Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineers
Facility: American II WWTP
Outfall: Effluent 001
Revised Report Date: 4/22/22
Ohio EPA Permit No.: 2PH00006*KD
Alloway Lab #: L22-13127-01

Report of Chronic Biomonitoring

Samples Collected on:

4/4/22
4/6/22
4/8/22

Pimephales promelas
Ceriodaphnia dubia

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

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Outfall: Effluent 001
Report Date: 4/22/22
Ohio EPA Permit No.: 2PH00006*KD
Alloway Lab #: L22-13127-01

Date: 4/22/22

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.

Radoslaw Bolek
Technical Director

Megan Hurd
Special Projects Manager

Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 4/22/22
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	Megan Hurd
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 _____

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineers
Facility: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*KD
Date of *P. promelas* Test: 4/5/22 - 4/12/22
Date of *C. dubia* Test: 4/5/22 - 4/11/22

Summary of Chronic Toxicity Test Results
Pimephales promelas

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, *Pimephales promelas* - TUc	AA	1.0	
Acute Toxicity, *Pimephales promelas* - TUa	AA	0.2	
Upstream:			
96 Hr. Acute Toxicity, *Pimephales promelas* - % Affected	AA	2.5	
7 day Chronic Toxicity, *Pimephales promelas* - % Affected	2.5	2.5	

Summary of Chronic Toxicity Test Results
Ceriodaphnia dubia

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, *Ceriodaphnia dubia* - TUc	AA	1.0	
Acute Toxicity, *Ceriodaphnia dubia* - TUa	AA	0.2	
Upstream:			
48 Hr. Acute Toxicity, *Ceriodaphnia dubia* - % Affected	AA	10.0	
7 day Chronic Toxicity, *Ceriodaphnia dubia* - % Affected	AA	10.0	

AA = below detectable
AE = analytical data not valid

Conclusion/Comments:
For the C. dubia test, the PMSD of the dilution series was below the lower limit of 13% when compared to the primary lab control. The PMSD being biased low indicates the test is overly sensitive to toxicity, this test resulted in a non-detect and therefore the low PMSD had no effect on the test results.

Customer: Allen County Sanitary Engineers
Facility: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*KD
Date of Test: 4/5/22 - 4/12/22

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 Lab	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.461	0.038	
Secondary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.403	0.033	
20%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.409	0.058	
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.367	0.010	*
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	10.0% (10.0%)	17.5% (17.5%)	17.5% (17.5%)	0.337	0.069	*
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.367	0.038	*
100%	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.383	0.033	*

LC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Survival** <1.0
95% Confidence Limits	LL								
	UL								
EC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Growth (100/IC25)** <1.0
95% Confidence Limits	LL								
	UL								

7-Day NOEC for Mortality (%)	80		7-Day NOEC for Growth (%)	100	**Method(s) used to determine Values:**
7-Day LOEC for Mortality (%)	100		7-Day LOEC for Growth (%)	>100	
Chronic Value for Mortality (%)	89.4		Chronic Value for Growth (%)	>100	
IC25 (%)	>100		IC50 (%)	>100	IC Pin, Dunnett's Test

*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:

OEPA Permit No.: 2PH00006*KD (American II WWTP)

PIMEPHALES PROMELAS CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	*Pimephales promelas* < 24 hours old
2. Test Type and Duration:	Static renewal; 7 days
3. Test Dates:	4/5/22 - 4/12/22
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. 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 - 4/4/22 ; day 2, 3 - 4/6/22 ; day 4, 5, 6 - 4/8/22
15. Dilution Control Water:	Lab rearing unit water
16. Secondary Control Water:	Upstream grab from the receiving stream
17. 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:	

Customer: Allen County Sanitary Engineers
Facility: American II WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00006*KD
Date of Test: 4/5/22 - 4/11/22

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 Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	--- (---)	34.1
Secondary 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%)	--- (---)	39.9
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%)	--- (---)	37.6
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%)	--- (---)	37.9
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%)	--- (---)	38.3
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%)	--- (---)	37.9
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	38.8
LC50 Values (%):	>100	>100	>100	>100	>100	>100	---	**Calculated TUc Value for Survival**
95% Confidence Limits LL								
95% Confidence Limits UL								<1.0
EC50 Values (%):	>100	>100	>100	>100	>100	>100	---	**Calculated TUc Value for Reproduction**
95% Confidence Limits LL								
95% Confidence Limits UL								<1.0

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

For the C. dubia test, the PMSD of the dilution series was below the lower limit of 13% when compared to the primary lab control. The PMSD being biased low indicates the test is overly sensitive to toxicity, this test resulted in a non-detect and therefore the low PMSD had no effect on the test results.

OEPA Permit No.: 2PH00006*KD (American II WWTP)

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	*Ceriodaphnia dubia* 4.1 - 12.1 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:	4/5/22 - 4/11/22
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Fed 0.1 mL YCT and 0.1 mL (3.5 million cells) *Selenastrum capricornutum.*
8. Size of Test Vessel:	1 fl. oz. (30 mL) clear plastic cup
9. Volume and Depth of Test Solutions:	15 mL, 20 mm deep
10. No. of Test Organisms per Test Vessel:	1 per test vessel
11. No. of Test Vessels per Test Solution:	10 per solution tested
12. Total No. of Test Organisms per Test Solution:	10 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 4/4/22 ; day 2, 3 - 4/6/22 ; day 4, 5, 6 - 4/8/22
15. Dilution Control Water:	Lab rearing unit water
16. Secondary Control Water:	Upstream grab from the receiving stream
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:	

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Outfall: Effluent 001
Report Date: 4/22/22
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Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection Beginning	Sample Collection Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	4/3/22 @ 0830	4/4/22 @ 0830
Type (Grab/Comp):	Comp	2nd	4/5/22 @ 0840	4/6/22 @ 0840
Volume Collected:	5 Gal.	3rd	4/7/22 @ 0840	4/8/22 @ 0840
Upstream Station:				
Waterbody:	Dug Run			
Station No.:	801	1st		4/4/22 @ 1339
Type (Grab/Comp):	Grab	2nd		4/6/22 @ 1122
Volume Collected:	5 Gal.	3rd		4/8/22 @ 1258

Samples collected by: Alloway

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.

1/3

● 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: Mike Santaguida
Company: Allen County Sanitary Eng.
Address: 4140 Diller Rd.
Lima, OH 45805

Invoice To (If Different):
Name:
Company:
Address:

Notes/Comments:

L22-13127

Phone #: 419-996-4696
Fax #:
E-mail: msantaguida@allencountyohio.com
PO#:

Project Name: Am II Bioassay - Day One

Sampler: (Print) Michael S. Hawly (Signature)

Turnaround: (*Rush Charges May Apply*)
Next Day ☐ 3 Working Days ☐ Routine ☑
2 Working Days ☐ 5 Working Days ☐

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	4/03/22-4/04/22	08:30-08:30	X		WW	1	1	Toxicity	01
2	Upstream	4/24/22	13:39		X	SW	1	1	Toxicity	1
3										
4										
5										
6										
7										
8										

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

Received for Laboratory By: (circle one): Mansfield Lima Marion
(Signature) 4/24/22 13:54

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

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

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

Sample Receiving (*For Lab Use Only*)
Ice Present? Y ☑ N ☐
Proper Preservation? Y ☑ N ☐
Container Temperature: 8°C T-120 BDP

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

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

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.

2/3

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: Mike Santaguida
Company: Allen County Sanitary Eng.
Address: 4140 Diller Rd. 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 Two
Sampler: (Print) Michael S. Hanly (Signature)

Turnaround: (*Rush Charges May Apply*)
Next Day ☐ 3 Working Days ☐ Routine ☑
2 Working Days ☐ 5 Working Days ☐

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	4/05/22 4/06/22	08:40 - 08:40	X		WW	1	1	Toxicity	01
2	Upstream	4/06/22	11:22		X	SW	1	1	Toxicity	1
3										
4										
5										
6										
7										
8										

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

Received for Laboratory By: (circle one): Mansfield Lima Marion
(Signature) Date: 4/22/22 Time: 11:42

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

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

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

Sample Receiving (For Lab Use Only)
Ice Present? Y ☒ N ☐
Proper Preservation? Y ☒ N ☐
Container Temperature: 3°C T-120 8DP

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

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

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.

3/3

● 1101 North Cole Street, Lima, OH 45805
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○ 1502 W. Fourth Street, Mansfield, OH 44906
(P) 419-525-1644 (F) 419-524-5575

Report To:
Name: Mike Santaguida
Company: Allen County Sanitary Eng.
Address: 4140 Diller Rd.
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

Sampler: (Print) Michael S. Hanly (Signature)

Turnaround: (*Rush Charges May Apply*)
Next Day ☐ 3 Working Days ☐ Routine ☑
2 Working Days ☐ 5 Working Days ☐

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	4/07/22-4/08/22	08:40-08:40	X		WW	1	1	Toxicity	01
2	Upstream	4/08/22	12:58		X	SW	1	1	Toxicity	1
3										
4										
5										
6										
7										
8										

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

Received for Laboratory By: (circle one): Mansfield Lima Marion
(Signature)
Date: 4/08/22 Time: 13:24

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

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

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

Sample Receiving
(For Lab Use Only)
Ice Present? Y ☒ N ☐
Proper Preservation? Y ☒ N ☐
T-120
Container Temperature: 3.0°C

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

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

Appendix B

Raw Data Sheets

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/5/2022	Test ID:	AMII0422Pp	Sample ID:	L22-13127
End Date:	4/12/2022	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 Control	0.4840	0.4870	0.4690	0.4050
20	0.4190	0.3330	0.4750	0.4090
40	0.3740	0.3730	0.3700	0.3530
60	0.3840	0.3890	0.2400	0.3370
80	0.4040	0.3440	0.3270	0.3950
100	0.3450	0.4240	0.3730	0.3890

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Lab Control	0.4613	1.0000	0.4613	0.4050	0.4870	8.307	4				0.4613	1.0000
20	0.4090	0.8867	0.4090	0.3330	0.4750	14.279	4	1.634	2.410	0.0770	0.4090	0.8867
*40	0.3675	0.7967	0.3675	0.3530	0.3740	2.671	4	2.933	2.410	0.0770	0.3675	0.7967
*60	0.3375	0.7317	0.3375	0.2400	0.3890	20.472	4	3.871	2.410	0.0770	0.3626	0.7861
*80	0.3675	0.7967	0.3675	0.3270	0.4040	10.279	4	2.933	2.410	0.0770	0.3626	0.7861
*100	0.3828	0.8298	0.3828	0.3450	0.4240	8.614	4	2.456	2.410	0.0770	0.3626	0.7861

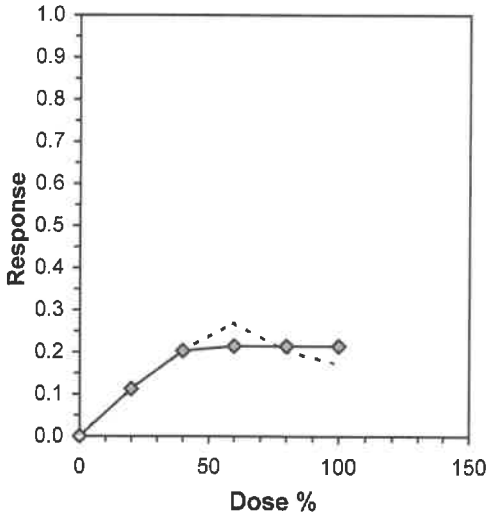
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.95536	0.916	-0.7309	0.43773
Bartlett's Test indicates equal variances (p = 0.16)	8.0092	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	20	40	28.2843	5	0.07704	0.16703	0.00738	0.00204	0.01948	5, 18
Treatments vs Lab Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	8.828	6.683	1.699	36.096	1.1167
IC10*	17.656	7.656	3.398	41.802	0.5581
IC15	28.163				
IC20	39.277				
IC25	>100				
IC40	>100				
IC50	>100				

* indicates IC estimate less than the lowest concentration



ToxCalc v5.0.32

Reviewed by: MLA

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: DJA, JDP, MHH
Beginning: Date 4/5/22 **Time** 1322
Ending: Date 4/12/22 **Time** 1120
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Lab Control

	Upstream Control					Lab Control					20% Effluent					40% Effluent				
Day	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
3	10G	10G	10G	10G	40G	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	10G	39G 1D	10G	10G	10G	10G	40G
4	10G	10G	10G	10G	40G	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	10G	39G 1D	10G	10G	10G	10G	40G
5	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	10G	39G 1D	10G	10G	10G	10G	40G
6	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	10G	39G 1D	10G	10G	10G	10G	40G
7	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	9G 1D	39G 1D	10G	9G 1D	10G	10G	39G 1D	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, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00006*KD

Person(s) Conducting Test: DJA, JDP, MHH
Beginning: Date 4/5/22 **Time** 1322
Ending: Date 4/12/22 **Time** 1120
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Lab Control

	60% Effluent					80% Effluent					100% Effluent									
Day	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	9G 1D	10G	10G	10G	39G 1D					
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	9G 1D	10G	10G	10G	39G 1D					
4	10G	10G	9G 1D	10G	39G 1D	10G	10G	10G	10G	40G	9G 1D	10G	10G	9G 1D	38G 2D					
5	10G	10G	7G 3D	9G 1D	36G 4D	9G 1D	10G	10G	10G	39G 1D	9G 1D	10G	10G	9G 1D	38G 2D					
6	10G	10G	5G 5D	8G 2D	33G 7D	9G 1D	10G	10G	10G	39G 1D	9G 1D	10G	10G	9G 1D	38G 2D					
7	10G	10G	5G 5D	8G 2D	33G 7D	9G 1D	10G	10G	10G	39G 1D	9G 1D	10G	10G	9G 1D	38G 2D					

* 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, Am. II WWTP
Date of Test: 4/5/22 - 4/12/22
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: _____
Analyst: _____

Balance Calibration Check: 1 gram weight = _____ grams
Date: _____
Analyst: _____
Dilution Water Used: Lab Control

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)	Average / Standard Deviation
Fish at Start of Test					10			
Upstream Control	1	9	1.09274	1.09630	10	3.56	0.356	
	2	10	1.10437	1.10869	10	4.32	0.432	
	3	10	1.09200	1.09614	10	4.14	0.414	0.403
	4	10	1.08799	1.09211	10	4.12	0.412	0.033
Lab Control	1	10	1.12319	1.12803	10	4.84	0.484	
	2	10	1.08487	1.08974	10	4.87	0.487	
	3	10	1.08118	1.08587	10	4.69	0.469	0.461
	4	10	1.09656	1.10061	10	4.05	0.405	0.038
20% Effluent	1	10	1.08670	1.09089	10	4.19	0.419	
	2	9	1.09159	1.09492	10	3.33	0.333	
	3	10	1.08742	1.09217	10	4.75	0.475	0.409
	4	10	1.10427	1.10836	10	4.09	0.409	0.058
40% Effluent	1	10	1.09844	1.10218	10	3.74	0.374	
	2	10	1.09869	1.10242	10	3.73	0.373	
	3	10	1.09813	1.10183	10	3.70	0.370	0.367
	4	10	1.12947	1.13300	10	3.53	0.353	0.010

Reviewed By: MMA

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: 4/5/22 - 4/12/22
Pimephales promelas Age at Start: < 24 Hours
Dilution Water Used: Lab Control

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)	Average / Standard Deviation
60% Effluent	1	10	1.10608	1.10992	10	3.84	0.384	
	2	10	1.09833	1.10222	10	3.89	0.389	
	3	5	1.09163	1.09403	10	2.40	0.240	0.337
	4	8	1.10199	1.10536	10	3.37	0.337	0.069
80% Effluent	1	9	1.09383	1.09787	10	4.04	0.404	
	2	10	1.08670	1.09014	10	3.44	0.344	
	3	10	1.15317	1.15644	10	3.27	0.327	0.367
	4	10	1.12602	1.12997	10	3.95	0.395	0.038
100% Effluent	1	9	1.10387	1.10732	10	3.45	0.345	
	2	10	1.08759	1.09183	10	4.24	0.424	
	3	10	1.16376	1.16749	10	3.73	0.373	0.383
	4	9	1.12664	1.13053	10	3.89	0.389	0.033
	1				10	0.00	0.000	
	2				10	0.00	0.000	
	3				10	0.00	0.000	0.000
	4				10	0.00	0.000	0.000

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
Date of Test: 4/5/22 - 4/12/22
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: 4/21/22
Analyst: DDP

Balance Calibration Check: 1 gram weight = 1.00000 / 1.00000 grams
Date: 4/19/22 / 4/21/22
Analyst: DDP / DDP
Dilution Water Used: Lab Control

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)	Average / Standard Deviation
Fish at Start of Test			1.07774		10			
Lab ~~Upstream~~ Control DDP 4/19/22	1	10	1.12319	1.12803	10	0.00	0.000	
	2	10	1.08487	1.08974	10	0.00	0.000	
	3	10	1.08118	1.08587	10	0.00	0.000	0.000
	4	9	1.09656	1.10061	10	0.00	0.000	0.000
Upstream ~~Lab~~ Control DDP 4/19/22	1	9	1.09274	1.09630	10	0.00	0.000	
	2	10	1.10437	1.10869	10	0.00	0.000	
	3	10	1.09200	1.09614	10	0.00	0.000	0.000
	4	10	1.08799	1.09211	10	0.00	0.000	0.000
20% Effluent	1	10	1.08670	1.09089	10	0.00	0.000	
	2	9	1.09159	1.09492	10	0.00	0.000	
	3	10	1.08742	1.09217	10	0.00	0.000	0.000
	4	10	1.10427	1.10836	10	0.00	0.000	0.000
40% Effluent	1	10	1.09844	1.10218	10	0.00	0.000	
	2	10	1.09869	1.10242	10	0.00	0.000	
	3	10	1.09813	1.10183	10	0.00	0.000	0.000
	4	10	1.12947	1.13300	10	0.00	0.000	0.000

Reviewed By: [illegible]

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: 4/5/22 - 4/12/22
Pimephales promelas Age at Start: < 24 Hours
Dilution Water Used: Lab Control

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)	Average / Standard Deviation
60% Effluent	1	10	1.10608	1.10992	10	0.00	0.000	
	2	10	1.09833	1.10222	10	0.00	0.000	
	3	5	1.09163	1.09403	10	0.00	0.000	0.000
	4	8	1.10199	1.10536	10	0.00	0.000	0.000
80% Effluent	1	9	1.09383	1.09787	10	0.00	0.000	
	2	10	1.08670	1.09014	10	0.00	0.000	
	3	10	1.15317	1.15644	10	0.00	0.000	0.000
	4	10	1.12602	1.12997	10	0.00	0.000	0.000
100% Effluent	1	9	1.10387	1.10732	10	0.00	0.000	
	2	10	1.08759	1.09183	10	0.00	0.000	
	3	10	1.16376	1.16749	10	0.00	0.000	0.000
	4	9	1.12664	1.13053	10	0.00	0.000	0.000
	1				10	0.00	0.000	
	2				10	0.00	0.000	
	3				10	0.00	0.000	0.000
	4				10	0.00	0.000	0.000

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	4/5/2022	Test ID:	AMII0422Cd	Sample ID:	L22-13127
End Date:	4/11/2022	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
Lab Control	36.000	40.000	34.000	35.000	36.000	33.000	35.000	35.000	36.000	21.000
20	38.000	37.000	36.000	37.000	35.000	40.000	40.000	39.000	35.000	39.000
40	35.000	38.000	41.000	36.000	39.000	37.000	37.000	44.000	36.000	36.000
60	35.000	38.000	38.000	39.000	38.000	39.000	37.000	40.000	40.000	39.000
80	39.000	37.000	38.000	35.000	39.000	37.000	39.000	38.000	37.000	40.000
100	38.000	40.000	38.000	38.000	40.000	40.000	38.000	39.000	38.000	39.000

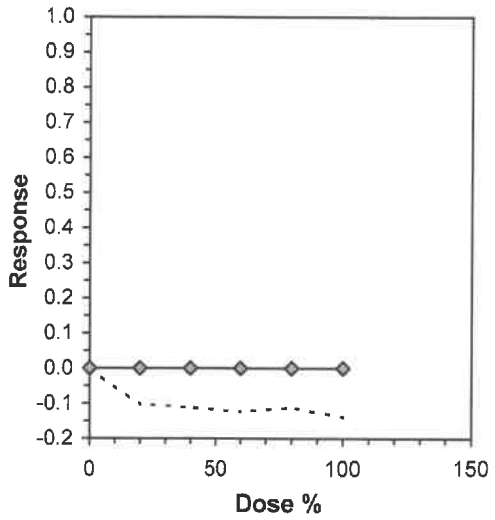
			Transform: Untransformed					Rank	1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	MSD	Mean	N-Mean
Lab Control	34.100	1.0000	34.100	21.000	40.000	14.529	10				37.433	1.0000
20	37.600	1.1026	37.600	35.000	40.000	5.046	10	135.50	75.00	2.472	37.433	1.0000
40	37.900	1.1114	37.900	35.000	44.000	7.300	10	138.00	75.00		37.433	1.0000
60	38.300	1.1232	38.300	35.000	40.000	3.902	10	141.50	75.00		37.433	1.0000
80	37.900	1.1114	37.900	35.000	40.000	3.824	10	141.00	75.00		37.433	1.0000
100	38.800	1.1378	38.800	38.000	40.000	2.368	10	146.50	75.00		37.433	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	1.25086	0.895	-2.0802	12.5494
Bartlett's Test indicates unequal variances (p = 7.97E-06)	31.3549	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDp
Steel's Many-One Rank Test	100	>100		1	
Treatments vs Lab Control					7.852

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



 ToxCalc v5.0.32 Reviewed by: MAHO

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: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1550
Age of *Ceriodaphnia dubia*: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	6 G	0 G	13 G	20 G		39	3
2				0	7	14	20		41	3
3					6	13	19		38	3
4					6	15	22		43	3
5				0	7	15	20		42	3
6				7	0	15	20		42	3
7				7	0	14	16		37	3
8				0	5	16	19		40	3
9					7	14	18		39	3
10	0 G	0 G	0 G	0 G	6 G +0	13 G	19 G		38	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									399	30
MEAN									39.9	3
S.D.									2.03	0.00

DJA
4/9/22

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

Reviewed by: MAH

CERIODAPHNIA DUBIA 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: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1550
Age of *Ceriodaphnia dubia*: 4.1 – 12.1 hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	5/G	14/G	17/G	/		36	3
2	/	/	/	/	7/	13/	20/	/		40	3
3	/	/	/	/	6/	14/	14/	/		34	3
4	/	/	/	/	6/	12/	17/	/		35	3
5	/	/	/	↓/	7/	13/	16/	/		36	3
6	/	/	/	0/	6/	13/	14/	/		33	3
7	/	/	/	6/	0/	15/	14/	/		35	3
8	/	/	/	0/	7/	12/	16/	/		35	3
9	/	↓/	↓/	↓/	7/↓	12/↓	17/G	/		36	3
10	0/G	0/G	0/G	0/	6/G	15/G	0/D	-/-	D	21	2
Total Adult Cond.	10G	10G	10G	10G	10G	10G	9G 1D				
									SUM	341	29
									MEAN	34.1	2.9
									S.D.	4.95	0.316

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Age of *Ceriodaphnia dubia*: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	8/G	12/G	18/G		38	3
2					7	13	17		37	3
3					7	12	17		36	3
4					6	14	17		37	3
5					7	13	15		35	3
6					7	14	19		40	3
7					6	15	19		40	3
8					7	13	19		39	3
9					6	11	18		35	3
10	0/G	0/G	0/G	0/G	7/G	13/G	19/G		39	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	376	30
								MEAN	37.6	3
								S.D.	1.90	0.00

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1550
Age of *Ceriodaphnia dubia*: 4.1 - 12.1 Hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	6/G	15/G	14/G	/	35	3
2	/	/	/	/	6/	14/	18/	/	38	3
3	/	/	/	/	6/	15/	20/	/	41	3
4	/	/	/	/	7/	13/	16/	/	36	3
5	/	/	/	/	6/	14/	19/	/	39	3
6	/	/	/	/	5/	15/	17/	/	37	3
7	/	/	/	/	6/	14/	17/	/	37	3
8	/	/	/	/	8/	15/	21/	/	44	3
9	/	/	/	/	6/	12/	18/	/	36	3
10	0/G	0/G	0/G	0/G	8/G	12/G	16/G	/	36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
									SUM 379	30
									MEAN 37.9	3
									S.D. 2.77	0.00

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1500
Age of *Ceriodaphnia dubia*: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	6/G	13/G	16/G		35	3
2					6	14	18		38	3
3					6	14	18		38	3
4					6	14	19		39	3
5					6	14	18		38	3
6					7	14	18		39	3
7					7	13	17		37	3
8					7	14	19		40	3
9					7	15	18		40	3
10	0/G	0/G	0/G	0/G	8/G	14/G	17/G		39	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									383	30
MEAN									38.3	3
S.D.									1.49	0.00

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Age of *Ceriodaphnia dubia*: 4.1 - 12.1 Hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	0/G	7/G	12/G	20/G	/	39	3
2	/	/	/	/	7/	13/	17/	/	37	3
3	/	/	/	/	6/	13/	19/	/	38	3
4	/	/	/	/	6/	11/	18/	/	35	3
5	/	/	/	/	6/	13/	20/	/	39	3
6	/	/	/	/	6/	12/	19/	/	37	3
7	/	/	/	/	8/	13/	18/	/	39	3
8	/	/	/	/	6/	13/	19/	/	38	3
9	/	/	/	/	6/	12/	19/	/	37	3
10	0/G	0/G	0/G	0/G	7/G	15/G	18/G	/	40	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	379	30
								MEAN	37.9	3
								S.D.	1.45	0.00

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PH00006*KD

Person(s) Conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Age of *Ceriodaphnia dubia*: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	0 G	7 G	15 G	16 G		38	3
2					6	14	20		40	3
3					6	13	19		38	3
4					6	13	19		38	3
5					6	16	18		~~40~~ 40	3
6					6	15	19		MHH 4/12/22 ~~38~~ 40	3
7					6	13	19		~~39~~ 38	3
8					4	16	19		39	3
9					7	13	18		38	3
10	0 G	0 G	0 G	0 G	6 G	14 G	19 G		39	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
								SUM	388	30
								MEAN	38.8	3
								S.D.	0.919	0.00

Appendix C

Parameter Data

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: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

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)	24.4	25.1	24.8/25.4	24.8	24.8/25.5	25.0	24.5/25.2	24.5	24.2/~~25~~24.8	23.7	23.4/23.8	25.0	24.8/25.4	24.8/25.5
Dissolved Oxygen (mg/L)	8.0	7.7	6.6	7.0	5.8	7.7	4.5	7.8	6.4	7.6	4.7	7.8	6.7	7.2
pH (s.u.)	7.41	7.44	7.19	7.60	7.16	7.91	7.16	7.73	7.18	7.47	7.60	7.15	7.60	7.23
Conductivity (µmho/cm)	378	378	382	442	388	442	457	441	469	394	470	375	409	401
Hardness (mg/L $CaCO_3$)	137	-	-	171	-	-	-	154	DJA 4/9/22	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	86	-	-	103	-	-	-	103	-	-	-	-	-	-

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: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

Day	0	1		2		3 DJA 4/8/22		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	24.8	25.0	24.8/25.4	24.9	24.8/25.5	24.3	24.5/25.2	24.4	24.2/24.8	24.7	23.4/23.8	25.0	24.8/25.4	24.8/25.5
Dissolved Oxygen (mg/L)	8.6	8.2	6.1	8.9	4.5	8.3	5.5	10.9	6.3	8.2	4.9	8.6	6.4	6.2
pH (s.u.)	8.01	7.84	7.69	7.92	7.53	8.04	7.80	8.06	7.80	8.04	7.65	7.75	7.73	7.61
Conductivity (µmho/cm)	1071	1067	1053	1010	1074	1007	1012	962	1006	963	969	960	971	984
Hardness (mg/L $CaCO_3$)	325	-	-	376	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	188	-	-	205	-	-	-	239	-	-	-	-	-	-

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: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

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)	24.9	24.8	24.8 / 25.4	24.9	24.8 / 25.5	24.5	24.8 / 25.2	25.2	24.2 / 24.8	24.7	23.4 / 23.8	24.9	24.8 / 25.4	24.8 / 25.5
Dissolved Oxygen (mg/L)	8.1	8.2	6.5	7.9	5.1	8.4	4.7	8.2	4.7	7.3	4.7	7.0	6.2	6.9
pH (s.u.)	7.89	7.91	7.66	7.84	7.54	7.91	7.71	7.89	7.62	7.82	7.72	7.62	7.74	7.52
Conductivity (µmho/cm)	500	497	545	500	545	502	541	552	538	559	599	559	606	610
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

Solution Tested: 40% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

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)	24.8	24.8	24.8/25.4	24.8	24.8/25.5	24.6	24.8/25.2	24.1	24.2/24.8	24.9	23.4/23.8	24.8	24.8/25.4	24.8/25.5
Dissolved Oxygen (mg/L)	8.0	8.3	6.4	7.8	5.7	8.4	4.5	8.4	4.4	7.1	5.0	6.9	6.3	6.4
pH (s.u.)	7.73	7.78	7.61	7.61	7.44	7.75	7.57	7.67	7.49	7.71	7.63	7.52	7.67	7.52
Conductivity (µmho/cm)	620	619	625	632	647	631	645	651	647	651	679	650	680	688
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

Solution Tested: 60% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

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)	24.9	24.9	24.8/25.4	24.8	24.8/25.5	24.7	24.8/25.2	24.9	24.2/24.8	25.0	23.4/23.8	25.0	24.8/25.4	24.8/25.5
Dissolved Oxygen (mg/L)	8.1	8.2	6.5	7.9	4.6	8.1	4.1	8.6	4.2	7.2	5.2	6.5	6.5	6.0
pH (s.u.)	7.61	7.71	7.64	7.57	7.48	7.71	7.51	7.55	7.45	7.63	7.60	7.46	7.66	7.52
Conductivity (µmho/cm)	744	744	750	754	767	755	771	756	770	757	783	756	793	811
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

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)	24.8	24.9	24.8 / 25.4	24.9	24.8 / 25.5	24.9	24.8 / 25.2	24.8	24.2 / 24.8	24.8	23.4 / 23.8	25.0	24.8 / 25.4	24.8 / 25.5
Dissolved Oxygen (mg/L)	8.2	8.1	5.9	7.9	4.3	8.2	4.2	9.0	3.6	7.1	5.4	6.8	6.3	6.0
pH (s.u.)	7.53	7.67	7.69	7.54	7.55	7.68	7.55	7.47	7.47	7.58	7.62	7.45	7.68	7.57
Conductivity (µmho/cm)	859	858	866	852	887	853	869	856	870	856	886	856	884	907
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

Solution Tested: 100% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 4/5/22 Time: 1322
Ending Date: 4/12/22 Time: 1120
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Lab Control

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)	24.8	24.8	24.8 / 25.4	24.9	24.8 / 25.5	24.7	24.8 / 25.2	24.6	24.2 / 24.8	24.8	23.4 / 23.8	24.9	24.8 / 25.4	24.8 / 25.5
Dissolved Oxygen (mg/L)	8.4	8.1	6.3	8.1	5.4	8.3	4.1	9.2	6.1	7.6	5.3	8.1	5.9	6.9
pH (s.u.)	7.45	7.63	7.78	7.49	7.75 / ~~7.66~~	7.66	7.61	7.41	7.72	7.57	7.66	7.57	7.70	7.75 / ~~7.62~~
Conductivity (µmho/cm)	975	974	974	953	995	950	970	958	964	957	993	966	990	1008
Hardness (mg/L $CaCO_3$)	308	-	-	325	DJA 4/7/22	-	-	291	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	205	-	-	205	-	-	-	205	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA 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: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12.1 Hrs
Dilution Water Used: Lab Control

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)	24.4	25.1	24.7 / 24.8	24.8	24.6 / 24.7	25.0	24.8 / 25.0	24.5	24.5 / 25.0	23.7	24.5 / 24.8		24.8	
Dissolved Oxygen (mg/L)	8.0	7.7	7.9	7.0	7.6	7.7	7.9	7.8	7.6	7.6	7.2		8.1	
pH (s.u.)	7.41	7.44	7.27	7.60	7.23	7.91	7.80	7.73	7.79	7.47	7.37		7.19	
Conductivity (µmho/cm)	378	378	380	442	381	442	391	441	450	394	443		408	
Hardness (mg/L $CaCO_3$)	137	-	-	171	-	-	-	154	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	86	-	-	103	-	-	-	103	-	-	-	-	-	-

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: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12.1 Hrs
Dilution Water Used: Lab Control

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)	24.8	25.0	24.7/24.8	24.9	24.6/24.7	24.3	24.8/25.0	24.4	24.5/25.0	24.7	24.5/24.8		24.8	
Dissolved Oxygen (mg/L)	8.6	8.2	7.9	8.9	7.5	8.3	7.9	10.9	7.5	8.2	7.3		8.1	
pH (s.u.)	8.01	7.84	8.03	7.92	8.08	8.04	8.19	8.06	8.12	8.04	8.24		8.02	
Conductivity (µmho/cm)	1071	1067	1067	1010	1056	1007	1010	962	1005	963	948		961	
Hardness (mg/L $CaCO_3$)	325	-	-	376	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	188	-	-	205	-	-	-	239	-	-	-	-	-	-

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: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

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)	24.9	24.8	24.7/24.8	24.9	24.6/24.7	24.5	24.8/25.0	25.2	24.5/25.0	24.7	24.5/24.8		24.8	
Dissolved Oxygen (mg/L)	8.1	8.2	8.0	7.9	7.7	8.4	8.0	8.2	7.6	7.3	7.4		8.1	
pH (s.u.)	7.89	7.91	8.10	7.84	8.09	7.91	8.18	7.89	8.05	7.82	8.20		8.09	
Conductivity (µmho/cm)	500	497	542	500	521	502	545	552	545	559	588		598	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

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)	24.8	24.8	24.7/24.8	24.8	24.6/24.7	24.6	24.8/25.0	24.1	24.5/25.0	24.9	24.5/24.8		24.8	
Dissolved Oxygen (mg/L)	8.0	8.3	8.0	7.8	7.7	8.4	8.0	8.4	7.6	7.1	7.5		8.2	
pH (s.u.)	7.73	7.78	8.03	7.61	8.02	7.75	8.11	7.67	8.01	7.71	8.15		8.04	
Conductivity (µmho/cm)	620	619	638	632	632	631	659	651	651	651	670		676	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

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)	24.9	24.9	24.7 / 24.8	24.8	24.6 / 24.7	24.7	24.8 / 25.0	24.9	24.5 / 25.0	25.0	24.5 / 24.8		24.8	
Dissolved Oxygen (mg/L)	8.1	8.2	8.0	7.9	7.7	8.1	7.9	8.6	7.6	7.2	7.5		8.1	
pH (s.u.)	7.61	7.71	8.02	7.57	8.04	7.71	8.17	7.55	8.07	7.63	8.16		8.05	
Conductivity (µmho/cm)	744	744	762	754	758	755	775	756	775	757	772		781	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11/2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12.1 Hrs.
Dilution Water Used: Lab Control

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)	24.8	24.9	24.7 / 24.8	24.9	24.6 / 24.7	24.9	24.8 / 25.0	24.8	24.5 / 25.0	24.8	24.5 / 24.8		24.8	
Dissolved Oxygen (mg/L)	8.2	8.1	8.0	7.9	7.7	8.2	7.9	9.0	7.6	7.1	7.5		8.1	
pH (s.u.)	7.53	7.67	8.05	7.54	8.11	7.68	8.27	7.47	8.12	7.58	8.19		8.09	
Conductivity (µmho/cm)	859	858	884	852	871	853	882	856	872	856	872		881	
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen County, Am. II WWTP
Address: 4140 Diller Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00006*KD

Person(s) conducting Test: JDP, DJA, MHH
Beginning Date: 4/5/22 Time: 1305
Ending Date: 4/11 /2022 Time: 1550
Test Organism: Ceriodaphnia dubia Age: 4.1-12 HRS.
Dilution Water Used: Lab Control

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)	24.8	24.8	24.7/24.8	24.9	24.6/24.7	24.7	24.8/25.0	24.6	24.5/25.0	24.8	24.5/24.8		24.8	
Dissolved Oxygen (mg/L)	8.4	8.1	8.0	8.1	7.7	8.3	7.9	9.2	7.6	7.6	7.5		8.1	
pH (s.u.)	7.45	7.63	8.13	7.49	8.19	7.66	8.30	7.41	8.25	7.57	8.25		8.15	
Conductivity (µmho/cm)	975	974	996	953	990	950	979	958	967	957	975		969	
Hardness (mg/L $CaCO_3$)	388	-	-	325	-	-	-	291	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	205	-	-	205	-	-	-	205	-	-	-	-	-	-

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: 4/5/22 -

1st Sample Collection

Date Samples Collected: 4/4/22 Date Samples Read: 4/5/22 Analyst: SDP

	100% Effluent	Upstream	-	-
Temperature (°C)	12.4	11.3		
Dissolved Oxygen (mg/L)	10.9	13.4		
pH (s.u.)	7.51	7.93		
Conductivity (µmhos)	980	1078		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Yellow	Tan		

2nd Sample Collection

Date Samples Collected: 4/6/22 Date Samples Read: 4/7/22 Analyst: SDP

	100% Effluent	Upstream	-	-
Temperature (°C)	11.0	10.9		
Dissolved Oxygen (mg/L)	10.7	12.7		
pH (s.u.)	7.72	8.24		
Conductivity (µmhos)	956	1009		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Yellow	Light Yellow		

3rd Sample Collection

Date Samples Collected: 4/8/22 Date Samples Read: 4/9/22 Analyst: DJA

	100% Effluent	Upstream	-	-
Temperature (°C)	9.8	11.5		
Dissolved Oxygen (mg/L)	10.4	16.1		
pH (s.u.)	7.66	8.23		
Conductivity (µmhos)	951	940		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Yellow	Light Yellow		

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 SDP 4/5/22 4
Type of Test: Chronic Date of Test: 4/5/22 - ~~3/11~~ /2022
Effluent Serial No.: 001 Permit No.: 2PH00006*KD

Investigator: SDP Culture No.: 204
Culture initiation date: 3/30/22 Culture ending date: 4/6/22
Origin of parental organisms: 202
Age at start of culture: <32 H
Culture water renewal frequency: minimum 3 times per week Culture water: Dechlorinated Lima
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)	A4	22	0100-0900	41	1
2)	A5	19		37	2
3)	A6	20		38	3
4)	B3	19		36	4
5)	B7	19		38	5
6)	B9	21		41	6
7)	C1	21		42	7
8)	C2	19		38	8
9)	C5	19		38	9
10)	C6	20	0100-0900	39	10

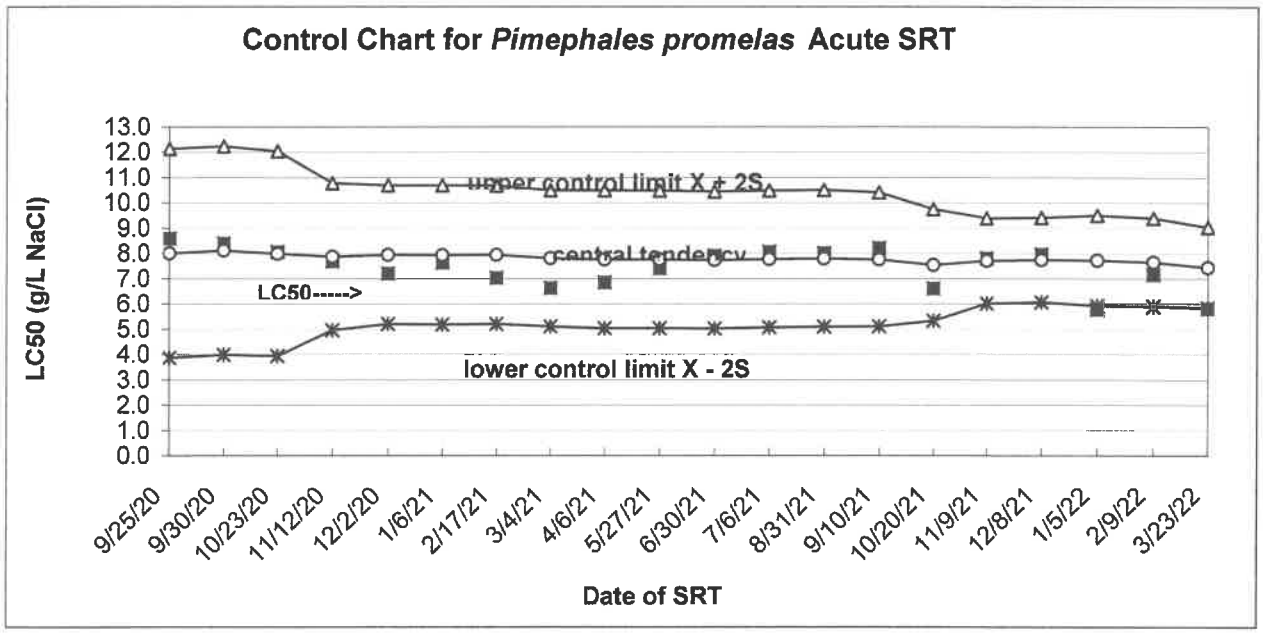
COMMENTS: Hatched on Tuesday (4/5)

Appendix D

SRT Data

96-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]
9/25/20	8.6	8.0	2.1	12.1	3.9	0.26	14.2	1.8	TSK
9/30/20	8.4	8.1	2.1	12.2	4.0	0.25	14.3	1.9	TSK
10/23/20	8.1	8.0	2.0	12.0	3.9	0.25	14.1	1.9	Probit
11/12/20	7.7	7.9	1.5	10.8	5.0	0.18	12.2	3.5	Probit
12/2/20	7.2	7.9	1.4	10.7	5.2	0.17	12.1	3.8	Probit
1/6/21	7.6	7.9	1.4	10.7	5.2	0.17	12.1	3.8	TSK
2/17/21	7.0	7.9	1.4	10.7	5.2	0.17	12.0	3.8	Probit
3/4/21	6.6	7.8	1.3	10.5	5.1	0.17	11.8	3.8	Probit
4/6/21	6.9	7.8	1.4	10.5	5.0	0.18	11.9	3.7	TSK
5/27/21	7.4	7.8	1.4	10.5	5.0	0.18	11.9	3.7	Probit
6/30/21	7.9	7.7	1.4	10.4	5.0	0.18	11.8	3.7	Probit
7/6/21	8.1	7.8	1.4	10.5	5.1	0.17	11.8	3.7	Probit
8/31/21	8.0	7.8	1.4	10.5	5.1	0.17	11.9	3.7	Probit
9/10/21	8.2	7.8	1.3	10.4	5.1	0.17	11.7	3.8	TSK
10/20/21	6.6	7.5	1.1	9.7	5.3	0.15	10.9	4.2	Probit
11/9/21	7.8	7.7	0.8	9.4	6.0	0.11	10.2	5.2	Probit
12/8/21	8.0	7.7	0.8	9.4	6.1	0.11	10.3	5.2	Probit
1/5/22	5.8	7.7	0.9	9.5	5.9	0.12	10.4	5.0	Probit
2/9/22	7.2	7.6	0.9	9.4	5.9	0.11	10.3	5.0	TSK
3/23/22	5.8	7.4	0.8	9.0	5.8	0.11	9.8	5.0	TSK

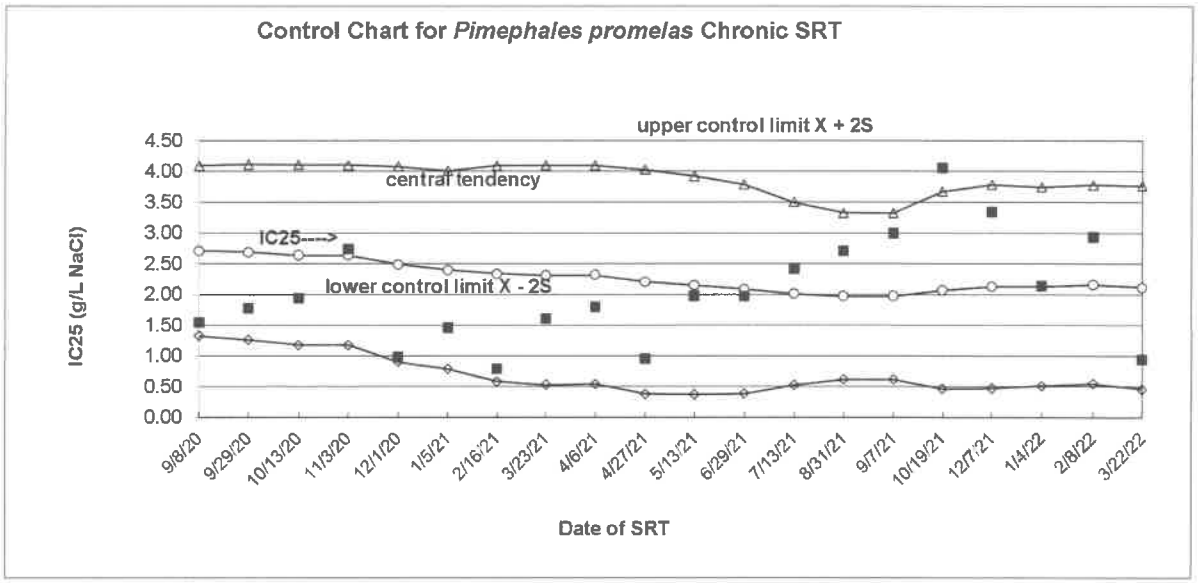


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.19
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X}$ ± 3SD = Well outside expected range.

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

IC25 for *Pimephales promelas* Chronic NaCl SRT

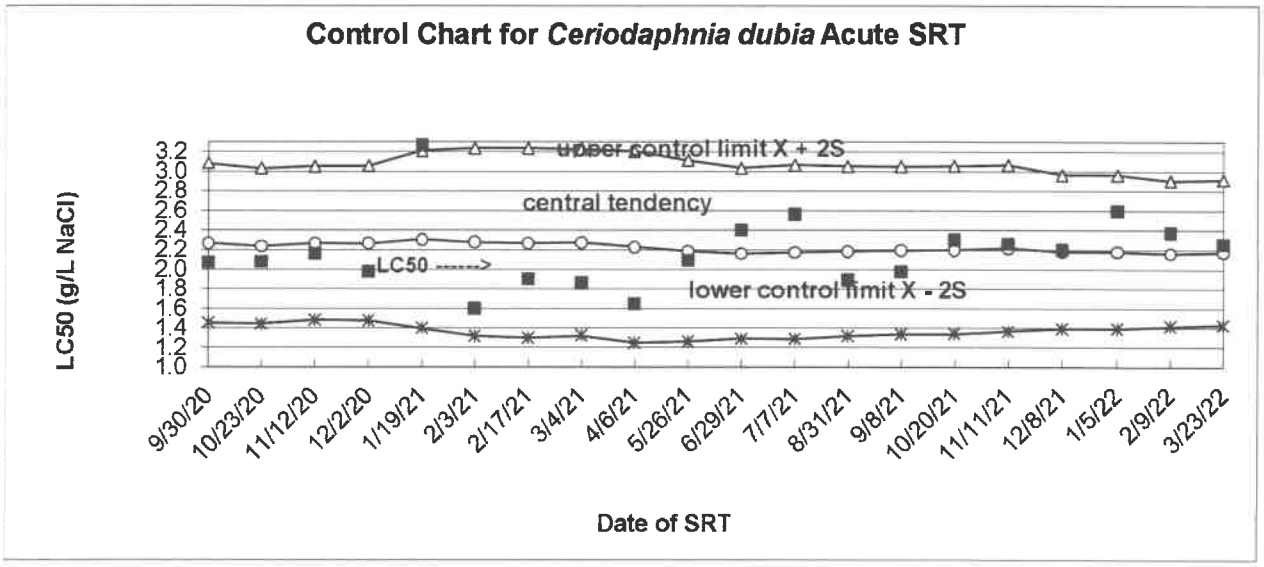
Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	CV Warning Limit**	CV Control Limit**
9/8/20	1.54	2.71	0.69	4.09	1.32	0.26	4.79	0.63		
9/29/20	1.77	2.69	0.72	4.12	1.26	0.27	4.83	0.54		
10/13/20	1.93	2.64	0.73	4.10	1.18	0.28	4.83	0.44		
11/3/20	2.73	2.64	0.73	4.10	1.17	0.28	4.83	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		
4/27/21	0.95	2.20	0.91	4.03	0.38	0.41	4.94	-0.53	0.84	0.99
5/13/21	1.98	2.15	0.89	3.92	0.37	0.41	4.81	-0.52	0.82	0.97
6/29/21	1.96	2.08	0.85	3.79	0.38	0.41	4.64	-0.47	0.79	0.94
7/13/21	2.42	2.01	0.74	3.50	0.52	0.37	4.24	-0.22		
8/31/21	2.70	1.97	0.68	3.32	0.61	0.34	4.00	-0.06		
9/7/21	2.99	1.97	0.68	3.32	0.61	0.34	4.00	-0.06		
10/19/21	4.06	2.07	0.80	3.67	0.46	0.39	4.47	-0.34		
12/7/21	3.34	2.13	0.83	3.78	0.47	0.39	4.61	-0.36		
1/4/22	2.14	2.13	0.81	3.74	0.51	0.38	4.55	-0.30		
2/8/22	2.93	2.16	0.81	3.78	0.54	0.37	4.58	-0.27		
3/22/22	0.93	2.11	0.83	3.77	0.45	0.39	4.60	-0.37		



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

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

Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
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
5/26/21	2.1	2.2	0.5	3.1	1.3	0.21	3.6	0.8	Probit
6/29/21	2.4	2.2	0.4	3.0	1.3	0.20	3.5	0.9	Probit
7/7/21	2.6	2.2	0.4	3.1	1.3	0.20	3.5	0.8	Probit
8/31/21	1.9	2.2	0.4	3.1	1.3	0.20	3.5	0.9	Probit
9/8/21	2.0	2.2	0.4	3.1	1.3	0.20	3.5	0.9	Probit
10/20/21	2.3	2.2	0.4	3.1	1.3	0.20	3.5	0.9	Probit
11/11/21	2.3	2.2	0.4	3.1	1.4	0.19	3.5	0.9	Probit
12/8/21	2.2	2.2	0.4	3.0	1.4	0.18	3.4	1.0	Probit
1/5/22	2.6	2.2	0.4	3.0	1.4	0.18	3.4	1.0	Probit
2/9/22	2.4	2.2	0.4	2.9	1.4	0.17	3.3	1.0	Probit
3/23/22	2.3	2.2	0.4	2.9	1.4	0.17	3.3	1.1	Probit

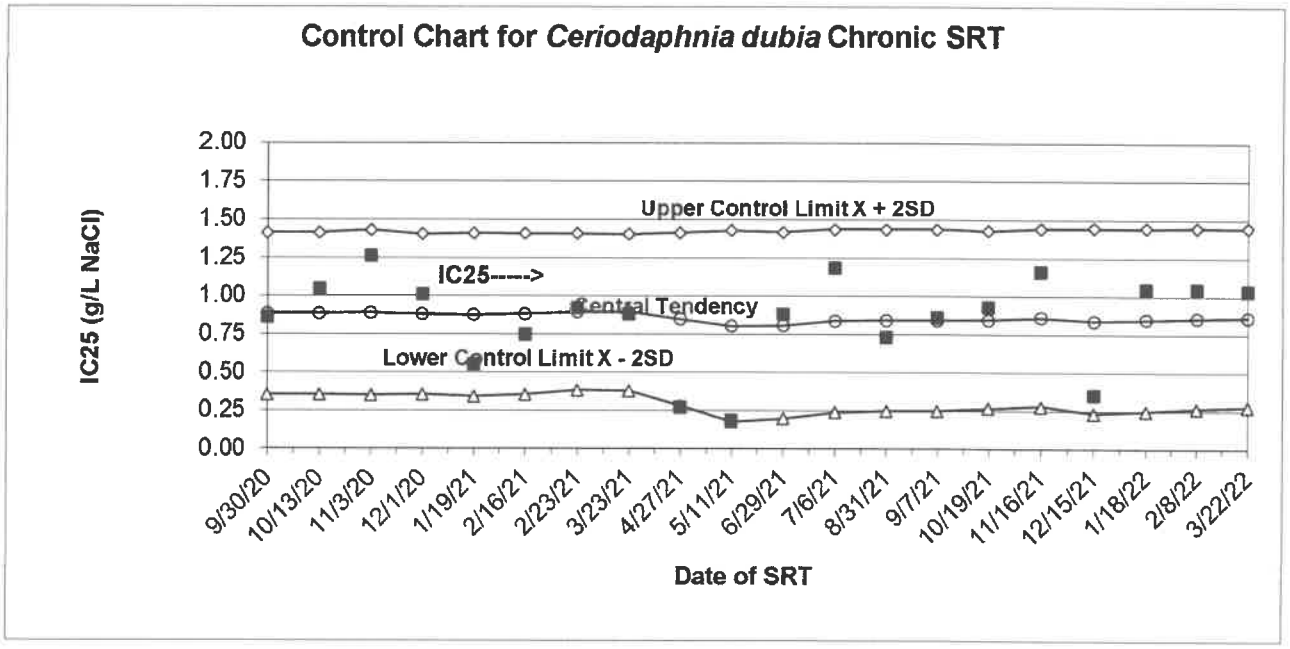


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	cv*	X+3SD	X-3SD
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
4/27/21	0.27	0.85	0.28	1.41	0.28	0.33	1.70	0.00
5/11/21	0.19	0.80	0.31	1.43	0.18	0.39	1.74	-0.14
6/29/21	0.88	0.81	0.31	1.42	0.19	0.38	1.72	-0.11
7/6/21	1.19	0.84	0.30	1.44	0.24	0.36	1.74	-0.06
8/31/21	0.73	0.84	0.30	1.44	0.25	0.35	1.73	-0.05
9/7/21	0.86	0.84	0.30	1.44	0.25	0.35	1.73	-0.05
10/19/21	0.93	0.85	0.29	1.43	0.27	0.34	1.72	-0.03
11/16/21	1.16	0.86	0.29	1.44	0.28	0.34	1.73	-0.01
12/15/21	0.35	0.84	0.30	1.45	0.23	0.36	1.75	-0.07
1/18/22	1.05	0.85	0.30	1.45	0.25	0.35	1.75	-0.05
2/8/22	1.05	0.86	0.30	1.45	0.26	0.35	1.74	-0.03
3/22/22	1.03	0.86	0.29	1.45	0.28	0.34	1.74	-0.02



*- 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.