

Customer: Allen County Sanitary Engineers
Facility: American II WWTP
Outfall: Effluent 001
Revised Report Date: 4/25/20
Ohio EPA Permit No.: 2PH00006*JD
Alloway Lab #: L20-12960-01

Report of Chronic Biomonitoring

Samples Collected on:

4/6/20
4/8/20
4/10/20

Pimephales promelas
Ceriodaphnia dubia

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

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Report Date: 4/25/20
Ohio EPA Permit No.: 2PH00006*JD
Alloway Lab #: L20-12960-01

Date: 4/25/20

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

Dear Mr. Santaguida:

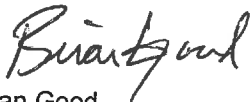
Enclosed is the revised report of the biomonitoring on the sample listed above. The only revision was made to the TUC for *Pimephales promelas*. The objective of the chronic biomonitoring was to determine the effects, both the acute endpoint (mortality) and chronic endpoints (mortality and growth, or 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: 4/25/20
Ohio EPA Permit No.: 2PH00006*JD

- | | |
|--|------------------------------------|
| 1. Facility Name | American II WWTP |
| 2. Facility Address | 4140 Diller Road
Lima, OH 45805 |
| 3. Ohio EPA Permit No. | 2PH00006*JD |
| 4. NPDES No. | OH0037338 |
| 5. Facility Contact | Mike Santaguida |
| 6. Telephone No. | 419-996-4696 |
| 7. Consulting Laboratory | Alloway |
| 8. Lab Contact | Brian Good |
| 9. Lab Telephone No. | 419-223-1362 |
| 10. Receiving water(s) of discharge | Dug Run |
| 11. Outfall(s) tested | Effluent 001 |
| 12. Is your current Standard Operating Procedure (SOP) Manual on file with Ohio EPA? Yes | |
| If yes, indicate date in which SOP was submitted to the EPA: <u>6/19/2017</u> | |

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

ML Santaguida
Signature

Mike Santaguida
Name (typed or printed)

5/11/20
Date

Superintendent
Title

Customer: Allen County Sanitary Engineers
 Facility: American II WWTP
 Outfall: Effluent 001
 Ohio EPA Permit No.: 2PH00006*JD
 Date of *P. promelas* Test: 4/7-14/20
 Date of *C. dubia* Test: 4/7-13/20

Summary of Chronic Toxicity Test Results *Pimephales promelas*

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

Summary of Chronic Toxicity Test Results *Ceriodaphnia dubia*

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

AA = below detectable

Conclusion/Comments:

Unacceptable *Ceriodaphnia dubia* survival was observed in the primary lab control. Mortality cannot exceed 20% in a control. The 100% effluent was compared to the secondary upstream control. Reproduction and survival in the 100% effluent were not significantly less than in the upstream control.

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Outfall: Effluent 001
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Date of Test: 4/7-14/20

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 Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	7.5% (7.5%)	15.0% (15.0%)	15.0% (15.0%)	0.541	0.178
Secondary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.691	0.037
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.626	0.046
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.613	0.088
60%	0.0% (0.0%)	0.0% (0.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	5.0% (5.0%)	0.564	0.044
80%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.604	0.049
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	5.0% (5.0%)	5.0% (5.0%)	0.634	0.059
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) <1.0	
95% Confidence Limits	LL								
	UL								
7-Day NOEC for Mortality (%)	100				7-Day NOEC for Growth (%)	100		Method(s) used to determine Values: IC Pin, Steel's Many-One Rank Test	
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			

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

Chronic Value = square root of (NOEC x LOEC)

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Date of Test: 4/7-13/20

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 Lab	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	20.0% (20.0%)	40.0% (40.0%)	40.0% (40.0%)	---	12.6
Secondary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	---	19.4
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%)	---	31.1
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%)	---	28.8
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	10.0% (10.0%)	10.0% (10.0%)	---	23.7
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%)	---	27.9
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	---	29.1
LC50 Values (%):		>100	>100	>100	>100	>100	>100	Calculated TUC Value for Survival <1.0
95% Confidence Limits	LL							
	UL							
EC50 Values (%):		>100	>100	>100	>100	>100	>100	Calculated TUC Value for Reproduction <1.0
95% Confidence Limits	LL							
	UL							
NOEC for Mortality (%)		100		NOEC for Reprod (%)			100	Method(s) used to determine Values:
LOEC for Mortality (%)		>100		LOEC for Reprod (%)			>100	
Chronic Value for Mortality (%)		>100		Chronic Value for Reprod (%)			>100	
IC25 (%)		>100		IC50 (%)			>100	IC Pin, Wilcoxon Rank Sum Test

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

Chronic Value = square root of (NOEC x LOEC)

COMMENTS:

Unacceptable *Ceriodaphnia dubia* survival was observed in the primary lab control. Mortality cannot exceed 20% in a control. The 100% effluent was compared to the secondary upstream control. Reproduction and survival in the 100% effluent were not significantly less than in the upstream control.

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Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection	
			Beginning	Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	4/5/20 @ 08:20	4/6/20 @ 08:20
Type (Grab/Comp):	Comp	2nd	4/7/20 @ 08:20	4/8/20 @ 08:20
Volume Collected:	5 Gal.	3rd	4/9/20 @ 08:20	4/10/20 @ 08:20
Upstream Station:				
Waterbody:	Dug Run			
Station No.:	801	1st		4/6/20 @ 11:02
Type (Grab/Comp):	Grab	2nd		4/8/20 @ 11:31
Volume Collected:	5 Gal.	3rd		4/10/20 @ 11:59
Samples collected by: Alloway				

PIMEPHALES PROMELAS CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	<i>Pimephales promelas</i> < 24 hours old
2. Test Type and Duration:	Static renewal; 7 days
3. Test Dates:	4/7-14/20
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 - 4/6/20 ; day 2, 3 - 4/8/20 ; day 4, 5, 6 - 4/10/20
15. Dilution Control Water:	Lab rearing unit water
16. Secondary Control Water:	Upstream grab from the receiving stream
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and 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> 16-23 hours
2. Test Type and Duration:	Static renewal; Until 60% or more of surviving control females have three broods (maximum test duration 8 days)
3. Test Dates:	4/7-13/20
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 - 4/6/20 ; day 2, 3 - 4/8/20 ; day 4, 5, 6 - 4/10/20
15. Dilution Control Water:	Lab rearing unit water
16. Secondary Control Water:	Upstream grab from the receiving stream
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data



Alloway
Your Resource for Defensible Data

Chain of Custody Record

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

1101 North Cole Street, Lima, OH 45805
(P) 419-223-1362 (F) 419-227-3792
1776 Marion-Waldo Road, Marion OH 43302
(P) 740-389-5991 (F) 740-389-1481
4906

Report To: Rick Boerger
Name: Allen County Sanitary Eng.
Company: 3226 N. Cole St.
Address: Lima, OH 45805

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

Phone #: 419-225-8048 Fax #:
E-mail: PO#:

Project Name: Am II Bioassay - Day One

Sampler	Customer Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code	Number of Containers	Preservation Code #	Analysis Required	Alloway LIMS # For Lab Use Only
1	Effluent	4/05/20-4/06/20	08:30-08:30	X		WW	1	1	Toxicity	
2	Upstream	4/06/20	11:02		X	SW	1	1	Toxicity	
3										
4										
5										
6										
7										
8										

Relinquished by: Received by: Date: Time: Method of Delivery: Matrix Codes: Preservation Codes: Sample Receiving (For Lab Use Only)

1	WW - wastewater	1 - None	7 - Sodium Thiosulfate	13 - Zinc Acetate	Ice Present? ☒ N ☐
2	dw - drinking water	2 - HNO ₃	8 - Ascorbic Acid	14 - Sodium Sulfite	Proper Preservation? ☒ N ☐
3	SW - surface water	3 - H ₂ SO ₄	9 - Maleic Acid	15 - Potassium Dihydrogen Citrate	Container Temperature: 7°C - 120
4	W - water	4 - HCl	10 - EDA	16 - Sodium Sulfite/Sulfate	
5	oil - oil	5 - NaOH	11 - Ammonium Chloride		
6	S - solid	6 - NaOH & Zinc Acetate	12 - (NH ₄) ₂ SO ₄ & NH ₄ OH		
	sg - sludge				
	l - leachate				
	a - acid				
	p - product				
	o - other				

Received for Laboratory By: (circle one) Mansfield Lima Marion

(Signature)

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

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



Alloway
Your Resource for Defensible Data

Chain of Custody Record

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

Report To: Rick Boerger Name: Allen County Sanitary Eng. Company: 3226 N. Cole St. Address: Lima, OH 45805		Invoice To (if Different): Name: Company: Address:		Notes/Comments:	
Phone #: 419-225-8048		Fax #:			
E-mail:		PDI#:			
Project Name Am II Bioassay - Day Two					
Sampler (Print) <i>Michael S. Haul</i> (Signature) <i>[Signature]</i>					
Sample ID / Sample Location	Sample Date	Sample Time	Composite	Grab	Matrix Code
1 Effluent	4/27/20	08:20	X		WW
2 Upstream	4/28/20	11:31		X	SW
3					
4					
5					
6					
7					
8					
Relinquished by:		Received by:		Date	
1		2		3	
4		5		6	
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667		668		669	
670		671		672	
673		674		675	
676		677		678	
679		680		681	
682		683		684	
685		686		687	
688		689		690	
691		692		693	
694		695		696	
697		698		699	
700		701		702	
703		704		705	
706		707		708	
709		710		711	
712		713		714	
715		716		717	
718		719		720	
721		722		723	
724		725		726	
727		728		729	
730		731		732	
733		734		735	
736		737		738	
739		740		741	
742		743		744	
745		746		747	
748		749		750	
751		752		753	
754		755		756	
757		758		759	
760		761		762	
763		764		765	
766		767		768	
769		770		771	
772		773		774	
775		776		777	
778		779		780	
781		782		783	
784		785		786	
787		788		789	
790		791		792	
793		794		795	
796		797		798	
799		800		801	
802		803		804	
805		806		807	
808		809		810	
811		812		813	
814		815		816	
817		818		819	
820		821		822	
823		824		825	
826		827		828	
829		830		831	
832		833		834	
835		836		837	
838		839		840	
841		842		843	
844		845		846	
847		848		849	
850		851		852	
853		854		855	
856		857		858	
859		860		861	
862		863		864	
865		866		867	
868		869		870	
871		872		873	
874		875		876	
877		878		879	
880		881		882	
883		884		885	
886		887		888	
889		890		891	
892		893		894	
895		896		897	
898		899		900	
901		902		903	
904		905		906	
907		908		909	
910		911		912	
913		914		915	
916		917		918	
919		920		921	
922		923		924	
925		926		927	
928		929			



Alloway
Your Resource for Defensible Data

Chain of Custody Record

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

- 1101 North Cole Street, Lima, OH 45805
(P) 419-223-1362 (F) 419-227-3792
1776 Marion-Waldo Road, Marion OH 43302
(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 Eng. Company: 3226 N. Cole St. Address: Lima, OH 45805		Invoice To (if Different): Name: Company: Address:		Notes/Comments:	
Phone #: 419-225-8048		Fax #:			
E-mail:		PO#:			
Project Name: Am II Bioassay - Day Three					
Sampler: Michael S. Hanley					
Customer: Sample ID / Sample Location		Sample Date		Sample Time	
1 Effluent		4/19/20		08:20	
2 Upstream		4/19/20		11:59	
3					
4					
5					
6					
7					
8					
Relinquished by:		Received by:			
1		Date		Time	
2		Date		Time	
3		Date		Time	
4		Date		Time	
5		Date		Time	
6		Date		Time	
Received for Laboratory By: (circle one) Mansfield		Lima		Marion	
(Signature)		4/19/20		12:35	
Matrix Codes:		Method of Delivery		Preservation Codes:	
1 - None		UPS		7 - Sodium Thiosulfate	
2 - HNO ₃		Fed Ex		8 - Ascorbic Acid Sulfite	
3 - H ₂ SO ₄		Client		9 - Maleic Acid Citrate	
4 - HCl		Alloway Pick Up		10 - EDA	
5 - NaOH		Alloway Sampling		11 - Ammonium Chloride	
6 - NaOH & Zinc Acetate		Other		12 - (NH ₄) ₂ SO ₄ & NH ₄ OH	
Ice Present? ☒ N ☐				Proper Preservation? ☒ N ☐	
Container Temperature: 60 F					

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

Transported to: Lima
By: 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. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001 NPDES Permit No.: 2PH000006*JD

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

Beginning: Date 4/7/20 Time 1320

Ending: Date 4/14/20 Time 1410

Age of Pimephales promelas: < 24 Hours

Dilution Water Used: Lab

Day	Upstream Control					Lab 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	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
4	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	9G 1D	9G 1D	10G	38G 2D	9G 1D	10G	10G	10G	39G 1D
5	9G 1D	10G	10G	10G	39G 1D	10G	7G 3D	10G	10G	37G 3D	10G	9G 1D	9G 1D	10G	38G 2D	9G 1D	10G	10G	10G	39G 1D
6	9G 1D	10G 5D	10G	10G	39G 1D	10G	5G 5D	9G 1D	10G	34G 6D	10G	9G 1D	9G 1D	10G	38G 2D	9G 1D	10G	10G	10G	39G 1D
7	9G 1D	10G	10G	10G	39G 1D	10G	5G 5D	9G 1D	10G	34G 6D	10G	9G 1D	9G 1D	10G	38G 2D	9G 1D	10G	10G	10G	39G 1D

EMS 4/13

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

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

JDP
4/11

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

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

Person(s) Conducting Test: BG, JDP, EMS, TAG
Beginning: Date 4/7/20 Time 1320
Ending: Date 4/14/20 Time 1410
Age of *Pimephales promelas*: < 24 Hours
Dilution Water Used: Lab

Day	60% Effluent					80% Effluent					100% Effluent					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	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	9G 1D	10G	10G	38G 2D	10G	10G	10G	9G 1D	39G 1D	10G	10G	10G	10G	40G	
4	9G 1D	9G 1D	10G	10G	38G 2D	10G	10G	10G	9G 1D	39G 1D	10G	10G	10G	10G	40G	
5	9G 1D	9G 1D	10G	10G	38G 2D	10G	10G	10G	9G 1D	39G 1D	10G	10G	10G	10G	40G	
6	9G 1D	9G 1D	10G	10G	38G 2D	10G	10G	10G	9G 1D	39G 1D	10G	10G	10G	10G	40G	
7	9G 1D	9G 1D	10G	10G	38G 2D	10G	10G	10G	9G 1D	39G 1D	10G	10G	10G	10G	40G	

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

WEIGHT OF FISH IN REPLICATE TEST VESSELS

7-DAY PIMEPHALES PROMELAS CHRONIC TOXICITY TEST

Discharge: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

Drying Temperature (C°): 60

Drying Time (h): 24

NPDES Permit No.: 2PH00006*JD

Date Weighed: 4/23/20

Pimephales promelas Age at Start: < 24 Hours

Analyst: TAG

Date of Test: 4/7/20 - 4/14/20

Balance Calibration Check: 1 gram weight = 0.99995 grams

Date: 4/23/20

Analyst: TAG

Dilution Water Used:

Lab

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			1.07773		10		
Upstream Control	1	9	1.09270	1.09885	1.09918 10	6.15 6.48	0.615 0.648
	2	10	1.10435	1.11147 44	10	6.72 7.09	0.672 0.709
	3	10	1.09195	1.09885	1.09921 10	6.20 7.32	0.620 0.732
	4	10	1.08802	1.09907	1.09480 10	2.04 6.76	0.204 0.676
Lab Control	1	10	1.12314	1.10230	1.13006 10	4.60 6.90	0.460 0.690
	2	5	1.08483	1.08757	1.08765 10	2.74 2.82	0.274 0.282
	3	9	1.08115	1.08682	1.08701 10	5.67 5.86	0.567 0.586
	4	10	1.09453	1.09905	1.10259 10	6.06	0.606
20% Effluent	1	10	1.08668	1.09231	10	5.63	0.563
	2	9	1.09158	1.09785	10	6.27	0.627
	3	9	1.08752	1.09423	10	6.71	0.671
	4	10	1.10430	1.11075	10	6.45	0.645
40% Effluent	1	9	1.09834	1.10488	10	6.52	0.652
	2	10	1.09845	1.10428	10	5.63	0.563
	3	10	1.09810	1.10331	10	5.21	0.521
	4	10	1.12939	1.13454	10	7.17	0.717

Reviewed By: 86 4/24/20

86 4/24/20

WEIGHT OF FISH IN REPLICATE TEST VESSELS

7-DAY PIMEPHALES PROMELAS CHRONIC TOXICITY TEST

Discharge: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

Pimephales promelas Age at Start: < 24 Hours

Drying Temperature (C°):

60

Drying Time (h):

24

Date of Test:

4/7/20 - 4/14/20

Dilution Water Used: Lab

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	9	1.10404	1.11195	10	5.89	0.589
	2	9	1.09824	1.10430	10	6.08	0.608
	3	10	1.09143	1.09713	10	5.60	0.560
	4	10	1.10201	1.10709	10	5.08	0.508
80% Effluent	1	10	1.09384	1.09982	10	5.98	0.598
	2	10	1.08662	1.09321	10	6.59	0.659
	3	10	1.15330	1.15948	10	6.18	0.618
	4	9	1.12599	1.13140	10	5.41	0.541
100% Effluent	1	10	1.10385	1.10990	10	6.05	0.605
	2	ADD 9	1.08756	1.09327	10	5.71	0.571
	3	4/23 10	1.14372	1.17080	10	7.08	0.708
	4	4/23 9	1.12663	1.13315	10	6.52	0.652
	1				10		
	2				10		
	3				10		
	4				10		

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 4/7/2020 Test ID: AmI0420p Sample ID: L20-12960-01
 End Date: 4/14/2020 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:

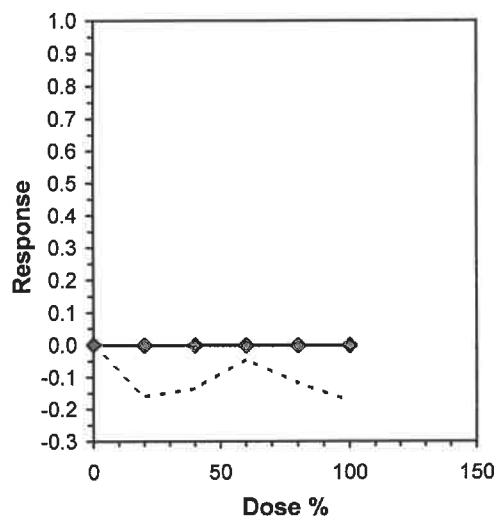
Conc-%	1	2	3	4
Lab Control	0.6900	0.2820	0.5860	0.6060
20	0.5630	0.6270	0.6710	0.6450
40	0.6520	0.5630	0.5210	0.7170
60	0.5890	0.6080	0.5500	0.5080
80	0.5980	0.6590	0.6180	0.5410
100	0.6050	0.5710	0.7080	0.6520

Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
Lab Control	0.5410	1.0000	0.5410	0.2820	0.6900	32.985	4			0.5971	1.0000
20	0.6265	1.1580	0.6265	0.5630	0.6710	7.346	4	20.00	10.00	0.5971	1.0000
40	0.6133	1.1335	0.6133	0.5210	0.7170	14.371	4	19.00	10.00	0.5971	1.0000
60	0.5638	1.0421	0.5638	0.5080	0.6080	7.862	4	17.00	10.00	0.5971	1.0000
80	0.6040	1.1165	0.6040	0.5410	0.6590	8.126	4	19.00	10.00	0.5971	1.0000
100	0.6340	1.1719	0.6340	0.5710	0.7080	9.380	4	20.00	10.00	0.5971	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.05$)	0.91226	0.916	-1.2397	3.72713
Bartlett's Test indicates equal variances ($p = 0.09$)	9.55897	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Lab Control				

Linear Interpolation (200 Resamples)

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



CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control

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

Industry/Toxicant: Allen County, Am. II WWTP

Beginning Date: 4/7/20

Time: 1450

Address: 4140 Diller Road, Lima, OH 45805

Ending Date: 4/13/20

Time: 231600

Effluent Serial No.: 001

Age of *Ceriodaphnia dubia*: 11-18 hrs. 16-23 h

NPDES Permit No.: 2PH00006*JD

Dilution Water Used: Lab

BG 4/24/20

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	1 / G	10 / G	9 / G	/		20	3
2	/	/	/	/	3 /	1 / D	- /	- /	D	4	2
3	/	/	/	/	4 /	Abort / D	- /	- /	D	4	1 2
4	/	/	/	/	3 /	8 / G	3 / G	/		14	3
5	/	/	/	0 / G	4 / G	9 / G	10 / G	/		23	3
6	/	/	/	- /	- /	- /	- /	- /	D	0	0
7	/	/	/	0 / G	0 / G	8 / G	9 / G	/		17	2
8	/	/	/	/	0 / D	- /	- /	- /	D	0	0
9	/	/	/	/	1 / G	9 / G	8 / G	/		18	3
10	0 / G	0 / G	0 / G	0 / G	3 / G	11 / G	12 / G	/		26	3
Total Adult Cond.	10G	10G	9G 1D	9G 1D	8G 2D	6G 4D	6G 4D				
									SUM	126	20 21
									MEAN	12.6	20 2.1
									S.D.	9.8	1.2

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

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>Upstream Control</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, TAG</u>
Industry/Toxicant: <u>Allen County, Am. II WWTP</u>	Beginning Date: <u>4/7/20</u> Time: <u>1450</u>
Address: <u>4140 Diller Road, Lima, OH 45805</u>	Ending Date: <u>4/13/20</u> Time: <u>1600</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>16-23 d</u>
NPDES Permit No.: <u>2PH00006*JD</u>	Dilution Water Used: <u>Lab</u>

NO. YOUNG PRODUCED / CONDITION OF ADULT*									TOTAL PER REP	
Rep.	DAY of TEST								No. Young	No. Broods
	0	1	2	3	4	5	6	7		
1	0/G	0/G	0/G	0/G	3/G	6/G	11/G	/	20	3
2	/	/	/	/	2/	6/	12/	/	20	3
3	/	/	/	/	3/	7/	10/	/	20	3
4	/	/	/	/	3/	6/	9/	/	18	3
5	/	/	/	/	3/	6/	10/	/	19	3
6	/	/	/	/	2/	7/	9/	/	18	3
7	/	/	/	/	3/	6/	13/	/	22	3
8	/	/	/	/	3/	7/	17/	/	27	3
9	/	/	/	/	1/	2/	6/	/	9	3
10	0/G	0/G	0/G	0/G	2/G	8/G	11/G	/	21	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G		SUM 194	30
									MEAN 19.4	3.0
									S.D. 4.5	0.0

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

Reviewed by: BG 4/25/20

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>20% Effluent</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, TAG</u>
Industry/Toxicant: <u>Allen County, Am. II WWTP</u>	Beginning Date: <u>4/7/20</u> Time: <u>1450</u>
Address: <u>4140 Diller Road, Lima, OH 45805</u>	Ending Date: <u>4/13/20</u> Time: <u>1600</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>16-23H</u>
NPDES Permit No.: <u>2PH00006*JD</u>	Dilution Water Used: <u>Lab</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	6 / G	0 / G	14 / G	18 / G		38	3
2				3 / G	1 / G	11 / G	14 / G		29	4 3
3				0 / G	6 / G	15 / G	16 / G		37	3
4				0 / G	4 / G	10 / G	11 / G		25	3
5				5 / G	0 / G	10 / G	12 / G		27	3
6				4 / G	0 / G	10 / G	16 / G		30	3
7				0 / G	4 / G	11 / G	13 / G		28	3
8				6 / G	0 / G	13 / G	15 / G		34	3
9				0 / G	5 / G	11 / G	16 / G		32	3
10	0 / G	0 / G	0 / G	0 / G	4 / G	12 / G	15 / G		31	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G		SUM 311	30
									MEAN 31.1	3.0
									S.D. 4.2	0.0

BG
4/24/20

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>40% Effluent</u> Industry/Toxicant: <u>Allen County, Am. II WWTP</u> Address: <u>4140 Diller Road, Lima, OH 45805</u> Effluent Serial No.: <u>001</u> NPDES Permit No.: <u>2PH00006*JD</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, TAG</u> Beginning Date: <u>4/7/20</u> Time: <u>1450</u> Ending Date: <u>4/13/20</u> Time: <u>1600</u> Age of <i>Ceriodaphnia dubia</i> : <u>16-23H</u> Dilution Water Used: <u>Lab</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	4/G	0/G	13/G	17/G	/	34	3
2	/	/	/	0	6	11	10	/	27	3
3	/	/	/	0	5	13	17	/	35	3
4	/	/	/	0	3	10	11	/	24	3
5	/	/	/	1	0	8	7	/	16	3
6	/	/	/	5	0	12	16	/	33	3
7	/	/	/	0	5	11	12	/	28	3
8	/	/	/	0	5	10	15	/	30	3
9	/	/	/	0	5	11	14	/	30	3
10	0/G	0/G	0/G	0/G	5/G	11/G	15/G	/	31	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G			
SUM									288	30
MEAN									28.8	3.0
S.D.									5.6	0.0

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

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>60% Effluent</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, TAG</u>
Industry/Toxicant: <u>Allen County, Am. II WWTP</u>	Beginning Date: <u>4/7/20</u> Time: <u>1450</u>
Address: <u>4140 Diller Road, Lima, OH 45805</u>	Ending Date: <u>4/13/20</u> Time: <u>1600</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>16-23 h</u>
NPDES Permit No.: <u>2PH00006*JD</u>	Dilution Water Used: <u>Lab</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	4/G	0/G	10/G	17/G	/	31	3
2	/	/	/	0	5	10	13	/	28	3
3	/	/	/	0	0	3	4	/	7	2
4	/	/	/	4	0	12	13	/	29	3
5	/	/	/	0	4	9	9	/	22	3
6	/	/	/	5	0/G	10/G	14/G	/	29	3
7	/	/	/	0	6/D	-	-	-	6	1
8	/	/	/	0	4/G	12/G	12/G	/	28	3
9	/	/	/	0	4/G	11/G	12	/	27	3
10	0/G	0/G	0/G	4/G	0/G	12/G	14/G	/	30	3
Total Adult Cond.	10G	10G	10G	10G	9G 1D	9G 1D	9G 1D			
SUM									237	27
MEAN									23.7	2.7
S.D.									9.4	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 DUBIA CHRONIC TOXICITY TEST

Solution Tested: <u>80% Effluent</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, TAG</u>
Industry/Toxicant: <u>Allen County, Am. II WWTP</u>	Beginning Date: <u>4/7/20</u> Time: <u>1450</u>
Address: <u>4140 Diller Road, Lima, OH 45805</u>	Ending Date: <u>4/13/20</u> Time: <u>1600</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>16-23d</u>
NPDES Permit No.: <u>2PH00006*JD</u>	Dilution Water Used: <u>Lab</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	5/G	0/G	12/G	15/G	/	32	3
2	/	/	/	4/	0/	11/	18/	/	33	3
3	/	/	/	0/	5/	9/	14/	/	28	3
4	/	/	/	0/	4/	9/	12/	/	25	3
5	/	/G	0/G	5/G	0/G	10/G	13/G	/	28	3
6	/	LA	LA	-	-	-	-	LA	-	-
7	/	/G	0/G	0/G	4/G	10/G	11/G	/	25	3
8	/	/	/	0/	4/	10/	9/	/	23	3
9	/	/	/	0/	5/	12/	10/	/	27	3
10	0/G	0/G	0/G	0/G	4/G	11/G	15/G	/	30	3
Total Adult Cond.	10G	9G 1LA	10G 9G 1LA	9G 1LA	9G 1LA	9G 1LA	9G 1LA		n=9 SUM MEAN S.D.	
										251
										27
										27.9
										3.0
										3.3
										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: <u>100% Effluent</u>	Person(s) Conducting Test: <u>BG, JDP, EMS, TAG</u>
Industry/Toxicant: <u>Allen County, Am. II WWTP</u>	Beginning Date: <u>4/7/20</u> Time: <u>1450</u>
Address: <u>4140 Diller Road, Lima, OH 45805</u>	Ending Date: <u>4/13/20</u> Time: <u>1600</u>
Effluent Serial No.: <u>001</u>	Age of <i>Ceriodaphnia dubia</i> : <u>16-23H</u>
NPDES Permit No.: <u>2PH00006*JD</u>	Dilution Water Used: <u>Lab</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	5 / G	0 / G	13 / G	19 / G	/	37	3	
2	/	/	/	5 /	0 /	10 /	12 /	/	27	3	
3	/	/	/	0 /	6 /	11 /	18 /	/	35	3	
4	/	/	/	0 /	4 /	10 /	11 /	/	25	3	
5	/	/	/	0 /	4 /	11 /	11 /	/	26	3	
6	/	/	/	5 /	0 /	11 /	13 /	/	29	3	
7	/	/	/	0 /	5 /	12 /	14 /	/	31	3	
8	/	/	/	4 /	0 /	11 /	12 /	/	27	3	
9	/	/	/	0 /	3 /	10 /	14 /	/	27	3	
10	0 / G	0 / G	0 / G	0 / G	4 / G	10 / G	13 / G	/	27	3	
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G		SUM	291	30
									MEAN	29.1	3.0
									S.D.	4.0	0.0

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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 4/7/2020 Test ID: AmlI0420c Sample ID: L20-12960-01
 End Date: 4/13/2020 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:

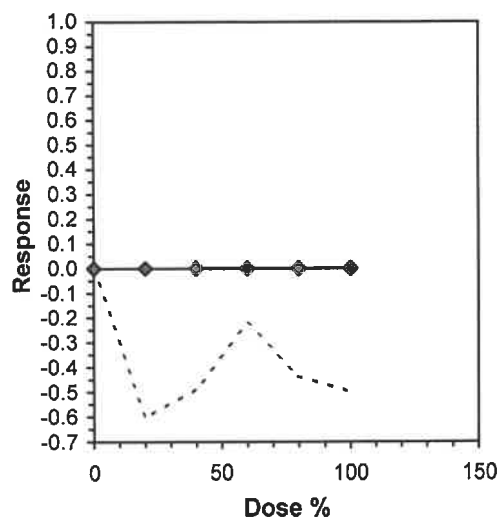
Conc-%	1	2	3	4	5	6	7	8	9	10
Upstr Control	20.000	20.000	20.000	18.000	19.000	18.000	22.000	27.000	9.000	21.000
20	38.000	29.000	37.000	25.000	27.000	30.000	28.000	34.000	32.000	31.000
40	34.000	27.000	35.000	24.000	16.000	33.000	28.000	30.000	30.000	31.000
60	31.000	28.000	7.000	29.000	22.000	29.000	6.000	28.000	27.000	30.000
80	32.000	33.000	28.000	25.000	28.000	25.000	23.000	27.000	30.000	
100	37.000	27.000	35.000	25.000	26.000	29.000	31.000	27.000	27.000	27.000

Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
Upstr Control	19.400	1.0000	19.400	9.000	27.000	23.078	10			26.665	1.0000
20	31.100	1.6031	31.100	25.000	38.000	13.596	10	153.50	74.00	26.665	1.0000
40	28.800	1.4845	28.800	16.000	35.000	19.422	10	144.50	74.00	26.665	1.0000
60	23.700	1.2216	23.700	6.000	31.000	39.584	10	133.00	74.00	26.665	1.0000
80	27.889	1.4376	27.889	23.000	33.000	11.952	9	131.50	61.00	26.665	1.0000
100	29.100	1.5000	29.100	25.000	37.000	13.789	10	151.00	74.00	26.665	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution ($p \leq 0.05$)	0.99556	0.895	-1.2543	2.52567
Bartlett's Test indicates equal variances ($p = 0.02$)	13.1161	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	100	>100		1
Treatments vs Upstr Control				

Linear Interpolation (200 Resamples)

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



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

Discharger/Toxicant: Allen County, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Type of Test: Chronic Date of Test: 4/7/20 - 4/13/20
 Effluent Serial No.: 001 Permit No.: 2PH00006*JD
 Investigator: BL Culture No.: 32
 Culture initiation date: 4/1/20 Culture ending date: 4/8/20
 Origin of parental organisms: Mins.-controls
 Age at start of culture: ~ 32H
 Culture water renewal frequency: minimum 3 times per week Culture water: 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: 97.5
 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>A1</u>	<u>18</u>	<u>0853-1600</u>	<u>27</u>	<u>1</u>
2)	<u>A2</u>	<u>16</u>		<u>24</u>	<u>2</u>
3)	<u>A3</u>	<u>19</u>		<u>29</u>	<u>3</u>
4)	<u>B1</u>	<u>14</u>		<u>24</u>	<u>4</u>
5)	<u>B3</u>	<u>16</u>		<u>23</u>	<u>5</u>
6)	<u>B4</u>	<u>13</u>		<u>21</u>	<u>6</u>
7)	<u>B5</u>	<u>14</u>		<u>23</u>	<u>7</u>
8)	<u>B9</u>	<u>17</u>		<u>31</u>	<u>8</u>
9)	<u>B10</u>	<u>16</u>		<u>25</u>	<u>9</u>
10)	<u>C1</u>	<u>16</u>	<u>0853-1600</u>	<u>25</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. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

Permit No.: 2PH00006*JD

Type of Test: Chronic

Date of Test: 4/7/20 - 4/14/20

1st Sample Collection

Date Samples Collected: 4/6/20

Date Samples Read: 4/6/20 Analyst: EMS

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	10.5	10.4		
Dissolved Oxygen (mg/L)	12.0	12.6		
pH (s.u.)	7.44	8.30		
Conductivity (µmhos)	780	457		
Chlorine, total (mg/L)	20.62	0.2		
Visual Description	clear	yellow		

2nd Sample Collection

Date Samples Collected: 4/8/20

Date Samples Read: 4/9/20 Analyst: JDP

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	3.4	4.9		
Dissolved Oxygen (mg/L)	9.8	10.6		
pH (s.u.)	7.39	7.70		
Conductivity (µmhos)	722	335		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Light Stain	Dark Stain		

3rd Sample Collection

Date Samples Collected: 4/10/20

Date Samples Read: 4/10/20 Analyst: JDP

	<u>100% Effluent</u>	<u>Upstream</u>	-	-
Temperature (°C)	8.4	10.9		
Dissolved Oxygen (mg/L)	10.8	12.3		
pH (s.u.)	7.36	8.03		
Conductivity (µmhos)	756	750		
Chlorine, total (mg/L)	<0.02	<0.02		
Visual Description	Yellow Tint	Yellow Tint		

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

Solution Tested: Lab Control

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

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

Beginning Date: 4/7/20

Time: 1320

Ending Date: 4/14/20

Time: 1416

Test Organism: Pimephales promelas

Age: < 24 Hours

Dilution Water Used: Lab

Day	0	1		2		3		4		5		6		7
		New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	
Solution														
Temperature (°C)	24.0	25.0	24.5 25.3	24.5	24.8 25.2	24.0	24.6 25.1	24.5	24.4 24.7	25.3	24.3 25.0	24.9	24.8 25.2	24.5 25.0
Dissolved Oxygen (mg/L)	7.5	7.5	7.1	7.7	5.5	7.6	5.8	8.1	6.7	7.4	5.7	8.0	6.0	6.4
pH (s.u.)	7.96	7.66	7.25	8.07	7.27	8.03	7.38	8.00	7.52	7.66	7.68	8.06	7.39	7.44
Conductivity (µmho/cm)	313	312	319	333	321	335	343	339	356	339	355	342	303	349
Hardness (mg/L CaCO ₃)	103	-	-	103	-	-	-	103	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	103	-	-	103	-	-	-	120	-	-	-	-	-	-

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

Solution Tested: Upstream Control
 Industry/Toxicant: Allen County, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Effluent Serial No.: 001
 NPDES Permit No.: 2PH000006*JD
 Person(s) conducting Test: BG, JDP, EMS, TAG
 Beginning Date: 4/7/20 Time: 1:20
 Ending Date: 4/14/20 Time: 4:10
 Test Organism: Pimephales promelas Age: < 24 Hours
 Dilution Water Used: Lab

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)	25.3	25.4	24.5 25.3	25.2	24.8 25.2	24.6 25.1	25.0	24.6 25.1	25.1	24.4 24.7	24.5	24.3 25.0	25.0	24.8 25.2	24.5 25.0	24.5 25.0
Dissolved Oxygen (mg/L)	7.7	8.0	6.7	7.7	5.8	5.5	7.9	5.5	8.4	6.4	7.9	5.2	9.0	5.9	5.9	5.9
pH (s.u.)	8.10	7.90	7.47	7.70	7.45	7.54	7.79	7.54	8.03	7.67	8.17	8.02	8.20	8.01	7.97	7.97
Conductivity (µmho/cm)	468	468	466	341	477	361	342	361	757	361	754	724	760	768	761	761
Hardness (mg/L CaCO ₃)	154	-	-	120	-	-	-	-	274	-	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	154	-	-	154	-	-	-	-	291	-	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

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

Beginning Date: 4/7/20 Time: 1320

Ending Date: 4/14/20 Time: 1410

Test Organism: *Pimephales promelas* Age: < 24 Hours

Dilution Water Used:

[illegible]

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

Solution Tested: 100% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

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

Beginning Date: 4/7/20 Time: 1320

Ending Date: 4/14/20 Time: 1410

Test Organism: Pimephales promelas Age: < 24 Hours

Dilution Water Used: Lab

Day	0	1		2		3		4		5		6		7
		New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	
Solution														
Temperature (°C)	25.4	25.4	24.5 25.3	25.2	24.8 25.2	24.9	24.6 25.1	25.1	24.4 24.7	24.6	24.3 25.0	25.1	24.8 25.2	24.5 25.0
Dissolved Oxygen (mg/L)	8.2	8.7	7.0	7.9	5.9	8.5	5.2	8.5	6.8	8.0	4.6	9.0	5.8	5.8
pH (s.u.)	7.69	7.89	7.90	7.46	7.83	7.75	7.73	7.54	7.92	7.80	7.81	7.97	7.93	7.92
Conductivity (µmho/cm)	783	783	801	712	796	716	738	760	786	758	764	759	798	788
Hardness (mg/L CaCO ₃)	205	-	-	205	-	-	-	257	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	205	-	-	205	-	-	-	257	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
 Industry/Toxicant: Allen County, Am. II WWTP
 Address: 4140 Diller Road, Lima, OH 45805
 Effluent Serial No.: 001
 NPDES Permit No.: 2PH00006*JD
 Person(s) conducting Test: BG, JDP, EMS, TAG
 Beginning Date: 4/7/20 Time: 1450
 Ending Date: 4/13/20 Time: 1600
 Test Organism: Ceriodaphnia dubia Age: 11-16 days
 Dilution Water Used: Lab 16-27H

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	24.8 25.3	25.0	24.6 25.1	24.9	24.4 24.9	24.9	24.5 24.9	24.8	24.5		24.4 24.7	
Dissolved Oxygen (mg/L)	7.3	7.9	7.2	7.5	7.2	7.3	7.4	7.9	8.0	7.4	7.4		7.1	
pH (s.u.)	5.80	5.75	7.55	5.65	7.55	8.17	6.92	7.74	7.81	7.88	7.82		7.82	
Conductivity (µmho/cm)	406	407	324	407	324	354	420	353	357	351	352		367	
Hardness (mg/L CaCO ₃)	86	-	-	86	-	-	-	86	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	120	-	-	51	-	-	-	120	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

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

Person(s) conducting Test: BG, JDP, EMS, TAG
 Beginning Date: 4/7/20
 Ending Date: 4/13/20
 Test Organism: Ceriodaphnia dubia
 Dilution Water Used: Lab

Time: 1450
 Time: 1600
 Age: 16-23H

Day	0	1		2		3		4		5		6		7
		New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	
Solution														
Temperature (°C)	24.6	24.8	24.8 25.3	24.8	24.6 25.1	24.5	24.4 24.9	24.3	24.5 24.9	24.4	24.5		24.6 24.7	
Dissolved Oxygen (mg/L)	8.0	8.1	7.6	7.7	7.3	7.7	7.5	8.6	7.9	7.9	7.5		7.4	
pH (s.u.)	8.12	8.00	7.70	7.70 7.64	7.58	7.81	7.51	8.11	7.94	8.16	8.38		8.31	
Conductivity (µmho/cm)	465	461	471	540 340	471	339	358	755	342	751	732		785	
Hardness (mg/L CaCO ₃)	154	-	-	120	-	-	-	274	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	154	-	-	154	-	-	-	291	-	-	-	-	-	-

PHYSICAL-CHEMICAL DATA

Solution Tested: 20% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

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

Beginning Date: 4/7/20 Time: 1450

Ending Date: 4/13/20 Time: 1:00

Test Organism: *Ceriodaphnia dubia* Age: 16-23 wk

Dilution Water Used:

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 40% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

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

Beginning Date:

Ending Date: 4/13/20

Test Organism: Ceriodaphnia dubia

Dilution Water Used:

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

Time: 450

Time: 1600

Age: 16-23 H

[illegible]

PHYSICAL-CHEMICAL DATA

Solution Tested: 60% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

Effluent Serial No.: 001

NPDES Permit No.: 2PH00006*JD

Person(s) conducting Test:

Beginning Date:

Ending Date:

Test Organism: *Ceriodaphnia dubia*

Dilution Water Used:

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

4/7/20 Time:

Time: 4/13/20

16-7341

450

1100

16-7

[illegible]

Solution Tested: 80% Effluent

Address: 4140 Diller Road, Lima, OH 45805

NPDES Permit No.: 2PH00006*ID

Dilution Water Used:

Age: _____

[illegible]

PHYSICAL-CHEMICAL DATA CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent

Industry/Toxicant: Allen County, Am. II WWTP

Address: 4140 Diller Road, Lima, OH 45805

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

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

Beginning Date: 4/7/20

Time: 1450

Ending Date: 4/13/20

Time: 1600

Test Organism: Ceriodaphnia dubia

Age: 16-23H

Dilution Water Used: Lab

Day	0	1		2		3		4		5		6		7
		New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	
Temperature (°C)	24.8	25.0	24.8 25.3	24.9	24.6 25.1	24.2	24.4 24.9	24.8	24.5 24.9	24.4	24.5		24.6 24.7	
Dissolved Oxygen (mg/L)	8.0	8.4	7.9	7.7	7.5	8.2	7.5	8.4	8.1	7.7	7.5		7.4	
pH (s.u.)	7.71	7.95	7.91	7.57	8.07	7.87	8.03	7.69	8.22	8.02	8.12		8.24	
Conductivity (µmho/cm)	784	781	797	713	816	714	734	758	729	757	769		805	
Hardness (mg/L CaCO ₃)	205	-	-	205	-	-	-	257	-	-	-	-	-	-
Alkalinity (mg/L CaCO ₃)	205	-	-	205	-	-	-	257	-	-	-	-	-	-

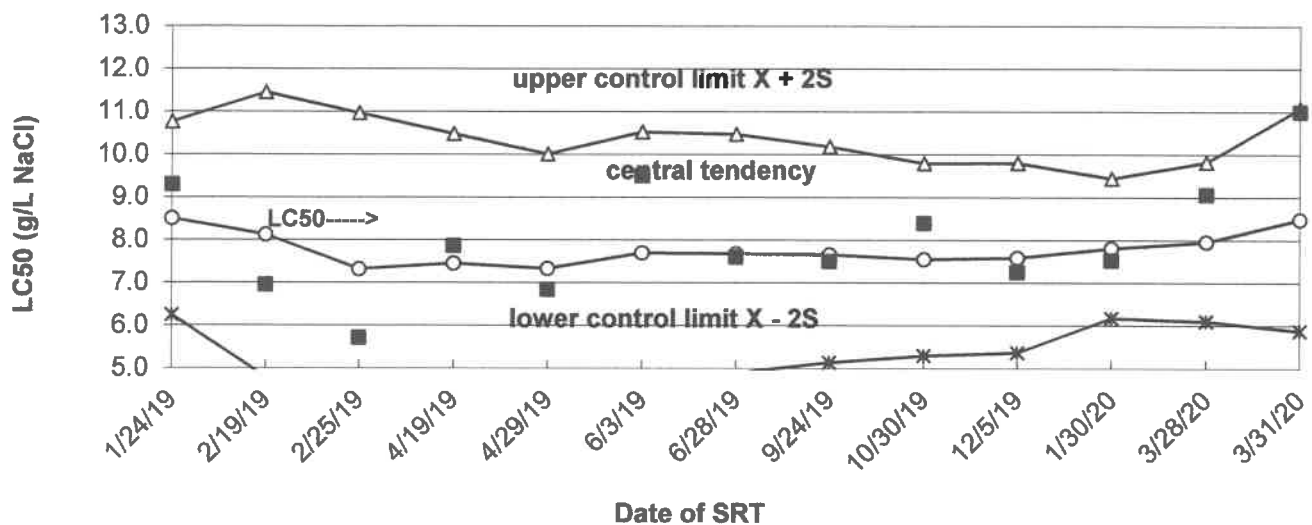
Appendix D

SRT Data

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

Date of Test	Cumulative LC50	LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method ¹
1/24/19	9.3	8.5	1.1	10.8	6.2	0.13	11.9	5.1	Probit
2/19/19	6.9	8.1	1.7	11.4	4.8	0.20	13.1	3.1	Probit
2/25/19	5.7	7.3	1.8	11.0	3.7	0.25	12.8	1.8	Probit
4/19/19	7.9	7.5	1.5	10.5	4.4	0.20	12.0	2.9	TSK
4/29/19	6.8	7.3	1.3	10.0	4.7	0.18	11.3	3.3	TSK
6/3/19	9.5	7.7	1.4	10.5	4.9	0.18	11.9	3.4	TSK
6/28/19	7.6	7.7	1.4	10.5	4.9	0.18	11.9	3.5	TSK
9/24/19	7.5	7.7	1.3	10.2	5.1	0.16	11.4	3.9	Probit
10/30/19	8.4	7.5	1.1	9.8	5.3	0.15	10.9	4.2	TSK
12/5/19	7.3	7.6	1.1	9.8	5.4	0.15	10.9	4.3	TSK
1/30/20	7.5	7.8	0.8	9.4	6.2	0.10	10.3	5.4	Probit
3/28/20	9.1	8.0	0.9	9.8	6.1	0.12	10.7	5.2	Probit
3/31/20	11.0	8.5	1.3	11.1	5.9	0.15	12.4	4.6	Probit

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

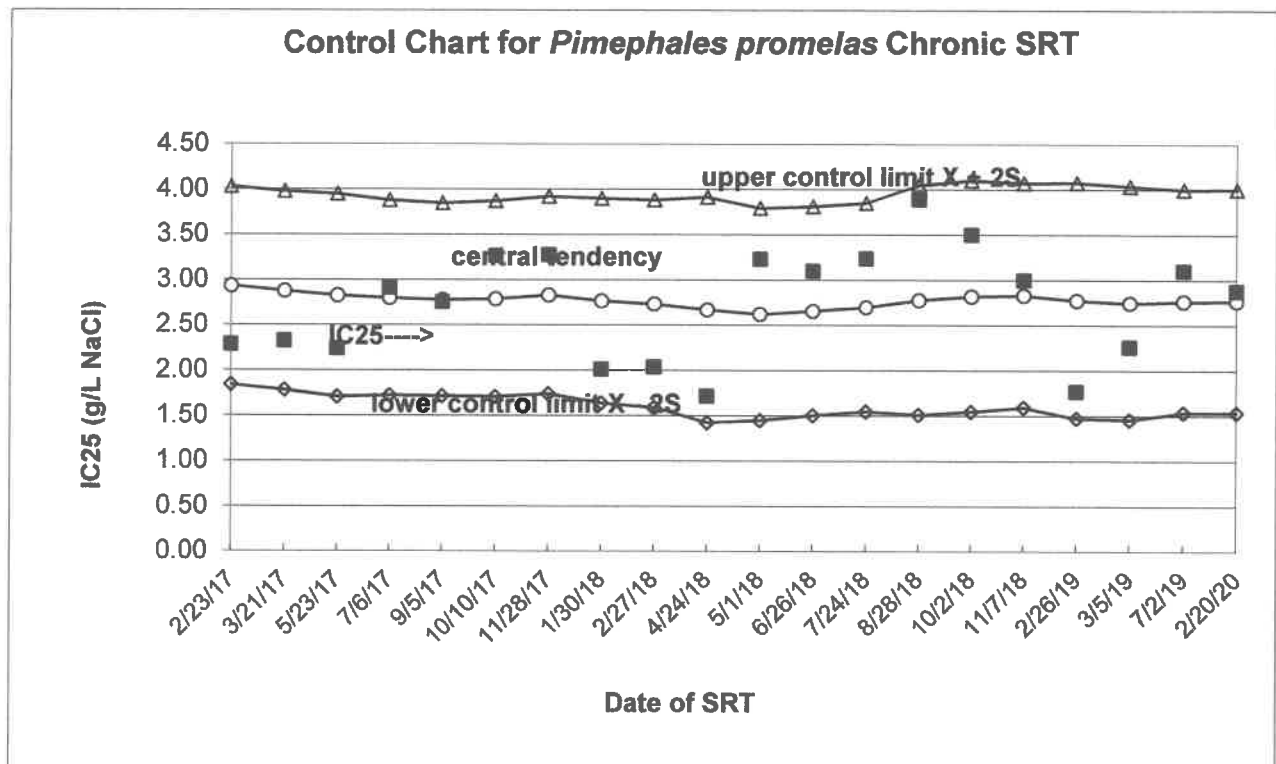
Calculated CV should not exceed 75th percentile of CVs.

$\bar{X} \pm 3SD$ = 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
2/23/17	2.29	2.94	0.55	4.03	1.84	0.19	4.58	1.29
3/21/17	2.32	2.88	0.55	3.98	1.78	0.19	4.53	1.23
5/23/17	2.24	2.83	0.56	3.95	1.71	0.20	4.51	1.15
7/6/17	2.91	2.80	0.54	3.88	1.72	0.19	4.42	1.18
9/5/17	2.76	2.78	0.53	3.85	1.71	0.19	4.38	1.18
10/10/17	3.27	2.79	0.54	3.87	1.71	0.19	4.41	1.17
11/28/17	3.27	2.83	0.55	3.92	1.74	0.19	4.47	1.19
1/30/18	2.01	2.77	0.57	3.90	1.64	0.20	4.46	1.07
2/27/18	2.04	2.73	0.57	3.88	1.59	0.21	4.45	1.01
4/24/18	1.72	2.67	0.62	3.91	1.42	0.23	4.53	0.80
5/1/18	3.23	2.62	0.59	3.79	1.45	0.22	4.38	0.86
6/26/18	3.10	2.66	0.58	3.81	1.50	0.22	4.39	0.93
7/24/18	3.24	2.70	0.58	3.85	1.55	0.21	4.42	0.97
8/28/18	3.89	2.78	0.63	4.05	1.51	0.23	4.68	0.88
10/2/18	3.50	2.82	0.64	4.10	1.55	0.23	4.74	0.91
11/7/18	3.00	2.83	0.62	4.07	1.59	0.22	4.69	0.97
2/26/19	1.77	2.77	0.65	4.08	1.47	0.23	4.73	0.82
3/5/19	2.26	2.75	0.64	4.04	1.46	0.23	4.68	0.81
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



*- 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

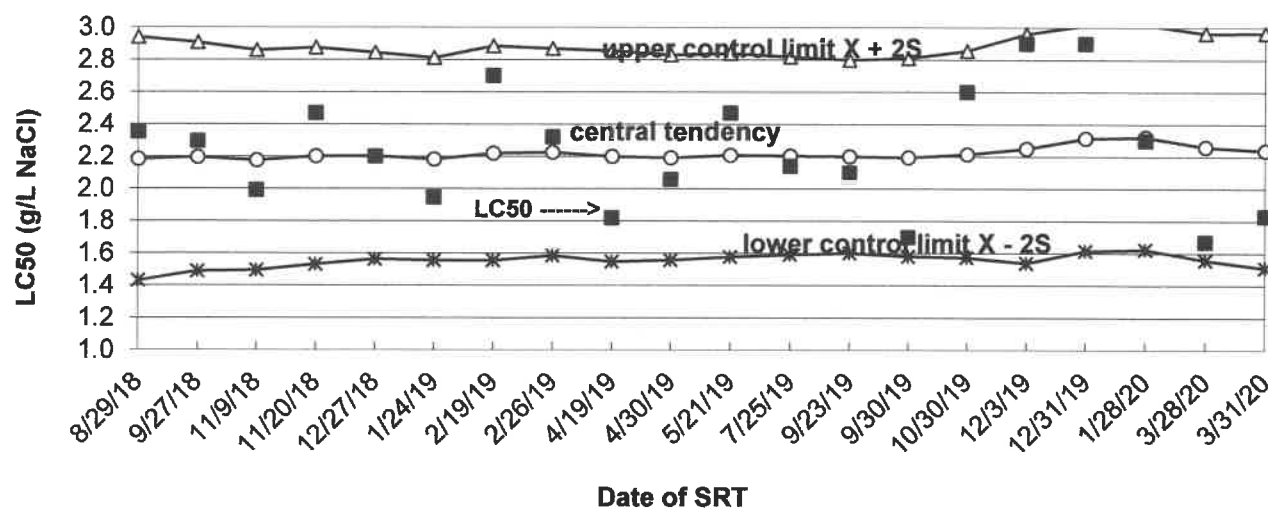
Calculated CV should not exceed 75th percentile of CVs.

$\bar{X} \pm 3SD$ = 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
8/29/18	2.4	2.2	0.4	2.9	1.4	0.17	3.3	1.1	SK
9/27/18	2.3	2.2	0.4	2.9	1.5	0.16	3.3	1.1	Probit
11/9/18	2.0	2.2	0.3	2.9	1.5	0.16	3.2	1.2	Probit
11/20/18	2.5	2.2	0.3	2.9	1.5	0.15	3.2	1.2	SK
12/27/18	2.2	2.2	0.3	2.8	1.6	0.15	3.2	1.2	Probit
1/24/19	1.9	2.2	0.3	2.8	1.6	0.14	3.1	1.2	Probit
2/19/19	2.7	2.2	0.3	2.9	1.6	0.15	3.2	1.2	Probit
2/26/19	2.3	2.2	0.3	2.9	1.6	0.14	3.2	1.3	Probit
4/19/19	1.8	2.2	0.3	2.9	1.5	0.15	3.2	1.2	Probit
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	0.16	3.3	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.

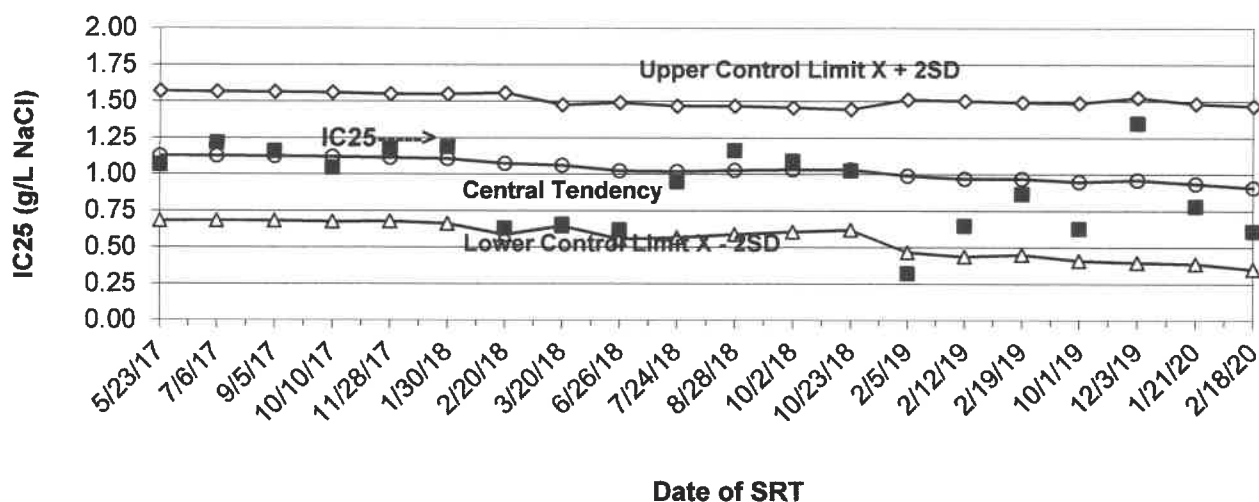
$\bar{X} \pm 3SD$ = Well outside expected range.

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
5/23/17	1.07	1.13	0.22	1.57	0.68	0.20	1.79	0.46
7/6/17	1.22	1.12	0.22	1.57	0.68	0.20	1.79	0.46
9/5/17	1.16	1.12	0.22	1.56	0.68	0.20	1.78	0.46
10/10/17	1.04	1.12	0.22	1.56	0.68	0.20	1.78	0.45
11/28/17	1.18	1.11	0.22	1.55	0.68	0.20	1.77	0.46
1/30/18	1.19	1.10	0.22	1.55	0.66	0.20	1.77	0.44
2/20/18	0.63	1.07	0.24	1.56	0.58	0.23	1.80	0.34
3/20/18	0.66	1.06	0.21	1.47	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

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.