Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: American II WWTP
Outfall: Effluent 001
Report Date: 4/22/14
Ohio EPA Permit No.: 2PH00006*ID
Alloway Lab #: L14-12598-01

Report of Chronic Biomonitoring

Samples Collected on:

4/7/14
4/9/14
4/11/14

Pimephales promelas
Ceriodaphnia dubia

Allen County Sanitary Engineer
American II WWTP
4140 Diller Road
Lima, OH 45805

Alloway
Your Resource for Defensible Data

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Facility: American II WWTP
Outfall: Effluent 001
Report Date: 4/22/14
Ohio EPA Permit No.: 2PH00006*ID
Alloway Lab #: L14-12598-01

Date: 4/22/14

Mike Santaguida
Allen County Saitary Engineers
American II WWTP
4140 Diller Road
Lima, OH 45805

Dear Mr. Santaguida:

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: 4/22/14
Ohio EPA Permit No.: 2PH00006*ID

1. Facility Name	American II WWTP
2. Facility Address	4140 Diller Road Lima, OH 45805
3. Ohio EPA Permit No.	2PH00006*ID
4. NPDES No.	OH0037338
5. Facility Contact	Mike Santaguida
6. Telephone No.	419-996-4696
7. Consulting Laboratory	Alloway
8. Lab Contact	Paul Crerar
9. Lab Telephone No.	419-223-1362
10. Receiving water(s) of discharge	Dug 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: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*ID
Date of Test: 4/8-15/14

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:

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

508 Bissman Ct. • Mansfield, Ohio 44903
419.525.1644 • Fax 419.524.5575
800.635.3222

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

www.alloway.com

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*ID
Date of Test: 4/8-14/14

Summary of Chronic Toxicity Test 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	AA	10.0	

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
Facility: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*ID
Date of Test: 4/8-15/14

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%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.841	0.103
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%)	0.850	0.074
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%)	0.899	0.070
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%)	0.801	0.021
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%)	0.826	0.019
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%)	0.866	0.051
100%	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%)	0.849	0.079
LC50 Values (%):	>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Survival** <1.0	
95% Confidence Limits LL									
95% Confidence Limits UL									
EC50 Values (%):	>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Growth (100/IC25)** <1.0	
95% Confidence Limits LL									
95% Confidence Limits UL									

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

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

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
Facility: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*ID
Date of Test: 4/8-14/14

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%)	— (—)	28.9
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (10.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	— (—)	1.9
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	— (—)	31.7
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%)	— (—)	31.3
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	— (—)	29.6
80%	0.0% (0.0%)	0.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	— (—)	25.9
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	— (—)	32.0
LC50 Values (%):	>100	>100	>100	>100	>100	>100	—	Calculated TUc Value for Survival <1.0
95% Confidence Limits LL								
95% Confidence Limits UL								
EC50 Values (%):	>100	>100	>100	>100	>100	>100	—	Calculated TUc Value for Reproduction <1.0
95% Confidence Limits LL								
95% Confidence Limits UL								

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

*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: American II WWTP
Outfall: Effluent 001
Report Date: 4/22/14
Ohio EPA Permit No.: 2PH00006*ID

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection Beginning	Sample Collection Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	4/6/14 @ 08:45	4/7/14 @ 08:45
Type (Grab/Comp):	Comp	2nd	4/8/14 @ 08:45	4/9/14 @ 08:45
Volume Collected:	5 Gal.	3rd	4/10/14 @ 08:30	4/11/14 @ 08:30
Upstream Station:				
Waterbody:	Dug Run			
Station No.:	801	1st		4/7/14 @ 09:36
Type (Grab/Comp):	Grab	2nd		4/9/14 @ 09:22
Volume Collected:	5 Gal.	3rd		4/11/14 @ 09:10

Samples collected by: Alloway

OEPA Permit No.: 2F..J0006*ID Allen Co. (American ll 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:	4/8-15/14
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 - 4/7; day 2, 3 - 4/9; day 4, 5, 6 - 4/11
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.: 2PH00006*ID Allen Co. (American II 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* 21-23 hours
2. Test Type and Duration:	Static renewal; 6 to 8 days (6 days)
3. Test Dates:	4/8-14/14
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 - 4/7; day 2, 3 - 4/9; day 4, 5, 6 - 4/11
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water (MHSF)
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data

Alloway
Your Resource for Defensible Data

Chain of Custody Record

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

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

Project: L14-12598

Phone #: ~~419-225-8048~~ Fax #:
E-mail: msantaguida@allencountyohio.com; rboerger@allencountyohio.com; jmotycka@allencountyohio.com PO#:
Project Name: Chronic Bioassay - AM II - Day One
Sampler (Print): Michael S Hardy (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	4/06/14 - 4/07/14	08:45 - 08:45	x		WW	1	1	Toxicity	
2	Upstream	4/07/14	09:36		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: 4/07/14 Time: 09:58

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: Room Temp

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 II **Field Technicians:** MSH

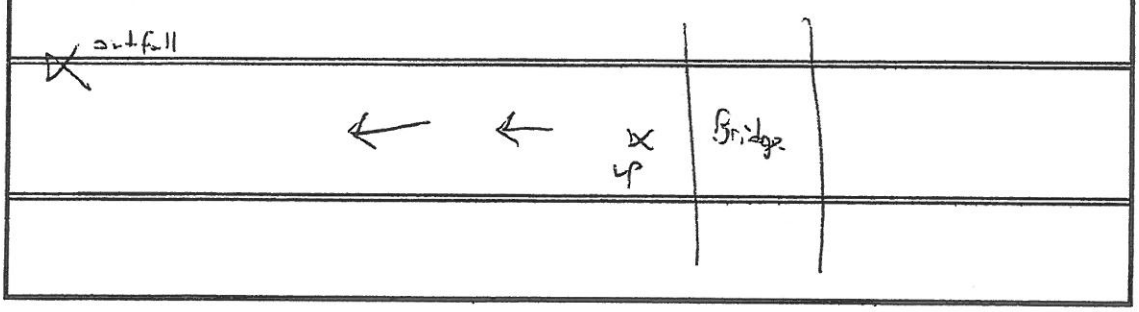
Date: 4/07/14

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	08:58	8.8	837	11.11	7.33
Upstream	09:36	6.0	787	11.62	7.87

Diagram of Collection Sites

~ Width of stream= 15'
~ Depth of stream= 2' > Approx.



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
- ☐ _____

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- 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 PO#:
rboerger@allencountyohio.com; jmotycka@allencountyohio.com
Project Name: AMT 5.0. - Day Two
Sampler (Print): Michael J. Hanky (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	4/08/14-4/09/14	08:45-08:45	x		ww	1	1	Toxicity	
2	Upstream	4/09/14	09:22		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) 4/09/14 09:38

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: Room Temp

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 II **Field Technicians:** NSH

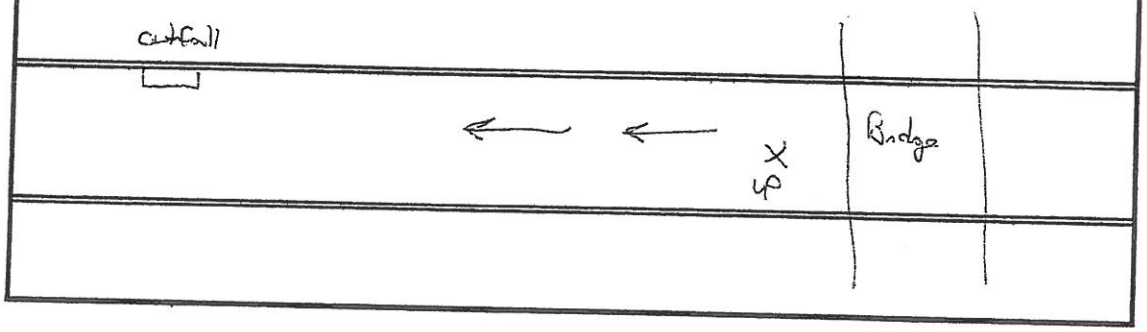
Date: 4/09/14

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	08:36	8.9	716	10.44	7.31
Upstream	09:22	6.9	623	10.93	7.74

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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Report To:
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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: Bio Day 3
Sampler: (Print) Mark Zwiebel (Signature) [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	UPSTream	4-11-14			x	RW	1	1	Toxicity	
2	Effluent	4-10-14-4-11-14	08:30-08:30	X		WW	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) [signature] 4-11-14 10:55

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: AM 2 **Field Technicians:** M 2

Date: 4-11-14

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)	
Effluent	08:40	10.0	823	11.05	7.62	16.1
Upstream	09:10	9.8	867	10.00	7.86	13.1

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 II WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning: Date 4/8/14 **Time** 1415
Ending: Date 4/15/14 **Time** 1415
Age of *Pimephales promelas*: <24H #3
Dilution Water Used: Upstream

Day	Lab Control					Upstream Control					20% Effluent					40% Effluent				
	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; 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. (Americantt WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning: Date 4/8/14 **Time** 1415
Ending: Date 4/15/14 **Time** 1415
Age of *Pimephales promelas*: 24H #3
Dilution Water Used: Upstream

Day	60% Effluent					80% Effluent					100% Effluent									
	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	10G	10G	40G					
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	10G	39G 1D					

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

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

Discharge: Allen Co. (American II WWTP) Date of Test: 4/8-15/14
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PA00006* *Pimephales promelas* Age at Start: <24H #3
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 4/17/14 Analyst: PSC

Balance Calibration Check: 1 gram weight = 1.00000 grams Date: 4/17/14 Analyst: PSC

AFA

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	1.07768	1.07856	10	0.88	0.088
Lab Control	1	10	1.12298	1.13223	10	9.25	0.925
	2	10	1.08482	1.09276	10	7.94	0.794
	3	10	1.08100	1.09002	10	9.02	0.902
	4	10	1.09645	1.10423	10	7.78	0.778
Upstream Control	1	10	1.09269	1.09959	10	6.90	0.690
	2	10	1.10420	1.11322	10	9.02	0.902
	3	10	1.09197	1.10103	10	9.06	0.906
	4	10	1.08790	1.09654	10	8.64	0.864
20% Effluent	1	10	1.08662	1.09600	10	9.38	0.938
	2	10	1.09157	1.10128	10	9.71	0.971
	3	10	1.08735	1.09607	10	8.72	0.872
	4	10	1.10395	1.11208	10	8.13	0.813
40% Effluent	1	10	1.09835	1.10612	10	7.77	0.777
	2	10	1.09856	1.10664	10	8.08	0.808
	3	10	1.09804	1.10598	10	7.94	0.794
	4	10	1.12927	1.13753	10	8.26	0.826

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

Discharge: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
Drying Temperature (C°): 60 Drying Time (h): 24
NPDES Permit No.: 2PH00006*
Date Weighed: 4/17/14
Date of Test: 4/8-15/14
Pimephales promelas Age at Start: <24H 43
Analyst: PSC

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	1.10598	1.11438	10	8.40	0.840
	2	10	1.09830	1.10668	10	8.38	0.838
	3	10	1.09138	1.09937	10	7.99	0.799
	4	10	1.10197	1.11022	10	8.25	0.825
80% Effluent	1	10	1.09376	1.10272	10	8.96	0.896
	2	10	1.08661	1.09506	10	8.45	0.845
	3	10	1.13230	1.14149	10	9.19	0.919
	4	10	1.12593	1.13398	10	8.05	0.805
100% Effluent	1	10	1.10382	1.11243	10	8.61	0.861
	2	10	1.08754	1.09597	10	8.43	0.843
	3	9	1.09138	1.09888	10	7.50	0.750
	4	10	1.12633	1.13576	10	9.43	0.943
	1				10		
	2				10		
	3				10		
	4				10		

EPA ICP 6/94

RDC 4/22/14 PJCV

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	.690	.938	.777	.840	.896	.861
Response 2	.902	.971	.808	.838	.845	.843
Response 3	.906	.872	.794	.799	.919	.750
Response 4	.864	.813	.826	.825	.805	.943

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American II WWTP 001
Test Start Date: 4/8/14 Test Ending Date: 4/15/14
Test Species: P. promelas
Test Duration: 7 days
DATA FILE: Ame0414p.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	0.840	0.102	0.870
2	4	20.000	0.899	0.070	0.870
3	4	40.000	0.801	0.021	0.836
4	4	60.000	0.826	0.019	0.836
5	4	80.000	0.866	0.051	0.836
6	4	100.000	0.849	0.079	0.836

*** 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 II WWTP 001 P. promelas 4/8-15/14 Growth mg KIA 4/22/14
File: AMER0414.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	0.8405	0.8405		
2	20 %	0.8985	0.8985	-1.2694	
3	40 %	0.8013	0.8013	0.8590	
4	60 %	0.8255	0.8255	0.3283	
5	80 %	0.8663	0.8663	-0.5636	
6	100 %	0.8493	0.8493	-0.1915	

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

Title: American II WWTP 001 P. promelas 4/8-15/14 Growth mg
File: AMER0414.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	0.1101	13.1	-0.0580
3	40 %	4	0.1101	13.1	0.0392
4	60 %	4	0.1101	13.1	0.0150
5	80 %	4	0.1101	13.1	-0.0257
6	100 %	4	0.1101	13.1	-0.0087

TOXSTAT 3.5

PJRV

Title: American II WWTP 001 P. promelas 4/8-15/14 Growth mg

ETR 4/22/14

File: AMER0414.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	4	0.6900	0.9060	0.8405
2	20 %	4	0.8130	0.9710	0.8985
3	40 %	4	0.7770	0.8260	0.8013
4	60 %	4	0.7990	0.8400	0.8255
5	80 %	4	0.8050	0.9190	0.8663
6	100 %	4	0.7500	0.9430	0.8493

Title: American II WWTP 001 P. promelas 4/8-15/14 Growth mg

File: AMER0414.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	0.0104	0.1021	0.0511	12.1479
2	20 %	0.0049	0.0703	0.0352	7.8249
3	40 %	0.0004	0.0208	0.0104	2.5968
4	60 %	0.0004	0.0189	0.0094	2.2867
5	80 %	0.0026	0.0512	0.0256	5.9129
6	100 %	0.0063	0.0792	0.0396	9.3256

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: ~~1356~~ 1355
Ending Date: 4/14/14 Time: 1358
Age of *Ceriodaphnia dubia*: 21-23 H ECT#4
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	4 / G	0 / G	Abort 0 / G	Abort 0 / G			4	3
2				Abort 0	0	0	0		♂	—	—
3				1	1	0	Abort 0			2	3
4				4	0	0	0			4	1
5				2	0	Abort 0	Abort 0			2	3
6				0	Abort 0	Abort 0	Abort 0			0	3
7				0	Abort 0	Abort 0	Abort 0			0	3
8				0	Abort 0	0	Abort 0			0	2
9				2	0	0	0			2	1
10				3	0	3	0			3	2
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—	1=9	SUM 17	21
										MEAN 1.9	2.3
										S.D. 1.6	—

2 Rep small all conc.

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: 1255
Ending Date: 4/14/14 Time: 1255
Age of *Ceriodaphnia dubia*: 21-23 h
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	3/G	0/G	9/G	17/G	/		29	3
2	/	/	0/	0/	0/	0/	0/	/	♂	—	—
3	/	/	1/	0/	8/	12/	19/AL	/		21	3
4	/	/	0/	2/	10/	0/	17/	/		29	3
5	/	/	/	3/	3/	9/	17/	/		32	3
6	/	/	/	4/	0/	9/	18/	/		31	3
7	/	/	/	4/	0/	11/	19/	/		34	3
8	/	/	/	3/	0/	8/	8/	/		19	3
9	/	/	/	4/	1/	10/	19/	/		34	3
10	/	/	/	3/	0/	12/	16/	/		31	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—	n=9		
									SUM	260	27
									MEAN	28.9	3.0
									S.D.	5.4	—

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/19/14 Time: 1355
Age of *Ceriodaphnia dubia*: 21-23 h
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	4/G	0/G	10/D	-/-	-/-	D	14	2
2	/	/	0/	0/	0/	0/G	0/G	/	♂	-	—
3	/	/	3/	0/	7/	19/	0/	/		29	3
4	/	/	0/	4/	10/	0/	20/	/		34	3
5	/	/	/	4/	0/	12/	23/	/		39	3
6	/	/	/	4/	0/	11/	22/	/		37	3
7	/	/	/	4/	1/	8/	19/	/		32	3
8	/	/	/	4/	0/	9/	18/	/		31	3
9	/	/	/	4/	0/	10/	19/	/		33	3
10	/	/	/	4/	0/	12/	20/	/		36	3
Total Adult Cond.	10G	10G	10G	10G	10G	9G 1D	9G 1D	—	n=9	SUM 285	26
										MEAN 31.7	2.9
										S.D. 7.3	—

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1350
Age of *Ceriodaphnia dubia*: 21-23H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	4/G	0/G	10/G	20/G	/		34	3
2	/	/	/	0/	0/	0/	0/	/	♂	—	—
3	/	/	/	0/	7/	7/	1/	/		15	3
4	/	/	/	2/	10/	0/	20/	/		32	3
5	/	/	/	4/	0/	10/	19/	/		33	3
6	/	/	/	4/	0/	12/	21/	/		37	3
7	/	/	/	0/	3/	9/	18/	/		30	3
8	/	/	/	4/	0/	8/	18/	/		30	3
9	/	/	/	4/	0/	12/	~~12~~ 21/LA	/		37	3
10	/	/	/	4/	0/	11/	19/	/		34	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–	n=9 SUM	282	27
									MEAN	31.3	3.0
									S.D.	6.6	–

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1355
Age of *Ceriodaphnia dubia*: 21-23 H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	4/G	0/G	12/G	20/G	/		36	3
2	/	/	/	0/	0/	0/	0/	/	8♂	—	—
3	/	/	/	4/	12/	0/	21/	/		37	3
4	/	/	/	4/	Abort 0/	0/	~~17~~ 1/	/		5	3
5	/	/	/	4/	0/	10/	20/	/		34	3
6	/	/	/	4/	0/	9/	19/	/		32	3
7	/	/	/	4/	0/	11/	20/	/		36	3
8	/	/	/	3/	0/	8/	15/	/		26	3
9	/	/	/	3/	0/	8/	~~10~~ 19/	/		30	3
10	/	/	/	4/	0/	8/	18/	/		30	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—	n=9	SUM 266	27
										MEAN 29.6	3.0
										S.D. 9.9	—

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1358
Age of *Ceriodaphnia dubia*: 21-23H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	6/G	0/G	10/G	23/G	/		39	3
2	/	/	/	0/	0/	0/	0/	/	♂	—	—
3	/	/	/	2/	9/	17/	0/	/		28	3
4	/	/	/	3/	9/	0/	3/	/		15	3
5	/	/	/	4/	0/	10/	19/	/		33	3
6	/	/	/	4/	0/	7/	19/	/		30	3
7	/	/	/A	0/D	—/—	—/—	—/—	—/—	D	0	0
8	/	/	/G	4/G	0/G	7/G	16/G	/		27	3
9	/	/	/	4/	0/	7/	19/G	/		30	3
10	/	/	/	4/	0/	9/	18/G	/		31	3
Total Adult Cond.	10G	10G	9G 1A	9G 1D	9G 1D	9G 1D	9G 1D	—	N=9	SUM 233	24
										MEAN 25.9	2.7
										S.D. 11.6	—

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen Co (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1355
Age of *Ceriodaphnia dubia*: ~~1~~ 21-23H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	6/G	0/G	11/G	22/G	/		39	3
2	/	/	/	0/	0/	0/	0/	/	♂	—	—
3	/	/	/	0/	3/	13/	19/	/		35	3
4	/	/	/	3/	10/	0/	17/	/		30	3
5	/	/	/	6/	0/	3/	19/	/		28	3
6	/	/	/	5/	0/	7/	20/	/		32	3
7	/	/	/	6/	0/	9/	20/	/		35	3
8	/	/	/	3/	0/	9/	18/	/		30	3
9	/	/	/	5/	0/	8/	19/	/		32	3
10	/	/	/	2/	0/	8/	17/	/		27	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G ~~10G~~	10G	–	n = 9 SUM	288	27
									MEAN	32.0	3.0
									S.D.	3.8	—

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

TOXSTAT 3.5

Title: American II WWTP 001 C. dubia 04/8-14/14 Reproduction XIX 4/22/14 AEV
File: AMER0414.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	28.8889				
2	20 %	31.6667	101.50	60.00	9.00	
3	40 %	31.3333	100.50	60.00	9.00	
4	60 %	29.5556	96.50	60.00	9.00	
5	80 %	25.8889	78.00	60.00	9.00	
6	100 %	32.0000	97.00	60.00	9.00	

Critical values are 1 tailed (k = 5)

EPA ICP 6/94 RER 4/22/14 PKW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	29	14	34	36	39	39
Response 2	21	29	15	37	28	35
Response 3	29	34	32	5	15	30
Response 4	32	39	33	34	33	28
Response 5	31	37	37	32	30	32
Response 6	34	32	30	36	0	35
Response 7	19	31	30	26	27	30
Response 8	34	33	37	30	30	32
Response 9	31	36	34	30	31	27

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American II WWTP 001
Test Start Date: 4/8/14 Test Ending Date: 4/14/14
Test Species: C. dubia
Test Duration: 6 days
DATA FILE: Ame0414c.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	9	0.000	28.889	5.372	30.630
2	9	20.000	31.667	7.314	30.630
3	9	40.000	31.333	6.633	30.630
4	9	60.000	29.556	9.876	29.556
5	9	80.000	25.889	11.602	28.944
6	9	100.000	32.000	3.808	28.944

*** 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 II WWTP 001 C. dubia 04/8-14/14 Reproduction
File: AMER0414.CDR Transform: NO TRANSFORMATION

REN 4/22/14

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	9	19.0000	34.0000	28.8889
2	20 %	9	14.0000	39.0000	31.6667
3	40 %	9	15.0000	37.0000	31.3333
4	60 %	9	5.0000	37.0000	29.5556
5	80 %	9	0.0000	39.0000	25.8889
6	100 %	9	27.0000	39.0000	32.0000

Title: American II WWTP 001 C. dubia 04/8-14/14 Reproduction
File: AMER0414.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	28.8611	5.3723	1.7908	18.5963
2	20 %	53.5000	7.3144	2.4381	23.0980
3	40 %	44.0000	6.6332	2.2111	21.1699
4	60 %	97.5278	9.8756	3.2919	33.4137
5	80 %	134.6111	11.6022	3.8674	44.8154
6	100 %	14.5000	3.8079	1.2693	11.8996

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

Discharger/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Type of Test: Chronic Date of Test: 4/8-15/14
Effluent Serial No.: 001 Permit No.: 2PH00006*

Investigator: [illegible] Culture No.: ECT #4
Culture initiation date: Culture ending date:
Origin of ~~parental~~ organisms: ECT Rec. 4/8/14
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	11	1500 to 1700 4/7/14		1
2)	2	9			2
3)	3	8			3
4)	4	8			4
5)	5	9			5
6)	6	11			6
7)	7	9			7
8)	8	12			8
9)	9	13			9
10)	10	8			10

COMMENTS:

Appendix C

Parameter Data

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

Industry/Toxicant: Allen Co (American II WWTP) Type of Test: chronic
Address: Lima, OH Date of Test: 4/8-15/14
Effluent Serial No.: 001 Permit No.: 2PH00006*

1st Sample Collection

Date Samples Collected: 4/7/14 Date Samples Read: 4/7/14 Analyst: RFA

	100% Effluent	Upstream
Temperature (°C)	8.5	8.6
Dissolved Oxygen (mg/L)	12.6	12.8
pH (s.u.)	7.53	7.84
Conductivity (µmhos)	859	870
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light brown stain

2nd Sample Collection

Date Samples Collected: 4/9/14 Date Samples Read: 4/9/14 Analyst: RSC

	100% Effluent	Upstream
Temperature (°C)	10.0	10.5
Dissolved Oxygen (mg/L)	11.1	11.5
pH (s.u.)	7.30	7.54
Conductivity (µmhos)	678	652
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light brown

3rd Sample Collection

Date Samples Collected: 4/11/14 Date Samples Read: 4/11/14 Analyst: RSC

	100% Effluent	Upstream
Temperature (°C)	11.8	11.7
Dissolved Oxygen (mg/L)	10.6	11.2
pH (s.u.)	7.47	7.83
Conductivity (µmhos)	840	927
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light tan stain

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

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1415
Test Organism: *Pimephales promelas* Age: <24 H #3
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 25.5	25.4	25.0 to 25.1	25.4	25.1 to 25.5	25.3	25.0 to 25.7	24.9	25.2 to 25.7	25.1	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	8.2	8.6	5.4 ~~5.9~~ mL	8.1	6.1	8.7	6.1	8.0	5.8	8.6	5.0	8.9	5.1	6.5
pH (s.u.)	7.89	7.28	6.95 ~~7.09~~ mL	7.81	7.20	7.45	7.15	7.77	7.30	7.56	7.34	7.30	7.33	7.47
Conductivity (µmho/cm)	332	332	351 ~~358~~ mL	334	345	334	348	341	345	341	347	341	366	358
Hardness (mg/L $CaCO_3$)	137			137				137						
Alkalinity (mg/L $CaCO_3$)	68			68				86						

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

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1415
Test Organism: *Pimephales promelas* Age: <24H-2D
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 25.5	25.3	25.0 to 25.1	25.3	25.1 to 25.5	25.2	25.0 to 25.3	25.2	25.2 to 25.7	25.2	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	8.2	8.7	6.1	8.6	5.5	8.9	5.3	8.2	5.1	8.9	5.3	8.9	4.4	6.5
pH (s.u.)	7.83	7.79	7.63	7.77	7.74	7.77	7.65	7.90	7.71	7.93	7.89	7.88	7.75	7.89
Conductivity (µmho/cm)	882	871	882	667	899	663	704	924	686	924	928	924	950	956
Hardness (mg/L $CaCO_3$)	410			274				359						
Alkalinity (mg/L $CaCO_3$)	222			188				222						

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

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1405
Test Organism: *Pimephales promelas* Age: <24H #3
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 25.5	25.3	25.0 to 25.1	25.3	25.1 to 25.5	25.2	25.0 to 25.3	25.2	25.2 to 25.7	25.2	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	9.0	8.7	5.4	8.5	6.0	8.9 ~~5.1~~	5.3	8.2	5.0	8.9	4.9	8.9	5.3	6.9
pH (s.u.)	7.82	7.81	7.70	7.74	7.84	7.79 ~~7.82~~	7.67	7.90	7.70	7.93	7.84	7.90	7.77	7.93
Conductivity (µmho/cm)	870	870	882	669	899	669 ~~911~~	711	908	689	908	910	909	943	950
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1415
Test Organism: *Pimephales promelas* Age: <24H #3
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 25.5	25.4	25.0 to 25.1	25.3	25.1 to 25.5	25.3	25.0 to 25.3	25.2	25.2 to 25.7	25.3	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	9.0	8.7	6.0	8.6	6.1	8.6	5.3	8.2	5.4	9.0	5.3	8.6	4.7	6.6
pH (s.u.)	7.75	7.79	7.77	7.64	7.82	7.75	7.69	7.81	7.72	7.85	7.85	7.82	7.73	7.91
Conductivity (µmho/cm)	868	867	895	673	911	673	723	892	701	895	907	893	928	935
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1415
Test Organism: *Pimephales promelas* Age: <24H #3
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 25.5	25.4	25.0 to 25.1	25.3	25.1 to 25.5	25.3	25.0 to 25.3	25.3	25.2 to 25.7	25.3	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	8.8	8.7	5.9	8.2	5.6	8.7	5.5	8.6	5.4	9.1	4.8	8.7	4.8	6.6
pH (s.u.)	7.67	7.68	7.73	7.65	7.76	7.74	7.66	7.74	7.71	7.79	7.79	7.77	7.70	7.89
Conductivity (µmho/cm)	865	866	886	677	900	676	717	876	698	879	885	877	896	904
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1405
Test Organism: *Pimephales promelas* Age: 24 hr #3
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 25.5	25.3	25.0 to 25.1	25.2	25.1 to 25.5	25.2	25.0 to 25.3	25.3	25.2 to 25.7	25.3	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	9.0	8.6	6.1	8.4	5.2	8.5	5.7	8.0	5.4	8.5	5.1	8.4	4.5	6.5
pH (s.u.)	7.60	7.65	7.77	7.59	7.73	7.69	7.72	7.72	7.71	7.75	7.79	7.76	7.67	7.86
Conductivity (µmho/cm)	862	864	879	680	903	680	726	859	705	860	861	861	880	899
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 4/8/14 Time: 1415
Ending Date: 4/15/14 Time: 1415
Test Organism: *Pimephales promelas* Age: <24H #3
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	25.1	25.1 to 25.5	25.3	25.0 to 25.1	25.3	25.1 to 25.5	25.2	25.0 to 25.3	25.2	25.2 to 25.7	25.4	25.2 to 25.5	24.4 to 24.7
Dissolved Oxygen (mg/L)	9.0	8.7	5.8	8.3	6.1	8.8	5.4	8.3	5.0	8.6	4.7	8.7	4.7	6.4
pH (s.u.)	7.58	7.62	7.73	7.55	7.77	7.67	7.72	7.60	7.69	7.66	7.78	7.88	7.67	7.83
Conductivity (µmho/cm)	861	841	889	684	903	684	738	843	718	843	862	846	876	899
Hardness (mg/L $CaCO_3$)	325			291				342						
Alkalinity (mg/L $CaCO_3$)	205			205				205						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 4/8/14 Time: ~~1~~ 1355
Ending Date: 4/14/14 Time: 1355
Test Organism: *Ceriodaphnia dubia*: Age: 21-23H Ext #4
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	24.9 to 25.3	25.4	24.9 to 25.2	25.3	24.6 to 24.9	25.2	24.9 to 25.3	24.9	25.0 to 25.2	25.2	24.9 to 25.2	—
Dissolved Oxygen (mg/L)	8.3	8.3	7.7	8.5	7.8	8.3	8.0	8.2	7.5	8.4	7.7	8.3	8.4	
pH (s.u.)	7.69	7.56	7.49	7.52	7.50	7.45	7.65	7.59	7.57	7.49	7.61	7.44	7.41	
Conductivity (µmho/cm)	290	290	288	291	296	288	297	282	295	281	290	282 PJC ~~[illegible]~~	297	
Hardness (mg/L $CaCO_3$)	103			103				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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1350
Test Organism: *Ceriodaphnia dubia*: Age: 21-23 H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.3	25.2	24.9 to 25.3	25.3	24.9 to 25.2	25.3	24.6 to 24.9	25.2	24.9 to 25.3	25.2	25.0 to 25.2	25.2	24.9 to 25.2	–
Dissolved Oxygen (mg/L)	8.2	8.7	7.9	8.6	7.8	8.9	7.9	8.2	7.6	8.9	7.7	8.9	8.2	
pH (s.u.)	7.83	7.79	8.04	7.77	7.97	7.77	7.85	7.90	7.90	7.93	8.14	7.88	7.98	
Conductivity (µmho/cm)	882	871	858	667	869	663	669	924	670	924	921	921	839	
Hardness (mg/L $CaCO_3$)	410			274				359						
Alkalinity (mg/L $CaCO_3$)	222			188				222						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co (American II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1358
Test Organism: *Ceriodaphnia dubia*: Age: 21-23H
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	24.9 to 25.3	25.3	24.9 to 25.2	25.3	24.6 to 24.9	25.2	24.9 to 25.3	25.2	25.0 to 25.2	25.2	24.9 to 25.2	—
Dissolved Oxygen (mg/L)	9.0	8.7	7.9	8.5	7.8	8.9	8.0	8.2	7.5	8.9	7.7	8.9	8.1	
pH (s.u.)	7.82	7.81	8.06	7.74	7.99	7.79	7.92	7.90	7.92	7.93	8.16	7.40	8.00	
Conductivity (µmho/cm)	870	870	875	669	886	669	692	908	686	908	932	909	940	
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 9/8/14 Time: 1355
Ending Date: 9/14/14 Time: 1355
Test Organism: *Ceriodaphnia dubia*: Age: 21-23H
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	24.9 to 25.2	25.4	24.9 to 25.2	25.3	24.6 to 24.9	25.3	24.9 to 25.3	25.2	25.0 to 25.2	25.3	24.9 to 25.2	—
Dissolved Oxygen (mg/L)	9.0	8.7	7.8	8.6	7.8	8.6	7.9	8.2	7.5	9.0	7.7	8.6	8.1	
pH (s.u.)	7.75	7.79	8.04	7.64	7.90	7.75	7.93	7.81	7.92	7.85	8.16	7.82	8.00	
Conductivity (µmho/cm)	868	867	870	673	888	673	694	892	690	895	909	893	922	
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1358
Test Organism: *Ceriodaphnia dubia*: Age: 21-23H
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	24.9 to 25.3	25.4	24.9 to 25.2	25.3	24.6 to 24.9	25.3	24.9 to 25.3	25.3	25.0 to 25.2	25.3	24.9 to 25.2	—
Dissolved Oxygen (mg/L)	8.8	8.7	7.9	8.2	7.8	8.7	8.0	8.6	7.5	9.1	7.6	8.7	8.0	
pH (s.u.)	7.67	7.68	8.01	7.65	7.96	7.74	7.92	7.74	7.92	7.79	8.14	7.77	8.02	
Conductivity (µmho/cm)	865	866	868	677	883	676	697	876	688	879	885	877	900	
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1358
Test Organism: Ceriodaphnia dubia: Age: 21-23H
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)	25.3	25.1	24.9 to 25.3	25.3	24.9 to 25.2	25.2	24.6 to 24.9	25.2	24.9 to 25.3	25.3	25.0 to 25.2	25.3	24.9 to 25.2	—
Dissolved Oxygen (mg/L)	9.0	8.6	8.0	8.4	7.7	8.5	8.0	8.0	7.6	8.5	7.7	8.4	7.9	
pH (s.u.)	7.60	7.65	7.99	7.59	7.96	7.69	7.92	7.72	7.93	7.75	8.13	7.76	8.01	
Conductivity (µmho/cm)	862	864	866	680	880	680	703	859	694	860	877	861	885	
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 II WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 4/8/14 Time: 1355
Ending Date: 4/14/14 Time: 1355
Test Organism: *Ceriodaphnia dubia*: Age: 21-23 H
Dilution Water Used: upstream

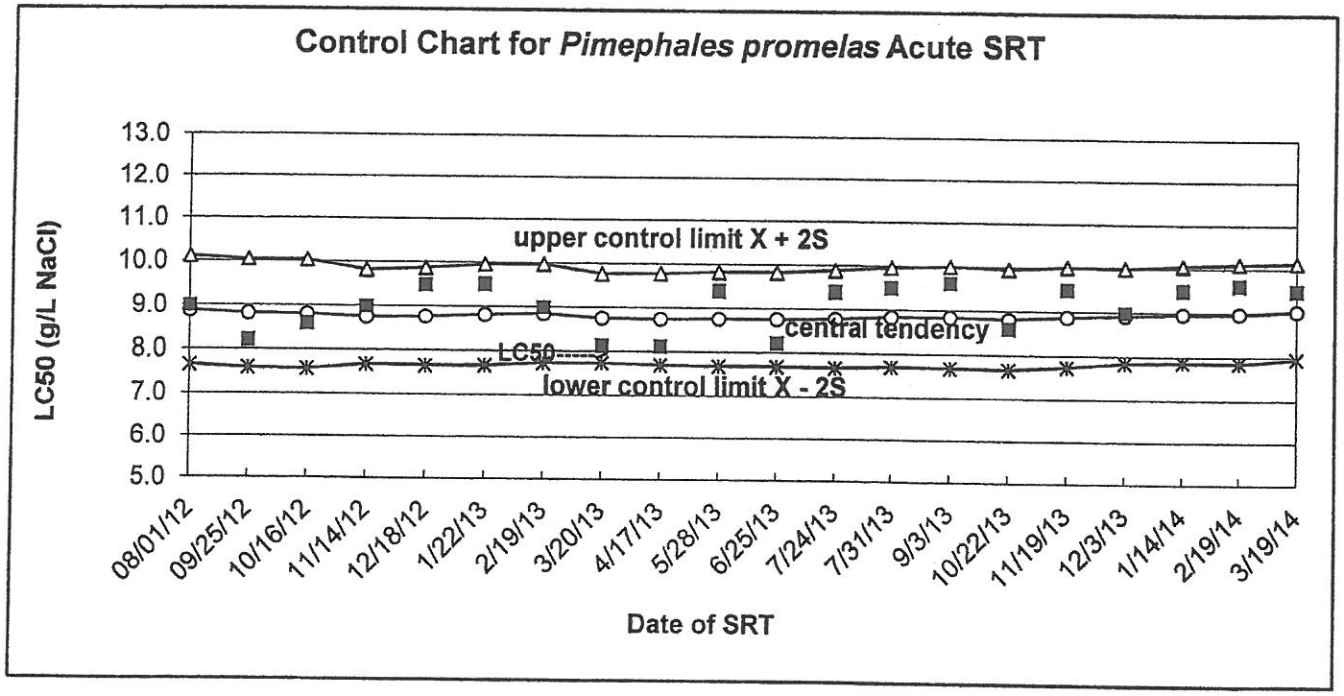
Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.1	25.1	24.9 to 25.8	25.3	24.9 to 25.2	25.3	24.6 to 24.9	25.2	24.9 to 25.3	25.2	25.0 to 25.2	25.4	24.9 to 25.2	—
Dissolved Oxygen (mg/L)	9.0	8.7	7.8	8.3	7.7	8.8	7.9	8.3	7.6	8.6	7.6	8.7	7.9	
pH (s.u.)	7.58	7.62	7.97	7.55	7.95	7.67	7.92	7.60	7.92	7.66	8.12	7.88	8.00	
Conductivity (µmho/cm)	861	861	862	684	874	684	706	843	700	843	862	846	862	
Hardness (mg/L $CaCO_3$)	325			291				342						
Alkalinity (mg/L $CaCO_3$)	205			205				205						

Appendix D

SRT Data

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

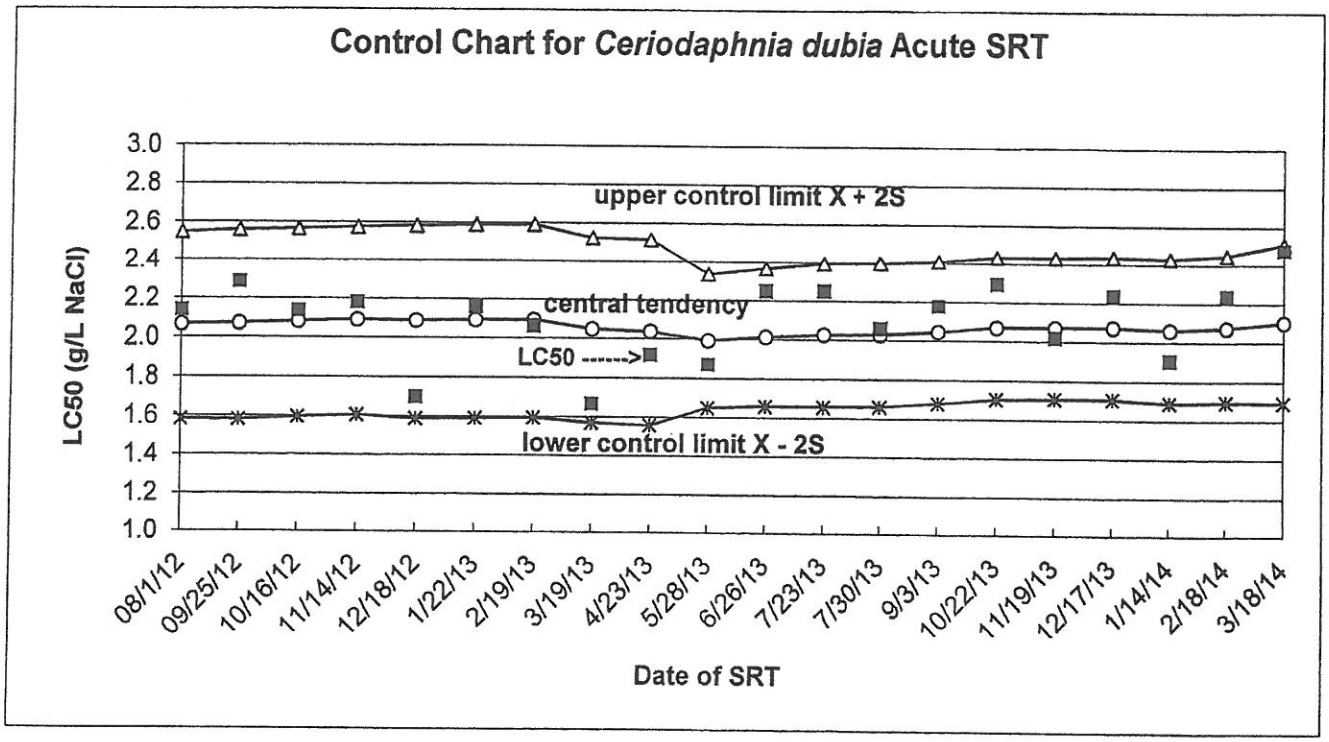
Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
08/01/12	9.0	8.9	0.6	10.1	7.6	0.07	10.7	7.0	SK
09/25/12	8.2	8.8	0.6	10.0	7.6	0.07	10.7	7.0	SK
10/16/12	8.6	8.8	0.6	10.0	7.6	0.07	10.7	6.9	SK
11/14/12	9.0	8.7	0.5	9.8	7.7	0.06	10.4	7.1	SK
12/18/12	9.5	8.8	0.6	9.9	7.6	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
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



*- 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.

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]
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
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

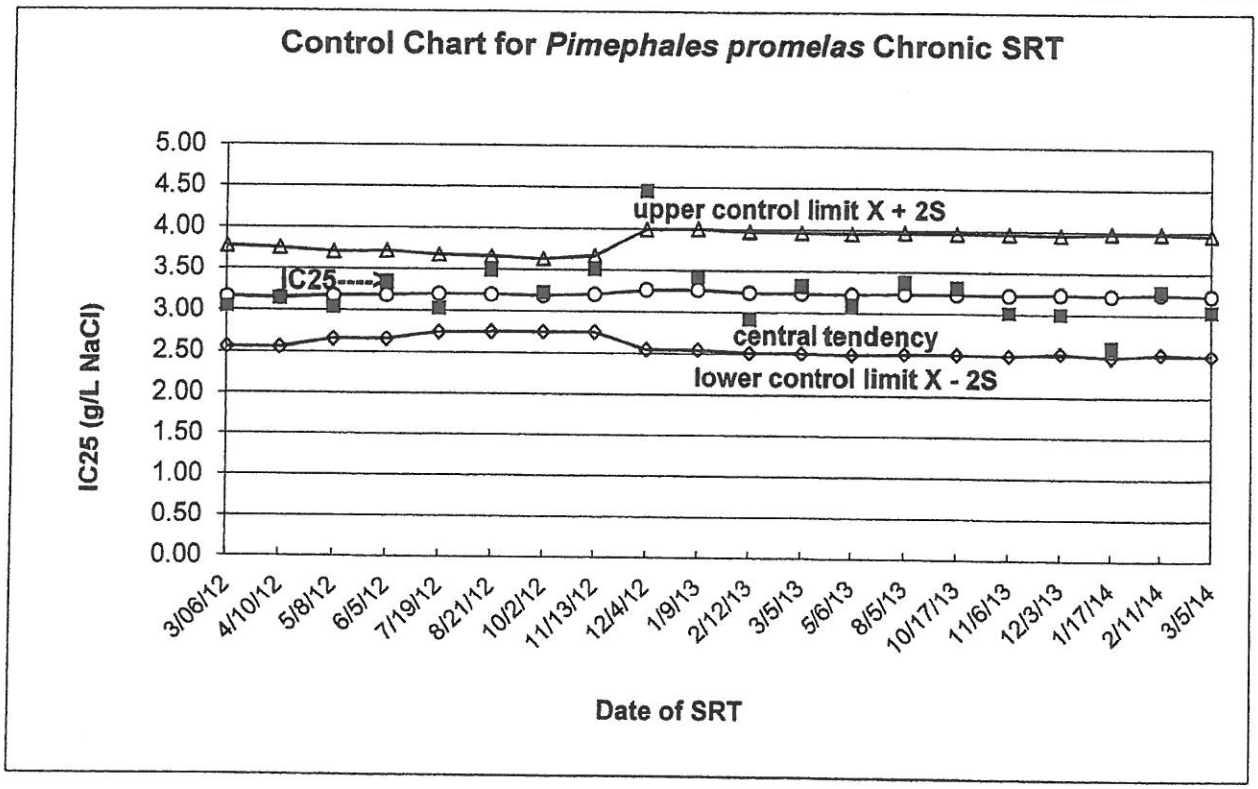


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

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

IC25 for *Pimephales promelas* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
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
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



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

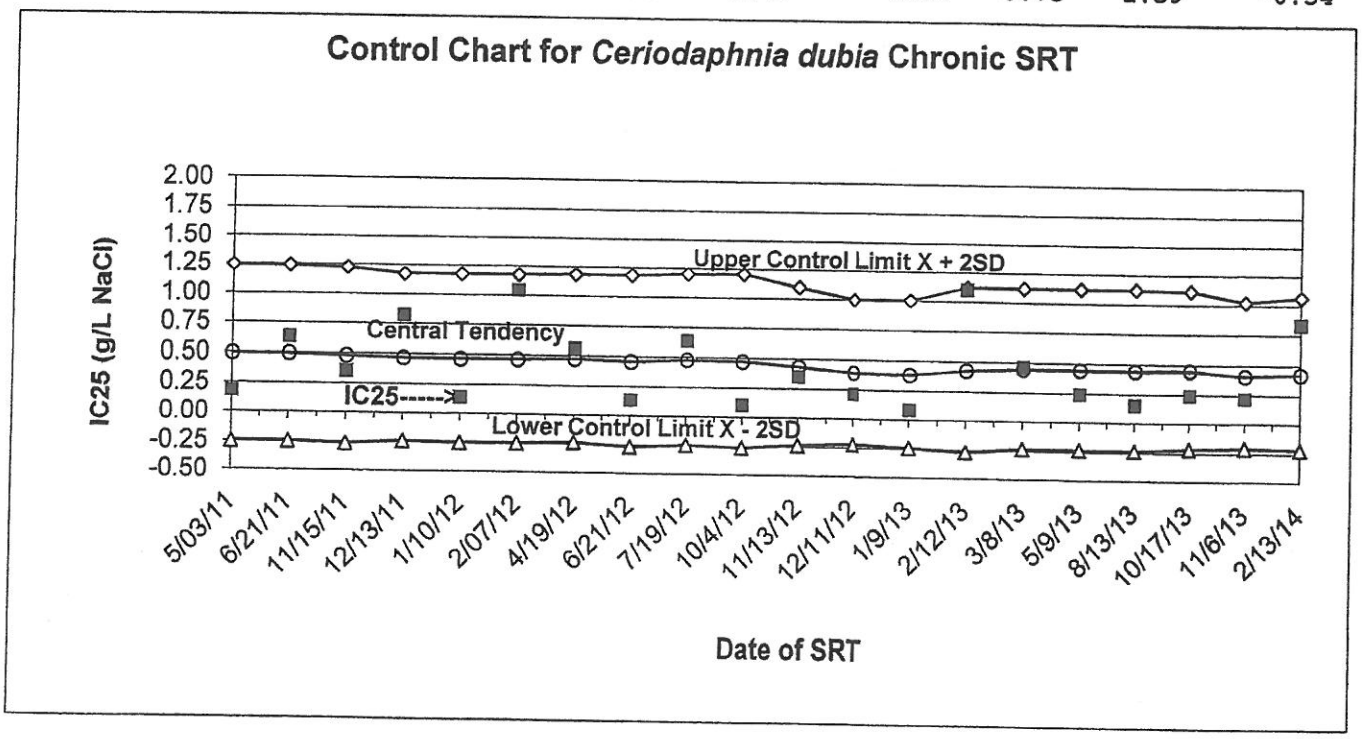
75th percentile of CVs for IC25 = 0.38

Calculated CV should not exceed 75th percentile of CVs.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
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
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



*- 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.