# Monitoring the Tidal Delaware River for Ambient Toxicity 2013 and 2014 Narrative Report

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

**Delaware River Basin Commission** 

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## 1.0 SUMMARY

This report is an update on studies to assess ambient waters in urbanized areas of the tidal Delaware River and its tributaries. The objective of the 2013 and 2014 surveys were to determine whether chronic lethal or sublethal toxicity was present in surface water samples, as measured in short-term laboratory experiments with the freshwater test species *Pimephales promelas*, *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata* or the salinity tolerant test species *Americamysis bahia, Menidia beryllina* and *Hyalella Azteca*. Endpoints appropriate for each test species including survival, growth, or reproduction were measured. As a follow-up to screening level toxicity tests conducted previously in 2012, confirmatory samples were collected from DRBC Water Quality Zone 5 in the main stem Delaware River. Red Lion Creek in Delaware was sampled, in 2013, concurrent with a DNREC and USEPA study of that Delaware River tributary. In 2014, the Schuylkill River in Pennsylvania and Crosswicks Creek in New Jersey were sampled. Based on the test species tested and the measured endpoints evaluated, 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). Fish consumption advisories are in place for segments of the study area due to existing concentrations of polychlorinated biphenyl (PCB), dioxins, furans, mercury and chlorinated pesticides (DRBC, 2010). In addition, sediment toxicity, elevated contaminant levels in sediment, and degraded benthic communities have been

observed within the study area (Costa and Sauer, 1994; Hartwell and Claflin, 2005; Hall *et al.*, 2005; USACE, 2013; and USEPA, 2004). 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 is therefore an essential component of programs designed to protect this valued resource. The objective of this study was to assess the potential for chronic lethal or sublethal toxicity to aquatic life in water samples collected from sampling stations in the tidal Delaware River and its tributaries.

A number of programs monitor chemical contaminants and toxicity in permitted wastewater discharges, water, sediment and benthic organisms in the Delaware Estuary (PDE, 2012; USEPA, 2004). 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. Sixteen main stem sites and thirty-seven sites in tributaries have been included in DRBC ambient

toxicity surveys from 2005 to 2014 (Figure 1). Results from surveys prior to 2013 and 2014 have been previously reported (DRBC, 2013; MacGillivray *et al.*, 2011).

#### 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  $\leq$  1750  $\mu$ mhos/cm or  $\leq$  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*).

Some of the sampling sites selected experience changes in salinity due to river flow and tidal conditions. The selection of test species and appropriate controls was complicated by this changing salinity gradient. For water samples with salinity >1 ppt, 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 three salinity tolerant species selected were a mysid, *Americamysis bahia* (formerly *Mysidopsis bahia*); a fish, *Menidia beryllina* (inland silverside); and an amphipod, *Hyalella azteca*. Acclimation of *A. bahia* to lower salinities during culturing prior to testing was needed to obviate or limit the need for major salinity adjustment of river water

samples from as low as 1 ppt to the standard test conditions at 20 ppt. *A. bahia* have been reported to meet test acceptance criteria in 7- and 28-d toxicity tests when tests were conducted with salinity as low as 10 ppt (Ward et al., 2006). MacGillivray *et al.* (2011) provide additional information on species selection and acclimation of mysids to lower salinity.

## 3.2 Study Design

Evaluations of all sampling sites from tributaries in 2013 and 2014 were made with tests using 100% ambient water. Since Zone 5 samples were a follow-up confirmation of screening level toxicity tests conducted in 2012, dilution series at 100%, 50%, 25%, 12.5% and 6.25% ambient water were run. Results from these tests were compared to controls of reconstituted laboratory water formulated to mimic freshwater (salinity < 1 ppt) and brackish water (salinities of 5, 10, 15 or 25 ppt). In 2013, water samples were collected from five sites in Zone 5 of the main stem in the tidal Delaware River (T2, T3, T4, T5 and T6) and four sites in Red Lion Creek. In 20014, water samples were collected in six sites in both the Schuylkill River and Crosswicks Creek (Figure 1). The sampling was not designed to characterize any potential near-field toxicity issues immediately surrounding point source discharges or other contaminant sources. USEPA shortterm chronic toxicity methods were used to evaluate toxicity and sublethal effects in ambient samples with Pimephales promelas, Americamysis bahia, 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 an artificial substrate (Nylon coiled-web material) 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 each main stem sampling site, a single grab sample was collected in the navigation channel for each location. All samples were collected at a depth of 0.6 of the water column using a 10 liter Niskin sampling bottle (Model 1010-1.2, General Oceanics, Miami, FL) configured to collect a vertical sample. Water was collected on two sampling days. Due to a forecast of inclement weather a third sampling day was not possible so sufficient water was collected on the second sampling day to complete the tests. 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, III). 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 4). 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 Salinity Adjustments and Controls

In toxicity tests with salinity tolerant species *A. bahia*, *M. beryllina*, and *H. azteca*, the test salinity adjustment was based on the ambient salinity of the first sample collected at each site. If the

ambient water salinity was lower on subsequent sampling days by greater than 2 ppt from the initial sample, the salinity was adjusted to the initial sample day conditions. No salinity adjustment was performed if salinity increased between sampling days. *A. bahia* was tested at ambient salinities when salinity was  $\geq 10$  ppt. If <10 ppt, the sample was adjusted to 10 ppt. The *A. bahia* tests included controls at salinities of 10 ppt and 25 ppt. *Menidia beryllina* were tested at ambient salinities if the salinity was  $\geq 5$  ppt. If the ambient salinity was <5 ppt, the sample was adjusted to 5 ppt. The *M. beryllina* tests included controls at salinities of 5 ppt and 10 ppt. *Hyalella azteca* was tested at the ambient salinity up to 10 ppt. Ambient water for the *H. azteca* tests did not need salinity adjustment. *H. azteca* tests were conducted with three controls at salinities of 1 ppt, 5 ppt, 10 ppt and 15 ppt.

## 3.4 Hydrology and Tides

Low flow conditions were targeted for sampling to assess the effects of wastewater effluents on receiving waters and to be within the range of flows used to regulate contaminants in surface waters coinciding with critical exposure conditions for aquatic life. Slack tide was targeted to facilitate sampling while tidal velocities are smaller. The mean daily average flows for the Delaware River at Trenton, NJ were 3,547 and 5,229 cfs on sampling days October 7 and 9, 2013 with the highest flow on the second day of sampling. Flows in the Delaware River have been lower than 5,229 cfs less than 25% of the time (http://waterdata.usgs.gov). Figures 2 and 3 show sampling locations, dates, and times aligned with NOAA predicted tides and currents for the Delaware River at Delaware DE and Marcus Hook. PA City, (http://tidesandcurrents.noaa.gov/ofs/dbofs/dbofs.html). In Red Lion Creek, low flow conditions and low slack tide were targeted for the sampling to maximize collection of tributary water and minimize the influence of Delaware River water. Insufficient data are available to accurately quantitate flows at sampling times in this tributary. Figures 4 shows sampling locations, dates, and times aligned with NOAA predicted tides and currents for the Delaware River at Delaware City, DE. The recorded discharge at USGS gage 01474500 on the Schuylkill River at Philadelphia, PA was 675 cfs on August 8, 2014; 1650 cfs on August 13, 2014 (rising hydrograph) and 1530 cfs on August 15, 2014. At that site on the Schuylkill River, the 25th percentile for flow is 460 cfs, and 75th percentile for flow is 1470 cfs. Figure 5 shows sampling locations, dates, and times aligned with NOAA predicted tides and currents for the Schuylkill River. (http://tidesandcurrents.noaa.gov/ofs/dbofs/dbofs.html). The mean daily average flows recorded at USGS gage 01464500 on Crosswicks Creek at Extonville, NJ were 66 cfs on October 6, 2014; 52 cfs on October 8, 2014 and 42 cfs on October 10, 2014. At that site on the Crosswicks Creek, the 25th percentile for flow is 74 cfs, and 75th percentile for flow is 178 cfs. Figure 6 shows sampling locations, dates, and times aligned with NOAA predicted tides and currents for the Delaware River at Trenton, NJ.

#### 3.5 Statistical Analysis

Statistical comparisons were made between the salinity control closest to the ambient sample salinity at 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<sub>25</sub>). To assure that differences between

controls and treatment were biologically significant as well as a statistically significant difference, 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 with 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  $\alpha = 0.20$  for *C. dubia* and *P. promelas*,  $\alpha = 0.25$  for *M. beryllina* and *P. subcapitata*, and at  $\alpha = 0.15$  for *A. bahia* (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 the No Observed Effect Concentration (NOEC) for survival, growth and reproduction and an additional tests for significant toxicity confirmed the lack of significant chronic lethal or sublethal effects at five sites sampled on October 7 and 9, 2013 between River Mile 55 and 75 in Zone 5 of the main stem Delaware River (T2, T3, T4, T5 and T6) (Table 5); six sites sampled on August 11, 13 and 15, 2014 in the Schuylkill River (Table 7) or six sites sampled on October 6, 8 and 10, 2014 in Crosswicks Creek (Table 8). In Red Lion Creek, water sampled on September 23, 24 and 25, 2013 from two sites (tide gate (TG) and Route 9) indicated no chronic toxicity for any of the three test species based on all methods used to analyze the data. Although the Route 7

site indicated a statistically significant difference from the control for one test (acclimated A. bahia), but did not indicate biologically significant toxicity based on the test for significant toxicity (TST) using the Welch's t test at the recommended b value for chronic tests of 0.75 and alpha level for A. bahia at  $\alpha = 0.15$ . Finally, the Route 1 site indicated a statistically significant difference from the control for all three species. However, the differences between ambient water and control for the Route 1 site were less < 20%, the percent minimum statistical difference (PMSD) were low for two species (C. dubia at 11.7% and P. subcapitata at 8.9%), and the TST indicated that the sample was not toxic for any of the test species at the recommended significance levels. Therefore, an evaluation of the data indicates the tests measured a statistical significant difference but not a biologically significant difference (Table 6).

## **5.0 CONCLUSIONS**

The objective of the 2013 and 2014 surveys were to determine the potential for chronic lethal or sublethal toxicity to aquatic life in ambient water samples collected from sampling stations in Zone 5 of the tidal Delaware River and other triburaries to the river. These surveys consisted of water column toxicity tests on samples collected during periods of low flow and low slack tide. Six species were used in the surveys including *Pimephales promelas, Americamysis bahia, 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 samples from all sites in the main stem of the Delaware River and three tributaries (Schuylkill River, Red

Lion Creek, and Crosswicks Creek) did not indicate chronic toxicity to aquatic life at a biologically significant level in any the water samples evaluated.

#### **6.0 ACKNOWLEGEMENTS**

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## **8.0 FIGURES AND TABLES**

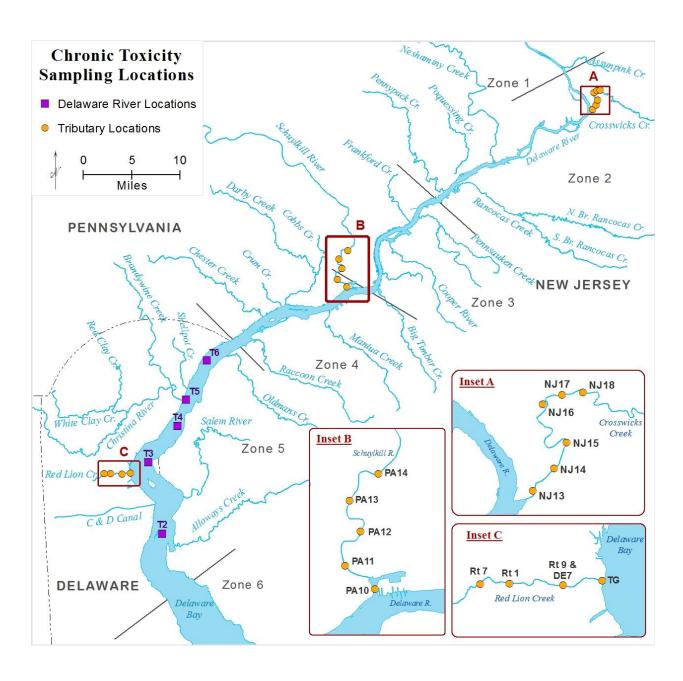


Figure 1. Sample sites in 2013 and 2014

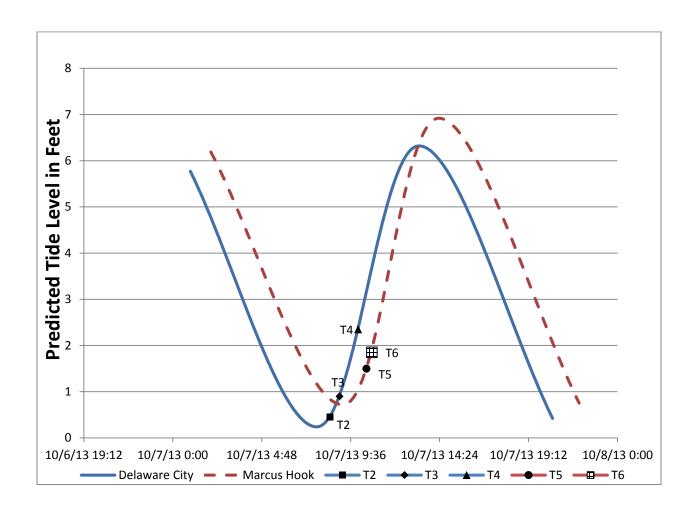


Figure 2. Tidal conditions at Delaware River sampling sites on October 7, 2013

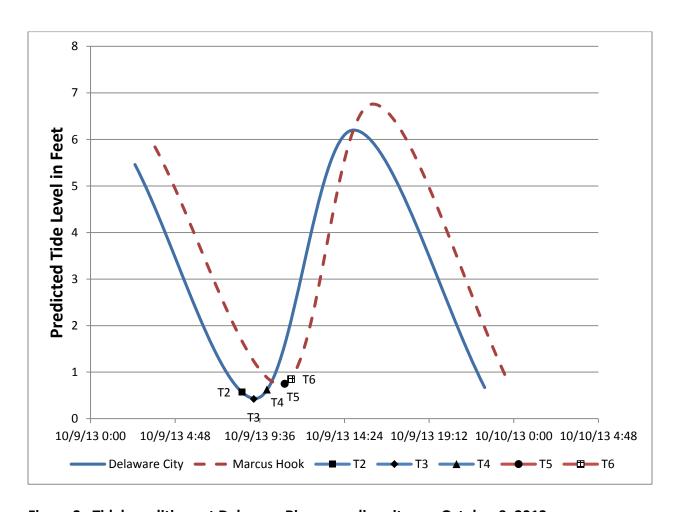


Figure 3. Tidal conditions at Delaware River sampling sites on October 9, 2013

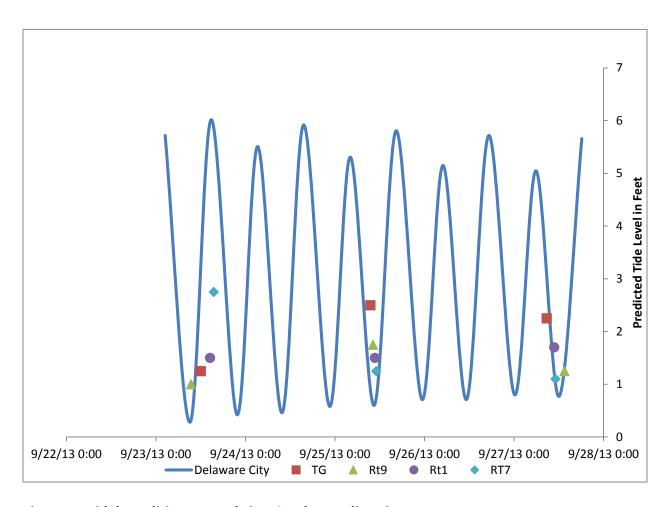


Figure 4. Tidal conditions at Red Lion Creek sampling sites.

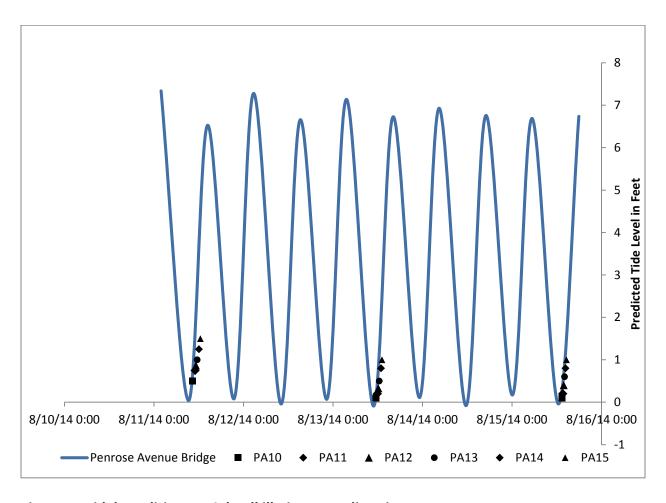


Figure 5. Tidal conditions at Schuylkill River sampling sites.

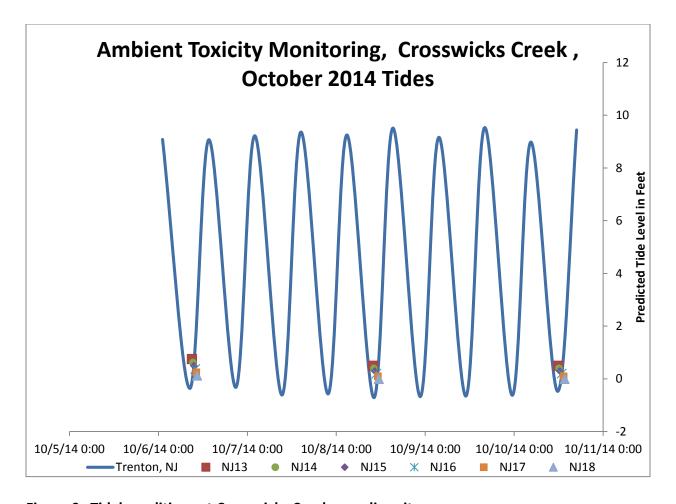


Figure 6. Tidal conditions at Crosswicks Creek sampling sites.

 Table 1. Physical-chemical data Mains Stem Sites 2013

| Sample                             | Time | Temp  | Н    | DO    | Specific              | pН   | Turbidity | Salinity |
|------------------------------------|------|-------|------|-------|-----------------------|------|-----------|----------|
|                                    |      | °C    | mg/l | % sat | Conductivity<br>uS/ml |      | NTU       | ppt      |
| EST-T2-100713<br>Reedy Island      | 0829 | 22.30 | 7.19 | 86.0  | 12,250                | 7.58 | 78.3      | 8.3      |
| EST-T3-100713<br>N of Pea Patch Is | 0900 | 22.08 | 7.02 | 82.1  | 7,757                 | 7.53 | 35.6      | 5.07     |
| EST-T4-100713<br>S of De Mem Br    | 1000 | 22.05 | 6.82 | 79.1  | 4,439                 | 7.48 | 39.8      | 2.8      |
| EST-T5-100713<br>N of De Mem Br    | 1027 | 22.10 | 6.79 | 78.1  | 2,463                 | 7.46 | 47.9      | 1.5      |
| EST-T6-100713<br>Oldmans Pt        | 1045 | 22.11 | 6.59 | 75.6  | 1,007                 | 7.4  | 47.8      | 0.6      |
| EST-T2-100913<br>Reedy Island      | 0835 | 21.38 | 7.19 | 85.0  | 13,380                | 7.64 | 67.9      | 8.99     |
| EST-T3-100913<br>N of Pea Patch Is | 0915 | 21.10 | 7.51 | 85.9  | 7,905                 | 7.64 | 46.6      | 5.06     |
| EST-T4-100913<br>S of De Mem Br    | 1000 | 21.09 | 7.13 | 81.2  | 4,509                 | 7.54 | 60.2      | 2.8      |
| EST-T5-100913<br>N of De Mem Br    | 1100 | 21.14 | 7.02 | 79.6  | 2,470                 | 7.52 | 46.5      | 1.5      |
| EST-T6-100913<br>Oldmans Pt        | 1122 | 21.28 | 6.65 | 75.0  | 1,052                 | 7.46 | 66.3      | 0.62     |

 Table 2. Physical-chemical data Red Lion Creek sites 2013

| Site                          | Time | Temp  | Н     | DO    | Specific              | pН   | Turbidity | Salinity |
|-------------------------------|------|-------|-------|-------|-----------------------|------|-----------|----------|
|                               |      | °C    | mg/l  | % sat | Conductivity<br>uS/ml |      | NTU       | ppt      |
| RL-TG-<br>092313<br>Tide Gate | 1200 | 18.47 | 9.35  | 100   | 2,967                 | 7.1  | 33.4      | 1.5      |
| RL-9-092313<br>Route 9        | 0923 | 16.82 | 5.01  | 49.5  | 929                   | 6.99 | 27.8      | 0.48     |
| RL-1-092313<br>Route 1        | 1430 | 17.60 | 9.23  | 96.6  | 243                   | 7.3  | 5.3       | 0.13     |
| RL-7-092313<br>Route 7        | 1530 | 16.21 | 9.34  | 95.1  | 170                   | 7.25 | 10.7      | 0.08     |
| RL-TG-<br>092513<br>Tide Gate | 0930 | 20.28 | 7.90  | 89.1  | 5,769                 | 6.98 | NA        | 3.14     |
| RL-9-092513<br>Route 9        | 1010 | 16.44 | 6.4   | 67.6  | 1,451                 | 7.38 | NA        | 0.73     |
| RL-1-092513<br>Route 1        | 1040 | 14.14 | 11.43 | 110.6 | 269                   | 7.41 | NA        | 0.13     |
| RL-7-092513<br>Route 7        | 1105 | 13.88 | 11.75 | 113.5 | 189                   | 7.26 | NA        | 0.09     |
| RL-TG-<br>092713<br>Tide Gate | 0845 | 22.3  | 9.00  | 104.9 | 6,031                 | 7.84 | NA        | 3.28     |
| RL-9-092713<br>Route 9        | 0130 | 20.85 | 6.21  | 71.0  | 5,390                 | 7.85 | NA        | 2.93     |
| RL-1-092713<br>Route 1        | 1046 | 15.56 | 10.04 | 100   | 275                   | 7.87 | NA        | 0.13     |
| RL-7-092713<br>Route 7        | 1106 | 15.49 | 11.25 | 112   | 203                   | 7.71 | NA        | 0.1      |

Table 3. Physical-chemical data Schuylkill River sites 2014

| Site        | Time | Temp  | Н    | DO    | Specific              | pН   | Turbidity |
|-------------|------|-------|------|-------|-----------------------|------|-----------|
|             |      | °C    | mg/l | % sat | Conductivity<br>uS/ml |      | NTU       |
| PA10-081114 | 1021 | 25.50 | 4.72 | 58    | 302.4                 | 7.10 | 13.7      |
| PA11-081114 | 1100 | NA    | 7.0  | NA    | NA                    | NA   | 14.8      |
| PA12-081114 | 1115 | NA    | 7.0  | NA    | NA                    | NA   | 15.3      |
| PA13-081114 | 1134 | NA    | 7.0  | NA    | NA                    | NA   | 9.36      |
| PA14-081114 | 1202 | NA    | 7.2  | NA    | NA                    | NA   | 6.32      |
| PA15-081114 | 1225 | NA    | 7.0  | NA    | NA                    | NA   | 3.3       |
| PA10-081314 | 1135 | NA    | 3.88 | 66.2  | 318.1                 | 7.22 | 12.3      |
| PA11-081314 | 1152 | 26.0  | 4.63 | 79.0  | 357.6                 | 7.32 | 10.8      |
| PA12-081314 | 1208 | 24.5  | 4.49 | 76.5  | 351.8                 | 7.29 | 7.29      |
| PA13-081314 | 1223 | 25.0  | 4.64 | 79.0  | 358.9                 | 7.32 | 21.7      |
| PA14-081314 | 1253 | 24.0  | 5.10 | 86.2  | 372.2                 | 7.43 | 6.0       |
| PA15-081314 | 1307 | 24.0  | 5.49 | 92.5  | 378.1                 | 7.52 | 3.1       |
| PA10-081514 | 1320 | 25.09 | 5.32 | 64.5  | 448.2                 | 7.24 | 7.92      |
| PA11-081514 | 1337 | 24.91 | 6.26 | 76    | 528.5                 | 7.42 | 8.81      |
| PA12-081514 | 1353 | 24.5  | 4.68 | 78.9  | 376.6                 | 7.45 | 9.22      |
| PA13-081514 | 1405 | 24.33 | 6.89 | 82.6  | 525.4                 | 7.55 | 6.09      |
| PA14-081514 | 1419 | 24.54 | 7.61 | 91.4  | 513.6                 | 7.70 | 3.41      |
| PA15-081514 | 1435 | 23.95 | 8.11 | 96.6  | 495.2                 | 7.78 | 4.22      |

Table 4. Physical-chemical data Crosswicks Creek sites 2014

| Site        | Time     | Temp  | Н    | DO    | Specific              | pН   | Turbidity |
|-------------|----------|-------|------|-------|-----------------------|------|-----------|
| Time        |          | °C    | mg/l | % sat | Conductivity<br>uS/ml |      | NTU       |
| NJ13-100614 | 09:02:32 | 15.48 | 8.9  | 89.4  | 264.6                 | 7.59 | 7.90      |
| NJ14-100614 | 09:16:32 | 15.03 | 7.02 | 69.8  | 263.4                 | 7.3  | 7.74      |
| NJ15-100614 | 09:33:49 | 14.73 | 7.02 | 69.3  | 260.9                 | 7.24 | 7.69      |
| NJ16-100614 | 09:56:00 | 14.78 | 8.09 | 80.0  | 265.6                 | 7.27 | 9.91      |
| NJ17-100614 | 10:11:39 | 14.32 | 8.44 | 82.6  | 232.7                 | 7.28 | 8.21      |
| NJ18-100614 | 10:30:33 | 14.57 | 8.67 | 85.4  | 232.3                 | 7.23 | 9.21      |
| NJ13-100814 | 10:01:57 | 17.07 | 6.59 | 68.5  | 267                   | 7.3  | 8.69      |
| NJ14-100814 | 10:11:31 | 17    | 6.45 | 66.9  | 267.4                 | 7.27 | 9.32      |
| NJ15-100814 | 10:23:32 | 16.92 | 6.32 | 65.5  | 267.7                 | 7.22 | 8.78      |
| NJ16-100814 | 10:50:01 | 16.53 | 7.33 | 75.3  | 257.7                 | 7.18 | 9.66      |
| NJ17-100814 | 11:13:21 | 16.51 | 7.83 | 80.3  | 232.9                 | 7.21 | 8.61      |
| NJ18-100814 | 11:27:43 | 16.82 | 8.07 | 83.4  | 237.3                 | 7.21 | 9.41      |
| NJ13-101014 | 11:52:46 | 16.01 | 6.59 | 67    | 272.1                 | 7.24 | 6.55      |
| NJ14-101014 | 12:06:07 | 15.75 | 6.5  | 65.7  | 272.8                 | 7.2  | 6.31      |
| NJ15-101014 | 12:18:21 | 15.54 | 6.71 | 67.4  | 268.6                 | 7.16 | 7.79      |
| NJ16-101014 | 12:50:26 | 15.52 | 8.04 | 80.8  | 245.1                 | 7.22 | 6.81      |
| NJ17-101014 | 13:20:57 | 15.33 | 8.27 | 82.7  | 226.1                 | 7.18 | 9.50      |
| NJ18-101014 | 13:36:39 | 15.38 | 8.46 | 84.8  | 222.5                 | 7.21 | 6.87      |

Table 5. Main Stem Toxicity Tests October 7 and 9, 2013

| Zone | Site                                  | River Mile Latitude Longitude  | A. bahia shrimp Survival and growth IC25/TST | M. beryllina fish Survival and growth  IC25/TST | H. azteca amphipod Survival and growth IC25 |
|------|---------------------------------------|--------------------------------|--|---|---|
| 5    | T2<br>Reedy<br>Island                 | 55<br>39.51250<br>-75.55111    | 100%/Pass                                    | 100%/Pass                                       | 100%  |
|      | T3<br>North of<br>Pea Patch<br>Island | 63<br>39.61310<br>-75.57722    | 100%/ Pass                                   | 100%/ Pass                                      | 100%  |
|      | T4 South of Del Mem. Brige            | 68<br>39.67556<br>-75.53028    | 100%/ Pass                                   | 100%/ Pass                                      | 100%  |
|      | T5 North of Del. Mem. Bridge          | 70.8<br>39.71908<br>-75.50425  | 100% /Pass                                   | 100%/ Pass                                      | 100%  |
|      |                                       |                                | P. promelas                                  | C. dubia  | Р.  |
|      |                                       |                                | <b>fish</b> Survival and growth              | invertebrate Survival and reproduction          | subcapitata algae growth                    |
|      |                                       |                                | IC25/TST                                     | IC25/TST  | IC25/TST                                    |
|      | T6<br>Oldman's<br>Point               | <b>75.1</b> 39.76750 -75.47389 | 100%/PASS                                    | 100%/PASS                                       | 100%/Pass                                   |

 $<sup>^1</sup>$ A. bahia were acclimated from salinity at 25 ppt to 10 ppt. Salinity of ambient water was adjusted upward to 10 ppt. All controls met acceptable test criteria of 80% survival and  $\geq$  0.20 mg mean dry weight.

Dilution series short-term chronic tests with ambient water.

NOEC = No Observed Effect Concentration; TST = Tests of Significant Toxicity

 $<sup>^{\</sup>rm 2}$  TST was not calculated with data from  $\it H.~azteca.$ 

Table 6. Red Lion Creek Toxicity Tests Sept 23, 24 and 25, 2013

| Site               | Latitude<br>Longitude | A. bahia <sup>1</sup> shrimp Survival and growth | M. beryllina fish Survival and growth        | H. azteca <sup>2</sup> amphipod Survival and growth |
|--------------------|-----------------------|--|--|---|
|                    |                       | IC25/TST   | IC25/TST                                     | IC25  |
| TG<br>tide<br>gate | 39.60687<br>-75.61367 | 100%/Pass  | 100%/Pass                                    | 100%  |
|                    |                       | P. promelas fish Survival and                    | C. dubia invertebrate Survival and           | P. subcapitata algae growth                         |
|                    |                       | growth IC25/TST                                  | reproduction                                 | IC25/TST  |
| Rt 9               | 39.60524<br>-75.63007 | 100%/Pass  | 100%/Pass                                    | 100%/Pass   |
|                    |                       | NOEC/TST   | NOEC/TST                                     | NOEC/TST  |
| Rt 1               | 39.60571<br>-75.65261 | <100%/Pass<br>16% <sup>4</sup>                   | <100% <sup>3</sup> /Pass<br>16% <sup>4</sup> | <100% <sup>3</sup> /Pass<br>11% <sup>4</sup>        |
| Rt 7               | 39.60551<br>-75.66479 | <100%/Pass<br>18% <sup>4</sup>                   | 100%/Pass                                    | 100%/Pass   |

<sup>&</sup>lt;sup>1</sup>A. bahia were acclimated from salinity at 25 ppt to 10 ppt. Salinity of ambient water was adjusted upward to 10 ppt. All controls met acceptable test criteria.

- Sites TG and Rt 9 are dilution series short-term chronic tests with ambient water reporting as Inhibitory Concentration of 25% reduction in the most sensitive endpoint of test organism (IC25)
- Sites Rt 1 and Rt 7 are single concentration short-term chronic tests of 100% ambient water reporting as No Observed Effect Concentration (NOEC). Rt 1 and Rt 7 samples exceed minimal quality control requirements, differences between treatment and control are < 20%.

<sup>&</sup>lt;sup>2</sup> Test of Significant Toxicity (TST) is not available for data from tests with H. azteca. 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.

<sup>&</sup>lt;sup>3</sup>Low PMSD in *C. dubia* at 11.7% *and P. subcapitatia* at 8.9% indicate that tests results may not be biologically significant.

<sup>&</sup>lt;sup>4</sup> Percent difference in sub-lethal response from site water versus control.

Table 7. Schuylkill River Toxicity Tests August 11, 13 and 15, 2014

| Site | Latitude<br>Longitude | P. promelas fish Survival and growth | C. dubia invertebrate Survival and reproduction | P. subcapitata algae growth |
|------|-----------------------|--------------------------------------|---|-----------------------------|
|      |                       | IC25/TST                             | IC25/TST  | IC25/TST                    |
| PA10 | 39.88790<br>-75.19478 | 100%/PASS                            | 100%/PASS                                       | 100%/Pass                   |
| PA11 | 39.89909<br>-75.21300 | 100%/PASS                            | 100%/PASS                                       | 100%/Pass                   |
| PA12 | 39.91527<br>-75.20376 | 100%/PASS                            | 100%/PASS                                       | 100%/Pass                   |
| PA13 | 39.92970<br>-75.21038 | 100%/PASS                            | 100%/PASS                                       | 100%/Pass                   |
| PA14 | 39.94254<br>-75.19293 | 100%/PASS                            | 100%/PASS                                       | 100%/Pass                   |
| PA15 | 39.95328<br>-75.18089 | 100%/PASS                            | 100%/PASS                                       | 100%/Pass                   |

Screening level tests with a single test concentration at 100% ambient water

Table 8. Crosswicks Creek Toxicity Tests October 6, 8 and 10, 2014

| Site | T 4'4 1                 | P. promelas         | C. dubia                  | <b>P</b> .      |
|------|-------------------------|---------------------|---------------------------|-----------------|
|      | Latitude<br>Longitude   | fish                | invertebrate              | subcapitata     |
|      |                         | Survival and growth | Survival and reproduction | algae<br>growth |
|      |                         | IC25/TST            | IC25/TST                  | IC25/TST        |
| NJ13 | 40.15395<br>-74.71398   | 100%/PASS           | 100%/PASS                 | 100%/Pass       |
| NJ14 | 40.160531<br>-74.707706 | 100%/PASS           | 100%/PASS                 | 100%/Pass       |
| NJ15 | 40.16807<br>-74.70411   | 100%/PASS           | 100%/PASS                 | 100%/Pass       |
| NJ16 | 40.17921<br>-74.71106   | 100%/PASS           | 100%/PASS                 | 100%/Pass       |
| NJ17 | 40.18198<br>-74.70538   | 100%/PASS           | 100%/PASS                 | 100%/Pass       |
| NJ18 | 40.18262<br>-74.69934   | 100%/PASS           | 100%/PASS                 | 100%/Pass       |

Screening level tests with a single test concentration at 100% ambient water