



Alloway[®]
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineers

Facility: American II WWTP

Outfall: Effluent 001

Revised Report Date: 5/7/21

Ohio EPA Permit No.: 2PH00006*KD

Alloway Lab #: L21-13631-01

Report of Chronic Biomonitoring

Samples Collected on:

4/19/21

4/21/21

4/23/21

Pimephales promelas

Ceriodaphnia dubia

Allen County Sanitary Engineers

American II WWTP

4140 Diller Road

Lima, OH 45805



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Report Date: 5/7/21

Ohio EPA Permit No.: 2PH00006*KD

Alloway Lab #: L21-13631-01

Date: 5/7/21

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

Dear Mr. Santaguida:

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

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

Dissolved oxygen, pH, and temperature were recorded daily at each renewal and at the end of each 24-hour exposure period. Results may be found in Appendix C "Parameter Data Sheets". Samples were analyzed for alkalinity, hardness and conductivity. These results may also be found in Appendix C.

Alloway (Lima, Ohio) performs biomonitoring testing in accordance with procedures established by the Environmental Protection Agency. The Standard Operating Procedures for the testing is on file with the Ohio EPA.

Please call if you have any questions or need additional information.

Schelby Gabel
Life Sciences Department Manager



Outfall: Effluent 001
Report Date: 5/7/21
Ohio EPA Permit No.: 2PH00006*KD

1. Facility Name American II WWTP
2. Facility Address 4140 Diller Road
Lima, OH 45805
3. Ohio EPA Permit No. 2PH00006*KD
4. NPDES No. OH0037338
5. Facility Contact Mike Santaguida
6. Telephone No. 419-996-4696
7. Consulting Laboratory Alloway
8. Lab Contact Schelby Gabel
9. Lab Telephone No. 419-223-1362
10. Receiving water(s) of discharge Dug Run
11. Outfall(s) tested Effluent 001

12. Is your current Standard Operating Procedure (SOP) Manual on file with Ohio EPA? Yes
If yes, indicate date in which SOP was submitted to the EPA: 6/19/2017

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



Customer: Allen County Sanitary Engineers

Facility: American II WWTP

Outfall: Effluent 001

Ohio EPA Permit No.: 2PH00006*KD

Date of *P. promelas* Test: 4/20/21 - 4/27/21Date of *C. dubia* Test: 4/20/21 - 4/26/21

Summary of Chronic Toxicity Test Results

Pimephales promelas

	4500 Form Value	MDL	4500 Comments Required
--	-----------------------	-----	---------------------------

Effluent/Outfall:

Chronic Toxicity, <i>Pimephales promelas</i> - TUC	AE	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	25.0	2.5

Summary of Chronic Toxicity Test Results

Ceriodaphnia dubia

	4500 Form Value	MDL	4500 Comments Required
--	-----------------------	-----	---------------------------

Effluent/Outfall:

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

AE = analytical data not valid

Conclusion/Comments:

Unacceptable *Pimephales promelas* survival was observed in the primary upstream control. Mortality cannot exceed 20% in a control. The effluent was compared to the secondary lab control. The TUC for survival was <1.0, but the growth test was considered insufficiently sensitive to detect toxic effects due to the percent minimum significant difference (PMSD) exceeding the upper PMSD limit of 30%. A new test must be conducted on a freshly collected sample.

Unacceptable *Ceriodaphnia dubia* survival was observed in the secondary lab control. Mortality cannot exceed 20% in a control. The primary upstream control passed QC requirements. The associated results should not be affected.

Customer: Allen County Sanitary Engineers
 Facility: American II WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PH00006*KD
 Date of Test: 4/20/21 - 4/27/21

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%)	17.5% (17.5%)	25.0% (25.0%)	25.0% (25.0%)	0.446	0.062		
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.6% (2.6%)	2.6% (2.6%)	5.1% (5.1%)	7.7% (7.7%)	0.519	0.169		
20%	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	10.0% (10.0%)	12.5% (12.5%)	0.487	0.132		
40%	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	10.0% (10.0%)	10.0% (10.0%)	12.5% (12.5%)	0.422	0.043		
60%	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	15.0% (15.0%)	15.0% (15.0%)	15.0% (15.0%)	15.0% (15.0%)	0.422	0.065		
80%	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	12.5% (12.5%)	12.5% (12.5%)	15.0% (15.0%)	0.429	0.064		
100%	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	25.0% (25.0%)	30.0% (30.0%)	37.5% (37.5%)	0.403	0.169		
Tertiary Control Lab - DMW	2.6% (2.6%)	2.6% (2.6%)	2.6% (2.6%)	2.6% (2.6%)	2.6% (2.6%)	7.7% (7.7%)	12.8% (12.8%)	0.480	0.035		
LC50 Values (%):	>100	>100	>100	>100	>100	>100	>100	Calculated TUC for Survival <1.0			
95% Confidence Limits LL											
95% Confidence Limits UL											
EC50 Values (%):	>100	>100	>100	>100	>100	>100	>100	Calculated TUC for Growth (100/IC25) AE			
95% Confidence Limits LL											
95% Confidence Limits UL											
7-Day NOEC for Mortality (%)	80	7-Day NOEC for Growth (%) 7-Day LOEC for Growth (%) Chronic Value for Growth (%)	>100 >100 >100	100	Method(s) used to determine Values: IC Pin, Dunnett's Test, Probit, Homoscedastic t Test						
7-Day LOEC for Mortality (%)	100										
Chronic Value for Mortality (%)	89.4										
IC25 (%)	>100	IC50 (%)	>100								

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

Chronic Value = square root of (NOEC x LOEC)

AE = analytical data not valid

COMMENTS:

Unacceptable *Pimephales promelas* survival was observed in the primary upstream control. Mortality cannot exceed 20% in a control. The effluent was compared to the secondary lab control. The TUC for survival was <1.0, but the growth test was considered insufficiently sensitive to detect toxic effects due to the percent minimum significant difference (PMSD) exceeding the upper PMSD bound of 30%. A new test must be conducted on a freshly collected sample.

Customer: Allen County Sanitary Engineers
 Facility: American II WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PH00006*KD
 Date of Test: 4/20/21 - 4/26/21

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	
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%)	---	34.8
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	20.0% (20.0%)	20.0% (20.0%)	30.0% (30.0%)	30.0% (30.0%)	---	13.1
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%)	---	34.5
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%)	---	34.5
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%)	---	35.5
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	---	31.4
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%)	---	34.3
LC50 Values (%):	>100	>100	>100	>100	>100	>100	---	Calculated TUC Value for Survival <1.0
	LL							
95% Confidence Limits	UL							
EC50 Values (%):	>100	>100	>100	>100	>100	>100	---	Calculated TUC Value for Reproduction <1.0
	LL							
95% Confidence Limits	UL							
NOEC for Mortality (%)	100		NOEC for Reprod (%) LOEC for Reprod (%) Chronic Value for Reprod (%)				100	Method(s) used to determine Values: IC Pin, Steel's Many-One Rank Test
LOEC for Mortality (%)	>100						>100	
Chronic Value for Mortality (%)	>100						>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:

Unacceptable *Ceriodaphnia dubia* survival was observed in the secondary lab control. Mortality cannot exceed 20% in a control. The primary upstream dilution water passed QC requirements. The associated results should not be affected.

Customer: Allen County Sanitary Engineers
Facility: American II WWTP
Outfall: Effluent 001
Report Date: 5/7/21
Ohio EPA Permit No.: 2PH00006*KD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description	Sample	Sample Collection Beginning	Sample Collection Ending
Final Effluent:			
Outfall No.:	Effluent 001	1st	4/18/21 @ 10:30
Type (Grab/Comp):	Comp	2nd	4/20/21 @ 07:30
Volume Collected:	5 Gal.	3rd	4/22/21 @ 07:30
Upstream Station:			
Waterbody:	Dug Run		
Station No.:	801	1st	4/19/21 @ 11:15
Type (Grab/Comp):	Grab	2nd	4/21/21 @ 10:40
Volume Collected:	5 Gal.	3rd	4/23/21 @ 10:44

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:	4/20/21 - 4/27/21
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Feed 0.1 g (0.1 to 0.2 mL) newly hatched brine shrimp nauplii three times daily (M-F) at 4-h intervals. On weekends 0.1g of nauplii are fed at the beginning of the workday, after renewal, and at the end of the workday. Sufficient larvae are added to provide an excess. Fish are not fed during the final 12-h of the test.
8. Size of Test Vessel:	500 mL plastic cup
9. Volume and Depth of Test Solutions:	250 mL, 6.7 cm deep
10. No. of Test Organisms per Test Vessel:	10 per test vessel
11. No. of Test Vessels per Test Solution:	4 per solution tested
12. Total No. of Test Organisms per Test Solution:	40 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 4/19/21 ; day 2, 3 - 4/21/21 ; day 4, 5, 6 - 4/23/21
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Tertiary Control Water:	Lab - diluted mineral water (DMW)
18. Aeration During Test:	None
19. Endpoints Measured:	Mortality, and atypical behavior or appearance; and growth (dry weight).
20. 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> 6-14 hours
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:	4/20/21 - 4/26/21
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 - 4/19/21 ; day 2, 3 - 4/21/21 ; day 4, 5, 6 - 4/23/21
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data



Chain of Custody Record

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

- 1101 North Cole Street, Lima, OH 45805
(P) 419-223-1362 (F) 419-227-3792
- 1776 Marion-Waldo Road, Marion OH 43302
(P) 740-389-5991 (F) 740-389-1481
- 1502 W. Fourth Street, Mansfield, OH 44906
(P) 419-525-1644 (F) 419-524-5575

Report To: Name: Company: Address:		Invoice To (If Different): Name: Company: Address:		Notes/Comments: <i>L21-13631</i>								
Phone #: 419-996-4696 E-mail: msantaguida@allencountyohio.com		Fax #: _____ PO#:										
Project Name	Am II Bioassay - Day One							Turnaround: (Rush Charges May Apply)				
Sampler	<i>(Print) Michael J. Honley (Signature)</i>							Next Day <input type="checkbox"/>	3 Working Days <input type="checkbox"/>	Routine <input checked="" type="checkbox"/>		
								2 Working Days <input type="checkbox"/>	5 Working Days <input type="checkbox"/>			
Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required			Alloway LIMS # For Lab Use Only	
1 Effluent	<i>4/18/21</i>	<i>10:30 - 10:30</i>	X		WW	1	1	Toxicity				
2 Upstream	<i>4/19/21</i>	<i>11:15</i>	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		
1						UPS <input type="checkbox"/>	ww - wastewater gw - groundwater	1 - None	7 - Sodium Thiosulfate	13 - Zinc Acetate	(For Lab Use Only)	
2						Fed Ex <input type="checkbox"/>	dw - drinking water sw - surface water	2 - HNO ₃	8 - Ascorbic Acid	14 - Sodium Sulfite		
3						Client <input type="checkbox"/>	w - water oil - oil	3 - H ₂ SO ₄	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate		
4						Alloway Pick Up <input type="checkbox"/>	s - solid sg - sludge	4 - HCl	10 - EDA	16 - Sodium Sulfite/Sodium Bisulfite		
5						Alloway Sampling <input checked="" type="checkbox"/>	t - leachate a - acid	5 - NaOH	11 - Ammonium Chloride			
6						Other <input type="checkbox"/>	p - product o - other	6 - NaOH & Zinc Acetate	12 - (NH ₄) ₂ SO ₄ & NH ₄ OH			
Received for Laboratory By: (circle one): Mansfield <input checked="" type="radio"/> Lima <input type="radio"/> Marion				<i>4/19/21</i>	<i>11:45</i>						Container Temperature: <i>10°C F190</i>	
(Signature) <i>[Signature]</i>												

Transported to: Lima
Marion

By: _____

Received By: _____

Date: _____ Time: _____

Transported to: Lima
Marion

By: _____

Received By: _____

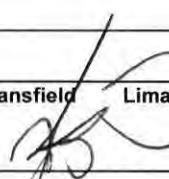
Date: _____ Time: _____



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(P) 419-525-1644 (F) 419-524-5575

Report To: Name: Mike Santaguida Company: Allen County Sanitary Eng. Address: 3226 N. Cole St. Lima, OH 45805		Invoice To (If Different): Name: Company: Address:		Notes/Comments:								
Phone #: 419-996-4696 E-mail: msantagiuda@allencountyohio.com		Fax #: PO#:										
Project Name: Am II Bioassay - Day Two				Turnaround: (Rush Charges May Apply) Next Day <input type="checkbox"/> 2 Working Days <input type="checkbox"/> 3 Working Days <input type="checkbox"/> 5 Working Days <input type="checkbox"/>								
Sampler: (Print) Michael S. Hanly (Signature) 									Routine <input checked="" type="checkbox"/>			
	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required			Alloway LIMS # For Lab Use Only
1	Effluent	4/20/21 4/21/21	07:30 ~ 07:30	X		WW	1	1	Toxicity			
2	Upstream	4/21/21	10:40		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	
1						UPS <input type="checkbox"/>	wv - wastewater gw - groundwater	1 - None	7 - Sodium Thiosulfate	13 - Zinc Acetate	(For Lab Use Only)	
2						Fed Ex <input type="checkbox"/>	dw - drinking water sw - surface water	2 - HNO ₃	8 - Ascorbic Acid	14 - Sodium Sulfite		
3						Client <input type="checkbox"/>	w - water oil - oil	3 - H ₂ SO ₄	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate		
4						Alloway Pick Up <input type="checkbox"/>	s - solid sg - sludge	4 - HCl	10 - EDA	16 - Sodium Sulfite/Sodium Bisulfite		
5						Alloway Sampling <input checked="" type="checkbox"/>	l - leachate a - acid	5 - NaOH	11 - Ammonium Chloride			
6						Other <input type="checkbox"/>	p - product o - other	6 - NaOH & Zinc Acetate	12 - (NH ₄) ₂ SO ₄ & NH ₄ OH			
Ice Present? <input checked="" type="checkbox"/> N <input type="checkbox"/>												
Proper Preservation? <input checked="" type="checkbox"/> N <input type="checkbox"/>												
Container Temperature: 4°C T-100 DDP												
Received for Laboratory By: (circle one): Mansfield <input checked="" type="radio"/> Lima <input type="radio"/> Marion				4/21/21	10:58							
(Signature) 												

Transported to: Lima
Marion

By: _____

Received By: _____

Date: _____ Time: _____

Transported to: Lima
Marion

By: _____

Received By: _____

Date: _____ Time: _____



Chain of Custody Record

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Report To: Name: Mike Santaguida Company: Allen County Sanitary Eng. Address: 3226 N. Cole St. Lima, OH 45805		Invoice To (If Different): Name: Company: Address:		Notes/Comments:								
Phone #: 419-996-4696 Fax #: _____ E-mail: msantaguida@allencountyohio.com		PO#:										
Project Name	Am II Bioassay - Day Three							Turnaround: (Rush Charges May Apply)				
Sampler	<i>Michael S. Hanley</i>							Next Day <input type="checkbox"/>	3 Working Days <input type="checkbox"/>	Routine <input checked="" type="checkbox"/>		
(Print)	(Signature)							2 Working Days <input type="checkbox"/>	5 Working Days <input type="checkbox"/>			
Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite <input checked="" type="checkbox"/>	Grab <input type="checkbox"/>	Matrix Code <input type="checkbox"/>	Number of Containers <input type="checkbox"/>	Preservation Code # <input type="checkbox"/>	Analysis Required			Alloway LIMS # For Lab Use Only	
1 Effluent	4/22/21- 4/23/21	07:30- 07:30	X		WW	1	1	Toxicity				
2 Upstream	4/23/21	10:44		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		
1						UPS <input type="checkbox"/>	wv - wastewater gw - groundwater	1 - None	7 - Sodium Thiosulfate	13 - Zinc Acetate	(For Lab Use Only)	
2						Fed Ex <input type="checkbox"/>	dw - drinking water sw - surface water	2 - HNO ₃	8 - Ascorbic Acid	14 - Sodium Sulfite		
3						Client <input type="checkbox"/>	w - water oil - oil	3 - H ₂ SO ₄	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate	Ice Present? <input checked="" type="checkbox"/> N <input type="checkbox"/>	
4						Alloway Pick Up <input type="checkbox"/>	s - solid sg - sludge	4 - HCl	10 - EDA	16 - Sodium Sulfite/Sodium Bisulfite		
5						Alloway Sampling <input checked="" type="checkbox"/>	l - leachate a - acid	5 - NaOH	11 - Ammonium Chloride		Proper Preservation? <input checked="" type="checkbox"/> N <input type="checkbox"/>	
6						Other <input type="checkbox"/>	p - product o - other	6 - NaOH & Zinc Acetate	12 - (NH ₄) ₂ SO ₄ & NH ₄ OH			
Received for Laboratory By: (circle one): <input checked="" type="radio"/> Mansfield <input type="radio"/> Lima <input type="radio"/> Marion											Container Temperature: <i>9°C T-120°F DP</i>	
(Signature)				4/23/21	11:07							

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, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: BG, DJA, JDP
 Beginning: Date 4/20/21 Time 1209
 Ending: Date 4/27/21 Time 1228
 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	9G 10	10G	39G 10	10G	9G 10	10G	10G	39G 10
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	9G 10	10G	39G 10	10G	9G 10	10G	10G	39G 10
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	9G 10	10G	39G 10	10G	8G 2D	10G	10G	38G 2D
4	10G	10G	9G 10	10G	39G 10	10G	10G	10G	9G 10	39G 10	10G	10G	9G 2D	10G	38G 2D	10G	8G 2D	10G	9G 10	37G 3D
5	10G	10G	9G 10	10G	39G 10	7G 3D	10G	8G 2D	8G 2D	33G 7D	10G	10G	7G 3D	10G	37G 3D	9G 10	8G 2D	10G	9G 10	36G 4D
6	10G	9G 10	9G 10	10G	38G 2D	7G 3D	8G 2D	8G 3D	7G 10D	30G 10D	10G	10G	6G 4D	10G	36G 4D	9G 10	8G 2D	10G	9G 10	36G 4D
7	10G	8G 2D	9G 10	10G	37G 3D	7G 3D	8G 2D	8G 3D	7G 10D	30G 10D	9G 10	10G	6G 4D	10G	35G 5D	8G 2D	8G 2D	10G	9G 10	35G 5D

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

DJA
 4/24/21

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

Industry/Toxicant: Allen County, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: BG, DJA, JDP
 Beginning: Date 4/20/21 Time 1209
 Ending: Date 4/27/21 Time 1228
 Age of *Pimephales promelas*: < 24 Hours
 Dilution Water Used: Upstream

Day	60% Effluent					80% Effluent					100% Effluent					Lab Control - DW				
	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	9G ID	10G	10G	10G	39G 10	10G	10G	10G	9G ID	39G 10
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	9G ID	10G	10G	10G	39G 10	10G	10G	10G	9G ID	39G 10
3	9G ID	9G ID	10G	10G	38G 20	10G	10G	9G ID	9G ID	38G 20	7G ID	10G	10G	10G	39G 10	10G	10G	10G	9G ID	39G 10
4	8G 20	7G 30	9G ID	10G	34G	9G ID	10G	9G ID	9G ID	37G	9G ID	10G	10G	9G ID	38G 20	10G	10G	10G	9G ID	39G 10
5	8G 20	7G 30	9G ID	10G	34G	8G 20	10G	8G 20	9G ID	35G	7G ID	7G 30	10G	6G ID	20G 20D	10G	10G	10G	9G ID	39G 10
6	8G 20	7G 30	9G ID	10G	34G	8G 20	10G	8G 20	9G ID	35G	6G ID	7G 30	10G	5G ID	28G 20	10G	10G	8G 20	9G ID	37G 30
7	8G 20	7G 30	9G ID	10G	34G	8G 20	9G ID	8G 20	9G ID	34G	6G ID	5G 40	10G	4G ID	25G 15D	7G 10A	9G ID	7G 30	9G ID	35G 5D

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

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

DJA
4/26/21

34G 1LA
TD P 4/29/21

VS. upstream

SG 5/6/21

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 4/20/2021 Test ID: Alle0421p Sample ID: L21-13631-01
 End Date: 4/27/2021 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
Upstream	0.7000	0.8000	0.8000	0.7000
20	0.9000	1.0000	0.6000	1.0000
40	0.8000	0.8000	1.0000	0.9000
60	0.8000	0.7000	0.9000	1.0000
80	0.8000	0.9000	0.8000	0.9000
100	0.6000	0.5000	1.0000	0.4000

Conc-%	Transform: Arcsin Square Root						1-Tailed			
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Upstream	0.7500	1.0000	1.0492	0.9912	1.1071	6.383	4			
20	0.8750	1.1667	1.2398	0.8861	1.4120	20.004	4	-1.372	2.410	0.3349
40	0.8750	1.1667	1.2188	1.1071	1.4120	11.906	4	-1.221	2.410	0.3349
60	0.8500	1.1333	1.1898	0.9912	1.4120	15.281	4	-1.012	2.410	0.3349
80	0.8500	1.1333	1.1781	1.1071	1.2490	6.954	4	-0.928	2.410	0.3349
100	0.6250	0.8333	0.9421	0.6847	1.4120	34.384	4	0.771	2.410	0.3349

Auxiliary Tests		Statistic		Critical		Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)		0.96695		0.916		0.50467	1.32941			
Bartlett's Test indicates equal variances (p = 0.13)		8.50014		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	0.32261	0.42918	0.05397	0.03862	0.2721	5, 18
Treatments vs Upstream										

Mortality in upstream control >20%

vs. secondary lab control

SL 5/6/21

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 4/20/2021 Test ID: Alle0421p Sample ID: L21-13631-01
 End Date: 4/27/2021 Lab ID: ALL-ALLOWAY Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: EPAFW02-EPA/821/R-02-01 Test Species: PP-Pimephales promelas

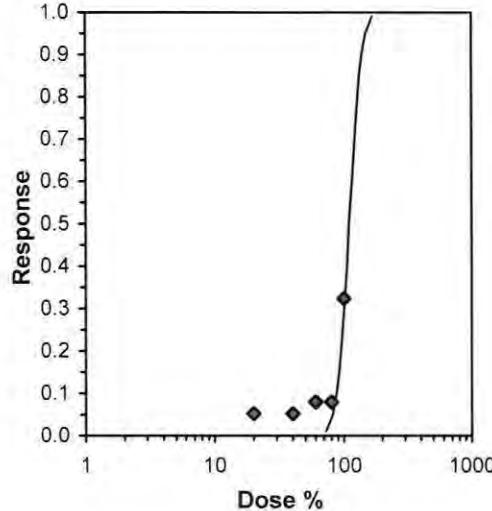
Comments:

Conc-%	1	2	3	4
Lab	1.0000	0.8000	0.9000	1.0000
20	0.9000	1.0000	0.6000	1.0000
40	0.8000	0.8000	1.0000	0.9000
60	0.8000	0.7000	0.9000	1.0000
80	0.8000	0.9000	0.8000	0.9000
100	0.6000	0.5000	1.0000	0.4000

Conc-%	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
Lab	0.9250	1.0000	1.2929	1.1071	1.4120	11.192	4				3	39
20	0.8750	0.9459	1.2398	0.8861	1.4120	20.004	4	0.369	2.410	0.3466	5	40
40	0.8750	0.9459	1.2188	1.1071	1.4120	11.906	4	0.515	2.410	0.3466	5	40
60	0.8500	0.9189	1.1898	0.9912	1.4120	15.281	4	0.717	2.410	0.3466	6	40
80	0.8500	0.9189	1.1781	1.1071	1.2490	6.954	4	0.798	2.410	0.3466	6	40
*100	0.6250	0.6757	0.9421	0.6847	1.4120	34.384	4	2.439	2.410	0.3466	15	40

Auxiliary Tests		Statistic		Critical		Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)		0.97946		0.916		0.42721	0.79942			
Bartlett's Test indicates equal variances (p = 0.36)		5.5009		15.0863						
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	80	100	89.4427	1.25	0.2666	0.2883	0.05959	0.04137	0.25765	5, 18
Treatments vs Lab										

Parameter	Value	SE	Maximum Likelihood-Probit		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			95% Fiducial Limits	95% Fiducial Limits							
Slope	12.7837	9.25234	-5.3509	30.9183		0.07692	0.38128	7.81472	0.94408	2.04327	0.07822
Intercept	-21.121	18.425	-57.234	14.9924							
TSCR	0.11916	0.02591	0.06837	0.16994							
Point	Probits	%	95% Fiducial Limits								
EC01	2.674	72.6595									
EC05	3.355	82.1489									
EC10	3.718	87.7043									
EC15	3.964	91.6632									
EC20	4.158	94.9367									
EC25	4.326	97.8381									
EC40	4.747	105.548									
EC50	5.000	110.476									
EC60	5.253	115.635									
EC75	5.674	124.747									
EC80	5.842	128.56									
EC85	6.036	133.151									
EC90	6.282	139.161									
EC95	6.645	148.572									
EC99	7.326	167.976									



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

Discharge: Allen County, Am. II WWTP

Date of Test: 4/20/21 - 4/27/21

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Pimephales promelas Age at Start: < 24 Hours

Drying Temperature (C°): 60

Drying Time (h): 24

Date Weighed:

S/4/21 Analyst: SKG

Balance Calibration Check: 1 gram weight = 0.99997 grams Date: 4/20/21 Analyst: JDP Dilution Water Used: Upstream
 1.00002 S/4/21 SKG

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			1.07775		10		
Lab Control	1	10	1.12321	1.13021	10	4.00707.00	0.700
	2	8	1.08486	1.08818	10	3.32	0.332
	3	9	1.08122	1.08738	10	6.16	0.616
	4	9	1.09160	1.100416	JDP 7.9	3.84	0.429
Upstream Control	1	7	1.09275	1.091652	10	3.77	0.377
	2	8	1.10435	1.10905	10	4.70	0.470
	3	8	1.09198	1.09717	10	5.19	0.519
	4	7	1.08801	1.09217	10	4.16	0.416
20% Effluent	1	9	1.08674	1.09158	10	4.84	0.484
	2	10	1.09161	1.09814	10	6.53	0.653
	3	6	1.08751	1.09087	10	3.30	0.330
	4	10	1.10423	1.10908	10	4.80	0.480
40% Effluent	1	8	1.09845	1.10297	10	4.52	0.452
	2	8	1.09870	1.10247	1.0075+1.10243	3.73	0.373
	3	10	1.09812	1.10247	1.00275+1.10243	4.63	0.463
	4	9	1.12942	1.13340	10	3.98	0.398

Reviewed By: SG 5/6/21

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

Discharge: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Drying Temperature (C°): 60

Drying Time (h): 24

Date of Test: 4/20/21 - 4/27/21

Pimephales promelas Age at Start: < 24 Hours

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	1.10607	1.10979	10	3.72	0.372
	2	7	1.09829	1.10213	10	3.84	0.384
	3	9	1.09160	1.09676	10	5.16	0.516
	4	10	1.10199	1.10616	10	4.17	0.417
80% Effluent	1	8	1.09385	1.09796	10	4.11	0.411
	2	9	1.08668	1.09071	10	4.03	0.403
	3	8	1.15315	1.15693	10	3.78	0.378
	4	9	1.12603	1.13125	10	5.22	0.522
100% Effluent	1	6	1.10388	1.10706	10	3.18	0.318
	2	10	1.08755	1.09406	10	5.61	0.561
	3	5	1.16371	1.16735	10	3.64	0.364
	4	4	1.12664	1.12942	10	2.78	0.278
Lab Control -DMW	1	9	1.09781	1.10235	10	4.54	0.504
	2	9	1.11562	1.12009	10	4.47	0.447
	3	7	1.08128	1.08644	10	5.16	0.516
	4	9	1.09925	1.10377	10	4.52	0.452

VS upstream

SG 5/6/21

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 4/20/2021 Test ID: Alle0421p Sample ID: L21-13631-01
 End Date: 4/27/2021 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:

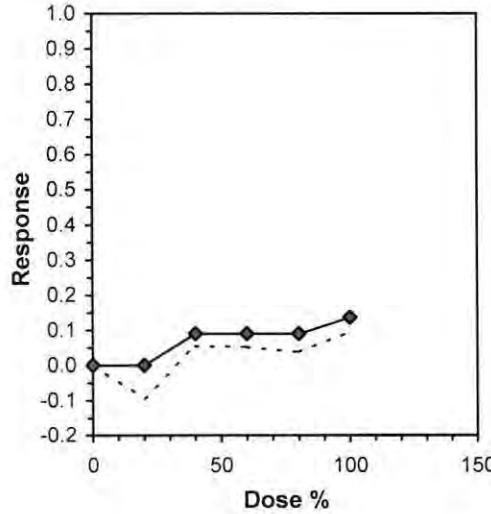
Conc-%	1	2	3	4
Upstream	0.3770	0.4700	0.5190	0.4160
20	0.4840	0.6530	0.3300	0.4800
40	0.4520	0.3730	0.4630	0.3980
60	0.3720	0.3840	0.5160	0.4170
80	0.4110	0.4030	0.3780	0.5220
100	0.3180	0.6510	0.3640	0.2780

Conc-%	Transform: Untransformed						t-Stat	Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%				Mean	N-Mean
Upstream	0.4455	1.0000	0.4455	0.3770	0.5190	13.937	4			0.4661	1.0000
20	0.4868	1.0926	0.4868	0.3300	0.6530	27.116	4	-0.583	2.410	0.1706	0.4661 1.0000
40	0.4215	0.9461	0.4215	0.3730	0.4630	10.211	4	0.339	2.410	0.1706	0.4241 0.9098
60	0.4223	0.9478	0.4223	0.3720	0.5160	15.472	4	0.329	2.410	0.1706	0.4241 0.9098
80	0.4285	0.9618	0.4285	0.3780	0.5220	14.912	4	0.240	2.410	0.1706	0.4241 0.9098
100	0.4028	0.9040	0.4028	0.2780	0.6510	42.008	4	0.604	2.410	0.1706	0.4028 0.8640

Auxiliary Tests		Statistic	Critical	Skew	Kurt					
Shapiro-Wilk's Test indicates normal distribution ($p > 0.05$)		0.93028	0.916	1.00986	1.79509					
Bartlett's Test indicates equal variances ($p = 0.19$)		7.40988	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	0.17056	0.38285	0.00337	0.01002	0.88412	5, 18

Treatments vs Upstream

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)	Skew	
IC05	31.087				
IC10	84.285				
IC15	>100				
IC20	>100				
IC25	>100				
IC40	>100				
IC50	>100				



Mortality in upstream control >20%

~~Secondary~~
~~Primary~~ Lab Control

SG 5/6/21

Earval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/20/2021	Test ID:	Alle0421p	Sample ID:	L21-13631-01
End Date:	4/27/2021	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
Lab	0.7000	0.3320	0.6160	0.4290
20	0.4840	0.6530	0.3300	0.4800
40	0.4520	0.3730	0.4630	0.3980
60	0.3720	0.3840	0.5160	0.4170
80	0.4110	0.4030	0.3780	0.5220
100	0.3180	0.6510	0.3640	0.2780

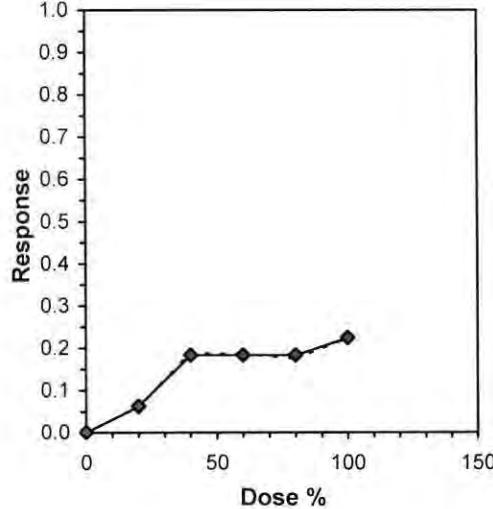
Conc-%	Transform: Untransformed						t-Stat	Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%				Mean	N-Mean
Lab	0.5193	1.0000	0.5193	0.3320	0.7000	32.462	4			0.5193	1.0000
20	0.4868	0.9374	0.4868	0.3300	0.6530	27.116	4	0.387	2.410	0.2024	0.4868 0.9374
40	0.4215	0.8117	0.4215	0.3730	0.4630	10.211	4	1.164	2.410	0.2024	0.4241 0.8167
60	0.4223	0.8132	0.4223	0.3720	0.5160	15.472	4	1.155	2.410	0.2024	0.4241 0.8167
80	0.4285	0.8252	0.4285	0.3780	0.5220	14.912	4	1.080	2.410	0.2024	0.4241 0.8167
100	0.4028	0.7756	0.4028	0.2780	0.6510	42.008	4	1.387	2.410	0.2024	0.4028 0.7756

Auxiliary Tests		Statistic		Critical		Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)		0.95764		0.916		0.58141	0.31148			
Bartlett's Test indicates equal variances (p = 0.18)		7.56405		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	0.20243	0.38985	0.00829	0.01411	0.70949	5, 18
Treatments vs Lab						39.0%				

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)	Skew	
IC05*	15.977				
IC10	26.199				
IC15	34.485				
IC20	88.141				
IC25	>100				
IC40	>100				
IC50	>100				

* indicates IC estimate less than the lowest concentration

PMSD over limit



Secondary lab control vs. 100%

SG 5/7/21

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/20/2021	Test ID:	Alle0421p	Sample ID:	L21-13631-01
End Date:	4/27/2021	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	
Lab	0.7000	0.3320	0.6160	0.4290	
100	0.3180	0.6510	0.3640	0.2780	

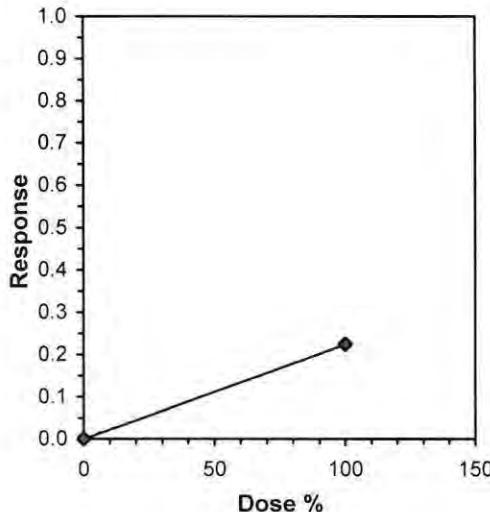
Conc-%	Transform: Untransformed						t-Stat	1-Tailed		Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%		Critical	MSD	Mean	N-Mean
Lab	0.5193	1.0000	0.5193	0.3320	0.7000	32.462	4	0.976	1.943	0.2320	0.5193 1.0000
100	0.4028	0.7756	0.4028	0.2780	0.6510	42.008	4				0.4028 0.7756

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.91367	0.818	0.60808	-1.1448
F-Test indicates equal variances (p = 1.00)	1.00746	47.4683		
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE
Homoscedastic t Test indicates no significant differences	0.23204	0.44688	0.02714	0.02852
Treatments vs Lab			44.7%	0.36694
				1, 6

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05*	22.285			
IC10*	44.571			
IC15*	66.856			
IC20*	89.142			
IC25	>100			
IC40	>100			
IC50	>100			

* indicates IC estimate less than the lowest concentration

PMSD over limit



CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested:	Lab Control - ECT	Person(s) Conducting Test:	BG, JDP, DJA
Industry/Toxicant:	Allen County, Am. II WWTP	Beginning Date:	4/20/21
Address:	4140 Diller Road, Lima, OH 45805	Ending Date:	4/26/21
Effluent Serial No.:	001	Age of Ceriodaphnia dubia:	6-14H
NPDES Permit No.:	2PH00006*KD	Dilution Water Used:	Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	0 G	0 G	0 G	0/G	6/G	10 G	/	16	2
2	/	/	/	0/G	4/G	5/ ↓	10 G	/	19	3
3	/	/	/	0/G	0/G	5/G	9/G	/	14	2
4	/	/	/	0/D	-/-	-/-	-/-	-/-	0	0
5	/	/	/	0/G	0/G	6/G	10 G	/	16	2
6	/	/	/	0/G	0/G	6/ ↓	11 G	/	17	2
7	/	/	/	0/ ↓	5/ ↓	8/G	9/G	/	22	3
8	/	/	/	0/G	0/G	5/D	-/-	-/-	5	1
9	/	/	/	0/D	-/-	-/-	-/-	-/-	0	0
10	0 G	0 G	0 G	0 G	4/G	7/G	11 G	/	22	3
Total Adult Cond.	10G	10G	10G	8G 2D	8G 2D	7G 3D	16 3D			
								SUM	131	18
								MEAN	13.1	1.8
								S.D.	8.4	1.1

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

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

Reviewed by: SG 5/6/21

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control Person(s) Conducting Test: BG, JDP, DJA
 Industry/Toxicant: Allen County, Am. II WWTP Beginning Date: 4/20/21 Time: 1401
 Address: 4140 Diller Road, Lima, OH 45805 Ending Date: 4/26/21 Time: 1315
 Effluent Serial No.: 001 Age of Ceriodaphnia dubia: 6-14H
 NPDES Permit No.: 2PH00006*KD Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	0 G	0 G	4 G	0 G	11 G	19 G	/	34	3
2	/	/	/	6	0	13	19	/	38	3
3	/	/	/	0	4	11	15	/	30	3
4	/	/	/	0	7	13	16	/	36	3
5	/	/	/	0	6	11	14	/	31	3
6	/	/	/	0	8	14	18	/	40	3
7	/	/	/	0	6	13	21	/	40	3
8	/	/	/	0	4	12	15	/	31	3
9	/	/	/	0	6	13	15	/	34	3
10	0 G	0 G	0 G	0 G	6 G	11 G	17 G	/	34	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	348	30
								MEAN	11.6	3.0
								S.D.	3.6	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: 20% Effluent Person(s) Conducting Test: BG, JDP, DJA
 Industry/Toxicant: Allen County, Am. II WWTP Beginning Date: 4/20/21 Time: 1407
 Address: 4140 Diller Road, Lima, OH 45805 Ending Date: 4/26/21 Time: 1315
 Effluent Serial No.: 001 Age of *Ceriodaphnia dubia*: 6-14H
 NPDES Permit No.: 2PH00006*KD Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	0 G	0 G	6 G	0/G	13 G	18 G	/	37	3
2	/	/	/	0	6	15	17	/	38	3
3	/	/	/	0	7	13	16	/	36	3
4	/	/	/	0	6	12	16	/	34	3
5	/	/	/	0	6	11	15	/	32	3
6	/	/	/	0	7	12	16	/	35	3
7	/	/	/	0	5	10	16	/	31	3
8	/	/	/	0	6	11	15	/	32	3
9	/	/	/	0	5	15	17	/	37	3
10	0 G	0 G	0 G	0 G	6/G	12 G	15 G	/	33	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G		345	30
								SUM	345	3.0
								MEAN	34.5	
								S.D.	2.5	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, JDP, DJA
Industry/Toxicant:	Allen County, Am. II WWTP	Beginning Date:	4/20/21
Address:	4140 Diller Road, Lima, OH 45805	Ending Date:	4/26/21
Effluent Serial No.:	001	Age of Ceriodaphnia dubia:	6-14 H
NPDES Permit No.:	2PH00006*KD	Dilution Water Used:	Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	D G	0 G	0 G	6/G	12 G	16 G	/	34	3
2	/	/	/	0	6	13	14	/	33	3
3	/	/	/	0	7	14	17	/	38	3
4	/	/	/	0	6	13	15	/	34	3
5	/	/	/	0	6	11	16	/	33	3
6	/	/	/	0	4	9	17	/	30	3
7	/	/	/	0	7	H	15	/	36	3
8	/	/	/	0	7	12	15	/	34	3
9	/	/	/	0	6	13	18	/	37	3
10	0 G	0 G	0 G	0 G	6/G	13/G	17 G	/	36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	345	30
								MEAN	34.5	3.0
								S.D.	23	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: 60% Effluent Person(s) Conducting Test: BG, JDP, DJA
 Industry/Toxicant: Allen County, Am. II WWTP Beginning Date: 4/20/21 Time: 1407
 Address: 4140 Diller Road, Lima, OH 45805 Ending Date: 4/26/21 Time: 1315
 Effluent Serial No.: 001 Age of Ceriodaphnia dubia: 6-14 H
 NPDES Permit No.: 2PH00006*KD Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	0 G	0 G	0 G	6 G	12 G	18 G		36	3
2				0	6	14	17		37	3
3				0	7	14	14		35	3
4				0	5	10	17		32	3
5				0	6	11	17		34	3
6				0	6	14	14		34	3
7				0	6	13	16		35	3
8				0	6	13	18		37	3
9				0	7	15	17		39	3
10	0 G	0 G	0 G	0 G	6 G	14 G	16 G		36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G		SUM 355	30
									MEAN 35.5	3.0
									S.D. 2.0	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: 80% Effluent Person(s) Conducting Test: BG, JDP, DJA
 Industry/Toxicant: Allen County, Am. II WWTP Beginning Date: 4/20/21 Time: 1407
 Address: 4140 Diller Road, Lima, OH 45805 Ending Date: 4/26/21 Time: 1315
 Effluent Serial No.: 001 Age of *Ceriodaphnia dubia*: 6-14 H
 NPDES Permit No.: 2PH00006*KD Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	0 G	0 G	0 G	6 G	13 G	13 G		32	3
2				0	6	13	16		35	3
3				0	8	12	17		37	3
4				0	0	6	12		18	2
5				0	6	12	11		29	3
6				0	9	14	18		41	3
7				0	4	9	14		27	3
8				0	6	12	17		35	3
9				0	6	11	14		31	3
10	0 G	0 G	0 G	0 G	4 G	7 G	15 G		26	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	314	29
								MEAN	31.4	2.9
								S.D.	6.6	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: 100% Effluent Person(s) Conducting Test: BG, JDP, DJA
 Industry/Toxicant: Allen County, Am. II WWTP Beginning Date: 4/20/21 Time: 1407
 Address: 4140 Diller Road, Lima, OH 45805 Ending Date: 4/26/21 Time: 1315
 Effluent Serial No.: 001 Age of Ceriodaphnia dubia: 6-14 H
 NPDES Permit No.: 2PH00006*KD Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST								TOTAL PER REP	
	0	1	2	3	4	5	6	7	No. Young	No. Broods
1	0 G	0 G	0 G	8 G	0/G	4 G	20 G	/	32	3
2	/	/	/	0	6	11	16	/	36	3
3	/	/	/	6	0	13	19	/	38	3
4	/	/	/	0	5	14	17	/	36	3
5	/	/	/	0	6	10	16	/	32	3
6	/	/	/	0	5	10	15	/	30	3
7	/	/	/	0	7	12	17	/	36	3
8	/	/	/	0	8	14	14	/	36	3
9	↓	↓	↓	0	7	14	14	↓	35	3
10	0 G	0 G	0 G	0 G	6/G	10 G	16 G	/	32	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
	SUM	343	30							
	MEAN	34.3	3.0							
	S.D.	2.6	0							

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

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

SB 5/5/21

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 4/20/2021 Test ID: Alle0421c Sample ID: L21-13631-01
 End Date: 4/26/2021 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
Upstream	34.000	38.000	30.000	36.000	31.000	40.000	40.000	31.000	34.000	34.000
20	37.000	38.000	36.000	34.000	32.000	35.000	31.000	32.000	37.000	33.000
40	34.000	33.000	38.000	34.000	33.000	30.000	36.000	34.000	37.000	36.000
60	36.000	37.000	35.000	32.000	34.000	34.000	35.000	37.000	39.000	36.000
80	32.000	35.000	37.000	18.000	29.000	41.000	27.000	35.000	34.000	26.000
100	32.000	36.000	38.000	36.000	32.000	30.000	36.000	36.000	35.000	32.000

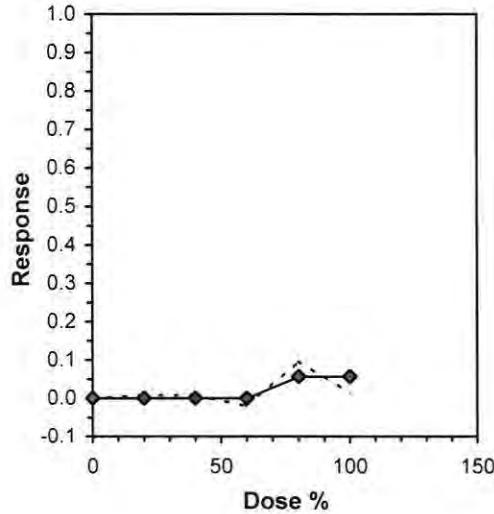
Conc-%	Transform: Untransformed						Rank Sum	1-Tailed Critical	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%			Mean	N-Mean
Upstream	34.800	1.0000	34.800	30.000	40.000	10.475	10		34.825	1.0000
20	34.500	0.9914	34.500	31.000	38.000	7.133	10	75.00	3.702	34.825 1.0000
40	34.500	0.9914	34.500	30.000	38.000	6.729	10	75.00	34.825	1.0000
60	35.500	1.0201	35.500	32.000	39.000	5.515	10	75.00	34.825	1.0000
80	31.400	0.9023	31.400	18.000	41.000	20.975	10	75.00	32.850	0.9433
100	34.300	0.9856	34.300	30.000	38.000	7.534	10	75.00	3.702	32.850 0.9433

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ($p > 0.05$)	0.5963	0.895	-0.6229	3.09393
Bartlett's Test indicates unequal variances ($p = 1.10E-03$)	20.2969	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDP
Steel's Many-One Rank Test	100	>100		1	10.6%

Treatments vs Upstream

Linear Interpolation (200 Resamples) SS21SK6				
Point	%	SD	95% CL	Skew
IC05	77.633			
IC10	>100			
IC15	>100			
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, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Type of Test: Chronic Date of Test: 4/20/21 - 4/26/21

Effluent Serial No.: 001 Permit No.: 2PH00006*KD

Investigator: BG Culture No.: 60

Culture initiation date: 4/14/21 Culture ending date: 4/21/21

Origin of parental organisms: ECT

Age at start of culture: <48H

Culture water renewal frequency: minimum 3 times per week Culture water: ECT MHSF

Culture temperature: 25°C Fed Daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

Number of brood organisms at start of culture: 40

Percent survival of parental organisms after 7 days: 100

Percent of non-reproducing adults after 7 days: 0

Average young production after 7 days of brood adults for test: _____

BROOD ADULT NO.	BROOD SIZE	HATCHING TIME WINDOW	TOTAL; YOUNG PROD. (7 DAYS)	USED IN REP. NO.
1) A6	16	2400-0800	35	1
2) A8	14		35	2
3) A9	15		36	3
4) C1	16		39	4
5) C10	16		35	5
6) D2	15		39	6
7) D4	17		35	7
8) D5	16		40	8
9) D6	15		39	9
10) D7	18	2400-0800	41	10

COMMENTS: _____

Appendix C

Parameter Data

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

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001 Permit No.: 2PH00006*KD

Type of Test: Chronic Date of Test: 4/20/21 - 4/27/21

1st Sample Collection

Date Samples Collected: 4/19/2021 Date Samples Read: 4/19/21 Analyst: DJA

	100% Effluent	Upstream
Temperature (°C)	12.7	11.6
Dissolved Oxygen (mg/L)	11.5	11.7
pH (s.u.)	7.53	8.01
Conductivity (μmhos)	945	1145
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	Light yellow stain	light yellow stain

2nd Sample Collection

Date Samples Collected: 4/21/21 Date Samples Read: 4/21/21 Analyst: BG

	100% Effluent	Upstream
Temperature (°C)	7.7	7.6
Dissolved Oxygen (mg/L)	10.4	10.7
pH (s.u.)	7.53	7.93
Conductivity (μmhos)	936	934
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light yellow	tan

3rd Sample Collection

Date Samples Collected: 4-23-21 Date Samples Read: 4-23-21 Analyst: BG

	100% Effluent	Upstream
Temperature (°C)	10.7	10.8
Dissolved Oxygen (mg/L)	10.0	10.5
pH (s.u.)	7.57	8.05
Conductivity (μmhos)	957	1050
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light yellow stain	tan

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

Solution Tested: Lab Control
 Industry/Toxicant: Allen County, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, DJA, JDP
 Beginning Date: 4/20/21 Time: 1209
 Ending Date: 4/27/21 Time: 1228
 Test Organism: Pimephales promelas Age: < 24 Hours
 Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	Old										
Temperature (°C)	25.7	25.3	25.2 25.6	25.1	25.4 25.8	24.8	24.8 25.1	25.1	24.4 25.4	25.4	24.6 25.3	25.6	24.4 25.5	25.2 26.0
Dissolved Oxygen (mg/L)	5.8	7.0	5.4	6.9	4.8	7.2	5.6	7.6	5.2	6.5	4.4	6.3	5.0	4.4
pH (s.u.)	7.57	7.84	7.24	7.84	7.22	8.95	7.32	8.27	7.40	7.59	7.19	7.55	7.32	7.28
Conductivity (μmho/cm)	322	326	336	327	343	336	339	339	356	340	364	340	364	364
Hardness (mg/L CaCO ₃)	120	-	-	120	-	-	-	171	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	86	-	-	51	-	-	-	103	-	-	-	-	-	-

SSP
4/24/21

DJA
4/26/21

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

Solution Tested: Upstream Control

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

Time: 1204

Ending Date: 4/27/21

Time: 1228

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	Old										
Temperature (°C)	26.3	25.1	25.2 25.6	25.0	25.4 25.8	25.4	24.8 25.1	24.7	24.4 25.4	25.6	24.6 25.3	25.2	24.4 25.5	25.2 26.0
Dissolved Oxygen (mg/L)	6.5	7.2	5.1	7.9	4.2	7.4	5.4	8.2	5.0	8.1	3.4	7.1	4.7	4.4
pH (s.u.)	7.97	8.05	7.90	7.91	7.86	7.96	7.61	8.00	7.59	7.91	7.44	8.03	7.60	7.71
Conductivity (μmho/cm)	1130	1130	1136	940	1125	936	944	1045	961	1042	1057	1044	1057	1057
Hardness (mg/L CaCO ₃)	410	-	-	222	-	-	-	376	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	222	-	-	137	-	-	-	222	-	-	-	-	-	-

DJA
4/26/21

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

Solution Tested: 20% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

me: 1209

Ending Date: 4/27/21

me: T223

Test Organism: Pimephales promelas

ge: < 24 Hous

Dilution Water Used: **Upstream**

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

Solution Tested: 40% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

Time: 1209

Ending Date: 4/27/21

Time: 12:38

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Upstream

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

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

Time: 1209

Ending Date: 4/27/21

Time: 1228

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Upstream

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

Solution Tested: 80% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

Time: 1209,

Ending Date: 4/27/21

Time: 10:23

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Upstream

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

Solution Tested: 100% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

Time: 1209

Ending Date: 4/27/21

Time: 1208

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)	25.3	25.3	25.2 25.6	25.3	25.4 25.8	25.7	24.8 25.1	25.0	24.4 25.4	24.7 25.3	24.6 25.3	25.5	24.4 25.5	25.2 26.0
Dissolved Oxygen (mg/L)	7.4	7.9	4.7	7.8	3.9	7.7	5.0	8.5	4.9	8.2	4.1	7.5	5.2	4.5
pH (s.u.)	7.54	7.77	7.70	7.57	7.69	7.87	7.73	7.56	7.74	7.49	7.57	7.77	7.71	7.71
Conductivity (μmho/cm)	936	939	957	931	976	933	971	956	982	951	990	954	976	982
Hardness (mg/L CaCO ₃)	239	-	-	257	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	171	-	-	120	-	-	-	205	-	-	-	-	-	-

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

Solution Tested: Lab Control-DMW

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, DJA, JDP

Beginning Date: 4/20/21

Time: 1209

Ending Date: 4/27/21

Time: 1228

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	Old										
Temperature (°C)	25.8	25.7	25.2	25.6	25.4	24.7	24.8	25.2	24.4	25.5	24.6	24.2	24.4	25.2
			25.6		25.8		25.1		25.4		25.3		25.5	26.0
Dissolved Oxygen (mg/L)	6.2	7.2	4.9	6.9	3.6	7.7	5.1	7.6	5.0	7.5	3.9	7.1	5.4	4.6
pH (s.u.)	7.86	7.68	7.59	7.89	7.50	8.03	7.66	7.96	7.67	7.85	7.46	7.88	7.63	7.62
Conductivity (μmho/cm)	167	169	211	168	219	167	220	167	217	168	227	161	207	207
Hardness (mg/L CaCO ₃)	120	-	-	86	-	-	-	120	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	86	-	-	103	-	-	-	103	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control - ECT

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA

Beginning Date: 4/20/21

Time: 1407

Ending Date: 4/26/21

Time: 1315

Test Organism: Ceriodaphnia dubia

Age: 6-14+1

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)	26.0	26.3	25.8	25.2	25.4 25.7	26.0	25.4 25.9	25.6	25.5 26.1	25.4 25.5	26.0 26.1			25.6 26.2
Dissolved Oxygen (mg/L)	6.0	7.3	7.4	6.7	6.7	7.2	7.3	6.7	7.0	6.6	7.1			6.4
pH (s.u.)	7.87	7.84	7.76	7.75	7.83	7.92	7.74	7.72	7.82	7.57	7.54			7.40
Conductivity (μmho/cm)	311	310	323	311	327	340	319	338	362	336	356			340
Hardness (mg/L CaCO ₃)	103	-	-	120	-	-	-	120	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	103	-	-	86	-	-	-	120	-	-	-	-	-	-

AJF
4/25

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
 Industry/Toxicant: Allen County, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA
 Beginning Date: 4/20/21 Time: 1401
 Ending Date: 4/26/21 Time: 1315
 Test Organism: Ceriodaphnia dubia Age: 10-14 H
 Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.3	25.1	25.8	25.0	25.4 25.7	25.4	25.4 25.9	24.7	25.5 26.1	25.6	26.0 26.1		25.6 26.2	
Dissolved Oxygen (mg/L)	6.5	7.2	7.5	7.9	6.7	7.4	7.3	8.2	7.0	8.1	7.2		6.7	
pH (s.u.)	7.97	8.05	8.16	7.91	8.26	7.96	7.88	8.00	7.88	7.91	7.84		7.91	
Conductivity (μmho/cm)	1130	1130	1096	940	1118	936	930	1045	944	1042	1059		1055	
Hardness (mg/L CaCO ₃)	410	-	-	222	-	-	-	376	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	222	-	-	137	-	-	-	222	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA

Beginning Date: 4/20/21

Time: 1407

Ending Date:

4/26/21

ime: 1315

Test Organism: Ceriodaphnia dubia

Dilution Water Used: Upstream

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA

Beginning Date: 4/20/21 Time: 1407

Ending Date: 4/26/21 Time: 1315

Test Organism: Ceriodaphnia dubia Age: 10-14 H

Dilution Water Used: Upstream

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA

Beginning Date: 4/20/21

Time: 1407

Ending Date:

4/2/21

ime: 1315

Test Organism: Ceriodaphnia dubia

Age: 14

Dilution Water Used: Upstream

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA

Beginning Date: 4/20/21

Time: 1407

Ending Date: 4/26/21

Time: 12:15

Test Organism: Ceriodaphnia dubia

Age: 13-14 H

Dilution Water Used: Upstream

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: BG, JDP, DJA

Beginning Date:

4/20/21

Time:

1407

Ending Date:

4/26/21

Time:

1315

Test Organism: Ceriodaphnia dubia

Age:

6-14 H

Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.3	25.3	25.8	25.3	25.4 25.7	25.7	25.4 25.9	26.0	25.5 26.1	25.7	26.0 26.1		25.6 26.2	
Dissolved Oxygen (mg/L)	7.4	7.9	7.4	7.8	6.6	7.7	7.4	8.5	7.2	8.2	7.3		7.1	
pH (s.u.)	7.54	7.77	8.01	7.57	8.15	7.87	7.99	7.66	8.02	7.49	7.82		7.94	
Conductivity (μmho/cm)	936	939	958	931	988	933	969	956	987	951	1006		990	
Hardness (mg/L CaCO ₃)	239	-	-	257	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	171	-	-	120	-	-	-	205	-	-	-	-	-	-

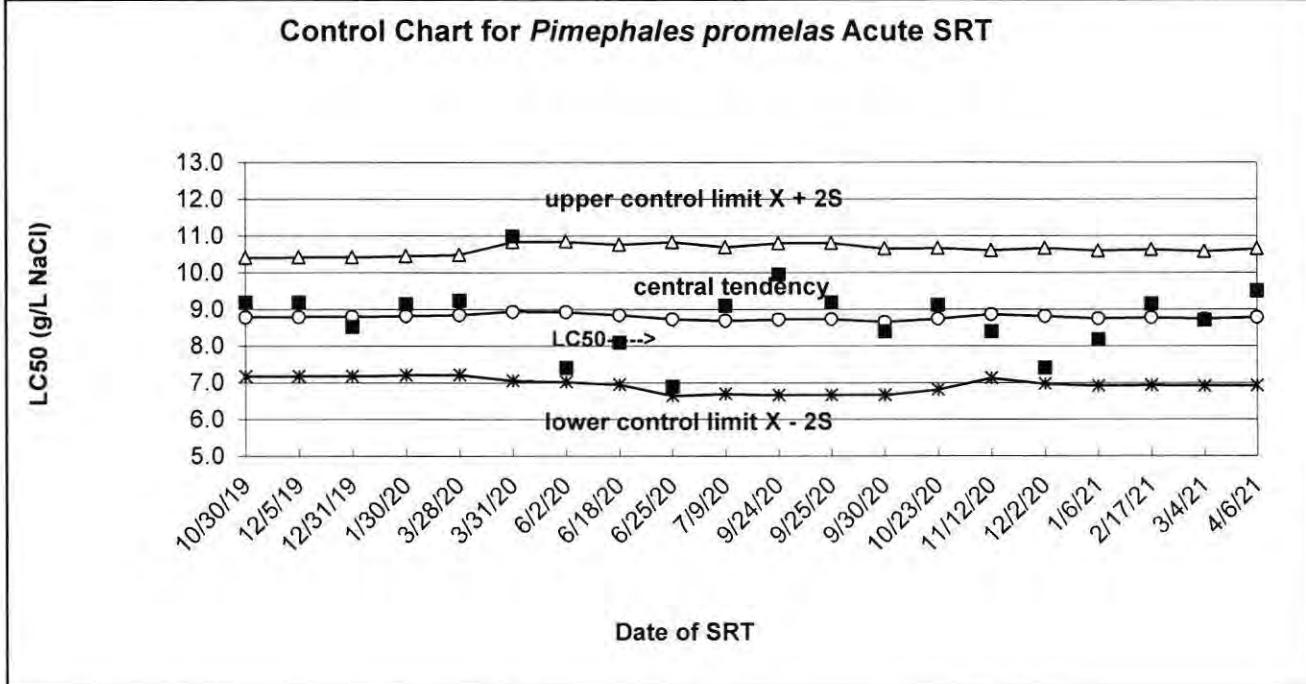
Appendix D

SRT Data

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

Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method ¹
10/30/19	9.2	8.8	0.8	10.4	7.2	0.09	11.2	6.4	TSK
12/5/19	9.2	8.8	0.8	10.4	7.2	0.09	11.2	6.4	TSK
12/31/19	8.5	8.8	0.8	10.4	7.2	0.09	11.2	6.4	Probit
1/30/20	9.1	8.8	0.8	10.5	7.2	0.09	11.3	6.4	Probit
3/28/20	9.2	8.9	0.8	10.5	7.2	0.09	11.3	6.4	Probit
3/31/20	11.0	8.9	0.9	10.8	7.1	0.11	11.8	6.1	Probit
6/2/20	7.4	8.9	1.0	10.8	7.0	0.11	11.8	6.1	Probit
6/18/20	8.1	8.9	1.0	10.8	6.9	0.11	11.7	6.0	Probit
6/25/20	6.9	8.7	1.0	10.8	6.7	0.12	11.9	5.6	Probit
7/9/20	9.1	8.7	1.0	10.7	6.7	0.11	11.7	5.7	Probit
9/24/20	10.0	8.7	1.0	10.8	6.7	0.12	11.8	5.6	Graphical
9/25/20	9.2	8.7	1.0	10.8	6.7	0.12	11.8	5.6	TSK
9/30/20	8.4	8.7	1.0	10.7	6.7	0.12	11.7	5.7	TSK
10/23/20	9.1	8.7	1.0	10.7	6.8	0.11	11.6	5.8	Probit
11/12/20	8.4	8.9	0.9	10.6	7.1	0.10	11.5	6.3	TSK
12/2/20	7.4	8.8	0.9	10.7	7.0	0.10	11.6	6.0	Probit
1/6/21	8.2	8.8	0.9	10.6	6.9	0.11	11.5	6.0	Probit
2/17/21	9.1	8.8	0.9	10.6	6.9	0.11	11.6	6.0	Probit
3/4/21	8.7	8.7	0.9	10.6	6.9	0.10	11.5	6.0	TSK
4/6/21	9.5	8.8	0.9	10.7	6.9	0.11	11.6	6.0	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.

X ± 3SD = Well outside expected range.

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

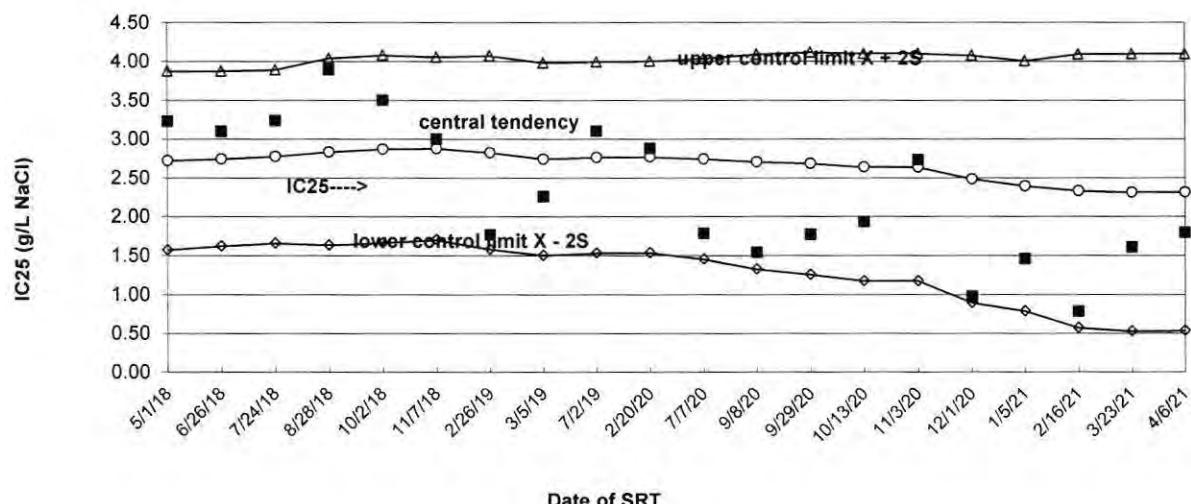
IC25 for *Pimephales promelas* Chronic NaCl SRT

Date of

Cumulative

Test	IC25	Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	CV Warning Limit**	CV Control Limit**
5/1/18	3.23	2.72	0.57	3.87	1.57	0.21	4.45	1.00		
6/26/18	3.10	2.75	0.56	3.87	1.62	0.21	4.44	1.06		
7/24/18	3.24	2.78	0.56	3.89	1.66	0.20	4.45	1.10		
8/28/18	3.89	2.84	0.60	4.04	1.63	0.21	4.64	1.03		
10/2/18	3.50	2.87	0.60	4.08	1.66	0.21	4.69	1.06		
11/7/18	3.00	2.88	0.59	4.06	1.70	0.20	4.65	1.11		
2/26/19	1.77	2.83	0.62	4.07	1.58	0.22	4.70	0.96		
3/5/19	2.26	2.74	0.62	3.98	1.50	0.23	4.60	0.88		
7/2/19	3.10	2.77	0.62	4.00	1.53	0.22	4.61	0.92		
2/20/20	2.88	2.77	0.62	4.00	1.54	0.22	4.62	0.92		
7/7/20	1.78	2.75	0.65	4.04	1.45	0.24	4.68	0.81		
9/8/20	1.54	2.71	0.69	4.09	1.32	0.26	4.79	0.63		
9/29/20	1.77	2.69	0.72	4.12	1.26	0.27	4.83	0.54		
10/13/20	1.93	2.64	0.73	4.10	1.18	0.28	4.83	0.44		
11/3/20	2.73	2.64	0.73	4.10	1.17	0.28	4.83	0.44		
12/1/20	0.98	2.49	0.80	4.08	0.89	0.32	4.88	0.10		
1/5/21	1.46	2.40	0.81	4.01	0.79	0.34	4.81	-0.02		
2/16/21	0.78	2.33	0.88	4.09	0.58	0.38	4.97	-0.30		
3/23/21	1.60	2.31	0.89	4.10	0.53	0.39	4.99	-0.36	0.88	1.04
4/6/21	1.79	2.32	0.89	4.10	0.54	0.38	4.99	-0.35		

Control Chart for *Pimephales promelas* Chronic SRT



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

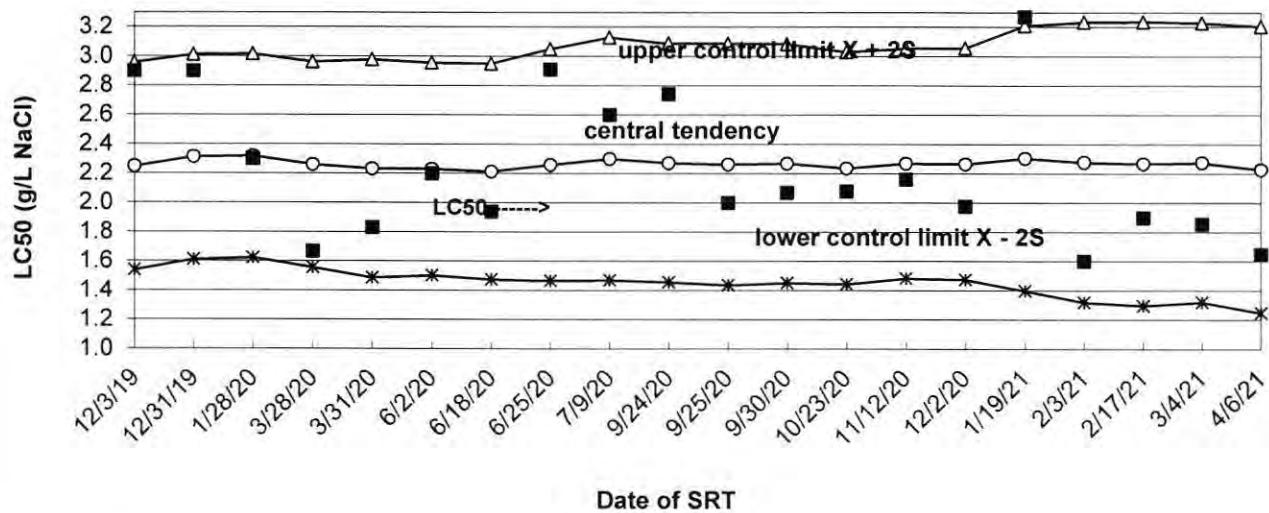
75th percentile of CVs for IC25 = 0.38

**Calculated CV should not exceed 75th percentile of CVs. If it does, warning and control limits are established using the 75th percentile (0.38) and 90th percentile (0.45) of CVs.
 $X \pm 3SD$ = Well outside expected range.

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

Date of Test	LC50	Cumulative							LC50
		LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	Method ¹
12/3/19	2.9	2.2	0.4	3.0	1.5	0.16	3.3	1.2	Probit
12/31/19	2.9	2.3	0.3	3.0	1.6	0.15	3.4	1.3	Probit
1/28/20	2.3	2.3	0.3	3.0	1.6	0.15	3.4	1.3	Probit
3/28/20	1.7	2.3	0.4	3.0	1.6	0.16	3.3	1.2	TSK
3/31/20	1.8	2.2	0.4	3.0	1.5	0.17	3.4	1.1	Probit
6/2/20	2.2	2.2	0.4	3.0	1.5	0.16	3.3	1.1	Probit
6/18/20	1.9	2.2	0.4	2.9	1.5	0.17	3.3	1.1	Probit
6/25/20	2.9	2.3	0.4	3.1	1.5	0.18	3.4	1.1	Probit
7/9/20	2.6	2.3	0.4	3.1	1.5	0.18	3.5	1.1	Probit
9/24/20	2.7	2.3	0.4	3.1	1.5	0.18	3.5	1.0	Probit
9/25/20	2.0	2.3	0.4	3.1	1.4	0.18	3.5	1.0	Probit
9/30/20	2.1	2.3	0.4	3.1	1.5	0.18	3.5	1.0	Probit
10/23/20	2.1	2.2	0.4	3.0	1.4	0.18	3.4	1.0	Probit
11/12/20	2.2	2.3	0.4	3.1	1.5	0.17	3.4	1.1	Probit
12/2/20	2.0	2.3	0.4	3.1	1.5	0.17	3.4	1.1	TSK
1/19/21	3.3	2.3	0.5	3.2	1.4	0.20	3.7	0.9	Probit
2/3/21	1.6	2.3	0.5	3.2	1.3	0.21	3.7	0.8	Probit
2/17/21	1.9	2.3	0.5	3.2	1.3	0.21	3.7	0.8	Probit
3/4/21	1.9	2.3	0.5	3.2	1.3	0.21	3.7	0.8	Probit
4/6/21	1.7	2.2	0.5	3.2	1.2	0.22	3.7	0.8	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.

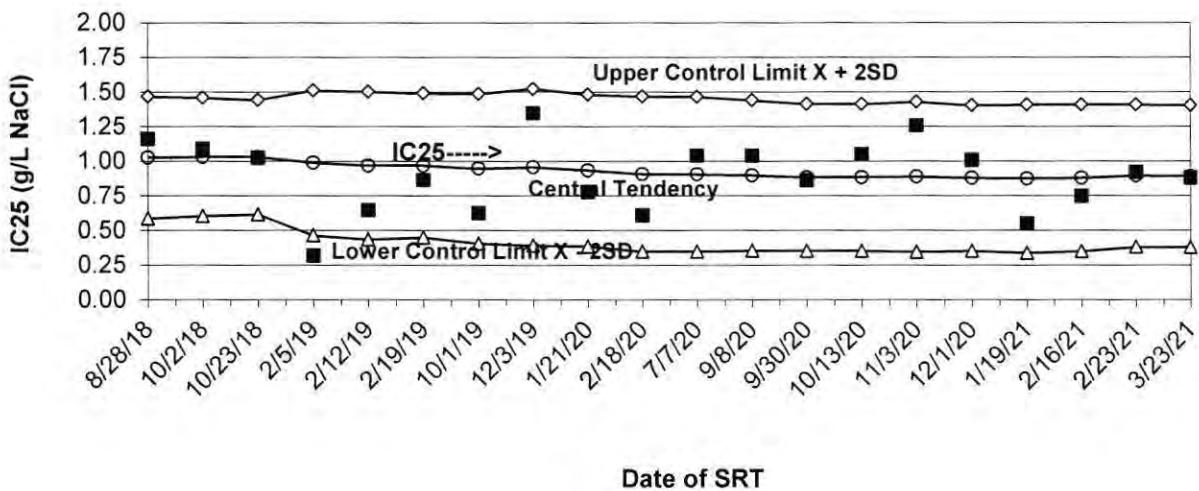
X ± 3SD = Well outside expected range.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	cv*	X+3SD	X-3SD
7/24/18	0.95	1.02	0.23	1.47	0.57	0.22	1.69	0.34
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	1.44	0.62	0.20	1.65	0.41
2/5/19	0.32	0.99	0.26	1.51	0.47	0.26	1.77	0.20
2/12/19	0.65	0.97	0.27	1.50	0.44	0.27	1.77	0.17
2/19/19	0.87	0.97	0.26	1.49	0.45	0.27	1.75	0.19
10/1/19	0.63	0.95	0.27	1.49	0.41	0.28	1.76	0.14
12/3/19	1.35	0.96	0.28	1.53	0.39	0.29	1.81	0.11
1/21/20	0.78	0.93	0.27	1.48	0.39	0.29	1.76	0.11
2/18/20	0.61	0.91	0.28	1.47	0.35	0.31	1.75	0.07
7/7/20	1.04	0.91	0.28	1.46	0.35	0.31	1.74	0.07
9/8/20	1.04	0.90	0.27	1.44	0.36	0.30	1.71	0.08
9/30/20	0.86	0.88	0.26	1.41	0.35	0.30	1.68	0.09
10/13/20	1.05	0.88	0.26	1.41	0.35	0.30	1.68	0.09
11/3/20	1.26	0.89	0.27	1.43	0.35	0.30	1.70	0.08
12/1/20	1.01	0.88	0.26	1.40	0.35	0.30	1.67	0.09
1/19/21	0.55	0.88	0.27	1.41	0.34	0.31	1.68	0.07
2/16/21	0.75	0.88	0.26	1.41	0.35	0.30	1.67	0.09
2/23/21	0.92	0.90	0.26	1.41	0.38	0.29	1.66	0.13
3/23/21	0.88	0.89	0.26	1.40	0.38	0.29	1.66	0.12

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.

X ± 3SD = Well outside expected range.