ACUTE TOXICITY TEST

Pimephales promelas

DELAWARE RIVER BASIN COMMISSION
AAT JOB # 123-01-17

DYBERRY CREEK

REPORT PREPARED BY:

AMERICAN AQUATIC TESTING, INC.
890 NORTH GRAHAM STREET
ALLENTOWN, PA 18109

BIOMONITORING REPORT FORM: ACUTE TOXICITY

NJPDES #: N/A Dyberry Creek / Flowback

FACILITY NAME: Delaware River Basin Commission

FACILITY LOCATION: 25 State Police Drive, P.O. Box 7360
West Trenton, N.J. 08628-0360

LABORATORY: American Aquatic Testing, Inc.

ACUTE TOXICITY CERTIFICATION NUMBER: PA682

TEST SPECIFICATIONS:

EFFLUENT TYPE: Flowback
TEST TYPE: Static Daily Renewal

TEST RESULTS:

Test start date: 07/17/12 Test completion date: 07/21/12

Test endpoint (check one): LC_{50} X NMAT ____ EC_{50} ____.

LC_{50}/ EC_{50}:	0.63%

Highest % mortality in any test concentration (if applicable): 100%

Test concentration: 2%

Test organism: *Pimephales promelas* (scientific name) Fathead minnow (common name)

QUALITY CONTROL SUMMARY:

Control mortality:	0%
Temperature maintained at 20 + / 2 ° C?	Yes
Dissolved 0_2 levels always > than 40 % saturation?	Yes
Two or more concentrations exhibit a trend deviation?	No
Were there any deviations from specified methodologies?	No

CERTIFICATION:

Report certified for accuracy:

Christopher J. Nally 08/01/12
President, Laboratory Director Date

TEST ORGANISM DATA:

Test organism source:

Cultured: ____ Commercial hatchery: __X__ (ABS,Inc.)

TEST ORGANISM ACCLIMATION

Is the culture water and test dilution water the same and are the culture water temp and dilution water temp the same? No

If yes, proceed to Test Design Section

Fish and Grass shrimp

INITIAL NUMBER OF ORGANISMS:		300
TOTAL ACCLIMATION PERIOD:	0 DAYS	0 HOURS
ACCLIMATION PERIOD TO 100% DILUTION WATER AT SPECIFIED TEST TEMPERATURE (HRS):		N/A
# OF MORTALITIES (48 HRS PRIOR TO TEST):		N/A
TEST ORGANISM AGE AT START OF TEST (DAYS):		9

Mysids and Daphnids

INITIAL NUMBER OF ORGANISMS:	N/A
TEST ORGANISM AGE AT START OF TEST (DAYS):	N/A
CULTURE WATER SOURCE:	N/A
CULTURE WATER SALINITY:	N/A
CULTURE WATER TEMPERATURE:	N/A
DILUTION WATER SOURCE:	N/A
DILUTION WATER SALINITY UPON COLLECTION:	N/A
# OF MORTALITIES:	N/A

TEST DESIGN:

# OF EFFLUENT CONCENTRATIONS:	05
# OF REPLICATES / CONCENTRATION:	02
# OF TEST ORGANISMS / REPLICATE:	10
VOLUME OF TEST CHAMBERS (LITERS):	1.0
FLOW-THROUGH BIOASSAY EXCHANGE RATE; #/DAY:	N/A

EFFLUENT SAMPLING:

PLANT SAMPLING LOCATION:	Flowback water from natural gas drilling operation
EFFLUENT TYPE:	untreated
DISCHARGE (check one):	Continuous ___ Intermittent ___.
EFFLUENT SAMPLE TYPE:	___grab sample
MAXIMUM HOLDING TIME:	N/A
TESTING LOCATION:	On-site ____ Remote laboratory _X_.

Beginning Date & Time	Ending Date & Time	Dissolved Oxygen (ppm)	pH (Std. Units)	Use Date	Time
07/16/12 1045	07/16/12 1045	N/A	4.9	07/17-20/12	1900

Revised 09/96

EFFLUENT SAMPLE ADJUSTMENTS

ARE SALINITY ADJUSTMENTS ANTICIPATED? No
IF YES, SPECIFY SOURCE OF SALT, BRINE OR WATER USED: N/A

WERE ANY pH ADJUSTMENTS MADE? No
IF YES, SPECIFY REAGENT USED AND AMOUNT: N/A
THE pH LEVEL UPON SAMPLE COLLECTION (INITIAL pH): N/A
THE pH LEVEL AFTER THE ADDITION OF SEA SALTS (DRIFTED pH): N/A
THE ADJUSTED pH LEVEL: N/A

SAMPLE FILTERED: NO IF YES, MESH SIZE (MM): N/A

WERE ANY ADJUSTMENTS TO THE CHLORINE LEVELS MADE? No
IF YES, SPECIFY REAGENT TO BE USED AND THE AMOUNT: N/A
WAS AN ADDITIONAL CONTROL INCLUDED IN THE TEST CONTAINING THE REAGENT? N/A

DILUTION WATER:

EFFLUENT RECEIVING WATER: Dyberry Creek
DILUTION WATER SOURCE: N/A
IF SUBSTITUTE DILUTION WATER WAS USED, HAS ITS USE BEEN APPROVED BY THE NJDEP IN THE METH. QUESTIONNAIRE? N/A

COLLECTION LOCATION: N/A
COLLECTION DATES: 07/16/12

BIOASSAY RESULTS:

Time	24 hour	48 hour	72 hour	96 hour
LC_{50}/EC_{50} (% effluent)				0.63% x

CALCULATION METHOD:

PROBIT METHOD	X
GRAPHICAL INTERPOLATION	
VISUAL INSPECTION	
TRIMMED SPARMAN-KARBER	

NOTE: Attach the statistical printouts used to determine the LC_{50}/EC_{50} value and the mortality data sheets

Is the calculated LC_{50}/EC_{50} valid according to the specifications of the method used? Yes

MISCELLANEOUS:

Were any exposure chambers aerated during the test? No

If yes, specify conc., duration, & lowest % saturation reached prior to aeration & the time: N/A

Were test organisms observed for appearance & behavior at least daily? Yes

NOTE: Attach a copy of the acute toxicity test bench sheets with coded observations for each day.

NOTE: Attach a copy of the raw data sheets for physical-chemical measurements taken during the test during to the report form.

Acute Test

American Aquatic Testing, Inc.

Job #: 123-01-17
Species: P. promelas
Hatch Date: 7-9-12

DYBERRY Creek

Start Date & Time: 7-17-12 RP
End Date & Time: 7-21-12 1900
Dilution Water: Dyberry Creek

Concentration %	Rep.	Live Count					Appearance & Behavior				
		0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Control	A	10	10	10	10	10	1	1	1	1	1
	B	10	10	10	10	10	1	1	1	1	1
0.25	A	10	10	10	10	8[2]	1	1	3	3	3
	B	10	10	9[1]	8[1]	7[1]	1	1	3	3	3
0.5	A	10	10	8[2]	6[2]	5[1]	1	1	3	3	3,2
	B	10	10	8[2]	6[2]	5[1]	1	1	3	3	3,2
1.0	A	10	10	8[2]	8	5[3]	1	1	3	3	3,2
	B	10	10	9[1]	7[2]	5[2]	1	1	3	3	3,2
2.0	A	10	10	8[2]	7[1]	43	1	1	3	3	3,2
	B	10	8[2]	3[5]	3	03	1	1	3	3	3,2
4.0	A	10	2[8]	0[2]	—	—	1	1	3	3	—
	B	10	1[9]	0[1]	—	—	1	1	—	—	—
Initials		BJ	MJF	MJF	MJF	MNP	BJ	MJF	MJF	MJF	MNP
Date		07/17	7-18	7-19	7-20	7/21	07/17	7-18	7-19	7-20	7/21

Observation Key: 1–Normal, 2–Inactive, 3–Irritated, 4–Surfacing, 5–Abnormal body orientation, 6–Abnormal skin color, 7–Abnormal skin condition, 8–Abnormal respiration

LC50 .68%

Weight and Length Data

Length in Millimeters				Weight in Grams	
1	8	11	8	1 0.00300	11 0.00186
2	9	12	9	2 0.00296	12 0.00172
3	8	13	7	3 0.00309	13 0.00469
4	9	14	10	4 0.00176	14 0.00198
5	8	15	7	5 0.00311	15 0.00246
6	9	16	8	6 0.00288	16 0.00153
7	9	17	9	7 0.00512	17 0.00210
8	10	18	11	8 0.00240	18 0.00450
9	8	19	10	9 0.00325	19 0.00161
10	7	20	8	10 0.00310	20 0.00236

Initials	MNP
Date	7/21

Chamber Volume (L): 2.5
Average Weight (g): 0.00277
Standard Dev.: 0.00103
Loading Factor (g/L): 0.055
Length of Shortest Fish (mm): 7
Length of Longest Fish (mm): 11
Mean Length (mm): 8.6
Standard Deviation: 1.095

Freshwater Acute Test

American Aquatic Testing, Inc.

Job #: 123-01-17

Species: P. promelas

Dilution Water: Dyberry Creek

Start Date & Time: 7-17-12 1900

End Date & Time: 7-21-12 1900

Test Type: 96 hr. SDR

Concentration %	Rep.	Dissolved Oxygen (mg/L)					Temperature (C)				
		0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Control	A	10.1	8.6	8.3	8.6	8.3	20.5	21.0	21.0	21.0	20.0
	B	10.1	8.6	8.3	8.6	8.3	20.5	21.5	21.0	21.0	20.0
0.25	A	10.1	8.7	8.4	8.7	8.2	20.5	21.5	21.0	21.0	20.0
	B	10.1	8.6	8.4	8.7	8.1	20.5	21.5	21.0	21.0	20.0
0.5	A	10.1	8.6	8.4	8.7	8.2	20.5	21.5	21.0	21.0	20.0
	B	10.1	8.6	8.4	8.7	8.2	20.5	21.5	21.0	21.0	20.0
1.0	A	10.1	8.5	8.4	8.8	8.0	20.5	21.5	21.0	21.0	20.0
	B	10.1	8.7	8.4	8.8	8.1	20.5	21.5	21.0	21.0	20.0
2.0	A	10.1	8.6	8.4	8.7	7.8	20.5	21.5	21.0	21.0	20.0
	B	10.1	8.7	8.5	8.8	8.2	20.5	21.5	21.0	21.0	20.0
4.0	A	10.3	8.7	7.7 (1)	–	–	20.5	21.5	20.5 (1)	–	–
	B	10.3	8.7	7.8 (1)	–	–	20.5	21.5	20.5 (1)	–	–
Initials		JTAP	MTF	JMTF	MTF	MMP	JTAP	MTF	JMTF	MTF	MMP
Date		7/17	7-18	7/19	7-20	7-21	7/17	7-18	7/19	7-20	7-21

Concentration %	pH (std units)					Conductivity (umhos)				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Control	7.7	7.6	7.7	7.7	7.7	70	133	69	69	70
0.25	7.6	7.5	7.4	7.4	7.3	1011	915	941	922	905
0.5	7.5	7.4	7.4	7.3	7.3	1909	1661	1834	1852	1814
1.0	7.4	7.3	7.4	7.3	7.3	4038	3640	3529	3573	3508
2.0	7.3	7.1	7.3	7.2	7.2	6480	6410	6620	6790	6660
4.0	7.2	7.0	7.1 (1)	–	–	12330	12570	12440 (1)	–	–
Initials	JTAP	MTF	JMTF	MTF	MMP	JTAP	MTF	JMTF	MTF	MMP
Date	7/17	7-18	7/19	7-20	7-21	7/17	7-18	7/19	7-20	7-21

Concentration	Alkalinity (mg/L)					Hardness (mg/L)				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Control	60					40				
100%	50					3260				
Initials	JKAK					JKAK				
Date	7/17					7/17				

Concentration	Chlorine (mg/L)			
	Sample 1	Sample 2	Sample 3	Sample 4
Control	0.01			
100%	0.02			
Initials	JKAK			
Date	7/17			

Observations: (1) Readings done due to Total NH3/NH4 - MTF 7-19-12

ACFWPAR.WK3

Acute Fish Test-96 Hr Survival

Start Date:	7/17/2012	Test ID:	1230117ppd	Sample ID:	Dyberry Creek & Flowback
End Date:	7/21/2012	Lab ID:	AAT, INC.	Sample Type:	GRAB
Sample Date:		Protocol:	EPAA 91-EPA/600/4-90/027F	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2
Dyberry Creek	1.0000	1.0000
0.25	0.8000	0.7000
0.5	0.5000	0.5000
1	0.5000	0.5000
2	0.4000	0.0000
4	0.0000	0.0000

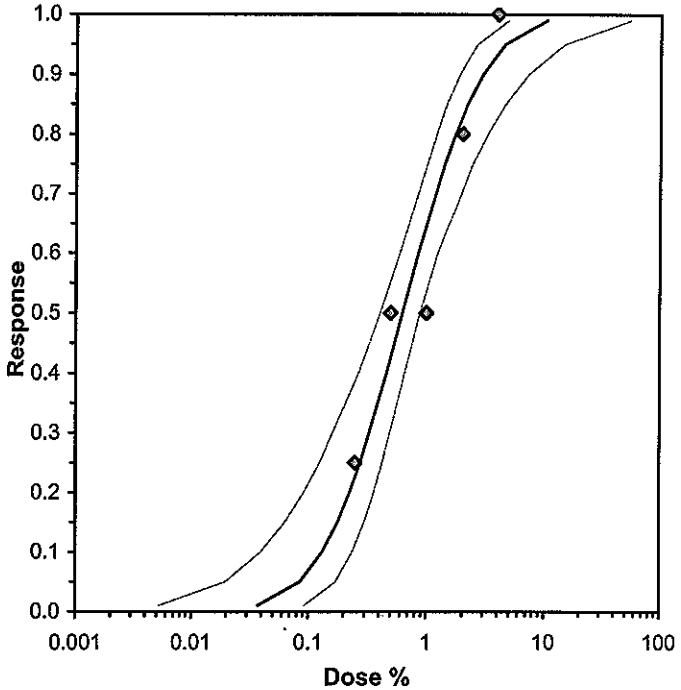
			Transform: Untransformed					Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
Dyberry Creek	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	2	0	20
0.25	0.7500	0.7500	0.7500	0.7000	0.8000	9.428	2	5	20
0.5	0.5000	0.5000	0.5000	0.5000	0.5000	0.000	2	10	20
1	0.5000	0.5000	0.5000	0.5000	0.5000	0.000	2	10	20
2	0.2000	0.2000	0.2000	0.0000	0.4000	141.421	2	16	20
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	2	20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.89974	0.37137	1.17186	2.62762	0	3.96714	7.81472	0.27	-0.202	0.52639	3
Intercept	5.38368	0.1458	5.09792	5.66944							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.03745	0.00523	0.09252
EC05	3.355	0.08555	0.01963	0.17089
EC10	3.718	0.13287	0.03951	0.23831
EC15	3.964	0.17884	0.0631	0.29942
EC20	4.158	0.22647	0.09122	0.36028
EC25	4.326	0.27733	0.12467	0.42381
EC40	4.747	0.46203	0.26668	0.65555
EC50	5.000	0.62811	0.40715	0.88195
EC60	5.253	0.85387	0.59514	1.23933
EC75	5.674	1.42258	1.00567	2.42667
EC80	5.842	1.74202	1.2054	3.25507
EC85	6.036	2.20597	1.472	4.63623
EC90	6.282	2.96912	1.87169	7.31635
EC95	6.645	4.61174	2.63683	14.5791
EC99	7.326	10.5341	4.91356	54.2407



ACUTE TOXICITY TEST

Ceriodaphnia dubia

DELAWARE RIVER BASIN COMMISSION
AAT JOB # 123-01-17

DYBERRY CREEK

REPORT PREPARED BY:

AMERICAN AQUATIC TESTING, INC.
890 NORTH GRAHAM STREET
ALLENTOWN, PA 18109

BIOMONITORING REPORT FORM: ACUTE TOXICITY

NJPDES #: N/A Dyberry Creek / Flowback

FACILITY NAME: Delaware River Basin Commission
FACILITY LOCATION: 25 State Police Drive, P.O. Box 7360
West Trenton, N.J. 08628-0360

LABORATORY: American Aquatic Testing, Inc.

ACUTE TOXICITY CERTIFICATION NUMBER: PA682

TEST SPECIFICATIONS:

EFFLUENT TYPE: Flowback
TEST TYPE: Static Daily Renewal

TEST RESULTS:

Test start date: 07/17/12 Test completion date: 07/19/12

Test endpoint (check one): LC_{50} X NMAT ____ EC_{50} ____.

LC_{50}:	0.59%

Highest % mortality in any test concentration (if applicable): 100%

Test concentration: 1.0, 2.0, 4.0%

Test organism: *Ceriodaphnia dubia* (scientific name) Water flea (common name)

QUALITY CONTROL SUMMARY:

Control mortality:	00%
Temperature maintained at 20 + / 2 ° C?	Yes
Dissolved 0_2 levels always > than 40 % saturation?	Yes
Two or more concentrations exhibit a trend deviation?	No
Were there any deviations from specified test methodologies?	No

CERTIFICATION:

Report certified for accuracy:

Christopher J. Nally
President, Laboratory Director

08/01/12
Date

Revised 09/96

TEST ORGANISM DATA:

Test organism source:

Cultured: X Commercial hatchery: (specify)

TEST ORGANISM ACCLIMATION

Is the culture water and test dilution water the same and are the culture water temp and dilution water temp the same? No

If yes, proceed to Test Design Section

Fish and Grass shrimp

INITIAL NUMBER OF ORGANISMS:	N/A
TOTAL ACCLIMATION PERIOD:	N/A
ACCLIMATION PERIOD TO 100% DILUTION WATER AT SPECIFIED TEST TEMPERATURE (HRS):	N/A
# OF MORTALITIES (48 HRS PRIOR TO TEST):	N/A
TEST ORGANISM AGE AT START OF TEST (DAYS):	N/A

Mysids and Daphnids

INITIAL NUMBER OF ORGANISMS:	300
TEST ORGANISM AGE AT START OF TEST (HOURS):	<24
CULTURE WATER SOURCE:	Moderately hard recon water
CULTURE WATER SALINITY:	N/A
CULTURE WATER TEMPERATURE:	20.0
DILUTION WATER SOURCE:	Dyberry Creek
DILUTION WATER SALINITY UPON COLLECTION:	N/A
# OF MORTALITIES:	N/A

TEST DESIGN:

# OF EFFLUENT CONCENTRATIONS:	05
# OF REPLICATES / CONCENTRATION:	02
# OF TEST ORGANISMS / REPLICATE:	10
VOLUME OF TEST CHAMBERS (LITERS):	0.25
FLOW-THROUGH BIOASSAY EXCHANGE RATE; #/DAY:	N/A

EFFLUENT SAMPLING:

PLANT SAMPLING LOCATION:	Flowback water from natural gas drilling operation
EFFLUENT TYPE:	untreated
DISCHARGE (check one):	Continuous ___ Intermittent ___.
EFFLUENT SAMPLE TYPE:	grab sample
MAXIMUM HOLDING TIME:	N/A
TESTING LOCATION:	On-site ____ Remote laboratory _X_.

Beginning Date & Time	Ending Date & Time	Dissolved Oxygen (ppm)	pH (Std. Units)	Use Date	Time
07/16/12 1045	07/16/12 1045	N/A	4.9	07/17-18/12	1800

2evised 09/96

EFFLUENT SAMPLE ADJUSTMENTS

ARE SALINITY ADJUSTMENTS ANTICIPATED? No
IF YES, SPECIFY SOURCE OF SALT, BRINE OR WATER USED: N/A

WERE ANY pH ADJUSTMENTS MADE? No
IF YES, SPECIFY REAGENT USED AND AMOUNT: N/A
THE pH LEVEL UPON SAMPLE COLLECTION (INITIAL pH): N/A
THE pH LEVEL AFTER THE ADDITION OF SEA SALTS (DRIFTED pH): N/A
THE ADJUSTED pH LEVEL: N/A

SAMPLE FILTERED: NO IF YES, MESH SIZE (MM): N/A

WERE ANY ADJUSTMENTS TO THE CHLORINE LEVELS MADE? No
IF YES, SPECIFY REAGENT TO BE USED AND THE AMOUNT: N/A
WAS AN ADDITIONAL CONTROL INCLUDED IN THE TEST CONTAINING THE REAGENT? N/A

DILUTION WATER:

EFFLUENT RECEIVING WATER: Dyberry Creek
DILUTION WATER SOURCE: N/A
IF SUBSTITUTE DILUTION WATER WAS USED, HAS ITS USE BEEN APPROVED BY THE NJDEP IN THE METH. QUESTIONNAIRE? N/A

COLLECTION LOCATION: N/A

COLLECTION DATES: 07/16/12

BIOASSAY RESULTS:

Time	24 hour	48 hour	72 hour	96 hour
LC_{50} (% effluent)		0.59 x		

CALCULATION METHOD:

PROBIT METHOD	—
GRAPHICAL	—
SPEARMAN-KARBER	X
TRIMMED SPEARMAN-KARBER	—
VISUAL	—

NOTE: Attach the statistical printouts used to determine the LC_{50}/EC_{50} value and the mortality data sheets

Is the calculated LC_{50}/EC_{50} valid according to the specifications of the method used? Yes

MISCELLANEOUS:

Were any exposure chambers aerated during the test? No

If yes, specify conc., duration & the time: N/A

Were test organisms observed for appearance & behavior at least daily? Yes
NOTE: Attach a copy of the acute toxicity test bench sheets with coded observations for each day.

NOTE: Attach a copy of the raw data sheets for physical-chemical measurements taken during the test to the report form.

Acute Test

American Aquatic Testing, Inc.

Job #: 123-01-17
Species: C. dubia
Hatch Date: 7-17-12

DYBERRY CREEK

Start Date & Time: 7-17-12 1800
End Date & Time: 7-19-12 1200
Dilution Water: Dyberry Creek

Concentration %	Rep.	Live Count 0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	Appearance & Behavior 0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Control	A	10	10	10			1	1	1		
	B	10	10	10			1	1	1		
0.25	A	10	10	10			1	1	1		
	B	10	10	10			1	1	1		
0.5	A	10	10	8[2]			1	1	3		
	B	10	10	7[3]			1	1	3		
1.0	A	10	5[10]	0[5]			1	3	–		
	B	10	9[9]	0[9]			1	3	–		
2.0	A	10	0[10]	–			1	–	–		
	B	10	0[10]	–			1	–	–		
4.0	A	10	0[10]	–			1	–	–		
	B	10	0[10]	–			1	–	–		
Initials		MME	MNF	MNF			MME	MNF	MNF		
Date		7-17	7-18	7-19			7-17	7-18	7-19		

Observation Key: 1–Normal, 2–Inactive, 3–Irritated, 4–Surfacing, 5–Abnormal body orientation, 6–Abnormal skin color, 7–Abnormal skin condition, 8–Abnormal respiration

LC50 0.59%

Weight and Length Data

Length in Millimeters		Weight in Grams	
1	11	1	11
2	12	2	12
3	13	3	13
4	14	4	14
5	15	5	15
6	16	6	16
7	17	7	17
8	18	8	18
9	19	9	19
10	20	10	20

Initials	
Date	

Chamber Volume (L): _____ Average Weight (g): _____ Standard Dev.: _____

Loading Factor (g/L): _____

Length of Shortest Fish (mm): _____ Length of Longest Fish (mm): _____

Mean Length (mm): _____ Standard Deviation: _____

Freshwater Acute Test

American Aquatic Testing, Inc.

Job #: 123-01-17

Species: C. dubia

Dilution Water: Dyberry Creek

Start Date: 7-17-12 Time 1800

End Date / Start Time: 7-19-12 1200

Test Type: 48hr. SNR

Concentration %	Rep.	Dissolved Oxygen (mg/L)			Temperature (C)			Live Count		
		0 hr.	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.
Control	A	10.1	8.9	86	20.5	20.5	20.5			
	B									
0.25	A	10.1	8.9	85	20.5	20.5	20.5			
	B									
0.5	A	10.1	9.0	86	20.5	20.0	20.5			
	B									
1.0	A	10.1	9.0	87	20.5	20.0	20.0			
	B									
2.0	A	10.1	8.90	-	20.5	20.0	-			
	B									
4.0	A	10.3	8.90	-	20.5	20.0	-			
	B									
Initials		GTAP	MTF	MM	GTAP	MTF	MM			
Date		7/17	7-18	7/19	7/17	7-18	7/19			

Concentration	Alkalinity (mg/L)			Hardness (mg/L)			Chlorine (mg/L)
	0 hr.	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.	Sample 1
Control	60			40			0.01
100%	50			3260			0.02
Initials	GTAP			GKAK			GKAK
Date	7/17			7/17			7/17

Concentration %	pH (std units)			Conductivity (umhos)		
	0 hr.	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.
Control	7.7	7.9	7.8	70	123	120
0.25	7.6	7.4	7.5	1011	999	998
0.5	7.5	7.4	7.5	1909	1800	1820
1.0	7.4	7.2	7.4	4038	4032	4092
2.0	7.3	7.20	-	6480	64200	-
4.0	7.2	7.00	-	12330	123000	-
Initials	GTAP	MTF	MM	GTAP	MTF	MM
Date	7/17	7-18	7/19	7/17	7-18	7/19

Observations: ① Readings done due to total mortality. 7-18-12 MTF

ACF48PAR.WK3

Acute Fish Test-48 Hr Survival

Start Date:	7/17/2012	Test ID:	1230117cdd	Sample ID:	DYBERRY CREEK
End Date:	7/19/2012	Lab ID:	AAT, INC.	Sample Type:	GRAB
Sample Date:		Protocol:	EPAA 91-EPA/600/4-90/027F	Test Species:	Ceriodaphnia dubia
Comments:					

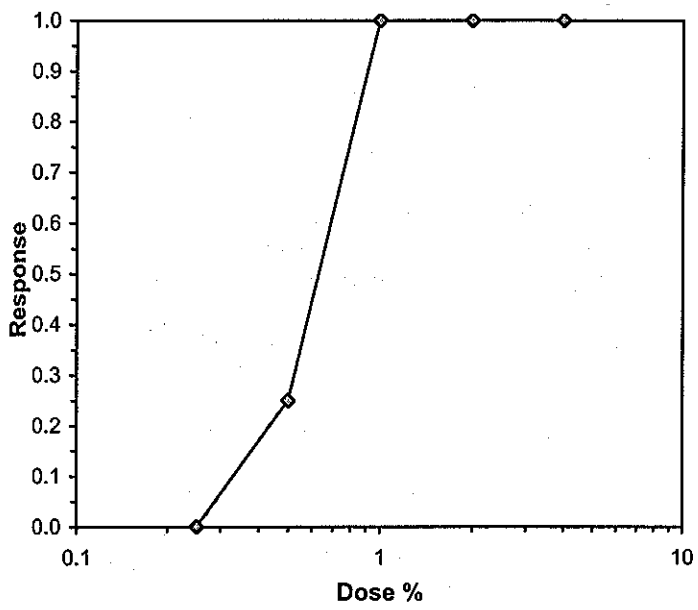
Conc-%	1	2
Dyberry Creek	1.0000	1.0000
0.25	1.0000	1.0000
0.5	0.8000	0.7000
1	0.0000	0.0000
2	0.0000	0.0000
4	0.0000	0.0000

			Transform: Arcsin Square Root					Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
Dyberry Creek	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
0.25	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
0.5	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5	20
1	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20
2	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20
4	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	0.5946	0.5199	0.6800
5.0%	0.6046	0.5195	0.7037
10.0%	0.6138	0.5128	0.7346
20.0%	0.6275	0.4708	0.8364
Auto-0.0%	0.5946	0.5199	0.6800



Reviewed by:

NJPDES BIOMONITORING REPORT FORM
FRESHWATER CHRONIC TOXICITY TEST

NJPDES #: N/A — Dyberry Creek / Flowback

FACILITY NAME: Delaware River Basin Commission
FACILITY LOCATION: 25 State Police Drive, P.O. Box 7360 West Trenton, N.J. 08628-0360

LABORATORY: American Aquatic Testing, Inc.

ACUTE TOXICITY CERTIFICATION NUMBER: PA682

SRT INFORMATION				
	DATE:	July 17, 2012	IC_{25}:	554.8 ppm
	CONTROL CHART		UCL:	638.0 ppm
	MEAN:	588.7 ppm	LCL:	539.4 ppm

TEST START DATE: July 24, 2012 — TEST END DATE: July 31, 2012

TEST TYPE AND RESULTS **(Check applicable test, circle applicable endpoint and fill in NOEC &/or IC_{25})**

X Fathead minnow, NOEC 0.031% (growth) IC_{25} 0.04%
PMSD 27.0%

Method 1000.0 (*Pimephales promelas* 7 day Larval Survival and Growth Test)

____ Cladoceran, (CN/CD) NOEL _____, IC_{25} _____

Method 1002.0 (*Ceriodaphnia dubia* 3 brood Survival and Reproduction Test)

CONTROL MORTALITY: **2.5 %**

Did the test meet the acceptability criteria for the test species as specified in Part III of the Chronic Methods Document? **YES**

ACCURACY OF REPORT CERTIFIED BY:

Christopher J. Nally — 08/01/12
President, Laboratory Director — Date

TEST DESIGN

NUMBER OF CONCENTRATIONS:	05
NUMBER OF REPLICATES / CONCENTRATION:	04
NUMBER OF TEST ORGANISMS / REPLICATE:	10
DILUTION SERIES:	0.5
TEST VESSEL SIZE:	1.0 L
TEST SOLUTION VOLUME:	500 mL
EXPLAIN ANY DEVIATIONS FROM SPECIFIED METHODS;	N/A

EFFLUENT SAMPLING

SAMPLE LOCATION:	Flowback water from natural gas drilling operation	
EFFLUENT TYPE:		untreated
DISCHARGE:		N/A
RETENTION TIME:		N/A
SAMPLE TYPE:	composite ____ Other __x__, (Grab)	

Sample Collection		Sample Data taken upon arrival at laboratory		Use in Toxicity Test	
Beginning Date & Time	Ending Date & Time	Dissolved Oxygen (ppm)	pH (Std. Units)	Date(s)	Time(s)
07/16/12, 1045	07/16/12, 1045	N/A	4.9	07/24-31/12	1630+/- 1 hr

Maximum holding time for any effluent sample: N/A

Describe any pretreatment of the effluent: N/A

TESTING LOCATION

REMOTE LABORATORY	X
ON-SITE LABORATORY	
ON-SITE COMMERCIAL LABORATORY	

DILUTION WATER

EFFLUENT RECEIVING WATER:	N/A
DILUTION WATER:	Dyberry Creek
DESCRIBE ANY ADJUSTMENT TO THE DILUTION WATER:	N/A
IF RECEIVING WATER IS USED AS DILUTION SOURCE, DESCRIBE COLLECTION LOCATION AND DATES OF COLLECTION:	07/16/12

SUMMARY SHEET FOR THE FATHEAD MINNOW, SHEEPSHEAD MINNOW, INLAND SILVERSIDE AND MYSID SHRIMP TESTS

Percent Effluent	Mean Percent Survival	Mean Dry Weight (mg.)	Percent of Surviving Females with Eggs
Control – White Clay Creek	97.5	0.4290	N/A
0.015	100	0.4588	N/A
0.031	92.5	0.4113	N/A
0.062	45	0.1918	N/A
0.125	7.5	0.0320	N/A
0.250	00	00	N/A

Organism source: __ cultured stock, _x_ commercial supplier

Name of supplier: ABS, Inc.

Hatch date: 07/23/12

Age of organisms (hours): < 48

Describe any aeration which was performed during the test: N/A

Describe any adjustments to the salinity of the test concentrations: N/A

How long after test termination were the organisms prepared for weighing/drying? Immediately

Was the average dry weight per test chamber determined by dividing the final dry weight by the number of original test organisms in the test chamber? Yes

Did the temp. in the test chambers vary by more than 01 ° Celsius each day? No

Did the salinity in the test chambers vary more than 2 ppt between replicates each day? N/A

How long after test termination were the mysids examined for eggs and sexes? * N/A

* Applies only to the Mysid shrimp test

Client/Toxicant: 123 Dyberry Creek
Project Number: 01-17R
Species: P. promelas

Beginning Date & Time: 7-24-12 1635
Ending Date & Time: 7/31/12 1100
Hatch Date: 7-23-12

Chronic Test
American Aquatic Testing, Inc.
Live Count

Conc.	Rep	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	10	10
	D	10	10	10	10	10	10	10	9
0.015%	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	10	10
	D	10	10	10	10	10	10	10	10
0.031%	A	10	10	10	10	10	9'	8'	7'
	B	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	10	10
	D	10	10	10	10	10	10	10	10
0.062%	A	10	10	10	10	10	~~8~~ 7 ③	6	6
	B	10	10	10	8^{2}	8	6^{2}	6	4'
	C	10	10	10	8^{2}	8	~~8~~ 7 ④	2	2
	D	10	10	10	10	10	~~8~~ 7 ③	6	6
0.125%	A	10	10	9'	5^{4}	5	~~7~~ 6 ⑤	1	1
	B	10	9	9	7'	7	~~5~~ 6 ⑥	2^{2}	2
	C	10	10	7^{3}	5^{1}	~~5~~ 4 ①	T	-	-
	D	10	10	9'	3^{5}	~~3~~ 2 ②	T	-	-
0.250%	A	10	10	9'	0^{9}	—	—	-	-
	B	10	9	8'	0^{8}	—	—	-	-
	C	10	10	10	0^{10}	—	—	-	-
	D	10	10	10	0^{10}	—	—	-	-
\	A								
	B								
	C								
	D								
Initials		MAF	MAF	WM	WM	WM	WM	MAF	WM
Date		7-24	7-25	7/26	7/27	7/28	7/29	7-30	7/31

Observations: ① 0^{5}
② 1^{2} WM 7/28
③ 6^{4}
④ 2^{6} WM 7/29
⑤ 4
⑥ 4^{3}

0.0421 %

CHFHLC.wk3

Client/Toxicant: 123 Dyberry Creek
Project Number: 01-17R
Species: P. promelas

Beginning Date & Time: 7-24-12 1635
Ending Date & Time: 7/31/12 1010
Hatch Date: 7-23-12

Chronic Test

American Aquatic Testing, Inc.

Weight Data

QC 0.01291

% Conc.	Rep	Pan #	A weight of boat (g)	B weight of boat & fish (g)	(B−A)*1000=C dry weight of fish (mg)	D # of surviving fish	C/D mean dry weight (mg)	C/E IC_{25} & NOEC calc. weight (mg)
Control	A	1	0.01288	0.01731	4.43	10	0.443	0.443
	B	2	0.01380	0.01801	4.21	10	0.421	0.421
	C	3	0.01363	0.01785	4.22	10	0.422	0.422
	D	4	0.01408	0.01838	4.30	9	0.478	0.430
0.015	A	5	0.01235	0.01643	4.08	10		0.408
	B	6	0.01236	0.01731	4.95	10		0.495
	C	7	0.01351	0.01836	4.85	10		0.485
	D	8	0.01380	0.01827	4.47	10		0.447
0.031	A	9	0.01164	0.01395	2.31	7		0.231
	B	10	0.01323	0.01764	4.41	10		0.441
	C	11	0.01311	0.01797	4.86	10		0.486
	D	12	0.01216	0.01703	4.87	10		0.487
0.062	A	13	0.01236	0.01501	2.65	6		0.265
	B	14	0.01080	0.01268	1.88	4		0.188
	C	15	0.01242	0.01326	0.84	2		0.084
	D	16	0.01278	0.01508	2.30	6		0.230
0.125	A	17	0.01200	0.01254	0.54	1		0.054
	B	18	0.01392	0.01466	0.74	2		0.074
	C	19	0.0					
	D	20	0.0					
0.250	A	21	—	—	—	—	—	—
	B	22	—	—	—	—	—	—
	C	23	—	—	—	—	—	—
	D	24	—	—	—	—	—	—
\	A							
	B							
	C							
	D							
Initials			thd	thd	thd	[illegible]	thd	thd
Date			7/31/12	8/1/12	8/1/12	7/31	8/1/12	8/1/12

QC 0.01294

QC 0.01294

E = Original number of organisms at test initiation, adjusted for losses.

Observations:

Date/Time In: 7/31/12 1640
Temp: 109.0

Date/Time Out: 8/1/12 5:00
Temp: 104.0

CHFHWT.wk3

Client/Toxicant: 123 Dyberry Creek
Job Number: 01-17R
Species: P. promelas
Beginning Date & Time: 7-24-12 1630
Ending Date & Time: 7/31/12 1000

Freshwater Chronic Test
American Aquatic Testing, Inc.,
Physical / Chemical Parameters
Initial Readings

Parameter	Concentration	Day 1	2	3	4	5	6	7	8
Dissolved Oxygen (mg/L)	Control	7.7	7.7	7.8	7.9	8.3	7.8	7.5	
	0.015%	7.6	7.6	7.8	7.9	8.3	7.8	7.6	
	0.031%	7.5	7.5	7.8	7.8	8.4	7.9	7.6	
	0.062%	7.9	7.4	7.7	7.8	8.4	7.9	7.6	
	0.125%	7.9	7.3	7.7	7.8	8.4	7.9	7.6	
	0.250%	7.9	7.3	7.7	–	–	–	–	
pH	Control	7.7	7.7	7.5	7.7	7.7	7.8	7.5	
	0.015%	7.6	7.6	7.5	7.6	7.7	7.7	7.5	
	0.031%	7.5	7.5	7.4	7.5	7.6	7.7	7.4	
	0.062%	7.5	7.4	7.4	7.4	7.5	7.6	7.3	
	0.125%	7.4	7.3	7.4	7.4	7.4	7.5	7.3	
	0.250%	7.3	7.3	7.4	–	–	–	–	
	Initials	WW	MF	KAK	WW	WW	KAK	WW	
	Date	7/24	7/25	7/26	7/27	7/28	7/29	7/30	

Conductivity (µmhos/cm)		
Date	Control	100%
7/24	713	1050
Initials	WW	WW

Alkalinity (mg/L as $CaCO_3$)		
Date	Control	100%
Initials		

Hardness (mg/L as $CaCO_3$)		
Date	Control	100%
Initials		

Final Readings

Parameter	Concentration	Day 1	2	3	4	5	6	7	8
T e m p °C	Control	25.0	25.0	25.0	25.5	25.5	25.0	25.5	
	0.015%	25.0	25.0	25.0	25.5	25.5	25.0	25.5	
	0.031%	25.0	25.0	25.0	25.5	25.5	25.0	25.5	
	0.062%	25.0	25.0	25.0	25.5	25.5	25.0	25.5	
	0.125%	25.0	25.0	25.0	25.5	25.5	25.0	25.5	
	0.250%	25.0	25.0	25.0	–	–	–		
Dissolved Oxygen (mg/L)	Control	7.4	7.3	6.9	6.9	7.0	6.6	6.6	
	0.015%	7.2	7.3	7.2	6.7	7.0	6.9	6.8	
	0.031%	7.2	7.4	7.1	6.8	7.1	6.8	6.7	
	0.062%	7.2	7.4	6.9	6.9	6.9	6.8	6.7	
	0.125%	7.3	7.3	6.8	6.8	7.3	7.2	7.0	
	0.250%	7.1	7.3	6.8	–	–	–		
pH	Control	7.7	7.8	7.7	7.8	7.9	7.7	7.7	
	0.015%	7.6	7.7	7.6	7.7	7.8	7.7	7.6	
	0.031%	7.6	7.6	7.6	7.6	7.7	7.6	7.6	
	0.062%	7.5	7.5	7.5	7.5	7.6	7.5	7.6	
	0.125%	7.5	7.4	7.5	7.5	7.5	7.5	7.5	
	0.250%	7.4	7.4	7.6	–	–	–		
	Initials	WW	MF	WW	KAK	KAK	TAP	MFP	
	Date	7/25	7/26	7/27	7/28	7/29	7/30	7/31	

Ammonia / Chlorine		
Date	NH_3	Res Cl_2
Initials		

Observations: ① 7.9 WW 7/24
② 8.0 MF 7/25

FWCHPAR.wk3

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	7/24/2012	Test ID:	1230117pdR	Sample ID:	Dyberry Creek
End Date:	7/31/2012	Lab ID:	AAT, INC.	Sample Type:	GRAB
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2	3	4
White Clay	1.0000	1.0000	1.0000	0.9000
0.015	1.0000	1.0000	1.0000	1.0000
0.031	0.7000	1.0000	1.0000	1.0000
0.062	0.6000	0.4000	0.2000	0.6000
0.125	0.1000	0.2000	0.0000	0.0000
0.25	0.0000	0.0000	0.0000	0.0000

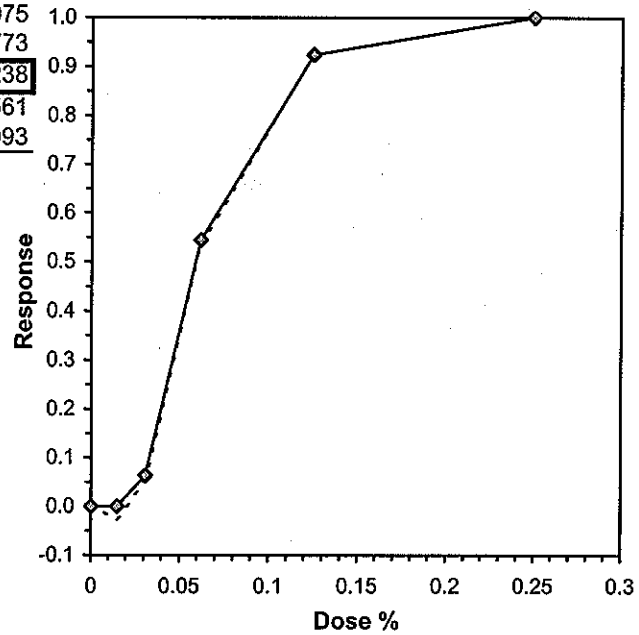
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
White Clay	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4				0.9875	1.0000
0.015	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	4	-0.383	2.360	0.2510	0.9875	1.0000
0.031	0.9250	0.9487	1.3068	0.9912	1.4120	16.103	4	0.606	2.360	0.2510	0.9250	0.9367
*0.062	0.4500	0.4615	0.7301	0.4636	0.8861	27.587	4	6.029	2.360	0.2510	0.4500	0.4557
*0.125	0.0750	0.0769	0.2757	0.1588	0.4636	53.294	4	10.302	2.360	0.2510	0.0750	0.0759
0.25	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92139	0.868	-0.9185	0.62548
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.031	0.062	0.04384	3225.81	0.1503	0.15644	0.99727	0.02262	4.0E-08	4, 15
Treatments vs White Clay										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	0.0276	0.0055	0.0134	0.0387	-0.2582
IC10	0.0334	0.0046	0.0159	0.0409	-0.7631
IC15	0.0366	0.0039	0.0199	0.0446	-0.7975
IC20	0.0398	0.0038	0.0239	0.0483	-0.6773
IC25	0.0430	0.0037	0.0309	0.0520	-0.3238
IC40	0.0527	0.0044	0.0419	0.0691	0.3561
IC50	0.0591	0.0065	0.0472	0.0851	0.6993



Larval Fish Growth and Survival Test-7 Day Biomass

Start Date:	7/24/2012	Test ID:	1230117pdR	Sample ID:	Dyberry Creek
End Date:	7/31/2012	Lab ID:	AAT, INC.	Sample Type:	GRAB
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2	3	4
White Clay	0.4430	0.4210	0.4220	0.4300
0.015	0.4080	0.4950	0.4850	0.4470
0.031	0.2310	0.4410	0.4860	0.4870
0.062	0.2650	0.1880	0.0840	0.2300
0.125	0.0540	0.0740	0.0000	0.0000
0.25	0.0000	0.0000	0.0000	0.0000

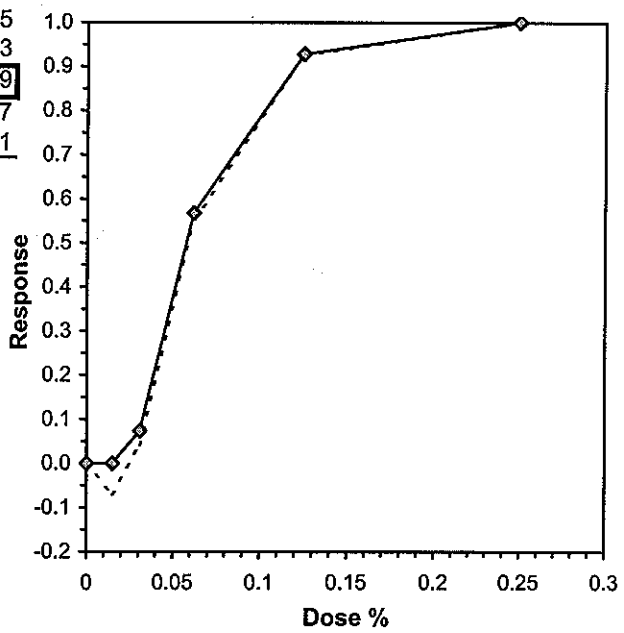
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
White Clay	0.4290	1.0000	0.4290	0.4210	0.4430	2.370	4				0.4439	1.0000
0.015	0.4588	1.0693	0.4588	0.4080	0.4950	8.643	4	-0.605	2.360	0.1160	0.4439	1.0000
0.031	0.4113	0.9586	0.4113	0.2310	0.4870	29.682	4	0.361	2.360	0.1160	0.4113	0.9265
*0.062	0.1918	0.4470	0.1918	0.0840	0.2650	40.901	4	4.827	2.360	0.1160	0.1918	0.4320
*0.125	0.0320	0.0746	0.0320	0.0000	0.0740	118.256	4	8.077	2.360	0.1160	0.0320	0.0721
0.25	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88129	0.868	-1.446	2.91783
Bartlett's Test indicates equal variances (p = 0.01)	12.749	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs White Clay	0.031	0.062	0.04384	3225.81	0.116	0.27039	0.13766	0.00483	7.5E-07	4, 15

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	0.0259	0.0064	0.0121	0.0393	-0.1906
IC10	0.0327	0.0062	0.0116	0.0404	-0.6932
IC15	0.0358	0.0060	0.0133	0.0437	-0.8025
IC20	0.0389	0.0060	0.0150	0.0470	-0.8933
IC25	0.0421	0.0059	0.0167	0.0503	-1.0139
IC40	0.0515	0.0053	0.0270	0.0625	-1.1487
IC50	0.0577	0.0054	0.0412	0.0763	0.2221



NJPDES BIOMONITORING REPORT FORM
FRESHWATER CHRONIC TOXICITY TEST

NJPDES #: N/A Dyberry Creek / Flowback

FACILITY NAME: Delaware River Basin Commission
25 State Police Drive, P.O. Box 7360
FACILITY LOCATION: West Trenton, N.J. 08628-0360

LABORATORY: American Aquatic Testing, Inc.

ACUTE TOXICITY CERTIFICATION NUMBER: PA682

SRT INFORMATION	DATE:	June 27, 2012	IC_{25}:	264.0 ppm
	CONTROL CHART		UCL:	397.8 ppm
	MEAN:	319.2 ppm	LCL:	240.5 ppm

TEST START DATE: July 17, 2012 TEST END DATE: July 23, 2012

TEST TYPE AND RESULTS **(Check applicable test, circle applicable endpoint and fill in NOEC &/or IC_{25})**

_____ Fathead minnow, (CN/FM) NOEL _____, IC_{25} _____

Method 1000.0 (*Pimephales promelas* 7 day Larval Survival and Growth Test)

_X_ Cladoceran, (CN/CD) NOEC 0.25% (Reproduction) IC_{25} 0.50%
PMSD 17.4%
Method 1002.0 (*Ceriodaphnia dubia* 3 brood Survival and Reproduction Test)

CONTROL MORTALITY: **00%**

Did the test meet the acceptability criteria for the test species as specified in Part III of the Chronic Methods Document? **YES**

ACCURACY OF REPORT CERTIFIED BY:

Christopher J. Nally 08/01/12
President, Laboratory Director Date

TEST DESIGN

NUMBER OF CONCENTRATIONS:	05
NUMBER OF REPLICATES / CONCENTRATION:	10
NUMBER OF TEST ORGANISMS / REPLICATE:	01
DILUTION SERIES:	0.5
TEST VESSEL SIZE:	30 mL
TEST SOLUTION VOLUME:	15 mL
EXPLAIN ANY DEVIATIONS FROM SPECIFIED METHODS;	N/A

EFFLUENT SAMPLING

SAMPLE LOCATION:	Flowback water from natural gas drilling operation	
EFFLUENT TYPE:		untreated
DISCHARGE:		N/A
RETENTION TIME:		N/A
SAMPLE TYPE:	Composite ___ Other ___x___, (Grab)	

Sample Collection		Sample Data taken upon arrival at laboratory		Use in Toxicity Test	
Beginning Date & Time	Ending Date & Time	Dissolved Oxygen (ppm)	pH (Std. Units)	Date(s)	Time(s)
07/16/12, 1045	07/16/12, 1045	N/A	4.9	07/17-22/12	1600+/- 1 hr

Maximum holding time for any effluent sample: N/A

Describe any pretreatment of the effluent: N/A

TESTING LOCATION

REMOTE LABORATORY	X
ON-SITE LABORATORY	
ON-SITE COMMERCIAL LABORATORY	

DILUTION WATER

EFFLUENT RECEIVING WATER:	N/A
DILUTION WATER:	Dyberry Creek
DESCRIBE ANY ADJUSTMENT TO THE DILUTION WATER:	N/A
IF RECEIVING WATER IS USED AS DILUTION SOURCE, DESCRIBE COLLECTION LOCATION AND DATES OF COLLECTION:	07/16/12

SUMMARY SHEET FOR THE CLADOCERAN CERIODAPHNIA DUBIA TEST

Percent Effluent	Mean Percent Survival	Mean Number of Young per Surviving Female	Percent of Females with Third Brood
Control – White Clay Creek	100	33.0	100
0.125	100	35.1	100
0.25	100	34.6	100
0.50	100	25.5	90
1.0	90	0.9	00
2.0	00	0.0	00

Organism source: _X_ cultured stock, __ commercial supplier

Name of supplier: N/A

Age of organisms (hours): < 24

Test organisms all released within an 08 hour period? Yes

Neonates obtained from: __ mass culture, X individually cultured organisms

Was the test terminated when 60 % of the surviving females in the controls had produced their third brood? Yes

Within how many hours after test termination were the organisms counted? Immediately

NUMBER OF MALES / EPHIPPIA

Percent Effluent	Number of Males	Number of Ephippia
Control	00	00
0.125	00	00
0.25	00	00
0.50	00	00
1.0	00	00
2.0	00	00

Did the number of males in the controls and/or the test concentrations influence the determination of the NOEC/IC_{25}? No

Project Number: 123-01-17 Dyberry Creek

Beginning Date & Time: 7-17-12 1600
Ending Date & Time: 7-23-12 1545

Ceriodaphnia dubia, Survival and Reproduction Test

American Aquatic Testing, Inc.,

Survival / Reproduction Data

Day	Conc. Control		Replicate 1		2		3		4		5		6		7		8		9		10		Initals
1	N	B	0		0		0		0		0		0		0		0		0		0		MP
2	N	B	0		0		0		0		0		0		0		0		0		0		TAO
3	N	B	6	1	4	1	4	1	6	1	6	1	4	1	4	1	4	1	4	1	4	1	TAO
4	N	B	16	2	12	2	14	2	12	2	12	2	12	2	12	2	8	2	12	2	10	2	MP
5	N	B	0		20	3	16	3	0		0		18	3	0		0		0		8	3	MP
6	N	B	20	3	0		0		14	3	14	3	0		16	3	18	3	20	3	0		TAO
7	N	B																					
8	N	B																					
	Tot N	Tot B	42	3	36	3	34	3	32	3	32	3	34	3	32	3	30	3	36	3	22	3	Tot A
																							10

Average Neonates per Female = 33.0 % Females with 3rd Brood = 100

Day	Conc. 0.125		Replicate 1		2		3		4		5		6		7		8		9		10		Initals
1	N	B	0		0		0		0		0		0		0		0		0		0		MP
2	N	B	0		0		0		0		0		0		0		0		0		0		TAO
3	N	B	6	1	6	1	6	1	4	1	4	1	4	1	4	1	4	1	6	1	4	1	TAO
4	N	B	12	2	12	2	14	2	12	2	14	2	0		14	2	14	2	14	2	12	2	MP
5	N	B	0		0		0		16	3	0		16	2	16	3	16	3	0		16	3	MP
6	N	B	16	3	12	3	22	3	0		18	3	16	3	0		0		21	3	0		TAO
7	N	B																					
8	N	B																					
	Tot N	Tot B	34	3	30	3	42	3	32	3	36	3	36	3	34	3	34	3	41	3	32	3	Tot A
																							10

Average Neonates per Female = 36.1 % Females with 3rd Brood = 100

Day	Conc. 0.25		Replicate 1		2		3		4		5		6		7		8		9		10		Initals
1	N	B	0		0		0		0		0		0		0		0		0		0		MP
2	N	B	0		0		0		0		0		0		0		0		0		0		TAO
3	N	B	4	1	4	1	6	1	6	1	4	1	3	1	4	1	4	1	4	1	4	1	TAO
4	N	B	12	2	12	2	18	2	12	2	10	2	11	2	12	2	10	2	16	2	10	2	MP
5	N	B	0		0		0		16	3	18	3	17	3	18	3	18	3	0		0		MP
6	N	B	16	3	20	3	18	3	0		0		0		0		0		20	3	19	3	TAO
7	N	B																					
8	N	B																					
	Tot N	Tot B	32	3	36	3	42	3	34	3	32	3	31	3	34	3	32	3	40	3	33	3	Tot A
																							10

Average Neonates per Female = 34.6 % Females with 3rd Brood = 100

(N=Neonates, B=Broods, A=Alive)

Observations:

CHCDLCRP.wk3

NOEC 0.5
REPRO IC25 0.495 %

Project Number: 123-01-17 Dyberry Creek

Beginning Date & Time: 7-17-12 1600
Ending Date & Time: 7-23-12 1545

Ceriodaphnia dubia, Survival and Reproduction Test

American Aquatic Testing, Inc.,

Survival / Reproduction Data

Day	Conc. 0.5		Replicate 1	2	3	4	5	6	7	8	9	10	Initals
1	N	B	0	0	0	0	0	0	0	0	0	0	MP
2	N	B	0	0	0	0	0	0	0	0	0	0	TRP
3	N	B	6 1	2 1	5 1	4 1	4 1	0	2 1	4 1	4 1	0	TRP
4	N	B	16 2	8 2	9 2	10 2	10 2	0	4 2	8 2	12 2	6 1	MP
5	N	B	0	0	0	12 3	0	4 1	6 2	10 3	0	12 2	MP
6	N	B	14 3	12 3	20 3	0	12 3	0	0	0	12 3	17 3	TRP
7	N	B											
8	N	B											
	Tot N	Tot B	36 3	32 3	34 3	46 3	26 3	4 1	12 2	22 3	28 3	35 3	Tot A 10

Average Neonates per Female = 25.5 % Females with 3rd Brood = 80

Day	Conc. 1.0		Replicate 1	2	3	4	5	6	7	8	9	10	Initals
1	N	B	0	0	0	0	0	0	0	0	0	0	MP
2	N	B	0	0	0	0	0	0	0	0	0	0	TRP
3	N	B	0	0	0	0	0	0	0	0	0	0	TRP
4	N	B	2 1	0	0	0	0	3 1	0	1 1	0	0	MP
5	N	B	0	0	0	0	D -	3 2	0	0	0	0	MP
6	N	B	0	0	0	0	- -	0	0	0	0	0	TRP
7	N	B											
8	N	B											
	Tot N	Tot B	2 1	0	10	0	0	6 2	0	1	0	0	Tot A 9

Average Neonates per Female = 0.9 % Females with 3rd Brood = 0

Day	Conc. 2.0		Replicate 1	2	3	4	5	6	7	8	9	10	Initals
1	N	B	0	0	0	D -	D -	0	D -	D -	D -	D -	MP
2	N	B	D -	D -	D -	- -	- -	D -	- -	- -	- -	- -	TRP
3	N	B											
4	N	B											
5	N	B											
6	N	B											
7	N	B											
8	N	B											
	Tot N	Tot B											Tot A 0

Average Neonates per Female = 0 % Females with 3rd Brood = 0

(N=Neonates, B=Broods, A=Alive)

Observations: _____

Client/Toxicant: 123
Job Number: 01-17 Dyberry Creek
Species: C. dubia

Beginning Date & Time: 7-17-12 1600
Ending Date & Time: 7-23-12 1545

Freshwater Chronic Test
American Aquatic Testing, Inc.,
Physical / Chemical Parameters
Initial Readings

Parameter	Concentration	Day 1	2	3	4	5	6	7	8
Dissolved Oxygen (mg/L)	Control	9.5	8.8	8.0	8.2	8.0	7.6		
	0.125	9.4	8.6	7.9	8.2	8.0	7.6		
	0.25	9.4	8.5	7.9	8.2	8.0	7.6		
	0.50	9.4	8.5	7.9	8.2	8.0	7.6		
	1.0	9.3	8.5	7.9	8.2	8.0	7.6		
	2.0	9.3	8.5	8.0	—	—	—		
pH	Control	7.8	7.8	7.7	7.5	7.5	7.7		
	0.125	7.6	7.6	7.4	7.4	7.4	7.7		
	0.25	7.4	7.5	7.4	7.3	7.4	7.6		
	0.5	7.3	7.4	7.3	7.3	7.3	7.5		
	1.0	7.2	7.3	7.3	7.2	7.3	7.4		
	2.0	7.0	7.2	7.2	—	—	—		
	Initials	TMP	TMP	TMP	TMP	MMP	MMP		
	Date	7/17	7/18	7/19	7/20	7/21	7/22		

Conductivity (µmhos/cm)		
Date	Control	100%
7/17	76	7160
Initials	TMP	TMP

Alkalinity (mg/L as $CaCO_3$)		
Date	Control	100%
7/17		
Initials		

Hardness (mg/L as $CaCO_3$)		
Date	Control	100%
7/17		
Initials		

Final Readings

Parameter	Concentration	Day 1	2	3	4	5	6	7	8
Temperature °C	Control	25.5	25.5	25.5	25.5	25.5	25.5		
	0.125	25.5	25.5	25.5	25.5	25.5	25.5		
	0.25	25.5	25.5	25.5	25.5	25.5	25.5		
	0.5	25.5	25.5	25.5	25.5	25.5	25.5		
	1.0	25.5	25.5	25.5	25.5	25.5	25.5		
	2.0	25.5	25.5	—	—	—	—		
Dissolved Oxygen (mg/L)	Control	8.0	7.6	8.2	8.1	7.8	8.3		
	0.125	8.0	7.6	8.2	8.1	7.8	8.1		
	0.25	7.9	7.6	8.2	8.1	7.8	8.1		
	0.5	8.1	7.5	8.2	8.1	7.8	8.1		
	1.0	8.1	7.5	8.2	8.1	7.8	8.1		
	2.0	8.1	7.6	—	—	—	—		
pH	Control	8.0	7.9	7.8	7.9	7.9	8.1		
	0.125	7.8	7.7	7.7	7.8	7.8	7.8		
	0.25	7.7	7.7	7.7	7.8	7.8	7.7		
	0.5	7.7	7.6	7.6	7.7	7.8	7.7		
	1.0	7.7	7.5	7.5	7.7	7.7	7.7		
	2.0	7.6	7.5	—	—	—	—		
	Initials	MMP	MTF	TMP	MMP	MMP	MTF		
	Date	7/18	7/19	7/20	7/21	7/22	7/23		

Ammonia / Chlorine		
Date	NH_3	Res Cl_2
Initials		

Observations:

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	7/17/2012	Test ID:	1230117cdc	Sample ID:	Dyberry Creek & Flowback
End Date:	7/23/2012	Lab ID:	AAT, INC	Sample Type:	GRAB
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

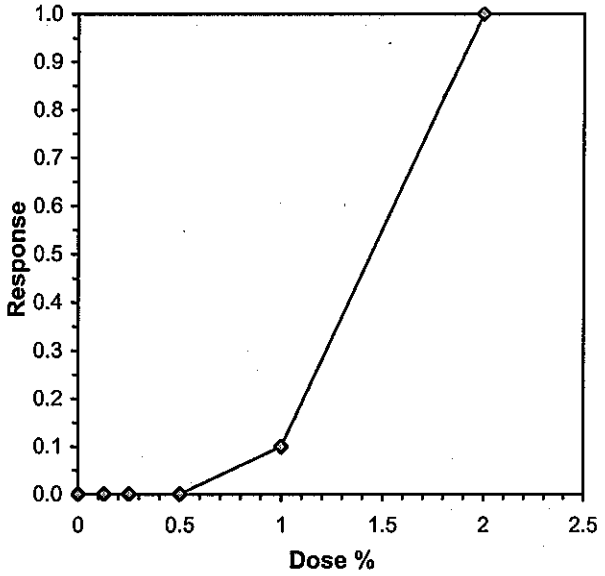
Conc-%	1	2	3	4	5	6	7	8	9	10
Dyberry Creek	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.125	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Isotonic Mean	Isotonic N-Mean
Dyberry Creek	1.0000	1.0000	0	10	10	10			1.0000	1.0000
0.125	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
0.25	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
0.5	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
1	0.9000	0.9000	1	9	10	10	0.5000	0.0500	0.9000	0.9000
2	0.0000	0.0000	10	0	10	10			0.0000	0.0000

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	1	2	1.41421	100
Treatments vs Dyberry Creek				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05	0.7500	0.1768	0.5833	1.0500	0.3289
IC10	1.0000	0.1457	0.6667	1.1000	-0.9232
IC15	1.0556	0.1217	0.7500	1.1500	-0.9887
IC20	1.1111	0.1000	0.8333	1.2000	-1.2344
IC25	1.1667	0.0925	0.9167	1.2500	-1.1999
IC40	1.3333	0.0737	1.1429	1.4000	-1.2582
IC50	1.4444	0.0614	1.2857	1.5000	-1.2582



Reviewed by:

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	7/17/2012	Test ID:	1230117cdc	Sample ID:	DYBERRY CR
End Date:	7/23/2012	Lab ID:	AAT, INC	Sample Type:	GRAB
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
Dyberry Creek	42.000	36.000	34.000	32.000	32.000	34.000	32.000	30.000	36.000	22.000
0.125	34.000	30.000	42.000	32.000	36.000	36.000	34.000	34.000	41.000	32.000
0.25	32.000	36.000	42.000	34.000	32.000	31.000	34.000	32.000	40.000	33.000
0.5	36.000	32.000	34.000	26.000	26.000	4.000	12.000	22.000	28.000	35.000
1	2.000	0.000	0.000	0.000	0.000	6.000	0.000	1.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

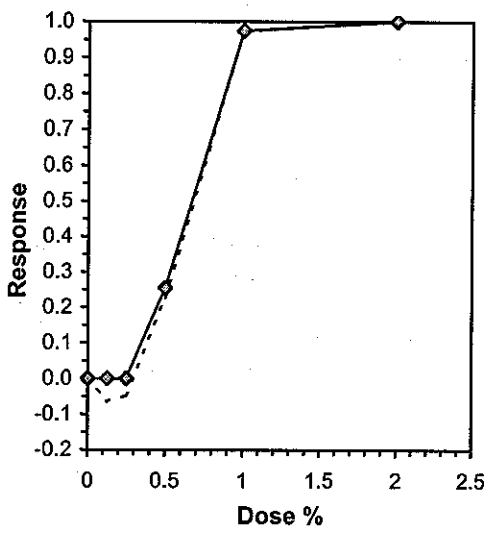
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Dyberry Creek	33.000	1.0000	33.000	22.000	42.000	15.452	10				34.233	1.0000
0.125	35.100	1.0636	35.100	30.000	42.000	10.948	10	-0.813	2.223	5.742	34.233	1.0000
0.25	34.600	1.0485	34.600	31.000	42.000	10.658	10	-0.620	2.223	5.742	34.233	1.0000
*0.5	25.500	0.7727	25.500	4.000	36.000	40.890	10	2.904	2.223	5.742	25.500	0.7449
*1	0.900	0.0273	0.900	0.000	6.000	212.439	10	12.429	2.223	5.742	0.900	0.0263
2	0.000	0.0000	0.000	0.000	0.000	0.000	10				0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.88078	0.93	-1.15	4.30868
Bartlett's Test indicates unequal variances (p = 3.54E-05)	25.7609	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs Dyberry Creek	0.25	0.5	0.35355	400	5.74196	0.174	2089.67	33.3489	8.1E-18	4, 45

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05	0.2990	0.0420	0.2213	0.3717	-0.8406
IC10	0.3480	0.0485	0.2919	0.4952	1.5487
IC15	0.3970	0.0555	0.3277	0.5270	0.8493
IC20	0.4460	0.0573	0.3581	0.5551	0.2181
IC25	0.4950	0.0563	0.3869	0.5832	-0.1877
IC40	0.6008	0.0532	0.4702	0.6687	-0.7559
IC50	0.6704	0.0480	0.5435	0.7275	-1.1520



Reviewed by:

NJPDES BIOMONITORING REPORT FORM
FRESHWATER CHRONIC TOXICITY TEST

NJPDES #: N/A — Dyberry Creek / Flowback

FACILITY NAME: Delaware River Basin Commission
FACILITY LOCATION: 25 State Police Drive, P.O. Box 7360 West Trenton, N.J. 08628-0360

LABORATORY: American Aquatic Testing, Inc.

ACUTE TOXICITY CERTIFICATION NUMBER: PA682

SRT INFORMATION				
	DATE:	July 19, 2012	IC_{25}:	2307.7 ppm
	CONTROL CHART		UCL:	3479.3 ppm
	MEAN:	2124.8 ppm	LCL:	902.8 ppm

TEST START DATE: July 17, 2012 TEST END DATE: July 21, 2012

TEST TYPE AND RESULTS **(Check applicable test, circle applicable endpoint and fill in NOEC &/or IC_{25})**

X *Selenastrum capricornutum*, NOEC <0.125% IC_{25} 0.08%
PMSD 8.44%

Method 1003.0 (Green Alga *Selenastrum capricornutum,* Growth Test)

____ Cladoceran, (CN/CD) NOEL _____, IC_{25} _____

Method 1001.0 (*Ceriodaphnia dubia* 3 brood Survival and Reproduction Test)

CONTROL GROWTH: **4.118 x 10^6 cells/mL**

Did the test meet the acceptability criteria for the test species as specified in Part III of the Chronic Methods Document? **YES**

ACCURACY OF REPORT CERTIFIED BY

Christopher J. Nally
President, Laboratory Director

08/01/12
Date

TEST DESIGN

NUMBER OF CONCENTRATIONS:	5
NUMBER OF REPLICATES / CONCENTRATION:	4
NUMBER OF TEST ORGANISMS / REPLICATE:	10,000 cells/mL
DILUTION SERIES:	0.5
TEST VESSEL SIZE:	125 mL
TEST SOLUTION VOLUME:	25 mL
EXPLAIN ANY DEVIATIONS FROM SPECIFIED METHODS;	N/A

EFFLUENT SAMPLING

SAMPLE LOCATION:	Flowback water from natural gas drilling operation
EFFLUENT TYPE:	untreated
DISCHARGE:	N/A
RETENTION TIME:	N/A
SAMPLE TYPE:	composite ___ Other ___X___, (grab)

Sample Collection		Sample Data taken upon arrival at laboratory		Use in Toxicity Test	
Beginning Date & Time	Ending Date & Time	Dissolved Oxygen (ppm)	pH (Std. Units)	Use Date	Time
07/16/12 1045	07/16/12 1045	N/A	4.9	07/17-20/12	1900

Maximum holding time for any effluent sample: N/A

Describe any pretreatment of the effluent: N/A

TESTING LOCATION

REMOTE LABORATORY	X
ON-SITE LABORATORY	
ON-SITE COMMERCIAL LABORATORY	

DILUTION WATER

EFFLUENT RECEIVING WATER:	N/A
DILUTION WATER:	Dyberry Creek
DESCRIBE ANY ADJUSTMENT TO THE DILUTION WATER:	N/A
IF RECEIVING WATER IS USED AS DILUTION SOURCE, DESCRIBE COLLECTION LOCATION AND DATES OF COLLECTION:	07/16/12

SUMMARY SHEET FOR THE FRESHWATER ALGA SELENASTRUM CAPRICORNUTUM

Percent Effluent	Mean Cells/mL x 10^6	Min Cells/mL x 10^6	Max Cells/mL x 10^6	C.V. %
White Clay Creek	4.118	3.831	4.357	5.518
0.125	2.408	2.185	2.586	7.494
0.25	1.281	0.871	1.700	31.082
0.5	0.540	0.508	0.583	5.843
1.0	0.423	0.385	0.461	9.060
2.0	0.468	0.414	0.559	14.164

Organism source: __ cultured stock, _x_ commercial supplier

Name of supplier: ABS, Inc.

Inoculation date: 07/12/12

Age of organisms (hours): 4-7 days

Describe any aeration which was performed during the test: N/A

Describe any adjustments to the salinity of the test concentrations: N/A

How long after test termination were the organisms prepared for analysis? Immediately

Did the temperature in the test chambers vary by more than 01° Celsius each day? No

Did the salinity in the test chambers vary more than 2 ppt between replicates each day? N/A

Freshwater Algae Test

American Aquatic Testing, Inc.

Job #: 123-01-17

Species: S. capricornutum

Dilution Water: Dyberry Creek

Start Date/Time: 7-19-12 1745

End Date/Time: 07/23/12 1640

Test Type: 96hr. SMR

Concentration %	pH (standard units)					Temperature (C)				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Control	7.7	8.1	8.3	8.7	8.8	25.0	25.5	25.5	25.5	25.6
0.125	7.4	8.0	8.4	8.8	8.6	25.0	25.6	25.5	25.5	25.5
0.25	7.4	8.0	8.2	8.5	8.1	26.0	26.5	25.5	25.5	25.5
0.5	7.3	7.9	8.1	8.2	8.0	26.0	26.6	25.5	25.5	25.5
1.0	7.3	7.8	7.9	7.9	7.8	25.0	26.5	25.5	25.5	25.5
2.0	7.2	7.8	7.9	7.9	7.7	25.0	26.5	25.5	25.5	25.5
Initials	TB		MP	MP				MP	MP	
Date	7/19	07/20	7/21	7/22	07/23	07/19	07/20	7/21	7/22	07/23

Concentration %	Conductivity (umhos)	Alkalinity (mg/l)	Hardness (mg/l)
Control	75		
0.125	428		
0.5	1651		
2.0	6880		
Initials	TAP		
Date	7/19		

Reading Date	Light Intensity (ft-c)
7/19	407
07/20	375
7/21	409
7/22	423
07/23	411
Initials	TB

Concentration %	Cell Density (1 x 10 cells/ml) Replicate 1	2	3	4	5	Mean Density (1 x 10 cells/ml)	Comments
Control	4.057	3.831	4.357	4.226		4.118	
0.125	2.342	2.185	2.586	2.517		(1)	
0.25	1.020	0.871	1.534	1.700		1.281	
0.5	0.537	0.558	0.583	0.530		0.540	
1.0	0.460	0.385	0.395	0.461		0.423	
2.0	0.424	0.414	0.476	0.689		0.468	
Initials							
Date	07/23	07/23	07/23	07/23		07/24	

Observations: ① 2.408 07/24

0.0752%

ACFWALGA.WK3

Phytoplankton Test-Growth-Cell Density

Start Date:	7/19/2012	Test ID:	1230117dsc	Sample ID:	DYBERRY CR
End Date:	7/23/2012	Lab ID:	AAT, INC	Sample Type:	GRAB
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	SC-Selenastrum capricornutum
Comments:					

Conc-%	1	2	3	4
Dyberry Creek	4057000	3831000	4357000	4226000
EPA Soft	4576000	4366000	4687000	4264000
0.125	2342000	2185000	2586000	2517000
0.25	1020000	871000	1534000	1700000
0.5	537000	508000	583000	530000
1	450000	385000	395000	461000
2	424000	414000	476000	559000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Dyberry Creek	4117750	0.9205	4117750	3831000	4357000	5.518	4	*			4117750	1.0000
EPA Soft	4473250	1.0000	4473250	4264000	4687000	4.310	4					
*0.125	2407500	0.5382	2407500	2185000	2586000	7.494	4	11.858	2.410	347597	2407500	0.5847
*0.25	1281250	0.2864	1281250	871000	1700000	31.082	4	19.666	2.410	347597	1281250	0.3112
*0.5	539500	0.1206	539500	508000	583000	5.843	4	24.809	2.410	347597	539500	0.1310
*1	422750	0.0945	422750	385000	461000	9.060	4	25.619	2.410	347597	445500	0.1082
*2	468250	0.1047	468250	414000	559000	14.164	4	25.303	2.410	347597	445500	0.1082

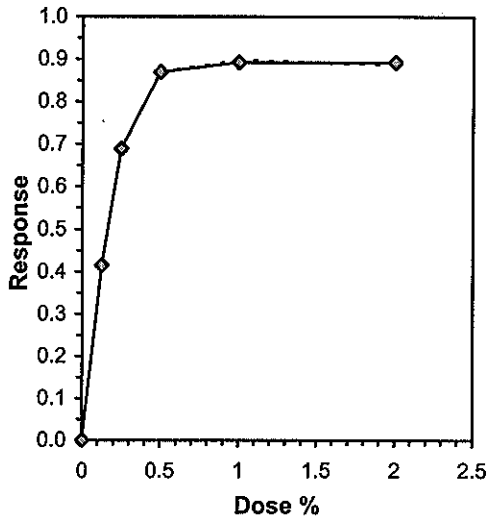
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95935	0.884	-0.0798	0.91736
Bartlett's Test indicates unequal variances (p = 7.13E-04)	21.2938	15.0863		
The control means are significantly different (p = 0.05)	2.38592	2.44691		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs Dyberry Creek	<0.125	0.125			347597	0.08441	8.7E+12	4.2E+10	2.8E-15	5, 18

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	0.0150	0.0009	0.0129	0.0181	0.4693
IC10*	0.0301	0.0018	0.0257	0.0362	0.4693
IC15*	0.0451	0.0027	0.0386	0.0543	0.4693
IC20*	0.0602	0.0036	0.0514	0.0724	0.4693
IC25*	0.0752	0.0045	0.0643	0.0905	0.4693
IC40*	0.1204	0.0078	0.1028	0.1517	0.6662
IC50	0.1637	0.0107	0.1336	0.2034	0.3259

* indicates IC estimate less than the lowest concentration



0.0752% = 752ppm

TEST ENDED EARLY – EXCESSIVE MORTALITY

NJPDES BIOMONITORING REPORT FORM
FRESHWATER CHRONIC TOXICITY TEST

NJPDES #: N/A — Dyberry Creek / Flowback

FACILITY NAME: Delaware River Basin Commission
FACILITY LOCATION: 25 State Police Drive, P.O. Box 7360 West Trenton, N.J. 08628-0360

LABORATORY: American Aquatic Testing, Inc.

ACUTE TOXICITY CERTIFICATION NUMBER: PA682

SRT INFORMATION	DATE:	July 17, 2012	IC_{25}:	554.8 ppm
	CONTROL CHART		UCL:	638.0 ppm
	MEAN:	588.7 ppm	LCL:	539.4 ppm

TEST START DATE: July 17, 2012 TEST END DATE: July 23, 2012

TEST TYPE AND RESULTS **(Check applicable test, circle applicable endpoint and fill in NOEC &/or IC_{25})**

__X__ Fathead minnow, NOEC <0.125% (Survival) IC_{25} 0.04% (estimate)
PMSD 3.6%

Method 1000.0 (*Pimephales promelas* 7 day Larval Survival and Growth Test)

____ Cladoceran, (CN/CD) NOEL _____, IC_{25} _____

Method 1002.0 (*Ceriodaphnia dubia* 3 brood Survival and Reproduction Test)

CONTROL MORTALITY: **15 %**

Did the test meet the acceptability criteria for the test species as specified in Part III of the Chronic Methods Document? **YES**

ACCURACY OF REPORT CERTIFIED BY:

Christopher J. Nally — Date 08/2/12
President, Laboratory Director

TEST ENDED EARLY – EXCESSIVE MORTALITY

TEST DESIGN

NUMBER OF CONCENTRATIONS:	05
NUMBER OF REPLICATES / CONCENTRATION:	04
NUMBER OF TEST ORGANISMS / REPLICATE:	10
DILUTION SERIES:	0.5
TEST VESSEL SIZE:	1.0 L
TEST SOLUTION VOLUME:	500 mL
EXPLAIN ANY DEVIATIONS FROM SPECIFIED METHODS;	N/A

EFFLUENT SAMPLING

SAMPLE LOCATION:	Flowback water from natural gas drilling operation	
EFFLUENT TYPE:		untreated
DISCHARGE:		N/A
RETENTION TIME:		N/A
SAMPLE TYPE:	composite ____ Other ___x__, (Grab)	

Sample Collection		Sample Data taken upon arrival at laboratory		Use in Toxicity Test	
Beginning Date & Time	Ending Date & Time	Dissolved Oxygen (ppm)	pH (Std. Units)	Date(s)	Time(s)
07/16/12, 1045	07/16/12, 1045	N/A	4.9	07/17-22/12	1800+/- 1 hr

Maximum holding time for any effluent sample: 72 hours

Describe any pretreatment of the effluent: N/A

TESTING LOCATION

REMOTE LABORATORY	X
ON-SITE LABORATORY	
ON-SITE COMMERCIAL LABORATORY	

DILUTION WATER

EFFLUENT RECEIVING WATER:	N/A
DILUTION WATER:	Dyberry Creek
DESCRIBE ANY ADJUSTMENT TO THE DILUTION WATER:	N/A
IF RECEIVING WATER IS USED AS DILUTION SOURCE, DESCRIBE COLLECTION LOCATION AND DATES OF COLLECTION:	07/16/12

TEST ENDED EARLY – EXCESSIVE MORTALITY

SUMMARY SHEET FOR THE FATHEAD MINNOW, SHEEPSHEAD MINNOW, INLAND SILVERSIDE AND MYSID SHRIMP TESTS

Percent Effluent	Mean Percent Survival	Mean Dry Weight (mg.)	Percent of Surviving Females with Eggs
Control – White Clay Creek	85	-	N/A
12.5	90	-	N/A
25.0	00	-	N/A
50.0	00	-	N/A
75.0	00	-	N/A
100.0	00	-	N/A

Organism source: __ cultured stock, _x_ commercial supplier

Name of supplier: ABS, Inc.

Hatch date: 07/16/12

Age of organisms (hours): < 48

Describe any aeration which was performed during the test: N/A

Describe any adjustments to the salinity of the test concentrations: N/A

How long after test termination were the organisms prepared for weighing/drying? Immediately

Was the average dry weight per test chamber determined by dividing the final dry weight by the number of original test organisms in the test chamber? Yes

Did the temp. in the test chambers vary by more than 01 ° Celsius each day? No

Did the salinity in the test chambers vary more than 2 ppt between replicates each day? N/A

How long after test termination were the mysids examined for eggs and sexes? * N/A

* Applies only to the Mysid shrimp test

Client/Toxicant: 123
Project Number: 01-17
Species: P. promelas

Beginning Date & Time: 7-17-12 1800
Ending Date & Time: 07/23/12 1200
Hatch Date: 7-16-12

Chronic Test
American Aquatic Testing, Inc.
Live Count

Conc.	Rep	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	
	C	10	10	10	5^{5} ①	4^{4} ①	4	4	
	D	10	10	10	10	10	10	10	
0.125	A	10	10	9^{1}	4^{5}	3^{2}	1^{2}	0^{1}	–
	B	10	10	10	7^{3}	5^{2}	3^{2}	1^{2}	
	C	10	10	10	8^{2}	6^{2}	3^{3}	1^{2}	
	D	10	10	10	7^{3}	5^{2}	3^{2}	2^{1}	
0.25	A	10	10	10	②$1^{9}$	1	0^{1}	–	–
	B	10	10	10	2^{8}	0^{2}	0	–	–
	C	10	10	9^{1}	2^{7}	2	0^{2}	–	–
	D	10	10	10	1^{9}	0^{1}	–	–	–
0.5	A	10	10	8^{2}	2^{6}	0^{2}	–	–	–
	B	10	10	10	0^{10}	–	–	–	–
	C	10	10	10	2^{8}	1	0^{1}	–	–
	D	10	10	9^{1}	3^{6}	0^{3}	–	–	–
1.0	A	10	10	8^{2}	1^{7}	0^{1}	–	–	–
	B	10	10	10	0^{10}	–	–	–	–
	C	10	10	10	3^{7}	0^{3}	–	–	–
	D	10	10	10	2^{8}	0^{2}	–	–	–
2.0	A	10	8^{2}	6^{2}	0^{6}	–	–	–	–
	B	10	8^{2}	10	0^{10}	–	–	–	–
	C	10	10	10	0^{10}	–	–	–	–
	D	10	10	9^{1}	0^{9}	–	–	–	–
\	A								
	B								
	C								
	D								
Initials			MMP		MTF	MMP	MMP	MTF	
Date		07/17	7/18	07/19	7-20	7-21	7/22	7-23	

Observations: ① DEAD FISH COVERED IN FUNGUS - REPLACED BEAKER - MTF 7-20-12
② 1 - MTF 7-20-12

S_{IC25} 0.0354% ~~0.0708%~~

Client/Toxicant: 123
Job Number: 01-17
Species: P. promelas

Beginning Date & Time: 7-17-12 1800
Ending Date & Time: 07/23/12 1800 1200

Dyberry Creek

Freshwater Chronic Test
American Aquatic Testing, Inc.,
Physical / Chemical Parameters
Initial Readings

Parameter	Concentration	Day 1	2	3	4	5	6	7	8
Dissolved Oxygen (mg/L)	Control	9.5	8.8	8.0	8.2				
	0.125	9.4	8.6	7.9	8.2				
	0.25	9.4	8.5	7.9	8.2				
	0.50	9.4	8.5	7.9	8.2				
	1.0	9.3	8.5	7.9	8.2				
	2.0	9.3	8.5	8.0	–				
pH	Control	7.8	7.8	7.7	7.5				
	0.125	7.6	7.6	7.4	7.4				
	0.25	7.4	7.5	7.4	7.3				
	0.5	7.3	7.4	7.3	7.3				
	1.0	7.2	7.3	7.3	7.2				
	2.0	7.0	7.2	7.2	–				
	Initials	KAK	TAP	TAP	TAP				
	Date	7/17	7/18	7/19	7/20				

Conductivity (µmhos/cm)		
Date	Control	100%
7/17	76	7160
Initials	TAP	KAK

Alkalinity (mg/L as $CaCO_3$)		
Date	Control	100%
Initials		

Hardness (mg/L as $CaCO_3$)		
Date	Control	100%
Initials		

Final Readings

Parameter	Concentration	Day 1	2	3	4	5	6	7	8
Temperature °C	Control	25.5	25.5	26.0	25.5	25.5	25.5		
	0.125	25.5	25.0	26.0	25.5	25.0	25.5		
	0.25	25.5	25.5	26.0	25.5	25.0	–		
	0.5	25.5	25.5	26.0	25.5	25.0	–		
	1.0	25.5	25.5	26.0	25.5	–	–		
	2.0	25.5	25.5	26.0	–	–	–		
Dissolved Oxygen (mg/L)	Control	7.6	6.9	6.9	7.5	6.3	6.6		
	0.125	7.6	7.0	6.8	7.6	6.9	7.0		
	0.25	7.5	6.8	6.8	7.7	7.0	–		
	0.5	7.5	6.6	7.2	7.7	6.9	–		
	1.0	7.4	6.7	7.1	7.4	–	–		
	2.0	7.5	6.6	7.3	–	–	–		
pH	Control	7.7	7.6	7.6	7.6	7.4	7.6		
	0.125	7.5	7.5	7.5	7.5	7.3	7.5		
	0.25	7.5	7.4	7.4	7.5	7.3	–		
	0.5	7.5	7.8	7.4	7.4	7.3	–		
	1.0	7.4	7.3	7.3	7.4	–	–		
	2.0	7.2	7.2	7.3	–	–	–		
	Initials	MP	DTAP	DL	MP	MP	MP		
	Date	7/18	7/19	07/20	7/21	7/22	7-23		

Ammonia / Chlorine		
Date	NH_3	Res Cl_2
Initials		

Observations:

Client/Toxicant: 123
Project Number: 01-17
Species: P. promelas

Beginning Date & Time: 7-17-12 1800
Ending Date & Time: 7/24/12 1200
Hatch Date: 7-16-12

Chronic Test

American Aquatic Testing, Inc.

Weight Data

Conc.	Rep	Pan #	A weight of boat (g)	B weight of boat & fish (g)	(B−A)*1000=C dry weight of fish (mg)	D # of surviving fish	C/D mean dry weight (mg)	C/E IC_{25} & NOEC calc. weight (mg)
Control	A							
	B							
	C							
	D							
0.125	A							
	B							
	C							
	D							
0.25	A							
	B							
	C							
	D							
0.5	A							
	B							
	C							
	D							
1.0	A							
	B							
	C							
	D							
2.0	A							
	B							
	C							
	D							
\	A							
	B							
	C							
	D							
	Initials							
	Date							

E = Original number of organisms at test initiation, adjusted for losses.

Observations:

Date/Time In:
Temp:

Date/Time Out:
Temp:

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	7/19/2012	Test ID:	1230117pdc	Sample ID:	Dyberry Creek & Flowback
End Date:	7/23/2012	Lab ID:	AAT, INC	Sample Type:	GRAB
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2	3	4
Dyberry Creek	1.0000	1.0000	0.4000	1.0000
0.125	0.0000	0.1000	0.1000	0.2000
0.25	0.0000	0.0000	0.0000	0.0000
0.5	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Dyberry Creek	0.8500	1.0000	1.2302	0.6847	1.4120	29.560	4				0.8500	1.0000
*0.125	0.1000	0.1176	0.3165	0.1588	0.4636	39.374	4	4.754	1.943	0.3735	0.1000	0.1176
0.25	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4				0.0000	0.0000
0.5	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4				0.0000	0.0000
1	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4				0.0000	0.0000
2	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.77548	0.749	-1.733	3.04159
F-Test indicates equal variances (p = 0.11)	8.51641	47.4683		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates significant differences Treatments vs Dyberry Creek	0.31734	0.3572	1.66973	0.07388	0.00315	1, 6

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	0.0071	0.0004	0.0063	0.0087	0.9300
IC10*	0.0142	0.0008	0.0125	0.0174	0.9300
IC15*	0.0213	0.0012	0.0188	0.0261	0.9300
IC20*	0.0283	0.0017	0.0251	0.0348	0.9300
IC25*	0.0354	0.0021	0.0314	0.0435	0.9300
IC40*	0.0567	0.0033	0.0502	0.0696	0.9300
IC50*	0.0708	0.0042	0.0627	0.0869	0.9300

* indicates IC estimate less than the lowest concentration