Monitoring in Tributaries of the Delaware River
for
Ambient Toxicity
2015 Narrative Report

Submitted to U.S. Environmental Protection Agency—Region III

Delaware River Basin Commission

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1.0 SUMMARY

The objective of the 2015 surveys was to determine the potential for chronic lethal or sublethal toxicity to aquatic life in ambient water samples collected from sampling stations in tributaries of the Delaware River. Five species were used in short-term laboratory experiments including the freshwater species *Pimephales promelas*, *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata* and the salinity tolerant test species *Menidia beryllina* and *Hyalella Azteca*. Endpoints appropriate for each test species including survival, growth, or reproduction were measured. Surface water samples from seven sites in the Christina River Basin and two sites in Shellpot Creek were collected in 2015 in cooperation with the Delaware Department of Natural Resources and Environmental Control (DNREC) Watershed Approach to Toxics Assessment & Restoration (WATAR) Program, a watershed-scale approach to the evaluation of contaminant sources, transport pathways and receptors. The long term goals of WATAR are to reduce toxic exposure to aquatic life in watersheds by identifying and controlling releases from land-based sources and creating innovative strategies to mitigate legacy contamination in sediment. Based on the test species tested and the measured endpoints, the water sampled did not indicate chronic toxicity to aquatic life at a biologically significant level.

2.0 INTRODUCTION

Potential sources of toxicity and water quality impairment in the Delaware Estuary include point and non-point sources, contaminated sites, tributaries, atmospheric deposition and contaminated sediment (Delaware Estuary Program, 1996). Based on existing water quality regulations for the estuary, no adverse effects should be observed in toxicity tests with undiluted ambient water (DRBC, 2012; USEPA, 1991). In 2000, the DRBC determined that the assimilative capacity of Zones 2 - 5 was exceeded for chronic toxicity and recommended continued monitoring to assess the cumulative effect of toxicity sources. Monitoring toxicity in the tidal Delaware River and its tributaries is therefore an essential component of programs designed to protect this valued resource.

A number of programs monitor chemical contaminants and toxicity in permitted wastewater discharges, water, sediment and benthic organisms in the Delaware Estuary (PDE, 2012). Since the DRBC monitoring program is the only on-going program to test for water column toxicity in the estuary, a cooperative effort was initiated by the DRBC through the formation of an Ambient Toxicity Workgroup to develop a scientifically sound sampling and analysis plan, with a holistic, broad, long-term view, to determine whether ambient toxicity occurs in the waters of the estuary. The Ambient Toxicity Workgroup includes personnel from the DRBC, U.S. Environmental Protection Agency (USEPA), basin states, municipal agencies, industry, and other interested parties. The Workgroup reviews and provides input on project plans for ambient toxicity monitoring as well as reviewing and commenting on the results from the toxicity testing. MacGillivray et al., 2011 reported on previous sampling and analysis of the Delaware River and its tributaries for ambient toxicity.

In response to the Ambient Toxicity Workgroup recommendation that the DRBC investigate toxicity in tributaries, surface water samples were collected for ambient toxicity testing in 2015 concurrently with activities scheduled within the DNREC - WATAR Program which has the goals of: 1) assessing the status, trends and sources of toxics in Delaware watersheds; 2) better coordinating efforts between water and waste site remediation programs; 3) identifying and implementing priority remediation and restoration projects; and 4) restoring Delaware's watersheds to a fishable status in the shortest timeframe possible.

http://www.dnrec.delaware.gov/dwhs/SIRB/Pages/WATAR.aspx

3.0 MATERIALS AND METHODS

3.1 Selection of Test Species

Toxicity in Delaware Estuary waters is assessed with standard test species used for testing effluents under the USEPA NPDES program; the same species have frequently been used to monitor receiving water toxicity (USEPA, 2002a and USEPA, 2002b). Three freshwater species were selected, for waters with conductivity ≤ 1750 µmhos/cm or ≤ 1 ppt salinity at 25 ^{o}C, a fish, *Pimephales promelas* (fathead minnow); an invertebrate, *Ceriodaphnia dubia* (water flea)*;* and a green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*).

Some of the sampling sites selected experience changes in salinity due to flow and tidal conditions. The selection of test species and appropriate controls was complicated by this changing salinity gradient. Additional test species were selected that were tolerant of salinity (1 to 15 ppt) and met the prescribed test acceptability requirements at ambient salinities. The species also had to be a standard toxicity test species and commercially available. The two salinity tolerant species used were a mysid, a fish, *Menidia beryllina* (inland silverside)*;* and an amphipod, *Hyalella azteca*.

3.2 Study Design

Evaluations of all sampling sites from tributaries in 2015 were made in dilution series at 100%, 50%, 25%, 12.5% and 6.25% ambient water. Results from these tests were compared to controls of reconstituted laboratory water formulated to mimic freshwater (salinity < 1 ppt) for *P. promelas*, *C. dubia*, and *P. subcapitata* or synthetic seawater diluted to mimic ambient estuarine water for *M. beryllina* and *H. Azteca*. In 2015, water samples were collected from seven sites in the Christina River Basin and two sites in Shellpot Creek (Figure 1). The sampling was designed to complement concurrent activities scheduled as part of the DNREC WATAR Program. USEPA short-term chronic toxicity methods were used to evaluate toxicity and sublethal effects in ambient samples with *Pimephales promelas*, *Ceriodaphnia dubia,* and *Menidia beryllina* in 7-day tests*; Pseudokirchneriella subcapitata* in a 96-hour test*;* and *Hyalella azteca* in a 10-day water-only test. Endpoints evaluated by these methods included survival, growth and reproduction (USEPA 2002a and USEPA 2002b). In the H. azteca tests, clean sand (Silica Company pool filter sand produced from Oriskany deposit in Berkeley Springs, WV, sieved to 1 mm and washed/dried) was used as a substrate and water was renewed daily (USEPA, 2000). Additional modifications to the toxicity test methods are described in the salinity adjustment and control section below.

At tributary sampling sites, water was collected on three days. At each sampling site, samples were collected below surface at a targeted depth of 0.6 of the water column using a Masterflex E/S portable sampler and C-Flex tubing L/S (Cole Parmer, Vernon Hills, Ill). On each day of sampling, in-field measurements were made for specific conductivity, salinity, water temperature, dissolved oxygen and pH using a Hydrolab or other appropriate meters (Table 1 to 3). Water samples for toxicity testing were transported to the laboratory in LDPE plastic cubitainers (VWR Int., Brisbane, CA) on ice in coolers to maintain the temperature at 4 ^{o}C ± 2 ^{o}C. Temperature inside the cooler was tracked during transport with a temperature logger.

3.3 Salinity Adjustments and Controls

In toxicity tests with salinity tolerant species *M. beryllina*, and *H. azteca*, the test salinity adjustment was based on the ambient salinity of the first sample collected at each site. Since the ambient salinity was <5 ppt, the sample was adjusted to 5 ppt for tests with *Menidia beryllina*. The *M. beryllina* tests included a control at a salinity of 5 ppt. *Hyalella azteca* was tested at the ambient salinity. Ambient water for the *H. azteca* tests did not need salinity adjustment. *H. azteca* tests were conducted with controls at salinities of 0.1 and 1 ppt.

3.4 Hydrology and Tides

Low flow conditions were targeted for the sampling. The recorded discharge at USGS gauge 01478245 on White Clay Creek at White Clay Creek Preserve ranging from 27 to 32 cfs on October

12 through 16, 2015 are low flows compared to a historic median flow of 79 cfs. The recorded discharge at USGS gauge 01480000 on Red Clay Creek at Wooddale, DE ranged from 21 to 28 cfs between October 12 and 16, 2015, and are low flows compared to a historic median flow of 63 cfs. The recorded discharge at USGS gauge 01481000 on Brandywine Creek at Wilmington, DE ranged from 127 to 138 cfs during the period October 19 through 23, 2015, and are low flows compared to a historic median flow of 495 cfs. Insufficient data are available to accurately quantitate flows at sampling times in Shellpot Creek or Christina River but, observationally the tributary flows were low while samples were collected.

Low slack tide was targeted for sampling in tidally influenced waters. Figure 2 shows the sampling location, dates, and times aligned with NOAA predicted tides and currents for Christina River samples (http://tidesandcurrents.noaa.gov/ofs/dbofs/dbofs.html). Insufficient data are available to accurately align sampling location, dates and times in the Brandywine Creek but, observationally the tide was low in the tributary while samples were collected.

3.5 Statistical Analysis

Statistical comparisons were made between the controls and treatments (dilutions) for each test site. All statistical analysis followed USEPA guidance for each test method (USEPA 2002a and USEPA 2002b) using ToxCalc v5.0 software (Tidepool Scientific Software, McKinnleyville, CA USA). Linear interpolation combined with bootstrapping was used to calculate the 25% inhibitory concentration point estimate (IC_{25}). To assure that differences between controls and treatment were biologically significant as well as statistically significant, a test was not considered positive for toxicity unless there was > 20 % difference observed between control and ambient water in the tests. In addition, a test for significant toxicity (TST) was conducted using results for 100% ambient water from sample sites compared to a control using the Welch's t test at a recommended b value for chronic tests of 0.75. The b value represents a fixed fraction of the control response that is compared to the response in the ambient water samples to evaluate the null hypothesis of no difference in the mean responses. Alpha levels for the TST test were set at $\alpha = 0.20$ for *C. dubia,* and *P. promelas*, and at $\alpha = 0.25$ for *M. beryllina*; and *P. subcapitata* (Denton *et al*., 2011; Shukla *et al*., 2000; USEPA, 2010). In the absence of recommended alpha values for *H. azteca*, the Welch's t test was not used with data from this species.

4.0 RESULTS AND DISCUSSION

Evaluation of IC_{25} for survival, growth and reproduction and additional tests for significant toxicity confirmed the lack of chronic lethal or sublethal effects for the species and endpoints tested based on methods used to analyze the data at the nine tributary sites sampled in 2015 (Tables 4, 5 and 6). An aberration occurred when fungal growth invalidated results for five *P. promelas* tests (DE5, DE13, DE14, DE15 and DE16).

5.0 CONCLUSIONS

The objective of the 2015 surveys was to determine the potential for chronic lethal or sublethal toxicity to aquatic life in ambient water samples collected from sampling stations in tributaries of the Delaware River. These surveys consisted of water column toxicity tests on samples collected during periods of low flow and low slack tide. Five species were used in the surveys including *Pimephales promelas*, *Menidia beryllina*, and *Ceriodaphnia dubia* in 7-day tests; *Pseudokirchneriella subcapitata* in a 96-hour test; and *Hyalella azteca* in a 10-day water-only test. Based on the measured endpoints appropriate for each test method including survival, growth, and reproduction, testing of surface water from sites in the Brandywine Creek, Christina River, Red Clay Creek, Shellpot Creek and White Clay Creek did not indicate chronic toxicity to aquatic life at a biologically significant level in any the water samples evaluated.

6.0 ACKNOWLEGEMENTS

This report was reviewed by John Yagecic, P.E. and Thomas Fikslin, Ph.D. (DRBC). Maps were prepared by Karen Reavy (DRBC). This project was supported by the Delaware River Basin Commission and United States Environmental Protection Agency 106 Grant.

7.0 REFERENCES

Delaware Estuary Program. 1996. The Scientific Characterization of the Delaware Estuary.

Delaware River Basin Commission. 2012. Delaware River and Bay Water Quality Assessment. http://www.nj.gov/drbc/library/documents/WQAssessmentReport2012.pdf

Denton, D, Diamond J and Zheng L. 2011. Test of significant toxicity: a statistical application for assessing whether an effluent or site water is truly toxic. Environ Toxicol and Chem 30: 1117-1126.

MacGillivray R, Russell DE, Brown S, Fikslin TJ, Greene R, Hoke R, Nally C, O'Donnell L (2011) Monitoring the tidal Delaware River for ambient toxicity. Integr Environ Assess Manag 7: 466-477.

Partnership for the Delaware Estuary. 2012. Technical Report for the Delaware Estuary and Basin. PDE Report No. 12-01. 255 pages. www.delawareestuary.org/science_programs_state_of_the_estuary.asp.

Shukla R., Wang Q, Fulk F, Deng C and Denton D. 2000. Bioequivalence approach for whole effluent toxicity testing. Environmental Toxicology and Chemistry 19:169-174.

U.S. Environmental Protection Agency. 1991. Technical Support Document For Water Quality-based Toxics Control. EPA/505/2-90-001.

U.S. Environmental Protection Agency. 2000. Section 11. Test Method 100.1. *Hyalella azteca* 10-d Survivial and Growth Test for Sediments. EPA 600/R-99/064.

U.S. Environmental Protection Agency, October 2002a. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Waters To Freshwater Organisms, Fourth Edition EPA-821-R-02-013.

U.S. Environmental Protection Agency, October 2002b. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Waters To Marine & Estuarine Organisms, Third Edition EPA-821-R-02-014.

U.S. Environmental Protection Agency, June 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document: An Additional Whole Effluent Toxicity Statistical Approach for Analyzing Acute and Chronic Test Data – EPA 833-R-10-003.

8.0 FIGURES AND TABLES

Figure 1. Sample sites in 2015

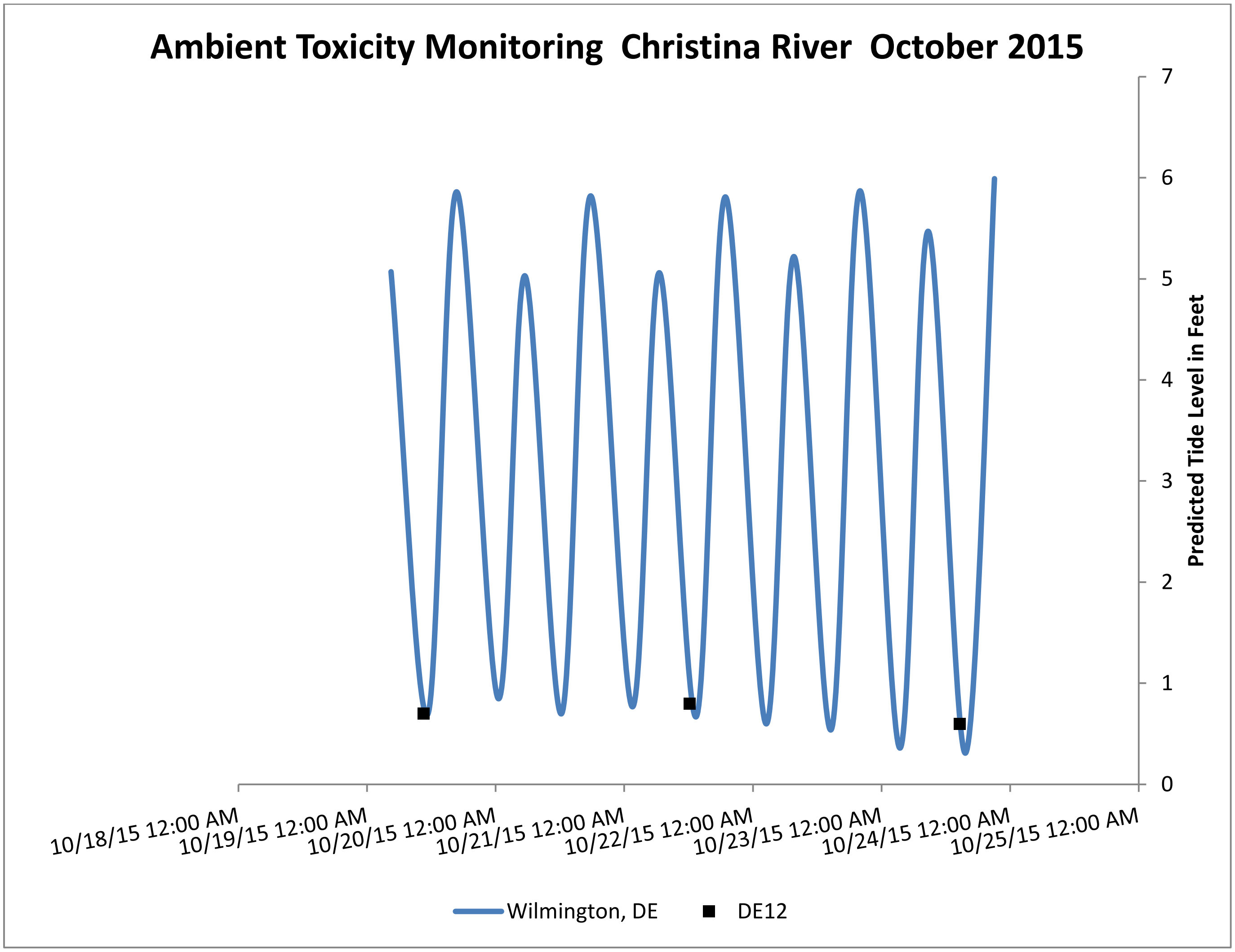

Figure 2. Tidal conditions during Christina River sampling

Table 1. Physical-chemical data for October 12, 14 and 16, 2015 samples

Sample	Time	Temp ºC	HDO		Specific Conductivity uS/ml	pH	Turbidity NTU	Salinity ppt
			mg/l	% sat				
DE-5-101215 Delaware Park White Clay Creek	1249	14.12	10.43	102.9	372.5	7.84	0.94	< 1 ppt
DE-13-101215 PA/DE state line Red Clay Creek	1041	12.99	11.70	112.7	513.4	8.18	1.08	< 1 ppt
DE14-101215 Wooddale Red Clay Creek	1426	15.01	12.12	122.0	394.7	8.49	0.94	< 1 ppt
DE-15-101215 Spencer Road East Br White Clay Creek (PA16)	1000	10.81	10.26	93.9	248.5	7.64	-	< 1 ppt
DE-16-101215 Chambers Rock Rd White Clay Creek	1140	11.93	10.81	101.6	397.9	7.88	0.80	< 1 ppt
DE-5-101415 Delaware Park White Clay Creek	1144	14.85	10.38	104.3	393.3	8.0	0.78	< 1 ppt
DE-13-101415 PA/DE state line Red Clay Creek	0952	14.23	10.35	102.6	537.3	7.82	0.91	< 1 ppt
DE14-101415 Wooddale Red Clay Creek	1239	14.84	11.92	119.8	438.5	8.43	1.33	< 1 ppt
DE-15-101415 Spencer Road East Br White Clay Creek (PA16)	0910	12.34	9.53	90.6	248.3	7.59	0.82	< 1 ppt
DE-16-101415 Chambers Rock Rd White Clay Creek	1048	13.66	10.24	100.2	414.4	7.85	0.62	< 1 ppt
DE-5-101615 Delaware Park White Clay Creek	1117	12.93	10.72	102.5	403.1	7.79	0.80	< 1 ppt
DE-13-101615 PA/DE state line Red Clay Creek	0943	12.08	10.22	96.1	536	7.73	0.69	< 1 ppt
DE14-101615 Wooddale Red Clay Creek	1212	12.97	12.0	114.9	448.1	8.28	0.71	< 1 ppt
DE-15-101615 Spencer Road East Br White Clay Creek (PA16)	0904	10.55	10.13	91.7	252.8	7.61	0.68	< 1 ppt
DE-16-101615 Chambers Rock Rd White Clay Creek	1032	11.32	10.78	99.4	421.9	7.86	0.94	< 1 ppt

Table 2. Physical-chemical data for October 19, 21 and 23, 2015 samples

Sample	Time	Temp ºC	HDO		Specific Conductivity uS/ml	pH	Turbidity NTU	Salinity ppt
			mg/l	% sat				
DE-11-101915 Brandywine Creek	1107	11.13	10.67	96.2	641.3	8.03	1.8	< 1 ppt
DE-12-101915 Christina River	1033	14.48	10.35	101.0	1999.0	7.63	19.9	1.01 ppt
DE-11-102115 Brandywine Creek	1253	11.05	10.82	98.0	485.7	8.18	2.9	< 1 ppt
DE-12-102115 Christina River	1214	13.81	11.89	115.0	1606.0	8.17	13.7	< 1 ppt
DE-11-102315 Brandywine Creek	1356	13.79	9.97	95.8	880.8	8.08	4.14	< 1 ppt
DE-12-102315 Christina River	1433	14.93	12.11	120.2	2061.0	8.17	16.4	1.05 ppt

Table 3. Physical-chemical data for November 2, 4 and 6, 2015 samples

Sample	Time	Temp ºC	HDO		Specific Conductivity uS/ml	pH	Turbidity NTU	Salinity ppt
			mg/l	% sat				
DE-1-110215 Shellpot Creek @ Hay Rd	1056	12.69	3.26	30.9	532.59	6.94	4.17	< 1 ppt
DE-17-110215 Shellpot Creek @ Rt 13	1019	12.85	8.79	77.6	533.09	7.21	0.90	< 1 ppt
DE-1-110415 Shellpot Creek @ Hay Rd	1103	13.35	10.30	98.9	797.1	6.71	9.86	< 1 ppt
DE-17-110415 Shellpot Creek @ Rt 13	1031	12.38	8.98	84.4	576.2	7.22	0.89	< 1 ppt
DE-1-110615 Shellpot Creek @ Hay Rd	1056	15.86	3.55	36.09	1212	7.07	1.66	< 1 ppt
DE-17-110615 Shellpot Creek @ Rt 13	1024	15.54	7.14	71.9	602.09	730	1.30	< 1 ppt

Table 4. Toxicity test results for October 12, 14 and 16, 2015 samples

Site	Latitude Longitude	*P. promelas* fish Survival and growth	*C. dubia* invertebrate Survival and reproduction	*P. subcapitata* algae growth
		IC25/TST	IC25/TST	IC25/TST
DE5 White Clay Creek @ Del Park	39.699083 -75.674944	NA	100%/PASS	100%/Pass
DE13 Red Clay Creek @ PA/DE line	39.808074 -75.681388	NA	100%/PASS	100%/Pass
DE14 Red Clay Creek @ Wooddale	39.762317 -75.636050	NA	100%/PASS	100%/Pass
DE15/PA16 White Clay Creek @ Stroud	39.858707 -75.783317	NA	100%/PASS	100%/Pass
DE16 White Clay Creek @ Chambers Rock	39.732889 -75.759639	NA	100%/PASS	100%/Pass

Inhibitory Concentration to 25% of test organisms (IC25)
Test for Significant Toxicity (TST) is recommended by USEPA because it incorporates a percent-based effects threshold and a false negative error rate absent from the NOEC calculations. Pass indicates TST declared sample concentration as not toxic.
TST is not available for data from tests with *H. azteca*.
NA – not available (fungal growth on fish)

Table 5. Toxicity tests results Oct 19, 21 and 23, 2015 samples

Site	Latitude Longitude	*C. dubia* invertebrate Survival and reproduction	*M. beryllina* fish Survival and growth	*H. azteca* amphipod Survival and growth
		IC25/TST	IC25/TST	IC25
DE11 Brandywine Creek	39.738033 -75.526950	100%/Pass	100%/Pass	100%
DE12 Christina River	39.734517 -75.549667	100%/Pass	100%/Pass	100%

Inhibitory Concentration to 25% of test organisms (IC25)
Test for Significant Toxicity (TST) is recommended by USEPA because it incorporates a percent-based effects threshold and a false negative error rate absent from the NOEC calculations. Pass indicates TST declared sample concentration as not toxic.
TST is not available for data from tests with *H. azteca*.

Table 6. Toxicity test results for November 2, 4 and 6, 2015 samples

Site	Latitude Longitude	***P. promelas* fish** Survival and growth	***C. dubia* invertebrate** Survival and reproduction	***P. subcapitata* algae** growth
		IC25/TST	**IC25/TST**	**IC25/TST**
DE1 Shellpot Creek @ Hay Rd	39.73901 -75.51076	100%/PASS	100%/PASS	100%/Pass
DE17 Shellpot Creek @ Rt 13	39.75298 -75.51591	100%/PASS	100%/PASS	100%/Pass

Inhibitory Concentration to 25% of test organisms (IC25)
Test for Significant Toxicity (TST) is recommended by USEPA because it incorporates a percent-based effects threshold and a false negative error rate absent from the NOEC calculations. Pass indicates TST declared sample concentration as not toxic.