Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Report Date: 8/17/22
Ohio EPA Permit No.: 2PK00002*KD
Alloway Lab #: L22-17547-01

Report of Chronic Biomonitoring

Samples Collected on:

7/25/22
7/27/22
7/29/22

Pimephales promelas
Ceriodaphnia dubia

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

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Outfall: Effluent 001
Report Date: 8/17/22
Ohio EPA Permit No.: 2PK00002*KD
Alloway Lab #: L22-17547-01

Date: 8/17/22

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

Dear Mr. Santaguida:

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

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

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

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

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

Megan Hurd

Megan Hurd
Special Projects Manager

Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 8/17/22
Ohio EPA Permit No.: 2PK00002*KD

1. Facility Name	Allen County Sanitary Engineer
2. Facility Address	Shawnee No. 2 WWTP 3640 Spencerville Road Lima, OH 45805
3. Ohio EPA Permit No.	2PK00002*KD
4. NPDES No.	OH0023850
5. Facility Contact	Mike Santaguida
6. Telephone No.	419-605-7560
7. Consulting Laboratory	Alloway
8. Lab Contact	Megan Hurd
9. Lab Telephone No.	419-223-1362
10. Receiving water(s) of discharge	Ottawa River
11. Outfall(s) tested	Effluent 001

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

ML Santaguida	8/19/2022
Signature	Date
Mike Santaguida	Superintendent
Name (typed or printed)	Title

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*KD
Date of *P. promelas* Test: 7/26/22 - 8/2/22
Date of *C. dubia* Test: 7/26/22 - 8/2/22

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	2.5	2.5	
7 day Chronic Toxicity, *Pimephales promelas* - % Affected	10.0	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

Conclusion/Comments:
For the *P. promelas* test, the PMSD of the dilution series exceeded the upper limit of 30% when compared to the primary upstream control. When the upstream control was compared to the 100% concentration only, the PMSD was within the method limits, indicating sufficient sensitivity to detect possible toxicity at the 100% concentration.

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*KD
Date of Test: 7/26/22 - 8/2/22

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 Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	10.0% (10.0%)	0.364	0.066
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.510	0.033
20%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	0.383	0.041
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.6% (2.6%)	5.1% (5.1%)	5.1% (5.1%)	0.386	0.040
60%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	10.0% (10.0%)	10.0% (10.0%)	0.375	0.087
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	7.5% (7.5%)	15.0% (15.0%)	0.369	0.108
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.388	0.032

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

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

Chronic Value = square root of (NOEC x LOEC)

COMMENTS:

For the *P. promelas* test, the PMSD of the dilution series exceeded the upper limit of 30% when compared to the primary upstream control. When the upstream control was compared to the 100% concentration only, the PMSD was within the method limits, indicating sufficient sensitivity to detect possible toxicity at the 100% concentration. The IC25 is >100.

OEPA Permit No.: 2PK00002*KD (Allen County Sanitary Engineer)

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:	7/26/22 - 8/2/22
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 - 7/25/22 ; day 2, 3 - 7/27/22 ; day 4, 5, 6 - 7/29/22
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and growth (dry weight).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PK00002*KD
Date of Test: 7/26/22 - 8/2/22

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 Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	40.6
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	38.9
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	42.1
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%)	42.9
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%)	0.0% (0.0%)	45.8
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	40.7
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	42.8

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

NOEC for Mortality (%)	100		NOEC for Reproduction (%)	100	**Method(s) used to determine Values:**
LOEC for Mortality (%)	>100		LOEC for Reproduction (%)	>100	
Chronic Value for Mortality (%)	>100		Chronic Value for Reprod (%)	>100	
IC25 (%)	>100		IC50 (%)	>100	IC Pin, Wilcoxon Bonferroni Adj Test

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

Chronic Value = square root of (NOEC x LOEC)

COMMENTS:

OEPA Permit No.: 2PK00002*KD (Allen County Sanitary Engineer)

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 - 7.4 hours old
2. Test Type and Duration:	Static renewal; Until 60% or more of surviving control females have three broods (maximum test duration 8 days)
3. Test Dates:	7/26/22 - 8/2/22
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 - 7/25/22 ; day 2, 3 - 7/27/22 ; day 4, 5, 6 - 7/29/22
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:	

Customer: Allen County Sanitary Engineer
Facility: Shawnee No. 2 WWTP
Outfall: Effluent 001
Report Date: 8/17/22
Ohio EPA Permit No.: 2PK00002*KD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection Beginning	Sample Collection Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	7/24/22 @ 1030	7/25/22 @ 1030
Type (Grab/Comp):	Comp	2nd	7/26/22 @ 0800	7/27/22 @ 0800
Volume Collected:	5 Gal.	3rd	7/28/22 @ 1245	7/29/22 @ 1245
Upstream Station:				
Waterbody:	Ottawa River			
Station No.:	801	1st		7/25/22 @ 1348
Type (Grab/Comp):	Grab	2nd		7/27/22 @ 1130
Volume Collected:	5 Gal.	3rd		7/29/22 @ 1200

Samples collected by: Alloway

Appendix A

Field Data

Alloway
Your Resource for Defensible Data

Chain of Custody Record

1/3

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: Matt Agner
Company: Allen County Sanitary Engineer
Address: 3226 North Cole St.
Lima, OH 45801

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

Notes/Comments:

L22-17547

Phone #: 419-996-4688
Fax #:
E-mail: magner@allencountyohio.com
PO#:

Project Name: Bioassay - Shawnee II - DAY 1

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	7/24/22-7/25/22	10:30-10:30	X		WW	1	1	Toxicity	01
2	Upstream	7/25/22	13:48		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) 7/25/22 14:12

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: 5.0°C

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

2/3

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: Matt Agner
Company: Allen County Sanitary Engineer
Address: 3226 North Cole St. Lima, OH 45801

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

Notes/Comments:

L22-17547

Phone #: 419-996-4688 Fax #:
E-mail: magner@allencountyohio.com PO#:

Project Name: Bioassay - Shawnee II - DAY 2

Sampler (Print) Tom Stephens (Signature) Tom Stephens

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	7-26-22 7-27-22	800 800	X		WW	1	1	Toxicity	01
2	Upstream	7-27-22	11:30		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) Tom Stephens 7-27-22 1500

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: 5.0°C

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 3/3

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: Matt Agner
Company: Allen County Sanitary Engineer
Address: 3226 North Cole St.
Lima, OH 45801

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

Notes/Comments:

L22-17547

Phone #: 419-996-4688
Fax #:
E-mail: magner@allencountyohio.com
PO#:

Project Name: Bioassay - Shawnee II - DAY 3

Sampler: (Print) Alex Fields (Signature)

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

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	7-28-22 7-29-22	12:45	X		WW	1	1	Toxicity	01
2	Upstream	7-29-22	12:00		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) 7-29-22 1955

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: 6.0 °C

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

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

Appendix B

Raw Data Sheets

CETIS Summary Report

Report Date: 17 Aug-22 17:21 (p 1 of 1)
Test Code/ID: L22-17547 Pp / 16-6546-8646

Fathead Minnow 7-d Larval Survival and Growth Test

Alloway

Batch ID:	21-0234-5057	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	26 Jul-22 16:31	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Upstream of Discharge
Ending Date:	02 Aug-22 11:56	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Test Length:	6d 19h	**Taxon:**	Actinopterygii	**Source:**	In-House Culture **Age:** <24

Sample ID:	08-9580-7831	**Code:**	L22-17547 Pp	**Project:**	WET Monthly Compliance Test (JUL)
Sample Date:	25 Jul-22 10:30	**Material:**	Industrial Effluent	**Source:**	Discharge Monitoring Report
Receipt Date:	25 Jul-22 14:12	**CAS (PC):**		**Station:**	001
Sample Age:	30h	**Client:**	ACSE - Shawnee		

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
19-2155-2408	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)		IC15	>100	---	---	<1	1
				IC20	>100	---	---	<1	
				IC25	>100	---	---	<1	
				IC40	>100	---	---	<1	
				IC50	>100	---	---	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits Lower	TAC Limits Upper	Overlap	Decision
19-2155-2408	Mean Dry Biomass-mg	Control Resp	0.03683	0.25	<<	Yes	Below Criteria

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	U	4	0.03637	0.02581	0.04694	0.0276	0.0424	0.003319	0.006638	18.25%	0.00%
20		4	0.03827	0.03172	0.04483	0.0327	0.0426	0.002059	0.004119	10.76%	-5.22%
40		4	0.04218	0.03973	0.04462	0.0404	0.044	0.000769	0.001537	3.64%	-15.95%
60		4	0.03748	0.0237	0.05125	0.0315	0.0499	0.004329	0.008659	23.11%	-3.02%
80		4	0.03685	0.01964	0.05406	0.0211	0.0445	0.005408	0.01082	29.35%	-1.31%
100		4	0.0388	0.03366	0.04394	0.0351	0.0425	0.001615	0.00323	8.32%	-6.67%

Mean Dry Biomass-mg Detail

MD5: 0C0E6F5E23D13CD4AC4AA6531E6B4FB4

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	U	0.035	0.0276	0.0405	0.0424
20		0.0385	0.0393	0.0327	0.0426
40		0.0404	0.0416	0.0427	0.044
60		0.0315	0.0316	0.0499	0.0369
80		0.0385	0.0433	0.0445	0.0211
100		0.0351	0.0425	0.0374	0.0402

CETIS Analytical Report

Report Date: 17 Aug-22 13:53 (p 1 of 2)
Test Code/ID: L22-17547 Pp / 16-6546-8646

Fathead Minnow 7-d Larval Survival and Growth Test — **Alloway**

Analysis ID:	05-6960-4529	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 13:53	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	17 Aug-22 13:45	**MD5 Hash:**	0C0E6F5E23D13CD4AC4AA6531E6B4FB	**Editor ID:**	006-582-221-1

Batch ID:	21-0234-5057	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	26 Jul-22 16:31	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Upstream of Discharge
Ending Date:	02 Aug-22 11:56	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Test Length:	6d 19h	**Taxon:**	Actinopterygii	**Source:**	In-House Culture **Age:** <24

Sample ID:	08-9580-7831	**Code:**	L22-17547 Pp	**Project:**	WET Monthly Compliance Test (JUL)
Sample Date:	25 Jul-22 10:30	**Material:**	Industrial Effluent	**Source:**	Discharge Monitoring Report
Receipt Date:	25 Jul-22 14:12	**CAS (PC):**		**Station:**	001
Sample Age:	30h	**Client:**	ACSE - Shawnee		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	MSDu	PMSD
Untransformed	C > T	100	>100	---	1	0.01133	31.14%

Dunnett Multiple Comparison Test

Control vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Upstream Control	20	6	-0.4037	2.407	0.01133	CDF	0.9251	Non-Significant Effect
	40	6	-1.232	2.407	0.01133	CDF	0.9910	Non-Significant Effect
	60	6	-0.2337	2.407	0.01133	CDF	0.8929	Non-Significant Effect
	80	6	-0.1009	2.407	0.01133	CDF	0.8613	Non-Significant Effect
	100	6	-0.5153	2.407	0.01133	CDF	0.9417	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
Control Resp	0.03638	0.25	<<	Yes	Below Criteria
PMSD	0.3114	0.12	0.3	Yes	Above Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.701E-05	1.740E-05	5	0.3928	0.8473	Non-Significant Effect
Error	0.0007973	4.43E-05	18			
Total	0.0008843		23			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	10.21	15.09	0.0695	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9717	0.884	0.7097	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	U	4	0.03637	0.02581	0.04694	0.03775	0.0276	0.0424	0.003319	18.25%	0.00%
20		4	0.03827	0.03172	0.04483	0.0389	0.0327	0.0426	0.002059	10.76%	-5.22%
40		4	0.04218	0.03973	0.04462	0.04215	0.0404	0.044	0.000769	3.64%	-15.95%
60		4	0.03748	0.0237	0.05125	0.03425	0.0315	0.0499	0.004329	23.11%	-3.02%
80		4	0.03685	0.01964	0.05406	0.0409	0.0211	0.0445	0.005408	29.35%	-1.31%
100		4	0.0388	0.03366	0.04394	0.0388	0.0351	0.0425	0.001615	8.32%	-6.67%

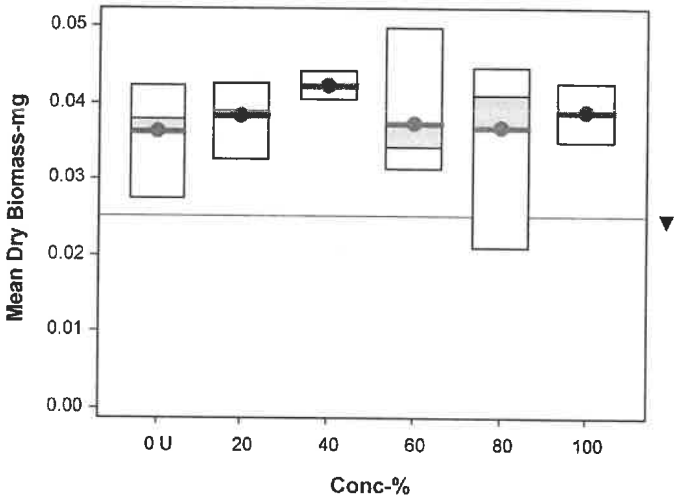
Mean Dry Biomass-mg Detail

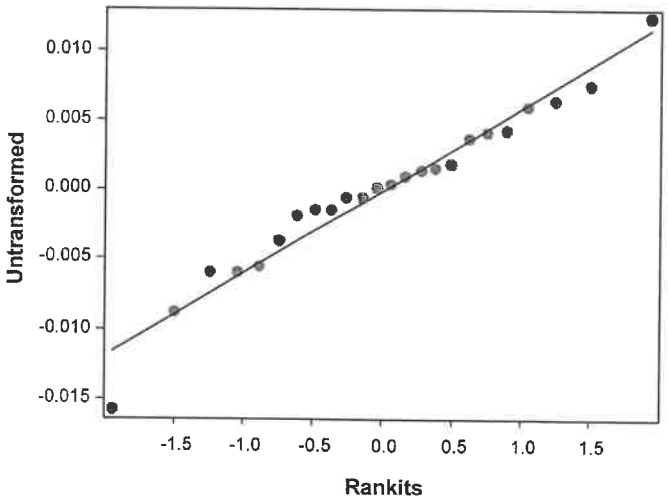
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	U	0.035	0.0276	0.0405	0.0424
20		0.0385	0.0393	0.0327	0.0426
40		0.0404	0.0416	0.0427	0.044
60		0.0315	0.0316	0.0499	0.0369
80		0.0385	0.0433	0.0445	0.0211
100		0.0351	0.0425	0.0374	0.0402

Fathead Minnow 7-d Larval Survival and Growth Test — **Alloway**

Analysis ID:	05-6960-4529	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 13:53	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	17 Aug-22 13:45	**MD5 Hash:**	0C0E6F5E23D13CD4AC4AA6531E6B4FB	**Editor ID:**	006-582-221-1

Graphics





CETIS Analytical Report

Report Date: 17 Aug-22 13:54 (p 1 of 2)
Test Code/ID: L22-17547 Pp / 16-6546-8646

Fathead Minnow 7-d Larval Survival and Growth Test — **Alloway**

Analysis ID:	17-0436-1864	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 13:53	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	17 Aug-22 13:45	**MD5 Hash:**	14EF2C702DC8E10AD442BCEEE5492777	**Editor ID:**	006-582-221-1

Batch ID:	21-0234-5057	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech		
Start Date:	26 Jul-22 16:31	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Upstream of Discharge		
Ending Date:	02 Aug-22 11:56	**Species:**	Pimephales promelas	**Brine:**	Not Applicable		
Test Length:	6d 19h	**Taxon:**	Actinopterygii	**Source:**	In-House Culture	**Age:**	<24

Sample ID:	08-9580-7831	**Code:**	L22-17547 Pp	**Project:**	WET Monthly Compliance Test (JUL)
Sample Date:	25 Jul-22 10:30	**Material:**	Industrial Effluent	**Source:**	Discharge Monitoring Report
Receipt Date:	25 Jul-22 14:12	**CAS (PC):**		**Station:**	001
Sample Age:	30h	**Client:**	ACSE - Shawnee		

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	100% passed mean dry biomass-mg endpoint	19.72%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Upstream Control		100	6	-0.657	1.943	0.007173	CDF	0.7322	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
Control Resp	0.03638	0.25	<<	Yes	Below Criteria
PMSD	0.1972	0.12	0.3	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.176E-05	1.176E-05	1	0.4316	0.5356	Non-Significant Effect
Error	0.0001635	2.725E-05	6			
Total	0.0001753		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	4.224	47.47	0.2674	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9525	0.6451	0.7366	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	U	4	0.03637	0.02581	0.04694	0.03775	0.0276	0.0424	0.003319	18.25%	0.00%
100		4	0.0388	0.03366	0.04394	0.0388	0.0351	0.0425	0.001615	8.32%	-6.67%

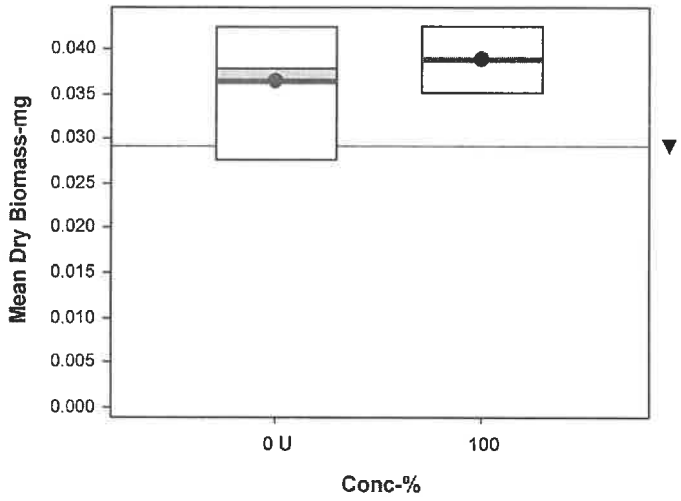
Mean Dry Biomass-mg Detail

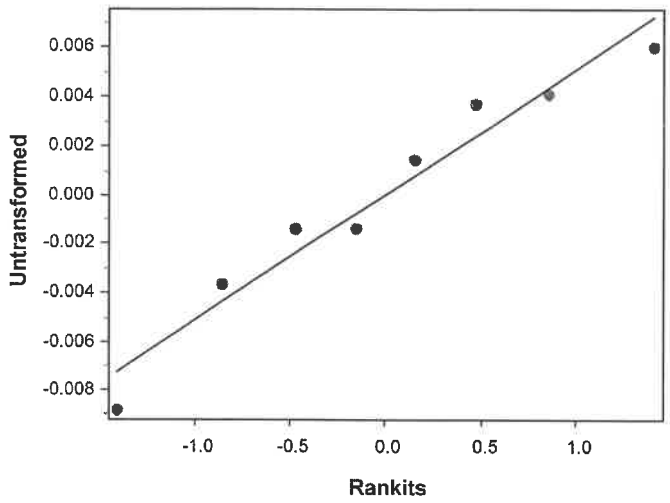
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	U	0.035	0.0276	0.0405	0.0424
100		0.0351	0.0425	0.0374	0.0402

Fathead Minnow 7-d Larval Survival and Growth Test — **Alloway**

Analysis ID:	17-0436-1864	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 13:53	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	17 Aug-22 13:45	**MD5 Hash:**	14EF2C702DC8E10AD442BCEEE5492777	**Editor ID:**	006-582-221-1

Graphics





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

Discharge: Allen County, Shawnee No. 2 WWTP Date of Test: 7/26/22 - 8/2/22

Address: 3640 Spencerville Road, Lima, OH 45805

Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*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: Upstream 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			
Lab Control	1	10	1.09678	1.10207	10	5.29	0.529	
	2	10	1.09741	1.10204	10	4.63	0.463	
	3	10	1.10295	1.10808	10	5.13	0.513	0.510
	4	10	1.08893	1.09428	10	5.35	0.535	0.033
Upstream Control	1	10	1.12475	1.12825	10	3.50	0.350	
	2	7	1.10460	1.10736	10	2.76	0.276	
	3	9	1.09454	1.09859	10	4.05	0.405	0.364
	4	10	1.13380	1.13804	10	4.24	0.424	0.066
20% Effluent	1	9	1.12595	1.12980	10	3.85	0.385	
	2	9	1.09299	1.09692	10	3.93	0.393	
	3	10	1.10103	1.10430	10	3.27	0.327	0.383
	4	10	1.07923	1.08349	10	4.26	0.426	0.041
40% Effluent	1	8	1.08516	1.08920	10	4.04	0.404	
	2	10	1.10358	1.10774	10	4.16	0.416	
	3	10	1.09319	1.09646	10	3.27	0.327	0.386
	4	9	1.12790	1.13146	9	3.56	0.396	0.040

Reviewed By: MAA

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

Discharge: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*KD
Drying Temperature (C°): 60
Drying Time (h): 24

Date of Test: 7/26/22 - 8/2/22
Pimephales promelas Age at Start: < 24 Hours
Dilution Water Used: Upstream 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	8	1.12912	1.13227	10	3.15	0.315	
	2	9	1.08628	1.08944	10	3.16	0.316	
	3	9	1.13231	1.13730	10	4.99	0.499	0.375
	4	10	1.10700	1.11069	10	3.69	0.369	0.087
80% Effluent	1	10	1.12399	1.12784	10	3.85	0.385	
	2	9	1.08960	1.09393	10	4.33	0.433	
	3	9	1.09709	1.10154	10	4.45	0.445	0.369
	4	6	1.08335	1.08546	10	2.11	0.211	0.108
100% Effluent	1	10	1.09983	1.10334	10	3.51	0.351	
	2	10	1.09873	1.10298	10	4.25	0.425	
	3	10	1.09790	1.10164	10	3.74	0.374	0.388
	4	10	1.08655	1.09057	10	4.02	0.402	0.032
	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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Date of Test: 7/26/22 - 8/2/22
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*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 = 0.99999 grams Date: 8/7/22 Analyst: SDP Dilution Water Used: Upstream 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			1.09888		10			
				DJS 8/3/22				
Lab Control	1	10	1.09678 1.10207	~~1.17303~~ 1.08308	10	0.00	0.000	
	2	10	1.09741 1.10204	~~1.16080~~	10	0.00	0.000	
	3	10	1.10295	1.1808	10	0.00	0.000	0.000
	4	10	1.08893	1.09428	10	0.00	0.000	0.000
Upstream Control	1	10	1.12475	1.12825	10	0.00	0.000	
	2	7	1.10460	1.10736	10	0.00	0.000	
	3	9	1.09454	1.09859	10	0.00	0.000	0.000
	4	10	1.13380	1.13804	10	0.00	0.000	0.000
20% Effluent	1	9	1.12595	1.12980	10	0.00	0.000	
	2	9	1.09299	1.09692	10	0.00	0.000	
	3	10	1.10103	1.10430	10	0.00	0.000	0.000
	4	10	1.07923	1.08349	10	0.00	0.000	0.000
40% Effluent	1	8	1.08516	1.08920	10	0.00	0.000	
	2	10	1.10358	1.10774	10	0.00	0.000	
	3	10	1.09319	1.09646	10	0.00	0.000	0.000
	4	9	1.12790	1.13146	~~8~~ 9	0.00	0.000	0.000

Reviewed By: [signature]

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

Discharge: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD
Drying Temperature (C°): 60 Drying Time (h): 24

Date of Test: 7/26/22 - 8/2/22
Pimephales promelas Age at Start: < 24 Hours
Dilution Water Used: Upstream 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	8	1.12912	1.13227	10	0.00	0.000	
	2	9	1.08628	1.08944	10	0.00	0.000	
	3	9	1.13231	1.13730	10	0.00	0.000	0.000
	4	10	1.10700	1.11069	10	0.00	0.000	0.000
80% Effluent	1	10	1.12399	1.12784	10	0.00	0.000	
	2	9	1.08960	1.09393	10	0.00	0.000	
	3	9	1.09709	1.10154	10	0.00	0.000	0.000
	4	6	1.08336	1.08546	10	0.00	0.000	0.000
100% Effluent	1	10	1.09983	1.10334	10	0.00	0.000	
	2	10	1.09873	1.10298	10	0.00	0.000	
	3	10	1.09790	1.10164	10	0.00	0.000	0.000
	4	10	1.08655	1.09057	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

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

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

Person(s) Conducting Test: DJA, JDP, MHH
Beginning: Date 7/26/22 **Time** 1631
Ending: Date 8/2/22 **Time** 1156
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Upstream Control

Day	Lab Control					Upstream Control					20% Effluent					40% Effluent				
	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D	10G	10G	10G	10G	40G
4	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D	10G	9G 1D	10G	10G	39G 1D	10G	10G	10G	10G	40G
5	10G	10G	10G	10G	40G	~~9G 1D~~ 10G	8G 2D	10G	10G	38G 2D	10G	9G 1D	10G	10G	39G 1D	9G 1D	10G	10G	10G	39G 1D
6	10G	10G	10G	10G	40G	7/31/22 10G	8G 2D	9G 1D	10G	37G 3D	9G 1D	9G 1D	10G	10G	38G 2D	8G 2D	10G	10G	10G	38G 2D
7	10G	10G	10G	10G	40G	10G	~~8G 2D~~ 7 3	9G 1D	10G	~~37G 3D~~ 4	9G 1D	9G 1D	10G	10G	~~38G 2D~~	8G 2D	10G	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).

9G LA 37G
2D LA
JDP
8/4/22

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

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

Person(s) Conducting Test: DJA, JDP, MHH
Beginning: Date 7/26/22 **Time** 1631
Ending: Date 8/2/22 **Time** 1166
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Upstream Control

Day	60% Effluent					80% Effluent					100% Effluent									
	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
3	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
4	9G 1D	9G 1D	10G	10G	38G 2D	10G	9G 1D	9G 1D	10G	38G 2D	10G	10G	10G	10G	40G					
5	8G 2D	9G 1D	10G	10G	37G 3D	10G	9G 1D	9G 1D	10G	38G 2D	10G	10G	10G	10G	40G					
6	~~7G 3D~~ 8G 2D	9G 1D	9G 1D	10G	~~35G 5D~~ 36G 4D	10G	9G 1D	9G 1D	9G 1D	37G 3D	10G	10G	10G	10G	40G					
7	8G 2D	9G 1D	9G 1D	10G	36G 4D	10G	9G 1D	9G 1D	6G 4D	34G 6D	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).

CETIS Analytical Report

Report Date:	17 Aug-22 17:22 (p 1 of 2)
Test Code/ID:	L22-17547 / 06-6768-0934

Ceriodaphnia 7-d Survival and Reproduction Test — **Alloway**

Analysis ID:	20-8375-2484	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 17:22	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	17 Aug-22 0:51	**MD5 Hash:**	01E904833C3F7E9C16ED4562E9977190	**Editor ID:**	006-582-221-1

Batch ID:	10-9592-1756	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Lab Tech	
Start Date:	26 Jul-22 16:03	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Dechlorinated Tap Water	
Ending Date:	02 Aug-22 11:50	**Species:**	Ceriodaphnia dubia	**Brine:**	Not Applicable	
Test Length:	6d 20h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:** 8 -

Sample ID:	10-9447-3372	**Code:**	L22-17547	**Project:**	WET Monthly Compliance Test (JUL)
Sample Date:	25 Jul-22 10:30	**Material:**	Industrial Effluent	**Source:**	Discharge Monitoring Report
Receipt Date:	25 Jul-22 14:12	**CAS (PC):**		**Station:**	001
Sample Age:	30h	**Client:**	ACSE - Shawnee		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1043320	1000	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits Lower	TAC Limits Upper	Overlap	Decision
Control Resp	40.6	15	<<	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC15	>100	---	---	<1	---	---
IC20	>100	---	---	<1	---	---
IC25	>100	---	---	<1	---	---
IC40	>100	---	---	<1	---	---
IC50	>100	---	---	<1	---	---

Reproduction Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	U	10	40.6	41.67	24	50	19.26%	0.00%	42.85	0.00%
20		9	42.11	44	29	47	12.76%	-3.72%	42.85	0.00%
40		10	42.9	43.33	28	52	14.60%	-5.67%	42.85	0.00%
60		10	45.8	45.67	43	51	5.62%	-12.81%	42.85	0.00%
80		10	40.7	43.5	30	46	12.69%	-0.25%	41.75	2.57%
100		10	42.8	43.75	39	46	5.26%	-5.42%	41.75	2.57%

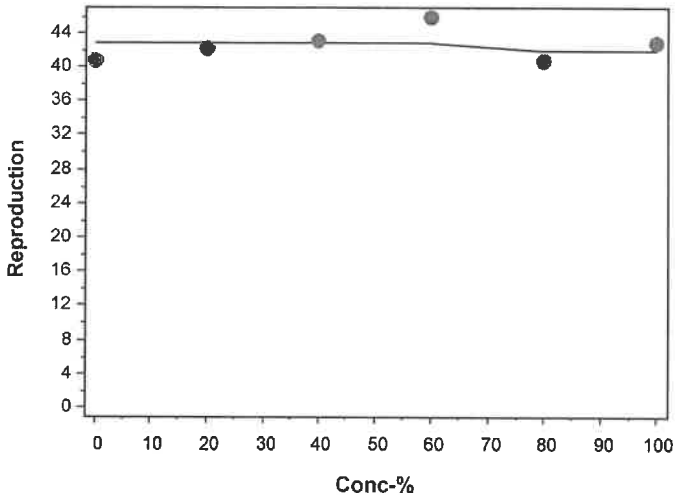
Reproduction Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	U	24	31	39	50	45	43	44	48	41	41
20		44	47	40	42	45	42	29	46	44	
40		45	52	43	28	41	45	40	48	44	43
60		51	45	46	46	43	44	44	48	48	43
80		40	44	44	36	45	46	42	30	44	36
100		40	44	41	43	42	44	45	46	39	44

Ceriodaphnia 7-d Survival and Reproduction Test — **Alloway**

Analysis ID:	20-8375-2484	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 17:22	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	17 Aug-22 0:51	**MD5 Hash:**	01E904833C3F7E9C16ED4562E9977190	**Editor ID:**	006-582-221-1

Graphics



CETIS Analytical Report

Report Date: 17 Aug-22 13:40 (p 1 of 2)
Test Code/ID: L22-17547 / 06-6768-0934

Ceriodaphnia 7-d Survival and Reproduction Test — **Alloway**

Analysis ID:	12-9178-0854	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 13:40	**Analysis:**	Nonparametric-Multiple Comparison	**Status Level:**	1
Edit Date:	17 Aug-22 0:51	**MD5 Hash:**	01E904833C3F7E9C16ED4562E9977190	**Editor ID:**	006-582-221-1

Batch ID:	10-9592-1756	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Lab Tech		
Start Date:	26 Jul-22 16:03	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Dechlorinated Tap Water		
Ending Date:	02 Aug-22 11:50	**Species:**	Ceriodaphnia dubia	**Brine:**	Not Applicable		
Test Length:	6d 20h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	8 -

Sample ID:	10-9447-3372	**Code:**	L22-17547	**Project:**	WET Monthly Compliance Test (JUL)
Sample Date:	25 Jul-22 10:30	**Material:**	Industrial Effluent	**Source:**	Discharge Monitoring Report
Receipt Date:	25 Jul-22 14:12	**CAS (PC):**		**Station:**	001
Sample Age:	30h	**Client:**	ACSE - Shawnee		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	MSDu	PMSD
Untransformed	C > T	100	>100	--	1	5.668	13.96%

Wilcoxon/Bonferroni Adj Test

Control vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Upstream Control	20	17	95.5	---	2	Exact	1.0000	Non-Significant Effect
	40	18	114	---	5	Exact	1.0000	Non-Significant Effect
	60	18	129.5	---	4	Exact	1.0000	Non-Significant Effect
	80	18	103	---	2	Exact	1.0000	Non-Significant Effect
	100	18	110	---	5	Exact	1.0000	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
Control Resp	40.6	15	<<	Yes	Passes Criteria
PMSD	0.1396	0.13	0.47	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	181.257	36.2514	5	1.299	0.2786	Non-Significant Effect
Error	1479.49	27.9149	53			
Total	1660.75		58			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	17.56	15.09	0.0036	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.889	0.9451	6.1E-05	Non-Normal Distribution

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	U	10	40.6	35.01	46.19	41.67	24	50	2.473	19.26%	0.00%
20		9	42.11	37.98	46.24	44	29	47	1.791	12.76%	-3.72%
40		10	42.9	38.42	47.38	43.33	28	52	1.98	14.60%	-5.67%
60		10	45.8	43.96	47.64	45.67	43	51	0.8138	5.62%	-12.81%
80		10	40.7	37.01	44.39	43.5	30	46	1.633	12.69%	-0.25%
100		10	42.8	41.19	44.41	43.75	39	46	0.7118	5.26%	-5.42%

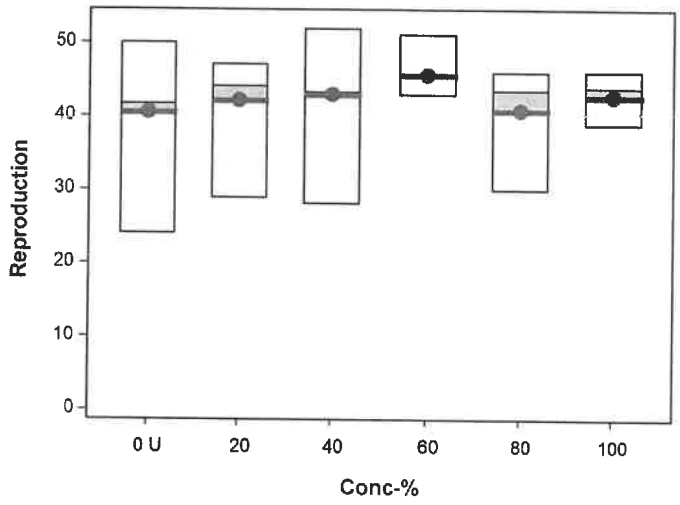
Reproduction Detail

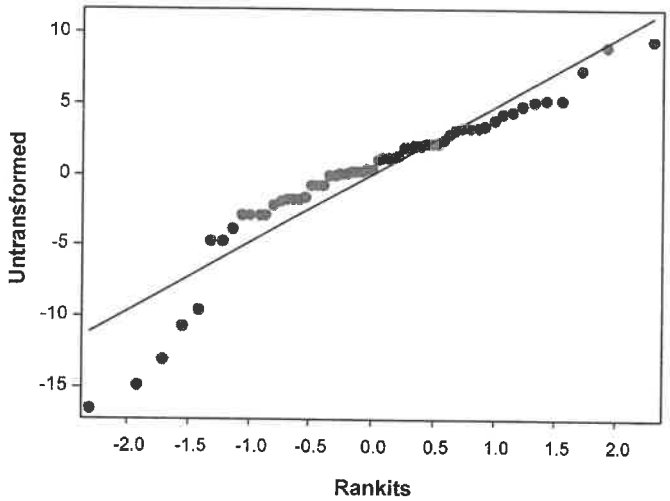
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	U	24	31	39	50	45	43	44	48	41	41
20		44	47	40	42	45	42	29	46	44	
40		45	52	43	28	41	45	40	48	44	43
60		51	45	46	46	43	44	44	48	48	43
80		40	44	44	36	45	46	42	30	44	36
100		40	44	41	43	42	44	45	46	39	44

Ceriodaphnia 7-d Survival and Reproduction Test — **Alloway**

Analysis ID:	12-9178-0854	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv2.1.1
Analyzed:	17 Aug-22 13:40	**Analysis:**	Nonparametric-Multiple Comparison	**Status Level:**	1
Edit Date:	17 Aug-22 0:51	**MD5 Hash:**	01E904833C3F7E9C16ED4562E9977190	**Editor ID:**	006-582-221-1

Graphics





CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

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

Person(s) Conducting Test: JDP, DJA, MHH
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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	11 G	0 G	19 G	36	3
2					6	15		20	41	3
3					7	14		20	41	3
4					7	11		19	37	3
5					8	14		19	41	3
6					7	15		19	41	3
7					7	12		17	36	3
8					6	12		20	38	3
9					8	13		18	39	3
10	0 G	0 G	0 G	0 G	8 G	12 G	0 G	19 G	39	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
								SUM	389	30
								MEAN	38.9	3.0
								S.D.	2.08	0.00

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

Reviewed by: MHH

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

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

Person(s) Conducting Test: JDP, DJA, MHH, BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1 - 7.4 Hrs.
Dilution Water Used: Upstream 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	17/G	1/D	-/-	D	24	2
2					7	1 Abort	23/G	0/G		31	3
3					5	14	0	20		39	3
4					7	19		24		50	3
5					7	14	1	24		45	3
6					7	15	0	21		43	3
7					6	17	21	0		44	3
8					5	14	0	26		48	3
9					6	15	0	20		41	3
10	0/G	0/G	0/G	0/G	7/G	12/G	0/G	22/G		41	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	9G 1D	9G 1D			
									SUM	406	29
									MEAN	40.6	2.9
									S.D.	7.82	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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*KD

Person(s) Conducting Test: JDP, DJA, MHH, GG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2 /22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1-7.4 hrs.
Dilution Water Used: Upstream 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	17/G	0/G	20/G		44	3
2	/	/	/	/	6/	16/	/	25/		47	3
3	/	/	/	/	7/	13/	/	20/		40	3
4	/	/	/	/	6/	13/	/	23/		42	3
5	/	/	/	/	8/	15/	/	22/		45	3
6	/	/	/	/	7/	16/	/	19/		42	3
7	/	/G	0/G	0/G	6/G	16/G	0/G	7/G		29	3
8	/	/LA	-/-	-/-	-/-	-/-	-/-	-/-	LA	-	-
9	/	/G	0/G	0/G	8/G	16/G	0/G	22/G		46	3
10	0/G	0/G	0/G	0/G	7/G	15/G	0/G	22/G		44	3
Total Adult Cond.	10G	9G 1LA	9G 1LA	9G 1LA	9G 1LA	9G 1LA	9G 1LA	9G 1LA			
									SUM	379	27
									MEAN	42.1	3.0
									S.D.	5.37	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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*KD

Person(s) Conducting Test: JDP, DJA, MHH, BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/ 2 /22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1 -7.4 Hrs.
Dilution Water Used: Upstream 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	0/G	25/G	45	3
2					8	17		27	52	3
3					6	15		22	43	3
4					8	16		4	28	3
5					8	12	0	21	41	3
6					7	0	16	22	45	3
7					7	14	0	19	40	3
8					6	19	0	23	48	3
9					7	15	0	22	44	3
10	0/G	0/G	0/G	0/G	8/G	14/G	21/G	0/G	43	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
								SUM	429	30
								MEAN	42.9	3.0
								S.D.	6.26	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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*KD

Person(s) Conducting Test: JDP, DJA, MHH, BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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	17 G	0 G	27 G	51	3
2					5	15		25	45	3
3					7	14		25	46	3
4					7	16		23	44	3
5					7	15	0	21	43	3
6					6	0	15	23	44	3
7					5	16	0	23	44	3
8					8	15		25	48	3
9					8	16		24	48	3
10	0 G	0 G	0 G	0 G	7 G	14 G	0 G	22 G	43	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
								SUM	458	30
								MEAN	45.8	3.0
								S.D.	2.57	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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*KD

Person(s) Conducting Test: JDP, DJA, MHH BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1-7.4 HRS.
Dilution Water Used: Upstream 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	14/G	0/G	18/G	40	3
2	/	/	/	/	7/	15/	/	22/	44	3
3	/	/	/	/	6/	16/	/	22/	44	3
4	/	/	/	/	7/	13/	/	16/	36	3
5	/	/	/	/	7/	15/	/	23/	45	3
6	/	/	/	/	4/	16/	/	26/	46	3
7	/	/	/	/	7/	13/	/	22/	42	3
8	/	/	/	/	5/	15/	/	10/	30	3
9	/	/	/	/	8/	13/	/	23/	44	3
10	0/G	0/G	0/G	0/G	6/G	14/G	0/G	16/G	36	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
								SUM	40.7	30
								MEAN	40.7	3.0
								S.D.	5.17	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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001
NPDES Permit No.: 2PK00002*KD

Person(s) Conducting Test: JDP, DJA, MHH, BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Age of *Ceriodaphnia dubia*: 4.1-7.4 hrs
Dilution Water Used: Upstream 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	13 / G	20 / G	0 / G	40	3
2	/	/	/	/	7 /	16 /	0 /	21 /	44	3
3	/	/	/	/	7 /	14 /	0 /	20 /	41	3
4	/	/	/	/	8 /	14 /	0 /	21 /	43	3
5	/	/	/	/	7 /	14 /	21 /	0 /	42	3
6	/	/	/	/	8 /	14 /	0 /	22 /	44	3
7	/	/	/	/	8 /	14 /	/	23 /	45	3
8	/	/	/	/	8 /	15 /	/	23 /	46	3
9	/	/	/	/	7 /	13 /	/	19 /	39	3
10	0 / G	0 / G	0 / G	0 / G	8 / G	16 / G	0 / G	20 / G	44	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	10G		
									SUM 428	30
									MEAN 42.8	3.0
									S.D. 2.25	0.00

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

Appendix C

Parameter Data

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

Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 Permit No.: 2PK00002*KD
Type of Test: Chronic Date of Test: 7/26/22 - 8/2/22

1st Sample Collection

Date Samples Collected: 7/25/22 Date Samples Read: 7/26/22 Analyst: JDP

	100% Effluent	Upstream	-	-
Temperature (°C)	11.0	11.2		
Dissolved Oxygen (mg/L)	6.0	8.6		
pH (s.u.)	7.43	8.24		
Conductivity (µmhos)	727	878		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Tan	Light Yellow		

2nd Sample Collection

Date Samples Collected: 7/27/22 Date Samples Read: 7/28/22 Analyst: JDP

	100% Effluent	Upstream	-	-
Temperature (°C)	10.4	9.7		
Dissolved Oxygen (mg/L)	8.2	8.9		
pH (s.u.)	7.52	8.07		
Conductivity (µmhos)	755	1031		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Yellow	Light Yellow		

3rd Sample Collection

Date Samples Collected: 7/29/22 Date Samples Read: 7/30/22 Analyst: DJA

	100% Effluent	Upstream	-	-
Temperature (°C)	10.4	16.1		
Dissolved Oxygen (mg/L)	6.8	8.4		
pH (s.u.)	7.40	8.15		
Conductivity (µmhos)	760	1180		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Stain	Light Stain		

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

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

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1186
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	24.7	25.0	24.3 / 24.7	24.5	24.3 / 24.5	24.3	24.5 / 25.1	24.9	24.7 / 24.9	25.2	23.8 / 24.1	25.2	25.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	7.5	7.9	5.3	7.6	5.3	7.9	5.6	7.7	5.4	7.2	5.8	7.6	5.8	5.8
pH (s.u.)	7.61	7.72	7.45	7.62	7.35	7.62	7.43	7.67	7.44	7.62	7.42	7.90	JDP 8/1/22 ~~7.35~~ 7.49	7.42
Conductivity (µmho/cm)	369	368	372	369	392	372	382	366	389	368	379	367	384	382
Hardness (mg/L $CaCO_3$)	137	-	-	137	-	-	-	137	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	86	-	-	86	-	-	-	103	-	-	-	-	-	-

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

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

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1156
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	25.0	25.1	24.3 / 24.7	24.9	24.3 / 24.5	24.7	24.5 / 25.1	24.7	24.7 / 24.9	25.2	23.8 / 24.1	25.1	25.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	7.8	8.4	5.6	7.5	5.4	7.7	5.1	8.0	5.1	8.0	5.8	7.7	5.5	5.6
pH (s.u.)	8.11	8.04	8.01	7.95	7.91	7.91	7.88	8.03	7.85	8.07	7.86	8.10	7.92	7.88
Conductivity (µmho/cm)	884	886	882	1027	897	1027	1017	1171	1045	1169	1152	1168	1166	1172
Hardness (mg/L $CaCO_3$)	308	-	-	359 / 368	MHH 8/16/22	-	-	410	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	222	-	-	205	-	-	-	239	-	-	-	-	-	-

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

Solution Tested: 20% Effluent
Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1156
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	25.2	25.2	24.3 / 24.7	25.0	24.3 / 24.5	24.5	24.5 / 25.1	24.4	24.7 / 24.9	25.4	23.8 / 24.1	25.2	25.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	7.9	8.3	5.3	8.2	4.9	8.3	5.3	8.0	5.6	8.0	5.9	7.9	5.7	5.8
pH (s.u.)	7.92	7.91	7.97	7.89	7.87	7.91	7.95	7.90	7.98	7.96	7.92	8.08	7.99	7.94
Conductivity (µmho/cm)	853	857	875	971	886	974	1011	1089	1022	1092	1116	1092	1131	1137
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1156
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	25.1	25.1	24.3 / 24.7	25.0	24.3 / ~~25.2~~ 24.5	24.4	24.5 / 25.1	24.5	24.7 / 24.9	25.2	23.8 / 24.1	25.2	25.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	7.8	8.4	5.6	8.0	4.5	8.5	5.0	7.8	6.0	8.0	6.1	8.1	LA	5.6
pH (s.u.)	7.79	7.90	8.00	7.80	7.84	7.87	7.94	7.77	8.03	7.87	7.99	8.01	LA	7.93
Conductivity (µmho/cm)	821	825	846	916	862	919	957	1009	965	1007	1041	1069	LA	1062
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1156
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	25.3	25.4	24.3 / 24.7	25.3	24.3 / 24.5	25.1	24.5 / 25.1	24.7	24.7 / 24.9	25.3	23.8 / 24.1	25.5	26.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	7.7	8.5	5.9	7.9	4.6	8.4	6.2	7.6	6.1	8.0	6.2	7.9	6.2	5.3
pH (s.u.)	7.69	7.82	8.03	7.74	7.86	7.86	8.07	7.68	8.03	7.82	7.98	7.96	8.05	7.91
Conductivity (µmho/cm)	789	793	809	861	821	864	891	923	966	923	954	925	974	970
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1156
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	25.2	25.2	24.3 / 24.7	25.2	24.3 / 24.5	24.8	24.5 / 25.1	24.9	24.7 / 24.9	25.3	23.8 / 24.1	25.3	25.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	7.6	8.5	5.7	7.9	5.1	8.3	5.7	7.3	5.4	8.0	5.6	8.0	5.7	5.5
pH (s.u.)	7.57	7.74	7.96	7.65	7.89	7.82	8.00	7.56	8.01	7.72	7.88	7.85	7.95	7.90
Conductivity (µmho/cm)	760	761	774	809	785	809	841	843	852	842	869	843	882	885
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: DJA, JDP, MHH
Beginning Date: 7/26/22 Time: 1631
Ending Date: 8/2/22 Time: 1156
Test Organism: Pimephales promelas Age: < 24 Hours
Dilution Water Used: Upstream 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)	25.1	25.3	24.3 / 24.7	25.3	24.3 / 24.5	24.5	24.5 / 25.1	24.5	24.7 / 24.9	25.2	23.8 / 24.1	25.3	25.0 / 25.1	24.1
Dissolved Oxygen (mg/L)	6.9	8.4	5.9	7.7	4.4	8.3	5.5	6.8	5.9	7.7	5.7	7.8	5.5	5.6
pH (s.u.)	7.42	7.65	7.97	7.56	7.82	7.79	7.99	7.45	8.03	7.59	7.88	7.81	7.93	7.90
Conductivity (µmho/cm)	727	729	744	752	754	754	790	757	803	760	793	762	797	805
Hardness (mg/L $CaCO_3$)	274	-	-	308	-	-	-	291	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	205	-	-	205	-	-	-	239	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

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

Person(s) conducting Test: JDP, DJA, MHH, BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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)	24.7	25.0	24.7 / 24.8	24.5	24.8 / 24.9	24.3	24.3 / 24.5	24.9	24.8 / 25.1	25.2	24.9 / 25.1	25.2	24.9 - 25.1	25.0 - 25.3
Dissolved Oxygen (mg/L)	7.5	7.9	7.1	7.6	7.2	7.9	7.9	7.7	7.7	7.2	7.6	7.6	7.1	6.8
pH (s.u.)	7.61	7.72	7.71	7.62	7.68	7.62	7.71	7.67	7.63	7.62	7.75	7.90	7.67	7.55
Conductivity (µmho/cm)	369	368	382	369	387	372	383	366	396	368	385	367	383	392
Hardness (mg/L $CaCO_3$)	137	-	-	137	-	-	-	137	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	86	-	-	86	-	-	-	103	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

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

Person(s) conducting Test: JDP, DJA, MHH, DG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2 /22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1 - 7.4 Hrs.
Dilution Water Used: Upstream 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)	26.0	25.1	24.7 / 24.8	24.9	24.8 / 24.9	24.7	24.3 / 24.5	24.7	24.8 / 25.1	25.2	24.9 / 25.1	25.1	24.9 - 25.1	25.0 - 25.3
Dissolved Oxygen (mg/L)	7.8	8.4	7.2	7.5	7.2	7.7	8.0	8.0	7.8	8.0	7.6	7.7	7.1	6.7
pH (s.u.)	8.11	8.04	8.17	7.95	8.11	7.91	8.27	8.03	8.26	8.07	8.24	8.10	8.21	8.05
Conductivity (µmho/cm)	884	886	888	1027	885	1027	1033	1171	1034	1169	1162	1168	1172	1163
Hardness (mg/L $CaCO_3$)	308	-	-	359	-	-	-	410	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	222	-	-	205	-	-	-	239	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: JDP, DJA, MHH, BC
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2 /22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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)	25.2	25.2	24.7 / 24.8	25.0	24.8 / 24.9	24.5	24.3 / 24.5	24.4	24.8 / 25.1	25.4	24.9 / 25.1	25.2	24.9 - 25.1	25.0 - 25.3
Dissolved Oxygen (mg/L)	7.9	8.3	7.2	8.2	7.3	8.3	8.1	8.0	7.9	8.0	7.6	7.9	7.2	6.8
pH (s.u.)	7.92	7.91	8.18	7.89	8.12	7.91	8.28	7.90	8.29	7.96	8.25	8.08	8.24	8.08
Conductivity (µmho/cm)	853	857	883	971	896	974	1018	1089	1030	1092	1121	1092	1128	1126
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: JDP, DJA, MHH, BW
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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)	25.1	25.1	24.7 / 24.8	25.0	24.8 / 24.9	24.4	24.3 / 24.5	24.5	24.8 / 25.1	25.2	24.9 / 25.1	25.2	24.9 - 25.1	25.0 / 25.3
Dissolved Oxygen (mg/L)	7.8	8.4	7.1	8.0	7.2	8.5	8.0	7.8	7.8	8.0	7.6	8.1	7.2	6.8
pH (s.u.)	7.79	7.90	8.17	7.80	8.11	7.87	8.29	7.77	8.30	7.87	8.26	8.01	8.24	8.09
Conductivity (µmho/cm)	821	825	862	916	868	919	977	1009	979	1007	1050	1009	1050	1056
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: JDP, DJA, MHH, BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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)	25.3	25.4	24.7 / 24.8	25.3	24.8 / 24.9	25.1	24.3 / 24.5	24.7	24.8 / 25.1	25.3	24.9 / 25.1	25.5	24.9 - 25.1	25.0 - 25.3
Dissolved Oxygen (mg/L)	7.7	8.5	7.2	7.9	7.3	8.4	8.0	7.6	8.0	8.0	7.6	7.9	7.2	7.0
pH (s.u.)	7.69	7.82	8.18	7.74	8.12	7.86	8.30	7.68	8.31	7.82	8.27	7.96	8.25	8.10
Conductivity (µmho/cm)	789	793	834	861	828	864	92 / 916	923	912	923	954	925	957	969
Hardness (mg/L $CaCO_3$)	-	-	-	-	-	-	DJA 7/29/22	-	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: JDP, DJA, MHH, BV
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/ 2 /22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1-7.4 Hrs.
Dilution Water Used: Upstream 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)	25.2	25.2	24.7 / 24.8	25.2	24.8 / 24.9	24.8	24.3 / 24.5	24.9	24.8 / 25.1	25.3	24.9 / 25.1	25.3	24.9 - 25.1	25.0 / 25.3
Dissolved Oxygen (mg/L)	7.6	8.5	7.0	7.9	7.4	8.3	8.0	7.3	8.0	8.0	7.6	8.0	7.2	6.9
pH (s.u.)	7.57	7.74	8.17	7.65	8.13	7.82	8.30	7.56	8.31	7.72	8.27	7.85	8.25	8.10
Conductivity (µmho/cm)	760	761	803	809	797	809	860	843	863	842	866	843	877	889
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, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PK00002*KD

Person(s) conducting Test: JDP, DJA, MHH BG
Beginning Date: 7/26/22 Time: 1603
Ending Date: 8/2/22 Time: 1150
Test Organism: Ceriodaphnia dubia Age: 4.1-7.44hrs.
Dilution Water Used: Upstream 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)	25.1	25.3	24.7 / 24.8	25.3	24.8 / 24.9	24.5	24.3 / 24.5	24.5	24.8 / 25.1	25.2	24.9 / 25.1	25.3	24.9 - 25.1	25.0 - 25.3
Dissolved Oxygen (mg/L)	6.9	8.4	7.2	7.7	7.3	8.3	8.0	6.8	7.9	7.7	7.6	7.8	7.2	6.9
pH (s.u.)	7.42	7.65	8.19	7.56	8.13	7.79	8.31	7.45	8.32	7.59	8.27	7.81	8.24	8.10
Conductivity (µmho/cm)	727	729	755	752	762	754	784	757	782	760	785	762	787	798
Hardness (mg/L $CaCO_3$)	274	-	-	308	-	-	-	291	-	-	-	-	-	-
Alkalinity (mg/L $CaCO_3$)	205	-	-	205	-	-	-	239	-	-	-	-	-	-

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

Discharger/Toxicant: Allen County, Shawnee No. 2 WWTP
Address: 3640 Spencerville Road, Lima, OH 45805
Type of Test: Chronic
Date of Test: 7/26/22 - 8/2 /22
Effluent Serial No.: 001
Permit No.: 2PK00002*KD

Investigator: JDP
Culture No.: 5
Culture initiation date: 7/20/22
Culture ending date: 7/27/22
Origin of parental organisms: 3
Age at start of culture: <24 Hrs
Culture water renewal frequency: minimum 3 times per week
Culture water: Dechlor 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)	C1	21	0842-1200	46	1
2)	C2	NLHH 8/16/22 ~~X~~ 19		44	2
3)	C3	20		44	3
4)	C4	20		43	4
5)	C9	22		48	5
6)	D2	20		45	6
7)	D3	19		45	7
8)	D5	18		42	8
9)	D6	21		47	9
10)	D10	19	0842-1200	43	10

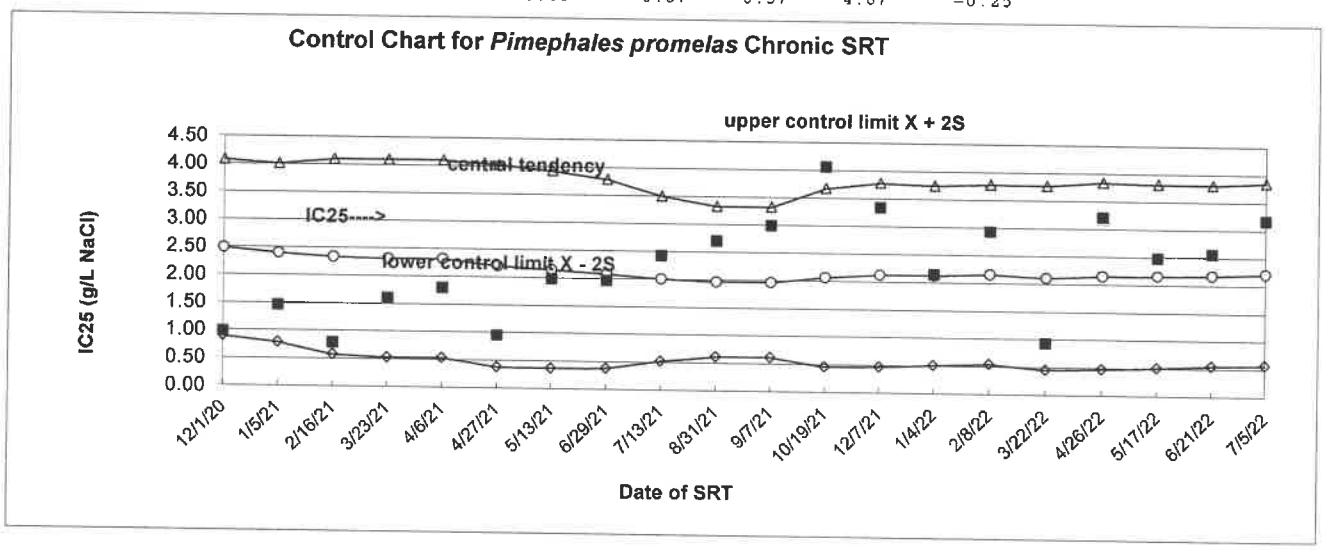
COMMENTS:

Appendix D

SRT Data

IC25 for *Pimephales promelas* Chronic NaCl SRT

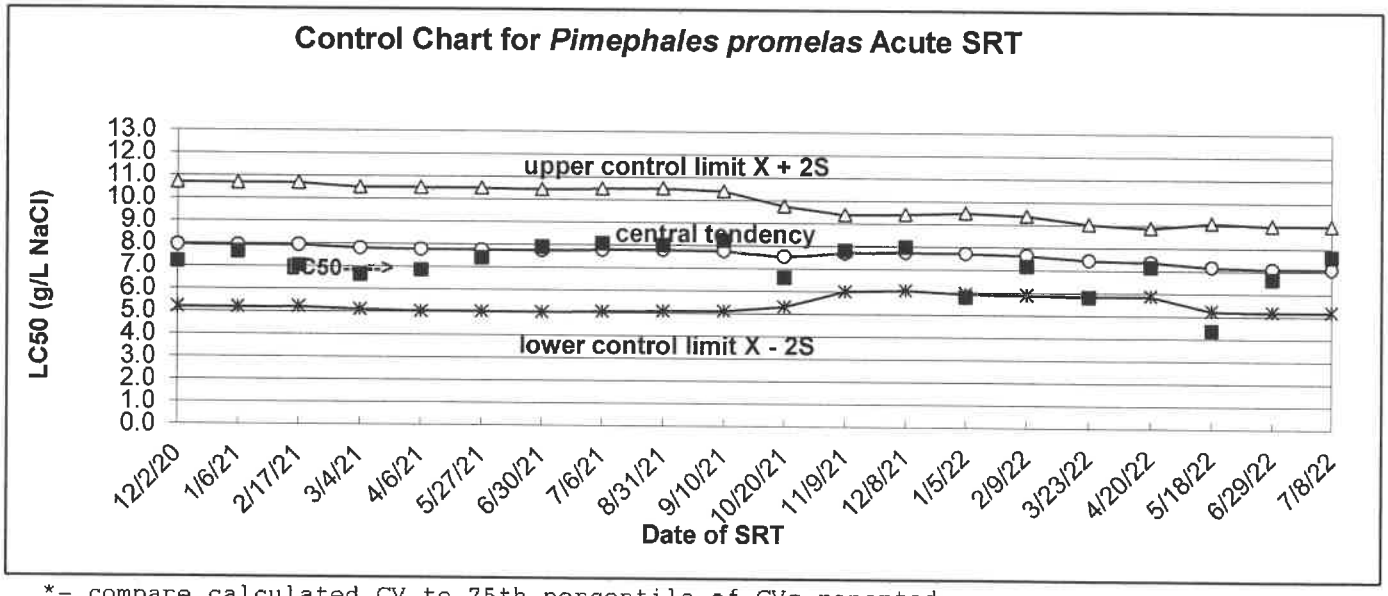
Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	CV Warning Limit**	CV Control Limit**
12/1/20	0.98	2.49	0.80	4.08	0.89	0.32	4.88	0.10		
1/5/21	1.46	2.40	0.81	4.01	0.79	0.34	4.81	-0.02		
2/16/21	0.78	2.33	0.88	4.09	0.58	0.38	4.97	-0.30		
3/23/21	1.60	2.31	0.89	4.10	0.53	0.39	4.99	-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		
4/26/22	3.21	2.15	0.84	3.83	0.47	0.39	4.67	-0.37		
5/17/22	2.48	2.17	0.83	3.82	0.51	0.38	4.64	-0.31		
6/21/22	2.56	2.18	0.81	3.81	0.55	0.37	4.62	-0.26		
7/5/22	3.17	2.21	0.82	3.85	0.57	0.37	4.67	-0.25		



*- 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 ± 3SD = Well outside expected range.

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]
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
4/20/22	7.1	7.4	0.8	8.9	5.9	0.10	9.6	5.1	Probit
5/18/22	4.4	7.2	1.0	9.1	5.2	0.14	10.1	4.3	Probit
6/29/22	6.6	7.1	1.0	9.0	5.2	0.13	10.0	4.2	Probit
7/8/22	7.6	7.1	1.0	9.0	5.2	0.13	9.9	4.2	Probit

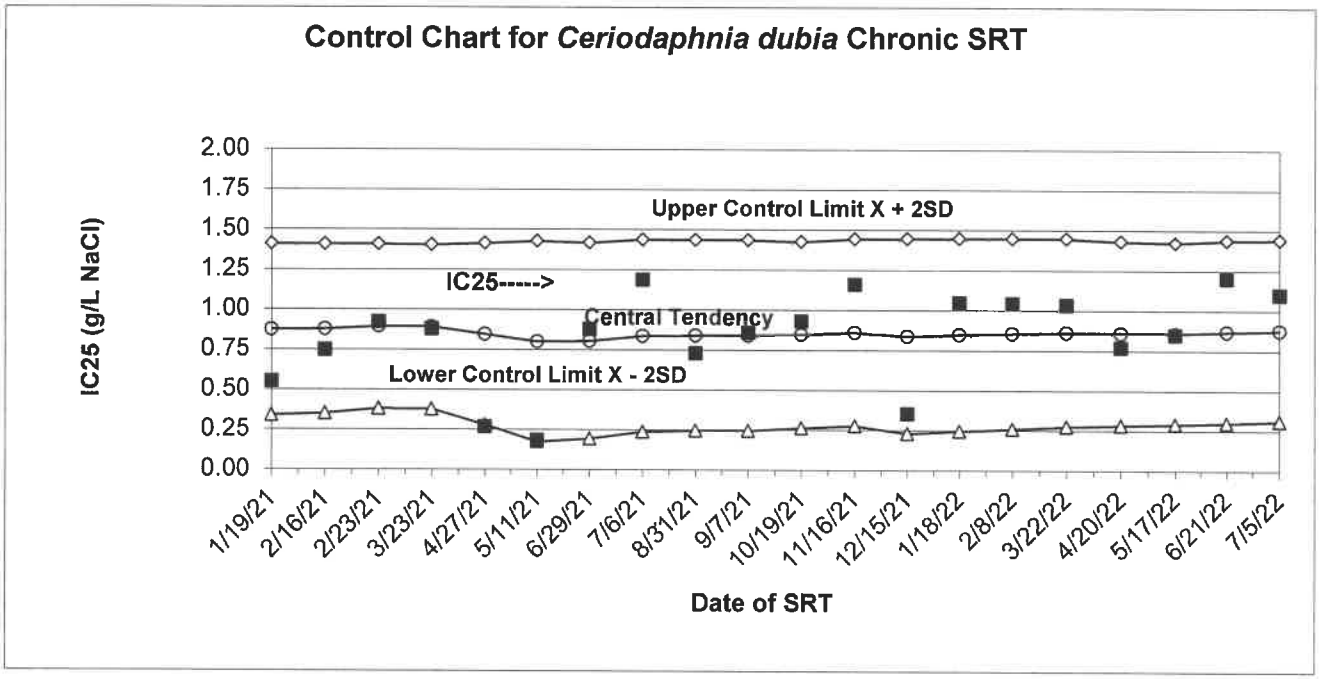


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003.
75th percentile of CVs for LC50 = 0.19
Calculated CV should not exceed 75th percentile of CVs.
X ± 3SD = Well outside expected range.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

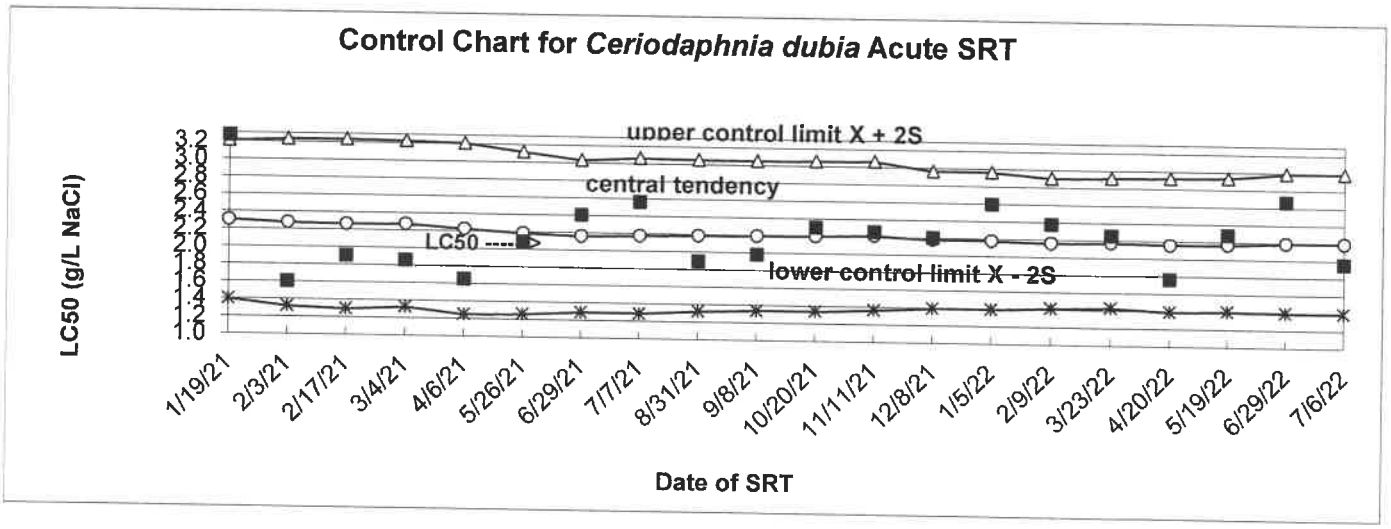
Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
1/19/21	0.55	0.88	0.27	1.41	0.34	0.31	1.68	0.07
2/16/21	0.75	0.88	0.26	1.41	0.35	0.30	1.67	0.09
2/23/21	0.92	0.90	0.26	1.41	0.38	0.29	1.66	0.13
3/23/21	0.88	0.89	0.26	1.40	0.38	0.29	1.66	0.12
4/27/21	0.27	0.85	0.28	1.41	0.28	0.33	1.70	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
4/20/22	0.77	0.86	0.29	1.43	0.28	0.33	1.72	0.00
5/17/22	0.85	0.86	0.28	1.42	0.29	0.33	1.70	0.01
6/21/22	1.20	0.87	0.28	1.44	0.30	0.33	1.72	0.02
7/5/22	1.10	0.88	0.28	1.44	0.31	0.32	1.72	0.03



*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-2 in EPA/833/R-00/003 .
75th percentile of CVs for IC25 = 0.45
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

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]
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
4/20/22	1.8	2.2	0.4	2.9	1.4	0.18	3.3	1.0	Probit
5/19/22	2.3	2.2	0.4	2.9	1.4	0.18	3.3	1.0	Probit
6/29/22	2.7	2.2	0.4	3.0	1.4	0.18	3.4	1.0	Probit
7/6/22	2.0	2.2	0.4	3.0	1.4	0.18	3.4	1.0	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.
X ± 3SD = Well outside expected range.

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