Toxicity Tests
For
Baseline Monitoring of Water Quality
In
Upper Delaware River Basin

Summary Report

DELAWARE RIVER BASIN COMMISSION

WEST TRENTON, NEW JERSEY

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INTRODUCTION

To protect the water resources of the Delaware River Basin during the construction and operation of natural gas (NG) development projects, DRBC monitoring initiatives in anticipation of natural gas development include biological monitoring, continuous conductivity monitoring, reanalysis of archived surface water samples for chemical parameters associated with NG flowback and produced waters, and ambient toxicity testing. Biological monitoring, continuous conductivity monitoring and reanalysis of archived surface water samples for chemical parameters are not covered in this report. Assessing the potential impact during natural gas development includes the knowledge of: 1) pre-alteration water quality including baseline information on water chemistry and ambient toxicity; 2) appropriate monitoring parameters for both additives used in the hydraulic fracturing process and contaminants released from the geologic formation in produced water; as well as 3) measures of long-term trends in ambient water conditions. Mixtures of numerous chemical additives are used in the hydraulic fracturing process (PADEP, 2010). Flowback and produced water from the fractured wells will contain additives from the fracturing process as well as salts, metals, and radioisotopes from the geological formation (GTI, 2009). Characterization of flowback and produced water includes measurements of physical-chemical composition of the water and assessment of hazard to the environment from this complex mixture. Toxicity tests are an effective way to measure and control toxic effects from mixtures in receiving streams (Pellston Workshop, 1995). Based on federal and state water quality regulations, no adverse effects should be observed in toxicity tests with undiluted surface water. Monitoring toxicity using standardized test methods is an essential component of programs designed to assess compliance with regulatory standards (MacGillivray *et al*., 2011). In addition to standard USEPA species of fish, daphnid and algae, benthic macroinvertebrates were chosen for this toxicity study involving ambient water and water produced from natural gas drilling because benthic macrionvertebrates are an ecologically important group of aquatic organisms that is commonly included in water quality assessment programs (Hellawell 1986, Resh 2008), and because they have provided water quality assessment programs with valuable insight for more than 100 years (Cairns and Pratt 1993). The presence or conspicuous absence of certain macroinvertebrate species at a site is a meaningful record of environmental conditions during the recent past (Weber 1973, Barbour et al. 1999). Among the aquatic macroinvertebrates, mayflies were chosen for this study because they are known to be relatively sensitive to changes in water quality and play an important role in the commonly used EPT Index (Lenat and Penrose 1996). Although mayflies represent an important and vulnerable group of organisms inhabiting streams and rivers they are not generally included in standard toxicity tests of effluents and receiving waters (Sweeney et al., 1993).

This study used standard toxicity test organisms of fish, daphnid, and algal, as well as non-standard mayfly species that are indigenous to the Marcellus shale region as test species. A compounding factor to be considered in this study is the low hardness and alkalinity of surface waters in the Delaware River Basin that will be potentially exposed to releases of fracking fluids and produced water. These soft waters are not typically used in standard wastewater and receiving water toxicity tests. Therefore, the test design controlled for these different water types.

This study evaluated whether or not selected toxicity test methods and species are appropriate to measure baseline ambient water conditions, to monitor cumulative effects of natural gas development in surface water including accidental releases, and to monitor wastewater from treatment plants receiving flowback/production waters. The objective of this project was to develop methodologies and procedures to demonstrate compliance with the applicable basin-wide effluent limitations and basin-wide stream quality objectives in current and proposed DRBC water quality regulations (DRBC, 2010a and 2010b). These regulations include: 1) the toxicity in Basin waters must not exceed 0.3 Toxic Units (acute), except in small mixing areas; 2) the toxicity in Basin waters must not exceed 1.0 Toxic Units (chronic); 3) TDS concentration, except intermittent streams, shall not exceed 133% of background; and 4) no measurable change to Existing Water Quality (EWQ) parameters at control points identified in Special Protection Water (SPW) regulations.

The project included the following tasks:

- Testing of ambient stream water directly using standard toxicity methods and test species *Pimephales promelas*, *Ceriodaphnia dubia*, and *Pseudokirchneriella subcapitata* (USEPA 2002a, and 2002b) as well as three non-standard mayfly species *Centroptilum triangulifer, Procloeon rivulare and Pseudocloeon frondale*. The test design included controls for soft and moderately hard water, in-field measurement of surface water parameters, and chemical analysis of ambient water.

- Testing of a standard reference toxicant (sodium chloride) in toxicity tests using standard and non-standard species.

- Testing of a sample of flowback/produced water from natural gas drilling using standard USEPA acute and short-term chronic methods with fish, daphnid, and algae as well as non-standard species of mayfly in acute and whole-life cycle methods with controls for water hardness. Flowback/produced water was also analyzed for physical chemical parameters.

The ambient water sample sites in Wayne County, PA, an area likely to first experience NG development in the Delaware River Basin, were chosen based on land use (forested, agricultural, urban/suburban) and water quality characterization. Sampling sites included locations on Dyberry Creek and the Lackawaxen River. Dyberry Creek (a high water quality site in this study) has a history of both PADEP and DRBC bioassessment sampling sites. The watershed has little land use alteration and the site has little influence by roads. Dyberry Creek water is classified as Exceptional Value (EV). The West Branch of the Lackawaxen River has a history of both PADEP and DRBC bioassessment sampling sites and was considered to be a medium water quality site for this study. The West Branch of the Lackawaxen River has a USGS gage and was monitored in 2011 as part of the DRBC pre-drilling conductivity monitoring. The study area does not contain a stream on the Pennsylvania Clean Water Act Section 305(b) impaired waters list. However, the Lackawaxen River south of the Honesdale Borough Wastewater Treatment Plant was sampled as a potential low water quality site.

A flowback/produced water sample was obtained from a treatment facility located in the Susquehanna River Basin. The sample was collected from a storage tank containing a composite of wastewater collected from a natural gas production site. The duration of time after fracturing for the generation of the produced water was not known. When compared to brine from other gas wells in the Marcellus Shale, produced-water in this study had high levels of total dissolved solids (TDS), chloride, barium, sodium, and strontium (GTI, 2009 and Haluszczak et al., 2013). The chemistry of of produced water is influenced by the location of the well and time from hydraulic fracturing.

Mortality was measured in acute toxicity tests. Chronic endpoints measured in the toxicity tests were survival and reproduction for the *Ceriodaphnia* tests, survival and growth for the fathead minnow tests; and survival, development time, growth, instantaneous growth rate, and population growth rate for the three mayfly species.

Both surface water samples and produced water were analyzed for baseline parameters selected for monitoring water quality during natural gas development (total dissolved solids, chloride, sodium, calcium, sulfate, barium and strontium, Na, Mg, Ca, K, bromide, sulfate, total alkalinity, total hardness, gross alpha and beta, volatile organic compounds and ethylene glycol). These parameters are indicators of contamination from natural gas drilling and can be used to predict toxicity from common inorganic ions in aquatic systems (GTI, 2009; USGS, 2009; Tietge, et al., 1994; Pillard, et al., 1999).

REPORTS

A complete description of test methods and test results for the study are provided in the following reports:

1) Ecotoxicity Study for Mayflies Exposed to Ambient Stream Water for the Upper Delaware River Basin, Reference Toxicant, and to Produced-Water From Natural Gas Drilling. Stroud Water Research Center, Avondale, PA, August, 2013.
2) Sodium Chloride Standard Reference Toxicant Tests: USEPA Methods. American Aquatic Testing, Allentown, PA, June, 2012.
3) Ambient Water Toxicity: USEPA Methods. American Aquatic Testing, Allentown, PA, March, 2012.

4) Produced Water Toxicity in White Clay Creek: USEPA Methods. American Aquatic Testing, Allentown, PA, July, 2012.
5) Produced Water Toxicity in Dyberry Creek: USEPA Methods. American Aquatic Testing, Allentown, PA, July, 2012.

SUMMARY

The standard USEPA species *P. promelas*, *C. dubia*, and *P. subcapitata* met all test acceptability criteria for acute and short-term toxicity methods in the ambient source waters tested. Although test acceptability criteria have not been established for mayfly life-cycle toxicity tests, there was no statistically significant difference among ambient source waters within species for *C. triangulifer, P. rivulare and P. frondale*. The range of hardness in the water samples used in the study (19 to 105 mg/L) did not adversely affect any of the test species for the endpoints measured in the test methods (Table 1). Ambient water collected from Dyberry Creek, Delaware River, West Branch of the Lackawaxen River, Lackawaxen River and White Clay Creek did not cause significant toxicity to any of the six species tested based on the endpoints measured (Table1). This result was expected because the study area does not contain impaired waters.

Using sodium chloride as a reference toxicant, all the test species indicated substantial sensitivity to sodium chloride (Table 2). *C. dubia* and *C. triangulifer* had similar results for acute and chronic toxicity. *P. promelas* was somewhat less sensitive to sodium chloride while *P. rivulare* and *P. frondale* were generally more sensitive. Mayfly species were more responsive to differences in the hardness of ambient water with a single toxicant (NaCl), but this sensitivity was not apparent in the toxicity of a mixture (produced water).

Not surprisingly, since the produced water had a high chloride concentration of 121,000 mg/L, all of the test species measured significant toxicity with LC_{50} endpoints $< 2\%$ and IC_{25} endpoints $\leq 1\%$ in the sample of produced water tested (Table 3). The *P. promelas* results in the produced water toxicity test were surprising in that the P. *promelas* was more sensitive to the produced water than *C. dubia* which is the inverse of predicted toxicity based on calculations in the Gas Research Institute Freshwater Salinity/ Toxicity (GRI FW STR) Model and data from tests with sodium chloride as the reference toxicant. *C. dubia* response to exposure to produced water was consistent with predicted toxicity from the freshwater salinity toxicity model. *P. promelas* response to exposure to produced water was more sensitive than expected and warrants further assessment. *C. triangulifer* was the most sensitive of the mayfly species in chronic tests measuring developmental time and growth. *P. frondale* was more sensitive than the other mayfly species in acute tests and population growth rate (PGR) although *P. frondale* survival in chronic tests was low (4 to 38%). Mayfly species, especially *C. triangulifer*, have potential for toxicity testing in ambient waters of the Delaware River Basin.

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Table 1. Chronic toxicity tests in ambient stream water

TEST SPECIES (TEST DURATION)		Dyberry Creek	Del R @Callicoon	West Branch Lackawaxen River	Lackawaxen River @ Honesdale	White Clay Creek
Hardness (mg/L)		22	19	26	28	105
Pimephales promelas (screening level) (7d)	NOEC	<100% [1]	<100% [2]	100%	100%	100%
Ceriodaphnia dubia (screening level) (7d)	NOEC	100%	100%	100%	100%	100%
Pseudokirchneriella subcapitata (screening level) (96h)	NOEC	<100% [3]	<100% [3]	100%	<100% [3]	<100% [3]
Centroptilum triangulifer (30 to 60d)	SURV	50%[4]	75%[4]	80%[4]	40%[4]	65%[4]
Procloeon rivulare (30 to 60d)	SURV	10%[4]	16%[4]	22%[4]	26%[4]	8%[4]
Pseudocloeon frondale (30 to 60d)	SURV	4%[4]	8%[4]	2%[4]	38%[4]	4%[4]

Endpoints: NOEC – No Observed Effect Concentration; SURV - survivorship

[1] - Not a biologically significant effect. Survival is 100%. Growth exceeds acceptable level at 0.25 mg.

[2] - Fungal infection observed

[3] - Not a biologically significant effect. Mean cell density exceeded acceptable level of $1x10^6$ cells/ml.

[4] - No statistically significant difference among ambient source waters within species.

Table 2. Tests with reference toxicant sodium chloride mg/L (ppm)

	acute	acute	acute	chronic	chronic	chronic
	Dyberry Creek LC_{50}	White Clay Creek LC_{50}	EPA ECOTOX mod hard water LC_{50}	Dyberry Creek	White Clay Creek	EPA ECOTOX mod hard water
Pimephales promelas 8-10d old	7787	7071	7050-8700	NA	NA	NOEC 1378 IC_{25} 1422
Ceriodaphnia dubia <24h old	2672	2736	1530	NA	NA	NOEC 644 IC_{25} 908
Centroptilum triangulifer 1st instar	2346	4054	NA	IC_{25} 959	IC_{25} 799	NA
Centroptilum triangulifer middle instar	4771	5527	NA	NA	NA	NA
Procloeon rivulare 1st instar	787	2476	NA	NA	NA	NA
Pseudocloeon frondale 1st instar	1626	3012	NA	NA	NA	NA
CV	0.71	0.70				

Table 3. Toxicity tests of a natural gas drilling produced water sample

	acute tests			chronic tests		
Test Species	Endpoint (Test Duration)	Dyberry Creek Water	White Clay Creek Water	Endpoint (Test Duration)	Dyberry Creek Water	White Clay Creek Water
Pimephales promelas	LC50 (96h)	0.63%	0.97%	IC25 (7d)	0.04% growth	0.08% growth
Ceriodaphnia dubia	LC50 (48h)	0.59%	1.0%	IC25 (7d)	0.5% reproduction	0.55% reproduction
Pseudokirchneriella subcapitata	NA	NA	NA	IC25 (96h)	0.08% growth	0.06% growth
Centroptilum triangulifer 1st instar	LC50 (48h)	1.764%	1.988%	IC25 (30 to 60d)	0.289% growth[1]	0.690% growth[1]
Centroptilum triangulifer middle instar	LC50 (48h)	1.704%	1.496%	IC25 (30 to 60d)	NA	NA
Procloeon rivulare 1st instar	LC50 (48h)	0.782%	0.735%	IC25 (30 to 60d)	0.711% F 1.020% M growth[1]	0.690% F 0.678% M growth[1]
Pseudocloeon frondale 1st instar	LC50 (48h)	0.251%	0.272%	IC25 (30 to 60d)	0.370% F 0.292% M growth[1]	0.491% F 0.428% M growth[1]
Pimephales promelas	PREDICTED LC50 (96h)	GRI FW STR V1.01[2]	4%	IC25	NA	NA
Ceriodaphnia dubia	PREDICTED LC50 (48h)	GRI FW STR V1.01[2]	2%	IC25	NA	NA
CV		0.71	0.70		0.79	0.56

[1] -Additional endpoints (mortality, development time and population growth rate) are reported for mayfly species in Stroud WRC report.
[2] - Gas Research Institute Freshwater Salinity/Toxicity Relationship (GRI FW STR) Model v1.01 predicted toxicity based on calcium, chloride, magnesium, potassium, sodium, sulfate, and total alkalinity concentrations measured in the produced water sample.