

Customer: Allen County Sanitary Engineer
Facility: American-Bath WWTP
Outfall: Effluent 001
Report Date: 11/30/20
Ohio EPA Permit No.: 2PH00007*LD
Alloway Lab #: L20-20904-01

Report of Chronic Biomonitoring

Samples Collected on:

11/16/20
11/18/20
11/20/20

Pimephales promelas
Ceriodaphnia dubia

Allen County Sanitary Engineer
American-Bath WWTP
3226 North Cole Street
Lima, OH 45805

Customer: Allen County Sanitary Engineer
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Outfall: Effluent 001
Report Date: 11/30/20
Ohio EPA Permit No.: 2PH00007*LD
Alloway Lab #: L20-20904-01

Date: 11/30/20

Rick Boerger
Allen County Sanitary Engineer
3226 North Cole Street
Lima, OH 45805

Dear Mr. Boerger:

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

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

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

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

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



Brian Good
Bioassay Lab Manager

Outfall: Effluent 001
Report Date: 11/30/20
Ohio EPA Permit No.: 2PH00007*LD

1. Facility Name	Allen County Sanitary Engineer
2. Facility Address	American-Bath WWTP 3226 North Cole Street Lima, OH 45805
3. Ohio EPA Permit No.	2PH00007*LD
4. NPDES No.	OH0023841
5. Facility Contact	Rick Boerger
6. Telephone No.	419-225-8084
7. Consulting Laboratory	Alloway
8. Lab Contact	Brian Good
9. Lab Telephone No.	419-223-1362
10. Receiving water(s) of discharge	Pike 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: <u>6/19/2017</u>	

I certify under penalty of law that I have personally examined and am familiar with the information submitted in this document and based on my inquiry of those individuals immediately responsible for obtaining information, I believe the submitted information is true, accurate and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment.

Signature

Date

Name (typed or printed)

Title

Customer: Allen County Sanitary Engineer
 Facility: American-Bath WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PH00007*LD
 Date of *P. promelas* Test: 11/17-24/20
 Date of *C. dubia* Test: 11/17-23/20

Summary of Chronic Toxicity Test Results

Pimephales promelas

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, <i>Pimephales promelas</i> - TUc	1.8	1.0	Growth
Acute Toxicity, <i>Pimephales promelas</i> - TUa	AA	0.2	
Upstream:			
96 Hr. Acute Toxicity, <i>Pimephales promelas</i> - % Affected	2.5	2.5	
7 day Chronic Toxicity, <i>Pimephales promelas</i> - % Affected	10.0	2.5	

Summary of Chronic Toxicity Test Results

Ceriodaphnia dubia

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, <i>Ceriodaphnia dubia</i> - TUc	1.5	1.0	Reproduction
Acute Toxicity, <i>Ceriodaphnia dubia</i> - TUa	AA	0.2	
Upstream:			
48 Hr. Acute Toxicity, <i>Ceriodaphnia dubia</i> - % Affected	AA	10.0	
7 day Chronic Toxicity, <i>Ceriodaphnia dubia</i> - % Affected	AA	10.0	

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
Facility: American-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*LD
Date of Test: 11/17-24/20

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%)	2.5% (2.5%)	5.0% (5.0%)	10.0% (10.0%)	0.356	0.047
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	7.5% (7.5%)	0.362	0.034
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	12.5% (12.5%)	15.0% (15.0%)	20.0% (20.0%)	0.296	0.013
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	7.5% (7.5%)	12.5% (12.5%)	0.325	0.065
60%	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	5.0% (5.0%)	7.5% (7.5%)	7.5% (7.5%)	10.0% (10.0%)	0.254	0.030
80%	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	12.5% (12.5%)	15.0% (15.0%)	0.184	0.007
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%)	0.192	0.020
LC50 Values (%):		>100	>100	>100	>100	>100	>100	Calculated TUC for Survival <1.0	
95% Confidence Limits	LL								
	UL								
EC50 Values (%):		>100	>100	>100	>100	>100	>100	Calculated TUC for Growth (100/IC25) 1.8	
95% Confidence Limits	LL								
	UL								
7-Day NOEC for Mortality (%)		100					40	Method(s) used to determine Values: IC Pin, Dunnett's	
7-Day LOEC for Mortality (%)		>100					60		
Chronic Value for Mortality (%)		>100					49.0		
IC25 (%)		55.6					>100		
			7-Day NOEC for Growth (%)						
			7-Day LOEC for Growth (%)						
			Chronic Value for Growth (%)						
			IC50 (%)						

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

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
 Facility: American-Bath WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PH00007*LD
 Date of Test: 11/17-23/20

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%)	--- (---)	31.9
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	31.1
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	33.9
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%)	--- (---)	25.5
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	27.5
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	--- (---)	19.0 *
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%)	--- (---)	18.3 *
LC50 Values (%):		>100	>100	>100	>100	>100	>100	Calculated TUc Value for Survival <1.0
95% Confidence Limits	LL							
	UL							
EC50 Values (%):		>100	>100	>100	>100	>100	>100	Calculated TUc Value for Reproduction 1.5
95% Confidence Limits	LL							
	UL							
NOEC for Mortality (%)		100		NOEC for Reproduction (%)			60	Method(s) used to determine Values: IC Pin, Dunnett's
LOEC for Mortality (%)		>100		LOEC for Reproduction (%)			80	
Chronic Value for Mortality (%)		>100		Chronic Value for Reprod (%)			69.3	
IC25 (%)		64.9		IC50 (%)			>100	

*Indicates significant differences from the primary control with an $p < 0.05$

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
 Facility: American-Bath WWTP
 Outfall: Effluent 001
 Report Date: 11/30/20
 Ohio EPA Permit No.: 2PH00007*LD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection	
			Beginning	Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	11/15/20 @ 10:00	11/16/20 @ 10:00
Type (Grab/Comp):	Comp	2nd	11/17/20 @ 10:30	11/18/20 @ 10:30
Volume Collected:	5 Gal.	3rd	11/19/20 @ 10:30	11/20/20 @ 10:30
Upstream Station:				
Waterbody:	Pike Run			
Station No.:	801	1st		11/16/20 @ 11:08
Type (Grab/Comp):	Grab	2nd		11/18/20 @ 12:22
Volume Collected:	5 Gal.	3rd		11/20/20 @ 12:41

Samples collected by: Alloway

PIMEPHALES PROMELAS CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	<i>Pimephales promelas</i> < 24 hours old
2. Test Type and Duration:	Static renewal; 7 days
3. Test Dates:	11/17-24/20
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Feed 0.1 g (0.1 to 0.2 mL) newly hatched brine shrimp nauplii three times daily (M-F) at 4-h intervals. On weekends 0.1g of nauplii are fed at the beginning of the workday, after renewal, and at the end of the workday. Sufficient larvae are added to provide an excess. Fish are not fed during the final 12-h of the test.
8. Size of Test Vessel:	500 mL plastic cup
9. Volume and Depth of Test Solutions:	250 mL, 6.7 cm deep
10. No. of Test Organisms per Test Vessel:	10 per test vessel
11. No. of Test Vessels per Test Solution:	4 per solution tested
12. Total No. of Test Organisms per Test Solution:	40 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 11/16/20 ; day 2, 3 - 11/18/20 ; day 4, 5, 6 - 11/20/20
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and growth (dry weight).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	<i>Ceriodaphnia dubia</i> 6-14 hours
2. Test Type and Duration:	Static renewal; Until 60% or more of surviving control females have three broods (maximum test duration 8 days)
3. Test Dates:	11/17-23/20
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Fed 0.1 mL YCT and 0.1 mL (3.5 million cells) <i>Selenastrum capricornutum</i> .
8. Size of Test Vessel:	1 fl. oz. (30 mL) clear plastic cup
9. Volume and Depth of Test Solutions:	15 mL, 20 mm deep
10. No. of Test Organisms per Test Vessel:	1 per test vessel
11. No. of Test Vessels per Test Solution:	10 per solution tested
12. Total No. of Test Organisms per Test Solution:	10 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 11/16/20 ; day 2, 3 - 11/18/20 ; day 4, 5, 6 - 11/20/20
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data



Project: L20-20904

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



Report To: Rick Boerger Name: Allen County Sanitary Engineer Company: 3226 North Cole St. Address: Lima, OH 45805		Invoice To (if Different): Name: Company: Address:		Phone #: 419-225-8048 E-mail: rboerger@allencountyohio.com Fax #:		PO#:	
Project Name Bioassay - AM BATH				Turnaround: (Rush Charges May Apply) 3 Working Days ☐ 5 Working Days ☐			
Sampler (Print) Michael S. Hart		Signature: 		Routine ☒		Alloway LIMS # For Lab Use Only	
Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #
1 Effluent	11/15/20	10:00	X		WW	1	1
2 Upstream	11/16/20	11:08		X	SW	1	1
3							
4							
5							
6							
7							
8							

Relinquished by:		Received by:		Date		Time	
1		2		3		4	
2		3		4		5	
3		4		5		6	
4		5		6		7	
5		6		7		8	
6		7		8		9	

Received for Laboratory By: (circle one) (Signature)  Mansfield		Lima		Marion	
Transported to: Lima Marion		Received By:		Date:	
Transported to: Lima Marion		Received By:		Date:	

Matrix Codes: ww - wastewater gw - groundwater dw - drinking water sw - surface water w - water oil - oil s - solid sg - sludge l - leachate a - acid p - product o - other		Method of Delivery UPS ☐ Fed Ex ☐ Client ☐ Alloway Pick Up ☐ Alloway Sampling ☒ Other ☐		Preservation Codes: 1 - None 2 - HNO ₃ 3 - H ₂ SO ₄ 4 - HCl 5 - NaOH 6 - NaOH & Zinc Acetate 7 - Sodium Thiosulfate 8 - Ascorbic Acid Sulfite 9 - Maleic Acid Dihydrogen Citrate 10 - EDTA 11 - Ammonium Chloride 12 - (NH ₄) ₂ SO ₄ & NH ₄ OH 13 - Zinc Acetate 14 - Sodium Sulfite 15 - Potassium Dihydrogen Phosphate 16 - Sodium Sulfite/Sodium Bisulfite		Sample Receiving (For Lab Use Only) Ice Present? ☒ N ☐ Proper Preservation? ☒ N ☐ Container Temperature: 50.6-110	
--	--	---	--	--	--	--	--



Chain of Custody Record

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

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

Report To: Rick Boerger Name: Allen County Sanitary Engineer Company: 3226 North Cole St. Address: Lima, OH 45805				Invoice To (if Different): Name: Company: Address:				Notes/Comments:			
Phone #: 419-225-8048 Fax #:											
E-mail: rboerger@allencountyohio.com PCH#:											
Project Name		Bioassay - AM BATH									
Sampler	(Print) Michael S. Amby	(Signature)									
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	Turnaround: (Rush Charges May Apply) ☐ Next Day ☐ 3 Working Days ☒ Routine ☐ 2 Working Days ☐ 5 Working Days	
1	Effluent	11/17/20 ~ 10:30 11/18/20 10:30	X		WW	1	1	Toxicity			
2	Upstream	11/18/20 12:22		X	SW	1	1	Toxicity			
3											
4											
5											
6											
7											
8											
Relinquished by:		Received by:		Date		Time		Method of Delivery		Matrix Codes:	
1								UPS ☐		1 - None 7 - Sodium Thiosulfate 13 - Zinc Acetate 2 - HNO ₃ 8 - Ascorbic Acid Sulfoxide 14 - Sodium Dithionite 3 - H ₂ SO ₄ 9 - Maleic Acid Citrate 15 - Potassium Dichromate 4 - HCl 10 - EDTA 16 - Sodium Sulfate/Sodium Bisulfate 5 - NaOH 11 - Ammonium Chloride 6 - NaOH & Zinc Acetate 12 - (NH ₄) ₂ SO ₄ & NH ₄ OH	
2								Fed Ex ☐		Ice Present? ☒ N ☐	
3								Client ☐		Proper Preservation? ☒ N ☐	
4								Alloway Pick Up ☐		Container Temperature: 6°C T-128 DP	
5								Alloway Sampling ☒			
6								Other ☐			
Received for Laboratory By: (circle one) Mansfield Lima Marlon				Date: 11/18/20 Time: 12:34							
(Signature)											

**Transported to: Lima
Marion**

Received By:

Date: _____ Time: _____

Transported to: Lima
Marion

Received By:

Date: _____ Time: _____



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(P) 419-223-1362 (F) 419-227-3792
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- 1502 W. Fourth Street, Mansfield, OH 44806
(P) 419-525-1644 (F) 419-524-5575

Report To: Rick Boerger Name: Allen County Sanitary Engineer Company: 3226 North Cole St. Address: Lima, OH 45805		Invoice To (if different): Name: Company: Address:	
Phone #: 419-225-8048 E-mail: rboerger@allencountyohio.com		Fax #: PO#	
Project Name: Bioassay - AM BATH			
Sampler: (Print) M. Zahra J. Boerger (Signature)			
Sample ID / Sample Location	Sample Date	Sample Time	Grab
1 Effluent	11/19/10	10:30-10:35	X
2 Upstream	11/19/10	12:41	X
3			
4			
5			
6			
7			
8			
Relinquished by:		Received by:	Date
1			
2			
3			
4			
5			
6			
Received for Laboratory By: (circle one) Mansfield		Lima	Marion
(Signature)		11/20/10	R. J. B.

**Transported to: Lima
Marion**

Received By:

Date: _____ Time: _____

Transported to: Lima
Marion

Received By:

Date: _____ Time: _____

Appendix B
Raw Data Sheets

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

Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*LD

Person(s) Conducting Test: BG, JDP, EMS, DIA
Beginning: Date 11/17/20 Time 1304
Ending: Date 11/24/20 Time 1015
Age of Pimephales promelas: < 24 Hours
Dilution Water Used: Upstream

Day	Lab Control					Upstream Control					20% Effluent					40% Effluent				
	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
4	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
5	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
6	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
7	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G

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

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

Industry/Toxicant: Allen County, Am. - Bath WWTP
Address: 3226 North Cole St., Lima OH 45805
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*LD

Person(s) Conducting Test: BG, JDP, EMS, DJA
Beginning: Date 11/17/20 Time 2304
Ending: Date 11/24/20 Time 1615
Age of Pimephales promelas: < 24 Hours
Dilution Water Used: Upstream

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

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

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

Discharge: Allen County, Am. - Bath WWTP
 Address: 3226 North Cole St., Lima OH 45805

Date of Test: 11/17/20 - 11/24/20

NPDES Permit No.: 2PH00007*LD

Pimephales promelas Age at Start: < 24 Hours

Effluent Serial No.: 001

Drying Temperature (C°): 60

Drying Time (h): 24

Date Weighed: 11/30/20

Analyst: BG

Balance Calibration Check: 1 gram weight = 1.00000 grams
 1.00001

Analyst: EYVS
 BG

Dilution Water Used: Upstream

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
Fish at Start of Test			1.13039		10		
Lab Control	1	8	1.08970	1.09290	10	3.20	0.320
	2	10	1.13030	1.13427	10	3.97	0.397
	3	9	1.08281	1.08632	10	3.51	0.351
	4	10	1.10420	1.10806	10	3.80	0.380
Upstream Control	1	8	1.11081	1.11392	10	3.06	0.306
	2	9	1.13110	1.13452	10	3.42	0.342
	3	9	1.12702	1.13062	10	3.56	0.356
	4	10	1.09255	1.09673	10	4.18	0.418
20% Effluent	1	7	1.13212	1.13509	10	2.93	0.293
	2	9	1.09781	1.10092	10	3.11	0.311
	3	8	1.09184	1.09465	10	2.81	0.281
	4	8	1.12403	1.12764	10	3.01	0.301
40% Effluent	1	10	1.12999	1.13412	10	4.13	0.413
	2	8	1.12400	1.12696	10	2.96	0.296
	3	7	1.08343	1.08605	10	2.62	0.262
	4	10	1.08812	1.09141	10	3.29	0.329

Reviewed By: _____

WEIGHT OF FISH IN REPLICATE TEST VESSELS

7-DAY PIMEPHALES PROMELAS CHRONIC TOXICITY TEST

Discharge: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

Drying Temperature (C°):

60

Drying Time (h):

24

NPDES Permit No.: 2PH00007*LD

Pimephales promelas Age at Start: < 24 Hours

Date of Test:

11/17/20 - 11/24/20

Dilution Water Used: Upstream

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
60% Effluent	1	10	1.12898	1.13182	10	2.84	0.284
	2	8	1.09504	1.09730	10	2.26	0.226
	3	10	1.13249	1.13525	10	2.76	0.276
	4	8	1.09098	1.09328	10	2.30	0.230
80% Effluent	1	7	1.10027	1.10220	10	1.83	0.183
	2	8	1.09099	1.09286	10	1.87	0.187
	3	9	1.10109	1.10360	10	1.91	0.191
	4	10	1.12505	1.12739	10	1.74	0.174
100% Effluent	1	10	1.12134	1.12336	10	2.02	0.202
	2	10	1.08408	1.08580	10	1.72	0.172
	3	10	1.12335	1.12551	10	2.16	0.216
	4	10	1.09989	1.10169	10	1.80	0.180
	1				10		
	2				10		
	3				10		
	4				10		

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 11/17/2020 Test ID: Bath1120p Sample ID: L20-20904-01
 End Date: 11/24/2020 Lab ID: ALL-ALLOWAY Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: EPAFW02-EPA/821/R-02-01 Test Species: PP-Pimephales promelas
 Comments:

Conc-%	1	2	3	4
Upstr Control	0.3060	0.3420	0.3560	0.4180
20	0.2930	0.3110	0.2810	0.3010
40	0.4130	0.2960	0.2620	0.3290
60	0.2840	0.2260	0.2760	0.2300
80	0.1830	0.1870	0.1910	0.1740
100	0.2020	0.1720	0.2160	0.1800

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Upstr Control	0.3555	1.0000	0.3555	0.3060	0.4180	13.133	4				0.3555	1.0000
20	0.2965	0.8340	0.2965	0.2810	0.3110	4.279	4	2.299	2.410	0.0619	0.3108	0.8741
40	0.3250	0.9142	0.3250	0.2620	0.4130	19.917	4	1.188	2.410	0.0619	0.3108	0.8741
*60	0.2540	0.7145	0.2540	0.2260	0.2840	11.907	4	3.955	2.410	0.0619	0.2540	0.7145
*80	0.1838	0.5169	0.1838	0.1740	0.1910	3.959	4	6.692	2.410	0.0619	0.1881	0.5292
*100	0.1925	0.5415	0.1925	0.1720	0.2160	10.472	4	6.351	2.410	0.0619	0.1881	0.5292

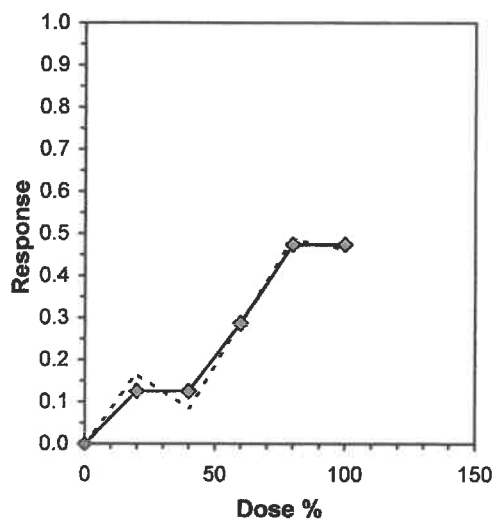
Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.94519	0.916	0.76146	1.895			
Bartlett's Test indicates equal variances (p = 0.02)					13.8029	15.0863					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		40	60	48.9898	2.5	0.06185	0.17399	0.01977	0.00132	7.0E-06	5, 18
Treatments vs Upstr Control											

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	7.944	9.731	2.430	65.146	2.4530
IC10*	15.888	14.857	4.860	67.228	0.4503
IC15	43.022	14.502	0.000	62.156	-0.5550
IC20	49.286	11.204	0.000	69.507	-1.4143
IC25	55.551	6.715	31.911	72.392	-0.5843
IC40	72.357				
IC50	>100				

* indicates IC estimate less than the lowest concentration

$$TU_c = 1.8$$



CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>Lab Control</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, DJA</u>
Industry/Toxicant: <u>Allen County, Am. - Bath WWTP</u>	Beginning Date: <u>11/17/20</u> Time: <u>1350</u>
Address: <u>3226 North Cole St., Lima OH 45805</u>	Ending Date: <u>11/23/20</u> Time: <u>1430</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>6-14H</u>
NPDES Permit No.: <u>2PH00007*LD</u>	Dilution Water Used: <u>Upstream</u>

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP		
Rep.	DAY of TEST								No. Young	No. Broods	
	0	1	2	3	4	5	6	7			
1	0 / G	0 / G	0 / G	3 / G	0 / G	13 / G	16 / G	/	32	3	
2	/	/	/	0 /	6 /	9 /	14 /	/	29	3	
3	/	/	/	/	5 /	12 /	16 /	/	33	3	
4	/	/	/	/	4 /	13 /	15 /	/	32	3	
5	/	/	/	/	6 /	11 /	14 /	/	31	3	
6	/	/	/	/	5 /	0 /	14 /	/	19	2	
7	/	/	/	/	6 /	12 /	14 /	/	32	3	
8	/	/	/	/	7 /	11 /	15 /	/	33	3	
9	/	/	/	/	6 /	13 /	15 /	/	34	3	
10	0 / G	0 / G	0 / G	0 / G	7 / G	15 / G	14 / G	/	36	3	
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G				
									SUM	311	30
									MEAN	31.1	3.0
									S.D.	4.6	0.0

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

Reviewed by: _____

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control

Person(s) Conducting Test: BG, JDP, EMS, DJA

Industry/Toxicant: Allen County, Am. - Bath WWTP

Beginning Date: 11/17/20

Time: 1350

Address: 3226 North Cole St., Lima OH 45805

Ending Date: 11/23/20

Time: 1430

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 6-14H

NPDES Permit No.: 2PH00007*LD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	6/G	0/G	13/G	17/G	/	36	3
2	/	/	/	0/G	6/G	13/G	16/G	/	35	3
3	/	/	/	/	5/G	12/G	16/G	/	33	3
4	/	/	/	/	6/G	9/G	13/G	/	28	3
5	/	/	/	/	7/G	15/G	17/G	/	39	3
6	/	/	/	/	6/G	5/G	0/G	/	11	2
7	/	/	/	/	5/G	14/G	17/G	/	36	3
8	/	/	/	/	7/G	12/G	16/G	/	35	3
9	/	/	/	/	7/G	13/G	16/G	/	36	3
10	0/G	0/G	0/G	0/G	6/G	10/G	14/G	/	30	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									319	29
MEAN									31.9	2.9
S.D.									8.0	0.3

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent

Person(s) Conducting Test: BG, JDP, EMS, DJA

Industry/Toxicant: Allen County, Am. - Bath WWTP

Beginning Date: 11/17/20

Time: 1350

Address: 3226 North Cole St., Lima OH 45805

Ending Date: 11/23/20

Time: 1430

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 6-14H

NPDES Permit No.: 2PH00007*LD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	7/G	0/G	16/G	20/G	/	43	3
2	/	/	/	0/G	6/G	12/G	14/G	/	32	3
3	/	/	/	/	6/G	13/G	16/G	/	35	3
4	/	/	/	/	8/G	9/G	16/G	/	33	3
5	/	/	/	/	6/G	14/G	11/G	/	31	3
6	/	/	/	/	6/G	11/G	14/G	/	31	3
7	/	/	/	/	6/G	10/G	17/G	/	33	3
8	/	/	/	/	6/G	13/G	16/G	/	35	3
9	/	/	/	0/G	8/G	9/G	17/G	/	34	3
10	0/G	0/G	0/G	5/G	0/G	12/G	15/G	/	32	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									339	30
MEAN									33.9	3.0
S.D.									3.5	0.0

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent

Person(s) Conducting Test: BG, JDP, EMS, DJA

Industry/Toxicant: Allen County, Am. - Bath WWTP

Beginning Date: 11/17/20

Time: 1350

Address: 3226 North Cole St., Lima OH 45805

Ending Date: 11/23/20

Time: 1430

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 6-14H

NPDES Permit No.: 2PH00007*LD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	0/G	6/G	11/G	14/G	/	31	3
2	/	/	/	/	6/	10/	13/	/	29	3
3	/	/	/	/	7/	13/	16/	/	36	3
4	/	/	/	/	6/	14/	14/	/	34	3
5	/	/	/	/	7/	11/	14/	/	32	3
6	/	/	/	/	4/	9/	6/	/	19	3
7	/	/	/	/	5/	10/	0/	/	15	2
8	/	/	/	/	5/	8/	0/	/	13	2
9	/	/	/	/	6/	15/	3/	/	24	3
10	0/G	0/G	0/G	0/G	7/G	14/G	1/G	/	22	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									255	28
MEAN									25.5	2.8
S.D.									8.1	0.4

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent

Person(s) Conducting Test: BG, JDP, EMS, DJA

Industry/Toxicant: Allen County, Am. - Bath WWTP

Beginning Date: 11/17/20

Time: 1350

Address: 3226 North Cole St., Lima OH 45805

Ending Date: 11/23/20

Time: 1430

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 6-14 h

NPDES Permit No.: 2PH00007*LD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	6/G	0/G	8/G	15/G	/	29	3
2	/	/	/	0/G	5/G	7/G	15/G	/	27	3
3	/	/	/	/	8/G	10/G	7/G	/	25	3
4	/	/	/	/	7/G	11/G	15/G	/	33	3
5	/	/	/	/	5/G	11/G	4/G	/	20	3
6	/	/	/	/	5/G	11/G	15/G	/	31	3
7	/	/	/	/	6/G	12/G	10/G	/	28	3
8	/	/	/	/	6/G	10/G	8/G	/	24	3
9	/	/	/	/	8/G	12/G	11/G	/	31	3
10	0/G	0/G	0/G	0/G	6/G	9/G	12/G	/	27	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									275	30
MEAN									27.5	3.0
S.D.									3.8	0.0

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent

Person(s) Conducting Test: BG, JDP, EMS, DJA

Industry/Toxicant: Allen County, Am. - Bath WWTP

Beginning Date: 11/17/20

Time: 1356

Address: 3226 North Cole St., Lima OH 45805

Ending Date: 11/23/20

Time: 1430

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 6-14H

NPDES Permit No.: 2PH00007*LD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

TOTAL PER REP

Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	0/G	7/G	12/G	4/G	/	23	3
2	/	/	/	/	4/G	11/G	15/G	/	30	3
3	/	/	/	/	4/G	0/D	-/-	-/-	4	1
4	/	/	/	/	3/G	8/G	5/G	/	16	3
5	/	/	/	/	6/G	8/G	11/G	/	25	3
6	/	/	/	/	6/G	10/G	8/G	/	24	3
7	/	/	/	/	0/G	7/G	0/G	/	7	1
8	/	/	/	/	4/G	7/G	0/G	/	11	2
9	/	/	/	/	6/G	9/G	6/G	/	21	3
10	0/G	0/G	0/G	0/G	5/G	10/G	14/G	/	29	3
Total Adult Cond.	10G	10G	10G	10G	10G	9G 1D	9G 1D			
SUM									190	25
MEAN									19.0	2.5
S.D.									9.1	0.8

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent

Person(s) Conducting Test: BG, JDP, EMS, DJA

Industry/Toxicant: Allen County, Am. - Bath WWTP

Beginning Date: 11/17/20

Time: 1350

Address: 3226 North Cole St., Lima OH 45805

Ending Date: 11/23/20

Time: 1430

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 6-14H

NPDES Permit No.: 2PH00007*LD

Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP	
Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	0/G	4/G	9/G	13/G	/	26	3
2	/	/	/	/	6	10	3	/	19	3
3	/	/	/	/	2	0	0	/	2	1
4	/	/	/	/	6	8	13	/	29 27	3
5	/	/	/	/	7	13	1	/	27 21	3 ₂
6	/	/	/	/	1	12	0	/	13	2
7	/	/	/	/	4	0	10	/	14	2
8	/	/	/	/	5	12	3	/	20	3
9	/	/	/	/	5	9	11	/	25	3
10	0/G	0/G	0/G	0/G	6/G	10/G	0/G	/	16	2
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
									SUM	183
									MEAN	18.3
									S.D.	7.5
										24
										2.4
										0.7

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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 11/17/2020 Test ID: Bath1120c Sample ID: L20-20904-01
 End Date: 11/23/2020 Lab ID: ALL-ALLOWAY Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: EPAFW02-EPA/821/R-02-01 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-%	1	2	3	4	5	6	7	8	9	10
Upstr Control	36.000	35.000	33.000	28.000	39.000	11.000	36.000	35.000	36.000	30.000
20	43.000	32.000	35.000	33.000	31.000	31.000	33.000	35.000	34.000	32.000
40	31.000	29.000	36.000	34.000	32.000	19.000	15.000	13.000	24.000	22.000
60	29.000	27.000	25.000	33.000	20.000	31.000	28.000	24.000	31.000	27.000
80	23.000	30.000	4.000	16.000	25.000	24.000	7.000	11.000	21.000	29.000
100	26.000	19.000	2.000	27.000	21.000	13.000	14.000	20.000	25.000	16.000

Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Upstr Control	31.900	1.0000	31.900	11.000	39.000	25.098	10				32.900	1.0000
20	33.900	1.0627	33.900	31.000	43.000	10.355	10	-0.637	2.287	7.181	32.900	1.0000
40	25.500	0.7994	25.500	13.000	36.000	31.765	10	2.038	2.287	7.181	26.500	0.8055
60	27.500	0.8621	27.500	20.000	33.000	13.953	10	1.401	2.287	7.181	26.500	0.8055
*80	19.000	0.5956	19.000	4.000	30.000	47.853	10	4.108	2.287	7.181	19.000	0.5775
*100	18.300	0.5737	18.300	2.000	27.000	41.058	10	4.331	2.287	7.181	18.300	0.5562

Auxiliary Tests						Statistic	Critical	Skew	Kurt		
Kolmogorov D Test indicates normal distribution (p > 0.05)						0.79392	0.895	-0.8851	0.98999		
Bartlett's Test indicates equal variances (p = 0.03)						12.0427	15.0863				
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		60	80	69.282	1.66667	7.18122	0.22512	416.017	49.313	6.0E-06	5, 54
Treatments vs Upstr Control											

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL		Skew
IC05	25.141	3.475	19.321	33.767	1.4596
IC10	30.281	6.999	24.796	60.429	2.6830
IC15	35.422	11.654	29.426	63.912	1.2085
IC20	60.480	13.854	33.262	72.176	0.1011
IC25	64.867	11.560	36.801	80.670	-0.9290
IC40	78.027				
IC50	>100				

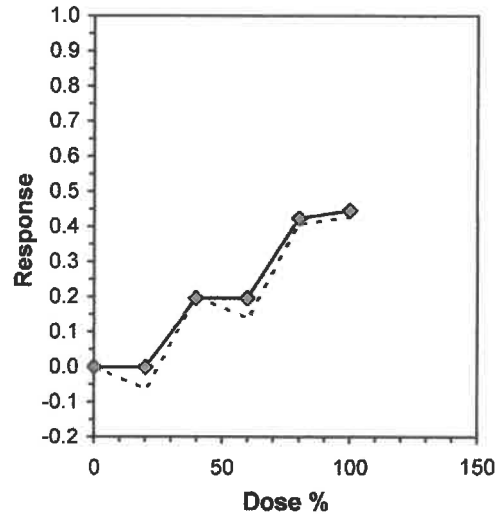
1.0

0.9

0.8

0.7

$\tau u_c = 1.5$



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

Discharger/Toxicant: Allen County, Am. - Bath WWTP
 Address: 3226 North Cole St., Lima OH 45805
 Type of Test: Chronic Date of Test: 11/17/20 - 11/23/20
 Effluent Serial No.: 001 Permit No.: 2PH00007*LD
 Investigator: B6 Culture No.: 99
 Culture initiation date: 11/11/20 Culture ending date: 11/19/20
 Origin of parental organisms: 97
 Age at start of culture: 432H
 Culture water renewal frequency: minimum 3 times per week Culture water: 2.8 MHSF
 Culture temperature: 25°C Fed Daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

Number of brood organisms at start of culture: 40
 Percent survival of parental organisms after 7 days: 100
 Percent of non-reproducing adults after 7 days: 0
 Average young production after 7 days of brood adults for test: _____

<u>BROOD ADULT NO.</u>	<u>BROOD SIZE</u>	<u>HATCHING TIME WINDOW</u>	<u>TOTAL; YOUNG PROD. (7 DAYS)</u>	<u>USED IN REP. NO.</u>
1) <u>A3</u>	<u>18</u>	<u>2400-0800</u>	<u>32</u>	<u>1</u>
2) <u>A4</u>	<u>16</u>	↓	<u>29</u>	<u>2</u>
3) <u>A5</u>	<u>16</u>		<u>30</u>	<u>3</u>
4) <u>A6</u>	<u>17</u>		<u>29</u>	<u>4</u>
5) <u>A7</u>	<u>17</u>		<u>33</u>	<u>5</u>
6) <u>A8</u>	<u>17</u>		<u>30</u>	<u>6</u>
7) <u>A10</u>	<u>17</u>		<u>30</u>	<u>7</u>
8) <u>B2</u>	<u>18</u>	↓	<u>31</u>	<u>8</u>
9) <u>B3</u>	<u>17</u>		<u>31</u>	<u>9</u>
10) <u>B4</u>	<u>16</u>	<u>2400-0800</u>	<u>31</u>	<u>10</u>

COMMENTS: _____

Appendix C

Parameter Data

PHYSICAL-CHEMICAL DATA OF SAMPLES WHEN RECEIVED BY LAB

For Chronic Toxicity Testing

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

Permit No.: 2PH00007*LD

Type of Test: Chronic

Date of Test: 11/17/20 - 11/24/20

1st Sample Collection

Date Samples Collected: 11/16/20

Date Samples Read: 11/16/20

Analyst: EMS

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	7.3	5.7		
Dissolved Oxygen (mg/L)	10.3	11.5		
pH (s.u.)	7.70	7.12		
Conductivity (µmhos)	791	392		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	light stain	dark yellow		

2nd Sample Collection

Date Samples Collected: 11/18/20

Date Samples Read: 11/19/20

Analyst: BDP

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	12.3	8.7		
Dissolved Oxygen (mg/L)	9.5	11.7		
pH (s.u.)	7.63	8.00		
Conductivity (µmhos)	761	665		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Stain	Dark Yellow		

3rd Sample Collection

Date Samples Collected: 11/20/20

Date Samples Read: 11/20/20

Analyst: BDP

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	13.8	9.1		
Dissolved Oxygen (mg/L)	8.6	10.6		
pH (s.u.)	7.56	7.88		
Conductivity (µmhos)	796	752		
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	Person(s) conducting Test: BG, JDP, EMS, DJA
Industry/Toxicant: Allen County, Am. - Bath WWTP	Beginning Date: 11/17/20 Time: 2304
Address: 3226 North Cole St., Lima OH 45805	Ending Date: 11/24/20 Time: 1615
Effluent Serial No.: 001	Test Organism: Pimephales promelas Age: < 24 Hours
NPDES Permit No.: 2PH00007*LD	Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	24.0	25.2	25.2	25.2	25.2	25.6	24.0	24.8	24.0	21.50	24.3	24.2	23.1	24.1
														20.9
Dissolved Oxygen (mg/L)	7.5	7.3	7.2	7.1	6.7	7.0	6.1	7.3	6.3	7.2	5.8	8.1	4.8	4.0
pH (s.u.)	7.99	7.77	7.77	8.22	7.54	7.78	7.12	7.91	7.43	7.78	7.40	8.40	7.68	7.50
										8.00				
Conductivity (µmho/cm)	330	354	389	334	358	337	342	336	346	339	349	340	364	354
										438				
Hardness (mg/L CaCO ₃)	137	-	-	137	-	-	-	137	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	103	-	-	103	-	-	-	103	-	-	-	-	-	-

EMS
11/22

JDP
11/18

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

Solution Tested: Upstream Control	Person(s) conducting Test: BG, JDP, EMS, DJA
Industry/Toxicant: Allen County, Am. - Bath WWTP	Beginning Date: 11/17/20 Time: 2304
Address: 3226 North Cole St., Lima OH 45805	Ending Date: 11/24/20 Time: 1615
Effluent Serial No.: 001	Test Organism: Pimephales promelas Age: < 24 Hours
NPDES Permit No.: 2PH00007*LD	Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
		New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	
Temperature (°C)	24.9	25.0	22.9 24.2	25.0	23.6 24.4	24.8	24.0 24.8	25.1	24.0 25.1	25.1	24.3 25.2	24.8	23.1 24.5	24.1 24.9
Dissolved Oxygen (mg/L)	7.6	7.2	6.2	7.9	6.5	7.1	5.3	8.0	5.9	8.2	5.6	8.6	4.5	4.0
pH (s.u.)	7.77	7.77	7.57	7.88	7.56	7.86	7.28	7.86	7.46	7.87	7.50	7.97	7.61	7.46
Conductivity (µmho/cm)	389	389	411	659	399	660	650	747	669	746	745	745	760	754
Hardness (mg/L CaCO ₃)	171	-	-	222	-	-	-	257	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	120	-	-	188	-	-	-	154	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Solution Tested: 20% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date: 11/17/20 Time: 2304

Ending Date: 11/24/20 Time: 16:15

Test Organism: Pimephales promelas Age: < 24 Hour

Dilution Water Used: Upstream

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 40% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.:

2PH00007*LD

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

Beginning Date: 11/17/20 Time: 7:00 AM

Ending Date: 11/24/20 Time:

Test Organism: *Pimephales promelas*

Dilution Water Used: Upstream

DJA

Time: 2304

Time: 10:15

Age: <74 Hours

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

Person(s) conducting Test:

Beginning Date:

Ending Date:

Test Organism: Pimephales promelas

Dilution Water Used: Upstream

BG, JDP, EMS, DJA

11/17/20 Time:

Time: 11/24/20

Age: <24 Hours

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 80% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date:	11/17/20	Time:
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Ending Date: 11/24/20

Test Organism: Pimephales promelas Age: _____

Dilution Water Used: Upstream

[illegible]

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

Solution Tested: 100% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH000007*LD

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

Beginning Date: 11/17/20

Time: 2304

Ending Date: 11/24/20

Time: 1615

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.1	25.0	22.9 24.2	25.1	23.6 24.4	25.1	24.0 24.8	25.2	24.0 25.1	25.0	24.3 25.2	24.3	23.1 24.5	24.1 24.9
Dissolved Oxygen (mg/L)	8.3	8.1	6.4	8.5	6.1	8.0	5.7	8.1	5.8	8.4	6.0	8.4	5.0	4.3
pH (s.u.)	7.74	7.88	7.95	7.70	7.64	7.85	7.70	7.76	7.67	7.76	7.82	7.89	7.73	7.63
Conductivity (µmho/cm)	778	777	812	768	783	757	774	792	796	794	818	792	811	807
Hardness (mg/L CaCO ₃)	274	-	-	274	-	-	-	325	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	222	-	-	188	-	-	-	188	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date: 11/17/20 Time: 1350

Ending Date: 11/23/20 Time: 1430

Test Organism: Ceriodaphnia dubia Age: 6-14H

Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	24.1	24.5	24.6 24.9	24.9	25.0 25.4	25.3	25.2 25.6	24.8	24.8 25.1	24.5	24.4 25.3		24.8 24.9	
Dissolved Oxygen (mg/L)	7.3	7.4	7.3	7.2	7.3	7.1	7.3	7.3	7.2	7.0	7.2		6.8	
pH (s.u.)	7.97	7.87	7.96	7.70	7.96	7.93	8.06	7.73	7.89	7.78	7.87		7.72	
Conductivity (µmho/cm)	477	476	473	436	483	434	446	438	444	438	447		463	
Hardness (mg/L CaCO ₃)	205	-	-	205	-	-	-	171	-	-	-		-	-
Alkalinity (mg/L CaCO ₃)	171	-	-	171	-	-	-	137	-	-	-		-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date: 11/17/20

Time: 1350

Ending Date: 11/23/20

Time: 1430

Test Organism: Ceriodaphnia dubia

Age: 6-14h

Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	24.9	25.0	24.6 24.9	25.0	25.0 to 25.4	24.8	25.2 to 25.6	25.1	24.8	25.1	24.4 25.3		24.8 24.9	
Dissolved Oxygen (mg/L)	7.6	7.2	7.5	7.9	7.3	7.1	7.3	8.0	7.4	8.2	7.4		6.9	
pH (s.u.)	7.77	7.77	7.95	7.88	7.89	7.86	8.05	7.86	7.82	7.87	8.04		7.86	
Conductivity (µmho/cm)	389	389	404	659	405	660	672	747	675	746	702		757	
Hardness (mg/L CaCO ₃)	111	-	-	222	-	-	-	257	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	120	-	-	188	-	-	-	154	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date: 11/17/20

Ending Date: 11/23/20

Test Organism: Ceriodaphnia dubia

Dilution Water Used: Upstream

Time: 1350

Time: 1430

Age: 6-14H

Day	0		1		2		3		4		5		6		7	
	Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.9	25.0	24.6 24.9	25.0 25.4	25.0	25.4	24.8	25.2 to 25.6	25.1	24.8 25.1	25.1	24.6 25.3		24.8 24.9		
Dissolved Oxygen (mg/L)	7.9	7.9	7.5	7.3	8.6	7.3	7.6	7.4	8.2	7.4	8.5	7.4		7.1		
pH (s.u.)	7.76	7.84	7.85	7.85	7.86	7.85	7.90	8.06	7.87	7.88	7.87	8.07		7.90		
Conductivity (µmho/cm)	472	472	482	483	679	483	681	701	755	714	750	780		797		
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. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date: 11/17/20

Ending Date: 11/23/20

Test Organism: Ceriodaphnia dubia

Dilution Water Used: Upstream

Time: 1350

Time: 1430

Age: 6-14H

Day	0		1		2		3		4		5		6		7	
	Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Temperature (°C)		24.9	25.1	$\frac{24.6}{24.9}$	25.1	$\frac{25.0}{25.4}$	25.0	$\frac{25.2}{25.6}$	25.2	$\frac{24.8}{25.1}$	25.0	$\frac{24.4}{25.3}$		$\frac{24.8}{24.9}$		
Dissolved Oxygen (mg/L)		8.2	7.9	7.5	8.7	7.4	7.8	7.4	8.2	7.5	8.4	7.4		7.3		
pH (s.u.)		7.75	7.87	7.85	7.82	7.87	7.87	8.06	7.85	7.91	7.85	8.09		8.01		
Conductivity (µmho/cm)		552	551	561	699	563	700	724	763	739	766	788		795		
Hardness (mg/L CaCO ₃)		-	-	-	-	-	-	-	-	-	-	-		-		-
Alkalinity (mg/L CaCO ₃)		-	-	-	-	-	-	-	-	-	-	-		-		-

PHYSICAL-CHEMICAL DATA

Solution Tested: 80% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date:	Time:
11/17/20	

Ending Date: 11/23/20 Time: _____

Test Organism: *Ceriodaphnia dubia* Age:

Dilution Water Used: Upstream

[illegible]

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent

Industry/Toxicant: Allen County, Am. - Bath WWTP

Address: 3226 North Cole St., Lima OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00007*LD

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

Beginning Date: 11/17/20

Time: 1350

Ending Date: 11/23/20

Time: 1430

Test Organism: Ceriodaphnia dubia

Age: 1-14d

Dilution Water Used: Upstream

Day	0		1		2		3		4		5		6		7	
	Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Temperature (°C)		25.1	25.0	24.6 24.9	25.0 25.1	25.0 25.4	25.1	25.2 25.6	25.2	24.8 25.1	25.0	24.6 25.3		24.8 24.9		
Dissolved Oxygen (mg/L)		8.3	8.1	7.6	8.5	7.4	8.0	7.4	8.1	7.5	8.4	7.5		7.5		
pH (s.u.)		7.74	7.88	7.94	7.75 7.70	7.98	7.85	8.10	7.76	7.97	7.76	8.11		8.06		
Conductivity (µmho/cm)		778	777	788	737 758	791	757	780	792	796	794	809		819		
Hardness (mg/L CaCO ₃)		274	-	-	274	-	-	-	325	-	-	-		-		-
Alkalinity (mg/L CaCO ₃)		222	-	-	188	-	-	-	188	-	-	-		-		-

BDP
11/19

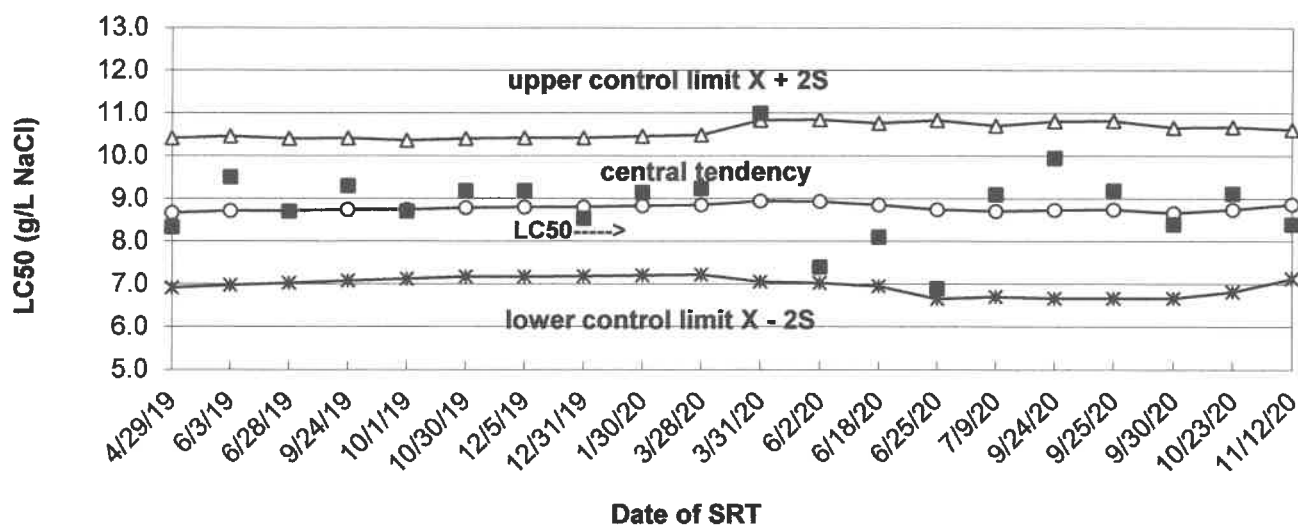
Appendix D

SRT Data

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

Date of	Cumulative								LC50
Test	LC50	LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	Method ¹
4/29/19	8.3	8.7	0.9	10.4	6.9	0.10	11.3	6.0	Probit
6/3/19	9.5	8.7	0.9	10.5	7.0	0.10	11.3	6.1	TSK
6/28/19	8.7	8.7	0.8	10.4	7.0	0.10	11.2	6.2	TSK
9/24/19	9.3	8.7	0.8	10.4	7.1	0.10	11.2	6.2	TSK
10/1/19	8.7	8.7	0.8	10.4	7.1	0.09	11.2	6.3	Probit
10/30/19	9.2	8.8	0.8	10.4	7.2	0.09	11.2	6.4	TSK
12/5/19	9.2	8.8	0.8	10.4	7.2	0.09	11.2	6.4	TSK
12/31/19	8.5	8.8	0.8	10.4	7.2	0.09	11.2	6.4	Probit
1/30/20	9.1	8.8	0.8	10.5	7.2	0.09	11.3	6.4	Probit
3/28/20	9.2	8.9	0.8	10.5	7.2	0.09	11.3	6.4	Probit
3/31/20	11.0	8.9	0.9	10.8	7.1	0.11	11.8	6.1	Probit
6/2/20	7.4	8.9	1.0	10.8	7.0	0.11	11.8	6.1	Probit
6/18/20	8.1	8.9	1.0	10.8	6.9	0.11	11.7	6.0	Probit
6/25/20	6.9	8.7	1.0	10.8	6.7	0.12	11.9	5.6	Probit
7/9/20	9.1	8.7	1.0	10.7	6.7	0.11	11.7	5.7	Probit
9/24/20	10.0	8.7	1.0	10.8	6.7	0.12	11.8	5.6	Graphical
9/25/20	9.2	8.7	1.0	10.8	6.7	0.12	11.8	5.6	TSK
9/30/20	8.4	8.7	1.0	10.7	6.7	0.12	11.7	5.7	TSK
10/23/20	9.1	8.7	1.0	10.7	6.8	0.11	11.6	5.8	Probit
11/12/20	8.4	8.9	0.9	10.6	7.1	0.10	11.5	6.3	TSK

Control Chart for *Pimephales promelas* Acute SRT



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

75th percentile of CVs for LC50 = 0.19

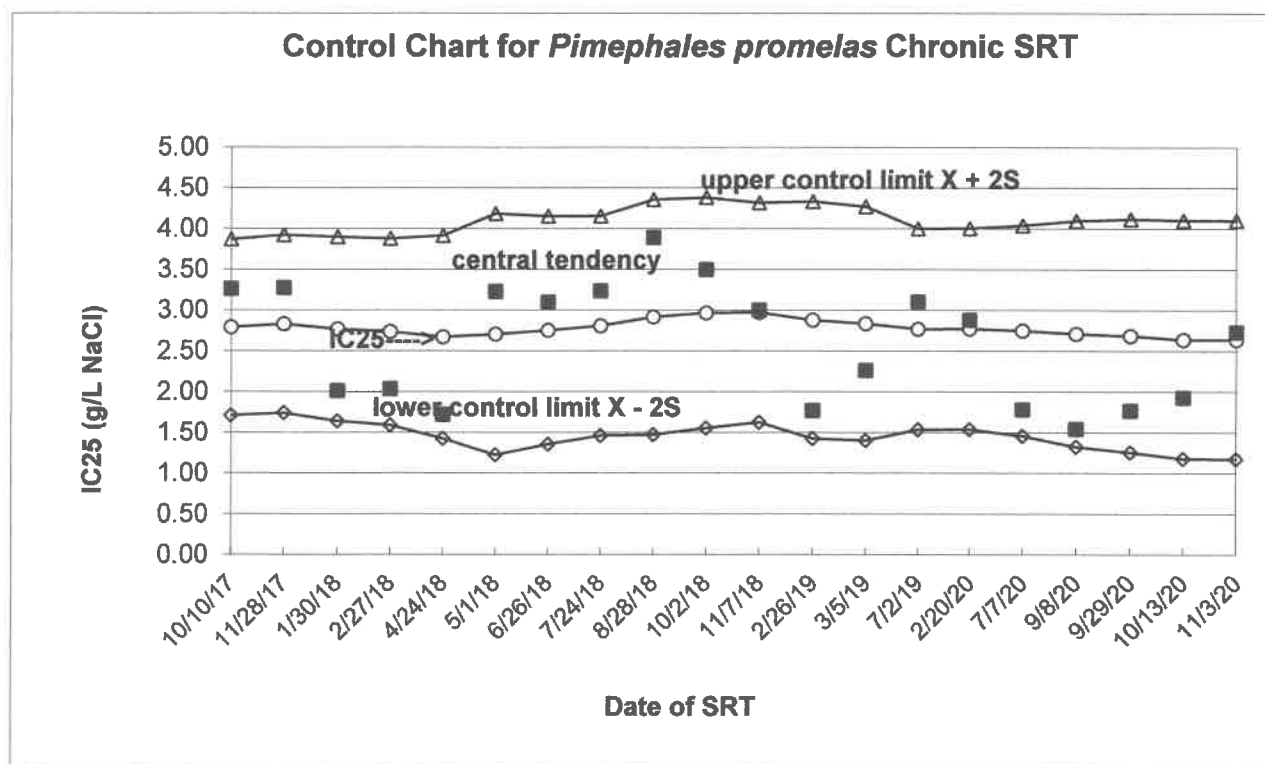
Calculated CV should not exceed 75th percentile of CVs.

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

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

IC25 for *Pimephales promelas* Chronic NaCl SRT

Date of	Cumulative							
Test	IC25	IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
10/10/17	3.27	2.79	0.54	3.87	1.71	0.19	4.41	1.17
11/28/17	3.27	2.83	0.55	3.92	1.74	0.19	4.47	1.19
1/30/18	2.01	2.77	0.57	3.90	1.64	0.20	4.46	1.07
2/27/18	2.04	2.73	0.57	3.88	1.59	0.21	4.45	1.01
4/24/18	1.72	2.67	0.62	3.91	1.42	0.23	4.53	0.80
5/1/18	3.23	2.70	0.74	4.18	1.22	0.27	4.92	0.48
6/26/18	3.10	2.75	0.70	4.15	1.35	0.25	4.85	0.65
7/24/18	3.24	2.81	0.67	4.15	1.46	0.24	4.83	0.78
8/28/18	3.89	2.91	0.72	4.36	1.47	0.25	5.08	0.75
10/2/18	3.50	2.97	0.71	4.38	1.55	0.24	5.09	0.85
11/7/18	3.00	2.97	0.67	4.32	1.62	0.23	4.99	0.95
2/26/19	1.77	2.88	0.73	4.33	1.42	0.25	5.06	0.70
3/5/19	2.26	2.83	0.72	4.27	1.40	0.25	4.99	0.68
7/2/19	3.10	2.77	0.62	4.00	1.53	0.22	4.61	0.92
2/20/20	2.88	2.77	0.62	4.00	1.54	0.22	4.62	0.92
7/7/20	1.78	2.75	0.65	4.04	1.45	0.24	4.68	0.81
9/8/20	1.54	2.71	0.69	4.09	1.32	0.26	4.79	0.63
9/29/20	1.77	2.69	0.72	4.12	1.26	0.27	4.83	0.54
10/13/20	1.93	2.64	0.73	4.10	1.18	0.28	4.83	0.44
11/3/20	2.73	2.64	0.73	4.10	1.17	0.28	4.83	0.44



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

75th percentile of CVs for IC25 = 0.38

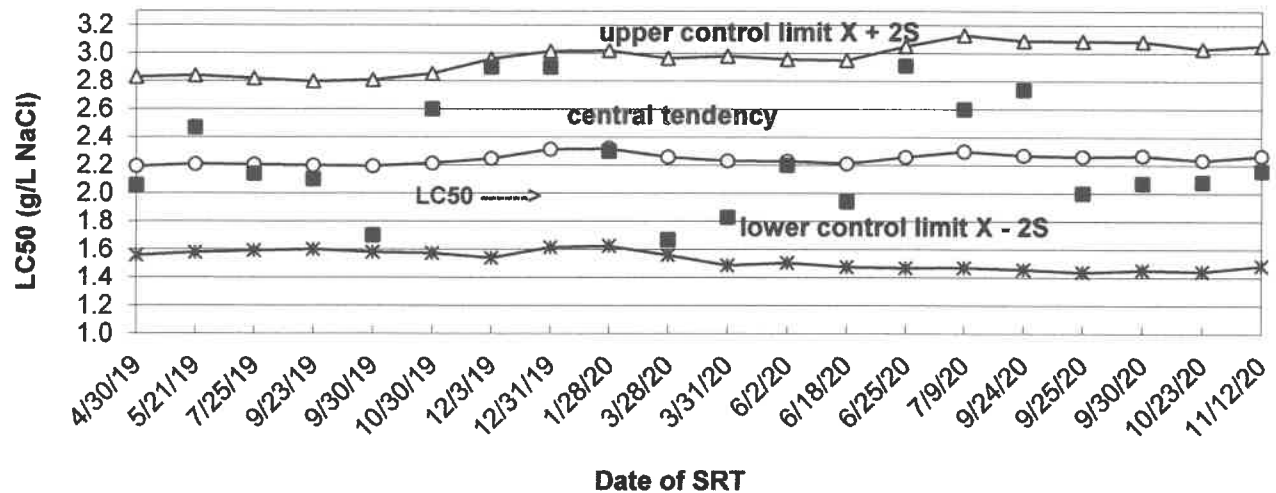
Calculated CV should not exceed 75th percentile of CVs.

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

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

Date of	Cumulative								LC50
Test	LC50	LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	Method ¹
4/30/19	2.1	2.2	0.3	2.8	1.6	0.15	3.1	1.2	Probit
5/21/19	2.5	2.2	0.3	2.8	1.6	0.14	3.2	1.3	TSK
7/25/19	2.1	2.2	0.3	2.8	1.6	0.14	3.1	1.3	Probit
9/23/19	2.1	2.2	0.3	2.8	1.6	0.14	3.1	1.3	Probit
9/30/19	1.7	2.2	0.3	2.8	1.6	0.14	3.1	1.3	Probit
10/30/19	2.6	2.2	0.3	2.9	1.6	0.14	3.2	1.3	Probit
12/3/19	2.9	2.2	0.4	3.0	1.5	0.16	3.3	1.2	Probit
12/31/19	2.9	2.3	0.3	3.0	1.6	0.15	3.4	1.3	Probit
1/28/20	2.3	2.3	0.3	3.0	1.6	0.15	3.4	1.3	Probit
3/28/20	1.7	2.3	0.4	3.0	1.6	0.16	3.3	1.2	TSK
3/31/20	1.8	2.2	0.4	3.0	1.5	0.17	3.4	1.1	Probit
6/2/20	2.2	2.2	0.4	3.0	1.5	0.16	3.3	1.1	Probit
6/18/20	1.9	2.2	0.4	2.9	1.5	0.17	3.3	1.1	Probit
6/25/20	2.9	2.3	0.4	3.1	1.5	0.18	3.4	1.1	Probit
7/9/20	2.6	2.3	0.4	3.1	1.5	0.18	3.5	1.1	Probit
9/24/20	2.7	2.3	0.4	3.1	1.5	0.18	3.5	1.0	Probit
9/25/20	2.0	2.3	0.4	3.1	1.4	0.18	3.5	1.0	Probit
9/30/20	2.1	2.3	0.4	3.1	1.5	0.18	3.5	1.0	Probit
10/23/20	2.1	2.2	0.4	3.0	1.4	0.18	3.4	1.0	Probit
11/12/20	2.2	2.3	0.4	3.1	1.5	0.17	3.4	1.1	Probit

Control Chart for *Ceriodaphnia dubia* Acute SRT



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

75th percentile of CVs for LC50 = 0.29

Calculated CV should not exceed 75th percentile of CVs.

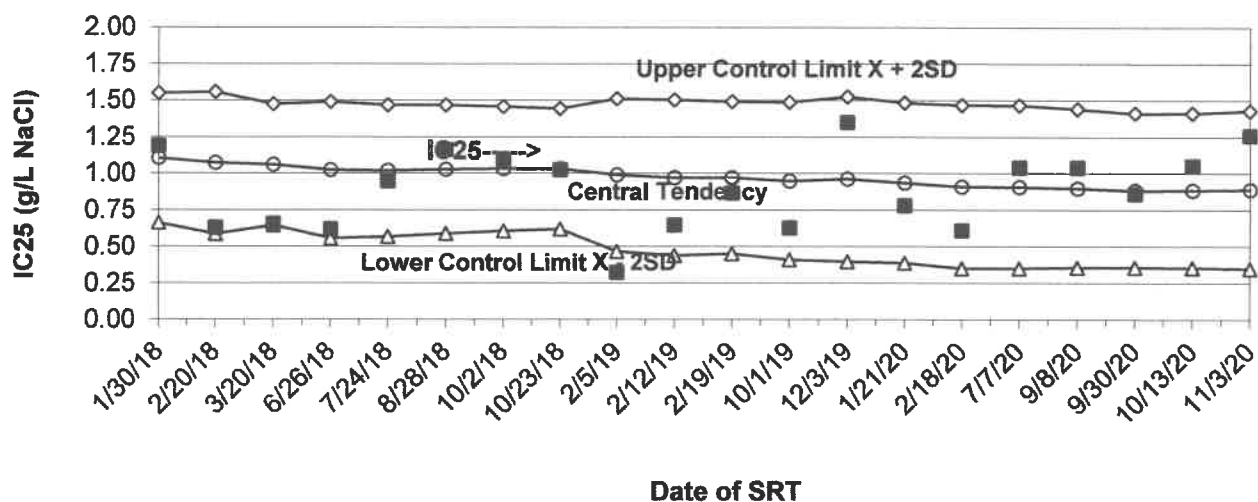
$\bar{X} \pm 3SD$ = Well outside expected range.

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

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
1/30/18	1.19	1.10	0.22	1.55	0.66	0.20	1.77	0.44
2/20/18	0.63	1.07	0.24	1.56	0.58	0.23	1.80	0.34
3/20/18	0.66	1.06	0.21	1.47	0.64	0.20	1.68	0.44
6/26/18	0.62	1.02	0.23	1.49	0.56	0.23	1.72	0.32
7/24/18	0.95	1.02	0.23	1.47	0.57	0.22	1.69	0.34
8/28/18	1.16	1.03	0.22	1.47	0.59	0.21	1.69	0.37
10/2/18	1.09	1.03	0.21	1.46	0.61	0.21	1.67	0.39
10/23/18	1.02	1.03	0.21	1.44	0.62	0.20	1.65	0.41
2/5/19	0.32	0.99	0.26	1.51	0.47	0.26	1.77	0.20
2/12/19	0.65	0.97	0.27	1.50	0.44	0.27	1.77	0.17
2/19/19	0.87	0.97	0.26	1.49	0.45	0.27	1.75	0.19
10/1/19	0.63	0.95	0.27	1.49	0.41	0.28	1.76	0.14
12/3/19	1.35	0.96	0.28	1.53	0.39	0.29	1.81	0.11
1/21/20	0.78	0.93	0.27	1.48	0.39	0.29	1.76	0.11
2/18/20	0.61	0.91	0.28	1.47	0.35	0.31	1.75	0.07
7/7/20	1.04	0.91	0.28	1.46	0.35	0.31	1.74	0.07
9/8/20	1.04	0.90	0.27	1.44	0.36	0.30	1.71	0.08
9/30/20	0.86	0.88	0.26	1.41	0.35	0.30	1.68	0.09
10/13/20	1.05	0.88	0.26	1.41	0.35	0.30	1.68	0.09
11/3/20	1.26	0.89	0.27	1.43	0.35	0.30	1.70	0.08

Control Chart for *Ceriodaphnia dubia* Chronic SRT



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