Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/18/13
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L13-15188-01

Report of Chronic Biomonitoring

Samples Collected on:

7/8/13
7/10/13
7/12/13

Pimephales promelas
Ceriodaphnia dubia

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

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/18/13
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L13-15188-01

Date: 7/18/13

Melanie Marshall
Allen County Sanitary Engineer
3226 North Cole Street
Lima, OH 45805

Dear Ms. Marshall:

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.

Paul J. Crerar
Biology Lab Manager

Richard F. Rupp
Senior Biologist

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: 7/9-16/13

Summary of Chronic Toxicity Test Results
Pimephales promelas

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
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	AA	2.5	

AA = below detectable

Conclusion/Comments:

Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 7/18/13
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	Melanie Marshall
6. Telephone No.	419-225-8084
7. Consulting Laboratory	Alloway
8. Lab Contact	Paul Crerar
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: 7/9-15/13

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	Reproduction
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	10	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: 7/9-16/13

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%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.937	0.062
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%)	0.995	0.078
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%)	0.963	0.090
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%)	0.0% (0.0%)	0.984	0.073
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%)	0.0% (0.0%)	0.978	0.044
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%)	0.0% (0.0%)	0.972	0.039
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	0.926	0.048

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: 7/9-15/13

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%)	10.0% (10.0%)	--- (---)	26.8
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	20.0% (20.0%)	20.0% (20.0%)	30.0% (30.0%)	--- (---)	8.5
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%)	--- (---)	26.2
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%)	--- (---)	26.7
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%)	--- (---)	24.5
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%)	--- (---)	24.6
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	--- (---)	24.1
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								

7-Day NOEC for Mortality (%)	100		7-Day NOEC for Reprod (%)	100	Method(s) used to determine Values:
7-Day LOEC for Mortality (%)	>100		7-Day LOEC for Reprod (%)	>100	
Chronic Value for Mortality (%)	>100		Chronic Value for Reprod (%)	>100	IC Pin, Steel's Many-One Rank
IC25 (%)	>100		IC50 (%)	>100	

*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: 7/18/13
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	7/7/13 @ 10:00	7/8/13 @ 10:00
Type (Grab/Comp):	Comp	2nd	7/9/13 @ 10:00	7/10/13 @ 10:00
Volume Collected:	5 Gal.	3rd	7/11/13 @ 10:00	7/12/13 @ 10:00
Upstream Station:				
Waterbody:	Pike Run			
Station No.:	801	1st		7/8/13 @
Type (Grab/Comp):	Grab	2nd		7/10/13 @ 12:20
Volume Collected:	5 Gal.	3rd		7/12/13 @ 10:17

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:	7/9-16/13
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 - 7/8; day 2, 3 - 7/10; day 4, 5, 6 - 7/12
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* 18-20 hours
2. Test Type and Duration:	Static renewal; 6 to 8 days (6 days)
3. Test Dates:	7/9-15/13
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 - 7/8; day 2, 3 - 7/10; day 4, 5, 6 - 7/12
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

Chain of Custody Record

Alloway
Your Resource for Defensible Data

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
- 508 Bissman Court, Mansfield, OH 44903 (P) 419-525-1644 (F) 419-524-5575

Notes/Comments:

Project: L13-15188

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

Invoice To (If Different):
Name: Melanie Marshall
Company: Allen County Sanitary
Address: 3226 North Cole St.
Lima, OH 45805

Phone #: 419-996-4689 Fax #:
E-mail: mmarshall@allencountyohio.com PO#:

Project Name: Chronic Bioassay - Day One
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	7/07/13-7/08/13	10:00-10:00	x		WW	1	1	Toxicity	15188-01
2	Upstream	7/08/13			x	SW	1	1	"	↓
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) 7/08/13

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:** [illegible] Cnc

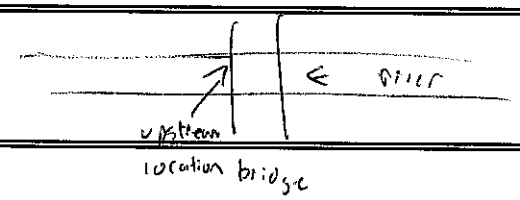
Date: 7/08/13

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	14:15	22.0	808	6.80	7.67
Upstream	14:40	21.9	464	6.07	7.78

Diagram of Collection Sites

~ Width of stream= [illegible]
~ Depth of stream= [illegible]



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
- 508 Bissman Court, Mansfield, OH 44903 (P) 419-525-1644 (F) 419-524-5575

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

Invoice To (If Different):
Name: Melanie Marshall
Company: Allen County Sanitary
Address: 3226 North Cole St.
Lima, OH 45805

Notes/Comments:

Phone #: ~~419-996-4689~~ Fax #:
E-mail: mmarshall@allencountyohio.com PO#:

Project Name: Chronic Bioassay - Day Two - Am Batch
Sampler: (Print) Michael S. Hanby (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	7/9/13-7/10/13	10:00-10:00	x		WW	1	1	Toxicity	
2	Upstream	7/10/13	12:20		x	SW	1	1	↓	
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: 7/10/13 Time: 15:54

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 Tap

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: 7/10/13

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	12:18	23.5	628	7.02	7.51
Upstream	12:29	22.4	505	7.15	7.80

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Chain of Custody Record

Alloway
Your Resource for Defensible Data

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
- 508 Bissman Court, Mansfield, OH 44903 (P) 419-525-1644 (F) 419-524-5575

Report To:		Invoice To (If Different):	
Name:	Melanie Marshall	Name:	Melanie Marshall
Company:	Allen County Sanitary Engineer	Company:	Allen County Sanitary
Address:	3226 North Cole St. Lima, OH 45805	Address:	3226 North Cole St. Lima, OH 45805

Notes/Comments:

Phone #: 419-996-4689 Fax #:

E-mail: mmarshall@allencountyohio.com PO#:

Project Name: Chronic Bioassay - Ann Bath - Day Three

Sampler: (Print) Michael S Hanks (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	7/11/13 7/12/13	10:00 10:00	X		WW	1	1	Toxicity	
2	Upstream	7/12/13	10:17		X	SW	1	1	↓ "	
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) 7/12/13 1032

Method of Delivery	Matrix Codes:	Preservation Codes:		
UPS ☐	ww - wastewater gw - groundwater	1 - None	7 - Sodium Thiosulfate	13 - Zinc Acetate
Fed Ex ☐	dw - drinking water sw - surface water	2 - HNO_3	8 - Ascorbic Acid	14 - Sodium Sulfite
Client ☐	w - water oil - oil	3 - H_2SO_4	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate
Alloway Pick Up ☐	s - solid sg - sludge	4 - HCl	10 - EDA	16 - Sodium Sulfite/Sodium Bisulfate
Alloway Sampling ☒	l - leachate a - acid	5 - NaOH	11 - Ammonium Chloride	
Other ☐	p - product o - other	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: 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:** MSH

Date: 7/12/13

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	10:35	21.2	770	7.82	7.52
Upstream	10:17	20.4	556	7.69	7.66

Diagram of Collection Sites

~ Width of stream= 20' > Approx
~ Depth of stream= 3'

WWTP

X UP

Bridge

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

Person(s) Conducting Test: JJC, RFR
Beginning: Date 7/9/13 **Time** 11:25
Ending: Date 7/16/13 **Time** 11:25
Age of *Pimephales promelas*: <24H EOT
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	10G	40G
5	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
6	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
7	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G

* 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: PJC, RFR
Beginning: Date 7/9/13 **Time** 11:25
Ending: Date 7/16/13 **Time** 11:25
Age of *Pimephales promelas*: <24H ECT
Dilution Water Used: Upstream

Day	60% Effluent					80% Effluent					100% Effluent					~~Far-field Mixing Zone~~				
	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	10G	10G	40G					
3	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					
5	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D					
6	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D					
7	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	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).

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

Discharge: Allen Co. (American - Bath WWTP) Date of Test: 7/9-16/13
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: <24H ECT
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7/18/13 Analyst: CRH

Balance Calibration Check: 1 gram weight = 1.00001 grams Date: 7/18/13 Analyst: CRH

PJCV 7/18/13

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	1.13022	1.13106	10	0.84	0.084
Lab Control	1	10	1.08949	1.09955	10	10.06	1.006
	2	10	1.13013	1.14114	10	11.01	1.101
	3	10	1.08264	1.09196	10	09.32	0.932
	4	10	1.10406	1.11348	10	09.42	0.942
Upstream Control	1	10	1.11062	1.11906	10	08.44	0.844
	2	10	1.13088	1.14048	10	09.60	0.960
	3	10	1.12683	1.13650	10	09.67	0.967
	4	10	1.09225	1.10202	10	09.77	0.977
20% Effluent	1	10	1.13200	1.14040	10	08.40	0.840
	2	10	1.09756	1.10707	10	09.51	0.951
	3	10	1.09167	1.10211	10	10.44	1.044
	4	10	1.12447	1.13462	10	10.15	1.015
40% Effluent	1	10	1.12982	1.14059	10	10.77	1.077
	2	10	1.12377	1.13328	10	09.51	0.951
	3	10	1.08325	1.09232	10	09.07	0.907
	4	10	1.08780	1.09781	10	10.01	1.001

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

Discharge: Allen Co. (American-Bath WWTP) Date of Test: 7/9-16/13
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: <24H ECT
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7/18/13 Analyst: CRH

ICV 7/18/13

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	1.12869	1.13826	10	09.57	0.957
	2	10	1.09483	1.10491	10	10.08	1.008
	3	10	1.13199	1.14125	10	09.26	0.926
	4	10	1.09088	1.10109	10	10.21	1.021
80% Effluent	1	10	1.10008	1.10992	10	09.84	0.984
	2	10	1.09081	1.10005	10	09.24	0.924
	3	10	1.10152	1.11168	10	10.16	1.016
	4	10	1.12547	1.13510	10	09.63	0.963
100% Effluent	1	10	1.12110	1.13069	10	09.59	0.959
	2	10	1.08400	1.09366	10	09.66	0.966
	3	9	1.12314	1.13232	10	09.18	0.918
	4	9	1.09964	1.10826	10	09.~~26~~62 BJC	0.~~826~~62 BJC
~~Far-field Mixing Zone~~	1		~~1.13266~~ CRH		10		
	2				10		
	3				10		
	4				10		

EPA ICP 6/94 PZR 7/18/13 PSW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	.844	.840	1.077	.957	.984	.959
Response 2	.960	.951	.951	1.008	.924	.966
Response 3	.967	1.044	.907	.926	1.016	.918
Response 4	.977	1.015	1.001	1.021	.963	.862

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP 001
Test Start Date: 7/9/13 Test Ending Date: 7/16/13
Test Species: P. promelas
Test Duration: 7 days
DATA FILE: amb0713p.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	0.937	0.062	0.967
2	4	20.000	0.963	0.090	0.967
3	4	40.000	0.984	0.073	0.967
4	4	60.000	0.978	0.044	0.967
5	4	80.000	0.972	0.039	0.967
6	4	100.000	0.926	0.048	0.926

*** 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 NIR 7/18/13

Title: American-Bath WWTP 001 P. promelas 7/9-16/13 Growth mg
File: AMBA0713.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	0.9370	0.9370		
2	20 %	0.9625	0.9625	-0.5809	
3	40 %	0.9840	0.9840	-1.0706	
4	60 %	0.9780	0.9780	-0.9339	
5	80 %	0.9718	0.9718	-0.7916	
6	100 %	0.9263	0.9263	0.2449	

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

Title: American-Bath WWTP 001 P. promelas 7/9-16/13 Growth mg
File: AMBA0713.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	0.1058	11.3	-0.0255
3	40 %	4	0.1058	11.3	-0.0470
4	60 %	4	0.1058	11.3	-0.0410
5	80 %	4	0.1058	11.3	-0.0348
6	100 %	4	0.1058	11.3	0.0107

TOXSTAT 3.5 XTR 7/18/13 PSW

Title: American-Bath WWTP 001 P. promelas 7/9-16/13 Growth mg
File: AMBA0713.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	4	0.8440	0.9770	0.9370
2	20 %	4	0.8400	1.0440	0.9625
3	40 %	4	0.9070	1.0770	0.9840
4	60 %	4	0.9260	1.0210	0.9780
5	80 %	4	0.9240	1.0160	0.9718
6	100 %	4	0.8620	0.9660	0.9263

Title: American-Bath WWTP 001 P. promelas 7/9-16/13 Growth mg
File: AMBA0713.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	0.0039	0.0624	0.0312	6.6586
2	20 %	0.0082	0.0904	0.0452	9.3961
3	40 %	0.0053	0.0729	0.0365	7.4115
4	60 %	0.0020	0.0443	0.0222	4.5322
5	80 %	0.0015	0.0386	0.0193	3.9699
6	100 %	0.0023	0.0478	0.0239	5.1584

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: RFR, RJC
Contact:
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Age of *Ceriodaphnia dubia*: 18-20H ECTA2
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	2 G	0 G	3 G			8	3
2			0	3	Abort 0	0	Abort 0 G			3	3
3			0	4	Abort 0	0	0 D		D	4	2
4			0	2	0 D	– –	– –	– –	D	2	1
5			0	4	0 D	–	– –	– –	D	4	1
6			2	0	Abort 0 G	10 G	0 G			12	3
7			1	0	Abort 0	8	0 G			9	3
8			0	4	Abort 0	0	10 G			14	3
9			0	4	Abort 0	11	0 G			15	3
10			0	4	3	0	7 G			14	3
Total Adult Cond.	10G	10G	10G	10G	8G 2D	8G 2D	7G 3D	–			
									SUM	85	25
									MEAN	8.5	2.5
									S.D.	5.0	–

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

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Age of *Ceriodaphnia dubia*: 18-20H
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	9 G	14 G	0 G			27	3
2			0	4	7	17	0 G			28	3
3			0	4	9	12	4 D		D	29	3
4			0	4	7	12	0 G			23	3
5			0	6	8	13	0			27	3
6			0	4	8	15	1			28	3
7			2	0	7	15	0			24	3
8			0	3	9	18	1			31	3
9			3	0	8	11	0			22	3
10			0	2	9	18	0			29	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	9G 10	—			
									SUM	268	30
									MEAN	26.8	3.0
									S.D.	2.9	—

* 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: RFR, MJC
Contact:
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Age of *Ceriodaphnia dubia*: 18-24H
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	9 / G	15 / G	0 / G	/	28	3
2	/	/	0 /	3 /	8 /	16 /	0 /	/	27 ~~25~~ pm	3
3	/	/	0 /	4 /	10 /	15 /	0 /	/	29	3
4	/	/	0 /	5 /	9 /	11 /	0 /	/	25	3
5	/	/	0 /	4 /	8 /	14 /	0 /	/	26	3
6	/	/	3 /	0 /	8 /	16 /	0 /	/	27	3
7	/	/	2 /	1 /	8 /	14 /	0 /	/	25	3
8	/	/	0 /	4 /	9 /	14 /	0 /	/	27	3
9	/	/	0 /	3 /	6 /	12 /	1 /	/	22	3
10	/	/	0 /	3 /	9 /	14 /	0 /	/	26	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—		
									SUM 262	30
									MEAN 26.2	3.0
									S.D. 1.9	—

* 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: RFR, MJC
Contact:
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/15/13 Time: 1125
Age of *Ceriodaphnia dubia*: 18-24H
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	7/G	0/G	17/G	/	28	3
2	/	/	0/	0/	8/	15/	0/	/	23	2
3	/	/	0/	3/	10/	18/	0/	/	31	3
4	/	/	4/	0/	7/	16/	0/	/	27	3
5	/	/	0/	4/	8/	15/	0/	/	27	3
6	/	/	0/	2/	9/	15/	0/	/	26	3
7	/	/	2/	0/	6/	17/	0/	/	25	3
8	/	/	0/	5/	9/	13/	0/	/	27	3
9	/	/	4/	0/	8/	16/	0/	/	28	3
10	/	/	0/	4/	8/	0/	13/	/	25	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–	SUM 267	~~30~~ 29
									MEAN 26.7	~~3.0~~ 2.9
									S.D. 2.2	—

* 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: RFR, PJC
Contact:
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/15/13 Time: 1125
Age of *Ceriodaphnia dubia*: 18-20H
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	9/G	16/G	0/G	/	29	3
2	/	/	0/	4/	7/	17/	0/	/	28	3
3	/	/	0/	4/	12/	11/	0/	/	27	3
4	/	/	0/	2/	9/	13/	0/	/	24	3
5	/	/	0/	4/	8/	11/	0/	/	23	3
6	/	/	0/	4/	7/	14/	0/	/	25	3
7	/	/	3/	0/	6/	10/	0/	/	19	3
8	/	/	0/	5/	9/	5/	0/	/	19	3
9	/	/	4/	0/	8/	15/	0/	/	27	3
10	/	/	0/	5/	11/	0/	8/	/	24	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	✓		
									SUM 245	30
									MEAN 24.5	3.0
									S.D. 3.5	—

* 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: RFR, PJC
Contact:
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/19/13 Time: 11:25
Age of *Ceriodaphnia dubia*: 18-20H
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	9/G	13/G	0/G	/	26	3
2	/	/	4/	0/	7/	15/	0/	/	26	3
3	/	/	0/	4/	9/	10/	0/	/	23	3
4	/	/	0/	6/	8/	15/	0/	/	29	3
5	/	/	0/	4/	8/	14/	1/	/	27	3
6	/	/	0/	5/	7/	18/	0/	/	30	3
7	/	/	2/	0/	8/	12/	0/	/	22	3
8	/	/	0/	3/	10/	17/	0/	/	30	3
9	/	/	0/	0/	9/	1/	0/	/	10	2
10	/	/	0/	5/	6/	1/	10/	/	23	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–		
								SUM	246	29
								MEAN	24.6	2.9
								S.D.	5.9	–

* 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: RFR, PJC
Contact:
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:28
Age of *Ceriodaphnia dubia*: 18-20H
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	6 G	0 D	–	–	D	9	2
2			0	4	7	12 G	0 G			23	3
3			0	4	9	17	0			30	3
4			0	5	8	14	0			27	3
5			0	4	9	12	0			25	3
6			4	0	6	11	0			21	3
7			0	0	7	12	0			19	2
8			0	4	14	13	0			31	3
9			4	0	7	16	0			27	3
10			0	5	8	16	0			29	3
Total Adult Cond.	10G	10G	10G	10G	10G	9G 1D	9G 1D	–	SUM	241	28
									MEAN	24.1	2.8
									S.D.	6.6	–

* 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).

TOXSTAT 3.5

Title: American-Bath WWTP 001 C. dubiqa 7/9-15/13 Reproduction DTA 7/15/13 PAW
File: AMBA0713.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	26.8000				
2	20 %	26.2000	93.50	75.00	10.00	
3	40 %	26.7000	99.00	75.00	10.00	
4	60 %	24.5000	86.50	75.00	10.00	
5	80 %	24.6000	94.50	75.00	10.00	
6	100 %	24.1000	94.00	75.00	10.00	

Critical values are 1 tailed (k = 5)

EPA ICV 6/94 RZA 7/16/13 PSW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	27	28	28	29	26	9
Response 2	28	27	23	28	26	23
Response 3	29	29	31	27	23	30
Response 4	23	25	27	24	29	27
Response 5	27	26	27	23	27	25
Response 6	28	27	26	25	30	21
Response 7	24	25	25	19	22	19
Response 8	31	27	27	19	30	31
Response 9	22	22	28	27	10	27
Response 10	29	26	25	24	23	29

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Allen Co. American-Bathw WWTP 001
Test Start Date: 7/9/13 Test Ending Date: 7/15/13
Test Species: C. dubia
Test Duration: 6 days
DATA FILE: amb0713c.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	26.800	2.898	26.800
2	10	20.000	26.200	1.932	26.450
3	10	40.000	26.700	2.163	26.450
4	10	60.000	24.500	3.472	24.550
5	10	80.000	24.600	5.892	24.550
6	10	100.000	24.100	6.574	24.100

*** 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 PZL 7/15/13 Rev

Title: American-Bath WWTP 001 C. dubiqa 7/9-15/13 Reproduction
File: AMBA0713.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	10	22.0000	31.0000	26.8000
2	20 %	10	22.0000	29.0000	26.2000
3	40 %	10	23.0000	31.0000	26.7000
4	60 %	10	19.0000	29.0000	24.5000
5	80 %	10	10.0000	30.0000	24.6000
6	100 %	10	9.0000	31.0000	24.1000

Title: American-Bath WWTP 001 C. dubiqa 7/9-15/13 Reproduction
File: AMBA0713.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	8.4000	2.8983	0.9165	10.8145
2	20 %	3.7333	1.9322	0.6110	7.3747
3	40 %	4.6778	2.1628	0.6839	8.1004
4	60 %	12.0556	3.4721	1.0980	14.1719
5	80 %	34.7111	5.8916	1.8631	23.9496
6	100 %	43.2111	6.5735	2.0787	27.2760

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: 7/9-16/13
Effluent Serial No.: 001 Permit No.: 2PH00007*

Investigator: RCA Culture No.: ECT # 2
Culture initiation date: _____ Culture ending date: _____
Origin of parental organisms: ECT # Rec 7/9/13
Age at start of culture: _____
Culture water renewal frequency: minimum 3 times per week Culture water: MHSF
Culture temperature: 25°C Fed daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

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

	BROOD ADULT NO.	BROOD SIZE	HATCHING TIME WINDOW	TOTAL; YOUNG PROD. (7 DAYS)	USED IN REP. NO.
1)	1	10	1500 to 1700 7/8/13		1
2)	2	9			2
3)	3	12			3
4)	4	12			4
5)	5	9			5
6)	6	9			6
7)	7	8			7
8)	8	12			8
9)	9	8			9
10)	10	12			10

COMMENTS: _____

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: 7/9-16/13
Effluent Serial No.: 001 Permit No.: 2PH00007*

1st Sample Collection

Date Samples Collected: 7/8/13 Date Samples Read: 7/8/13 Analyst: RJC

	100% Effluent	Upstream
Temperature (°C)	24.3	23.0
Dissolved Oxygen (mg/L)	6.4	7.7
pH (s.u.)	7.67	7.86
Conductivity (µmhos)	813	459
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	tan/brown stain

2nd Sample Collection

Date Samples Collected: 7/10/13 Date Samples Read: 7/10/13 Analyst: RKA

	100% Effluent	Upstream
Temperature (°C)	16.1	17.3
Dissolved Oxygen (mg/L)	8.1	8.2
pH (s.u.)	7.58	7.75
Conductivity (µmhos)	619	503
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light brown stain

3rd Sample Collection

Date Samples Collected: 7/12/13 Date Samples Read: 7/12/13 Analyst: ORA

	100% Effluent	Upstream
Temperature (°C)	21.8	21.3
Dissolved Oxygen (mg/L)	8.0	8.2
pH (s.u.)	7.89	8.09
Conductivity (µmhos)	682	556
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	stained	stained

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: PJC, RFR
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/16/13 Time: 11:25
Test Organism: *Pimephales promelas* Age: <24H old
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	24.8 to 25.0	25.3	25.0 to 25.2	25.1	25.0 to 25.1	25.4	24.9 to 25.2	25.3	24.7 to 24.9	25.2	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	8.2	9.0	6.3	8.2	5.9	8.5	5.6	8.0	6.0	8.7	5.9	8.8	5.7	6.8
pH (s.u.)	7.59	7.20	7.15	7.65	7.21	7.54	7.34	7.76	7.42	7.64	7.66	7.60	7.44	7.40
Conductivity (µmho/cm)	367	366	374	364	383	365	383	336	376	336	354	337	361	358
Hardness (mg/L $CaCO_3$)	154			120				137						
Alkalinity (mg/L $CaCO_3$)	86			86				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: PJC, RFR
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/16/13 Time: 1125
Test Organism: *Pimephales promelas* Age: <24H ECT
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	24.8 to 25.0	25.4	25.0 to 25.2	25.3	25.0 to 25.1	25.4	24.9 to 25.2	25.3	24.7 to 24.9	25.3	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	8.1	8.2	5.1	8.4	4.8	8.5	3.8	8.2	5.2	8.7	5.0	8.9	5.7	6.7
pH (s.u.)	7.94	7.77	7.43	7.90	7.55	7.99	7.64	8.01	7.67	7.95	7.69	7.98	7.67	7.71
Conductivity (µmho/cm)	460	459	474	503	474	503	520	555	574	556	570	556	576	573
Hardness (mg/L $CaCO_3$)	188			222				257						
Alkalinity (mg/L $CaCO_3$)	171			171				205						

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: PJC, RFR
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/16/13 Time: 1125
Test Organism: *Pimephales promelas* Age: <24H ECT
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	24.8 to 25.0	25.4	25.0 to 25.2	25.3	25.0 to 25.1	25.4	24.9 to 25.2	25.4	24.7 to 24.9	25.4	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	8.1	8.3	6.0	8.5	5.3	8.5	6.4	8.2	6.1	8.9	6.7	9.0	6.3	7.1
pH (s.u.)	7.97	7.74	7.54	7.91	7.56	8.02	7.84	8.00	7.78	8.00	7.90	8.01	7.76	7.79
Conductivity (µmho/cm)	531	532	548	527	554	527	551	581	548	581	590	581	614	611
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: PJC, RFR
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/16/13 Time: 1125
Test Organism: *Pimephales promelas* Age: <24 H ECI
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	25.1	24.8 to 25.0	25.3	25.0 to 25.2	25.2	25.0 to 25.1	25.4	24.9 to 25.2	25.3	24.7 to 24.9	25.4	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	7.9	8.2	4.9	8.5	5.4	8.5	3.4	8.1	5.1	8.9	5.6	9.1	5.5	7.0
pH (s.u.)	7.96	7.86	7.52	7.90	7.67	8.02	7.7	8.01	7.73	7.99	7.89	8.01	7.76	7.79
Conductivity (µmho/cm)	604	605	631	550	634	550	584	605	573	606	625 ~~658~~	606	644	644
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: PJC, RFR
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/16/13 Time: 1125
Test Organism: *Pimephales promelas* Age: <24H EC
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	24.8 to 25.0	25.4	25.0 to 25.2	25.3	25.0 to 25.1	25.4	24.9 to 25.2	25.4	24.7 to 24.9	25.3	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	7.8	8.4	6.1	8.6	5.8	8.5	6.6	8.2	6.0	8.2	6.0	8.8	6.0	7.1
pH (s.u.)	7.94	7.83	7.61	7.89	7.71	8.03	7.89	7.99	7.80	7.97	7.91	7.99	7.79	7.80
Conductivity (µmho/cm)	673	673	703	574	701	574	608	630	595	632	660	632	672	671
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: PJC, RFR
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/16/13 Time: 1125
Test Organism: *Pimephales promelas* Age: <24H EC
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	24.8 to 25.0	25.4	25.0 to 25.2	25.2	25.0 to 25.1	25.4	24.9 to 25.2	25.3	24.7 to 24.9	25.4	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	7.8	8.2	5.9	8.5	5.4	8.4	5.5	8.1	5.7	8.3	5.9	8.5	6.0	6.8
pH (s.u.)	7.94	7.85	7.61	7.86	7.71	8.02	7.83	7.99	7.81	7.95	7.92	7.95	7.81	7.81
Conductivity (µmho/cm)	743	743	756	596	761	596	676	655	671	658	667	658	684	686
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: PJC, RFR
Beginning Date: 7/9/13 Time: 1125
Ending Date: 7/16/13 Time: 1125
Test Organism: *Pimephales promelas* Age: <24 H EC
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)	25.1	25.2	24.8 to 25.0	25.3	25.0 to 25.2	25.2	25.0 to 25.1	25.4	24.9 to 25.2	25.3	24.7 to 24.9	25.5	25.2 to 25.4	24.7 to 25.0
Dissolved Oxygen (mg/L)	7.5	8.4	6.1	8.4	6.6	8.5	5.6	8.2	6.1	8.2	6.2	9.0	5.8	7.4
pH (s.u.)	7.85	7.80	7.65	7.83	7.78	8.02	7.83	7.96	7.79	7.92	7.94	7.94	7.80	7.84
Conductivity (µmho/cm)	813	813	832	619	841	678	661	681	646	681	698	682	719	697
Hardness (mg/L $CaCO_3$)	308			291				308						
Alkalinity (mg/L $CaCO_3$)	205			188				205						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

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

Person(s) Conducting Test: RFR, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Test Organism: *Ceriodaphnia dubia*: Age: 18-20H ECT #2
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)	25.2	25.0	25.2 to 25.8	25.4	25.0 to 25.4	25.2	25.1 to 25.4	25.2	25.0 to 25.4	25.2	25.0 to 25.2	25.4	25.0 to 25.2	—
Dissolved Oxygen (mg/L)	8.0	8.5	7.7	8.1	8.1	8.2	7.9	7.8	8.2	8.5	8.1	8.0	7.3	
pH (s.u.)	8.04	7.66	7.51	7.57	7.49	7.58	7.71	7.64	7.52	7.74	7.47	7.70 ~~7.4~~ BLS	7.67	
Conductivity (µmho/cm)	306	307	312	330	315	328	331	334	332	332	336	327	343	
Hardness (mg/L $CaCO_3$)	120			120				120						
Alkalinity (mg/L $CaCO_3$)	103			103				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: RFR, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Test Organism: *Ceriodaphnia dubia*: Age: 18-24H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		T
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.1	25.2 to 25.5	25.4	25.0 to 25.4	25.3	25.1 to 25.4	25.4	25.0 to 25.4	25.3	25.0 to 25.3	25.3	25.0 to 25.2	✓
Dissolved Oxygen (mg/L)	8.1	8.2	7.7	8.4	8.0	8.5	7.9	8.2	7.7	8.7	8.0	8.9	7.4	
pH (s.u.)	7.94	7.77	7.62	7.90	7.83	7.99	8.00	8.01	7.99	7.95	7.95	7.98	8.01	
Conductivity (µmho/cm)	460	459	465	503	472	503	513	555	507	556	565	550	570	
Hardness (mg/L $CaCO_3$)	188			222				257						
Alkalinity (mg/L $CaCO_3$)	171			171				205						

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, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:28
Test Organism: *Ceriodaphnia dubia*: Age: 18-20H
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	25.2 to 25.5	25.4	~~24.8~~ 25.0 to 25.4	25.3	25.1 to 25.4	25.4	25.0 to 25.4	25.4	25.0 to 25.3	25.4	25.0 to 25.2	–
Dissolved Oxygen (mg/L)	8.1	8.3	7.7	8.5	7.9	8.5	8.0	8.2	7.8	8.9	7.9	9.0	7.4	
pH (s.u.)	7.97	7.79	7.70	7.91	7.87	8.02	8.07	8.00	8.05	8.00	7.99	8.01	8.08	
Conductivity (µmho/cm)	531	532	540	527	549	527	543	581	534	581	596	581	600	
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, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Test Organism: *Ceriodaphnia dubia*: Age: 18-24
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	25.1	25.2 to 25.5	25.3	25.0 to 25.4	25.2	25.1 to 25.4	25.4	25.0 to 25.4	25.3	25.0 to 25.3	25.4	25.0 to 25.2	–
Dissolved Oxygen (mg/L)	7.9	8.2	7.7	8.5	7.9	8.5	7.9	8.1	7.8	8.9	7.9	9.1	7.4	
pH (s.u.)	7.96	7.86	7.74	7.90	7.91	8.02	8.02	8.01	8.06	7.99	8.04	8.01	8.10	
Conductivity (µmho/cm)	604	605	610	556	620	550	567	605	559	606	618	606	623	
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, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:28
Test Organism: *Ceriodaphnia dubia*: Age: 18-24h
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)	25.4	25.2	25.2 to 25.5	25.4	25.0 to 25.4	25.3	25.1 to 25.4	25.4	25.0 to 25.4	25.4	25.0 to 25.3	25.3	25.0 to 25.2	–
Dissolved Oxygen (mg/L)	7.8	8.4	7.8	8.6	8.0	8.5	8.0	8.2	7.8	8.2	7.8	8.8	7.5	
pH (s.u.)	~~6.74~~ 7.94	7.83	7.76	7.89	7.94	8.03	8.04	7.99	8.09	7.97	8.08	7.99	8.13	
Conductivity (µmho/cm)	673	673	679	574	696	574	594	630	585	632	639	632	651	
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, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Test Organism: *Ceriodaphnia dubia* Age: 18-20H
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	25.2 to 25.8	25.4	25.0 to 25.4	25.2	25.1 to 25.4	25.4	25.0 to 25.4	25.3	25.0 to 25.3	25.4	25.0 to 25.2	—
Dissolved Oxygen (mg/L)	7.8	8.2	7.8	8.5	7.9	8.4	7.9	8.1	7.8	8.3	8.0	8.5	7.5	
pH (s.u.)	7.94	7.85	7.79	7.86	7.97	8.02	8.05	7.99	8.10	7.95	8.11	7.95	8.14	
Conductivity (µmho/cm)	743	743	753	596	767	596	616	655	606	658	672	658	672	
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, PJC
Beginning Date: 7/9/13 Time: 11:25
Ending Date: 7/15/13 Time: 11:25
Test Organism: *Ceriodaphnia dubia*: Age: 18-24H
Dilution Water Used: Upstream

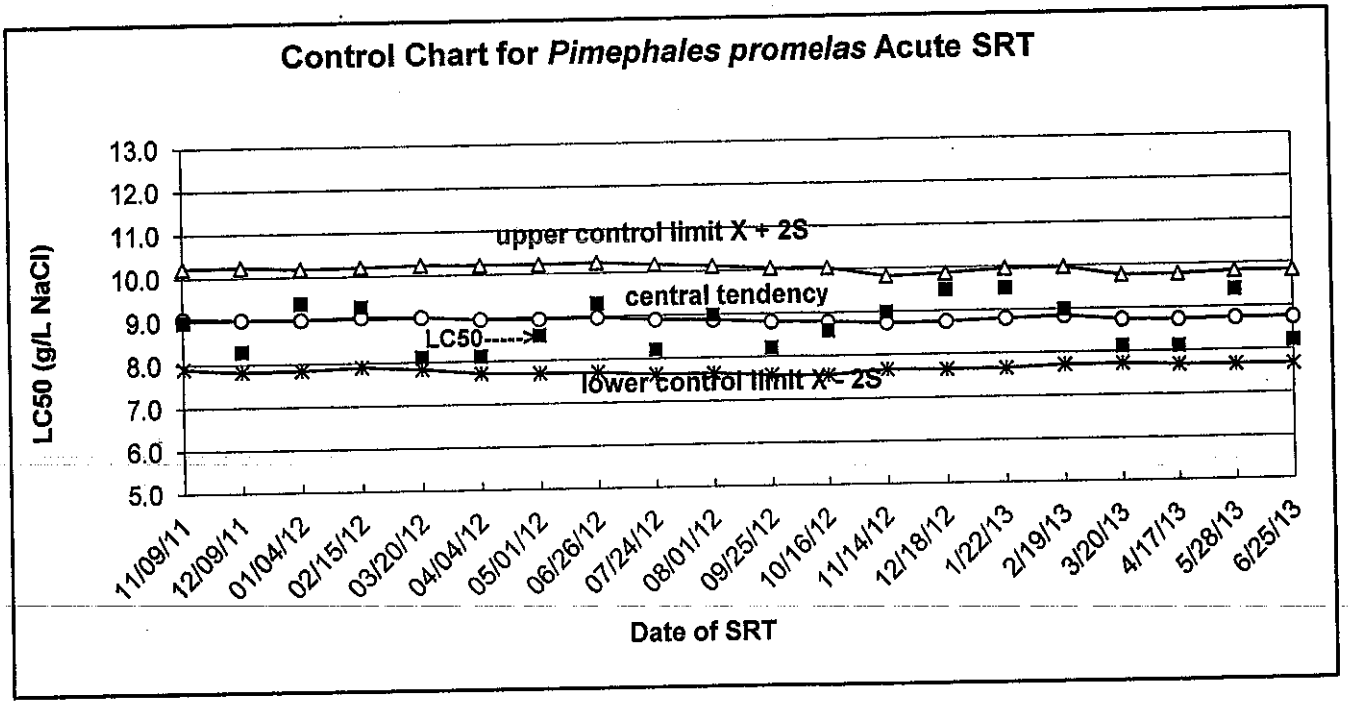
Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	~~Old~~
Temperature (°C)	25.1	25.2	25.2 to 25.5	25.3	25.0 to 25.4	25.2	25.1 & 25.4	25.4	25.0 to 25.4	25.3	25.0 to 25.3	25.5	25.0 to 25.2	-
Dissolved Oxygen (mg/L)	7.5	8.4	7.8	8.4	8.6	8.5	7.9	8.0	7.8	8.2	7.8	9.0	7.4	
pH (s.u.)	7.85	7.80	7.80	7.83	8.01	8.00	8.05	7.96	8.12	7.92	8.14	7.94	8.14	
Conductivity (µmho/cm)	813	813	817	614	833	618	642	681	624	681	695	682	700	
Hardness (mg/L $CaCO_3$)	308			291				308						
Alkalinity (mg/L $CaCO_3$)	205			188				205						

Appendix D

SRT Data

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

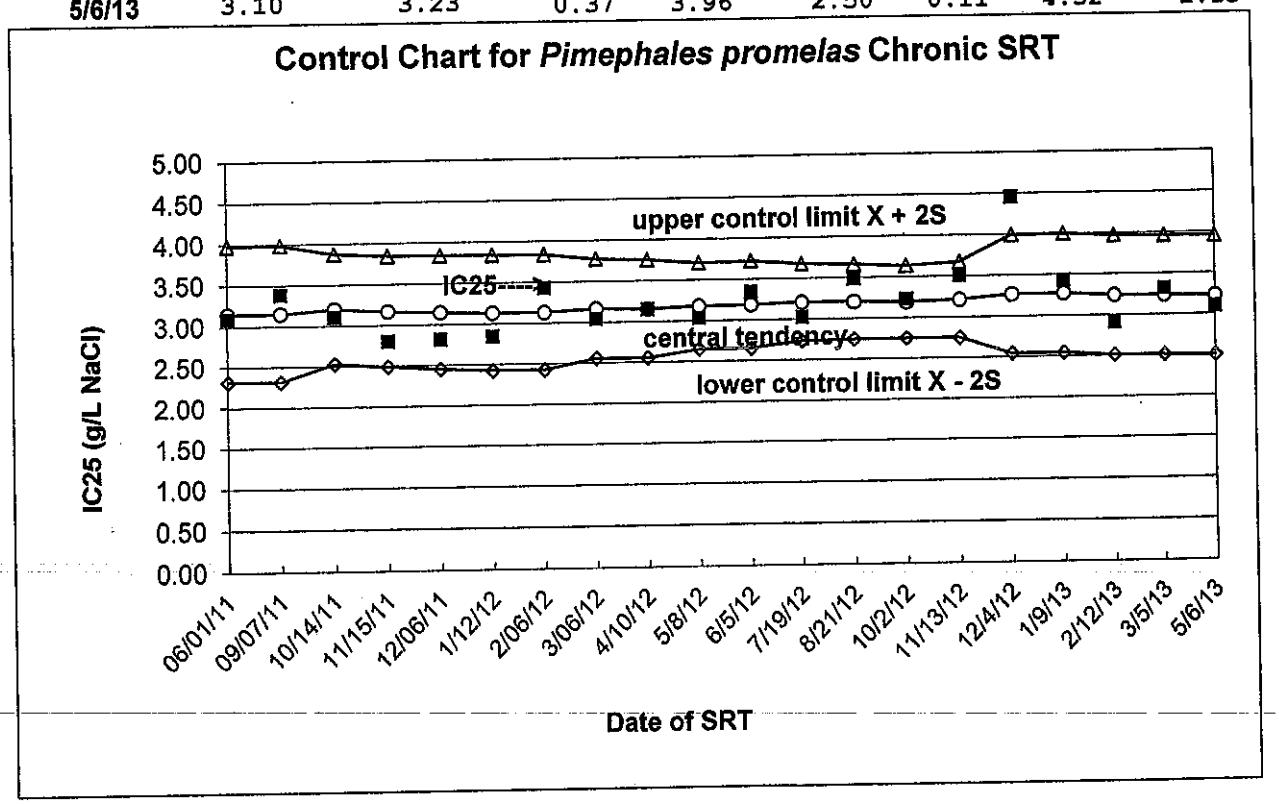
Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
11/09/11	9.0	9.1	0.6	10.2	7.9	0.06	10.8	7.3	SK
12/09/11	8.3	9.0	0.6	10.2	7.8	0.07	10.8	7.2	SK
01/04/12	9.4	9.0	0.6	10.2	7.8	0.06	10.8	7.3	SK
02/15/12	9.3	9.1	0.6	10.2	7.9	0.06	10.8	7.3	SK
03/20/12	8.1	9.0	0.6	10.2	7.8	0.07	10.8	7.2	SK
04/04/12	8.1	9.0	0.6	10.2	7.7	0.07	10.8	7.1	SK
05/01/12	8.6	9.0	0.6	10.2	7.7	0.07	10.8	7.1	SK
06/26/12	9.3	9.0	0.6	10.2	7.7	0.07	10.9	7.1	SK
07/24/12	8.2	8.9	0.6	10.2	7.6	0.07	10.8	7.0	SK
08/01/12	9.0	8.9	0.6	10.1	7.6	0.07	10.7	7.0	SK
09/25/12	8.2	8.8	0.6	10.0	7.6	0.07	10.7	7.0	SK
10/16/12	8.6	8.8	0.6	10.0	7.6	0.07	10.7	6.9	SK
11/14/12	9.0	8.7	0.5	9.8	7.7	0.06	10.4	7.1	SK
12/18/12	9.5	8.8	0.6	9.9	7.6	0.06	10.4	7.1	P
1/22/13	9.5	8.8	0.6	10.0	7.7	0.07	10.5	7.1	P
2/19/13	9.0	8.8	0.6	10.0	7.7	0.06	10.5	7.2	SK
3/20/13	8.1	8.7	0.5	9.8	7.7	0.06	10.3	7.2	P
4/17/13	8.1	8.7	0.5	9.8	7.7	0.06	10.3	7.2	SK
5/28/13	9.4	8.7	0.5	9.8	7.7	0.06	10.4	7.1	SK
6/25/13	8.2	8.7	0.5	9.8	7.7	0.06	10.4	7.1	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
06/01/11	3.09	3.15	0.42	3.98	2.32	0.13	4.40	1.90
09/07/11	3.39	3.15	0.42	3.99	2.32	0.13	4.41	1.90
10/14/11	3.11	3.20	0.34	3.88	2.53	0.10	4.21	2.20
11/15/11	2.81	3.17	0.34	3.85	2.49	0.11	4.19	2.15
12/06/11	2.82	3.15	0.35	3.84	2.45	0.11	4.19	2.11
1/12/12	2.85	3.13	0.35	3.84	2.43	0.11	4.20	2.07
2/06/12	3.43	3.13	0.35	3.84	2.43	0.11	4.19	2.07
3/06/12	3.05	3.17	0.30	3.78	2.56	0.10	4.08	2.26
4/10/12	3.15	3.16	0.30	3.76	2.56	0.10	4.06	2.26
5/8/12	3.04	3.18	0.26	3.71	2.66	0.08	3.97	2.39
6/5/12	3.35	3.19	0.27	3.72	2.66	0.08	3.99	2.39
7/19/12	3.03	3.21	0.23	3.68	2.74	0.07	3.91	2.51
8/21/12	3.49	3.21	0.23	3.66	2.75	0.07	3.89	2.52
10/2/12	3.23	3.19	0.22	3.64	2.75	0.07	3.86	2.53
11/13/12	3.51	3.21	0.23	3.68	2.75	0.07	3.91	2.52
12/4/12	4.46	3.27	0.36	3.99	2.55	0.11	4.36	2.18
1/9/13	3.43	3.27	0.36	4.00	2.55	0.11	4.37	2.18
2/12/13	2.92	3.24	0.37	3.97	2.51	0.11	4.34	2.15
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



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

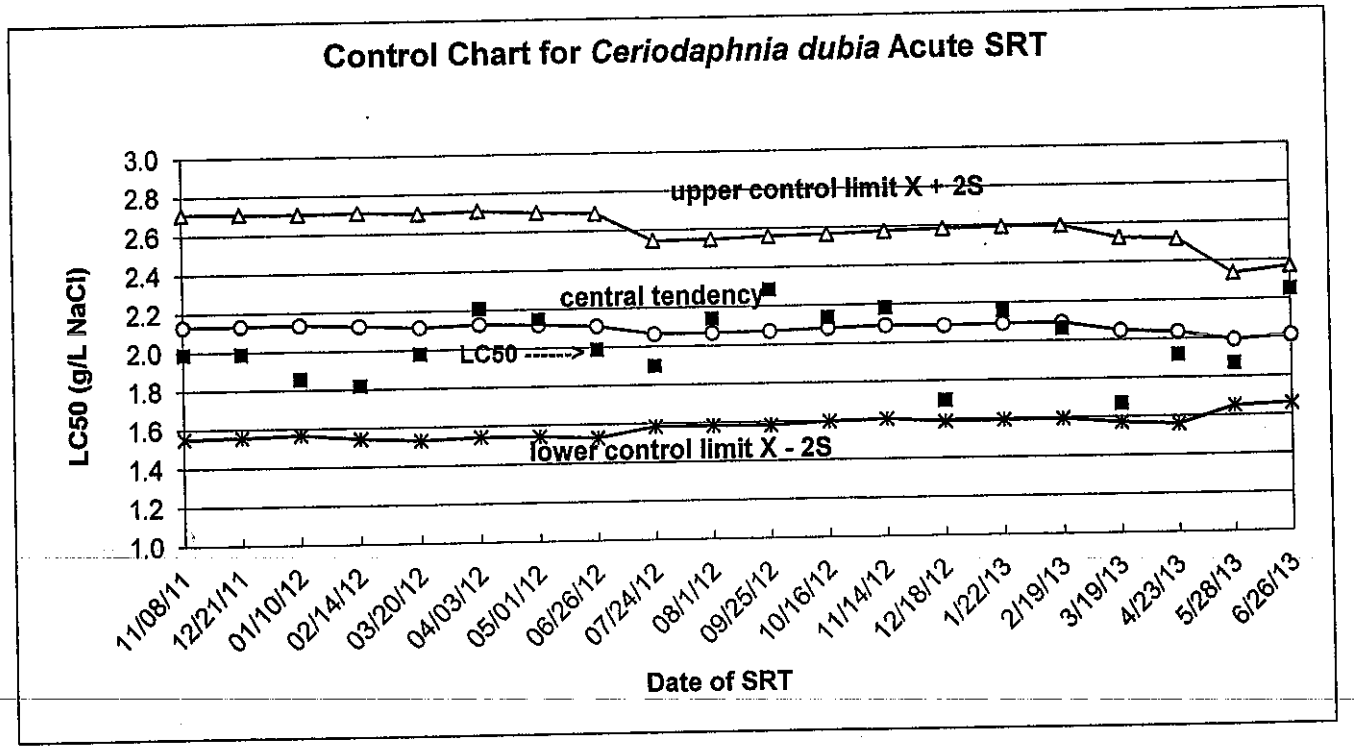
75th percentile of CVs for IC25 = 0.38

Calculated CV should not exceed 75th percentile of CVs.

$\overline{X} \pm 3SD$ = 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]
11/08/11	2.0	2.1	0.3	2.7	1.6	0.14	3.0	1.3	TSK
12/21/11	2.0	2.1	0.3	2.7	1.6	0.14	3.0	1.3	SK
01/10/12	1.9	2.1	0.3	2.7	1.6	0.13	3.0	1.3	Probit
02/14/12	1.8	2.1	0.3	2.7	1.5	0.14	3.0	1.3	SK
03/20/12	2.0	2.1	0.3	2.7	1.5	0.14	3.0	1.2	TSK
04/03/12	2.2	2.1	0.3	2.7	1.5	0.14	3.0	1.3	Probit
05/01/12	2.2	2.1	0.3	2.7	1.5	0.14	3.0	1.3	Probit
06/26/12	2.0	2.1	0.3	2.7	1.5	0.14	3.0	1.2	SK
07/24/12	1.9	2.1	0.2	2.5	1.6	0.12	2.8	1.3	SK
08/1/12	2.1	2.1	0.2	2.5	1.6	0.12	2.8	1.3	SK
09/25/12	2.3	2.1	0.2	2.6	1.6	0.12	2.8	1.3	Probit
10/16/12	2.1	2.1	0.2	2.6	1.6	0.12	2.8	1.4	Probit
11/14/12	2.2	2.1	0.2	2.6	1.6	0.12	2.8	1.4	Probit
12/18/12	1.7	2.1	0.2	2.6	1.6	0.12	2.8	1.3	Graphical
1/22/13	2.2	2.1	0.2	2.6	1.6	0.12	2.8	1.3	TSK
2/19/13	2.1	2.1	0.2	2.6	1.6	0.12	2.8	1.3	Probit
3/19/13	1.7	2.0	0.2	2.5	1.6	0.12	2.8	1.3	SK
4/23/13	1.9	2.0	0.2	2.5	1.6	0.12	2.8	1.3	TSK
5/28/13	1.9	2.0	0.2	2.3	1.7	0.09	2.5	1.5	Probit
6/26/13	2.3	2.0	0.2	2.4	1.7	0.09	2.5	1.5	TSK

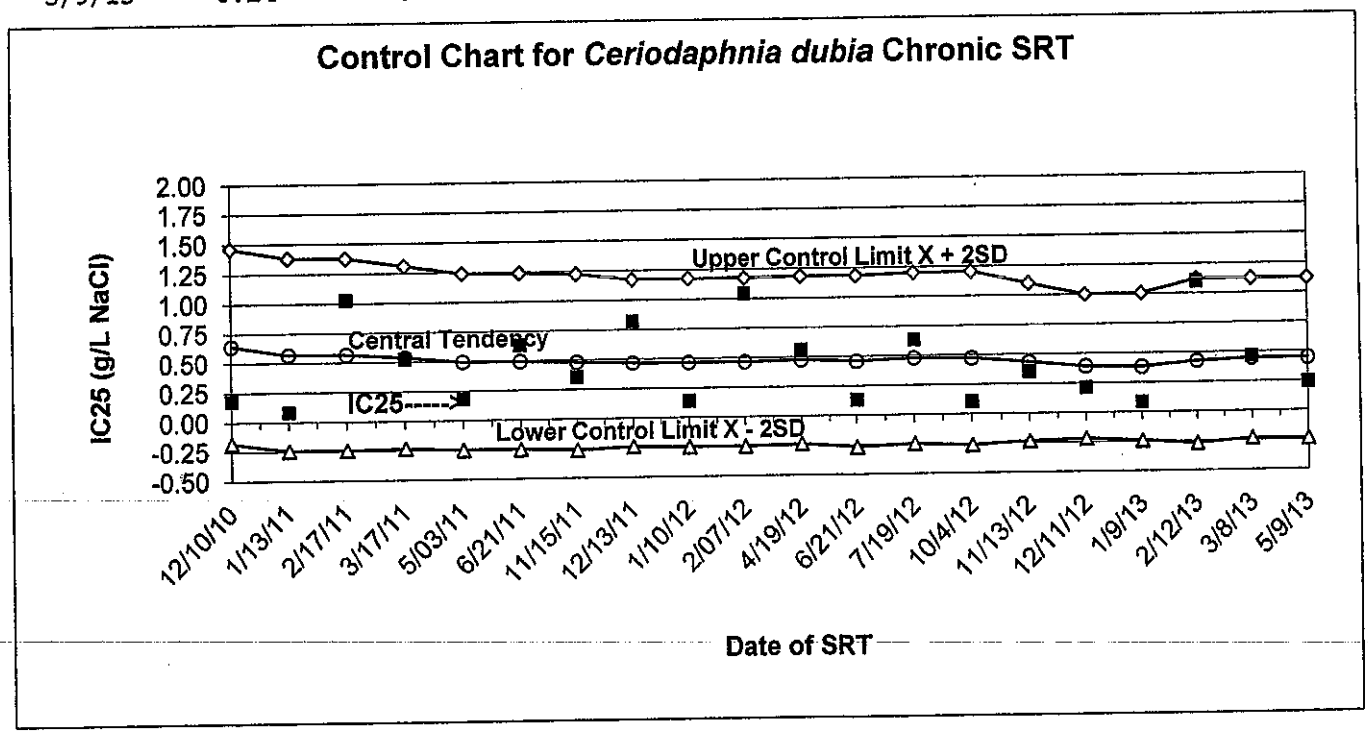


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\bar{X} \pm 3SD$ = Well outside expected range.

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
12/10/10	0.18	0.64	0.41	1.46	-0.18	0.64	1.88	-0.59
1/13/11	0.09	0.57	0.41	1.38	-0.24	0.71	1.79	-0.65
2/17/11	1.03	0.57	0.41	1.38	-0.24	0.71	1.78	-0.65
3/17/11	0.53	0.54	0.39	1.31	-0.23	0.71	1.70	-0.62
5/03/11	0.18	0.50	0.37	1.24	-0.25	0.75	1.61	-0.62
6/21/11	0.63	0.50	0.37	1.24	-0.25	0.75	1.61	-0.62
11/15/11	0.35	0.48	0.37	1.23	-0.26	0.77	1.60	-0.63
12/13/11	0.82	0.47	0.35	1.17	-0.24	0.75	1.53	-0.59
1/10/12	0.14	0.46	0.36	1.18	-0.25	0.77	1.53	-0.60
2/07/12	1.05	0.46	0.36	1.18	-0.25	0.77	1.53	-0.60
4/19/12	0.56	0.48	0.35	1.19	-0.23	0.75	1.54	-0.59
6/21/12	0.13	0.46	0.36	1.18	-0.27	0.79	1.55	-0.63
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



*- 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.