

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Report Date: 8/1/19
Ohio EPA Permit No.: 2PK00002*JD
Alloway Lab #: L19-16892-01

Report of Chronic Biomonitoring

Samples Collected on:

7/15/19
7/17/19
7/19/19

Pimephales promelas
Ceriodaphnia dubia

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



Alloway
Your Resource for Defensible Data

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Outfall: Effluent 001
Report Date: 8/1/19
Ohio EPA Permit No.: 2PK00002*JD
Alloway Lab #: L19-16892-01

Date: 8/1/19

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.


Brian Good
Bioassay Lab Manager

Outfall: Effluent 001
Report Date: 8/1/19
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	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>6/19/2017</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.

ML Santaguida
Signature

Mike Santaguida
Name (typed or printed)

8/9/2019
Date

Superintendent
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 *P. promelas* Test: 7/15-22/19

Date of *C. dubia* Test: 7/15-22/19

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	2.5	2.5	
7 day Chronic Toxicity, <i>Pimephales promelas</i> - % Affected	5.0	2.5	

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:

Unacceptable *Pimephales promelas* survival was observed in the secondary lab control. Mortality cannot exceed 20% in a control. The associated results were not affected.

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*JD
Date of Test: 7/15-22/19

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%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.686	0.124
Secondary Control Lab		0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (5.0%)	12.5% (15.0%)	27.5% (27.5%)	0.370	0.121
20%		0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	0.643	0.130
40%		2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	7.5% (7.5%)	0.596	0.068
60%		0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.604	0.052
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%)	0.642	0.023
100%		0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	0.624	0.123
LC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	Calculated TUC for Survival 	

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

Chronic Value = square root of (NOEC x LOEC)

COMMENTS:

Unacceptable *Pimephales promelas* survival was observed in the secondary lab control. Mortality cannot exceed 20% in a control. The associated results were not affected.

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*JD
Date of Test: 7/15-22/19

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%)	0.0% (0.0%)	32.3
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%)	28.9
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%)	32.4
40%		0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	29.7
60%		0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	28.6
80%		0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	27.7
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%)	0.0% (0.0%)	26.5
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 Test
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)

COMMENTS:

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
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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	7/14/19 @ 10:30	7/15/19 @ 10:30
Type (Grab/Comp):	Comp	2nd	7/16/19 @ 10:30	7/17/19 @ 10:30
Volume Collected:	5 Gal.	3rd	7/18/19 @ 10:30	7/19/19 @ 10:30
Upstream Station:				
Waterbody:	Ottawa River			
Station No.:	801	1st		7/15/19 @ 11:34
Type (Grab/Comp):	Grab	2nd		7/17/19 @ 11:31
Volume Collected:	3.5 to 5 Gal.	3rd		7/19/19 @ 11:20

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:	7/15-22/19
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 - 7/15/19 ; day 2, 3 - 7/17/19 ; day 4, 5, 6 - 7/19/19
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> <24 hours (8H window)
2. Test Type and Duration:	Static renewal; Until 60% or more of surviving control females have three broods (maximum test duration 8 days)
3. Test Dates:	7/15-22/19
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 - 7/15/19 ; day 2, 3 - 7/17/19 ; day 4, 5, 6 - 7/19/19
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data



Alloway®
Your Resource for Defensible Data

Chain of Custody Record

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

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

14906

Project: L19-16892

Report To: Rick Boerger Name: Allen County Sanitary Engineer Company: 3226 North Cole St. Address: Lima, OH 45805		Invoice To (if Different): Name: Company: Address:	
Phone #: 419-225-8048		Fax #:	
E-mail: rboerger@allencountyohio.com		PO#:	
Project Name Bioassay - Shawnee II - DAY 1			
Sampler	Customer Sample ID / Sample Location	Sample Date	Sample Time
1	Effluent	7/14/19	10:30
2	Upstream	7/15/19	11:34
3			
4			
5			
6			
7			
8			
Relinquished by:		Received by:	Date
1			
2			
3			
4			
5			
6			
Received for Laboratory By: (circle one): Mansfield <u>Lima</u> Marion		Received By:	
(Signature)		Received By:	
Transported to: Lima Marion		Received By: By:	
Transported to: Lima Marion		Received By: By:	
Date: _____		Time: _____	
Date: _____		Time: _____	

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	7/14/19	10:30	X		WW	1	1	Toxicity	
2	Upstream	7/15/19	11:34		X	SW	1	1	Toxicity	
3										
4										
5										
6										
7										
8										

Preservation Codes:	Matrix Codes:	Method of Delivery	Sample Receiving (For Lab Use Only)
1 - None	WW - wastewater	UPS ☐	Ice Present? ☒ N ☐
2 - HNO ₃	GW - groundwater	Fed Ex ☐	Proper Preservation? ☒ N ☐
3 - H ₂ SO ₄	SW - drinking water	Client ☐	Container Temperature: 22°C
4 - HCl	SW - surface water	Alloway Pick Up ☐	
5 - NaOH	W - water	Alloway Sampling ☒	
6 - NaOH & Zinc Acetate	Oil - oil	Other ☐	
7 - Sodium Thiosulfate	Sg - sludge		
8 - Ascorbic Acid	l - leachate		
9 - Maleic Acid	a - acid		
10 - EDA	p - product		
11 - Ammonium Chloride	o - other		
12 - (NH ₄) ₂ SO ₄ & NH ₄ OH			
13 - Zinc Acetate			
14 - Sodium Sulfite			
15 - Potassium Dihydrogen Citrate			
16 - Sodium Sulfite/Sodium Bisulfite			



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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
- 1502 W. Fourth Street, Mansfield, OH 44906
- (P) 419-525-1644 (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 E-mail: rboerger@allencountyohio.com		Fax #:			
Project Name: Bioassay - Shawnee II - DAY 2		PO#:			
Sampler	Customer Sample ID / Sample Location	Sample Date	Sample Time (Signature)	Grab	Composite
1	Effluent	7/17/19	10:30-10:30		X
2	Upstream	7/17/19	11:31	X	
3					
4					
5					
6					
7					
8					
Relinquished by:		Received by:	Date	Time	Method of Delivery
1					UPS ☐
2					Fed Ex ☐
3					Client ☐
4					Alloway Pick Up ☐
5					Alloway Sampling ☒
6					Other ☐
Received for Laboratory By: (circle one): Mansfield ☐ Marion ☒		Date		Time	Method of Delivery
(Signature)		7/17/19			
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 ₂ SO ₄ 4 - HCl 5 - NaOH 6 - NaOH & Zinc Acetate 7 - Sodium Thiosulfate 8 - Ascorbic Acid 9 - Maleic Acid 10 - EDTA 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: 11°C T-126	
Turnaround: (Rush Charges May Apply) Next Day ☐ 2 Working Days ☐ 3 Working Days ☐ 5 Working Days ☒ Routine ☒		Analysis Required Toxicity Toxicity		Alloway LIMS # For Lab Use Only	

Transported to: Lima
By: Marion

Received By:

Date:

Time:

Transported to: Lima
By: Marion

Received By:

Date:

Time:



Chain of Custody Record

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- ☐ 1502 W. Fourth Street, Mansfield, OH 44806
- ☐ (P) 419-525-1644 (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 Email: rboerger@allencountyohio.com		Fax #:													
Project Name: Bioassay - Shawnee II - DAY 3		PO#:													
Sampler	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	2 Working Days ☐ 3 Working Days ☐ 5 Working Days ☐ Routine ☒	Analysis Required	Alloway LIMS # For Lab Use Only				
1	Effluent	7/18/19	10:30	X		WW	1	1		Toxicity					
2	Upstream	7/19/19	11:20		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		1 - None		7 - Sodium Thiosulfate		13 - Zinc Acetate	
2								☐ Fed Ex		2 - HNO ₃		8 - Ascorbic Acid Sulfite		14 - Sodium Sulfite	
3								☐ Client		3 - H ₂ SO ₄		9 - Maleic Acid Dihydrogen Citrate		15 - Potassium Bisulfate	
4								☐ Alloway Pick Up		4 - HCl		10 - EDTA		16 - Sodium Sulfite/Sodium Bisulfate	
5								☒ Alloway Sampling		5 - NaOH		11 - Ammonium Chloride		Proper Preservation? ☒ N ☐	
6								☐ Other		6 - NaOH & Zinc Acetate		12 - (NH ₄) ₂ SO ₄ & NH ₄ OH		Container Temperature: 21°C T-120 NDM	
Received for Laboratory By: (circle one):		Mansfield		Lima		Marion									
(Signature)															

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

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, NDM, CBS, EMS
Beginning: Date 7/15/19 Time 1400
Ending: Date 7/22/19 Time 1614
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	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 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: BG, NDM, CBS, EMS
Beginning: Date 7/15/19 Time 1600
Ending: Date 7/22/19 Time 1614
Age of *Pimephales promelas*: < 24 Hours
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
start	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	10G	10G	40G
7	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:

7/25/19

Pimephales promelas Age at Start: < 24 Hours

Analyst: NDM

Date of Test: 7/16/19 - 7/23/19

Balance Calibration Check: 1 gram weight = 0.99999 grams

Date: 7/10/19

Analyst: NDM

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	1.09884	1.09949	10	0.065	0.0065
Lab Control	1	9	1.09674	1.09866	10	1.92	0.192
	2	7	1.09736	1.10199	10	4.63	0.463
	3	7	1.10299	1.10709	10	0.47410	0.47410
	4	6	1.08898	1.09313	10	9.15	0.415
Upstream Control	1	10	1.12477	1.13236	10	7.59	0.759
	2	10	1.10463	1.11226	10	7.63	0.763
	3	10	1.09451	1.10169	10	7.18	0.718
	4	8	1.13377	1.13879	10	5.02	0.502
20% Effluent	1	10	1.12595	1.13266	10	6.71	0.671
	2	10	1.09302	1.09957	10	6.55	0.655
	3	9	1.10103	1.10570	10	4.67	0.467
	4	10	1.07920	1.08699	10	7.79	0.779
40% Effluent	1	9	1.08518	1.09106	10	5.88	0.588
	2	10	1.10360	1.11000	10	6.40	0.640
	3	8	1.09322	1.09824	10	5.02	0.502
	4	10	1.12792	1.13445	10	6.53	0.653

Reviewed By: 8/1/19

W

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

Pimephales promelas Age at Start: < 24 Hours

Date of Test:

7/18/19 - 7/23/19

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	10	1.12914	1.13554	10	6.40	0.640
	2	10	1.08626	1.09233	10	6.57	0.657
	3	9	1.13232	1.13783	10	5.56	0.556
	4	9	1.10697	1.11261	10	5.64	0.564
80% Effluent	1	10	1.12395	1.13009	10	6.14	0.614
	2	10	1.08962	1.09608	10	6.46	0.646
	3	10	1.09717	1.10386	10	6.69	0.669
	4	9	1.08341	1.08917	10	5.76	0.576
100% Effluent	1	9	1.09971	1.10579	10	5.76	0.576
	2	8	1.09877	1.10349	10	4.12	0.412
	3	10	1.09788	1.10436	10	6.48	0.648
	4	10	1.08160	1.09430	10	7.70	0.770
	1				10		
	2				10		
	3				10		
	4				10		

0.556 0.6

0.640 0.6

0.608 0.6

0.472 0.6

B6 8/1/19

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 7/15/2019 Test ID: Shaw0719p Sample ID: L19-16892-01
 End Date: 7/22/2019 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	0.7590	0.7630	0.7180	0.5020
20	0.6710	0.6550	0.4670	0.7790
40	0.5880	0.6400	0.5020	0.6530
60	0.6400	0.6570	0.5560	0.5640
80	0.6140	0.6460	0.6690	0.6400
100	0.6080	0.4720	0.6480	0.7700

Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Upstr Control	0.6855	1.0000	0.6855	0.5020	0.7630	18.091	4				0.6855	1.0000
20	0.6430	0.9380	0.6430	0.4670	0.7790	20.158	4	0.627	2.410	0.1634	0.6430	0.9380
40	0.5958	0.8691	0.5958	0.5020	0.6530	11.501	4	1.324	2.410	0.1634	0.6167	0.8996
60	0.6043	0.8815	0.6043	0.5560	0.6570	8.551	4	1.199	2.410	0.1634	0.6167	0.8996
80	0.6423	0.9369	0.6423	0.6140	0.6690	3.519	4	0.638	2.410	0.1634	0.6167	0.8996
100	0.6245	0.9110	0.6245	0.4720	0.7700	19.666	4	0.900	2.410	0.1634	0.6167	0.8996

Auxiliary Tests

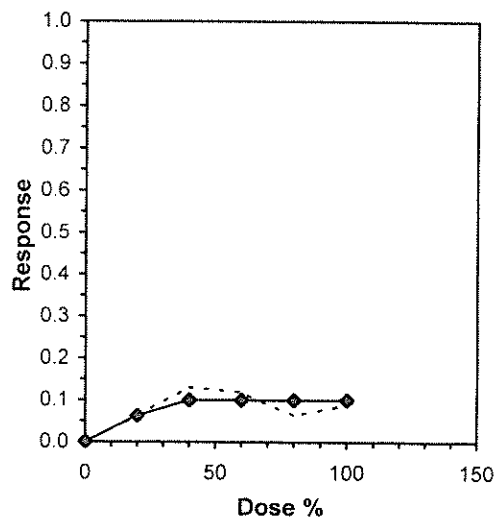
Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.05$)	0.92907	0.916	-0.7148
Bartlett's Test indicates equal variances ($p = 0.13$)	8.45491	15.0863	0.49741

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.16336	0.23831	0.00418	0.00919	0.80423	5, 18
Treatments vs Upstr Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	16.129			
IC10	39.800			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

* indicates IC estimate less than the lowest concentration



CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control Person(s) Conducting Test: BG, NDM, CBS, EMS
 Industry/Toxicant: Allen County, Shawnee No. 2 WWTP Beginning Date: 7/15/19 Time: 1651
 Address: 3640 Spencerville Road, Lima, OH 45805 Ending Date: 7/22/19 Time: 1700/1140
 Effluent Serial No.: 001 Age of *Ceriodaphnia dubia*: 224H ECT
 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	0 / G	4 / G	6 / G	9 / G	6 / G	19	3
2	/	/	/	/	7 /	12 /	14 /	/	33	3
3	/	/	/	/	4 /	8 /	2 /	13 /	27	3
4	/	/	/	/	5 /	11 /	16 /	/	32	3
5	/	/	/	/	6 /	10 /	12 /	/	28	3
6	/	/	/	/	5 /	11 /	13 /	/	29	3
7	/	/	/	/	6 /	10 /	14 /	/	30	3
8	/	/	/	/	5 /	10 /	16 /	/	31	3
9	/	/	/	/	6 /	8 /	14 /	/	28	3
10	0 / G	0 / G	0 / G	0 / G	6 / G	12 / G	2 / G	12 / G	32	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
									SUM	
									289	30
									MEAN	
									28.9	3.0
									S.D.	
									4.0	0.0

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

Reviewed by: NDM 8/1/19

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>Upstream Control</u>	Person(s) Conducting Test: <u>BG, NDM, CBS, EMS</u>
Industry/Toxicant: <u>Allen County, Shawnee No. 2 WWTP</u>	Beginning Date: <u>7/15/19</u> Time: <u>1651</u>
Address: <u>3640 Spencerville Road, Lima, OH 45805</u>	Ending Date: <u>7/21/19</u> Time: <u>1140</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>424H</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	0 / G	6 / G	9 / G	13 / G	0 / G	28	3
2	0 / G	0 / G	0 / G	0 / G	5 / G	14 / G	16 / G	0 / G	35	3
3	0 / G	0 / G	0 / G	0 / G	6 / G	10 / G	17 / G	0 / G	33	3
4	0 / G	0 / G	0 / G	0 / G	4 / G	8 / G	14 / G	0 / G	26	3
5	0 / G	0 / G	0 / G	0 / G	5 / G	13 / G	15 / G	0 / G	33	3
6	0 / G	0 / G	0 / G	0 / G	5 / G	7 / G	13 / G	0 / G	25	3
7	0 / G	0 / G	0 / G	0 / G	5 / G	10 / G	13 / G	0 / G	28	3
8	0 / G	0 / G	0 / G	0 / G	0 / G	15 / G	21 / G	0 / G	36	2
9	0 / G	0 / G	0 / G	0 / G	6 / G	15 / G	16 / G	0 / G	37	3
10	0 / G	0 / G	0 / G	0 / G	6 / G	10 / G	0 / G	20 / G	42	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
									SUM	
									323	29
									MEAN	
									32.3	2.9
									S.D.	
									5.4	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: <u>20% Effluent</u>	Person(s) Conducting Test: <u>BG, NDM, CBS, EMS</u>
Industry/Toxicant: <u>Allen County, Shawnee No. 2 WWTP</u>	Beginning Date: <u>7/15/19</u> Time: <u>1651</u>
Address: <u>3640 Spencerville Road, Lima, OH 45805</u>	Ending Date: <u>7/21/19</u> Time: <u>1140</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>24H</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	0 / G	4 / G	9 / G	13 / G	/ G	26	3
2	/	/	/	/	7 /	14 /	17 /	/	38	3
3	/	/	/	/	6 /	18 /	18 /	/	42	3
4	/	/	/	/	5 /	13 /	16 /	/	34	3
5	/	/	/	/	6 /	12 /	16 /	/	34	3
6	/	/	/	/	1 /	10 /	17 /	/	28	3
7	/	/	/	/	4 /	10 /	14 /	/	28	3
8	/	/	/	/	6 /	11 /	15 /	/	32	3
9	/	/	/	/	4 /	9 /	17 /	/	30	3
10	0 / G	0 / G	0 / G	0 / G	4 / G	14 / G	17 / G	/ G	32	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
									SUM	
									324	30
									MEAN	
									32.4	3.0
									S.D.	
									4.9	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: 40% Effluent

Person(s) Conducting Test: BG, NDM, CBS, EMS

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

Beginning Date: 7/15/19

Time: 1651

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/21/19

Time: 1140

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 24H

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	0/G	5/G	14/G	18/G	/G	37	3
2	/	/	/	/	5	13	14/G	/G	34	3
3	/	/	/	/	5	14	12/D	/-	31	3
4	/	/	/	/	4	11	12/G	/G	27	3
5	/	/	/	/	6	9	13	/	28	3
6	/	/	/	/	6	9	15	/	30	3
7	/	/	/	/	Q5	10	16	/	31	3
8	/	/	/	/	20	11	10	/	21	2
9	/	/	/	/	52	8	7	4	21	3
10	0/G	0/G	0/G	0/G	5/G	14/G	18/G	/G	37	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	96 10	96 10		
SUM									297	29
MEAN									29.7	2.9
S.D.									5.7	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: 60% Effluent

Person(s) Conducting Test: BG, NDM, CBS, EMS

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

Beginning Date: 7/15/19

Time: 1651

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/22/19

Time: 1140

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 424H

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	0 / G	4 / G	10 / G	18 / G	0 / G	32	3
2	0 / G	0 / G	0 / G	0 / G	6 / G	9 / G	16 / G	0 / G	31	3
3	0 / G	0 / G	0 / G	0 / G	5 / G	10 / G	18 / G	0 / G	33	3
4	0 / G	0 / G	0 / G	0 / G	5 / G	10 / G	16 / G	0 / G	31	3
5	0 / G	0 / G	0 / G	0 / G	5 / G	11 / G	13 / G	0 / G	29	3
6	0 / G	0 / G	0 / G	0 / G	5 / G	13 / G	16 / G	0 / G	34	3
7	0 / G	0 / G	0 / G	0 / G	4 / G	9 / G	15 / G	0 / G	28	3
8	0 / G	0 / G	0 / G	0 / G	3 / G	12 / G	20 / G	0 / G	35	3
9	0 / D	0 / D	0 / D	0 / D	- / -	- / -	- / -	- / -	0	0
10	0 / G	0 / G	0 / G	0 / G	5 / G	9 / G	0 / G	19 / G	33	3
Total Adult Cond.	10G	10G	10G	9G 1D	9G 1D	9G 1D	9G 1D	9G 1D		
SUM									286	27
MEAN									28.6	2.7
S.D.									10.3	0.9

* 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

Person(s) Conducting Test: BG, NDM, CBS, EMS

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

Beginning Date: 7/15/19

Time: 1651

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/22/19

Time: 1140

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 224H

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	0/G	4/G	8/G	0/G	16/G	28	3
2	/	/	/	/	4/G	9/G	14/G	/	27	3
3	/	/	/	/	5/G	14/G	21/G	/	40	3
4	/	/	/	/	5/G	10/G	19/G	/	34	3
5	/	/	/	/G	5/G	9/G	16/G	/G	30	3
6	/	/	/	D	-	-	-	-	0	0
7	/	/	/	/G	6/G	8/G	18/G	/G	32	3
8	/	/	/	/	0/G	8/G	16/G	/G	24	2
9	/	/	/	/	5/G	11/G	16/G	/G	32	3
10	0/G	0/G	0/G	0/G	6/G	9/G	11/G	4/G	30	3
Total Adult Cond.	10G	10G	10G	9G 1D	9G 1D	9G 1D	9G 1D	9G 1D		
SUM									277	24
MEAN									27.7	2.6
S.D.									10.6	1.0

* 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: 100% Effluent

Person(s) Conducting Test: BG, NDM, CBS, EMS

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

Beginning Date: 7/15/19

Time: 1651

Address: 3640 Spencerville Road, Lima, OH 45805

Ending Date: 7/22/19

Time: 1140

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 42 hr

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	0 / G	6 / G	10 / G	15 / G	/ G	31	3
2	/	/	/	/	6 /	11 /	17 /	/	34	3
3	/	/	/	/	6 /	10 /	15 /	/	31	3
4	/	/	/	/	4 /	9 /	11 /	/	24	3
5	/	/	/	/	5 /	11 /	14 /	/	30	3
6	/	/	/	/	4 /	7 /	10 /	/	21	3
7	/	/	/	/	3 /	9 /	12 /	/	24	3
8	/	/	/	/	4 /	17 /	10 /	14 /	29	3
9	/	/	/	/	3 /	10 /	1 /	11 /	25	3
10	0 / G	0 / G	0 / G	0 / G	4 / G	6 / G	1 / G	5 / G	16	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
SUM									265	30
MEAN									26.5	3.0
S.D.									5.5	0.0

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

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Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 7/15/2019 Test ID: Shaw0719c Sample ID: L19-16892-01
 End Date: 7/22/2019 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:

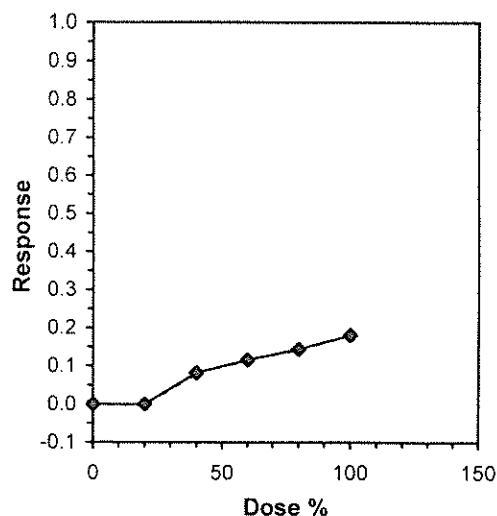
Conc-%	1	2	3	4	5	6	7	8	9	10
Upstr Control	28.000	35.000	33.000	26.000	33.000	25.000	28.000	36.000	37.000	42.000
20	26.000	38.000	42.000	34.000	34.000	28.000	28.000	32.000	30.000	32.000
40	37.000	34.000	31.000	27.000	28.000	30.000	31.000	21.000	21.000	37.000
60	32.000	31.000	33.000	31.000	29.000	34.000	28.000	35.000	0.000	33.000
80	28.000	27.000	40.000	34.000	30.000	0.000	32.000	24.000	32.000	30.000
100	31.000	34.000	31.000	24.000	30.000	21.000	24.000	29.000	25.000	16.000

Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
Upstr Control	32.300	1.0000	32.300	25.000	42.000	16.898	10			32.350	1.0000
20	32.400	1.0031	32.400	26.000	42.000	15.064	10	105.00	75.00	32.350	1.0000
40	29.700	0.9195	29.700	21.000	37.000	19.116	10	95.00	75.00	29.700	0.9181
60	28.600	0.8854	28.600	0.000	35.000	35.931	10	96.50	75.00	28.600	0.8841
80	27.700	0.8576	27.700	0.000	40.000	38.434	10	91.00	75.00	27.700	0.8563
100	26.500	0.8204	26.500	16.000	34.000	20.688	10	77.50	75.00	26.500	0.8192

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution ($p \leq 0.05$)					1.00154	0.895	-2.0179	6.53742
Bartlett's Test indicates equal variances ($p = 0.05$)					11.2201	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	32.208			
IC10	50.636			
IC15	83.375			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



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: 7/15/19 - 7/21/19
 Effluent Serial No.: 001 Permit No.: 2PK00002*JD
 Investigator: ECT Culture No.: ECT-LE₃
 Culture initiation date: _____ Culture ending date: _____
 Origin of parental organisms: _____
 Age at start of culture: _____
 Culture water renewal frequency: minimum 3 times per week Culture water: 1 L:9 MHSF
 Culture temperature: 25°C Fed Daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

Number of brood organisms at start of culture: _____
 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: _____

	<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>1</u>	<u>17</u>	<u>424 hours (8H window)</u>		<u>1</u>
2)	<u>2</u>	<u>11</u>			<u>2</u>
3)	<u>3</u>	<u>10</u>			<u>3</u>
4)	<u>4</u>	<u>17</u>			<u>4</u>
5)	<u>5</u>	<u>12</u>			<u>5</u>
6)	<u>6</u>	<u>15</u>			<u>6</u>
7)	<u>7</u>	<u>13</u>			<u>7</u>
8)	<u>8</u>	<u>17</u>			<u>8</u>
9)	<u>9</u>	<u>16</u>			<u>9</u>
10)	<u>10</u>	<u>15</u>	<u>424 H (8H window)</u>		<u>10</u>

COMMENTS: _____

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: 7/15/19 - 7/22/19

1st Sample Collection

Date Samples Collected: 7/15/19

Date Samples Read: 7/15/19

Analyst: EMS

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	20.9	25.7		
Dissolved Oxygen (mg/L)	6.5	6.5		
pH (s.u.)	7.18	7.75		
Conductivity (µmhos)	730	909		
Chlorine, total (mg/L)	20.02	20.02		
Visual Description	clear, light yellow tint	clear, light yellow tint		

2nd Sample Collection

Date Samples Collected: 7/17/19

Date Samples Read: 7/17/19

Analyst: EMS

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	12.3	23.4 23.4		
Dissolved Oxygen (mg/L)	7.1	6.2		
pH (s.u.)	7.17	7.48		
Conductivity (µmhos)	650	574		
Chlorine, total (mg/L)	20.02	20.02		
Visual Description	light yellow	dark yellow		

3rd Sample Collection

Date Samples Collected: 7/19/19

Date Samples Read: 7/19/19

Analyst: NDM

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	20.4	24.9		
Dissolved Oxygen (mg/L)	6.2	6.4		
pH (s.u.)	7.35	7.51		
Conductivity (µmhos)	701	674		
Chlorine, total (mg/L)	20.02			
Visual Description	med. yellow	dark yellow		

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, NDM, CBS, EMS
 Beginning Date: 7/15/19 Time: 1400
 Ending Date: 7/22/19 Time: 1614
 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	Old
Temperature (°C)	25.4 25.0	25.4 25.7	25.4	25.1 25.7	25.5	25.4	25.7	24.9 25.4	25.2	25.3 25.8	24.5	24.9 25.2	24.3	24.4 25.0	25.1 25.9
Dissolved Oxygen (mg/L)	6.7 7.9	6.7 7.9	6.7	5.5	7.3	2.2	7.5	6.1	6.4	4.3	7.0	4.8	7.7	5.3	4.4
pH (s.u.)	7.84 7.64	7.84 7.64	7.84	7.60	7.64	7.01	7.10	7.48	7.74	7.21	7.15	6.88	7.86	7.45	7.40
Conductivity (µmho/cm)	311 315	311 315	311	332	314	335	310	328	310	329	312	325	317	324	350
Hardness (mg/L CaCO ₃)	103	103	-	-	120	-	-	-	137	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	120	120	-	-	120	-	-	-	103	-	-	-	-	-	-

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, NDM, CBS, EMS

Beginning Date: 7/15/19

Time: 1400

Ending Date: 7/22/19

Time: 1614

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	Old
Temperature (°C)	25.8 25.4	25.4 25.7	25.8 25.7	25.4 25.7	25.3 25.7	25.1 25.7	25.0 25.7	24.9 25.4	25.1 25.8	25.3 25.8	25.0 25.2	24.9 25.2	25.0 25.0	24.4 25.0	25.1 25.9
Dissolved Oxygen (mg/L)	7.3 6.4	5.2	7.3	6.4	6.2 3.6	7.7	7.1	6.4	6.6	4.5	7.0	4.7	7.8	4.9	4.6
pH (s.u.)	7.82 7.79	7.67	7.82	7.67	7.38 7.00	7.54	7.54	7.61	7.49	7.50	7.70	7.04	7.27	7.71	7.66
Conductivity (µmho/cm)	968 972	956	968	956	575 971	575	575	588	677	575	674	665	671	669	688
Hardness (mg/L CaCO ₃)	342	-	-	-	239	-	-	-	342	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	205	-	-	-	171	-	-	-	171	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Person(s) conducting Test: BG, NDM, CBS, EMS

Beginning Date: 7/15/19 Time: 1600

Beginning Date: 7/15/19 Time: 1000
Ending Date: 7/22/19 Time: 1614

2PK00002*JD

Dilution Water Used: Upstream

[illegible]

PHYSICAL-CHEMICAL DATA

Person(s) conducting Test: BG, NDM, CBS, EMS

Beginning Date: 7/15/19 Time: 1000

Beginning Date: 7/13/19
Ending Date: 7/22/19
Time: 10:00
Time: 10:13

2PK00002*JD

Test Organism: Pimephales promelas

Dilution Water Used: Upstream

[illegible]

PHYSICAL-CHEMICAL DATA

Person(s) conducting Test: BG, NDM, CBS, EMS

Beginning Date: 7/15/19 Time: 1600

Beginning Date: 7/15/19 Time: 1000
Ending Date: 7/22/19 Time: 1614

2PK00002*JD

Test Organism: Pimephales promelas

Dilution Water Used: Upstream

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 80% 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, NDM, CBS, EMS

Beginning Date: 7/15/19

Ending Date: 7/22/19

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, NDM, CBS, EMS

Beginning Date: 7/15/19

Time: 1600

Ending Date: 7/22/19

Time: 1614

Test Organism: Pimephales promelas

Age: < 24 Hours

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)	25.8	25.8	25.4 25.7	25.9	25.1 25.7	25.4	24.9 25.4	25.4	25.3 25.8	25.4	24.9 25.2	25.4	24.4 25.0	25.1 25.9
Dissolved Oxygen (mg/L)	7.61	7.6	4.8	6.4	4.1	7.6	6.0	6.4	4.1	7.4	4.3	7.5	4.2	4.9
pH (s.u.)	7.71 7.37	7.71	7.81	7.36	7.69	7.57	7.81	7.47	7.74	7.58	7.62	7.96	7.87	7.84
Conductivity (µmho/cm)	729 731	729	752	650	745	653	687	709	664	708	712	704	714	723
Hardness (mg/L CaCO ₃)	239	-	-	257	-	-	-	239	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	205	-	-	188	-	-	-	239	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA 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, NDM, CBS, EMS

Beginning Date: 7/15/19

Time: 10:00 1651

Ending Date: 7/21/19

Time: 1140

Test Organism: Ceriodaphnia dubia

Age: 424H

Dilution Water Used: Upstream

Day	0		1		2		3		4		5		6		7
	Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.0	25.0	25.0	25.2 25.7	25.3	25.3 to 25.4	24.4	25.2	25.3	25.4 25.8	24.7	24.4 24.8	25.1	25.1 25.5	25.7 to 25.8
Dissolved Oxygen (mg/L)	7.9	7.0	7.0	6.2	7.2	6.7	7.6	7.6	7.3	6.8	6.9	7.0	7.5	7.4	7.2
pH (s.u.)	7.64	7.63	7.63	7.18	7.46	7.14	7.84	7.47	7.55	7.26	7.58	7.80	7.64	7.46	7.69
Conductivity (µmho/cm)	315	341	351	351	357	355	334	355	344	359	337	346	337	367	351
Hardness (mg/L CaCO ₃)	120	-	-	-	154	-	-	-	171	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	120	-	-	-	154	-	-	-	154	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Solution Tested: 20% 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, NDM, CBS, EMS

Beginning Date:

Ending Date:

Test Organism: Ceriodaphnia dubia

Dilution Water Used: Upstream

5/19 Time:

7/21/19

daphnia dubia

Upstream

[illegible]

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: BG, NDM, CBS, EMS
Beginning Date: 7/15/19 Time: 10:00
Ending Date: 7/21/19 Time: 11:40
Test Organism: Ceriodaphnia dubia Age: 4-24H
Dilution Water Used: Upstream

[illegible]

Solution Tested: 80% Effluent	Person(s) conducting Test: BG, NDM, CBS, EMS
Industry/Toxicant: Allen County, Shawnee No. 2 WWTP	Beginning Date: 7/15/19
Address: 3640 Spencerville Road, Lima, OH 45805	Ending Date: 7/21/19
Effluent Serial No.: 001	Test Organism: Ceriodaphnia dubia
	Dilution Water Used: Upstream
	NPDES Permit No.: 2PK00002*JD
	Time: 11:40
	Age: 2-24H

[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*JD

Person(s) conducting Test: BG, NDM, CBS, EMS

Beginning Date: 7/15/19

Time: 1651

Ending Date: 7/21/19

Time: 1146

Test Organism: Ceriodaphnia dubia

Age: 4-24H

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)	25.8	25.8	25.2 25.7	25.9	25.3 25.4	25.4	25.2	25.4	25.4 25.8	25.4	24.6 24.8	25.4	25.1 25.5	25.7 25.8
Dissolved Oxygen (mg/L)	7.1	7.6	7.0	6.4	7.0	7.6	7.5	6.4	7.2	7.4	7.3	7.5	7.6	7.5
pH (s.u.)	7.37	7.71	8.04	7.36	8.00	7.57	8.16	7.47	8.01	7.58	8.13	7.96	8.39	8.30
Conductivity (µmho/cm)	731	729	745	650	743	653	664	709	673	708	707	704	713	715
Hardness (mg/L CaCO ₃)	239	-	-	259	-	-	-	234	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	205	-	-	188	-	-	-	234	-	-	-	-	-	-

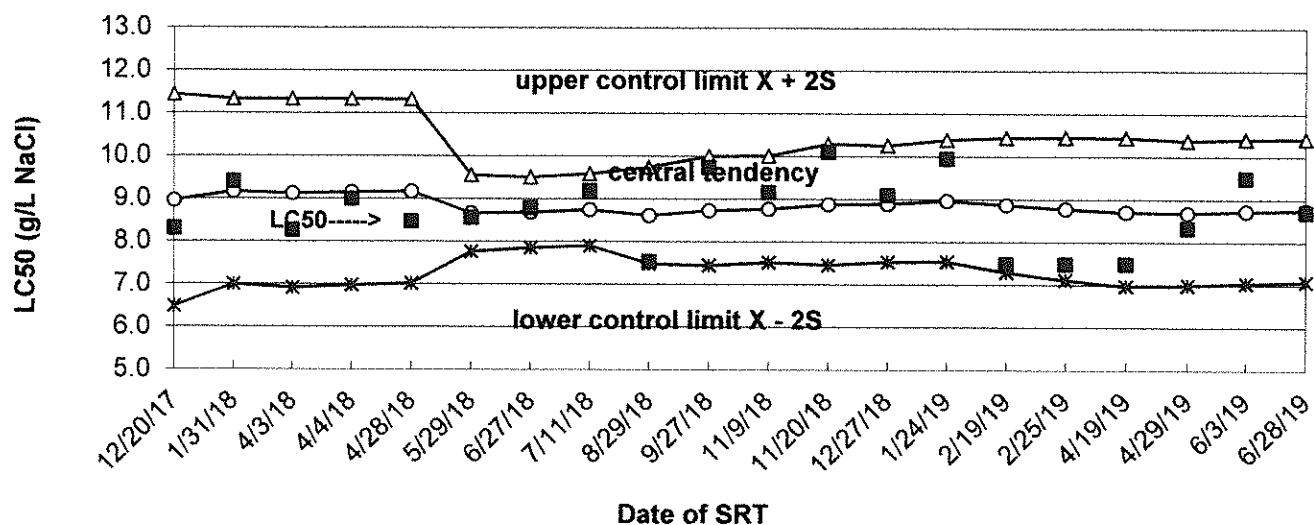
Appendix D

SRT Data

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

Date of	Cumulative								LC50
Test	LC50	LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	Method ¹
12/20/17	8.3	9.0	1.2	11.4	6.5	0.14	12.7	5.2	Probit
1/31/18	9.4	9.2	1.1	11.3	7.0	0.12	12.4	5.9	TSK
4/3/18	8.3	9.1	1.1	11.3	6.9	0.12	12.4	5.8	Probit
4/4/18	9.0	9.1	1.1	11.3	7.0	0.12	12.4	5.9	TSK
4/28/18	8.5	9.2	1.1	11.3	7.0	0.12	12.4	5.9	Probit
5/29/18	8.6	8.7	0.4	9.6	7.8	0.05	10.0	7.3	Probit
6/27/18	8.8	8.7	0.4	9.5	7.9	0.05	9.9	7.4	TSK
7/11/18	9.2	8.7	0.4	9.6	7.9	0.05	10.0	7.5	TSK
8/29/18	7.5	8.6	0.6	9.7	7.5	0.07	10.3	6.9	Probit
9/27/18	9.7	8.7	0.6	10.0	7.4	0.07	10.6	6.8	Probit
11/9/18	9.2	8.8	0.6	10.0	7.5	0.07	10.6	6.9	Probit
11/20/18	10.1	8.9	0.7	10.3	7.5	0.08	11.0	6.7	TSK
12/27/18	9.1	8.9	0.7	10.3	7.5	0.08	10.9	6.8	TSK
1/24/19	9.9	9.0	0.7	10.4	7.5	0.08	11.1	6.8	Probit
2/19/19	7.5	8.9	0.8	10.4	7.3	0.09	11.2	6.5	TSK
2/25/19	7.5	8.8	0.8	10.5	7.1	0.10	11.3	6.3	Probit
4/19/19	7.5	8.7	0.9	10.4	7.0	0.10	11.3	6.1	TSK
4/29/19	8.3	8.7	0.8	10.4	7.0	0.10	11.2	6.1	Probit
6/3/19	9.5	8.7	0.8	10.4	7.0	0.10	11.3	6.2	TSK
6/28/19	8.7	8.7	0.8	10.4	7.1	0.10	11.3	6.2	TSK

Control Chart for *Pimephales promelas* 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 = 0.19

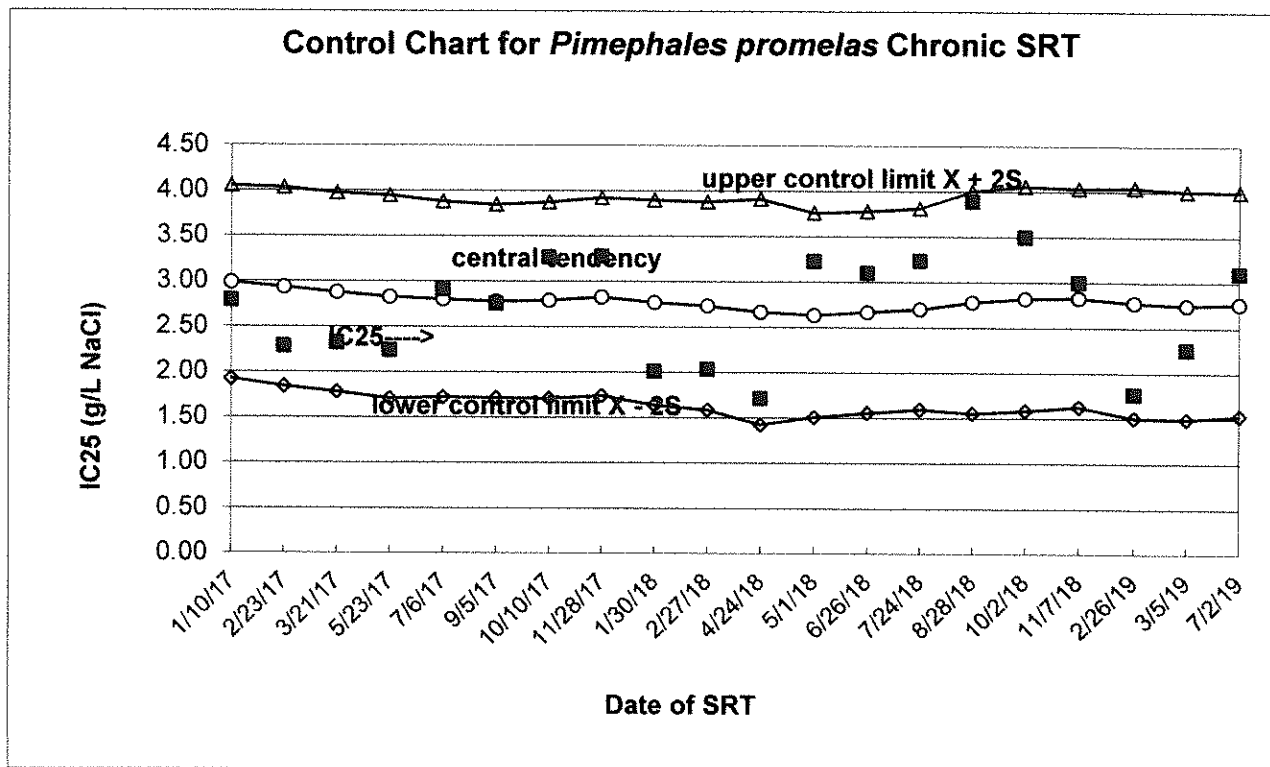
Calculated CV should not exceed 75th percentile of CVs.

$\bar{X} \pm 3SD$ = 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
1/10/17	2.79	2.99	0.53	4.05	1.92	0.18	4.58	1.39
2/23/17	2.29	2.94	0.55	4.03	1.84	0.19	4.58	1.29
3/21/17	2.32	2.88	0.55	3.98	1.78	0.19	4.53	1.23
5/23/17	2.24	2.83	0.56	3.95	1.71	0.20	4.51	1.15
7/6/17	2.91	2.80	0.54	3.88	1.72	0.19	4.42	1.18
9/5/17	2.76	2.78	0.53	3.85	1.71	0.19	4.38	1.18
10/10/17	3.27	2.79	0.54	3.87	1.71	0.19	4.41	1.17
11/28/17	3.27	2.83	0.55	3.92	1.74	0.19	4.47	1.19
1/30/18	2.01	2.77	0.57	3.90	1.64	0.20	4.46	1.07
2/27/18	2.04	2.73	0.57	3.88	1.59	0.21	4.45	1.01
4/24/18	1.72	2.67	0.62	3.91	1.42	0.23	4.53	0.80
5/1/18	3.23	2.63	0.56	3.76	1.51	0.21	4.32	0.94
6/26/18	3.10	2.67	0.56	3.78	1.56	0.21	4.33	1.00
7/24/18	3.24	2.70	0.56	3.81	1.59	0.21	4.37	1.04
8/28/18	3.89	2.78	0.61	4.00	1.55	0.22	4.62	0.94
10/2/18	3.50	2.82	0.62	4.06	1.58	0.22	4.68	0.97
11/7/18	3.00	2.83	0.60	4.03	1.63	0.21	4.64	1.03
2/26/19	1.77	2.77	0.63	4.04	1.51	0.23	4.68	0.87
3/5/19	2.26	2.75	0.63	4.00	1.49	0.23	4.63	0.87
7/2/19	3.10	2.77	0.62	4.00	1.53	0.22	4.61	0.92



*- 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

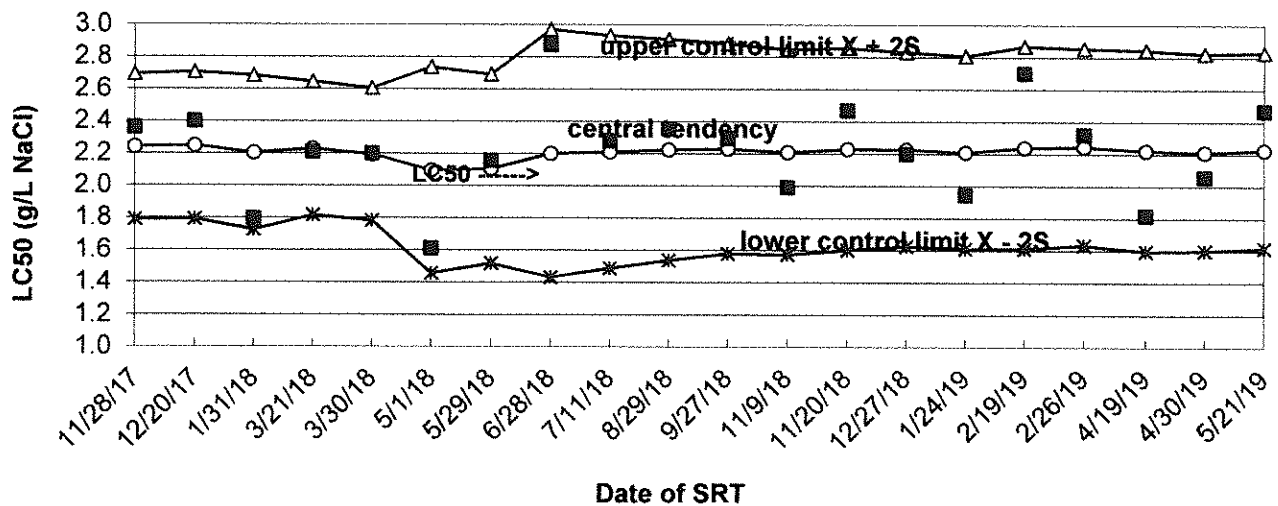
Calculated CV should not exceed 75th percentile of CVs.

$\bar{X} \pm 3SD$ = 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
11/28/17	2.4	2.2	0.2	2.7	1.8	0.10	2.9	1.6	Probit
12/20/17	2.4	2.2	0.2	2.7	1.8	0.10	2.9	1.6	SK
1/31/18	1.8	2.2	0.2	2.7	1.7	0.11	2.9	1.5	Probit
3/21/18	2.2	2.2	0.2	2.6	1.8	0.09	2.9	1.6	Probit
3/30/18	2.2	2.2	0.2	2.6	1.8	0.09	2.8	1.6	Probit
5/1/18	1.6	2.1	0.3	2.7	1.5	0.15	3.1	1.1	Probit
5/29/18	2.2	2.1	0.3	2.7	1.5	0.14	3.0	1.2	Probit
6/28/18	2.9	2.2	0.4	3.0	1.4	0.17	3.4	1.0	Probit
7/11/18	2.3	2.2	0.4	2.9	1.5	0.16	3.3	1.1	Probit
8/29/18	2.4	2.2	0.3	2.9	1.5	0.15	3.3	1.2	SK
9/27/18	2.3	2.2	0.3	2.9	1.6	0.15	3.2	1.3	Probit
11/9/18	2.0	2.2	0.3	2.8	1.6	0.14	3.2	1.3	Probit
11/20/18	2.5	2.2	0.3	2.9	1.6	0.14	3.2	1.3	SK
12/27/18	2.2	2.2	0.3	2.8	1.6	0.14	3.1	1.3	Probit
1/24/19	1.9	2.2	0.3	2.8	1.6	0.14	3.1	1.3	Probit
2/19/19	2.7	2.2	0.3	2.9	1.6	0.14	3.2	1.3	Probit
2/26/19	2.3	2.2	0.3	2.9	1.6	0.14	3.2	1.3	Probit
4/19/19	1.8	2.2	0.3	2.8	1.6	0.14	3.2	1.3	Probit
4/30/19	2.1	2.2	0.3	2.8	1.6	0.14	3.1	1.3	Probit
5/21/19	2.5	2.2	0.3	2.8	1.6	0.14	3.1	1.3	TSK

Control Chart for *Ceriodaphnia dubia* Acute SRT



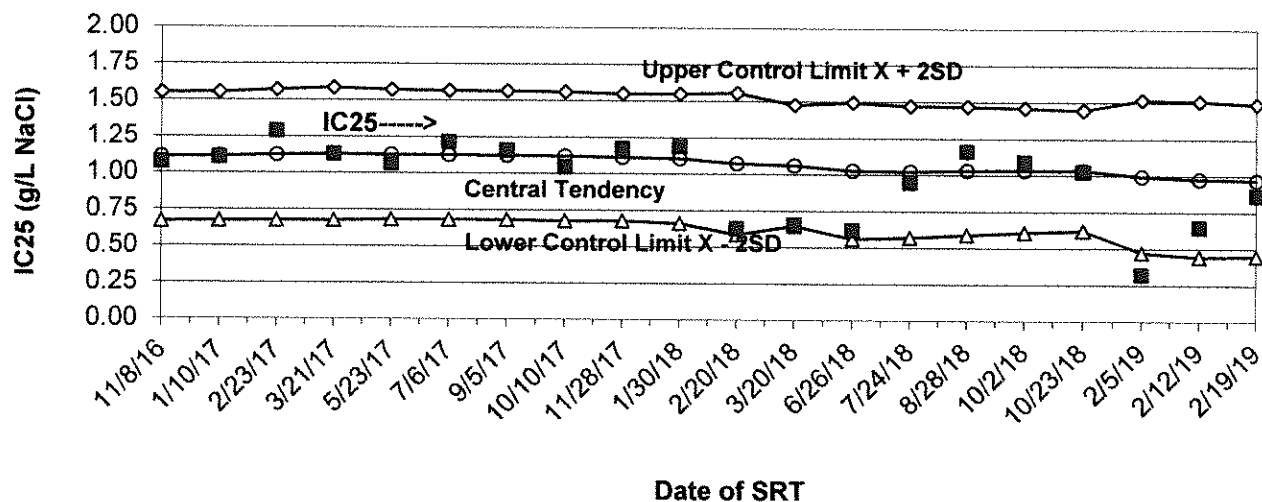
*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
 75th percentile of CVs for LC50 = 0.29
 Calculated CV should not exceed 75th percentile of CVs.
 $\bar{X} \pm 3SD$ = Well outside expected range.

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
11/8/16	1.07	1.11	0.22	1.55	0.67	0.20	1.77	0.45
1/10/17	1.11	1.11	0.22	1.55	0.67	0.20	1.77	0.45
2/23/17	1.29	1.12	0.22	1.57	0.68	0.20	1.79	0.45
3/21/17	1.13	1.13	0.23	1.58	0.67	0.20	1.81	0.45
5/23/17	1.07	1.13	0.22	1.57	0.68	0.20	1.79	0.46
7/6/17	1.22	1.12	0.22	1.57	0.68	0.20	1.79	0.46
9/5/17	1.16	1.12	0.22	1.56	0.68	0.20	1.78	0.46
10/10/17	1.04	1.12	0.22	1.56	0.68	0.20	1.78	0.45
11/28/17	1.18	1.11	0.22	1.55	0.68	0.20	1.77	0.46
1/30/18	1.19	1.10	0.22	1.55	0.66	0.20	1.77	0.44
2/20/18	0.63	1.07	0.24	1.56	0.58	0.23	1.80	0.34
3/20/18	0.66	1.06	0.21	1.48	0.65	0.20	1.68	0.44
6/26/18	0.62	1.03	0.23	1.49	0.56	0.23	1.73	0.33
7/24/18	0.95	1.02	0.23	1.47	0.57	0.22	1.70	0.35
8/28/18	1.16	1.03	0.22	1.47	0.59	0.21	1.69	0.37
10/2/18	1.09	1.03	0.21	1.46	0.61	0.21	1.67	0.39
10/23/18	1.02	1.03	0.21	1.45	0.62	0.20	1.65	0.41
2/5/19	0.32	0.99	0.26	1.52	0.47	0.26	1.78	0.21
2/12/19	0.65	0.98	0.27	1.51	0.44	0.27	1.77	0.18
2/19/19	0.87	0.97	0.26	1.49	0.45	0.27	1.75	0.19

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.