightly, approx 1 bobble 15 sec.
			fewer burrow notes Replicate
			199E had a Star PS
			present on the surface of the water.
/21/05	1700	144	Semple AT4-799 had petroleum smell and obvious nortality of test organisms. No

1/21/04 1200 M49

Simple AT4-799 had petroleum smell

and obvious nortality of test organisms

There were little of oil balls recovered

in the sieve. An oily shein was observed

spreading around the clumps ATS-T29

spreading around the clumps

				Lepto	cheirus plumi	ulosus 10-Day Test	
Start Date:	1/11/2005		Test ID:	TN-05-013	3	Sample ID:	DNREC
End Date:	1/21/2005		Lab ID:			Sample Type:	Sediments
Sample Date:			Protocol:	EPAA 91-I	EPA Acute	Test Species:	LP-Leptocheirus plumulosus
Comments:							·
Conc-%	1	2	3	4	5		
Control	0.9000	0.9500	0.9500	0.9500	0.9000		
Sed 01	0.9500	0.9500	0.9000	0.8500	0.9000		
Sed 02	0.9500	0.9000	0.9500	0.9500	1.0000		
Sed 03	0.3500	0.3500	0.2000	0.4000	0.3000		

			1-Tailed							
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.9300	1.0000	1.3068	1.2490	1.3453	4.034	5			
Sed 01	0.9100	0.9785	1.2724	1.1731	1.3453	5.772	5	0.852	1.860	0.0752
Sed 02	0.9500	1.0215	1.3487	1.2490	1.4588	5.509	5			
Sed 03	0.3200	0.3441	0.5988	0.4636	0.6847	14.062	5			

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90981		0.781		-0.2799	-1.3111
F-Test indicates equal variances (p = 0.54)	1.94133	_	23.1539			
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	0.04261	0.04572	0.00296	0.00409	0.41909	1, 8

ATTACHMENT II

Report Quality Assurance Record (2 pages)



REPORT QUALITY ASSURANCE RECORD

Client: Delaware DNREC F	Project Number: 7000	5.08
Author: While E	A Report Number: 46	91
REPORT CHEC	KLIST	
QA/QC ITEM	REVIEWER	DATE
Samples collected, transported, and received according to study plan requirements.	Millod	2/22/05
Samples prepared and processed according to study plan requirements.	he fullad	2/22/05
3. Data collected using calibrated instruments and equipment.	The fulled	2/22/05
4. Calculations checked: - Hand calculations checked	Me Cullal	2/22/05
- Documented and verified statistical procedure used.	MoJulled	2/22/05
5. Data input/statistical analyses complete and correct.	had Connelly	3/1/05
6. Reported results and facts checked against original sources.	hada Connelly	3/1/05
7. Data presented in figures and tables correct and in agreement with text.	land a - County	3/1/05
8. Results reviewed for compliance with study plan requirements.	refullad	2/22/05
	<u>AUTHOR</u>	DATE
9. Commentary reviewed and resolved.	rfillal	3/4/05
10. All study plan and quality assurance/control requirement approved:	ents have been met and the r	eport is
	CT MANAGER	3/4/05 DATE
Pic QUALIT	Rod A - County	3/1/65 DATE
SENIO	TECHNICAL REVIEWER	3/4/c5 DATE