

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PK00002*JD
Alloway Lab #: L16-15974-01

Report of Chronic Biomonitoring

Samples Collected on:

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

Pimephales promelas
Ceriodaphnia dubia

Allen County Sanitary Engineer
Shawnee No. 2 WWTP
3640 Spencerville Road
Lima, OH 45805



Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PK00002*JD
Alloway Lab #: L16-15974-01

Date: 7/30/16

Mike Santaguida
Allen County Sanitary Engineer
3640 Spencerville Road
Lima, OH 45805

Dear Mr. Santaguida:

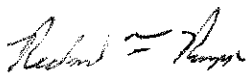
Enclosed is the report of the biomonitoring on the sample listed above. The objective of the chronic biomonitoring was to determine the effects, both the acute endpoint (mortality) and chronic endpoints (mortality and growth, or reproduction), from the test water. Fathead minnow larvae (*Pimephales promelas*)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

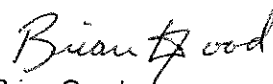
Acute toxicity was determined by locating the median lethal concentration (LC50) and the median effective concentration (EC50). Growth was determined from the dry weight of surviving *Pimephales promelas* larvae. Reproduction was monitored daily by counting the number of young produced by each female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


Richard F. Rupp
Bioassay Lab Manager


Brian Good
Bioassay Analyst



Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PK00002*JD

- | | |
|--|--|
| 1. Facility Name | Allen County Sanitary Engineer |
| 2. Facility Address | Shawnee No. 2 WWTP
3640 Spencerville Road
Lima, OH 45805 |
| 3. Ohio EPA Permit No. | 2PK00002*JD |
| 4. NPDES No. | OH0023850 |
| 5. Facility Contact | Mike Santaguida |
| 6. Telephone No. | 419-605-7560 |
| 7. Consulting Laboratory | Alloway |
| 8. Lab Contact | Dick Rupp or Brian Good |
| 9. Lab Telephone No. | 419-223-1362 |
| 10. Receiving water(s) of discharge | Ottawa River |
| 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: <u>1/21/2005</u> | |

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: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*JD
Date of Test: 7/12-19/16

Summary of Chronic Toxicity Test Results
Pimephales promelas

Effluent/Outfall:	4500 Form Value	MDL	4500 Comments Required
Chronic Toxicity, <i>Pimephales promelas</i> - TUc	AA	1.0	
Acute Toxicity, <i>Pimephales promelas</i> - TUa	AA	0.2	

Upstream:

96 Hr. Acute Toxicity, <i>Pimephales promelas</i> - % Affected	AA	2.5
7 day Chronic Toxicity, <i>Pimephales promelas</i> - % Affected	5.0	2.5

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
 Facility: Shawnee No. 2 WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PK00002*JD
 Date of Test: 7/12-18/16

Summary of Chronic Toxicity Test Results
Ceriodaphnia dubia

Effluent/Outfall:	4500 Form Value	MDL	4500 Comments Required
Chronic Toxicity, <i>Ceriodaphnia dubia</i> - TUC	AA	1.0	
Acute Toxicity, <i>Ceriodaphnia dubia</i> - TUA	AA	0.2	

Upstream:

48 Hr. Acute Toxicity, <i>Ceriodaphnia dubia</i> - % Affected	AA	10.0
7 day Chronic Toxicity, <i>Ceriodaphnia dubia</i> - % Affected	AA	10.0

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
 Facility: Shawnee No. 2 WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PK00002*JD
 Date of Test: 7/12-19/16

Chronic Toxicity Test Results

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

Test Solutions	Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)*							Mean Dry Weight*	
	Test Day							Mean	SD
	1	2	3	4	5	6	7		
Primary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	0.738	0.047
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	7.5% (7.5%)	0.659	0.061
20%	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	10.0% (10.0%)	0.660	0.084
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%)	2.5% (2.5%)	0.754	0.071
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%)	7.5% (7.5%)	0.712	0.102
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	10.0% (10.0%)	10.0% (10.0%)	0.731	0.052
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	7.5% (7.5%)	0.819	0.061
LC50 Values (%):		>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	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: IC Pin, Dunnett's	
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		

*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: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*JD
Date of Test: 7/12-18/16

Chronic Toxicity Test Results

Results of a Ceriodaphnia dubia Survival and Reproduction Test

Test Solutions		Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)*							Number of Young Produced Mean
		Test Day							
		1	2	3	4	5	6	7	
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%)	--- (---)	24.2
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%)	--- (---)	31.8
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%)	--- (---)	25.7
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%)	--- (---)	26.3
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%)	--- (---)	32.8
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%)	--- (---)	29.3
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%)	--- (---)	26.2
LC50 Values (%):		>100	>100	>100	>100	>100	>100	---	Calculated TUC Value for Survival <1.0
95% Confidence Limits	LL								
	UL								
EC50 Values (%):		>100	>100	>100	>100	>100	>100	---	Calculated TUC Value for Reproduction <1.0
95% Confidence Limits	LL								
	UL								
NOEC for Mortality (%)			100		NOEC for Reproduction (%)			100	Method(s) used to determine Values: IC Pin, Steel's Many-One Rank
LOEC for Mortality (%)			>100		LOEC for Reproduction (%)			>100	
Chronic Value for Mortality (%)			>100		Chronic Value for Reprod (%)			>100	
IC25 (%)			>100		IC50 (%)			>100	

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

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Report Date: 7/30/16
Ohio EPA Permit No.: 2PK00002*JD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection	
			Beginning	Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	7/10/16 @ 10:00	7/11/16 @ 10:00
Type (Grab/Comp):	Comp	2nd	7/12/16 @ 10:30	7/13/16 @ 10:30
Volume Collected:	5 Gal.	3rd	7/14/16 @ 11:30	7/15/16 @ 11:30
Upstream Station:				
Waterbody:	Ottawa River			
Station No.:	801	1st		7/11/16 @ 11:55
Type (Grab/Comp):	Grab	2nd		7/13/16 @ 11:20
Volume Collected:	3.5 to 5 Gal.	3rd		7/15/16 @ 12:32

Samples collected by: Alloway

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

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

Appendix A

Field Data



- 1101 North Cole Street, Lima, OH 45805
(P) 419-223-1362 (F) 419-227-3792
- 1776 Marion-Waldo Road, Marion OH 43302
(P) 740-389-5991 (F) 740-389-1481
- 1502 W. Fourth Street, Mansfield, OH 44906
(P) 419-526-1644 (F) 419-524-5575

Report To: Rick Boerger Name: Allen County Sanitary Engineer Company: 3226 North Cole St. Address: Lima, OH 45805		Invoice To (if Different): Name: Company: Address:		Notes/Comments:	
Phone #: 419-225-8048 Fax #:		PO#:		Turnaround: (Rush Charges May Apply) ☐ Next Day ☐ 3 Working Days ☐ 5 Working Days ☒ Routine	
Project Name Bioassay - Shawnee II - DAY 1		Sample ID / Sample Location Effluent		Analysis Required Toxicity	
Sampler Michael S. Hanley		Sample Date 7/10/16		Preservation Code # 1	
Sample ID / Sample Location Upstream		Sample Time 11:55		Analysis Required Toxicity	
Customer Sample ID / Sample Location		Composite X		Number of Containers 1	
Grab X		Matrix Code WW		Preservation Code # 1	
Date 7/10/16		Date 7/10/16		Time 12:30	
Relinquished by:		Received by:		Method of D ☐ UPS ☐ Fed Ex ☐ Client ☐ Alloway Pick Up ☒ Alloway Sampling ☐ Other	
Received for Laboratory By: (circle one): Mansfield		Received By:		Date:	
Transported to: Lima		Received By:		Date:	
Transported to: Marion		Received By:		Date:	

ACSE Biomonitoring Field Data Sheet

Client: Shawnee II

Field Technicians: MSH

Date: 7/11/16

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	12:20	23.5	729	11.70	7.16
Upstream	11:55	25.8	1104	10.52	7.66

Diagram of Collection Sites

~ Width of stream= ~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____



Alloway
Your Resource for Defensible Data

Chain of Custody Record

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

- ☐ 1101 North Cole Street, Lima, OH 45805
- ☐ (P) 419-223-1382 (F) 419-227-3792
- ☐ 1776 Marlon-Waldo Road, Marion OH 43302
- ☐ (P) 740-389-8991 (F) 740-389-1481
- ☐ 1502 W. Fourth Street, Mansfield, OH 44906
- ☐ (P) 419-525-1644 (F) 419-524-5575

Report To: Rick Boerger Company: Allen County Sanitary Engineer Address: 3226 North Cole St. Lima, OH 45805		Invoice To (If Different): Name: Company: Address:		Notes/Comments:	
Phone #: 419-225-8048 E-mail: rboerger@allencountyohio.com		Fax #: _____			
Project Name Bioassay - Shawnee II - DAY 2		PO# _____			
Sampler	Customer Sample ID / Sample Location Effluent Upstream	Sample Date 2/12/16 7/13/16 7/13/16	Sample Time 10:30 10:35 11:20	Signature (Print) Michael S. Hanley	
1	1	WW	1	1	Toxicity
2	1	SW	1	1	Toxicity
3					
4					
5					
6					
7					
8					
Relinquished by:		Received by:			
1					
2					
3					
4					
5					
6					
Received for Laboratory By: (circle one): Mansfield Lima Marion		Date 7/13/16		Time 12:48	
(Signature)					
Transported to: Lima Marion		By:		Received By:	
Transported to: Lima Marion		By:		Received By:	
				Date:	
				Time:	

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	Method of Delivery UPS ☐ Fed Ex ☐ Client ☐ Alloway Pick Up ☐ Alloway Sampling ☒ Other ☐	Preservation Codes: 1 - None 2 - HNO ₃ 3 - H ₂ SO 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) ₂ SO ₄ & NH ₄ OH 13 - Zinc Acetate 14 - Sodium Sulfite 15 - Potassium Dihydrogen Citrate 16 - Sodium Sulfite/Sodium Bisulfite	Sample Receiving (For Lab Use Only) Ice Present? ☒ N ☐ Proper Preservation? ☒ N ☐ Container Temperature: _____
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ACSE Biomonitoring Field Data Sheet

Client: Shawnee III

Field Technicians: MJH

Date: 7/13/16

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent		23.8	728	11.92	7.20
Upstream	11:20	25.8	1546	9.06	7.54

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

(12:18)



Chain of Custody Record

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- ☐ 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
- ☐ 1602 W. Fourth Street, Mansfield, OH 44906
- ☐ (P) 419-525-1644 (F) 419-624-5575

Report To: Rick Boerger Name: Allen County Sanitary Engineer Company: 3226 North Cole St. Address: Lima, OH 45805		Invoice To (if different): Name: Company: Address:		Notes/Comments:											
Phone #: 419-225-8048 E-mail: rboerger@allencountyohio.com		Fax #:		PO#:											
Project Name: Bioassay - Shawnee II - DAY 3		Turnaround: (Rush Charges May Apply) Next Day ☐ 3 Working Days ☐ Routine ☒ 2 Working Days ☐ 5 Working Days ☐													
Sampler	Customer (Print) Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only					
1	Effluent	7/14/16	11:30	X		WW	1	1	Toxicity						
2	Upstream	7/15/16	12:32		X	SW	1	1	Toxicity						
3															
4															
5															
6															
7															
8															
Relinquished by:		Received by:		Date		Time		Method of Delivery		Matrix Codes:		Preservation Codes:		Sample Receiving (For Lab Use Only)	
1								UPS ☐		ww - wastewater gw - groundwater		1 - None 7 - Sodium Thiosulfate		Ice Present? ☒ N ☐	
2								Fed Ex ☐		dw - drinking water sw - surface water		2 - HNO ₃ 8 - Ascorbic Acid Sulfite		Proper Preservation? ☒ N ☐	
3								Client ☐		w - water oil - oil		3 - H ₂ SO ₄ 9 - Maleic Acid		Container Temperature: _____	
4								Alloway Pick Up ☐		s - solid sg - sludge		4 - HCl 10 - EDTA			
5								Alloway Sampling ☒		li - leachate a - acid		5 - NaOH 11 - Ammonium Chloride			
6								Other ☐		p - product o - other		6 - NaOH & Zinc Acetate			
Received for Laboratory By: (circle one) Mansfield		Lima		Marion		7/15/16 13:14									
(Signature)															

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

ACSE Biomonitoring Field Data Sheet

Client: Shawnee II

Field Technicians: MSH

Date: 7/15/16

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	12:18	23.8	726	9.84	7.12
Upstream		26.2	1389	9.12	7.52

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

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

Stream Conditions

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

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Appendix B

Raw Data Sheets

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

Industry/Toxicant:

Allen County, Shawnee No. 2 WWTP

Address:

3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.:

001

NPDES Permit No.: 2PK00002*JD

Person(s) Conducting Test:

BG, SS, RFR

Beginning: Date

7/12/16

Time 1315

Ending: Date

7/19/16

Time 1315

Age of *Pimephales promelas*:

< 24 Hours

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	38G 2D	10G	10G	10G	10G	40G
4	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	38G 2D	10G	10G	10G	10G	40G
5	10G	10G	10G	10G	40G	9G	10G	10G	10G	39G 1D	10G	10G	10G	10G	38G 2D	10G	10G	10G	10G	40G
6	9G 1D	10G	10G	10G	39G 1D	9G	10G	10G	9G 1D	38G 2D	10G	10G	10G	10G	38G 2D	9G	10G	10G	10G	40G
7	9G 1D	10G	9G 1D	9G 1D	37G 3D	9G 1D	10G	10G	9G 1D	38G 2D	10G	10G	10G	10G	36G 4D	9G 1D	10G	10G	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).

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

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Address: 3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*JD

Person(s) Conducting Test: BG, SS, RFR

Beginning: Date 7/12/16 Time 1315

Ending: Date 7/19/16 Time 1315

Age of Pimephales promelas: < 24 Hours

Dilution Water Used: Upstream

Day	60% Effluent					80% Effluent					100% Effluent					Total				
	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	8G	2D	10G	10G	37G	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).

WEIGHT OF FISH IN REPLICATE TEST VESSELS

7-DAY PIMEPHALES PROMELAS CHRONIC TOXICITY TEST

Discharge: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001
 Drying Temperature (C°): 60
 Drying Time (h): 24
 NPDES Permit No.: 2PK00002*JD
 Date Weighed: 7/27/16
 Pimephales promelas Age at Start: < 24 Hours
 Analyst: SS
 Date of Test: 7/12/16 - 7/19/16

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

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
Fish at Start of Test		10	1.09875	1.09989	10	0.074	0.074
Lab Control	1	9	1.09659	1.10380	10	7.21	0.721
	2	10	1.09726	1.10424	10	6.98	0.698
	3	9	1.10286	1.10878	10	5.92	0.592
	4	9	1.08884	1.07513	10	0.24	0.624
Upstream Control	1	9	1.12472	1.13169	10	6.97	0.697
	2	10	1.10458	1.11254	10	8.01	0.801
	3	10	1.09447	1.10155	10	7.08	0.708
	4	9	1.13359	1.14105	10	7.46	0.746
20% Effluent	1	10	1.12582	1.13276	10	6.94	0.694
	2	10	1.09288	1.09989	10	7.01	0.701
	3	9	1.10092	1.10802	10	7.10	0.710
	4	7	1.07911	1.08446	10	5.35	0.535
40% Effluent	1	9	1.08503	1.09175	10	6.70	0.670
	2	10	1.10342	1.11183	10	8.41	0.841
	3	10	1.09314	1.10081	10	7.67	0.767
	4	10	1.12776	1.13506	10	7.36	0.736

Reviewed By: RAN

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

Discharge: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001
 Drying Temperature (C°): 60
 Drying Time (h): 24
 NPDES Permit No.: 2PK00002*JD
 Date Weighed: 7/27/16
 Date of Test: 7/12/16 - 7/19/16
 Pimephales promelas Age at Start: < 24 Hours
 Analyst: JS
 Balance Calibration Check: 1 gram weight = 0.9999 grams
 Date: 7/11/16
 Analyst: CE
 Dilution Water Used: Upstream

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
60% Effluent	1	8	1.12893	1.13518	10	6.25	0.625
	2	10	1.08615	1.09475	10	8.60	0.860
	3	9	1.13209	1.13894	10	6.76	0.676
	4	10	1.10676	1.11364	10	6.88	0.688
80% Effluent	1	9	1.12388	1.13090	10	7.06	0.706
	2	10	1.08745	1.09734	10	7.89	0.789
	3	9	1.09686	1.10492	10	7.56	0.756
	4	8	1.08334	1.09006	10	6.72	0.672
100% Effluent	1	9	1.09967	1.10828	10	8.61	0.861
	2	9	1.09859	1.10589	10	7.30	0.730
	3	9	1.09771	1.10599	10	8.28	0.828
	4	10	1.08638	1.09493	10	8.55	0.855
	1				10		
	2				10		
	3				10		
	4				10		

86 7/30/16

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 7/12/2016 Test ID: shaw0716p Sample ID: L16-15974-01
 End Date: 7/19/2016 Lab ID: ALL-ALLOWAY Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: EPAFW02-EPA/821/R-02-01 Test Species: PP-Pimephales promelas
 Comments:

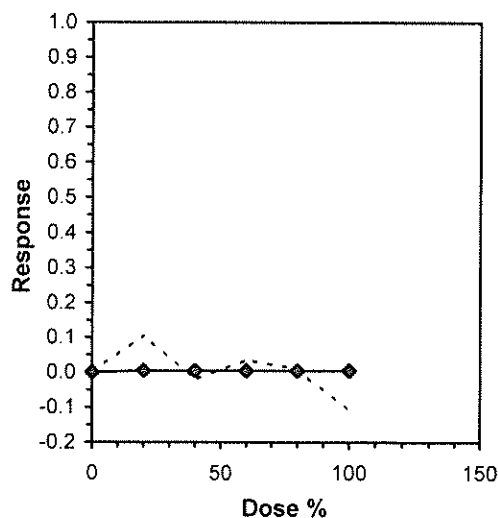
Conc-%	1	2	3	4
Upstr Control	0.6970	0.8010	0.7080	0.7460
20	0.6940	0.7010	0.7100	0.5350
40	0.6700	0.8410	0.7670	0.7360
60	0.6250	0.8600	0.6760	0.6880
80	0.7060	0.7890	0.7560	0.6720
100	0.8610	0.7300	0.8280	0.8550

Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Upstr Control	0.7380	1.0000	0.7380	0.6970	0.8010	6.362	4				0.7380	1.0000
20	0.6600	0.8943	0.6600	0.5350	0.7100	12.665	4	1.533	2.410	0.1226	0.7350	0.9959
40	0.7535	1.0210	0.7535	0.6700	0.8410	9.421	4	-0.305	2.410	0.1226	0.7350	0.9959
60	0.7123	0.9651	0.7123	0.6250	0.8600	14.351	4	0.506	2.410	0.1226	0.7350	0.9959
80	0.7308	0.9902	0.7308	0.6720	0.7890	7.108	4	0.142	2.410	0.1226	0.7350	0.9959
100	0.8185	1.1091	0.8185	0.7300	0.8610	7.419	4	-1.582	2.410	0.1226	0.7350	0.9959

Auxiliary Tests					Statistic		Critical		Skew	Kurt				
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.98217		0.916		0.08235	0.03335				
Bartlett's Test indicates equal variances (p = 0.80)					2.31077		15.0863							
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					100	>100		1	0.12262	0.16615	0.01079	0.00518	0.1149	5, 18
Treatments vs Upstr Control														

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew

IC05	>100
IC10	>100
IC15	>100
IC20	>100
IC25	>100
IC40	>100
IC50	>100



CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control

Person(s) Conducting Test: RFR, BG, SS

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Beginning Date: 7/12/2016

Time: 15:3

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/18/16

Time: 15:0

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 13-21 H

12/0

NPDES Permit No.: 2PK00002*JD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP	
Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0 / G	0 / G	0 / G	5 / G	0 / G	10 / G	17 / G	—	32	3
2	—	—	—	6 /	0 /	11 /	16 /	—	33	3
3	—	—	—	5 /	1 /	11 /	17 /	—	34	3
4	—	—	—	6 /	0 /	8 /	14 /	—	28	3
5	—	—	—	0 /	1 /	13 /	17 /	—	31	3
6	—	—	—	5 /	0 /	12 /	14 /	—	31	3
7	—	—	—	4 /	0 /	8 /	11 /	—	23	3
8	—	—	—	7 /	0 /	11 /	17 /	—	35	3
9	—	—	—	5 /	0 /	11 /	15 /	—	31	3
10	0 / G	8 / G	0 / G	7 / G	0 / G	16 / G	17 / G	—	40	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—		
									SUM	
									318	30
									MEAN	
									31.8	3.0
									S.D.	
									4.4	0.0

* Condition of Adult: G = Good Condition; D = Dead; A = Atypical Behavior or Appearance.

LA = Lab Accident (not used in analyses of data).

Reviewed by: AR

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control

Person(s) Conducting Test: RFR, BG, SS

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Beginning Date: 7/12/2016

Time: 1513

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/18/16

Time: 1510

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 13-21 H

NPDES Permit No.: 2PK00002*JD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	6/G	0/G	11/G	19/G	/	36	3
2	/	/	/	5	0	6	13	/	24	3
3	/	/	/	0	2	10	14	/	26	3
4	/	/	/	5	0	12	16	/	33	3
5	/	/	/	4	0	13	6	/	23	3
6	/	/	/	6	0	12	7	/	25	3
7	/	/	/	0	0	3	7	/	3	1
8	/	/	/	0	3	15	47/5	/	23	3
9	/	/	/	6	0	10	10	/	26	3
10	0/G	0/G	0/G	6/G	0/G	5/G	12/G	/	23	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-		
SUM									242	28
MEAN									24.2	2.8
S.D.									8.7	0.6

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent

Person(s) Conducting Test: RFR, BG, SS

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Beginning Date: 7/12/2016

Time: 15:3

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/18/16

Time: 18:0

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 13-21 d

NPDES Permit No.: 2PK00002*JD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0 / G	0 / G	0 / G	4 / G	0 / G	12 / G	16 / G		32	3
2				5 /	1 /	15 /	17 /		37	3
3				4 /	0 /	11 /	17 /		32	3
4				4 /	0 /	8 /	0 /		12	3
5				7 /	0 /	12 /	14 /		33	3
6				4 /	10 /	11 /	14 /		29	3
7				0 /	0 /	2 /	0 /		2	2
8				7 /	0 /	12 /	0 /		19	3
9				3 /	0 /	10 /	12 /		25	3
10	0 / G	5 / G	5 / G	5 / G	1 / G	11 / G	19 /		36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-		
SUM									257	29
MEAN									25.7	2.9
S.D.									11.4	0.3

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent

Person(s) Conducting Test: RFR, BG, SS

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Beginning Date: 7/12/2016

Time: 1513

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/18/16

Time: 1510

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 13-21 H

NPDES Permit No.: 2PK00002*JD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/6	0/6	0/6	5/6	0/6	13/6	16/6	/	34	3
2	/	/	/	4/	0/	11/	16/	/	31	3
3	/	/	/	5/	0/	11/	4/	/	20	3
4	/	/	/	5/	0/	9/	16/	/	30	3
5	/	/	/	0/	0/	8/	8/	/	8	2
6	/	/	/	5/	1/	7/	10/	/	23	3
7	/	/	/	4/	0/	12/	13/	/	29	3
8	/	/	/	4/	2/	8/	13/	/	27	3
9	/	/	/	4/	0/	12/	16/	/	32	3
10	0/6	0/6	0/6	4/6	0/6	11/6	14/6	/	29	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-		
SUM									263	29
MEAN									26.3	2.9
S.D.									7.7	0.3

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>60% Effluent</u>	Person(s) Conducting Test: <u>RFR, BG, SS</u>
Industry/Toxicant: <u>Allen County, Shawnee No. 2 WWTP</u>	Beginning Date: <u>7/12/2016</u> Time: <u>1513</u>
Address: <u>3640 Spencerville Road, Lima, OH 45805</u>	Ending Date: <u>7/18/16</u> Time: <u>1500</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>13-21 H</u>
NPDES Permit No.: <u>2PK00002*JD</u>	Dilution Water Used: <u>Upstream</u>

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP	
Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0 / G	0 / G	0 / G	5 / G	0 / G	14 / G	19 / G	/	38	3
2	/	/	/	6 /	0 /	12 /	17 /	/	35	3
3	/	/	/	5 /	0 /	12 /	11 /	/	28	3
4	/	/	/	6 /	0 /	14 /	16 /	/	36	3
5	/	/	/	5 /	1 /	14 /	16 /	/	36	3
6	/	/	/	5 /	0 /	9 /	13 /	/	27	3
7	/	/	/	4 /	0 /	9 /	16 /	/	29	3
8	/	/	/	6 /	0 /	15 /	19 /	/	40	3
9	/	/	/	5 /	0 /	10 /	15 /	/	30	3
10	0 / G	8 / G	8 / G	6 / G	0 / G	14 / G	9 / G	/	29	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-	SUM	328
									MEAN	32.8
									S.D.	4.7
										30
										3.0
										0.0

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>80% Effluent</u>	Person(s) Conducting Test: <u>RFR, BG, SS</u>
Industry/Toxicant: <u>Allen County, Shawnee No. 2 WWTP</u>	Beginning Date: <u>7/12/2016</u> Time: <u>1513</u>
Address: <u>3640 Spencerville Road, Lima, OH 45805</u>	Ending Date: <u>7/18/16</u> Time: <u>1810</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>13-21 H</u>
NPDES Permit No.: <u>2PK00002*JD</u>	Dilution Water Used: <u>Upstream</u>

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP	
Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	7/G	7/G	10/G	11/G	/	28	3
2	/	/	/	6/G	6/G	14/G	2/G	/	22	3
3	/	/	/	0/G	0/G	10/G	18/G	/	34	3
4	/	/	/	0/G	0/G	6/G	15/G	/	21	2
5	/	/	/	4/G	3/G	8/G	17/G	/	32	3
6	/	/	/	6/G	0/G	12/G	18/G	/	36	3
7	/	/	/	4/G	0/G	9/G	0/G	/	13	3
8	/	/	/	0/G	6/G	12/G	14/G	/	32	3
9	/	/	/	6/G	0/G	11/G	19/G	/	36	3
10	0/G	0/G	0/G	5/G	0/G	14/G	20/G	/	39	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-		
									SUM	293
									MEAN	29.3
									S.D.	8.2
										0.3

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>100% Effluent</u>	Person(s) Conducting Test: <u>RFR, BG, SS</u>
Industry/Toxicant: <u>Allen County, Shawnee No. 2 WWTP</u>	Beginning Date: <u>7/12/2016</u> Time: <u>1513</u>
Address: <u>3640 Spencerville Road, Lima, OH 45805</u>	Ending Date: <u>7/18/16</u> Time: <u>1510</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>13-21 H</u>
NPDES Permit No.: <u>2PK00002*JD</u>	Dilution Water Used: <u>Upstream</u>

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP		
Rep.	DAY of TEST								No. Young	No. Broods	
	0	1	2	3	4	5	6	7			
1	0 / G	0 / G	0 / G	5 / G	0 / G	11 / G	16 / G	/	32	3	
2	/	/	/	1 /	0 /	9 /	13 /	/	23	3	
3	/	/	/	2 /	0 /	8 /	19 /	/	34	3	
4	/	/	/	4 /	0 /	10 /	18 /	/	32	3	
5	/	/	/	0 /	5 /	4 /	5 /	/	14	3	
6	/	/	/	5 /	0 /	13 /	16 /	/	34	3	
7	/	/	/	4 /	0 /	9 /	12 /	/	25	3	
8	/	/	/	6 /	1 /	10 /	17 /	/	34	3	
9	/	/	/	5 /	1 /	1 /	0 /	/	7	3	
10	0 / G	0 / G	0 / G	0 / G	5 / G	11 / G	11 / G	/	21	3	
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	-			
									SUM	262	30
									MEAN	26.2	3.0
									S.D.	9.3	0.0

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

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

Discharger/Toxicant: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Type of Test: Chronic Date of Test: 7/12/16 - 7/18/16
 Effluent Serial No.: 001 Permit No.: 2PK00002*JD

Investigator: APR Culture No.: 121 B
 Culture initiation date: 7/6/16 Culture ending date: 7/13/16
 Origin of parental organisms: 112 Allouney
 Age at start of culture: > 24H
 Culture water renewal frequency: minimum 3 times per week Culture water: 1/1000 A. SMASH
 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: 32.2

	<u>BROOD ADULT NO.</u>	<u>BROOD SIZE</u>	<u>HATCHING TIME WINDOW</u>	<u>TOTAL; YOUNG PROD. (7 DAYS)</u>	<u>USED IN REP. NO.</u>
1)	<u>B1</u>	<u>18</u>	<u>1830 to 0230 7/11-12/16</u>	<u>35</u>	<u>1</u>
2)	<u>B2</u>	<u>14</u>		<u>30</u>	<u>2</u>
3)	<u>B3</u>	<u>18</u>		<u>32</u>	<u>3</u>
4)	<u>B4</u>	<u>15</u>		<u>30</u>	<u>4</u>
5)	<u>B5</u>	<u>14</u>		<u>29</u>	<u>5</u>
6)	<u>B6</u>	<u>15</u>		<u>31</u>	<u>6</u>
7)	<u>B7</u>	<u>19</u>		<u>32</u>	<u>7</u>
8)	<u>B8</u>	<u>17</u>		<u>32</u>	<u>8</u>
9)	<u>B9</u>	<u>20</u>		<u>36</u>	<u>9</u>
10)	<u>B10</u>	<u>18</u>		<u>34</u>	<u>10</u>

COMMENTS: _____

BL 7/24/16

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 7/12/2016 Test ID: shaw0716c Sample ID: L16-15974-01
 End Date: 7/18/2016 Lab ID: ALL-ALLOWAY Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: EPAFW02-EPA/821/R-02-01 Test Species: CD-Ceriodaphnia dubia
 Comments:

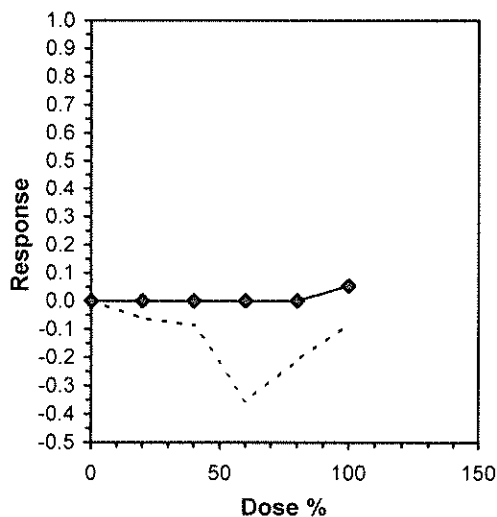
Conc-%	1	2	3	4	5	6	7	8	9	10
Upstr Control	36.000	24.000	26.000	33.000	23.000	25.000	3.000	23.000	26.000	23.000
20	32.000	37.000	32.000	12.000	33.000	29.000	2.000	19.000	25.000	36.000
40	34.000	31.000	20.000	30.000	8.000	23.000	29.000	27.000	32.000	29.000
60	38.000	35.000	28.000	36.000	36.000	27.000	29.000	40.000	30.000	29.000
80	28.000	22.000	34.000	21.000	32.000	36.000	13.000	32.000	36.000	39.000
100	32.000	23.000	34.000	32.000	14.000	34.000	25.000	34.000	7.000	27.000

Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
Upstr Control	24.200	1.0000	24.200	3.000	36.000	35.855	10			27.660	1.0000
20	25.700	1.0620	25.700	2.000	37.000	44.405	10	114.50	75.00	27.660	1.0000
40	26.300	1.0868	26.300	8.000	34.000	29.126	10	116.50	75.00	27.660	1.0000
60	32.800	1.3554	32.800	27.000	40.000	14.286	10	143.00	75.00	27.660	1.0000
80	29.300	1.2107	29.300	13.000	39.000	28.100	10	120.00	75.00	27.660	1.0000
100	26.200	1.0826	26.200	7.000	34.000	35.523	10	116.00	75.00	26.200	0.9472

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution ($p \leq 0.05$)	1.07487	0.895	-1.1488	1.00399
Bartlett's Test indicates equal variances ($p = 0.26$)	6.45417	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Upstr Control				

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL	Skew
IC05	98.945			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



Appendix C

Parameter Data

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

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001 Permit No.: 2PK00002*JD
 Type of Test: Chronic Date of Test: 7/12/16 - 7/19/16

1st Sample Collection

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

	100% Effluent	Upstream
Temperature (°C)	25.0	25.0
Dissolved Oxygen (mg/L)	6.7	6.7
pH (s.u.)	6.83	7.03
Conductivity (µmhos)	737	1122
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light tan	light tan

2nd Sample Collection

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

	100% Effluent	Upstream
Temperature (°C)	24.4	25.6
Dissolved Oxygen (mg/L)	6.7	6.6
pH (s.u.)	7.03	7.38
Conductivity (µmhos)	746	1562
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light stain

3rd Sample Collection

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

	100% Effluent	Upstream
Temperature (°C)	24.4	25.5
Dissolved Oxygen (mg/L)	6.5	6.5
pH (s.u.)	6.86	7.20
Conductivity (µmhos)	745	1428
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light stain	light stain

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

Solution Tested: Lab Control
 Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: BG, SS, RFR
 Beginning Date: 7/12/16 Time: 1315
 Ending Date: 7/19/16 Time: 1315
 Test Organism: Pimephales promelas Age: <24 Hours
 Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
		New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	
Temperature (°C)	6.3	25.2	25.1	25.5	25.4	25.4	24.6 to 24.4	25.4	25.4 to 26.0	25.4	25.0 to 25.9	25.2	25.3 to 25.6	25.2
Dissolved Oxygen (mg/L)	8.4	8.3	5.8	8.1	6.6	8.3	5.7	8.0	5.8	8.1	6.6	8.1	6.1	6.7
pH (s.u.)	7.23	7.22	7.08	7.33	7.13	7.18	6.93	7.36	6.96	7.27	7.15	7.00	7.06	7.00
Conductivity (µmho/cm)	327	328	346	323	344	324	343	308	332	307	328	308	325	331
Hardness (mg/L CaCO ₃)	120	-	-	120	-	-	-	120	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	68	-	-	68	-	-	-	68	-	-	-	-	-	-

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

Solution Tested: Upstream Control
 Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: BG, SS, RFR
 Beginning Date: 7/12/16 Time: 1315
 Ending Date: 7/19/16 Time: 1315
 Test Organism: Pimephales promelas Age: < 24 Hours
 Dilution Water Used: Upstream

Day	0		1		2		3		4		5		6		7	
Solution	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Temperature (°C)	25.4	25.1	25.4	25.8	25.5	25.4	25.4	24.6	25.4	25.4	25.7	25.9	25.7	25.5	25.7	25.6
Dissolved Oxygen (mg/L)	8.2	6.6	8.3	5.4	8.1	5.2	8.5	5.2	7.8	5.9	8.0	6.5	8.6	5.8	6.5	6.5
pH (s.u.)	7.59	7.31	7.41	7.47	7.46	7.30	7.58	7.30	7.53	7.31	7.57	7.03	7.46	7.13	7.01	7.01
Conductivity (µmho/cm)	1120	1121	1121	1133	1560	1577	1559	1577	1423	1568	1421	1415	1419	1468	1496	1496
Hardness (mg/L CaCO ₃)	376	-	-	-	445	-	-	-	445	-	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	188	-	-	-	171	-	-	-	171	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Solution Tested: 20% Effluent

Industry/Toxicant: Allen County, Shawnee No 2 WWTTP

Address: 3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: BG, SS, RFR

Beginning Date:

Ending Date:

Test Organism: *Pimephales promelas* Age: ≤ 24 Hours

Dilution Water Used: Upstream

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Address: 3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: BG, SS, RFR

Beginning Date:

Ending Date:

Test Organism: Pimentales promelas

Dilution Water Used: Upstream

Time: 13:5

Time: 1312

Age: 74 Years

[illegible]

PHYSICAL-CHEMICAL DATA

Person(s) conducting Test: BG, SS, RFR

Beginning Date: 7/12/16

Ending Date: 7/19/16

2PK00002*JD

Test Organism: Pimephales promelas

Dilution Water Used: Upstream

[illegible]

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

Solution Tested: 100% Effluent
 Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: BG, SS, RFR
 Beginning Date: 7/12/16 Time: 1315
 Ending Date: 7/19/16 Time: 1315
 Test Organism: Pimephales promelas Age: < 24 Hours
 Dilution Water Used: Upstream

Day	0		1		2		3		4		5		6		7	
Solution	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Temperature (°C)	25.4	25.7 + 25.9	25.4	25.4 + 25.8	25.3	24.6 24.9	25.4	25.4 + 26.0	25.4	25.4 + 26.0	25.4	25.0 + 25.9	26.5	25.5 + 25.8	25.2	25.6
Dissolved Oxygen (mg/L)	8.1	8.2	8.2	8.2	8.4	5.6	7.1	5.8	7.1	5.8	7.1	5.7	8.0	5.4	6.3	
pH (s.u.)	7.20	7.45	7.53	7.21	7.49	7.51	7.53	7.10	7.51	7.25	7.45	7.41	7.43	7.35		
Conductivity (µmho/cm)	750	740	762	744	782	748	802	745	782	780	781	744	788	795		
Hardness (mg/L CaCO ₃)	257	-	-	291	-	-	-	257	-	-	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	188	-	-	171	-	-	-	205	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Solution Tested: Lab Control

Industry/Toxicant: Allen County, Shawnee No 2 WWTB

Address: 3640 Spencerville Road, Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: RFR BG SS

Beginning Date: 7/12/2016 Time: 15:13

Ending Date: 7/18/16 Time: 1513

Test Organism: *Ceriodaphnia dubia*

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	
Temperature (°C)		25.4	25.6	24.9 to 25.4	25.8	25.2 to 25.6	25.3	25.1 to 25.5	25.4	25.1 to 25.4	25.2	25.1 to 25.4	-	25.0 to 25.4	-
Dissolved Oxygen (mg/L)		8.3	8.3	7.8	8.3	7.8	8.5	7.8	8.0	7.7	7.8	7.7		7.9	
pH (s.u.)		7.42	7.28	7.48	7.55	6.94	7.39	7.48	7.47	7.45	7.40	7.39		7.59	
Conductivity (µmho/cm)		330	336	333	334	336	333	344	326	341	324	349		761 768 768	
Hardness (mg/L CaCO ₃)		103	-	-	120	-	-	-	120	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)		120	-	-	103	-	-	-	103	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
 Industry/Toxicant: Allen County, Shawnee No. 2 WWT
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: RFR, BG, SS
 Beginning Date: 7/12/2016 Time: 1513
 Ending Date: 7/18/16 Time: 1510
 Test Organism: Ceriodaphnia dubia Age: 13-21H
 Dilution Water Used: Upstream

Day	0		1		2		3		4		5		6		7	
	Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Temperature (°C)	25.4	25.4	25.4	24.9 to 25.4	25.5	25.2 to 25.6	25.4	25.1 to 25.5	25.4	25.1 to 25.4	25.5	25.1 to 25.4	-	25.0 to 25.4	-	-
Dissolved Oxygen (mg/L)	8.2	8.3	7.8		8.1	7.8	8.5	7.7	7.8	7.5	8.0	7.5		8.0		
pH (s.u.)	7.55	7.31	7.56		7.46	7.56	7.58	7.62	7.53	7.73	7.57	7.63		7.64		
Conductivity (µmho/cm)	1120	1121	1106		1560	1113	1559	1562	1423	1561	1421	1434		912		
Hardness (mg/L CaCO ₃)	376	-	-		445	-	-	-	445	-	-	-		-	-	-
Alkalinity (mg/L CaCO ₃)	188	-	-		171	-	-	-	171	-	-	-		-	-	-

PHYSICAL-CHEMICAL DATA

Person(s) conducting Test: RFR, BG, SS

Time: 1513

Time: 1811

Age: 13-21

Emerging Date: 11/21/11
Test Organism: Ceriodaphnia dubia

$$\text{Ans. } \underline{17411}$$
[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 40% Effluent

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Address: 3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: RFR, BG, SS

Beginning Date: 7/12/2016

Ending Date: 7/18/16

Test Organism: *Ceriodaphnia dubia*

Dilution Water Used: Upstream

Time: 1513

Time: 7510

Age: 13-21 M

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP

Address: 3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PK00002*JD

Person(s) conducting Test: RFR, BG, SS

Beginning Date:

Ending Date: 7/18/16

Test Organism: *Ceriodaphnia dubia*

Dilution Water Used: _____

Time: 1513

Time: 1510

Age: 13-21 yr

[illegible]

PHYSICAL-CHEMICAL DATA

Person(s) conducting Test: RFR, BG, SS

Beginning Date: 7/12/2016

Ending Date: 7/18/16

Test Organism: Ceriodaphnia dubia

Dilution Water Used: _____ Upstream

Time: 1510

100

[illegible]

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
 Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
 Address: 3640 Spencerville Road, Lima, OH 45805
 Effluent Serial No.: 001
 NPDES Permit No.: 2PK00002*ID

Person(s) conducting Test: RFR, BG, SS
 Beginning Date: 7/12/2016
 Ending Date: 7/18/16
 Test Organism: Ceriodaphnia dubia
 Dilution Water Used: Upstream

Time: 1513
 Time: 1516
 Age: 13-21 H

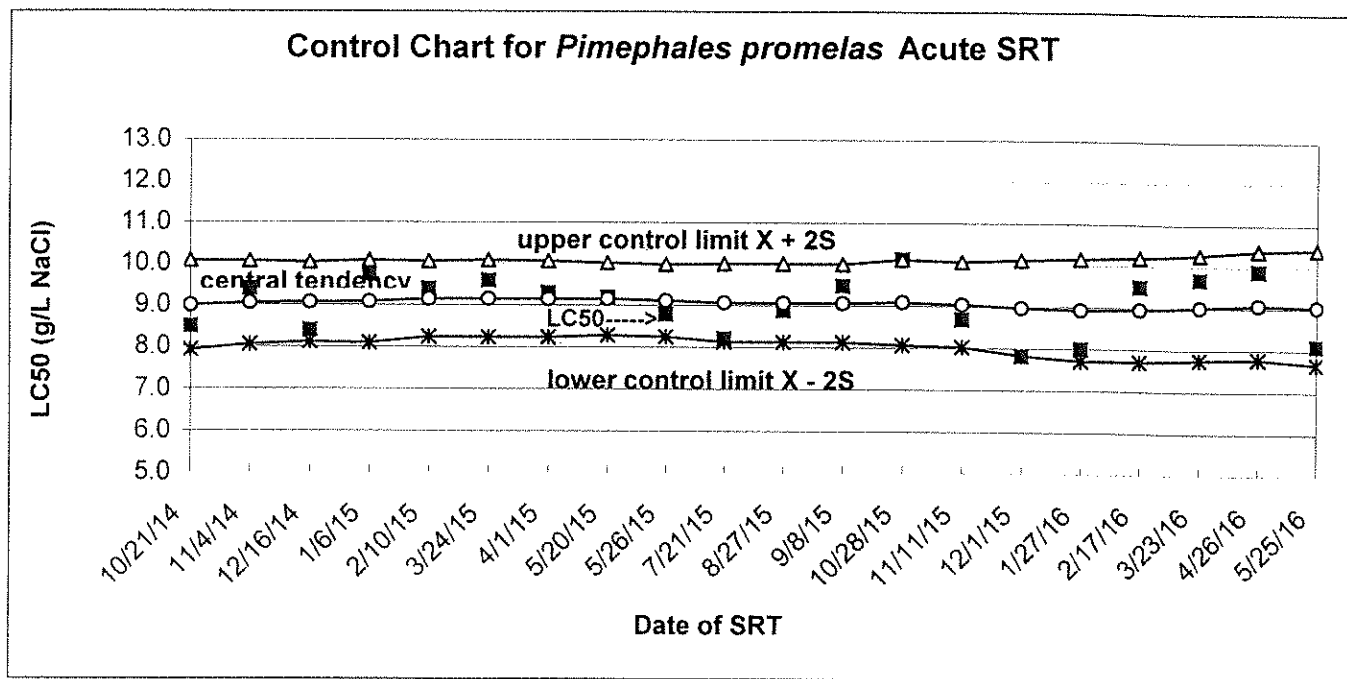
Day	0		1		2		3		4		5		6		7	
	Solution	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.4	24.9 to 25.4	25.5	25.2 to 25.6	25.3	25.1 to 25.5	25.4	28.1 to 25.4	25.4	25.1 to 25.4	25.4	25.0 to 25.4	-	25.4	-
Dissolved Oxygen (mg/L)	8.1	8.2	8.1	7.4	7.8	8.4	7.9	7.1	7.8	7.6	7.7	7.6	8.0			
pH (s.u.)	7.26	7.45	7.70	7.21	7.77	7.54	7.75	7.16	7.85	7.29	7.79	7.14	7.64			
Conductivity (µmho/cm)	738	740	761	744	758	748	783	745	780	743	781	11454	11454			
Hardness (mg/L CaCO ₃)	257	-	-	291	-	-	-	257	-	-	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	188	-	-	171	-	-	-	205	-	-	-	-	-	-	-	-

Appendix D

SRT Data

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

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



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

75th percentile of CVs for LC50 = 0.19

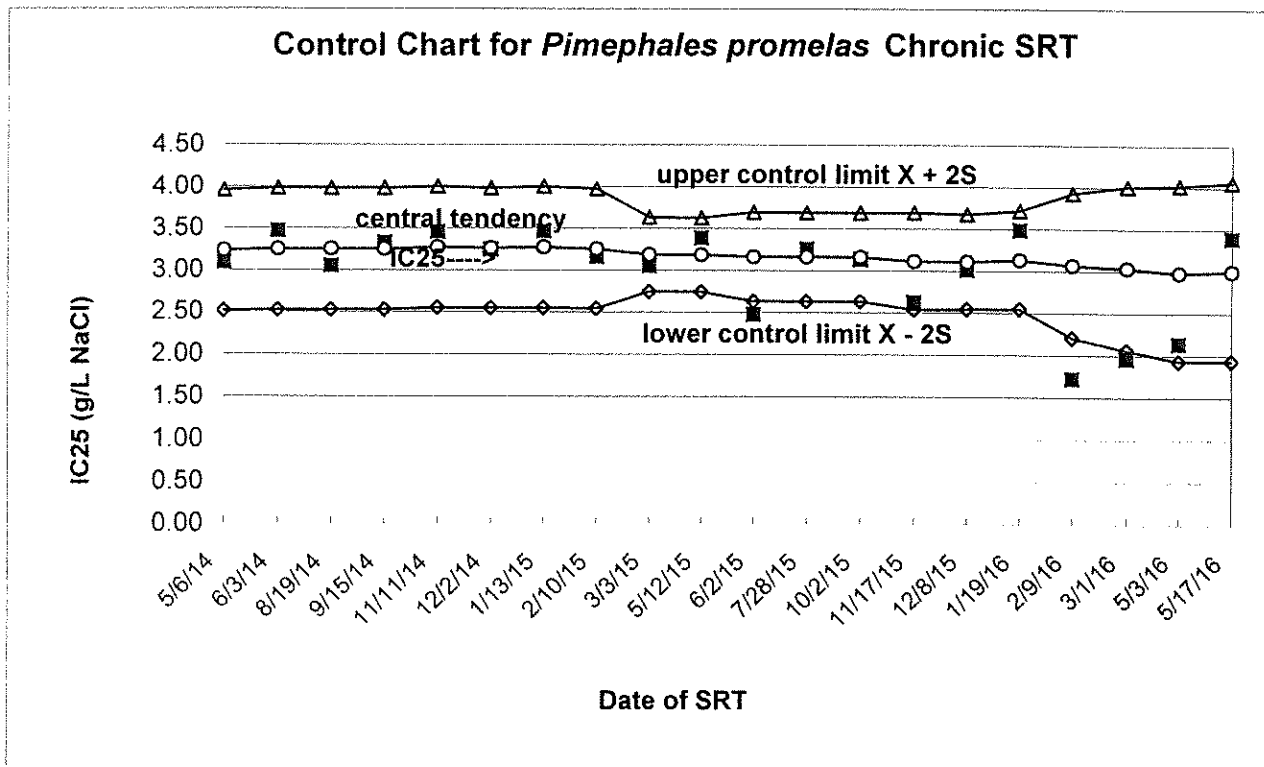
Calculated CV should not exceed 75th percentile of CVs.

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

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

IC25 for *Pimephales promelas* Chronic NaCl SRT

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



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

75th percentile of CVs for IC25 = 0.38

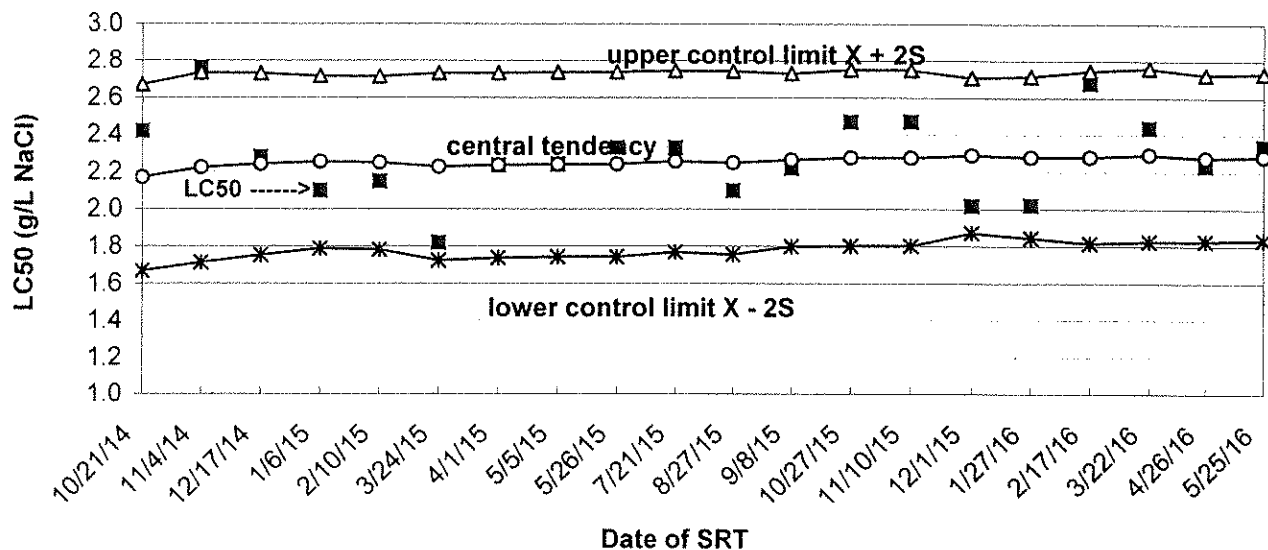
Calculated CV should not exceed 75th percentile of CVs.

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

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

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

Control Chart for *Ceriodaphnia dubia* Acute SRT



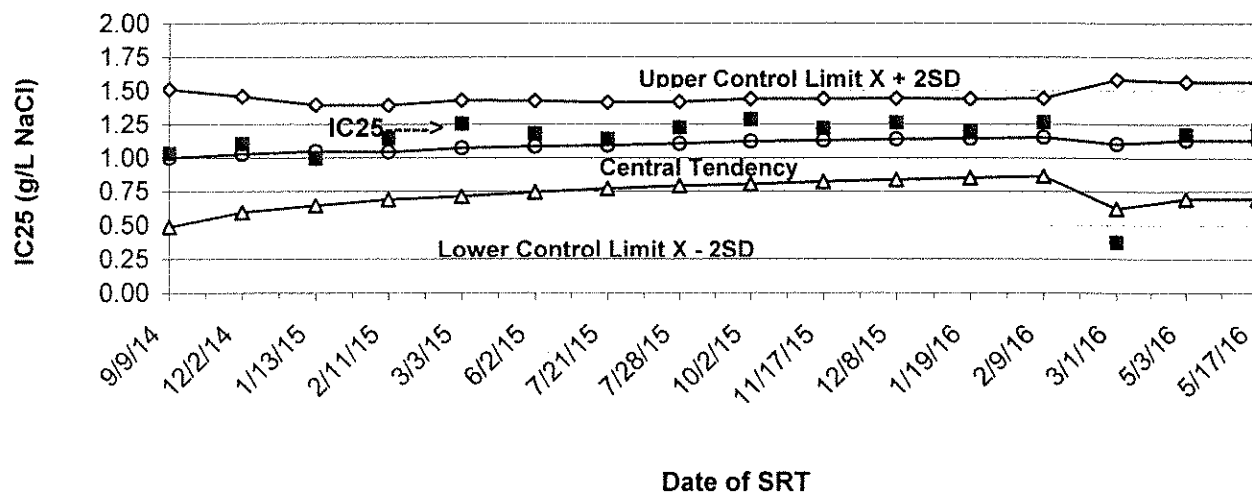
*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
 75th percentile of CVs for LC50 = 0.29
 Calculated CV should not exceed 75th percentile of CVs.
 $\bar{X} \pm 3SD$ = Well outside expected range.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

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

Control Chart for *Ceriodaphnia dubia* Chronic SRT



*- 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.

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