Monitoring in Tributaries of the Delaware River for
Ambient Toxicity
2016 Narrative Report

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

Delaware River Basin Commission

Contact: A. Ronald MacGillivray, Ph.D.

May, 2017
Table of Contents

1.0 SUMMARY .................................................................................................................................................. 3
2.0 INTRODUCTION ........................................................................................................................................ 3
3.0 MATERIALS AND METHODS .................................................................................................................. 5
   3.1 Selection of Test Species .................................................................................................................. 5
   3.2 Study Design ......................................................................................................................................... 5
   3.3 Hydrology and Tides ......................................................................................................................... 6
   3.4 Statistical Analysis .............................................................................................................................. 7
4.0 RESULTS AND DISCUSSION .................................................................................................................... 8
5.0 CONCLUSIONS ......................................................................................................................................... 8
6.0 ACKNOWLEDGEMENTS ........................................................................................................................ 9
7.0 REFERENCES ........................................................................................................................................... 10
8.0 FIGURES AND TABLES ............................................................................................................................ 11
   Figure 1. Sample sites .................................................................................................................................. 11
   Figure 2. Tidal conditions during sampling ............................................................................................... 12
   Table 1. Physical-chemical data for October 17, 2016 samples ............................................................ 13
   Table 2. Toxicity test results for October 17, 2016 samples .................................................................... 14
1.0 SUMMARY

The objective of the 2016 survey 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. Three species were used in short-term laboratory experiments including the freshwater species *Pimephales promelas, Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. Endpoints appropriate for each test species including survival, growth, or reproduction were measured. Surface water samples at four sites from Cedar Creek Watershed in northeastern Sussex County, DE were collected in 2016 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 species tested and the measured endpoints, overall 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 River and Bay include point and non-point sources, contaminated sites, tributaries, atmospheric deposition
and contaminated sediment (DRBC, 2016). Based on existing water quality regulations, 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 (DRBC, 2016). 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 2016.
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 °C, a fish, *Pimephales promelas* (fathead minnow); an invertebrate, *Ceriodaphnia dubia* (water flea); and a green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*).

3.2 Study Design

Evaluations of all sampling sites from tributaries in 2016 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 a *P. promelas* (fish), *C. dubia* (invertebrate), and *P. subcapitata* (algae). In 2016, water samples
were collected from four sites in the northeastern Sussex County, DE (Figure 1). The sampling was designed to complement concurrent activities scheduled as part of the DNREC WATAR Program (DNREC 2016). USEPA short-term chronic toxicity methods were used to evaluate toxicity and sublethal effects in ambient samples with *Pimephales promelas* and *Ceriodaphnia dubia* in 7-day tests and *Pseudokirchneriella subcapitata* in a 96-hour test. Endpoints evaluated by these methods included survival, growth and reproduction (USEPA 2002a).

At tributary sampling sites, water was collected on a single day due to the logistics of sampling and to keep samples concurrent with WATAR program samples. 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). In-field measurements were made for specific conductivity, salinity, water temperature, dissolved oxygen and pH (Table 1). 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 °C ± 2 °C. Temperature inside the cooler was tracked during transport with a temperature logger.

### 3.3 Hydrology and Tides

Insufficient data are available to accurately quantitate flows at sampling times in Slaughter Creek and Prime Hook Creek. The recorded discharge at the nearest USGS gauge 01484100 Beaverdam Branch at Houston was 5 cfs on October 17, 2015 compared to a Q-7-10 of 0.1 cfs for the period of record 1959 to 2016. Figure 2 shows the sampling location, dates, and times
aligned with NOAA predicted tides and currents for 2016 samples (http://tidesandcurrents.noaa.gov/ofsdbofs/dbofs.html).

3.4 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) 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 *P. subcapitata* (Denton *et al.*, 2011; Shukla *et al.*, 2000; USEPA, 2010).
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 all the species and endpoints tested based on methods used to analyze the data at tributary sites DE19, DE20 and DE21 sampled in 2016 (Table 2). The TST for site DE18 with one species (P. promelas) indicated a statistically significant difference from the control with a mean dry weight of 0.2370 mg in 100% ambient water that was 28.6% less than the mean dry weight of the control at 0.3320 mg, a result considered biologically significant. However, site DE18 tests with *C. daphnia* and *P. subcapitata* did not detect chronic lethal or sublethal effects (Table 2).

5.0 CONCLUSIONS

The objective of the 2016 survey 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. Three freshwater species were used in the survey including *Pimephales promelas*, *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. Endpoints measured included survival, growth, and reproduction. The Slaughter Creek surface water sample indicated a slight potential for sublethal toxic effects to fish but, no toxic effects were observed in the invertebrate or algal species tested. Surface water from three sites in Prime Hook Creek did not indicate chronic toxicity to aquatic life. Overall, the water samples did not indicate chronic toxicity to aquatic life at a biologically significant level.
6.0 ACKNOWLEDGEMENTS

This report was reviewed by John Yagecic, P.E. (DRBC) and Dr. Richard Greene (DNREC). 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 Department of Natural Resources and Environmental Control. 2016. Sampling and Analysis Plan 2016 WATAR Program.


8.0 FIGURES AND TABLES

Figure 1. Sample sites in 2016
Figure 2. Tidal conditions during 2016 sampling
<table>
<thead>
<tr>
<th>Site Time</th>
<th>Time</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Temp °C</th>
<th>HDO mg/L</th>
<th>Specific Conductivity uS/cm</th>
<th>pH</th>
<th>Turbidity NTU</th>
<th>Salinity ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE-18</td>
<td>15:00</td>
<td>38.860739</td>
<td>-75.291829</td>
<td>21.44</td>
<td>8.80</td>
<td>452.2</td>
<td>6.57</td>
<td>7.67</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Slaughter Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE-19</td>
<td>14:25</td>
<td>38.823535</td>
<td>-75.311203</td>
<td>18.52</td>
<td>9.19</td>
<td>153.3</td>
<td>6.87</td>
<td>1.14</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Prime Hook Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waples Pond</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE-20</td>
<td>09:35</td>
<td>38.835680</td>
<td>-75.257551</td>
<td>16.11</td>
<td>4.47</td>
<td>514.0</td>
<td>6.47</td>
<td>7.13</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Prime Hook Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Shop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE-21</td>
<td>12:21</td>
<td>38.827272</td>
<td>-75.280314</td>
<td>17.54</td>
<td>8.29</td>
<td>152.0</td>
<td>6.58</td>
<td>1.90</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Prime Hook Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Shooting Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Toxicity test results for October 17, 2016 samples

<table>
<thead>
<tr>
<th>Site</th>
<th><em>P. promelas</em> fish Survival and growth</th>
<th><em>C. dubia</em> invertebrate Survival and reproduction</th>
<th><em>P. subcapitata</em> algae growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC25/TST</td>
<td>IC25/TST</td>
<td>IC25/TST</td>
</tr>
<tr>
<td>DE18</td>
<td>94.6%/FAIL</td>
<td>100%/PASS</td>
<td>100%/Pass</td>
</tr>
<tr>
<td>DE19</td>
<td>100%/PASS</td>
<td>100%/PASS</td>
<td>100%/Pass</td>
</tr>
<tr>
<td>DE20</td>
<td>100%/PASS</td>
<td>100%/PASS</td>
<td>100%/Pass</td>
</tr>
<tr>
<td>DE21</td>
<td>100%/PASS</td>
<td>100%/PASS</td>
<td>100%/Pass</td>
</tr>
</tbody>
</table>

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 with PMSD < 12% are rejected.