Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L16-15975-01

Report of Chronic Biomonitoring

Samples Collected on:

7/11/16
7/13/16
7/15/16

Pimephales promelas
Ceriodaphnia dubia

Allen County Sanitary Engineer
America-Bath WWTP
3226 North Cole Street
Lima, OH 45805

Alloway
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Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L16-15975-01

Date: 7/30/16

Rick Boerger
Allen County Sanitary Engineer
3226 North Cole Street
Lima, OH 45805

Dear Mr. Boerger:

Enclosed is the report of the biomonitoring on the sample listed above. The objective of the chronic biomonitoring was to determine the effects, both the acute endpoint (mortality) and chronic endpoints (mortality and growth, or reproduction), from the test water. Fathead minnow larvae (*Pimephales promelas*) and neonate water fleas (*Ceriodaphnia dubia*) were the test organisms.

Acute toxicity was determined by locating the median lethal concentration (LC50) and the median effective concentration (EC50). Growth was determined from the dry weight of surviving *Pimephales promelas* larvae. Reproduction was monitored daily by counting the number of young produced by each female *Ceriodaphnia dubia*.

Dissolved oxygen, pH, and temperature were recorded daily at each renewal and at the end of each 24-hour exposure period. Results may be found in Appendix C "Parameter Data Sheets". Samples were analyzed for alkalinity, hardness and conductivity. These results may also be found in Appendix C.

Alloway (Lima, Ohio) performs biomonitoring testing in accordance with procedures established by the Environmental Protection Agency. The Standard Operating Procedures for the testing is on file with the Ohio EPA.

Please call if you have any questions or need additional information.

Richard F. Rupp
Bioassay Lab Manager

Brian Good
Bioassay Analyst

Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PH00007*KD

1. Facility Name	Allen County Sanitary Engineer
2. Facility Address	America-Bath WWTP 3226 North Cole Street Lima, OH 45805
3. Ohio EPA Permit No.	2PH00007*KD
4. NPDES No.	OH0023841
5. Facility Contact	Rick Boerger
6. Telephone No.	419-225-8084
7. Consulting Laboratory	Alloway
8. Lab Contact	Dick Rupp or Brian Good
9. Lab Telephone No.	419-223-1362
10. Receiving water(s) of discharge	Pike Run
11. Outfall(s) tested	Effluent 001

12. Is your current Standard Operating Procedure (SOP) Manual on file with Ohio EPA? Yes
If yes, indicate date in which SOP was submitted to the EPA: 1/21/2005

I certify under penalty of law that I have personally examined and am familiar with the information submitted in this document and based on my inquiry of those individuals immediately responsible for obtaining information, I believe the submitted information is true, accurate and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment.

Signature _____ Date _____

Name (typed or printed) _____ Title _____

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*KD
Date of Test: 7/12-19/16

Summary of Chronic Toxicity Test Results
Pimephales promelas

Effluent/Outfall:	4500 Form Value	MDL	4500 Comments Required
Chronic Toxicity, *Pimephales promelas* - TUc	AA	1.0	
Acute Toxicity, *Pimephales promelas* - TUa	AA	0.2	
Upstream:			
96 Hr. Acute Toxicity, *Pimephales promelas* - % Affected	AA	2.5	
7 day Chronic Toxicity, *Pimephales promelas* - % Affected	7.5	2.5	

AA = below detectable

Conclusion/Comments:

1101 N. Cole St. • Lima, Ohio 45805
419.223.1362 • Fax 419.227.3792
800.436.1243

1502 W. Fourth St. • Mansfield, Ohio 44906
419.525.1644 • Fax 419.524.5575
800.635.3222

1776 Marion-Waldo Rd. • Marion, Ohio 43302
740.389.5991 • Fax 740.389.1481
800.873.2835

www.alloway.com

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*KD
Date of Test: 7/12-18/16

Summary of Chronic Toxicity Tedt Results
Ceriodaphnia dubia

Effluent/Outfall:	4500 Form Value	MDL	4500 Comments Required
Chronic Toxicity, *Ceriodaphnia dubia* - TUc	AA	1.0	
Acute Toxicity, *Ceriodaphnia dubia* - TUa	AA	0.2	
Upstream:			
48 Hr. Acute Toxicity, *Ceriodaphnia dubia* - % Affected	AA	10.0	
7 day Chronic Toxicity, *Ceriodaphnia dubia* - % Affected	AA	10.0	

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*KD
Date of Test: 7/12-19/16

Chronic Toxicity Test Results

Results of a 7-Day Pimephales promelas Survival and Growth Test

Test Solutions	Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)* Test Day							Mean Dry Weight*	
	1	2	3	4	5	6	7	Mean	SD
Primary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	7.5% (7.5%)	7.5% (7.5%)	7.5% (7.5%)	0.733	0.079
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	12.5% (12.5%)	0.651	0.141
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.703	0.069
40%	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	7.5% (7.5%)	7.5% (7.5%)	10.0% (10.0%)	0.712	0.110
60%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.721	0.154
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.735	0.051
100%	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	12.5% (12.5%)	0.740	0.163

LC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Survival** <1.0
95% Confidence Limits	LL								
	UL								
EC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Growth (100/IC25)** <1.0
95% Confidence Limits	LL								
	UL								

7-Day NOEC for Mortality (%)	100		7-Day NOEC for Growth (%)	100	**Method(s) used to determine Values:**
7-Day LOEC for Mortality (%)	>100		7-Day LOEC for Growth (%)	>100	
Chronic Value for Mortality (%)	>100		Chronic Value for Growth (%)	>100	
IC25 (%)	>100		IC50 (%)	>100	IC Pin, Dunnett's

*indicates significant differences from the primary control with an * ($p<0.05$)

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*KD
Date of Test: 7/12-18/16

Chronic Toxicity Test Results

Results of a Ceriodaphnia dubia Survival and Reproduction Test

Test Solutions	Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)* Test Day							Number of Young Produced
	1	2	3	4	5	6	7	Mean
Primary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	26.8
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	28.0
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	30.2
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	29.4
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	32.7
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	31.7
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	28.0
LC50 Values (%):	>100	>100	>100	>100	>100	>100	---	**Calculated TUc Value for Survival** <1.0
95% Confidence Limits LL								
95% Confidence Limits UL								
EC50 Values (%):	>100	>100	>100	>100	>100	>100	---	**Calculated TUc Value for Reproduction** <1.0
95% Confidence Limits LL								
95% Confidence Limits UL								

NOEC for Mortality (%)	100		NOEC for Reproduction (%)	100	**Method(s) used to determine Values:**
LOEC for Mortality (%)	>100		LOEC for Reproduction (%)	>100	
Chronic Value for Mortality (%)	>100		Chronic Value for Reprod (%)	>100	
IC25 (%)	>100		IC50 (%)	>100	IC Pin, Wilcoxon Rank Sum Test

*indicates significant differences from the primary control with an * (p<0.05)

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PH00007*KD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection Beginning	Sample Collection Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	7/10/16 @ 08:30	7/11/16 @ 08:30
Type (Grab/Comp):	Comp	2nd	7/12/16 @ 08:30	7/13/16 @ 08:30
Volume Collected:	5 Gal.	3rd	7/14/16 @ 08:30	7/15/16 @ 08:30
Upstream Station:				
Waterbody:	Pike Run			
Station No.:	801	1st		7/11/16 @ 10:48
Type (Grab/Comp):	Grab	2nd		7/13/16 @ 10:10
Volume Collected:	5 Gal.	3rd		7/15/16 @ 11:13

Samples collected by: Alloway

OEPA Permit No.: 2PH00007*KD Allen Co. (American-Bath WWTP)

PIMEPHALES PROMELAS CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	*Pimephales promelas* < 24 hours old
2. Test Type and Duration:	Static renewal; 7 days
3. Test Dates:	7/12-19/16
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Feed 0.1 g (0.1 to 0.2 mL) newly hatched brine shrimp nauplii three times daily (M-F) at 4-h intervals. On weekends 0.1g of nauplii are fed at the beginning of the workday, after renewal, and at the end of the workday. Sufficient larvae are added to provide an excess. Fish are not fed during the final 12-h of the test.
8. Size of Test Vessel:	500 mL plastic cup
9. Volume and Depth of Test Solutions:	250 mL, 6.7 cm deep
10. No. of Test Organisms per Test Vessel:	10 per test vessel
11. No. of Test Vessels per Test Solution:	4 per solution tested
12. Total No. of Test Organisms per Test Solution:	40 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 7/11/16 ; day 2, 3 - 7/13/16 ; day 4, 5, 6 - 7/15/16
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and growth (dry weight).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

OEPA Permit No.: 2PH00007*KD Allen Co. (American-Bath WWTP)

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	*Ceriodaphnia dubia* 13-21 hours
2. Test Type and Duration:	Static renewal; 6 to 8 days (6 days)
3. Test Dates:	7/12-18/16
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Fed 0.1 mL YCT and 0.1 mL (3.5 million cells) *Selenastrum capricornutum.*
8. Size of Test Vessel:	1 fl. oz. (30 mL) clear plastic cup
9. Volume and Depth of Test Solutions:	15 mL, 20 mm deep
10. No. of Test Organisms per Test Vessel:	1 per test vessel
11. No. of Test Vessels per Test Solution:	10 per solution tested
12. Total No. of Test Organisms per Test Solution:	10 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 7/11/16 ; day 2, 3 - 7/13/16 ; day 4, 5, 6 - 7/15/16
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data

Alloway
Your Resource for Defensible Data

Chain of Custody Record

This is a legal document that authorizes Alloway to perform testing on samples submitted under this agreement.

- 1101 North Cole Street, Lima, OH 45805 (P) 419-223-1362 (F) 419-227-3792
- 1776 Marion-Waldo Road, Marion OH 43302 (P) 740-389-5991 (F) 740-389-1481
- 1502 W. Fourth Street, Mansfield, OH 44906

Project: L16-15975

Report To: Name:	Rick Boerger
Company:	Allen County Sanitary Engineer
Address:	3226 North Cole St. Lima, OH 45805
Invoice To (If Different): Name:	
Company:	
Address:	
Phone #:	419-225-8048
Fax #:	
E-mail:	rboerger@allencountyohio.com
PO#:	
Project Name	Bioassay - AM BATH - DAY 1
Sampler (Print)	Michael S. Hanly
(Signature)	
Next Day	☐
2 Working Days	☐

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	7/10/16 - 7/11/16	08:30 - 08:30	X		WW	1	1	Toxicity	
2	Upstream	7/11/16	10:48		X	SW	1	1	Toxicity	
3										
4										
5										
6										
7										
8										

	Relinquished by:	Received by:	Date	Time
1				
2				
3				
4				
5				
6				

Received for Laboratory By: (circle one): Mansfield Lima Marion

(Signature) 7/11/16 11:01

Method of Delivery: UPS ☐ Fed Ex ☐ Client ☐ Alloway Pick Up ☐ Alloway Sampling ☑ Other ☐

Matrix Codes: ww - wastewater; gw - groundwater; dw - drinking water; sw - surface water; w - water; oil - oil; s - solid; sg - sludge; l - leachate; a - acid; p - product; o - other

Preservation Codes: 1 - None; 2 - HNO_3; 3 - H_2SO_4; 4 - HCl; 5 - NaOH; 6 - NaOH & Zinc Acetate; 7 - Sodium Thiosulfate; 8 - Ascorbic Acid; 9 - Maleic Acid; 10 - EDA; 11 - Ammonium Chloride; 12 - $(NH_4)_2SO_4$ & NH_4OH; 13 - Zinc Acetate; 14 - Sodium Sulfite; 15 - Potassium Dihydrogen Citrate; 16 - Sodium Sulfite/Sodium Bisulfate

Sample Receiving (For Lab Use Only)

Ice Present? Y ☑ N ☐

Proper Preservation? Y ☑ N ☐

Container Temperature: _____

Transported to: Lima Marion By: _____ Received By: _____ Date: _____ Time: _____

Transported to: Lima Marion By: _____ Received By: _____ Date: _____ Time: _____

ACSE Biomonitoring Field Data Sheet

Client: Am. Bath **Field Technicians:** MSH

Date: 7/11/16

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	10:30	22.6	850	10.86	7.43
Upstream	10:48	21.5	702	10.90	7.67

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- ☐ Cloudy
- ☐ Heavy Rain
- ☐ Light Rain
- ☐ Clear Skies
- ☒ Sunny
- ☐ Snow
- ☐ _____

Stream Conditions

- ☒ Rapid and Complete Mixing
- ☐ Mixing is not Rapid and Complete

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Alloway
Your Resource for Defensible Data

Chain of Custody Record

This is a legal document that authorizes Alloway to perform testing on samples submitted under this agreement.

- 1101 North Cole Street, Lima, OH 45805 (P) 419-223-1362 (F) 419-227-3792
- 1776 Marion-Waldo Road, Marion OH 43302 (P) 740-389-5991 (F) 740-389-1481
- 1502 W. Fourth Street, Mansfield, OH 44906 (P) 419-525-1644 (F) 419-524-5575

Report To:
Name: Rick Boerger
Company: Allen County Sanitary Engineer
Address: 3226 North Cole St.
Lima, OH 45805

Invoice To (If Different):
Name:
Company:
Address:

Notes/Comments:

Phone #: 419-225-8048 Fax #:
E-mail: rboerger@allencountyohio.com PO#:

Project Name: Bioassay - AM BATH - DAY 2

Sampler: (Print) Michael S. Hanly (Signature)

Turnaround: (*Rush Charges May Apply*)
Next Day ☐ 3 Working Days ☐ Routine ☑
2 Working Days ☐ 5 Working Days ☐

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	7/12/16 - 7/13/16	08:30 - 08:30	X		WW	1	1	Toxicity	
2	Upstream	7/13/16	10:10		X	SW	1	1	Toxicity	
3										
4										
5										
6										
7										
8										

	Relinquished by:	Received by:	Date	Time
1				
2				
3				
4				
5				
6				

Received for Laboratory By: (circle one): Mansfield Lima Marion
(Signature) 7/13/16 10:26

Method of Delivery
UPS ☐
Fed Ex ☐
Client ☐
Alloway Pick Up ☐
Alloway Sampling ☑
Other ☐

Matrix Codes:
ww - wastewater
gw - groundwater
dw - drinking water
sw - surface water
w - water
oil - oil
s - solid
sg - sludge
l - leachate
a - acid
p - product
o - other

Preservation Codes:
1 - None
2 - HNO_3
3 - H_2SO_4
4 - HCl
5 - NaOH
6 - NaOH & Zinc Acetate
7 - Sodium Thiosulfate
8 - Ascorbic Acid
9 - Maleic Acid
10 - EDA
11 - Ammonium Chloride
12 - $(NH_4)_2SO_4$ & NH_4OH
13 - Zinc Acetate
14 - Sodium Sulfite
15 - Potassium Dihydrogen Citrate
16 - Sodium Sulfite/Sodium Bisulfate

Sample Receiving (*For Lab Use Only*)
Ice Present? Y ☒ N ☐
Proper Preservation? Y ☒ N ☐
Container Temperature: _____

Transported to: Lima Marion By: _____ Received By: _____ Date: _____ Time: _____

Transported to: Lima Marion By: _____ Received By: _____ Date: _____ Time: _____

ACSE Biomonitoring Field Data Sheet

Client: Am. Bath **Field Technicians:** MSH

Date: 7/13/16

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	09:50	24.2	856	11.08	7.38
Upstream	10:10	23.7	467	9.21	7.39

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- ☐ Cloudy
- ☐ Heavy Rain
- ☐ Light Rain
- ☒ Clear Skies
- ☐ Sunny
- ☐ Snow
- ☒ Hot

Stream Conditions

- ☒ Rapid and Complete Mixing
- ☐ Mixing is not Rapid and Complete

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Alloway
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Chain of Custody Record

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- 1776 Marion-Waldo Road, Marion OH 43302 (P) 740-389-5991 (F) 740-389-1481
- 1502 W. Fourth Street, Mansfield, OH 44906 (P) 419-525-1644 (F) 419-524-5575

Report To:
Name: Rick Boerger
Company: Allen County Sanitary Engineer
Address: 3226 North Cole St. Lima, OH 45805

Invoice To (If Different):
Name:
Company:
Address:

Notes/Comments:

Phone #: 419-225-8048 Fax #:
E-mail: rboerger@allencountyohio.com PO#:

Project Name: Bioassay - AM BATH - DAY 3

Sampler: (Print) Michael S. Hanley (Signature)

Turnaround: (*Rush Charges May Apply*)
Next Day ☐ 3 Working Days ☐ Routine ☑
2 Working Days ☐ 5 Working Days ☐

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	7/14/16 - 7/15/16	08:30 - 08:30	X		WW	1	1	Toxicity	
2	Upstream	7/15/16	11:13		X	SW	1	1	Toxicity	
3										
4										
5										
6										
7										
8										

	Relinquished by:	Received by:	Date	Time
1				
2				
3				
4				
5				
6				

Received for Laboratory By: (circle one): Mansfield / Lima / Marion
(Signature)
Date: 7/15/16 Time: 11:27

Method of Delivery: UPS ☐ Fed Ex ☐ Client ☐ Alloway Pick Up ☐ Alloway Sampling ☑ Other ☐

Matrix Codes: ww - wastewater; gw - groundwater; dw - drinking water; sw - surface water; w - water; oil - oil; s - solid; sg - sludge; l - leachate; a - acid; p - product; o - other

Preservation Codes: 1 - None; 2 - HNO_3; 3 - H_2SO_4; 4 - HCl; 5 - NaOH; 6 - NaOH & Zinc Acetate; 7 - Sodium Thiosulfate; 8 - Ascorbic Acid; 9 - Maleic Acid; 10 - EDA; 11 - Ammonium Chloride; 12 - $(NH_4)_2SO_4$ & NH_4OH; 13 - Zinc Acetate; 14 - Sodium Sulfite; 15 - Potassium Dihydrogen Citrate; 16 - Sodium Sulfite/Sodium Bisulfate

Sample Receiving (*For Lab Use Only*)
Ice Present? ☒ Y ☐ N
Proper Preservation? ☒ Y ☐ N
Container Temperature: _____

Transported to: Lima / Marion By: _____ Received By: _____ Date: _____ Time: _____

Transported to: Lima / Marion By: _____ Received By: _____ Date: _____ Time: _____

ACSE Biomonitoring Field Data Sheet

Client: Am. Booth **Field Technicians:** MSH

Date: 7/15/16

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	10:58	24.2	842	11.06	7.36
Upstream	11:13	23.7	558	10.84	7.46

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- ☐ Cloudy
- ☐ Heavy Rain
- ☐ Light Rain
- ☒ Clear Skies
- ☒ Sunny
- ☐ Snow
- ☐ _____

Stream Conditions

- ☒ Rapid and Complete Mixing
- ☐ Mixing is not Rapid and Complete

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Appendix B

Raw Data Sheets

CONDITION OF ORGANISMS* IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00007*KD

Person(s) Conducting Test: BG, SS, RFR
Beginning: Date 7/12/16 **Time** 1420
Ending: Date 7/19/16 **Time** ~~1420~~ 1205
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Upstream

Day	Lab Control 1	Lab Control 2	Lab Control 3	Lab Control 4	Lab Control Total	Upstream Control 1	Upstream Control 2	Upstream Control 3	Upstream Control 4	Upstream Control Total	20% Effluent 1	20% Effluent 2	20% Effluent 3	20% Effluent 4	20% Effluent Total	40% Effluent 1	40% Effluent 2	40% Effluent 3	40% Effluent 4	40% Effluent Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	9G 1D	39G 1D
3	10A	10A	10A	10A	40A	10A	10A	10A	10A	40A	10A	10A	10A	10A	40A	10A	10A	10A	8A 2D	38A 2D
4	10A	10A	10A	10A	40A	10A	10A	10A	10A	40A	10A	10A	10A	10A	40A	10A	10A	10A	8A 2D	38A 2D
5	9G 1D	10G	10G	10G	39G 1D	10G	9G 1D	9G 1D	9G 1D	37G 3D	10G	10G	10G	10G	40G	10G	9G 1D	10G	8G 2D	38G 3D
6	9G 1D	9G 1D	10G	10G	38G 2D	10G	9G 1D	9G 1D	9G 1D	37G 3D	10G	10G	10G	10G	40G	10G	9G 1D	10G	8G 2D	37G 3D
7	6G 4D	9G 1D	10G	9G 1D	35G 5D	10G	9G 1D	9G 1D	9G 1D	37G 3D	10G	10G	10G	10G	40G	9G 1D	9G 1D	10G	8G 2D	37G 3D

36A 4D

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CONDITION OF ORGANISMS* IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00007*KD

Person(s) Conducting Test: BG, SS, RFR
Beginning: Date 7/12/16 **Time** 1420
Ending: Date 7/19/16 **Time** 1205
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Upstream

	60% Effluent					80% Effluent					100% Effluent									
Day	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D					
3	10G	10G	10G	9G 1D	39G 1D	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D					
4	10G	10G	10G	8G 2D	38G 2D	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D					
5	10G	10G	10G	8G 2D	38G 2D	10G	8G 2D	10G	10G	38G ~~40G~~ 2D	10G	10G	9G 1D	10G	39G 1D					
6	10G	10G	10G	8G 2D	38G 2D	10G	8G 2D	10G	10G	38G 2D	10G	10G	9G 1D	10G	39G 1D					
7	10G	10G	10G	8G 2D	38G 2D	10G	8G 2D	10G	10G	38G 2D	9G ~~10G~~ 1D	10G	9G 1D	7G 3D	36G ~~4D~~					
											BG				35G 5D					

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

WEIGHT OF FISH IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Discharge: Allen County, Am. - Bath WWTP
Date of Test: 7/12/16 - 7/19/16
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*KD
Pimephales promelas Age at Start: < 24 Hours
Drying Temperature (C°): 60
Drying Time (h): 24
Date Weighed: 7/20/16
Analyst: ~~SS~~ CE

Balance Calibration Check: 1 gram weight = 0.99999 grams
Date: 7/11/16
Analyst: CE
Dilution Water Used: Upstream

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
Fish at Start of Test		10	1.13029	1.13116	10	0.870	0.087
Lab Control	1	6	1.08963	1.09411	10	4.48	0.448
	2	9	1.13017	1.13678	10	6.61	0.661
	3	10	1.08262	1.09010	10	7.48	0.748
	4	9	1.10407	1.11152	10	7.45	0.745
Upstream Control	1	10	1.11070	1.11824	10	7.54	0.754
	2	9	1.13089	1.13820	10	7.31	0.731
	3	9	1.12691	1.13510	10	8.19	0.819
	4	9	1.09240	1.09869	10	6.29	0.629
20% Effluent	1	10	1.13201	1.13965	10	7.64	0.764
	2	10	1.09763	1.10510	10	7.47	0.747
	3	10	1.09176	1.09868	10	6.92	0.692
	4	10	1.12442	1.13052	10	6.10	0.610
40% Effluent	1	9	1.12990	1.13614	10	6.24	0.624
	2	9	1.12383	1.13206	10	8.23	0.823
	3	10	1.08331	1.09120	10	7.89	0.789
	4	8	1.08790	1.09401	10	6.11	0.611

Reviewed By: BG 7-30-16

WEIGHT OF FISH IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Discharge: Allen County, Am. - Bath WWTP Date of Test: 7/12/16 - 7/19/16

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD *Pimephales promelas* Age at Start: < 24 Hours

Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7/26/16 Analyst: ~~SS~~ CE

Balance Calibration Check: 1 gram weight = 0.99999 grams Date: 7/11/16 Analyst: CE Dilution Water Used: Upstream

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
60% Effluent	1	10	1.12883	1.13546	10	6.63	0.663
	2	10	1.09488	1.10422	10	9.34	0.934
	3	10	1.13210	1.13928	10	7.18	0.718
	4	8	1.09083	1.09653	10	5.70	0.570
80% Effluent	1	10	1.10022	1.10743	10	7.71	0.771
	2	8	1.09081	1.09766	10	6.85	0.685
	3	10	1.10162	1.10949	10	7.87	0.787
	4	10	1.12541	1.13238	10	6.97	0.697
100% Effluent	1	9	1.12109	1.12872	10	7.63	0.763
	2	10	1.08394	1.09282	10	8.88	0.888
	3	9	1.12319	1.13119	10	8.00	0.800
	4	7	1.09966	1.10474	10	5.08	0.508
	1				10		
	2				10		
	3				10		
	4				10		

BG 7-30-16

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	7/12/2016	Test ID:	bath0716p	Sample ID:	L16-15975-01
End Date:	7/19/2016	Lab ID:	ALL-ALLOWAY	Sample Type:	DMR-Discharge Monitoring Report
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2	3	4
Upstr Control	0.7540	0.7310	0.8190	0.6290
20	0.7640	0.7470	0.6920	0.6100
40	0.6240	0.8230	0.7890	0.6110
60	0.6630	0.9340	0.7180	0.5700
80	0.7710	0.6850	0.7870	0.6970
100	0.7630	0.8880	0.8000	0.5080

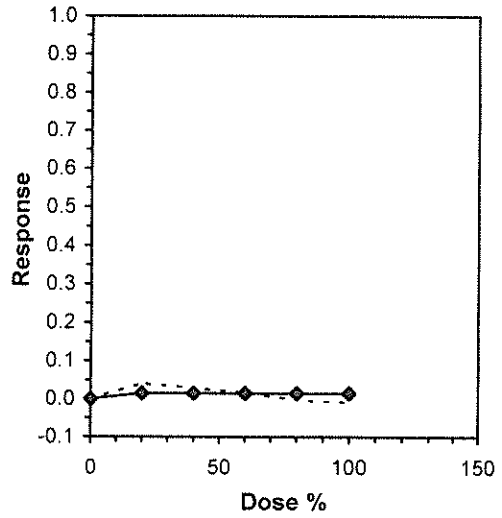
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Upstr Control	0.7333	1.0000	0.7333	0.6290	0.8190	10.755	4				0.7333	1.0000
20	0.7033	0.9591	0.7033	0.6100	0.7640	9.861	4	0.376	2.410	0.1921	0.7222	0.9849
40	0.7118	0.9707	0.7118	0.6110	0.8230	15.432	4	0.270	2.410	0.1921	0.7222	0.9849
60	0.7213	0.9836	0.7213	0.5700	0.9340	21.411	4	0.151	2.410	0.1921	0.7222	0.9849
80	0.7350	1.0024	0.7350	0.6850	0.7870	7.001	4	-0.022	2.410	0.1921	0.7222	0.9849
100	0.7398	1.0089	0.7398	0.5080	0.8880	22.055	4	-0.082	2.410	0.1921	0.7222	0.9849

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.98945	0.916	-0.1836	0.33576
Bartlett's Test indicates equal variances (p = 0.40)	5.13807	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.19205	0.26192	0.00083	0.0127	0.99656	5, 18
Treatments vs Upstr Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



ToxCalc v5.0.32

Reviewed by: _____

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*KD

Person(s) Conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 14:30
Ending Date: 7/18/16 Time: 14:00
Age of *Ceriodaphnia dubia*: 13-21H #121A
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	6 G	0 G	7 G	12 G		25	3
2				6	0	8	15		29	3
3				0	5	8	8		21	3
4				6	0	10	14		30	3
5				6	0	5	15		26	3
6				5	0	8	18		31	3
7				6	0	9	17		32	3
8				6	0	9	15		30	3
9				5	0	7	13		25	3
10	0 G	0 G	0 G	6 G	0 G	10 G	15 G		31	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–	SUM 280	30
									MEAN 28.0	3.0
									S.D. 3.6	0.0

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

Reviewed by: RFR

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Person(s) Conducting Test: RFR, BG, SS
Industry/Toxicant: Allen County, Am. - Bath WWTP
Beginning Date: 7/12/2016 Time: 14:30
Address: 3226 North Cole St., Lima OH 45805
Ending Date: 7/19/16 Time: 14:00
Effluent Serial No.: 001
Age of *Ceriodaphnia dubia*: 13-24 H
NPDES Permit No.: 2PH00007*KD
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	6 / G	0 / G	5 / G	16 / G	/	27	3
2	/	/	/	6 /	0 /	12 /	15 /	/	33	3
3	/	/	/	5 /	0 /	8 /	17 /	/	30	3
4	/	/	/	5 /	0 /	11 /	14 /	/	30	3
5	/	/	/	5 /	0 /	11 /	2 /	/	18	3
6	/	/	/	6 /	0 /	11 /	17 /	/	34	3
7	/	/	/	0 /	0 /	0 /	6 /	/	6	1
8	/	/	/	5 /	0 /	10 /	11 /	/	26	3
9	/	/	/	3 /	1 /	11 /	15 /	/	30	3
10	0 / G	0 / G	0 / G	4 / G	0 / G	10 / G	20 / G	/	34	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–	SUM 268	28
									MEAN 26.8	2.8
									S.D. 8.7	0.6

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Person(s) Conducting Test: RFR, BG, SS
Industry/Toxicant: Allen County, Am. - Bath WWTP
Beginning Date: 7/12/2016 Time: 14:30
Address: 3226 North Cole St., Lima OH 45805
Ending Date: 7/18/16 Time: 14:00
Effluent Serial No.: 001
Age of *Ceriodaphnia dubia*: 13-21 h
NPDES Permit No.: 2PH00007*KD
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	4 G	1 G	11 G	20 G		36	3
2				7	0	7	14		28	3
3				6	0	12	18		36	3
4				5	0	13	18		36	3
5				7	0	7	17		31	3
6				6	0	10	16		32	3
7				5	0	11	2		18	3
8				6	0	9	12		27	3
9				6	0	13	15		34	3
10	0 G	0 G	0 G	3 G	0 G	2 G	19 G		24	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–		
								SUM	302	30
								MEAN	30.2	3.0
								S.D.	6.0	0.0

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested:	40% Effluent	Person(s) Conducting Test:	RFR, BG, SS		
Industry/Toxicant:	Allen County, Am. - Bath WWTP	Beginning Date:	7/12/2016	Time:	1430
Address:	3226 North Cole St., Lima OH 45805	Ending Date:	7/18/16	Time:	1400
Effluent Serial No.:	001	Age of *Ceriodaphnia dubia*:	13-21H		
NPDES Permit No.:	2PH00007*KD	Dilution Water Used:	Upstream		

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	0 G (Abort)	0 G	7 G	16 G		23	3
2				6	0	13	18		37	3
3				5	0	9	18		32	3
4				4	0	11	19		34	3
5				5	0	12	18		35	3
6				5	0	5	17		27	3
7				5	0	10	0		15	2
8				6	0	14	17		37	3
9				5	0	9	18		32	3
10	0 G	0 G	0 G	5 G	0 G	7 G	10 G		22	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	294	29
								MEAN	29.4	2.9
								S.D.	7.4	0.3

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*KD

Person(s) Conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 14:30
Ending Date: 7/18/16 Time: 14:00
Age of *Ceriodaphnia dubia*: 18-21 H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	6 G	0 G	10 G	15 G			31	3
2				6	0	13	18			37	3
3				0	0	11	9			20	2
4				6	0	8	20			34	3
5				6	0	14	13			33	3
6				6 G	0 G	14 G	19 G			39	3
7				0 LA	— —	— —	— —	— —	LA	—	—
8				6 G	0 G	9 10 G	16 G			32	3
9				5	0 G	12	20			37	3
10	0 G	0 G	0 G	4 G	0 G	10 G	17 G			31	3
Total Adult Cond.	10G	10G	10G	9G 1LA	9G 1LA	9G 1LA	9G 1LA	—	n=9		
									SUM	294	26
									MEAN	32.7	2.9
									S.D.	5.5	0.3

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*KD

Person(s) Conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/19/16 Time: 1400
Age of *Ceriodaphnia dubia*: 13-24 H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	2 G	0 G	10 G	18 G		30	3
2				6	0	~~11~~ 14	17		37	3
3				5	0	~~12~~ 11	17		33	3
4				6	0	~~8~~ 11	19		36	3
5				5	0	~~3~~ 6	3		14	3
6				6	0	~~12~~ 14	13		33	3
7				6	0	~~12~~ 14	20		40	3
8				6	0	~~11~~ 12	15		33	3
9				4	0	12	13		29	3
10	0 G	0 G	0 G	5 G	0 G	~~8~~ 10 G	17 G		32	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—	SUM 317	30
									MEAN 31.7	3.0
									S.D. 7.0	0.0

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*KD

Person(s) Conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/18/16 Time: 1400
Age of *Ceriodaphnia dubia*: 13-21 H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	Abort 0 G	0 G	10 G	16 G		26	3
2				6	0	11	14		31	3
3				5	0	12	1		18	3
4				0	5	10	15		30	3
5				5	0	4	16		25	3
6				6	0	13	17		36	3
7				6	0	11	18		35	3
8				6	0	12	18		36	3
9				6	0	13	4		23	3
10	0 G	0 G	0 G	2 G	0 G	8 G	10 G		20	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—		
								SUM	280	30
								MEAN	28.0	3.0
								S.D.	6.6	0.0

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.
LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA BROOD STOCK INDIVIDUAL CULTURE INFORMATION FORM FOR *C. DUBIA* BROODS USED IN CHRONIC TESTS

Discharger/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Type of Test: Chronic Date of Test: 7/12/16 - 7/18/16
Effluent Serial No.: 001 Permit No.: 2PH00007*KD

Investigator: RCR Culture No.: 121 A
Culture initiation date: 7/6/16 Culture ending date: 7/13/16
Origin of parental organisms: 117 Allowy
Age at start of culture: >24H
Culture water renewal frequency: minimum 3 times per week Culture water: 1/2 mod. 9 MHSF
Culture temperature: 25°C Fed Daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

Number of brood organisms at start of culture: 40
Percent survival of parental organisms after 7 days: 100
Percent of non-reproducing adults after 7 days: 0
Average young production after 7 days of brood adults for test: 31.6

	BROOD ADULT NO.	BROOD SIZE	HATCHING TIME WINDOW	TOTAL; YOUNG PROD. (7 DAYS)	USED IN REP. NO.
1)	A1	16	=1830 to 0230 7/11-12/16	30	1
2)	A2	17		35	2
3)	A3	23		36	3
4)	A4	15		30	4
5)	A5	14		33	5
6)	A6	13		31	6
7)	A7	19		32	7
8)	A8	16		31	8
9)	A9	17		26	9
10)	A10	16		32	10

COMMENTS:

BG 7-30-16

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	7/12/2016	Test ID:	bath0716c	Sample ID:	L16-15975-01
End Date:	7/18/2016	Lab ID:	ALL-ALLOWAY	Sample Type:	DMR-Discharge Monitoring Report
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
Upstr Control	27.000	33.000	30.000	30.000	18.000	34.000	6.000	26.000	30.000	34.000
20	36.000	28.000	36.000	36.000	31.000	32.000	18.000	27.000	34.000	24.000
40	23.000	37.000	32.000	34.000	35.000	27.000	15.000	37.000	32.000	22.000
60	31.000	37.000	20.000	34.000	33.000	39.000	32.000	37.000	31.000	
80	30.000	37.000	33.000	36.000	14.000	33.000	40.000	33.000	29.000	32.000
100	26.000	31.000	18.000	30.000	25.000	36.000	35.000	36.000	23.000	20.000

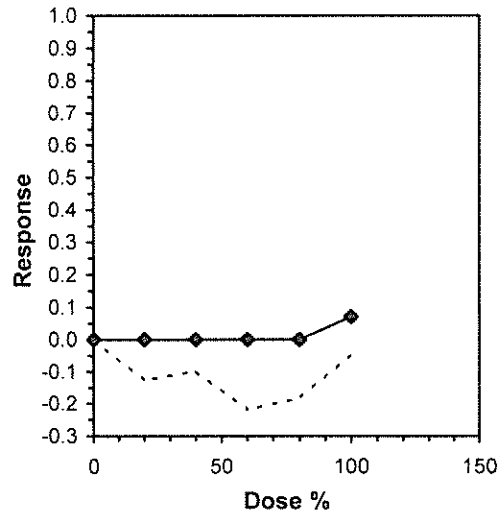
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
Upstr Control	26.800	1.0000	26.800	6.000	34.000	32.520	10			30.153	1.0000
20	30.200	1.1269	30.200	18.000	36.000	19.794	10	119.00	74.00	30.153	1.0000
40	29.400	1.0970	29.400	15.000	37.000	25.108	10	116.50	74.00	30.153	1.0000
60	32.667	1.2189	32.667	20.000	39.000	16.975	9	114.50	61.00	30.153	1.0000
80	31.700	1.1828	31.700	14.000	40.000	22.159	10	125.00	74.00	30.153	1.0000
100	28.000	1.0448	28.000	18.000	36.000	23.570	10	107.50	74.00	28.000	0.9286

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	0.95044	0.895	-1.1556	1.1104
Bartlett's Test indicates equal variances (p = 0.82)	2.17427	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	100	>100		1
Treatments vs Upstr Control				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	94.003			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



ToxCalc v5.0.32

Reviewed by: MM

Appendix C

Parameter Data

PHYSICAL-CHEMICAL DATA OF SAMPLES WHEN RECEIVED BY LAB
For Chronic Toxicity Testing

Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 Permit No.: 2PH00007*KD
Type of Test: Chronic Date of Test: 7/12/16 - 7/19/16

1st Sample Collection

Date Samples Collected: 7/11/16 Date Samples Read: 7/11/16 Analyst: SS

	100% Effluent	Upstream	-	-
Temperature (°C)	~~21.7~~ 22.7	21.7		
Dissolved Oxygen (mg/L)	7.8	7.2		
pH (s.u.)	7.00	7.11		
Conductivity (µmhos)	856	462		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	light stain	light stain		

2nd Sample Collection

Date Samples Collected: 7.13.16 Date Samples Read: 7.13.16 Analyst: BG

	100% Effluent	Upstream	-	-
Temperature (°C)	26.3	24.6		
Dissolved Oxygen (mg/L)	6.7	6.8		
pH (s.u.)	7.23	7.10		
Conductivity (µmhos)	860	465		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	light stain	light stain		

3rd Sample Collection

Date Samples Collected: 7.15.16 Date Samples Read: 7.15.16 Analyst: BG

	100% Effluent	Upstream	-	-
Temperature (°C)	25.2	23.3		
Dissolved Oxygen (mg/L)	6.9	6.5		
pH (s.u.)	7.39	7.32		
Conductivity (µmhos)	849	553		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	light stain	tan		

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.1	25.5 to 25.8	25.4	25.5 to 25.9	25.4	24.5 to 25.0	25.5	25.4 to 25.8	25.2	25.1 to 25.7	25.7	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.6	8.2	6.9	8.2	6.0	8.5	5.5	8.0	6.6	8.3	6.3	8.3	6.2	6.5
pH (s.u.)	7.29	7.33	7.12	7.24	7.00	6.98	6.90	7.23	7.07	7.15	7.05	7.17	7.16	7.14
Conductivity (µmho/cm)	327	321	335	323	342	324	341	306	333	305	316	306	321	327
Hardness (mg/L $CaCO_3$)	120	-	-	120	-	-	-	120	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	68	-	-	68	-	-	-	68	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.4	25.5 to 25.8	25.4	25.5 to 25.9	25.5	24.5 to 25.0 ~~25.6~~	25.4	25.4 to 25.8	25.4	25.1 to 25.7	25.6	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.2	8.3	6.4	8.0	5.6	8.4	5.6	8.0	6.6	8.3	6.2	8.8	6.7	6.6
pH (s.u.)	7.50	7.04	7.15	7.28	7.00	7.24	7.08 ~~7.36~~	7.44	7.37	7.43	7.04	7.48	7.17	7.04
Conductivity (µmho/cm)	462	463	549	465	475	465	492 ~~541~~	~~552~~ X	475	550	559	552	566 ~~556~~	563
Hardness (mg/L $CaCO_3$)	205	-	-	188	-	-	-	222	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	120	-	-	137	-	-	-	154	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.4	25.5 to 25.8	25.5	25.5 to 25.9	25.4	24.5 to 25.0	25.4	25.4 to 25.8	25.6	25.1 to 25.7	25.6	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.3	8.2	6.4	8.0	5.9	8.5	4.7	7.9	6.4	8.1	6.5	7.8	6.5	6.6
pH (s.u.)	7.54	7.30	7.30	7.46	7.19	7.34	7.18	7.50	7.39	7.52	7.21	7.55	7.20	7.07
Conductivity (µmho/cm)	543	545	561	547	567	547	590	613	565	611	630	613	633	635
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.4	25.5 to 25.8	25.4	25.5 to 25.9	25.5	24.5 to 25.0	25.4	25.4 to 25.8	25.4	25.1 to 25.7	25.6	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.3	8.2	6.6	8.0	5.9	8.4	4.6	7.9	6.2	8.3	6.6	8.6	6.5	6.1
pH (s.u.)	7.57	7.28	7.32	7.49	7.26	7.41	7.21	7.50	7.43	7.55	7.36	7.56	7.27	7.13
Conductivity (µmho/cm)	621	623	695	627	644	626	658	673	647	670	697	672	699	695
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.5	25.5 to 25.8	25.5	25.5 to 25.9	25.4	24.5 to 25.0	25.3	25.4 to 25.8	25.7	25.1 to 25.7	25.5	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.5	8.3	6.0	8.0	5.5	8.5	5.0	7.9	6.3	8.2	6.5	8.7	6.7	6.2
pH (s.u.)	7.56	7.34	7.35	7.47	7.32	7.43	7.25	7.58	7.48	7.56	7.35	7.59	7.32	7.19
Conductivity (µmho/cm)	701	703	709	706	727	706	741	732	728	730	753	733	768	755
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.5	25.5 to 25.8	25.5	25.5 to 25.9	25.4	24.5 to 25.0	25.5	25.4 to 25.8	25.4	25.1 to 25.7	25.5	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.3	8.3	6.1	7.9	5.4	8.4	5.1	7.8	6.4	8.5	6.3	9.2	6.1	6.4
pH (s.u.)	7.56	7.35	7.38	7.49	7.37	7.47	7.30	7.62	7.55	7.59	7.38	7.60	7.37	7.24
Conductivity (µmho/cm)	780	778	803	783	810	783	828	791	811	787	817	790	830	835
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: BG, SS, RFR
Beginning Date: 7/12/16 Time: 1420
Ending Date: 7/19/16 Time: 1205
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.5	25.5 to 25.8	25.5	25.5 to 25.9	25.5	24.5 to 25.0	25.4	25.4 to 25.8	25.4	25.1 to 25.7	25.3	25.0 to 25.4	24.8 to 25.8
Dissolved Oxygen (mg/L)	8.5	8.1	6.1	7.8	5.3	8.4	4.7	7.9	6.4	8.3	6.2	8.7	6.1	6.5
pH (s.u.)	7.55	7.46	7.42	7.45	7.42	7.45	7.33	7.61	7.57	7.60	7.41	7.63	7.40	7.28
Conductivity (µmho/cm)	854	852	876	860	880	861	903	849	893	845	883	848	897	892
Hardness (mg/L $CaCO_3$)	222	-	-	325	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	137	-	-	171	-	-	-	171	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 14:30
Ending Date: 7/18/16 Time: 14:00
Test Organism: Ceriodaphnia dubia Age: 13-24 H #14 A
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	~~New~~	Old	~~Old~~
Temperature (°C)	25.4	25.3	24.8 to 25.4	25.8	25.1 to 25.6 ~~25.4~~ m	25.5	25.1 to 25.4	25.4	25.1 to 25.6	25.1	25.1 to 25.4	–	25.0 to 25.4	–
Dissolved Oxygen (mg/L)	8.1	8.2	8.0	8.1	7.8 ~~8.0~~ m	8.3	7.9	7.5	7.6	8.3	7.4		7.5	
pH (s.u.)	7.35	7.34	7.51	7.41	7.47 ~~7.28~~ m	7.23	7.35	7.44	7.35	7.33	7.20		7.39	
Conductivity (µmho/cm)	334	336	339	333	339 ~~465~~ m	333	343	326	341	324	335	~~333~~ ce	335	
Hardness (mg/L $CaCO_3$)	103	-	-	120	-	-	-	120	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	120	-	-	103	-	-	-	103	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/19/16 Time: 1460
Test Organism: Ceriodaphnia dubia Age: 13-24 H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.4	24.8 to 25.4	25.4	25.1 to 25.6	25.5	25.1 to ~~25.4~~ 25.6	25.4	25.1 to 25.6	25.4	25.1 to 25.4	—	25.0 to 25.4	—
Dissolved Oxygen (mg/L)	8.2	8.3	8.1	8.0	7.8	8.4	7.9	8.0	7.6	8.3	7.5		7.8	
pH (s.u.)	7.50	7.04	7.42	7.28	7.45	7.24	7.35	7.44	7.50	7.43	7.44		7.57	
Conductivity (µmho/cm)	462	463	471	465	472	465	478 ~~547~~	552	417	550	567		567	
Hardness (mg/L $CaCO_3$)	205	-	-	188	-	-	-	222	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	120	-	-	137	-	-	-	154	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/18/16 Time: 1400
Test Organism: Ceriodaphnia dubia Age: 13-21 H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.4	24.8 to 25.4	25.5	25.1 to 25.6	25.6	25.1 to 25.4	25.4	25.1 to 25.6	26.6	25.1 to 25.4	—	25.0 to 25.4	—
Dissolved Oxygen (mg/L)	8.3	8.2	8.0	8.0	7.8	8.5	8.0	7.9	7.7	8.1	7.7		7.8	
pH (s.u.)	7.59	7.30	7.44	7.46	7.53	7.34	7.45	7.50	7.56	7.52	7.51		7.62	
Conductivity (µmho/cm)	543	545	550	547	560	547	570	613	560	611	633	~~631~~ CE	636	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 14:30
Ending Date: 7/18/16 Time: 1400
Test Organism: Ceriodaphnia dubia Age: 13-21H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.4	24.8 to 25.4	25.4	25.1 to 25.6	25.5	25.1 to 25.4	25.4	25.1 to 25.6	25.4	25.1 to 25.4	—	25.0 to 25.4	—
Dissolved Oxygen (mg/L)	8.3	8.2	8.0	8.0	7.8	8.4	8.0	7.9	7.7	8.3	7.4		7.7	
pH (s.u.)	7.57	7.28	7.50	7.49	7.57	7.41	7.51	7.55	7.61	7.55	7.56		7.66	
Conductivity (µmho/cm)	621	623	629	627	635	626	647	673	641	670	693	TCE	695	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/18/16 Time: 1400
Test Organism: Ceriodaphnia dubia Age: 13-21H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	~~New~~	Old	~~Old~~
Temperature (°C)	25.4	25.5	24.8 to 25.4	25.5	25.1 to 25.6	25.4	25.1 to 25.4	25.3	25.1 to 25.6	25.7	25.1 to 25.4	–	25.0 to 25.4	–
Dissolved Oxygen (mg/L)	8.5	8.3	8.0	8.0	7.8	8.5	8.0	7.9	7.7	8.2	7.5		7.9	
pH (s.u.)	7.56	7.34	7.56	7.47	7.46	7.43	7.56	7.58	7.66	7.56	7.63		7.71	
Conductivity (µmho/cm)	701	703	704	706	709	706	726	732	724	730	762	~~X~~ CE	753	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/18/16 Time: 1400
Test Organism: Ceriodaphnia dubia Age: 13-21 H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.5	25.5	24.8 to 25.4	25.5	25.1 to 25.6	25.4	25.1 to 25.4	25.5	25.1 to 25.6	25.4	25.1 to 25.4	—	25.0 to 25.4	-
Dissolved Oxygen (mg/L)	8.3	8.3	8.0	7.9	7.9	8.4	8.0	7.8	7.8	8.5	7.7		7.9	
pH (s.u.)	7.56	7.35	7.57	7.49	7.61	7.47	7.60	7.62	7.68	7.59	7.66		7.75	
Conductivity (µmho/cm)	780	778	793	783	796	783	804	791	801	787	814		814	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*KD

Person(s) conducting Test: RFR, BG, SS
Beginning Date: 7/12/2016 Time: 1430
Ending Date: 7/18/16 Time: 1400
Test Organism: Ceriodaphnia dubia Age: 13-24 H
Dilution Water Used: Upstream

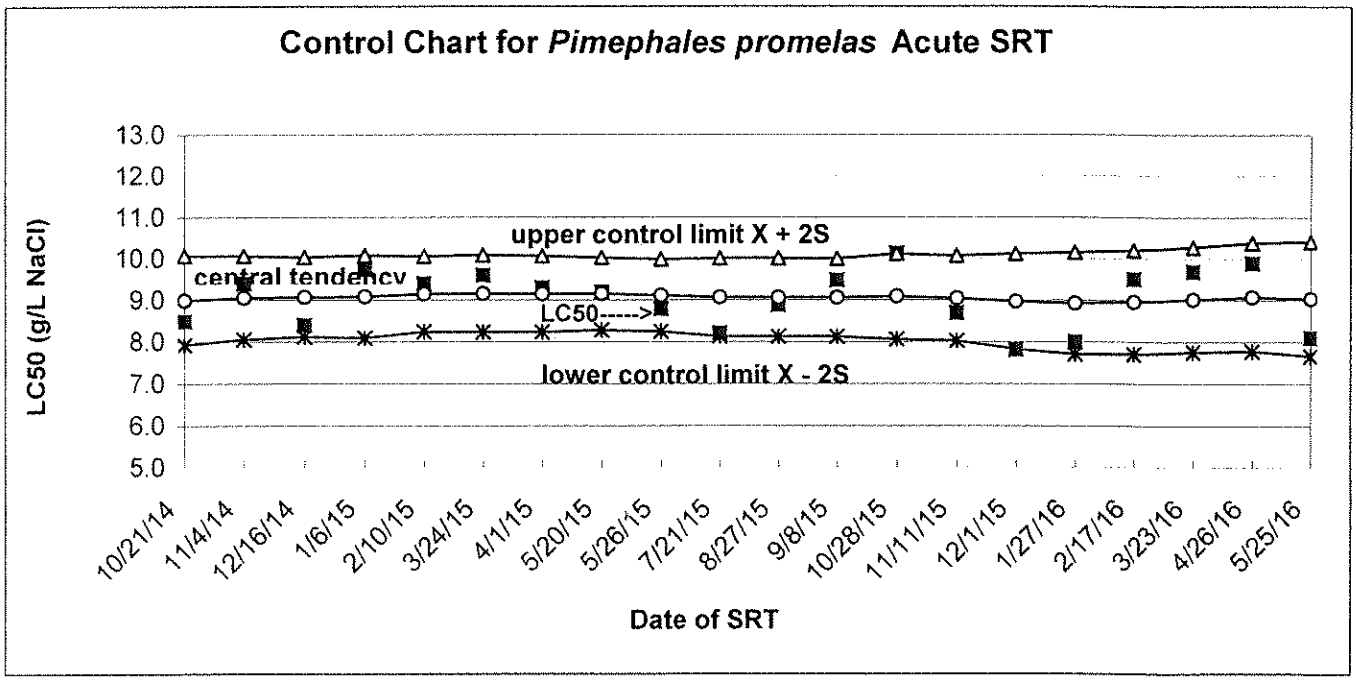
Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.5	24.8 to 25.4	25.5	25.1 to 25.6	25.5	25.1 to 25.4	25.4	25.1 to 25.6	25.4	25.1 to 25.4	—	25.0 to 25.4	—
Dissolved Oxygen (mg/L)	8.5	8.1	8.0	7.8	7.9	8.4	8.0	7.9	7.8	8.3	7.6		7.9	
pH (s.u.)	7.55	7.46	7.60	7.45	7.68	7.45	7.65	7.61	7.74	7.60	7.71		7.77	
Conductivity (µmho/cm)	854	852	871	860	872	861	889	849	882	845	875		869	
Hardness (mg/L $CaCO_3$)	222	-	-	325	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	137	-	-	171	-	-	-	171	-	-	-	-	-	-

Appendix D

SRT Data

48-H LC50 for *Pimephales promelas* Acute NaCl SRT

Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
10/21/14	8.5	9.0	0.5	10.1	7.9	0.06	10.6	7.4	SK
11/4/14	9.4	9.1	0.5	10.1	8.1	0.06	10.6	7.6	SK
12/16/14	8.4	9.1	0.5	10.0	8.1	0.05	10.5	7.6	SK
1/6/15	9.7	9.1	0.5	10.1	8.1	0.05	10.6	7.6	SK
2/10/15	9.4	9.1	0.5	10.1	8.2	0.05	10.5	7.8	SK
3/24/15	9.6	9.2	0.5	10.1	8.2	0.05	10.5	7.8	SK
4/1/15	9.3	9.1	0.5	10.1	8.2	0.05	10.5	7.8	SK
5/20/15	9.2	9.2	0.4	10.0	8.3	0.05	10.5	7.8	SK
5/26/15	8.8	9.1	0.4	10.0	8.2	0.05	10.4	7.8	SK
7/21/15	8.2	9.1	0.5	10.0	8.1	0.05	10.5	7.7	SK
8/27/15	8.9	9.1	0.5	10.0	8.1	0.05	10.5	7.7	Probit
9/8/15	9.5	9.1	0.5	10.0	8.1	0.05	10.5	7.7	SK
10/28/15	10.1	9.1	0.5	10.1	8.1	0.06	10.6	7.6	SK
11/11/15	8.7	9.0	0.5	10.1	8.0	0.06	10.6	7.5	SK
12/1/15	7.8	9.0	0.6	10.1	7.8	0.06	10.7	7.3	SK
1/27/16	8.0	8.9	0.6	10.1	7.7	0.07	10.8	7.1	G
2/17/16	9.5	9.0	0.6	10.2	7.7	0.07	10.8	7.1	SK
3/23/16	9.7	9.0	0.6	10.3	7.8	0.07	10.9	7.1	SK
4/26/16	9.9	9.1	0.7	10.4	7.8	0.07	11.0	7.1	G
5/25/16	8.1	9.0	0.7	10.4	7.7	0.08	11.1	7.0	SK

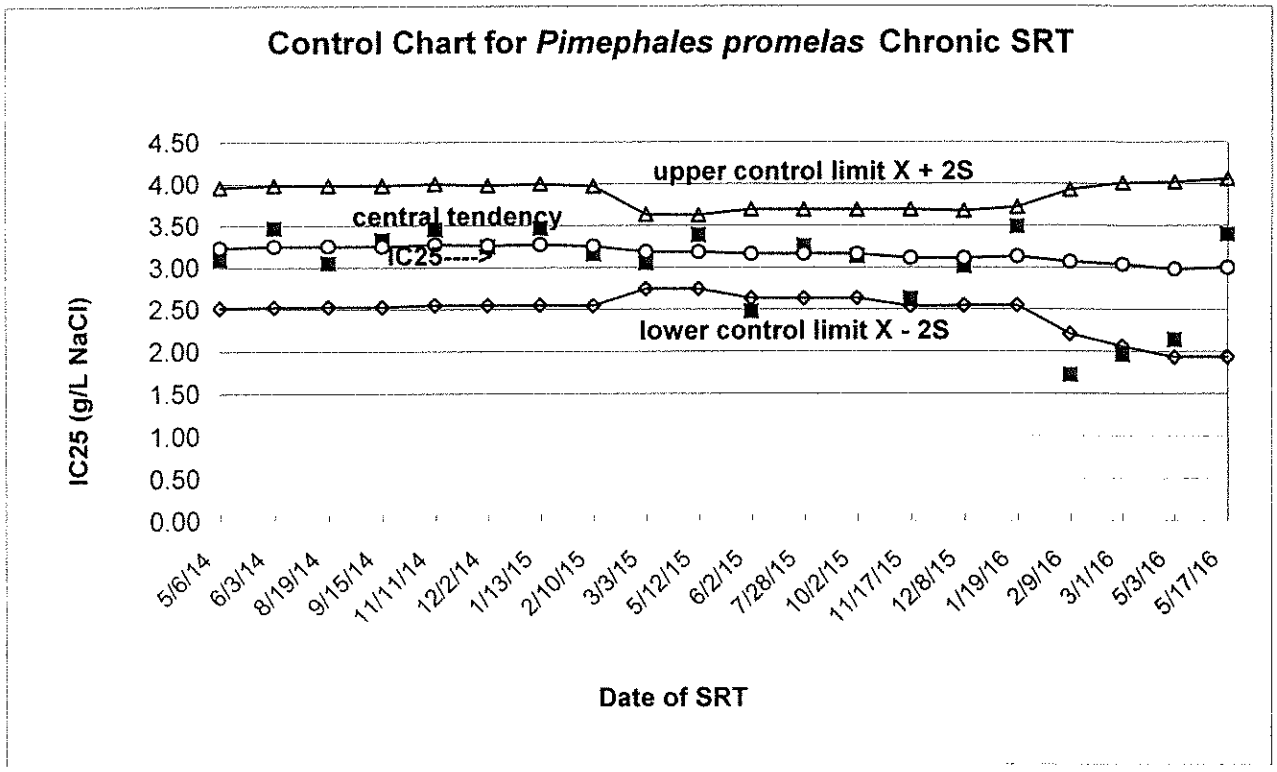


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.19
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

1 - P = Probit method, G = Graphical method, SK = Spearman-Karber method, TSK = Trimmed Spearman-Karber method.

IC25 for *Pimephales promelas* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
5/6/14	3.09	3.24	0.36	3.96	2.51	0.11	4.32	2.15
6/3/14	3.47	3.25	0.36	3.98	2.52	0.11	4.35	2.16
8/19/14	3.05	3.25	0.36	3.98	2.53	0.11	4.35	2.16
9/15/14	3.33	3.25	0.36	3.98	2.52	0.11	4.35	2.16
11/11/14	3.46	3.27	0.36	4.00	2.55	0.11	4.36	2.19
12/2/14	3.25	3.26	0.36	3.98	2.54	0.11	4.34	2.18
1/13/15	3.47	3.27	0.36	4.00	2.55	0.11	4.36	2.19
2/10/15	3.16	3.26	0.36	3.98	2.54	0.11	4.33	2.18
3/3/15	3.05	3.19	0.22	3.63	2.74	0.07	3.86	2.52
5/12/15	3.39	3.19	0.22	3.63	2.74	0.07	3.85	2.52
6/2/15	2.47	3.16	0.27	3.70	2.63	0.08	3.96	2.36
7/28/15	3.26	3.16	0.27	3.69	2.63	0.08	3.96	2.36
10/2/15	3.13	3.16	0.26	3.69	2.63	0.08	3.96	2.37
11/17/15	2.62	3.11	0.29	3.69	2.53	0.09	3.98	2.24
12/8/15	3.01	3.11	0.28	3.67	2.54	0.09	3.96	2.26
1/19/16	3.49	3.13	0.29	3.72	2.54	0.09	4.02	2.25
2/9/16	1.73	3.07	0.43	3.93	2.21	0.14	4.36	1.78
3/1/16	1.96	3.04	0.49	4.01	2.06	0.16	4.50	1.57
5/3/16	2.14	2.98	0.52	4.03	1.93	0.18	4.55	1.41
5/17/16	3.40	3.00	0.53	4.06	1.93	0.18	4.59	1.40



*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-2 in EPA/833/R-00/003 .

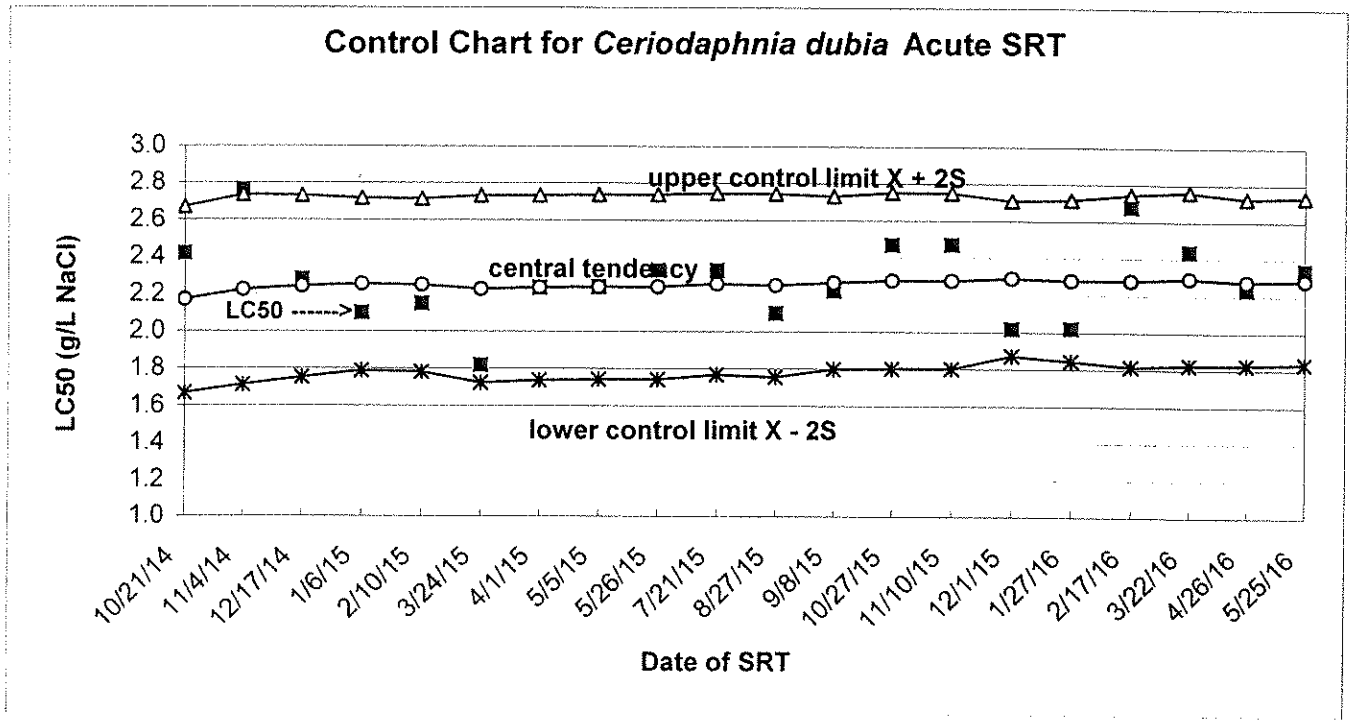
75th percentile of CVs for IC25 = 0.38

Calculated CV should not exceed 75th percentile of CVs.

$\bar{X} \pm 3SD$ = Well outside expected range.

48-H LC50 for *Ceriodaphnia dubia* Acute NaCl SRT

Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
10/21/14	2.4	2.2	0.3	2.7	1.7	0.12	2.9	1.4	SK
11/4/14	2.8	2.2	0.3	2.7	1.7	0.11	3.0	1.5	Probit
12/17/14	2.3	2.2	0.2	2.7	1.8	0.11	3.0	1.5	SK
1/6/15	2.1	2.3	0.2	2.7	1.8	0.10	2.9	1.6	SK
2/10/15	2.2	2.2	0.2	2.7	1.8	0.10	2.9	1.5	SK
3/24/15	1.8	2.2	0.3	2.7	1.7	0.11	3.0	1.5	SK
4/1/15	2.2	2.2	0.2	2.7	1.7	0.11	3.0	1.5	SK
5/5/15	2.2	2.2	0.2	2.7	1.7	0.11	3.0	1.5	Probit
5/26/15	2.3	2.2	0.2	2.7	1.7	0.11	3.0	1.5	SK
7/21/15	2.3	2.3	0.2	2.7	1.8	0.11	3.0	1.5	SK
8/27/15	2.1	2.2	0.2	2.7	1.8	0.11	3.0	1.5	Probit
9/8/15	2.2	2.3	0.2	2.7	1.8	0.10	3.0	1.6	Probit
10/27/15	2.5	2.3	0.2	2.8	1.8	0.10	3.0	1.6	SK
11/10/15	2.5	2.3	0.2	2.8	1.8	0.10	3.0	1.6	Probit
12/1/15	2.0	2.3	0.2	2.7	1.9	0.09	2.9	1.7	SK
1/27/16	2.0	2.3	0.2	2.7	1.8	0.10	2.9	1.6	SK
2/17/16	2.7	2.3	0.2	2.7	1.8	0.10	3.0	1.6	SK
3/22/16	2.4	2.3	0.2	2.8	1.8	0.10	3.0	1.6	Probit
4/26/16	2.2	2.3	0.2	2.7	1.8	0.10	3.0	1.6	Probit
5/25/16	2.3	2.3	0.2	2.7	1.8	0.10	3.0	1.6	Probit

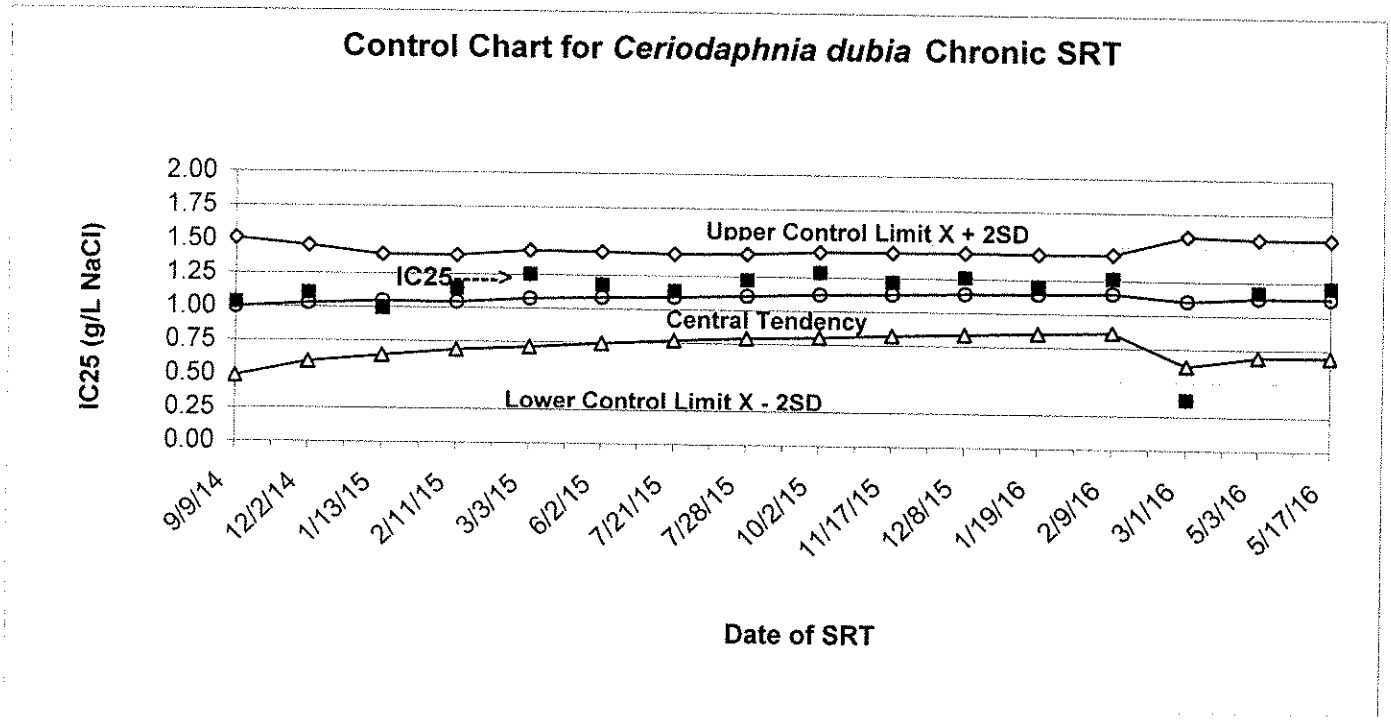


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\bar{X} \pm 3SD$ = Well outside expected range.

1 - P = Probit method, G = Graphical method, SK = Spearman-Karber method, TSK = Trimmed Spearman-Karber method.

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
9/9/14	1.04	1.00	0.26	1.51	0.49	0.26	1.77	0.23
12/2/14	1.11	1.03	0.22	1.46	0.59	0.21	1.67	0.38
1/13/15	0.99	1.05	0.19	1.39	0.65	0.18	1.58	0.46
2/11/15	1.14	1.04	0.18	1.39	0.69	0.17	1.57	0.52
3/3/15	1.25	1.07	0.18	1.43	0.71	0.17	1.61	0.53
6/2/15	1.18	1.08	0.17	1.42	0.74	0.16	1.60	0.57
7/21/15	1.14	1.09	0.16	1.41	0.77	0.15	1.57	0.61
7/28/15	1.22	1.10	0.16	1.42	0.79	0.14	1.57	0.63
10/2/15	1.29	1.12	0.16	1.44	0.80	0.14	1.60	0.64
11/17/15	1.22	1.13	0.15	1.44	0.82	0.14	1.59	0.67
12/8/15	1.26	1.14	0.15	1.44	0.84	0.13	1.59	0.68
1/19/16	1.20	1.14	0.15	1.44	0.85	0.13	1.58	0.70
2/9/16	1.27	1.15	0.15	1.44	0.86	0.13	1.59	0.72
3/1/16	0.38	1.10	0.24	1.58	0.62	0.22	1.82	0.38
5/3/16	1.17	1.13	0.22	1.57	0.70	0.19	1.78	0.48
5/17/16	1.21	1.13	0.22	1.56	0.70	0.19	1.78	0.48



*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-2 in EPA/833/R-00/003 .
75th percentile of CVs for IC25 = 0.45
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.