Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/17/14
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L14-15506-01

Report of Chronic Biomonitoring

Samples Collected on:

7/7/14
7/9/14
7/11/14

Pimephales promelas
Ceriodaphnia dubia

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

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Outfall: Effluent 001
Report Date: 7/17/14
Ohio EPA Permit No.: 2PH00007*KD
Alloway Lab #: L14-15506-01

Date: 7/17/14

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.

Paul J. Crerar
Biology Lab Manager

Richard F. Rupp
Senior Biologist

Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 7/17/14
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	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: 7/8-14/14

Summary of Chronic Toxicity Tedt Results
Ceriodaphnia dubia

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, *Ceriodaphnia dubia* - TUc	1.1	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	AA	10.0	

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*KD
Date of Test: 7/8-15/14

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%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.676	0.109
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%)	0.721	0.078
20%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	7.5% (7.5%)	0.584	0.093
40%	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	0.576	0.045
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	0.678	0.031
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%)	2.5% (2.5%)	0.658	0.031
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%)	2.5% (2.5%)	0.745	0.053

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: 7/8-14/14

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%)	--- (---)	30.8
Secondary Control Lab	0.0% (0.0%)	10.0% (10.0%)	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%)	--- (---)	31.8
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%)	--- (---)	28.1
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (10.0%)	--- (---)	27.6
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (10.0%)	--- (---)	25.7
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	--- (---)	24.~~1~~4 *
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 1.1
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 (%)	>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
Report Date: 7/17/14
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	7/6/14 @ 09:00	7/7/14 @ 09:00
Type (Grab/Comp):	Comp	2nd	7/8/14 @ 09:00	7/9/14 @ 08:15
Volume Collected:	5 Gal.	3rd	7/10/14 @ 09:00	7/11/14 @ 09:00
Upstream Station:				
Waterbody:	Pike Run			
Station No.:	801	1st		7/7/14 @ 10:00
Type (Grab/Comp):	Grab	2nd		7/9/14 @ 09:00
Volume Collected:	5 Gal.	3rd		7/11/14 @ 09:35

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:	7/8-15/14
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 - 7/7; day 2, 3 - 7/9; day 4, 5, 6 - 7/11
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* 9-17 hours
2. Test Type and Duration:	Static renewal; 6 to 8 days (6 days)
3. Test Dates:	7/8-14/14
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 - 7/7; day 2, 3 - 7/9; day 4, 5, 6 - 7/11
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

Report To: Name:	Rick Boerger
Company:	Allen County Sanitary Engineer
Address:	3226 North Cole St. Lima, OH 45805
Invoice To (If Different):	Rick Boerger
Company:	Allen County Sanitary
Address:	3226 North Cole St. Lima, OH 45805
Phone #:	419-225-8048
Fax #:	
E-mail:	msantaguida@allencountyohio.com; rboerger@allencountyohio.com; jmotycka@allencountyohio.com
PO#:	
Project Name	Bioassay - Am. Bath - Day 1
Sampler	(Print) Mark Zwick (Signature)

Notes/Comments: ~~ICP 200.7 Metals: Cd, Cr, Cu, Pb, Ni, Sr, Zn~~

Project: L14-15672

Project: L14-15506

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									TOXICITY	
2	Effluent	7-6-14 - 7-7-14	09:00 - 09:00	X		Waste water	1	1		
3										
4	Upstream	7-7-14	10:00		X	Surface water	1	1	"	
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) 7/7/14

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

Sample Receiving (For Lab Use Only)

Ice Present? Y ☒ N ☐

Proper Preservation? Y ☒ N ☐

Container Temperature: _____

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

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

ACSE Biomonitoring Field Data Sheet

Client: ACSE Bath **Field Technicians:** MZ

Date: 7/7/2014

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)	
Effluent	9:30	20.5	931	7.52	7.60	20.4
Upstream	10:00	21.2	802	5.29	8.05	22.0

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

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.

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

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

Notes/Comments:

Phone #: 419-225-8048
Fax #:
E-mail: msantaguida@allencountyohio.com; ~~rboerger@allencountyohio.com;~~ jmotycka@allencountyohio.com
PO#:
Project Name:
Sampler: (Print) Mark Zwiebel (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	7-8-14 - 7-9-14	09:00 - 08:15	X		ww	1	1	Toxicity	
2	Upstream	7-9-14	09:00		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) 7-9-14 12:55

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

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

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

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

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

ACSE Biomonitoring Field Data Sheet

Client: Bath Party

Field Technicians: MR

Date:

CMC

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	8:15	20.7	819	6.33	7.60
Upstream	9:00	20.5	615	5.24	7.93

21.9

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Chain of Custody Record

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- 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:	Rick Boerger Allen County Sanitary Engineer 3226 North Cole St. Lima, OH 45805	Invoice To (If Different):	Rick Boerger Allen County Sanitary 3226 North Cole St. Lima, OH 45805	Notes/Comments: ~~ICP 200.7 Metals: Cd, Cr, Cu, Pb, Ni, Se, Zn~~
Name:				
Company:				
Address:				
Phone #:	419-225-8048	Fax #:		
E-mail:	msantaguida@allencountyohio.com; rboerger@allencountyohio.com; jmotycka@allencountyohio.com	PO#:		
Project Name	Bioassay Am. Bath - Day 3			
Sampler	(Print) Mark Zwiel (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										
2	Effluent	7-10-14 - 7-11-14	09:00 - 09:00	X		Waste water	1	1	TOXICITY	
3										
4	Upstream	7-11-14	9:35		X	Surface Water	1	1	"	
5										
6										
7										
8										

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

Received for Laboratory By: (circle one): Mansfield Lima Marion
(Signature) 7-11-14 11:35

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

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

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

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

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

ACSE Biomonitoring Field Data Sheet

Client: Bath **Field Technicians:** MZ

Date: 7/11/2014

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	9:20	21.3	833	6.35	7.71
Upstream	9:35	19.5	820	6.72	8.08

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Appendix B

Raw Data Sheets

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

Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2IH00007*

Person(s) Conducting Test: PJC, RFR
Beginning: Date 7/8/14 **Time** 1140
Ending: Date 7/15/14 **Time** 1140
Age of *Pimephales promelas*: <24H ECT
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	9G 1D	10G	39G 1D	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	9G 1D	38G 2D
4	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	9G 1D	38G 2D
5	10G	10G	10G	10G	40G	10G	9G 1D	9G 1D	10G	38G 2D	9G 1D	10G	10G	9G 1D	38G 2D	10G	8G 2D	10G	8G 2D	36G 4D
6	10G	10G	10G	10G	40G	10G	9G 1D	9G 1D	10G	38G 2D	8G 2D	10G	10G	9G 1D	37G 3D	10G	8G 2D	10G	8G 2D	36G 4D
7	10G	10G	10G	10G	40G	10G	9G 1D	9G 1D	10G	38G 2D	8G 2D	10G	10G	9G 1D	37G 3D	10G	8G 2D	10G	8G 2D	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).

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: AJC, RFR
Beginning: Date 7/8/14 **Time** 1140
Ending: Date 7/15/14 **Time** 1140
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	9G 1D	39G 1D	10G	10G	10G	10G	40G	10G	10G	9G 1D	10G	39G 1D					
7	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	10G	39G 1D	10G	10G	9G 1D	10G	39G 1D					

* 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: 7/8-15/14
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: <24HFCT
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7/17/14 Analyst: cmc

Balance Calibration Check: 1 gram weight = 1.00000 grams Date: 7/17/14 Analyst: cmc

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	1.13034	1.13104	10	0.70	0.070
Lab Control	1	10	1.08964	1.09637	10	6.73	0.673
	2	10	1.13016	1.13786	10	7.70	0.770
	3	10	1.08265	1.09068	10	8.03	0.803
	4	10	1.10404	1.11041	10	6.37	0.637
Upstream Control	1	10	1.11070	1.11700	10	6.30	0.630
	2	9	1.13086	1.13866	10	7.80	0.780
	3	9	1.12697	1.13241	10	5.44	0.544
	4	10	1.09237	1.09986	10	7.49	0.749
20% Effluent	1	8	1.13197	1.13772	10	5.75	0.575
	2	10	1.09765	1.10319	10	5.54	0.554
	3	10	1.09181	1.09895	10	7.14	0.714
	4	9	1.12444	1.12937	10	4.93	0.493
40% Effluent	1	10	1.12989	1.13564	10	5.75	0.575
	2	8	1.12388	1.12914	10	5.26	0.526
	3	10	1.08335	1.08970	10	6.35	0.635
	4	8	1.08794	1.09363	10	5.69	0.569

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

Discharge: Allen Co. (American-Bath WWTP) Date of Test: 7/8-15/14
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: 7/17/14 Analyst: CMC

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	1.12899	1.13583	10	6.84	0.684
	2	10	1.09481	1.10169	10	6.88	0.688
	3	10	1.13209	1.13916	10	7.07	0.707
	4	9	1.09079	1.09713	10	6.34	0.634
80% Effluent	1	10	1.10023	1.10709	10	6.86	0.686
	2	9	1.09081	1.09698	10	6.17	0.617
	3	10	1.10161	1.10837	10	6.76	0.676
	4	10	1.12542	1.13195	10	6.53	0.653
100% Effluent	1	10	1.12114	1.12848	10	7.34	0.734
	2	10	1.08396	1.09126	10	7.30	0.730
	3	9	1.12320	1.13016	10	6.96	0.696
	4	10	1.09960	1.10781	10	8.21	0.821
~~Far-field Mixing Zone~~	1				10		
	2				10		
	3				10		
	4				10		

EPA ICP 6/94 TSR 7/12/14 PJW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80.0	100.0
Response 1	.630	.575	.575	.684	.686	.734
Response 2	.780	.554	.526	.688	.617	.730
Response 3	.544	.714	.635	.707	.676	.696
Response 4	.749	.493	.569	.634	.653	.821

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American- Bath WWTP 001
Test Start Date: 7/8/14 Test Ending Date: 7/15/14
Test Species: P. promelas
Test Duration: 7 days
DATA FILE: Amb0714p.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	0.676	0.109	0.676
2	4	20.000	0.584	0.093	0.648
3	4	40.000	0.576	0.045	0.648
4	4	60.000	0.678	0.031	0.648
5	4	80.000	0.658	0.031	0.648
6	4	100.000	0.745	0.053	0.648

*** 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.

TO XSTAT 35

Title: American-Bath WWTP 001 P>Romelas 7/8-15/14 PER 7/07/4 RSV
File: AMBA0714.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	0.6758	0.6758		
2	20 %	0.5840	0.5840	1.9209	
3	40 %	0.5763	0.5763	2.0832	
4	60 %	0.6783	0.6783	-0.0523	
5	80 %	0.6580	0.6580	0.3716	
6	100 %	0.7452	0.7452	-1.4551	

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

Title: American-Bath WWTP 001 P>Romelas 7/8-15/14
File: AMBA0714.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	0.1151	17.0	0.0918
3	40 %	4	0.1151	17.0	0.0995
4	60%	4	0.1151	17.0	-0.0025
5	80 %	4	0.1151	17.0	0.0177
6	100 %	4	0.1151	17.0	-0.0695

TOXSTAT-1.5

PKW

Title: American-Bath WWTP 001 P>Romelas 7/8-15/14
File: AMBA0714.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	4	0.5440	0.7800	0.6758
2	20 %	4	0.4930	0.7140	0.5840
3	40 %	4	0.5260	0.6350	0.5763
4	60 %	4	0.6340	0.7070	0.6783
5	80 %	4	0.6170	0.6860	0.6580
6	100 %	4	0.6960	0.8210	0.7452

Title: American-Bath WWTP 001 P>Romelas 7/8-15/14
File: AMBA0714.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	0.0119	0.1091	0.0545	16.1397
2	20 %	0.0087	0.0934	0.0467	15.9905
3	40 %	0.0020	0.0448	0.0224	7.7806
4	60	0.0010	0.0312	0.0156	4.5941
5	80 %	0.0009	0.0306	0.0153	4.6545
6	100 %	0.0028	0.0533	0.0267	7.1520

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control (1 Hand: 9 mist)
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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Age of *Ceriodaphnia dubia*: 9-17H A118 (1)
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	~~7~~		TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	0 / G	2 / G	4 / G	0 / G	/		6	2
2	/	/	/	0 /	4 /	10 /	11 /	/		25	3
3	/	/	/	0 /	4 /	11 /	12 /	/		27	3
4	/	/	/	0 /	4 /	5 /	17 /	/		26	3
5	/	/	/	0 /	6 /	9 /	14 /	/		29	3
6	/	/	/	0 /	5 /	5 /	8 /	/		18	3
7	/	/	/	0 /	1 /	9 /	14 /	/		24	3
8	/	/	/	0 /	6 /	8 /	15 /	/		29	3
9	/	/	/	0 /	5 /	10 /	17 /	/		32	3
10	/	/	/ D	– / –	– / –	– / –	– / –	– / –	D	0	0
Total Adult Cond.	10G	10G	9G 1D	9G 1D	9G 1D	9G 1D	9G 1D	–			
									SUM	216	26
									MEAN	21.6	2.6
									S.D.	10.6	–

* 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: 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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Age of *Ceriodaphnia dubia*: 9-12H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	0 / G	6 / G	10 / G	11 / G	/	27	3
2	/	/	/	5 /	0 /	10 /	15 /	/	30	3
3	/	/	/	0 /	5 /	9 /	15 /	/	29	3
4	/	/	/	0 /	5 /	11 /	17 /	/	33	3
5	/	/	/	0 /	6 /	9 /	15 /	/	30	3
6	/	/	/	0 /	6 /	13 /	14 /	/	33	3
7	/	/	/	0 /	6 /	9 /	16 /	/	31	3
8	/	/	/	0 /	5 /	10 /	10 /	/	25	3
9	/	/	/	0 /	7 /	10 /	17 /	/	34	3
10	/	/	/	0 /	6 /	12 /	18 /	/	36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—		
									SUM 308	30
									MEAN 30.8	3.0
									S.D. 3.3	—

* 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, RJC
Contact:
Beginning Date: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Age of *Ceriodaphnia dubia*: 11:30 8-12h
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	6/G	9/G	12/G	/	27	3
2	/	/	/	0/	6/	10/	15/	/	31	3
3	/	/	/	0/	6/	10/	18/	/	34	3
4	/	/	/	0/	5/	8/	12/	/	25	3
5	/	/	/	0/	5/	10/	18/	/	33	3
6	/	/	/	0/	6/	11/	16/	/	33	3
7	/	/	/	0/	7/	9/	16/	/	32	3
8	/	/	/	0/	7/	13/	14/	/	34	3
9	/	/	/	0/	5/	13/	17/	/	35	3
10	/	/	/	0/	6/	9/	19/	/	34	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—		
									SUM 318	30
									MEAN 31.8	3.0
									S.D. 3.3	—

* 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, PJC
Contact:
Beginning Date: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Age of *Ceriodaphnia dubia*: ~~11:30~~ 9-17H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	6/G	10/G	13/G	/	29	3
2	/	/	/	0/	5/	4/	16/	/	25	3
3	/	/	/	0/	5/	9/	8/	/	22	3
4	/	/	/	4/	~~8~~ 0/	9/	9/	/	22	3
5	/	/	/	0/	6/	10/	16/	/	32	3
6	/	/	/	0/	6/	8/	11/	/	25	3
7	/	/	/	0/	6/	10/	9/	/	25	3
8	/	/	/	0/	6/	15/	14/	/	35	3
9	/	/	/	0/	7/	9/	16/	/	32	3
10	/	/	/	0/	6/	10/	18/	/	34	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—		
									SUM	281 / 30
									MEAN	28.1 / 3.0
									S.D.	4.9 / —

* 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: 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: 7/8/14 Time: 1130
Ending Date: 7/14/14 Time: 1130
Age of *Ceriodaphnia dubia*: 9-12H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	6/G	9/G	11/A	M	A	26	3
2				0	6	10	14/G			30	3
3				0	5	9	12			26	3
4				0	5	10	7			22	3
5				0	6	11	14			31	3
6				0	5	10	14			29	3
7				0	3	12	6			21	3
8				0	4	11	16			31	3
9				0	4	10	15			29	3
10				0	6	8	17			31	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	~~10G~~ 9G 1A	—			
									SUM	276	30
									MEAN	27.6	3.0
									S.D.	3.7	—

M

* 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, RJC
Contact:
Beginning Date: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Age of *Ceriodaphnia dubia*: 9-17H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	0 G	4 G	6 G	4 G			14	3
2				0	6	6	15			27	3
3				0	5	10	9			24	3
4				1	0	8	15			24	3
5				0	6	10	3			19	3
6				0	5	10	13 A		A	28	3
7				0	6	9	15 G			30	3
8				0	0	11	18 G			29	2
9				0	3	9	17 G			29	3
10				0	5	11	17 G			33	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	9G 1LA	-			
									SUM	257	29
									MEAN	25.7	2.9
									S.D.	5.7	–

* 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 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, RJC
Contact:
Beginning Date: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Age of *Ceriodaphnia dubia*: 9-12H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	0 G	5 G	4 G	0 D		D	9	2
2				0	4	5	11 G			20	3
3				0	3	0	14			17	3
4				0	0	2	14			16	2
5				0	6	10	11			27	3
6				0	1	11	18			30	3
7				0	4	11	14			29	3
8				0	6	10	17			33	3
9				0	6	10	18			34	3
10				0	5	6	18			29	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	9G ~~10G~~ 1D	—			
									SUM	244	28
									MEAN	24.4	2.8
									S.D.	8.4	—

* 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 C. dubia 7/8-14/14 Reproduction
File: amba0714.cdr 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	30.8000	30.8000		
2	20 %	31.8000	31.8000	-0.4308	
3	40 %	28.1000	28.1000	1.1631	
4	60 %	27.6000	27.6000	1.3785	
5	80 %	25.7000	25.7000	2.1970	
6	100 %	24.4000	24.4000	2.7570	*

Dunnett critical value = 2.3100 (1 Tailed, alpha = 0.05, df [used] = 5,40)
(Actual df = 5,54)

Title: American- Bath WWTP C. dubia 7/8-14/14 Reproduction
File: amba0714.cdr 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	10			
2	20 %	10	5.3624	17.4	-1.0000
3	40 %	10	5.3624	17.4	2.7000
4	60 %	10	5.3624	17.4	3.2000
5	80 %	10	5.3624	17.4	5.1000
6	100 %	10	5.3624	17.4	6.4000

EPA ICP 6/94 PER 7/10/14 BW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	27	27	29	26	14	9
Response 2	30	31	25	30	27	20
Response 3	29	34	22	26	24	17
Response 4	33	25	22	22	24	16
Response 5	30	33	32	31	19	27
Response 6	33	33	25	29	28	30
Response 7	31	32	25	21	30	29
Response 8	25	34	35	31	29	33
Response 9	34	35	32	29	29	34
Response 10	36	34	34	31	33	29

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP
Test Start Date: 7/8/14 Test Ending Date: 7/14/14
Test Species: C. dubia
Test Duration: 6 days
DATA FILE: amb0714c.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	30.800	3.327	31.300
2	10	20.000	31.800	3.293	31.300
3	10	40.000	28.100	4.909	28.100
4	10	60.000	27.600	3.718	27.600
5	10	80.000	25.700	5.658	25.700
6	10	100.000	24.400	8.356	24.400

*** 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: American- Bath WWTP C. dubia 7/8-14/14 Reproduction
File: amba0714.cdr Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	10	25.0000	36.0000	30.8000
2	20 %	10	25.0000	35.0000	31.8000
3	40 %	10	22.0000	35.0000	28.1000
4	60 %	10	21.0000	31.0000	27.6000
5	80 %	10	14.0000	33.0000	25.7000
6	100 %	10	9.0000	34.0000	24.4000

Title: American- Bath WWTP C. dubia 7/8-14/14 Reproduction
File: amba0714.cdr Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	11.0667	3.3267	1.0520	10.8008
2	20 %	10.8444	3.2931	1.0414	10.3556
3	40 %	24.1000	4.9092	1.5524	17.4704
4	60 %	13.8222	3.7178	1.1757	13.4704
5	80 %	32.0111	5.6578	1.7892	22.0149
6	100 %	69.8222	8.3560	2.6424	34.2458

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: 7/8-15/14
Effluent Serial No.: 001 Permit No.: 2PH00007*

Investigator: RFR Culture No.: 118
Culture initiation date: 7/2/14 Culture ending date: 7/9/14 w
Origin of parental organisms: Allowy # 111 (1.9 Harrod)
Age at start of culture:
Culture water renewal frequency: minimum 3 times per week Culture water: ~~MHSF~~ / Harrod 0.9 MHSF
Culture temperature: 25°C Fed daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

Number of brood organisms at start of culture: 40
Percent survival of parental organisms after 7 days: 100
Percent of non-reproducing adults after 7 days: 0
Average young production after 7 days of brood adults for test: 31.9

	BROOD ADULT NO.	BROOD SIZE	HATCHING TIME WINDOW	TOTAL; YOUNG PROD. (7 DAYS)	USED IN REP. NO.
1)	D1	17	≈ 1800 to 0200 7/7-8/14	31	1
2)	D2	16		31	2
3)	D3	16		31	3
4)	D4	16		~~31~~ 30	4
5)	D5	16		31	5
6)	D6	17		34	6
7)	D7	16		29	7
8)	D8	20		38	8
9)	D9	18		32	9
10)	D10	18		32	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: 7/8-15/14
Effluent Serial No.: 001 Permit No.: 2PH00007*

1st Sample Collection

Date Samples Collected: 7/7/14 Date Samples Read: 7/7/14 Analyst: SS

	100% Effluent	Upstream
Temperature (°C)	15.9	20.0
Dissolved Oxygen (mg/L)	8.5	7.1
pH (s.u.)	7.70	7.94
Conductivity (µmhos)	920	836
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light brown

2nd Sample Collection

Date Samples Collected: 7/9/14 Date Samples Read: 7/9/14 Analyst: BG

	100% Effluent	Upstream
Temperature (°C)	17.2	18.4
Dissolved Oxygen (mg/L)	9.0	8.2
pH (s.u.)	7.76	7.88
Conductivity (µmhos)	841	604
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light brown

3rd Sample Collection

Date Samples Collected: 7/11/14 Date Samples Read: 7/11/14 Analyst: JC

	100% Effluent	Upstream
Temperature (°C)	17.1	17.8
Dissolved Oxygen (mg/L)	8.0	8.3
pH (s.u.)	7.80	8.00
Conductivity (µmhos)	823	824
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light green/yellow stain	light tan stain

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control (1 Hound: 9 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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H & 11-8H
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	24.6 to 25.3	25.3	24.9 to 25.1	25.3	24.9 to 25.3	25.1	24.9 to 25.3	25.3	24.9 to 25.3	25.1	24.9 to 25.3	–
Dissolved Oxygen (mg/L)	8.3	8.8	7.8	8.0	7.9	8.3	7.9	8.7	7.8	8.9	8.0	8.5	7.8	
pH (s.u.)	7.47	7.38	7.49	7.66	7.67	7.45	7.69	7.62	7.78	7.97	7.60	7.90	7.64	
Conductivity (µmho/cm)	296	292	313	359	305	357	374	357	369	355	363	346	372	
Hardness (mg/L $CaCO_3$)	103			120				103						
Alkalinity (mg/L $CaCO_3$)	86			86				86						

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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H
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	24.6 to 25.3	25.2	24.9 to 25.1	25.1	24.9 to 25.3	25.1	24.9 to 25.3	25.2	24.9 to 25.3	25.2	24.9 to 25.3	—
Dissolved Oxygen (mg/L)	8.2	8.5	7.7	8.3	7.9	8.3	8.0	8.2	7.8	8.3	8.0	8.5	7.9	
pH (s.u.)	7.87	7.87	7.94	7.94	8.02	7.81	7.96	8.05	8.09	8.02	8.02	8.05	7.89	
Conductivity (µmho/cm)	831	831	835	605	842	606	626	827	617	828	839	825	844	
Hardness (mg/L $CaCO_3$)	325			222				257						
Alkalinity (mg/L $CaCO_3$)	188			154				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: RFB, PJC
Beginning Date: 7/8/14 Time: 11:30
Ending Date: 7/ /14 Time:
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H
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	25.0	24.6 to 25.3	25.1	24.9 to 25.1	25.1	24.9 to 25.3	25.1	24.9 to 25.3	25.3	24.9 to 25.3	25.2	24.9 to 25.3	–
Dissolved Oxygen (mg/L)	8.1	8.4	7.6	8.2	7.8	8.7	8.0	8.1	7.6	8.6	7.8	8.3	8.0	
pH (s.u.)	7.94	7.80	7.95	7.93	8.06	7.87	7.95	8.04	8.14	7.96	8.06	8.04	7.94	
Conductivity (µmho/cm)	849	849	868	655	877	655	684	827	676	826	843	827	868	
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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H
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	25.2	24.6 to 25.3	25.1	24.9 to 25.1	25.2	24.9 to 25.3	25.2	24.9 to 25.3	25.3	24.9 to 25.3	25.3	24.9 to 25.3	–
Dissolved Oxygen (mg/L)	8.1	8.2	7.6	8.3	7.9	8.9	7.9	8.2	7.7	8.9	7.8	8.4	8.0	
pH (s.u.)	7.93	7.87	7.95	7.98	8.08	7.88	7.96	8.01	8.16	7.98	8.06	8.04	7.94 ~~8~~	
Conductivity (µmho/cm)	867	867	886	703	898	703	734	824	725	826	853	826	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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H
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	24.6 to 25.3	25.2	24.9 to 25.1	25.2	24.9 to 25.3	25.2	24.9 to 25.3	25.3	24.9 to 25.3	25.3	24.9 to 25.3	—
Dissolved Oxygen (mg/L)	8.3	8.3	7.7	8.0	7.9	8.6	8.0	8.3	7.8	8.7	7.8	8.2	7.8	
pH (s.u.)	7.87	7.83	7.93	7.92	8.09	7.87	7.98	7.99	8.16	7.95	8.06	8.02	7.93	
Conductivity (µmho/cm)	884	884	909	747	918	747	780	824	769	825	854	825	877	
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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H
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	25.2	24.6 to 25.3	25.1	24.9 to 25.1	25.2	24.9 to 25.3	25.2	24.9 to 25.3	25.3	24.9 to 25.3	25.4	24.9 to 25.3	–
Dissolved Oxygen (mg/L)	8.2	8.7	7.7	8.1	7.9	8.8	8.0	8.2	7.7	8.5	8.0	8.8	7.7	
pH (s.u.)	7.83	7.81	7.93	7.90	8.09	7.85	8.00	7.96	8.17	7.94	8.05	7.94	7.91	
Conductivity (µmho/cm)	901	902	919	792	823	791	818	823	815	822	851	825	859	
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: 7/8/14 Time: 11:30
Ending Date: 7/14/14 Time: 11:30
Test Organism: *Ceriodaphnia dubia*: Age: 9-17H
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	24.6 to 25.3	25.1	24.9 to 25.1	25.2	24.9 to 25.3	25.1	24.9 to 25.3	25.3	24.9 to 25.3	25.3	24.9 to 25.3	–
Dissolved Oxygen (mg/L)	8.4	8.4	7.5	8.2	7.9	8.9	8.0	8.2	7.7	8.5	8.0	8.1	7.8	
pH (s.u.)	7.80	7.79	7.92	7.90	8.08	7.84	8.00	7.98	8.18	7.94	8.02	7.98	7.90	
Conductivity (µmho/cm)	919	920	938	836	952	838	866	821	860	824	847	823	850	
Hardness (mg/L $CaCO_3$)	359			342				325						
Alkalinity (mg/L $CaCO_3$)	205			171				188						

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: RJC, RFR
Beginning Date: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
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)	25.6	25.1	25.1 to 25.3	25.1	25.2 to 25.4	25.0	25.1 to 25.4	25.2	25.0 to 25.1	25.3	24.9 to 25.2	25.4	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.0	8.4	6.4	8.0	5.7	8.5	5.9	7.8	5.3	8.8	5.8	8.4	5.5	6.0
pH (s.u.)	7.57	7.24	7.24	7.78	7.34	7.39	7.27	7.89	7.21	7.47	7.45	7.42	7.36	7.35
Conductivity (µmho/cm)	333	333	351	333	357	334	378	337	348	338	355	338	361	354
Hardness (mg/L $CaCO_3$)	103			103				103						
Alkalinity (mg/L $CaCO_3$)	86			86				86						

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: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
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)	25.3	25.1	25.1 to 25.3	25.2	25.2 to 25.4	25.1	25.1 to 25.4	25.1	25.0 to 25.1	25.2	24.9 to 25.2	25.2	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.2	8.5	5.4	8.3	5.9	8.3	6.0	8.2	5.5	8.3	5.1	8.5	6.7	5.5
pH (s.u.)	7.87	7.82	7.68	7.94	7.84	7.81	7.75	8.05	7.69	8.02	7.74	8.05	7.71	7.73
Conductivity (µmho/cm)	831	831	839	605	854	600	643	827	615	828	835	825	849	837
Hardness (mg/L $CaCO_3$)	325			222				257						
Alkalinity (mg/L $CaCO_3$)	188			154				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: RJC, RFR
Beginning Date: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
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)	25.3	25.0	25.1 to 25.3	25.1	25.2 to 25.4	25.1	25.1 to 25.4	25.1	25.0 to 25.1	25.3	24.9 to 25.2	25.2	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.1	8.4	6.2	8.2	5.3	8.7	5.5	8.1	5.6	8.6	4.6	8.3	6.3	5.5
pH (s.u.)	7.94	7.86	7.76	7.93	7.80	7.87	7.68	8.04	7.64	7.96	7.72	8.04	7.81	7.73
Conductivity (µmho/cm)	849	849	880	655	889	655	694	827	673	836	838	827	863	853
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: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
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)	25.3	25.2	25.1 to 25.3	25.1	25.2 to 25.4	25.2	25.1 to 25.4	25.2	25.0 to 25.1	25.3	24.9 to 25.2	25.3	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.1	8.2	4.5	8.3	5.5	8.9	4.9	8.7	5.4	8.9	5.3	8.4	5.7	5.6
pH (s.u.)	7.93	7.87	7.63	7.86	7.80	7.88	7.66	8.01	7.62	7.98	7.79	8.04	7.79	7.77
Conductivity (µmho/cm)	867	867	915	703	908	703	759	824	737	826	867	826	882	871
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: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
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)	25.3	25.1	25.1 to 25.3	25.2	25.2 to 25.4	25.2	25.1 to 25.4	25.2	25.0 to 25.1	25.3	24.9 to 25.2	25.3	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.3	8.3	6.4	8.0	5.5	8.0	6.5	8.3	5.0	8.7	6.0	8.2	6.2	5.1
pH (s.u.)	7.87	7.83	7.76	7.92	7.84	7.87	7.73	7.99	7.64	7.95	7.82	8.02	7.80	7.77
Conductivity (µmho/cm)	884	884	922	747	933	747	802	824	782	825	858	825	863	857
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: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
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)	25.2	25.2	25.1 to 25.3	25.1	25.2 to 25.4	25.2	25.1 to 25.4	25.2	25.0 to 25.1	25.3	24.9 to 25.2	25.4	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.2	8.7	6.4	8.1	6.1	8.8	6.3	8.2	5.0	8.5	5.5	8.8	7.4	4.9
pH (s.u.)	7.83	7.81	7.75	7.80	7.72	7.85	7.77	7.96	7.64	7.94	7.78	7.94	7.81	7.75
Conductivity (µmho/cm)	901	902	940	792	920	791	834	823	815	822	845	825	868	848
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.: 2PA00007*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 7/8/14 Time: 1140
Ending Date: 7/15/14 Time: 1140
Test Organism: *Pimephales promelas* Age: <24H ECT
Dilution Water Used: upstream

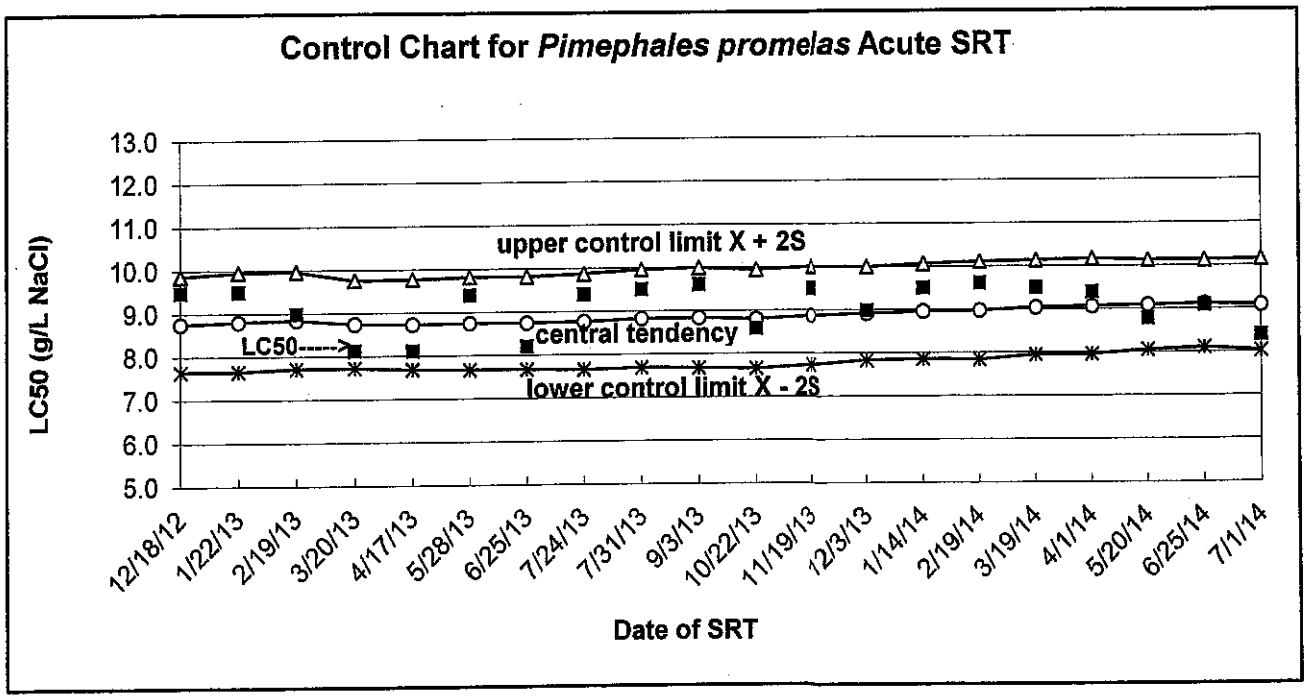
Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.3	25.1	25.1 to 25.3	25.1	25.2 to 25.4	25.2	25.1 to 25.4	25.1	25.0 to 25.1	25.3	24.9 to 25.2	25.3 ~~25.3~~	25.1 to 25.5	25.0 to 25.2
Dissolved Oxygen (mg/L)	8.4	8.4	5.6	8.2	5.0	8.9	6.1	8.2	5.5	8.5	5.1	8.1	6.1	5.3
pH (s.u.)	7.80	7.79	7.68	7.90	7.71	7.84	7.77	7.98	7.68	7.94	7.74	7.98	7.74	7.78
Conductivity (µmho/cm)	919	920	957	838	866	838	897	821	872	824	849	823	864	862
Hardness (mg/L $CaCO_3$)	359			342				325						
Alkalinity (mg/L $CaCO_3$)	205			171				185						

Appendix D

SRT Data

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

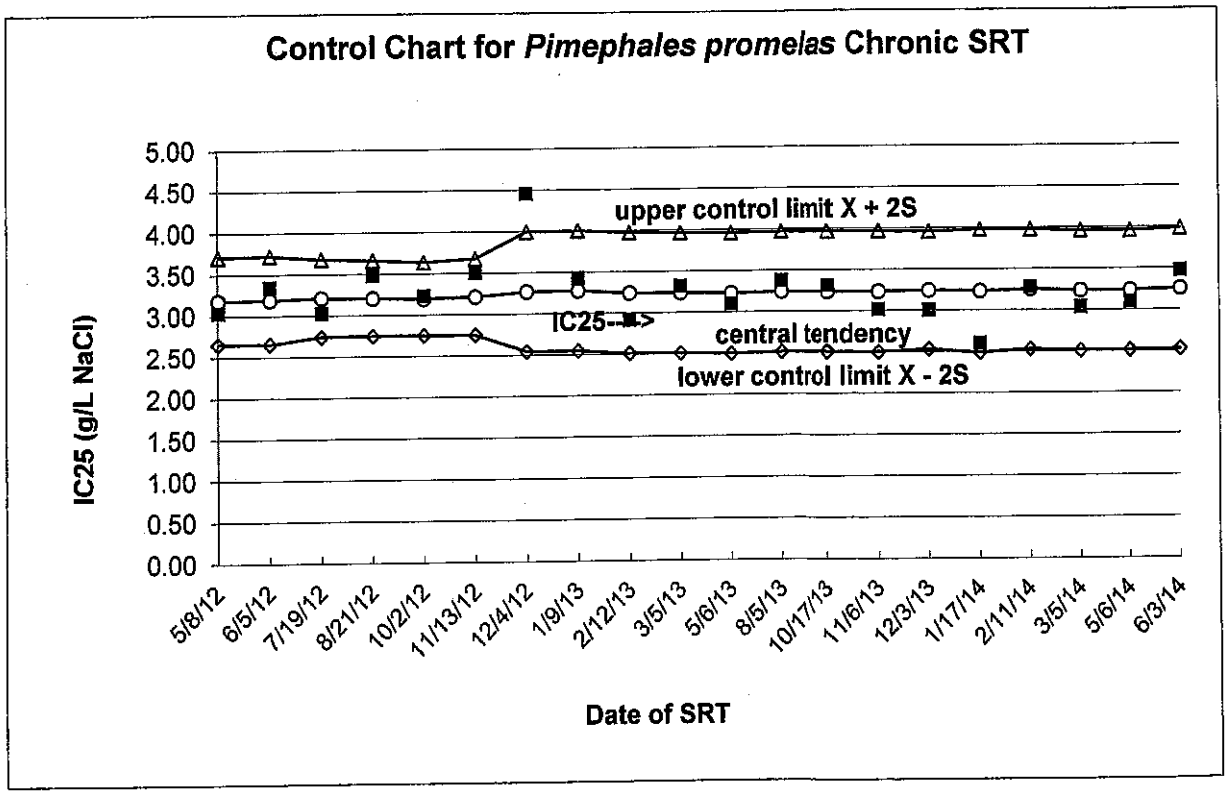
Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
12/18/12	9.5	8.8	0.6	9.9	7.6	0.06	10.4	7.1	P
1/22/13	9.5	8.8	0.6	10.0	7.7	0.07	10.5	7.1	P
2/19/13	9.0	8.8	0.6	10.0	7.7	0.06	10.5	7.2	SK
3/20/13	8.1	8.7	0.5	9.8	7.7	0.06	10.3	7.2	P
4/17/13	8.1	8.7	0.5	9.8	7.7	0.06	10.3	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
7/24/13	9.4	8.8	0.6	9.9	7.7	0.06	10.4	7.1	SK
7/31/13	9.5	8.8	0.6	10.0	7.7	0.06	10.5	7.1	SK
9/3/13	9.6	8.8	0.6	10.0	7.7	0.07	10.6	7.1	SK
10/22/13	8.6	8.8	0.6	10.0	7.7	0.07	10.5	7.1	SK
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



*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.19
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.
1 - P = Probit method, G = Graphical method, SK = Spearman-Karber method, TSK = Trimmed Spearman-Karber method.

IC25 for *Pimephales promelas* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
5/8/12	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
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



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

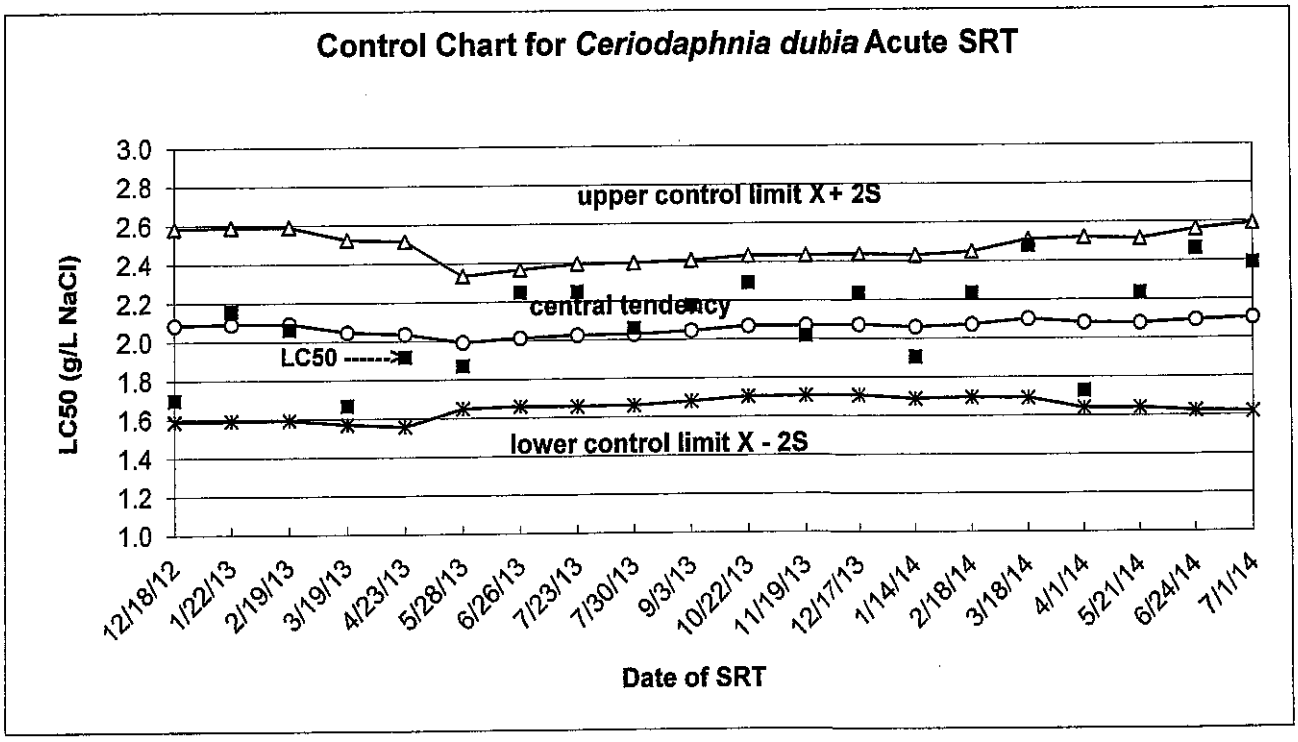
75th percentile of CVs for IC25 = 0.38

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]
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
7/23/13	2.3	2.0	0.2	2.4	1.7	0.09	2.6	1.5	Probit
7/30/13	2.1	2.0	0.2	2.4	1.7	0.09	2.6	1.5	Probit
9/3/13	2.2	2.0	0.2	2.4	1.7	0.09	2.6	1.5	Probit
10/22/13	2.3	2.1	0.2	2.4	1.7	0.09	2.6	1.5	Probit
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

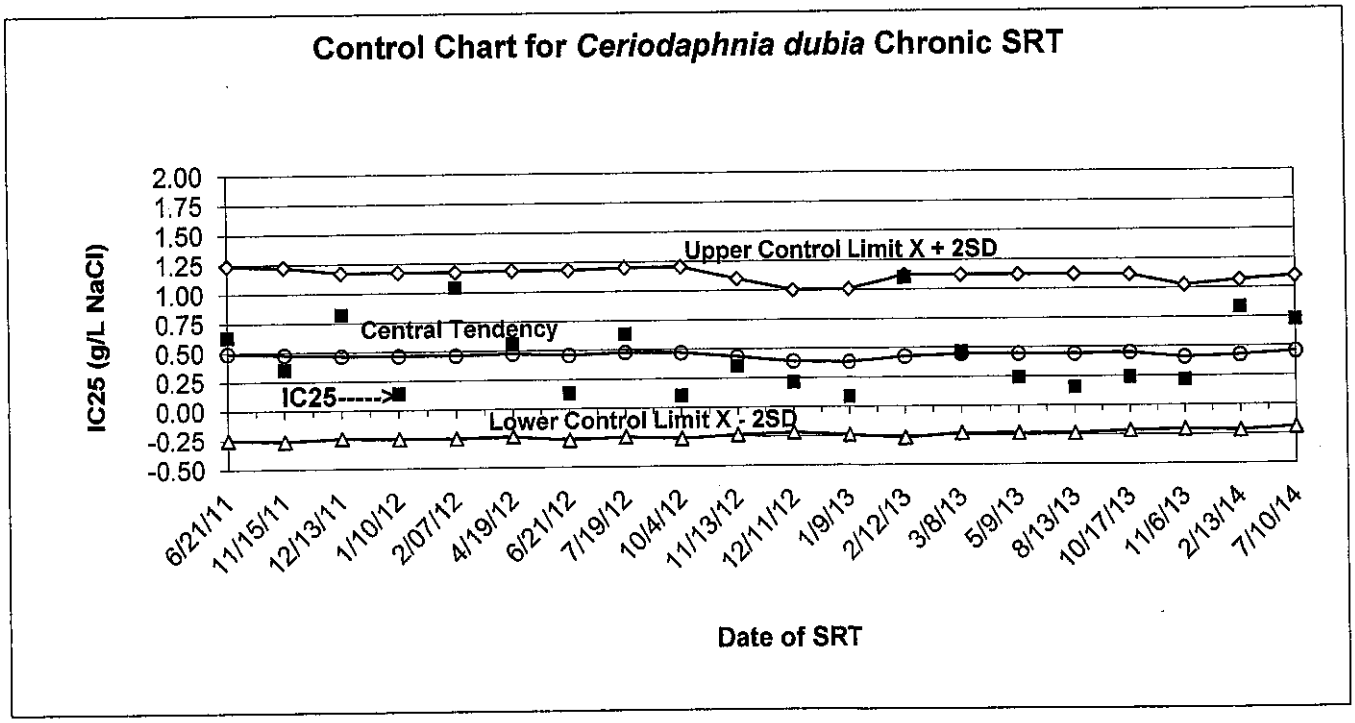


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

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
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
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



*- 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.