Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/23/15
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L1-15413-01

Report of Chronic Biomonitoring

Samples Collected on:

7/6/15
7/8/15
7/10/15

Pimephales promelas
Ceriodaphnia dubia

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

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Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/23/15
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L1-15413-01

Date: 7/23/15

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: 7/23/15
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
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

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: 7/7-14/15

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	Growth
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	2.5	2.5	

AA = below detectable

Conclusion/Comments:

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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: 7/7-13/15

Summary of Chronic Toxicity Tedt Results
Ceriodaphnia dubia

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
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:

There was a statistical difference between 100% and upstream control but no statistical difference between 100% and secondary control.

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Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*KD
Date of Test: 7/7-14/15

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%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.996	0.031
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%)	0.0% (0.0%)	1.082	0.052
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%)	2.5% (2.5%)	0.956	0.101
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	1.041	0.115
60%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.917	0.115
80%	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	10.0% (10.0%)	10.0% (10.0%)	0.887	0.092
100%	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	10.0% (10.0%)	0.983	0.146
LC50 Values (%):	>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Survival** <1.0	
95% Confidence Limits LL									
95% Confidence Limits UL									
EC50 Values (%):	>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Growth (100/IC25)** <1.0	
95% Confidence Limits LL									
95% Confidence Limits 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: 7/7-13/15

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%)	--- (---)	31.2
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	--- (---)	21.6
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%)	--- (---)	29.5
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%)	--- (---)	30.4
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%)	--- (---)	29.9
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	--- (---)	26.0
100%	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	--- (---)	22.9 *
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 (%)	80	**Method(s) used to determine Values:**
LOEC for Mortality (%)	>100		LOEC for Reproduction (%)	100	
Chronic Value for Mortality (%)	>100		Chronic Value for Reprod (%)	89.4	
IC25 (%)	96.8		IC50 (%)	>100	IC Pin, Steel's Many-One Rank

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

Chronic Value = square root of (NOEC x LOEC)

There was a statistical difference between 100% and upstream control but no statistical difference between 100% and secondary control.

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/23/15
Ohio EPA Permit No.: 2PH00007*KD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection Beginning	Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	7/5/15 @ 09:00	7/6/15 @ 09:00
Type (Grab/Comp):	Comp	2nd	7/7/15 @ 09:00	7/8/15 @ 09:00
Volume Collected:	5 Gal.	3rd	7/9/15 @ 09:00	7/10/15 @ 09:00
Upstream Station:				
Waterbody:	Pike Run			
Station No.:	801	1st		7/6/15 @ 09:57
Type (Grab/Comp):	Grab	2nd		7/8/15 @ 09:36
Volume Collected:	5 Gal.	3rd		7/10/15 @ 09:48

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:	7/7-14/15
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 - 7/6; day 2, 3 - 7/8; day 4, 5, 6 - 7/10
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* 18-21 hours
2. Test Type and Duration:	Static renewal; 6 to 8 days (6 days)
3. Test Dates:	7/7-13/15
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 - 7/6; day 2, 3 - 7/8; day 4, 5, 6 - 7/10
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.

Project: L15-15413

Report To: Rick Boerger **Name:** Allen County Sanitary Engineer **Company:** 3226 North Cole St. **Address:** 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. 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	7/05/15-7/06/15	09:00-09:00	X		WW	1	1	Toxicity	
2	Upstream	7/06/15	09:57		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)*		7/6/15	10:14

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	7 - Sodium Thiosulfate	13 - Zinc Acetate
2 - HNO_3	8 - Ascorbic Acid	14 - Sodium Sulfite
3 - H_2SO_4	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate
4 - HCl	10 - EDA	16 - Sodium Sulfite/Sodium Bisulfate
5 - NaOH	11 - Ammonium Chloride	
6 - NaOH & Zinc Acetate	12 - $(NH_4)_2SO_4$ & NH_4OH	

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: _____

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. Hawley (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	7/07/15-7/08/15	09:00-09:00	X		WW	1	1	Toxicity	
2	Upstream	7/08/15	09:36		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) 7/08/15 09:49

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	7 - Sodium Thiosulfate	13 - Zinc Acetate
2 - HNO_3	8 - Ascorbic Acid	14 - Sodium Sulfite
3 - H_2SO_4	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate
4 - HCl	10 - EDA	16 - Sodium Sulfite/Sodium Bisulfate
5 - NaOH	11 - Ammonium Chloride	
6 - NaOH & Zinc Acetate	12 - $(NH_4)_2SO_4$ & NH_4OH	

Sample Receiving (*For Lab Use Only*)
Ice Present? ~~YES~~ NO ☐
Proper Preservation? ~~YES~~ NO ☐
Container Temperature: Ran Ice

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: 7/08/15

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	09:18	19.1	763	9.00	7.69
Upstream	09:36	18.8	572	7.97	7.91

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- [x] Cloudy
- [ ] Heavy Rain
- [ ] Light Rain
- [ ] Clear Skies
- [ ] Sunny
- [ ] Snow
- [x] Cool

Stream Conditions

- [x] Rapid and Complete Mixing
- [ ] Mixing is not Rapid and Complete

Other Conditions

- [ ] Outfall Flooded Due to Heavy Rain
- [ ] _____

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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. Harley (Signature)

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

	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	7/9/15-7/10/15	09:00-09:00	X		WW	1	1	Toxicity	
2	Upstream	7/10/15	09:48		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)		7/10/15	10:02

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: Raw Ice

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:** MSN

Date: 7/10/15

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	09:30	18.8	554	8.15	7.60
Upstream	09:48	19.0	489	9.14	7.91

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- [x] Cloudy
- [ ] Heavy Rain
- [x] Light Rain
- [ ] Clear Skies
- [ ] Sunny
- [ ] Snow
- [ ] _____

Stream Conditions

- [x] 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 Co. (American - Bath WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning: Date 7/7/15 **Time** 1045
Ending: Date 7/14/15 **Time** 1240
Age of *Pimephales promelas*: < 24H
Dilution Water Used: upstream

	Lab Control					Upstream Control					20% Effluent					40% 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	10G	10G	10G	10G	40G
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
4	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	9G 1D	39G 1D
5	10G	10G	10G	10G	40G	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	9G 1D	39G 1D
6	10G	10G	10G	10G	40G	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	9G 1D	39G 1D
7	10G	10G	10G	10G	40G	9G 1D	10G	10G	10G	39G 1D	9G 1D	10G	10G	10G	39G 1D	10G	10G	9G 1D	9G 1D	38G 2D

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; 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 Co. (American-Bath WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00007*

Person(s) Conducting Test: BG, SS, RFM
Beginning: Date 7/7/15 **Time** 1045
Ending: Date 7/14/15 **Time** 1240
Age of *Pimephales promelas*: < 24H
Dilution Water Used: Upstream

	60% Effluent					80% Effluent					100% Effluent					~~Far-field Mixing Zone~~				
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	9G 1D	39G 1D	9G 1D	10G	10G	10G	39G 1D					
3	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	9G 1D	39G 1D	9G 1D	10G	10G	10G	39G 1D					
4	9G 1D	10G	10G	10G	39G 1D	9G 1D	10G	10G	9G 1D	38G 2D	9G 1D	9G 1D	10G	10G	38G 2D					
5	9G 1D	10G	10G	10G	39G 1D	9G 1D	10G	10G	9G 1D	38G 2D	9G 1D	9G 1D	10G	10G	38G 2D					
6	9G 1D	10G	10G	10G	39G 1D	9G 1D	9G 1D	9G 1D	9G 1D	36G 4D	9G 1D	9G 1D	10G	10G	38G 2D					
7	9G 1D	10G	10G	10G	~~39G 1D~~ 39G 1D	9G 1D	9G 1D	9G 1D	9G 1D	36G 4D	8G 2D	9G 1D	9G 1D	10G	36G 4D					

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; 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 Co. (American - Bath WWTP) Date of Test: 7/7-14/15
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: <24H
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7-16-15 Analyst: BG

Balance Calibration Check: 1 gram weight = 1.00002 grams Date: 7-16-15 Analyst: BG

per

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	1.13037	1.13123	10	0.86	0.086
Lab Control	1	10	1.08969	1.10074	10	11.05	1.105
	2	10	1.13027	1.14126	10	10.99	1.099
	3	10	1.08274	1.09278	10	10.04	1.004
	4	10	1.10410	1.11528	10	11.18	1.118
Upstream Control	1	9	1.11077	1.12072	10	9.95	0.995
	2	10	1.13096	1.14068	10	9.72	0.972
	3	10	1.12702	1.13741	10	10.39	1.039
	4	10	1.09265	1.10242	10	9.77	0.977
20% Effluent	1	9	1.13212	1.14080	10	8.68	0.868
	2	10	1.09770	1.10742	10	9.72	0.972
	3	10	1.09182	1.10073	10	8.91	0.891
	4	10	1.12455	1.13547	10	10.92	1.092
40% Effluent	1	10	1.13001	1.14087	10	10.86	1.086
	2	10	1.12389	1.13568	10	11.79	1.179
	3	9	1.08330	1.09310	10	9.80	0.980
	4	9	1.08796	1.09715	10	9.19	0.919

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

Discharge: Allen Co. (American-Bath WWTP) Date of Test: 7/7-14/15
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: < 24 H
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7-16-15 Analyst: BG

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	9	1.12892	1.13656	10	7.64	0.764
	2	10	1.09492	1.10434	10	9.42	0.942
	3	10	1.13215	1.14256	10	10.41	1.041
	4	10	1.09092	1.10012	10	9.20	0.920
80% Effluent	1	9	1.10033	1.10804	10	7.71	0.771
	2	9	1.09085	1.09982	10	8.97	0.897
	3	9	1.10169	1.11053	10	8.84	0.884
	4	9	1.12551	1.13547	10	9.96	0.996
100% Effluent	1	8	1.12120	1.12952	10	8.32	0.832
	2	9	1.08403	1.09338	10	9.35	0.935
	3	9	1.12330	1.13313	10	9.83	0.983
	4	10	1.09970	1.11151	10	11.81	1.181
~~Far-field Mixing Zone~~	1				10		
	2				10		
	3				10		
	4				10		

EPA ICP 6/94 APA 7/23/18

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	20	40	60	80	100
Response	1	.995	.868	1.086	.764	.771	.832
Response	2	.972	.972	1.179	.942	.897	.935
Response	3	1.039	.891	.980	1.041	.884	.983
Response	4	.977	1.092	.919	.920	.996	1.181

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP 001
Test Start Date: 7/7/15 Test Ending Date: 7/14/15
Test Species: P. promelas
Test Duration: 7 days
DATA FILE: Ame0715P.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	0.996	0.030	0.998
2	4	20.000	0.956	0.101	0.998
3	4	40.000	1.041	0.115	0.998
4	4	60.000	0.917	0.115	0.929
5	4	80.000	0.887	0.092	0.929
6	4	100.000	0.983	0.146	0.929

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

TOXSTAT 3.5

Title: Amer-Bath WWTP 001 P. promelas 7/7-14/15 Growth mg AFA 7/23/15
File: AMER0715.PPW Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Upst. Control	0.9958	0.9958		
2	20 %	0.9558	0.9558	0.5335	
3	40 %	1.0410	1.0410	-0.6035	
4	60 %	0.9168	0.9168	1.0537	
5	80 %	0.8870	0.8870	1.4505	
6	100 %	0.9828	0.9828	0.1734	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Amer-Bath WWTP 001 P. promelas 7/7-14/15 Growth mg
File: AMER0715.PPW Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Upst. Control	4			
2	20 %	4	0.1807	18.1	0.0400
3	40 %	4	0.1807	18.1	-0.0452
4	60 %	4	0.1807	18.1	0.0790
5	80 %	4	0.1807	18.1	0.1088
6	100 %	4	0.1807	18.1	0.0130

TOXSTAT 3.5

Title: Amer-Bath WWTP 001 P. promelas 7/7-14/15 Growth mg AFA 7/23/15
File: AMER0715.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	4	0.9720	1.0390	0.9958
2	20 %	4	0.8680	1.0920	0.9558
3	40 %	4	0.9190	1.1790	1.0410
4	60 %	4	0.7640	1.0410	0.9168
5	80 %	4	0.7710	0.9960	0.8870
6	100 %	4	0.8320	1.1810	0.9828

Title: Amer-Bath WWTP 001 P. promelas 7/7-14/15 Growth mg
File: AMER0715.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	0.0009	0.0305	0.0152	3.0608
2	20 %	0.0102	0.1012	0.0506	10.5879
3	40 %	0.0132	0.1150	0.0575	11.0469
4	60 %	0.0131	0.1146	0.0573	12.5037
5	80 %	0.0085	0.0921	0.0460	10.3831
6	100 %	0.0214	0.1464	0.0732	14.8981

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: LFW, BG, SS
Contact:
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Age of *Ceriodaphnia dubia*: 18-24 h #116①
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	3 G	0 G	10 G	18 G	/		31	3
2	/	/	/	2	0	8	11	/		21	3
3	/	/	/	2	4	9	12	/		27	3
4	/	/	/	Absent 0	0	3	16	/		19	3
5	/	/	/	0	6	7	13	/		26	3
6	/	/	/	5	0	5	15	/		25	3
7	/	/	/	0 D	-	-	-	-	D	0	0
8	/	/	/	Absent 0 G	0 G	6 G	18 G	/		24	3
9	/	/	/	2 G	0	8	10	/		20	3
10	/	/	/	3 G	0	3	17	/		23	3
Total Adult Cond.	10 G	10 G	10 G	9 G 1 D	9 G 1 D	9 G 1 D	9 G 1 D	-		SUM 216	27
										MEAN 21.6	2.7
										S.D. 8.4	0.95

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: KFH, BG, SS
Contact:
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
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	4 G	0 G	10 G	18 G	/	32	3
2	/	/	/	6 /	0 /	12 /	17 /	/	35	3
3	/	/	/	5 /	0 /	11 /	14 /	/	30	3
4	/	/	/	5 /	0 /	12 /	15 /	/	32	3
5	/	/	/	4 /	0 /	10 /	19 /	/	33	3
6	/	/	/	6 /	0 /	13 /	19 /	/	38	3
7	/	/	/	5 /	0 /	11 /	7 /	/	23	3
8	/	/	/	1 /	0 /	11 /	20 /	/	32	3
9	/	/	/	5 /	0 /	3 /	13 /	/	21	3
10	/ ↓	/ ↓	/ ↓	5 ↓	0 ↓	12 ↓	19 ↓	/	36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-	SUM 312	30
									MEAN 31.2	3.0
									S.D. 5.4	0.0

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: KFH, BG, SS
Contact: ~~20% Eff~~
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
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	0 (Abort) G	0 G	12 G	18 G	/	30	3
2	/	/	/	6 /	0 /	13 /	15 /	/	34	3
3	/	/	/	6 /	0 /	9 /	18 /	/	33	3
4	/	/	/	5 /	0 /	9 /	13 /	/	27	3
5	/	/	/	5 /	0 /	10 /	16 /	/	31	3
6	/	/	/	4 /	0 /	9 /	18 /	/	31	3
7	/	/	/	0 (Abort)	0 /	12 /	21 /	/	33	3
8	/	/	/	5 /	0 /	7 /	17 /	/	29	3
9	/	/	/	4 /	0 /	10 /	18 /	/	32	3
10	/ ↓	/ ↓	/ ↓	4 ↓	9 ↓	0 ↓	2 ↓	/	15	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-	SUM 295	30
									MEAN 29.5	3.0
									S.D. 5.8	0.0

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: KCH, BG, SS
Contact:
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
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	4 G	0 G	8 G	21 G	/	33	3
2	/	/	/	5 /	2 /	8 /	12 /	/	27	3
3	/	/	/	5 /	0 /	13 /	21 /	/	39	3
4	/	/	/	3 /	0 /	11 /	17 /	/	31	3
5	/	/	/	3 /	0 /	6 /	15 /	/	24	3
6	/	/	/	6 /	0 /	10 /	16 /	/	32	3
7	/	/	/	4 /	1 /	9 /	19 /	/	33	3
8	/	/	/	5 /	0 /	10 /	18 /	/	33	3
9	/	/	/	6 /	0 /	8 /	18 /	/	32	3
10	/	/	/	4 /	0 /	10 /	6 /	/	20	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–	SUM 304	30
									MEAN 30.4	3.0
									S.D. 5.4	0.0

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; 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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: ICH, BG, SS
Contact:
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
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	4 / G	0 / G	11 / G	14 / G	/	29	3
2	/	/	/	5 /	0 /	11 /	17 /	/	33	3
3	/	/	/	1 /	0 /	9 /	19 /	/	29	3
4	/	/	/	2 /	0 /	10 /	17 /	/	29	3
5	/	/	/	4 /	0 /	10 /	16 /	/	30	3
6	/	/	/	5 /	0 /	10 /	19 /	/	~~33~~ 34 AM	3
7	/	/	/	3 /	0 /	5 /	20 /	/	28	3
8	/	/	/	6 /	10 /	0 /	20 /	/	36	3
9	/	/	/	4 /	0 /	7 /	14 /	/	25	3
10	/ ↓	/ ↓	/ ↓	6 / ↓	0 ↓	12 ↓	8 ↓	/	26	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	/	SUM 299	30
									MEAN 29.9	3.0
									S.D. 3.5	0.0

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; 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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: LCH, BG, SS
Contact:
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
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	5/G	0/G	0/D	—/—	—/—	D	5	1
2	/	/	/	6/	0/	6/G	19/G	/		31	3
3	/	/	/	5/	0/	11/	14/	/		30	3
4	/	/	/	4/	0/	8/	16/	/		28	3
5	/	/	/	Abort 0/	0/	10/	18/	/		28	3
6	/	/	/	2/	0/	10/	17/	/		29	3
7	/	/	/	6/	0/	10/	20/	/		36	3
8	/	/	/	6/	0/	9/	16/	/		31	3
9	/	/	/	6/	0/	Abort 0/	3/	/		9	3
10	↓/↓	↓/↓	↓/↓	4/↓	0/↓	10/↓	19/↓	/		33	3
Total Adult Cond.	10G	10G	10G	10G	10G	9G ~~10G~~ 10	9G ~~10G~~ 10	—			
									SUM	260	28
									MEAN	26.0	2.8
									S.D.	10.3	0.6

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; 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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFH, BG, SS
Contact:
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Age of *Ceriodaphnia dubia*: 18-31 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	3/G	0/G	5/G	14/G	/		22	3
2	/	/	/	8/G	0/	4/	13/	/		17	3
3	/	/	/	0/	0/	9/	13/	/		22	2
4	/	/	/	3/	0/	9/	15/	/		27	3
5	/	/	/	0/	5/	3/	10/	/		18	3
6	/	/	/	5/	0/	9/	16/	/		30	3
7	/	/	/	1/D	-/	-/	-/	-/	D	1	1
8	/	/	/	4/G	8/G	0/G	14/G	/		26	3
9	/	/	/	6/	0/	12/	19/	/		37	3
10	/	/	/	4/	0/	8/	17/	/		29	3
Total Adult Cond.	10G	10G	10G	9G 1D	9G 1D	9G 1D	9G 1D	-		SUM 229	27
										MEAN 22.9	2.7
										S.D. 9.7	0.7

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; 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 Co. (American - Bath WWTP)
Address: Lima, OH
Type of Test: Chronic Date of Test: 7/7-14/15
Effluent Serial No.: 001 Permit No.: 2PH00007*

Investigator: AEA Culture No.: 116
Culture initiation date: 7/1/15 Culture ending date: 7/8/15
Origin of parental organisms: Allwy 113
Age at start of culture: >24h
Culture water renewal frequency: minimum 3 times per week Culture water: 9MHSF, 1 Aerad.
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:
Average young production after 7 days of brood adults for test: 30.2

	BROOD ADULT NO.	BROOD SIZE	HATCHING TIME WINDOW (1425 to 1700)	TOTAL; YOUNG PROD. (7 DAYS)	USED IN REP. NO.
1)	A9	16	7/6/15	30	1
2)	A10	17		30	2
3)	B7	14		29	3
4)	C3	15		32	4
5)	C4	18		31	5
6)	C7	15		28	6
7)	C10	16		28	7
8)	D1	16		31	8
9)	D4	17		30	9
10)	D5	17		32	10

COMMENTS:

TOXSTAT 3.5

Title: Amer-Bath WWTP 001 C. dubia 7/7-13/15 Reproduction AFK 7/23/15
File: AMER0715.CDR Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Upst. Control	31.2000				
2	20 %	29.5000	92.00	75.00	10.00	
3	40 %	30.4000	100.50	75.00	10.00	
4	60 %	29.9000	91.50	75.00	10.00	
5	80 %	26.0000	84.50	75.00	10.00	
6	100 %	22.9000	74.50	75.00	10.00	*

Critical values are 1 tailed (k = 5)

EPA ICP 6/94 MRA 7/28/15

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	32	30	33	29	5	22
Response 2	35	34	27	33	31	17
Response 3	30	33	39	29	30	22
Response 4	32	27	31	29	28	27
Response 5	33	31	24	30	28	18
Response 6	38	31	32	34	29	30
Response 7	23	33	33	28	36	1
Response 8	32	29	33	36	31	26
Response 9	21	32	32	25	9	37
Response 10	36	15	20	26	33	29

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP 001
Test Start Date: 7/7/15 Test Ending Date: 7/13/15
Test Species: C. dubia
Test Duration: 6 days
DATA FILE: Ame0715c.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	31.200	5.391	31.200
2	10	20.000	29.500	5.503	29.950
3	10	40.000	30.400	5.379	29.950
4	10	60.000	29.900	3.479	29.900
5	10	80.000	26.000	10.339	26.000
6	10	100.000	22.900	9.735	22.900

The Linear Interpolation Estimate: 96.7742 Entered P Value: 25

Number of Resamplings: 80Those resamples not used had estimates above the highest concentration/ %Effluent.
Resampling time in Seconds: 0.06 Random_Seed: 28379958

TOXSTAT 3.5 JCR 7/23/18

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	32	30	33	29	5	22
Response 2	35	34	27	33	31	17
Response 3	30	33	39	29	30	22
Response 4	32	27	31	29	28	27
Response 5	33	31	24	30	28	18
Response 6	38	31	32	34	29	30
Response 7	23	33	33	28	36	1
Response 8	32	29	33	36	31	26
Response 9	21	32	32	25	9	37
Response 10	36	15	20	26	33	29

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP 001
Test Start Date: 7/7/15 Test Ending Date: 7/13/15
Test Species: C. dubia
Test Duration: 6 days
DATA FILE: Ame0715c.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	31.200	5.391	31.200
2	10	20.000	29.500	5.503	29.950
3	10	40.000	30.400	5.379	29.950
4	10	60.000	29.900	3.479	29.900
5	10	80.000	26.000	10.339	26.000
6	10	100.000	22.900	9.735	22.900

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 50% of the control response mean.

TOXSTAT 3.5

Title: Amer-Bath WWTP 001 C. dubia 7/7-13/15 Reproduction AFA 7/23/15
File: AMER0715.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	10	21.0000	38.0000	31.2000
2	20 %	10	15.0000	34.0000	29.5000
3	40 %	10	20.0000	39.0000	30.4000
4	60 %	10	25.0000	36.0000	29.9000
5	80 %	10	5.0000	36.0000	26.0000
6	100 %	10	1.0000	37.0000	22.9000

Title: Amer-Bath WWTP 001 C. dubia 7/7-13/15 Reproduction
File: AMER0715.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	29.0667	5.3914	1.7049	17.2800
2	20 %	30.2778	5.5025	1.7401	18.6526
3	40 %	28.9333	5.3790	1.7010	17.6940
4	60 %	12.1000	3.4785	1.1000	11.6338
5	80 %	106.8889	10.3387	3.2694	39.7643
6	100 %	94.7667	9.7348	3.0784	42.5101

Appendix C

Parameter Data

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

Industry/Toxicant: Allen Co. (American - Bath WWTP) Type of Test: Chronic
Address: Lima, OH Date of Test: 7/7-14/15
Effluent Serial No.: 001 Permit No.: 2PH00007*

1st Sample Collection

Date Samples Collected: 7/6/15 Date Samples Read: 7/6/15 Analyst: SS

	100% Effluent	Upstream
Temperature (°C)	16.4	16.3
Dissolved Oxygen (mg/L)	8.4	8.8
pH (s.u.)	7.82	8.06
Conductivity (µmhos)	885	715
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light stain

2nd Sample Collection

Date Samples Collected: 7.8.15 Date Samples Read: 7.8.15 Analyst: EMT

	100% Effluent	Upstream
Temperature (°C)	15.7	20.2°C
Dissolved Oxygen (mg/L)	8.4	7.98 mg/L
pH (s.u.)	7.6	7.72
Conductivity (µmhos)	862	588
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light stain

3rd Sample Collection

Date Samples Collected: 7.10.15 Date Samples Read: 7.10.15 Analyst: EMT

	100% Effluent	Upstream
Temperature (°C)	13.9	15.7
Dissolved Oxygen (mg/L)	8.8	8.7
pH (s.u.)	7.46	7.76
Conductivity (µmhos)	548	506
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light stain

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

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <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)	25.0	25.3	24.9 to 25.5	25.5	24.8 to 25.3	25.3	24.7 to 25.3	~~25.3~~ ~~25.3~~	24.8 to 25.5	25.3	24.7 to 25.5	~~25.3~~ ~~25.2~~ ~~25.6~~	25.3 to 25.6	—
Dissolved Oxygen (mg/L)	7.8	8.2	4.8	8.0	5.1	8.4	6.6	8.2	5.5	8.4	6.3	8.3	~~9.2~~ ~~9.1~~ ~~8.3~~	6.6
pH (s.u.)	7.51	7.47	7.04	7.46	7.11	7.55	6.81	7.51	6.89	7.43	7.04	7.28	~~7.24~~ ~~7.28~~	7.39
Conductivity (µmho/cm)	326	326	343	326	338	326	334	332	350	333	346	332	~~352~~ ~~332~~	346
Hardness (mg/L $CaCO_3$)	120			103				154						
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <24H
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	25.2	24.9 to 25.5	25.4	24.8 to 25.3	25.3	24.7 to 25.3	25.3	24.8 to 25.5	25.3	24.7 to 25.5	~~25.3~~ ~~25.2~~ ~~25.6~~ 25.2	25.3 to 25.6	—
Dissolved Oxygen (mg/L)	8.5	8.0	4.2	8.0	4.7	8.2	5.5	8.6	4.7	9.1	5.4	8.1	5.0	6.0
pH (s.u.)	7.99	7.96	7.53	7.80	7.56	7.73	7.39	7.78	7.51	7.73	7.69	7.98	7.69	7.71
Conductivity (µmho/cm)	715	715	726	592	730	590	598	506	608	505	516	505	529	522
Hardness (mg/L $CaCO_3$)	325			222				257						
Alkalinity (mg/L $CaCO_3$)	205			171				222						

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

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <24H
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	25.3 New	Old	Old
Temperature (°C)	25.6	25.3	24.9 to 25.5	25.4	24.8 to 25.3	25.3	24.7 to 25.3	25.4	24.8 to 25.5	25.3	24.7 to 25.5	~~25.3~~ to ~~25.6~~	25.3 to 25.6	—
Dissolved Oxygen (mg/L)	8.5	8.0	4.6	8.0	4.6	8.3	5.7	8.4	4.2	8.9	5.9	8.1	6.4	5.8
pH (s.u.)	7.97	7.95	7.66	7.77	7.66	7.74	7.63	7.82	7.55	7.90	7.79	7.91	7.76	7.76
Conductivity (µmho/cm)	752	751	777	647	783	626	673	514	678	514	537	515	543	541
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <24H
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	25.1 New	Old	Old
Temperature (°C)	25.3	25.3	24.4 to 25.5	25.5	24.8 to 25.8	25.2	24.7 to 25.3	25.5	24.8 to 25.5	25.3	24.7 to 25.5	~~25.3~~ to 25.6	25.3 to 25.6	—
Dissolved Oxygen (mg/L)	8.7	8.1	4.2	8.1	5.5	8.3	5.8	8.4	5.0	8.7	5.4	8.1	6.1	5.5
pH (s.u.)	7.92	7.89	7.64	7.76	7.23	7.73	7.68	7.68	7.63	7.81	7.79	7.95	7.76	7.74
Conductivity (µmho/cm)	786	782	804	702	806	701	715	523	715	524	542	523	551	557
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <24H
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	25.3 New	Old	Old
Temperature (°C)	25.4	25.2	24.9 to 25.5	25.4	24.8 to 25.3	25.3	24.7 to 25.3	25.4	24.8 to 25.5	25.2	24.7 to 25.5	~~25.3 to~~ 25.6	25.3 to 25.6	–
Dissolved Oxygen (mg/L)	8.5	8.0	5.0	8.2	4.4	8.4	5.7	8.6	5.1	9.2	5.9	8.0	5.0	6.6
pH (s.u.)	7.92	7.91	7.71	7.74	7.65	7.73	7.70	7.67	7.66	7.82	7.84	7.96	7.69	7.82
Conductivity (µmho/cm)	821	816	860	756	860	754	783	532	787	532	571	532	571	555
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <24H
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	25.2 New	Old	Old
Temperature (°C)	25.4	25.3 to 25.5	24.9 to 25.5	25.4	24.8 to 25.3	25.2	24.7 to 25.3	25.4	24.8 to 25.5	25.4	24.7 to 25.5	~~25.3~~ to 25.6	25.3 to 25.6	—
Dissolved Oxygen (mg/L)	8.5	8.2	4.5	8.2	4.8	8.4	5.9	8.5	4.9	8.9	5.6	8.1	5.4	6.7
pH (s.u.)	7.89	7.89	7.68	7.79	7.69	7.81	7.73	7.61	7.65	7.80	7.80	8.00	7.69	7.82
Conductivity (µmho/cm)	855	851	878	806	879	805	828	541	840	540	572	540	546	570
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: BG, SS, RFR
Beginning Date: 7/7/15 Time: 1045
Ending Date: 7/14/15 Time: 1240
Test Organism: *Pimephales promelas* Age: <24H
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	25.3 New	Old	Old
Temperature (°C)	25.4	25.3	24.9 to 25.5	25.4	24.8 to 25.3	25.2	24.7 to 25.3	25.4	24.8 to 25.5	25.6	24.7 to 25.5	~~25.3~~ ~~to~~ ~~?~~	25.3 to 25.6	—
Dissolved Oxygen (mg/L)	8.2	8.0	5.3	8.2	5.1	8.5	5.9	8.4	5.5	9.1	6.0	8.1	5.4	5.4
pH (s.u.)	7.85	7.86	7.74	7.76	7.70	7.78	7.77	7.58	7.72	7.74	7.86	7.96	7.70	7.72
Conductivity (µmho/cm)	889	886	916	857	915	860	882	556	883	549	580	549 ~~548~~	576	581
Hardness (mg/L $CaCO_3$)	376			342				291						
Alkalinity (mg/L $CaCO_3$)	188			188				188						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, BG, SS
Beginning Date: 7/7/15 Time: 11:00
Ending Date: 7/13/15 Time: 1:00
Test Organism: *Ceriodaphnia dubia* Age: 18-21H #116
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	25.2	25.0 to 25.3	25.4	25.0 to 25.4	25.1	24.9 to 25.2	25.2	25.0 to 25.4	25	24.8 to 25.4	—	24.8 to 25.2	-
Dissolved Oxygen (mg/L)	8.3	8.4	7.9	8.3	7.5	8.3	7.9	8.4	8.2	8.4	8.4		8.0	
pH (s.u.)	7.90	7.62	7.57	7.60	7.54	7.54	7.58	7.57	7.70	7.38	7.51		7.49	
Conductivity (µmho/cm)	337	337	353	325	342	323	331	319	342	319	332		329	
Hardness (mg/L $CaCO_3$)	120			137				120						
Alkalinity (mg/L $CaCO_3$)	103			120				103						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, BS, SS
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Test Organism: *Ceriodaphnia dubia* Age: 18-21 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	25.2	25.0 to 25.3	25.4	25.0 to 25.4	25.3	24.9 to 25.2	25.3	25.0 to 25.4	25.3	24.8 to 25.4	–	24.8 to 25.2	–
Dissolved Oxygen (mg/L)	8.5	8.0	7.9	8.0	7.7	8.2	8.0	8.6	8.0	9.1	8.3		8.0	
pH (s.u.)	7.99	7.96	7.84	7.80	7.85	7.73 7.77	7.71	7.78	7.83	7.73	7.89		8.06	
Conductivity (µmho/cm)	715	715	719	592	719	590	598	506	604	505	523		520	
Hardness (mg/L $CaCO_3$)	325			222				257						
Alkalinity (mg/L $CaCO_3$)	205			171				222						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, BS, SS
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Test Organism: *Ceriodaphnia dubia* Age: 18-21 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)	25.6	25.3	25.0 to 25.3	25.4	25.0 to 25.4	25.3	24.9 to 25.2	25.4	25.0 to 25.4	25.3	24.8 to 25.4	—	24.8 to 25.2	—
Dissolved Oxygen (mg/L)	8.5	8.0	8.0	8.0	7.5	8.3	8.0	8.4	8.0	8.9	8.3		8.0	
pH (s.u.)	7.97	7.95	7.89	7.77	7.88	7.74	7.85	7.82	790	7.90	7.98		8.08	
Conductivity (µmho/cm)	752	751	770	647	765	640	666	574	669	514	539		534	
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 Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, BS, SS
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Test Organism: *Ceriodaphnia dubia* Age: 18-21 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)	25.3	25.3	25.0 to 25.3	25.5	25.0 to 25.4	25.2	24.9 to 25.2	25.5	25.0 to 25.4	25.3	24.8 to 25.4	—	24.8 to 25.2	—
Dissolved Oxygen (mg/L)	8.7	8.1	7.9	8.1	7.4	8.3	8.0	8.4	7.9	8.7	8.4		8.0	
pH (s.u.)	7.92	7.89	7.90	7.76	7.89	7.73	7.86	7.68	7.91	7.81	7.99		8.06	
Conductivity (µmho/cm)	786	782	806	702	801	~~702~~ 701	725	523	724	524	548		538	
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, BS, SS
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Test Organism: *Ceriodaphnia dubia* Age: 18-21 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)	25.4	25.2	25.0 to 25.3	25.4	25.0 to 25.4	25.3	24.9 to 25.2	25.4	25.0 to 25.4	25.2	24.8 to 25.4	—	24.8 to 25.2	—
Dissolved Oxygen (mg/L)	8.5	8.0	7.9	8.2	7.8	8.4	8.0	8.6	8.0	9.2	8.3		7.9	
pH (s.u.)	7.92	7.91	7.91	7.74	7.88	7.73	7.89	7.67	7.92	7.82	7.97		8.06	
Conductivity (µmho/cm)	821	816	841	750	837	754	783	532	778	532	556		546	
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, BS, SS
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Test Organism: *Ceriodaphnia dubia* Age: 18-31 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	25.3	25.0 to 25.3	25.4	25.0 to 25.4	25.2	24.9 to 25.2	25.4 ~~25~~	25.0 to 25.4	25.4	24.8 to 25.4	—	24.8 to 25.2	—
Dissolved Oxygen (mg/L)	8.5	8.2	7.9	8.2	7.8	8.4	8.0	8.5	8.0	8.9	8.3		8.0	
pH (s.u.)	7.89	7.89	7.90	7.79	7.89	7.81	7.88	7.61	7.93	7.80	7.96		~~8.0~~ 8.00	
Conductivity (µmho/cm)	855	851	865	806	873	805	830	541	827	540	566		558	
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 Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, BS, SS
Beginning Date: 7/7/15 Time: 1100
Ending Date: 7/13/15 Time: 1100
Test Organism: *Ceriodaphnia dubia* Age: 18-21 H
Dilution Water Used: Upstream

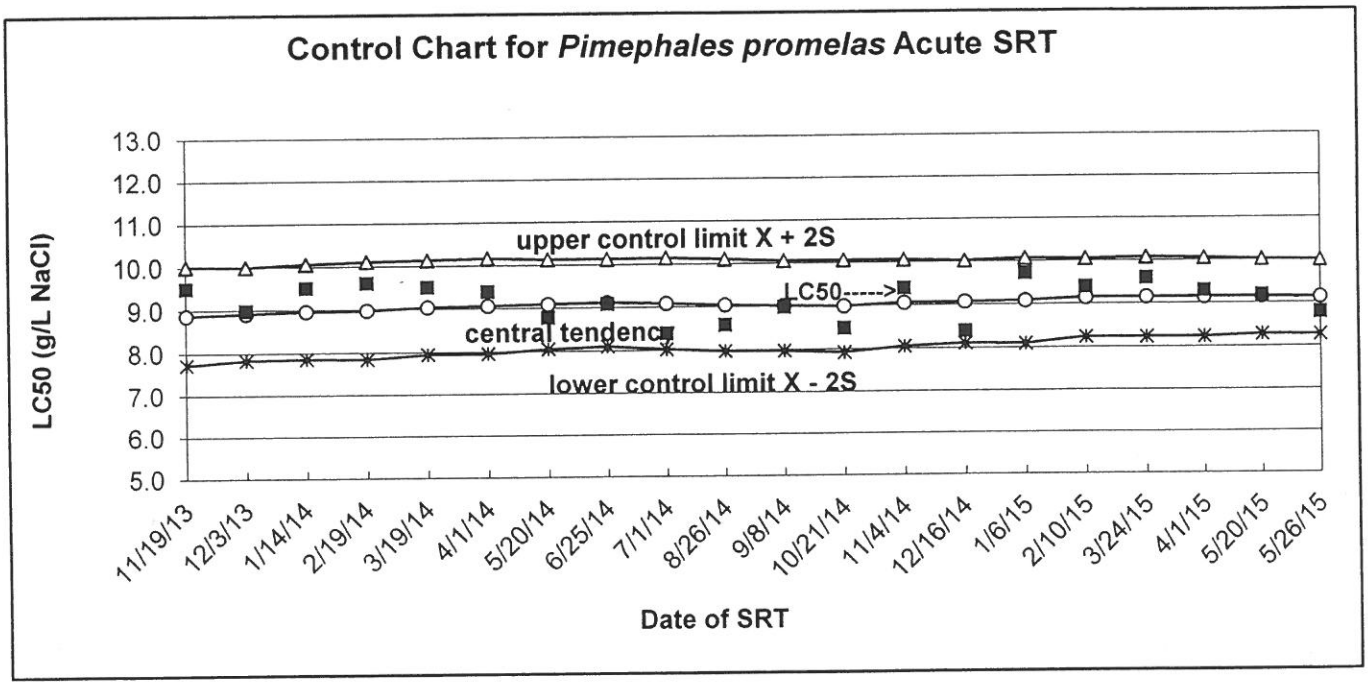
Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	~~New~~	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.1	25.4	25.0 to 25.4	25.2	24.9 to 25.2	25.4	25.0 to 25.4	25.6	24.8 to 25.4	—	24.8 to 25.2	—
Dissolved Oxygen (mg/L)	8.2	8.0	7.9	8.2	7.7	8.5	8.0	8.4	8.0	9.1	8.3		8.0	
pH (s.u.)	7.85	7.86	7.90	7.76	7.88	7.78	7.91	7.58	7.93	7.74	7.97		8.02	
Conductivity (µmho/cm)	889	886	906	857	907	860	886	850	884	549	576		570	
Hardness (mg/L $CaCO_3$)	376			342				291						
Alkalinity (mg/L $CaCO_3$)	188			188				188						

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]
11/19/13	9.5	8.9	0.6	10.0	7.7	0.06	10.6	7.2	SK
12/3/13	9.0	8.9	0.5	10.0	7.8	0.06	10.5	7.3	SK
1/14/14	9.5	9.0	0.6	10.1	7.9	0.06	10.6	7.3	SK
2/19/14	9.6	9.0	0.6	10.1	7.8	0.06	10.7	7.3	SK
3/19/14	9.5	9.0	0.5	10.1	7.9	0.06	10.7	7.4	SK
4/1/14	9.4	9.1	0.6	10.2	8.0	0.06	10.7	7.4	SK
5/20/14	8.8	9.1	0.5	10.1	8.1	0.06	10.7	7.5	SK
6/25/14	9.1	9.1	0.5	10.1	8.1	0.06	10.6	7.6	SK
7/1/14	8.4	9.1	0.5	10.2	8.0	0.06	10.7	7.5	SK
8/26/14	8.6	9.0	0.5	10.1	8.0	0.06	10.6	7.4	SK
9/8/14	9.0	9.0	0.5	10.1	8.0	0.06	10.6	7.5	SK
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	8.3	0.05	10.4	7.8	SK

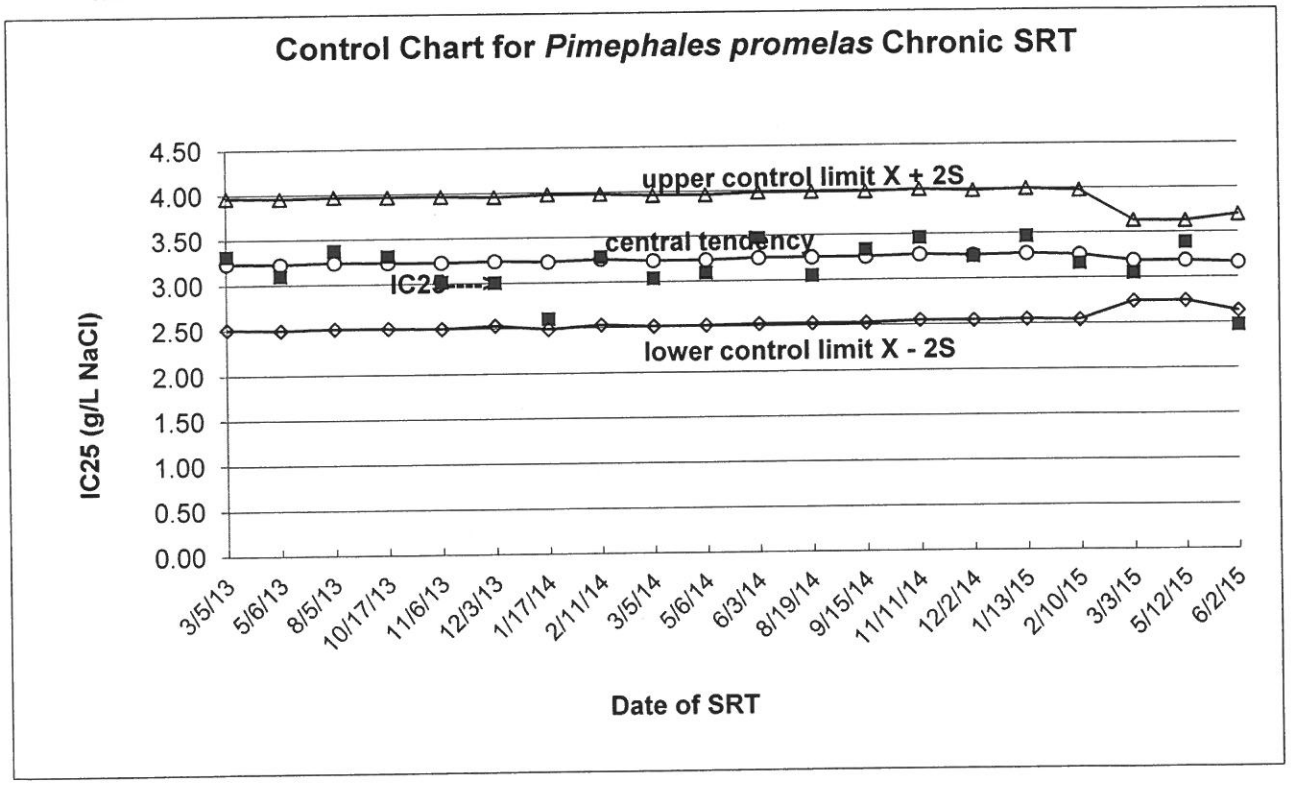


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.19
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

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

IC25 for *Pimephales promelas* Chronic NaCl SRT

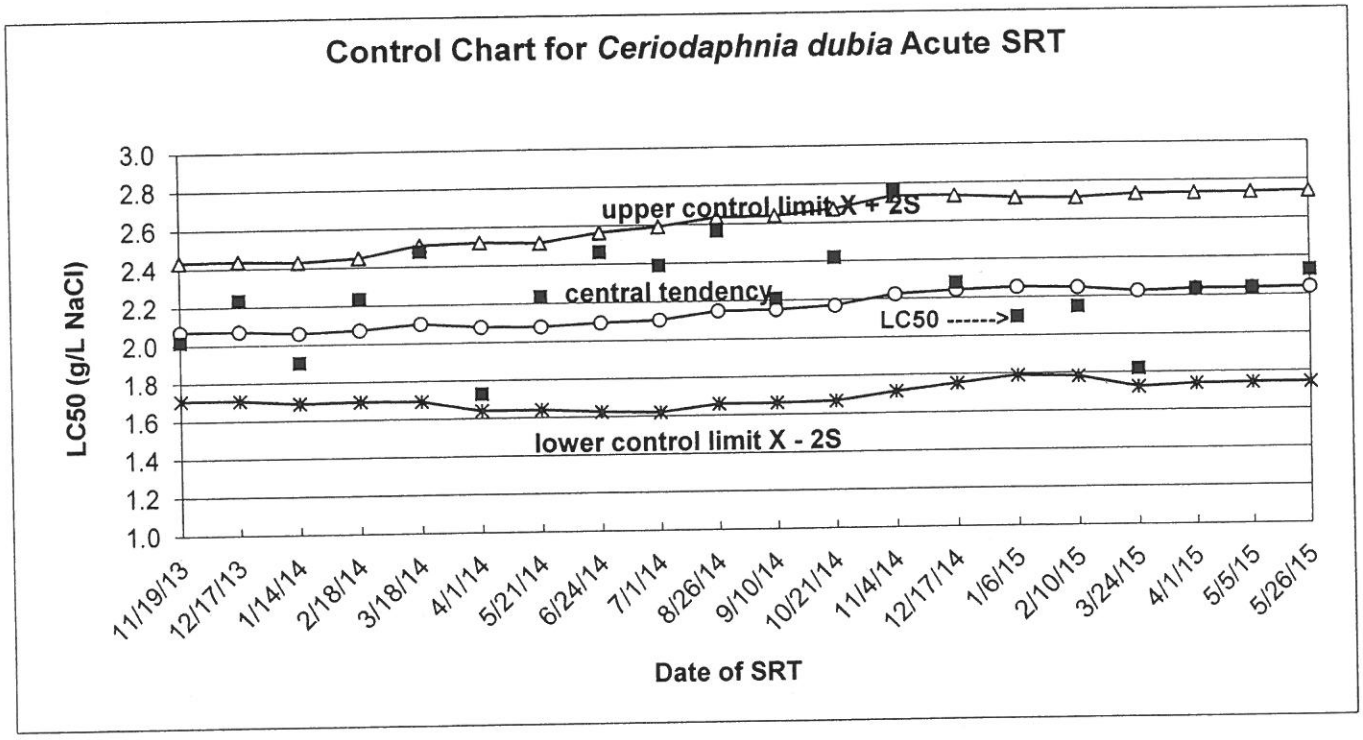
Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
3/5/13	3.32	3.24	0.36	3.97	2.51	0.11	4.33	2.15
5/6/13	3.10	3.23	0.37	3.96	2.50	0.11	4.32	2.13
8/5/13	3.38	3.24	0.37	3.97	2.51	0.11	4.34	2.15
10/17/13	3.31	3.24	0.36	3.97	2.51	0.11	4.33	2.15
11/6/13	3.02	3.23	0.37	3.97	2.50	0.11	4.33	2.14
12/3/13	3.01	3.24	0.36	3.96	2.53	0.11	4.31	2.17
1/17/14	2.60	3.23	0.37	3.98	2.49	0.12	4.35	2.11
2/11/14	3.28	3.25	0.36	3.98	2.53	0.11	4.34	2.17
3/5/14	3.04	3.23	0.36	3.96	2.51	0.11	4.32	2.15
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



*- 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.
$\bar{X}$ ± 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]
11/19/13	2.0	2.1	0.2	2.4	1.7	0.09	2.6	1.5	SK
12/17/13	2.2	2.1	0.2	2.4	1.7	0.09	2.6	1.5	Probit
1/14/14	1.9	2.1	0.2	2.4	1.7	0.09	2.6	1.5	Probit
2/18/14	2.2	2.1	0.2	2.4	1.7	0.09	2.6	1.5	Probit
3/18/14	2.5	2.1	0.2	2.5	1.7	0.10	2.7	1.5	TSK
4/1/14	1.7	2.1	0.2	2.5	1.6	0.11	2.7	1.4	Graphical
5/21/14	2.2	2.1	0.2	2.5	1.6	0.10	2.7	1.4	Probit
6/24/14	2.5	2.1	0.2	2.6	1.6	0.11	2.8	1.4	Probit
7/1/14	2.4	2.1	0.2	2.6	1.6	0.12	2.8	1.4	Probit
8/26/14	2.6	2.1	0.2	2.6	1.7	0.11	2.9	1.4	Probit
9/10/14	2.2	2.2	0.2	2.6	1.7	0.11	2.9	1.4	SK
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

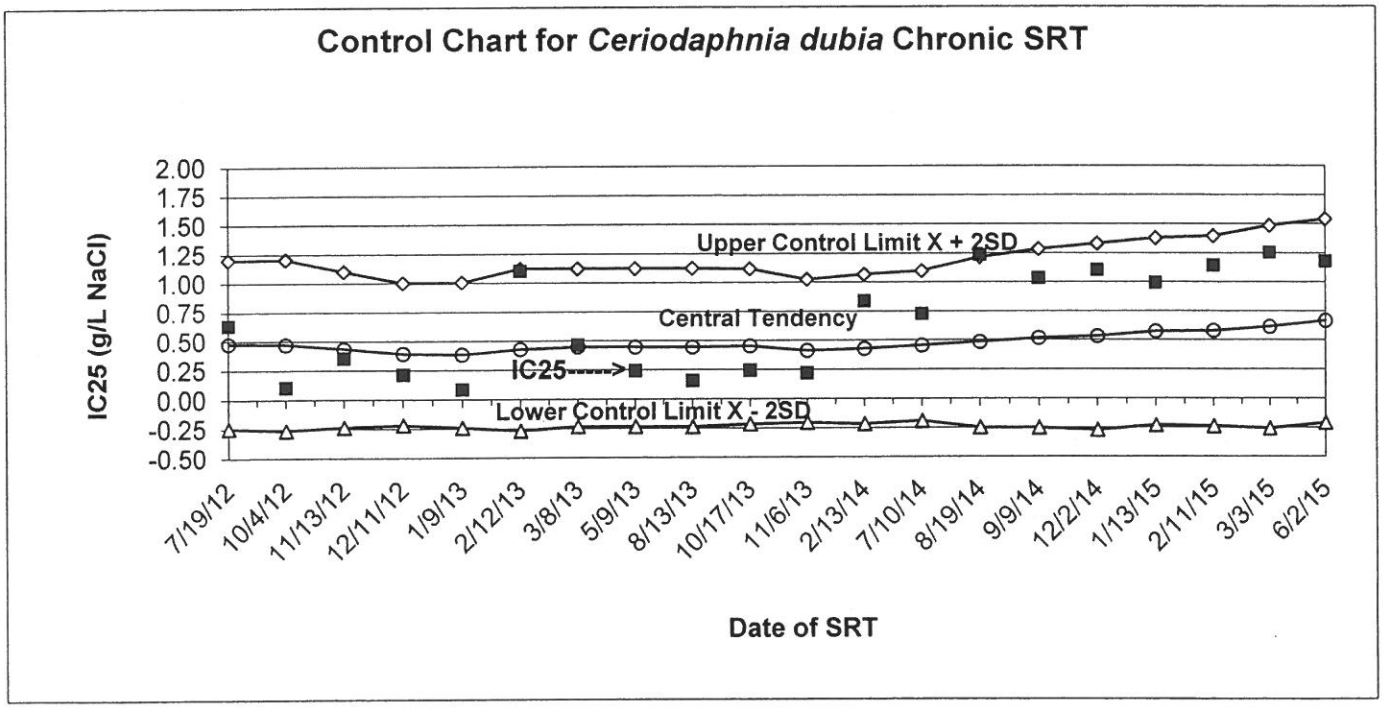


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
7/19/12	0.64	0.48	0.36	1.20	-0.24	0.76	1.56	-0.61
10/4/12	0.11	0.47	0.37	1.21	-0.26	0.78	1.57	-0.63
11/13/12	0.35	0.43	0.33	1.10	-0.23	0.77	1.44	-0.57
12/11/12	0.21	0.39	0.31	1.00	-0.22	0.78	1.31	-0.52
1/9/13	0.09	0.38	0.31	1.01	-0.24	0.81	1.32	-0.55
2/12/13	1.10	0.43	0.35	1.12	-0.27	0.81	1.47	-0.61
3/8/13	0.46	0.45	0.34	1.12	-0.23	0.76	1.46	-0.57
5/9/13	0.24	0.45	0.34	1.12	-0.23	0.76	1.46	-0.57
8/13/13	0.16	0.44	0.34	1.12	-0.24	0.77	1.46	-0.58
10/17/13	0.24	0.45	0.33	1.12	-0.21	0.74	1.45	-0.55
11/6/13	0.22	0.41	0.31	1.03	-0.20	0.75	1.33	-0.51
2/13/14	0.84	0.43	0.32	1.07	-0.22	0.75	1.39	-0.54
7/10/14	0.73	0.45	0.32	1.10	-0.19	0.71	1.42	-0.51
8/19/14	1.24	0.48	0.37	1.21	-0.25	0.75	1.58	-0.61
9/9/14	1.04	0.52	0.38	1.29	-0.25	0.74	1.67	-0.63
12/2/14	1.11	0.53	0.40	1.33	-0.27	0.75	1.73	-0.67
1/13/15	0.99	0.57	0.40	1.38	-0.23	0.70	1.78	-0.63
2/11/15	1.14	0.58	0.41	1.40	-0.24	0.71	1.81	-0.65
3/3/15	1.25	0.61	0.44	1.49	-0.26	0.71	1.92	-0.69
6/2/15	1.18	0.67	0.44	1.54	-0.21	0.66	1.98	-0.65



*- 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.