

Full Circle Lithium
Acute Toxicity Evaluation

With the Water Flea Species *Ceriodaphnia dubia*,
and the Fathead Minnow Species *Pimephales promelas*

FCL-X

Conducted For:

FULL CIRCLE LITHIUM

Conducted and Prepared By:

ENVIROSCIENCE, INC.
5070 Stow Road
Stow, OH 44224



_____, Technical Supervisor

January 17, 2025

Mr. Tyler Taylor
Full Circle Lithium

Re: FCL-X Toxicity Tests

Dear Mr. Taylor:

Enclosed is a copy of EnviroScience's final report for the following toxicity tests conducted with the product FCL-X:

- (1) 48-hour static, non-renewal acute bioassay using *Ceriodaphnia dubia* (water flea).
- (1) 96-hour static, non-renewal acute bioassay using *Pimephales promelas* (minnow).

The product identified as FCL-X was received in the EnviroScience laboratory in December 2024. The sample was tested for LC₅₀ values (i.e., the concentration that is lethal to 50% of the exposed organisms). The following report details the testing events for each species and the respective toxicity endpoints.

Acute endpoints for FCL-X		
	<i>Ceriodaphnia dubia</i>	<i>Pimephales promelas</i>
	48-hour LC ₅₀	96-hour LC ₅₀
FCL-X	17.68 g/L	8.15 g/L

Please call me if you have any questions.

Sincerely,



Alexandria M. Tite, Technical Supervisor
enclosures



5070 Stow Road
Stow, OH 44224

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1.0 INTRODUCTION

The toxicity of Full Circle Lithium product, FCL-X, was evaluated for two aquatic species common to freshwater toxicity test methods. These methods are used by the National Pollutant Discharge Elimination System (NPDES) regulatory program for monitoring discharges of industrial and municipal sources of wastewater in the United States.

One vertebrate species, *Pimephales promelas* (minnow), and one invertebrate species, *Ceriodaphnia dubia* (water flea), were exposed to FCL-X in separate tests using a static non-renewal procedure. This report describes the results of toxicity tests conducted at EnviroScience Inc., 5070 Stow Rd., Stow, OH 44224, during the period of December 12-16, 2024, December 31, 2024-January 4, 2025 and January 8-12, 2025.

2.0 METHODS

Toxicity test methods followed EnviroScience's written standard operating procedures (SOPs), which were derived from USEPA guidelines found in their documents titled *Methods for Measuring the Acute Toxicity of Effluents and Receiving waters to Freshwater and Marine Organisms* (EPA-821-R-02-012).

2.1 Test design

Table 2.1 lists testing conditions including test dates and product concentrations.

2.2 Preparation of test solutions

The tests used concentrations set up with aliquots of a stock solution of product FCL-X equal to 100 mg/L product. This served as the highest concentration tested for both species. For both species each concentration was made using a portion of stock solution that was measured out to 6.25, 12.5, 25, 50, and 100 mg/L and each was brought to 1000 milliliters utilizing MHRW. These concentrations were too low to determine an LC₅₀ for both species. A

second stock solution equal to 1600 mg/L was made. Each concentration was made using a portion of stock solution that was measured out to 100, 200, 400, 800, and 1600 mg/L and each was brought to 1000 milliliters. These concentrations were too low to determine an LC₅₀ for both species. A third stock solution equal to 25 g/L was made. Each concentration was made using a portion of stock solution that was measured out to 1.56, 3.13, 6.25, 12.5, and 25 g/L and each was brought to 1000 milliliters. The dilution water (MHRW) was prepared by dissolving four reagent grade salts (KCl, MgSO₄, CaSO₄·2H₂O, and NaHCO₃) in ELGA Chorus de-ionized water and was continuously aerated before use. Specimens exposed to an aliquot of diluent served as the test control. The stock solution was prepared by measuring the product and mixing it for 30 minutes with MHRW with a Teflon coated magnetic stir bar.

Labeled test vessels were filled with test solutions, covered with a sheet of plexiglass, and placed in an environmental chamber set to operate at the desired test conditions until solutions reached test temperature (25±1 °C for both species).

Approximately 50 milliliters of each test solution was poured into a labeled plastic beaker for analysis of the initial water quality (dissolved oxygen concentration, pH, and conductivity). Temperature was measured directly in test solutions immediately prior to loading specimens into the prepared test vessels.

2.3 Data collection

Tests were initiated as organisms were randomly selected and placed into test vessels. Each chamber was monitored daily at 24 ±2 hour intervals from the time of test initiation (±1 hour at test termination). The number of mortalities and the total number of adversely affected specimens (cumulative mortality plus specimens showing a behavioral effect) were recorded on bench sheets. Solution temperature was recorded from one replicate vessel per test level. Dissolved oxygen concentration, pH, and conductivity were monitored daily. The following methods and instruments were used in chemical analysis:

Dissolved Oxygen: APHA (1998, 20th ed.) 4500-O G., OX 4100L
pH: APHA (1998, 20th ed.) 4500-H⁺ B., Orion Star A211
Conductivity: APHA (1998, 20th ed.) 2510 B., Orion Star A212

2.4 Data analysis

The median lethal concentration (LC₅₀) was computed from mortality data using the Spearman-Kärber, trimmed Spearman-Kärber, or binomial methods with the computer program Cetis 2.1.5. The LC₅₀ endpoint represents the concentration of product that would be expected to cause 50% mortality during the specified exposure period, typically 48 hours for the *Ceriodaphnia dubia* and 96 hours for the *Pimephales promelas*.

Table 2.1. Summary of toxicity test conditions for testing with <i>Ceriodaphnia dubia</i> and <i>Pimephales promelas</i> .		
	<i>Ceriodaphnia dubia</i>	<i>Pimephales promelas</i>
1. Test period:	01/08/25-1320 to 01/10/25-1230	01/08/25-1330 to 01/12/25-1230
2. Test type and duration:	static, non-renewal, 48 hours	static, non-renewal, 96-hours
3. Age, source of test organisms:	<24 hours, ES 01/07/25-1600	9 days, ES 12/30/24-1315
4. Photoperiod:	16 hours light / 8 hours dark	16 hours light / 8 hours dark
5. Light quality:	fluorescent light, 50-100fc	fluorescent light, 50-100fc
6. Test solution temperatures, °C:	25±1	25±1
7. Feeding regime:	fed alga <i>Selenastrum capricornutum</i> and YAT prior to test only	fed approximately 500 brine shrimp/vessel at 48 hours
8. Size of test vessel:	30 ml plastic cup	600 mL glass beaker
9. Volume/depth of test solutions:	15 ml and 24 mm	300 ml and 5.8 cm
10. No. of test organisms/vessel:	5	10
11. No. of vessels per solution:	4	2
12. No. organisms/test level:	20	20
13. Test concentrations: g/L MHRW	1.56, 3.13, 6.25, 12.5, 25	1.56, 3.13, 6.25, 12.5, 25
14. Dilution and control water:	moderately hard reconstituted water (MHRW)	moderately hard reconstituted water (MHRW)
15. Aeration:	none	none
16. Test acceptability criterion:	≥90% survival in control	≥90% survival in control
17. Endpoints:	mortality - no movement with gentle prodding (LC ₅₀)	mortality - no movement with gentle prodding (LC ₅₀)

3.0 RESULTS

Toxicity test results and associated water quality data are summarized in Tables 3.1 through 3.3. Table 3.1 lists LC₅₀ endpoints. Table 3.2 lists the initial water quality data for samples of diluent and selected concentrations of freshly prepared test solutions. Water quality data collected at 24-hour intervals and at test termination are included in the Appendix. Table 3.3 lists percent mortality and percent adversely affected in test solutions for each 24-hour period and provides the 95% confidence interval (C.I.) estimates associated with the calculated LC₅₀ value for the acute tests.

Acute endpoints for FCL-X		
	<i>Ceriodaphnia dubia</i>	<i>Pimephales promelas</i>
	48-hour LC ₅₀	96-hour LC ₅₀
FCL-X	17.68 g/L	8.15 g/L

Table 3.2 Water quality data for dilution water and selected test concentrations for freshly prepared test solution.							
Test solution		Date of first use in tests	DO mg/L	pH s.u.	conductivity µmho/cm	alkalinity mg/L CaCO ₃	hardness mg/L CaCO ₃
MHRW 010725		01/08/25	8.6	7.3	302	52	92
FCL-X	25 g/L	01/08/25	8.6	9.0	481	NA	NA

Table 3.3A. Product FCL-X toxicity test results.
Cumulative mortality and percent affected, LC₅₀ and 95% confidence interval, and method of analysis

Concentration grams product/ L MHRW	<i>C. dubia</i> (water flea) tested <u>01/08/25</u> to <u>01/10/25</u>	
	24-hours % mortality (% affected)	48-hours % mortality (% affected)
0, MHRW	0 (0)	0 (0)
1.56	0 (0)	0 (0)
3.13	0 (0)	0 (0)
6.25	0 (0)	0 (0)
12.5	0 (0)	0 (0)
25	100 (100)	100 (100)
LC ₅₀		17.68 g/L
95% C.I.		12.50-25.0
Method		B
Comments: SK = Spearman-Kärber, TSK = Trimmed Spearman-Kärber, B = Binomial. Cetus 2.1.5		

Table 3.3B. Product FCL-X toxicity test results.
Cumulative mortality and percent affected, LC₅₀ and 95% confidence interval, and method of analysis

<i>P. promelas</i> (fathead minnow) tested <u>01/08/25</u> to <u>01/12/25</u>				
Conc. grams product/ L MHRW	24-hours % mortality (% affected)	48-hours % mortality (% affected)	72-hours % mortality (% affected)	96-hours % mortality (% affected)
0, MHRW	0 (0)	0 (0)	0 (0)	0 (0)
1.56	5 (5)	10 (10)	10 (10)	10 (10)
3.13	5 (5)	5 (5)	5 (5)	10 (10)
6.25	5 (5)	10 (10)	15 (15)	15 (15)
12.5	15 (15)	80 (80)	100 (100)	100 (100)
25	80 (80)	100 (100)	100 (100)	100 (100)
LC ₅₀				<u>8.15 g/L</u>
95% C.I.				7.02-9.46
Method				TSK
Comments: SK = Spearman-Kärber, TSK = Trimmed Spearman-Kärber, B = Binomial. Cetis 2.1.5				

APPENDIX

Bench Sheets
Data Analysis
Standard Reference Toxicant (SRT) Control Charts



ACUTE, STATIC-NON RENEWAL BIOASSAY:

CLIENT: CL-XSTART DATE: 010815 TIME: 1320END DATE: 011015 TIME: 1230DILUTION WATER: mnrORGANISM: L. dubiaORIGIN: B122924(A)HATCH: 010724 1600AGE: 424hrsORGANISMS PER VESSEL: 5

Test Levels		Biological Parameters			Chemistry and Physical Parameters											
		Ni	Dead / Affected		Temperature (Celsius)			Dissolved Oxygen (mg/l)			pH (s.u.)			Conductivity (µmhos/cm)		
Rep	24 hr		48 hr	0	24	48	0	24	48	0	24	48	0	24	48	
Control: mnr	A	5	0 / 0	0 / 0	24.0	24.2	25.2	8.6		8.4	7.2		7.4	314		346
	B	5	0 / 0	0 / 0	Alkalinity: _____ mL _____ (Mg/L CaCO ₃) Hardness: _____ mL _____ (Mg/L CaCO ₃)											
	C	5	0 / 0	0 / 0												
	D	5	0 / 0	0 / 0												
1.56 µg/L	A	5	0 / 0	0 / 0	24.0	24.6	25.6	8.4		8.6	7.8		7.2	309		351
	B	5	0 / 0	0 / 0												
	C	5	0 / 0	0 / 0												
	D	5	0 / 0	0 / 0												
3.13 µg/L	A	5	0 / 0	0 / 0	24.0	24.8	25.6	8.6		8.6	8.0		7.5	321		351
	B	5	0 / 0	0 / 0												
	C	5	0 / 0	0 / 0												
	D	5	0 / 0	0 / 0												
6.25 µg/L	A	5	0 / 0	0 / 0	24.0	24.8	25.6	8.4		8.6	8.3		7.4	314		355
	B	5	0 / 0	0 / 0												
	C	5	0 / 0	0 / 0												
	D	5	0 / 0	0 / 0												
12.5 µg/L	A	5	0 / 0	0 / 0	24.0	24.6	25.6	8.6		8.2	8.7		7.4	390		446
	B	5	0 / 0	0 / 0												
	C	5	0 / 0	0 / 0												
	D	5	0 / 0	0 / 0												
25 µg/L	A	5	5 / 5	5 / 5	24.0	24.2	-	8.6	8.6	-	9.0	7.9	-	481	501	-
	B	5	5 / 5	5 / 5												
	C	5	5 / 5	5 / 5												
	D	5	5 / 5	5 / 5												
Time	1320		1120	1230	1320	1120	1230	1400	1400	1230	1400	1400	1230	1400		
Tech	RL	1	TM	TM	RL	TM	TM	DA	DA	DA	DA	DA	DA	DA	DA	DA
Comments:					6	1	1	OX4100L			Orion Star A211 A			Orion Star A212		
					ID or √ instrument used			DA	DA	DA	DA	DA	DA	DA	DA	DA
								Other:			Orion Star A211 B			Other:		

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CETIS Analytical Report

Report Date: 17 Jan-25 11:54 (p 1 of 2)
Test Code/ID: 7BAD9FE1 / 20-7497-6225

Ceriodaphnia 48-h Acute Survival Test

EnviroScience

Analysis ID: 21-2919-6178 Endpoint: 48h Survival Rate CETIS Version: CETIS v2.1.5
Analyzed: 17 Jan-25 11:54 Analysis: Binomial-Method Status Level: 1
Edit Date: 17 Jan-25 0:00 MD5 Hash: BA224C1B0259349C96C2A48B5BDB08A4 Editor ID: 007-869-049-5

Batch ID: 10-1570-3818 Test Type: Survival (48h) Analyst:
Start Date: 08 Jan-25 13:20 Protocol: EPA/821/R-02-012 (2002) Diluent: Mod-Hard Synthetic Water
Ending Date: 10 Jan-25 12:30 Species: Ceriodaphnia dubia Brine:
Test Length: 47h Taxon: Branchiopoda Source: In-House Culture Age:

Sample ID: 15-9886-9613 Code: 5F4CD06D Project:
Sample Date: 08 Jan-25 Material: Not Applicable Source: Unknown Source
Receipt Date: 08 Jan-25 CAS (PC): Station:
Sample Age: 13h Client: Full Circle Lithium

Binomial/Graphical Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	4.247	0	17680	12500	25000

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	1	0.9	>>	Yes	Passes Criteria

48h Survival Rate Summary

Conc-mg/L	Code	Count	Calculated Variate(A/B)							Isotonic Variate	
			Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
1560		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
3130		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
6250		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
12500		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
25000		4	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/20	0.0000	100.00%

48h Survival Rate Detail

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
1560		1.0000	1.0000	1.0000	1.0000
3130		1.0000	1.0000	1.0000	1.0000
6250		1.0000	1.0000	1.0000	1.0000
12500		1.0000	1.0000	1.0000	1.0000
25000		0.0000	0.0000	0.0000	0.0000

48h Survival Rate Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
1560		5/5	5/5	5/5	5/5
3130		5/5	5/5	5/5	5/5
6250		5/5	5/5	5/5	5/5
12500		5/5	5/5	5/5	5/5
25000		0/5	0/5	0/5	0/5

CETIS Analytical Report

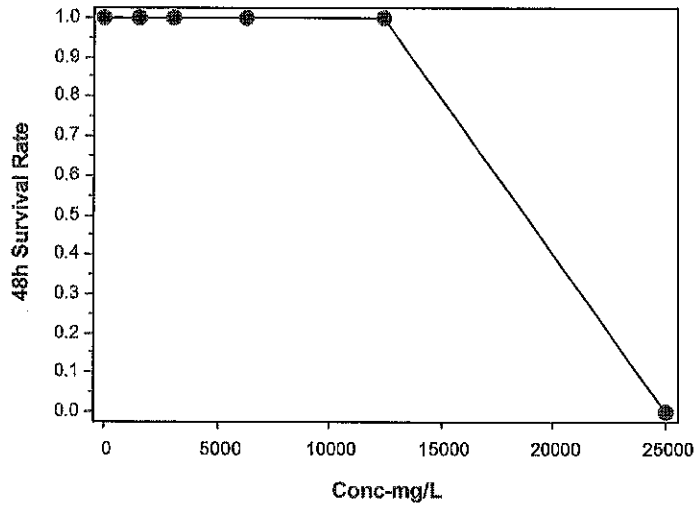
Report Date: 17 Jan-25 11:54 (p 2 of 2)
Test Code/ID: 7BAD9FE1 / 20-7497-6225

Ceriodaphnia 48-h Acute Survival Test

EnviroScience

Analysis ID: 21-2919-6178	Endpoint: 48h Survival Rate	CETIS Version: CETIS v2.1.5
Analyzed: 17 Jan-25 11:54	Analysis: Binomial Method	Status Level: 1
Edit Date: 17 Jan-25 0:00	MD5 Hash: BA224C1B0259349C96C2A48B5BDB08A4	Editor ID: 007-869-049-5

Graphics





ACUTE, STATIC, NON-RENEWAL BIOASSAY:
CLIENT: CL-X
START DATE: 0825 TIME: 1330
END DATE: 0825 TIME: 1330
DILUTION WATER: WMP

ORGANISM: P. promelas
ORIGIN: ES culture
HATCH: 123024 1315
AGE: 9 days old 10/17
ORGANISMS PER VESSEL: 10

Test Levels		Biological Parameters										Chemistry and Physical Parameters											
		Rep	Ni	Dead / Affected				Temperature (Celsius)			Dissolved Oxygen (mg/l)			pH (s.u.)			Conductivity						
				24h	48h	72h	96h	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	
Control: WMP	A 10	0'0	0'0	0'0	0'0	24.0	24.2	24.6	24.2	25.2	8.0	8.0	8.5	8.0	7.2	7.8	7.1	7.0	34	34	310	315	307
	B 10	0'0	0'0	0'0	0'0																		
1.56 mg/L	A 10	0'0	0'0	0'0	0'0	24.0	24.2	24.6	24.2	25.2	8.0	8.0	8.5	8.0	7.2	7.8	7.1	7.0	34	34	310	315	307
	B 10	1'1	2'2	2'2	2'2																		
3.13 mg/L	A 10	0'0	0'0	0'0	0'0	24.0	24.2	24.6	24.2	25.2	8.0	8.0	8.5	8.0	7.2	7.8	7.1	7.0	34	34	310	315	307
	B 10	1'1	1'1	1'1	1'1																		
6.25 mg/L	A 10	0'0	0'0	0'0	0'0	24.0	24.2	24.6	24.2	25.2	8.0	8.0	8.5	8.0	7.2	7.8	7.1	7.0	34	34	310	315	307
	B 10	1'1	2'2	2'2	2'2																		
12.5 mg/L	A 10	3'3	8'8	10'10	10'10	24.0	24.2	24.6	24.2	25.2	8.0	8.0	8.5	8.0	7.2	7.8	7.1	7.0	34	34	310	315	307
	B 10	0'0	8'8	10'10	10'10																		
25 mg/L	A 10	2'2	8'8	10'10	10'10	24.0	24.2	24.6	24.2	25.2	8.0	8.0	8.5	8.0	7.2	7.8	7.1	7.0	34	34	310	315	307
	B 10	2'2	8'8	10'10	10'10																		
Time	1330	1130	1330	1130	1230																		
Tech	CE	TM	TM	WB	sm																		
Comments:																							

JW

CETIS Analytical Report

Report Date: 17 Jan-25 12:36 (p 1 of 2)
Test Code/ID: 30844E15 / 08-1397-7109

Fathead Minnow 96-h Acute Survival Test

EnviroScience

Analysis ID: 03-9553-7454	Endpoint: 96h Survival Rate	CETIS Version: CETIS v2.1.5
Analyzed: 17 Jan-25 12:36	Analysis: Trimmed Spearman-Kärber	Status Level: 1
Edit Date: 17 Jan-25 0:00	MD5 Hash: F433B769E5F0B91D731785AA4DD9F9F7	Editor ID: 007-869-049-5
Batch ID: 07-5812-3318	Test Type: Survival (96h)	Analyst:
Start Date: 08 Jan-25 13:30	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 12 Jan-25 12:30	Species: Pimephales promelas	Brine:
Test Length: 95h	Taxon: Actinopterygii	Source: In-House Culture Age:
Sample ID: 04-8337-8222	Code: 1CCFC42E	Project:
Sample Date: 08 Jan-25	Material: Not Applicable	Source: Unknown Source
Receipt Date: 08 Jan-25	CAS (PC):	Station:
Sample Age: 14h	Client: Full Circle Lithium	

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	10.00%	3.911	0.03235	8147	7019	9456

Test Acceptability Criteria

		TAC Limits		Overlap	Decision
Attribute	Test Stat	Lower	Upper		
Control Resp	1	0.9	>>	Yes	Passes Criteria

96h Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-mg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	D	2	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
1560		2	0.9000	0.9000	0.8000	1.0000	15.71%	10.00%	18/20	0.9000	10.00%
3130		2	0.9000	0.9000	0.9000	0.9000	0.00%	10.00%	18/20	0.9000	10.00%
6250		2	0.8500	0.8500	0.8000	0.9000	8.32%	15.00%	17/20	0.8500	15.00%
12500		2	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/20	0.0000	100.00%
25000		2	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/20	0.0000	100.00%

96h Survival Rate Detail

Conc-mg/L	Code	Rep 1	Rep 2
0	D	1.0000	1.0000
1560		1.0000	0.8000
3130		0.9000	0.9000
6250		0.9000	0.8000
12500		0.0000	0.0000
25000		0.0000	0.0000

96h Survival Rate Binomials

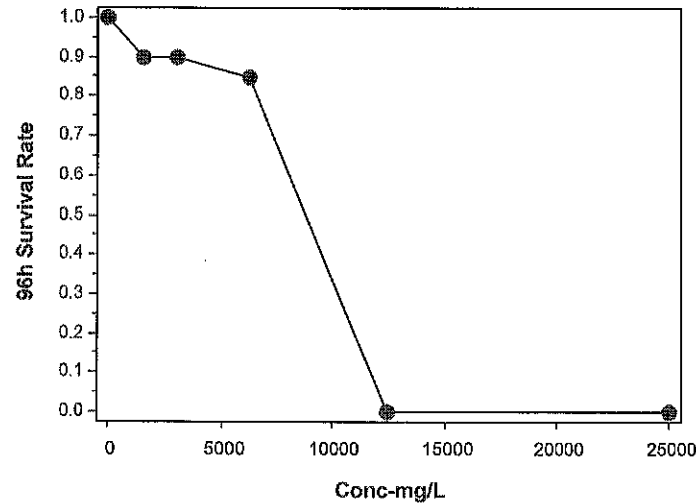
Conc-mg/L	Code	Rep 1	Rep 2
0	D	10/10	10/10
1560		10/10	8/10
3130		9/10	9/10
6250		9/10	8/10
12500		0/10	0/10
25000		0/10	0/10

CETIS Analytical Report

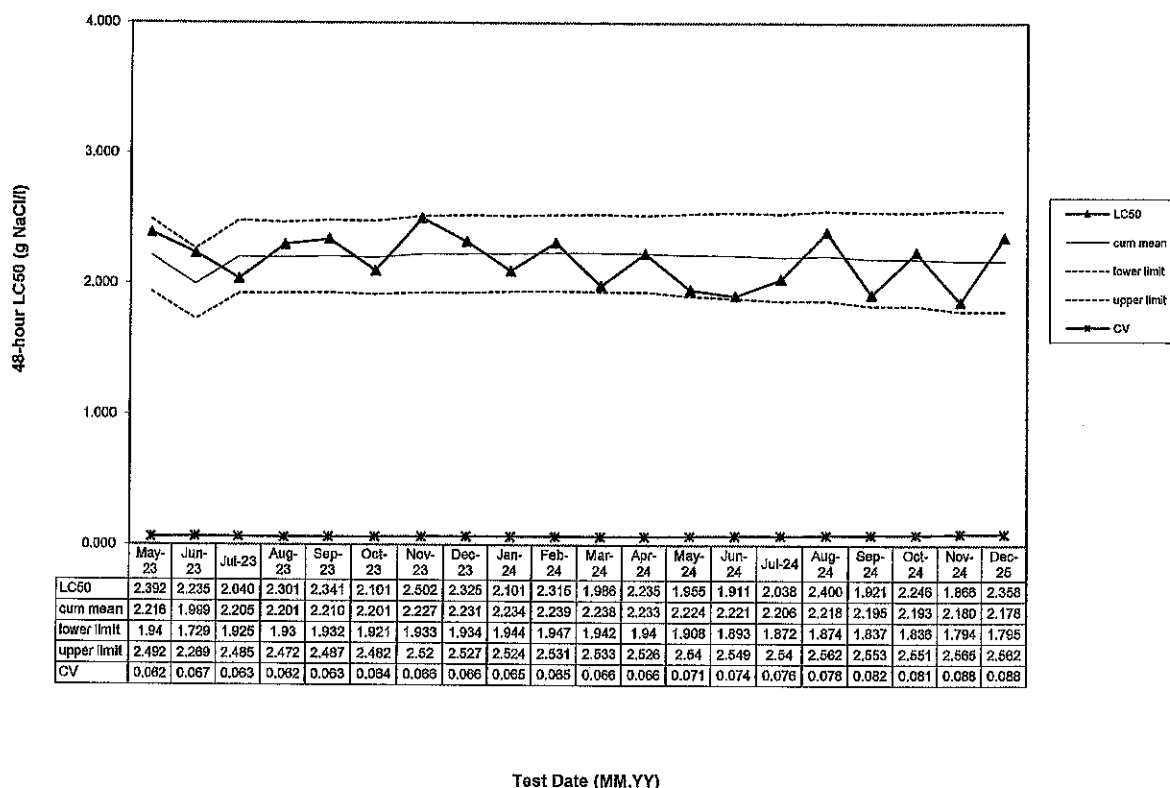
Report Date: 17 Jan-25 12:36 (p 2 of 2)
Test Code/ID: 30844E15 / 08-1397-7109

Fathead Minnow 96-h Acute Survival Test			EnviroScience	
Analysis ID: 03-9553-7454	Endpoint: 96h Survival Rate	CETIS Version: CETIS v2.1.5		
Analyzed: 17 Jan-25 12:36	Analysis: Trimmed Spearman-Kärber	Status Level: 1		
Edit Date: 17 Jan-25 0:00	MD5 Hash: F433B769E5F0B91D731785AA4DD9F9F7	Editor ID: 007-869-049-5		

Graphics



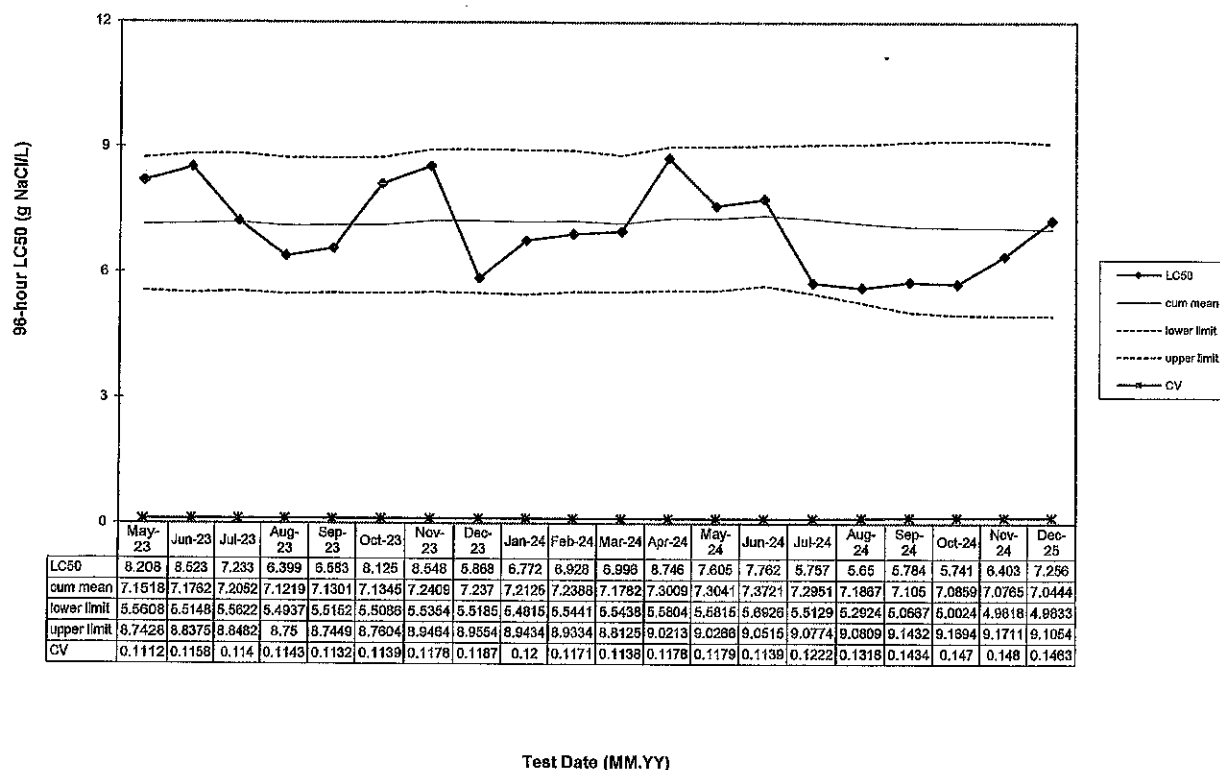
EnviroScience QC Chart - *Ceriodaphnia dubia*
Acute Toxicity endpoint - 25 C



Tests conducted at Stow, OH facility

EnviroScience QC/SRT Chart rev. 01/08/25

EnviroScience QC Chart - *Pimephales promelas*
Acute Toxicity Endpoint - 25 C



Tests conducted at Stow, OH facility

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