

Acute Toxicity Evaluation for the Ohio River WWTP

May 2025

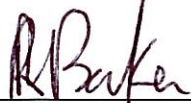
Prepared by:

Beckmar Environmental Laboratory
Biomonitoring Department
3251 Ruckriegel Parkway
Louisville, KY. 40299
(502) 266-6533

Submitted to:

Ohio River WWTP
1701 Landing Road
Goshen, KY. 40026

Released by: _____



Biomonitoring QA Officer

7-18-2025

Summary

Acute toxicity testing was performed on final effluent samples collected May 12, 2025 from the Ohio River Wastewater Treatment Plant. Testing was performed May 13 through 15, 2025 and upon termination, the following conclusions were reached:

For the 48-hour *Ceriodaphnia dubia* survival test, the LC_{50s} for survival for samples “A” and “B” were greater than 100% effluent, generating an acute toxicity value of less than 1.00 TUa (TUa=100/LC₅₀).

Introduction

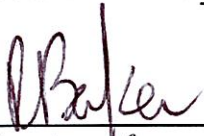
At the request of Ohio River WWTP, acute toxicity testing was performed on two grab effluent samples collected May 12, 2025 from the Ohio River Wastewater Treatment Plant in Goshen, KY. Information concerning plant and laboratory conditions can be found on the following pages.

The acute toxicity testing was performed in accordance with the US EPA methodology as defined in the US EPA manual “Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms” fifth edition, 2002 (EPA-821-R-02-012). The acute toxicity tests were performed in order to ascertain the LC₅₀ values for *Ceriodaphnia dubia* survival.

Test Type: Acute X Screen
Chronic Definitive X

TOXICITY TEST REPORT SHEET

- 1) Facility/Discharger: Ohio River WWTP Report Date: 7/8/2025
- 2) Address: 1701 Landing Road, Goshen KY 40026
- 3) NPDES Permit #: KY0106143 4) Receiving Stream: Ohio River @ mile pt. 391.5
- 5) Facility Contact:
- 6) Phone #:
- 7) Consultant/Testing Lab Name: Beckmar Environmental Laboratory
- 8) Lab Contact: Becky Barker Phone #: (502) 266-6533
- 9) Outfall(s) Tested: 1
- 10) Average Daily flow on day sampled (MGD) 1) na 2) na
3) na 4) 5) 6) 7)
- 11) Test Species: 1) Ceriodaphnia dubia 2)
- 12) Species Age: 1) < 24 hours old 2)
- 13) Organism Source: 1) lab culture 2)
- 14) Acclimation Procedures: 1) Reared at test conditions in synthetic water
2)
- 15) Test Conditions: Static: X Static-Renewal:
- 16) Dilution Water Type (Synthetic, Receiving Stream) synthetic - mhs
- 17) Aeration? (Before Test/During Test/None): no
- 18) Dechlorination? no Original Chlorine Level: <0.01 mg/L



(Signature of person filling out form)

Rhonda Baker

Name (Typed or Printed)

7/8/2025

Date

Biologist

Title

Materials and Methods

Sampling

Two effluent samples were collected 12 hours apart (Table I) and delivered to Beckmar Environmental Laboratory. Upon receipt, each sample went through standard log in procedures.

Control/Dilution Water

All chemicals used are reagent grade, obtained from Aldrich. 1.20 grams of CaSO_4 , 1.2 grams of MgSO_4 , 1.92 grams of NaHCO_3 , and 0.080 grams of KCl were dissolved in distilled water provided by a Barnstead Thermolyne distillation system and aerated for a minimum of 24 hours.

Test Containers

C. dubia tests were performed in 1 ounce plastic cups obtained from Plastics Inc. (St. Paul, MN).

Toxicity Testing

Samples were allowed to warm to room temperature (25°C) and were tested for residual chlorine immediately prior to dilution. Testing was then performed in accordance with US EPA methodology. Data was recorded on Beckmar generated lab sheets (Appendix I).

Chemical Analysis

All test dilutions as well as control/dilution water were tested to determine initial dissolved oxygen, temperature, and pH. At the end of 48 hours, the control/dilution water and test dilutions were again tested to determine final dissolved oxygen, temperature and pH. Also, specific conductance, hardness, and alkalinity analyses were performed on the initial control/dilution water and 100% effluent samples. Data was recorded on Beckmar generated lab sheet (Appendix I).

Statistical Analysis

Statistical data was generated using ToxCalc 5.0® (Tidepool Scientific software, McKinleyville, CA) and ToxStat® (USEPA, Cincinnati, OH) on a Pentium IV®, computer using Windows 98® Operating System.

Additional Toxicity Test Information

- 1) Submit copies of all bench sheets and statistical calculations/printouts obtained during the test(s). Data must be presented in tabular form and must include all physical and/or chemical measurements recorded during the test (e.g. temperature, conductivity, total residual chlorine, dissolved oxygen, etc.).

- 2) Methods/Instrumentation used in chemical analysis:

Dissolved Oxygen/temperature:	YSI Model 58
PH:	Orion Model 720A
Conductivity:	Oakton CON 300 Series
Alkalinity:	Standard Methods Titration
Hardness:	Standard Methods Titration
Total Chlorine Residual:	Fisher-Porter Titration
EPA Acute/Chronic Manual:	5 th Acute Edition, 2002

- 3) Indicate below any other relevant information that may aid in the evaluation of this report. Include any deviations from EPA methodology that was necessary for these tests as well as any sample manipulations that were performed, such as aeration, dechlorination with sodium thiosulfate, etc., and the justification for such manipulations or deviations. Attach additional pages as needed.
- 4) Sample temperature upon receipt may be greater than 4°C. Samples are picked up immediately after the final grab sample is collected. The samples are refrigerated; however it may be impossible to rapidly drop the effluent to 4°C.

TABLE I
Sampling Summary

Outfall	Sample Type	Volume	Collection Period	Rainfall	Sample Temp
1	Grab	1 gallon	05/12/25 @ 8:30 AM	NA	5.0 degrees C
	Grab	1 gallon	05/12/25 @ 8:30 PM	NA	5.0 degrees C

Dates/Times of Test Performance

Species #1: *Ceriodaphnia dubia*

Initiated: 05/13/25 @ 3:30 P.M.

Terminated 05/15/25 @ 3:30 P.M.

Results

In sample "A", *Ceriodaphnia dubia* test exhibited 100% survival in the control, 100% survival in the 20% dilution, 100% survival in the 40% dilution, 100% survival in the 60% dilution, 100% survival in the 80% dilution, and 100% survival in the 100% dilution.

In sample "B", *Ceriodaphnia dubia* test ran exhibited 100% survival in the control, 100% survival in the 20% dilution, 100% survival in the 40% dilution, 100% survival in the 60% dilution, 100% survival in the 80% dilution, and 100% survival in the 100% dilution.

For the 48-hour *Ceriodaphnia dubia* survival test, the LC50s for survival for samples "A" and "B" were greater than 100% effluent, generating an acute toxicity value of less than 1.00 TUa.

Appendix I

Ceriodaphnia dubia Data Sheets

Ceriodaphnia Survival and Reproduction Test-48 Hr Survival					
Start Date:	5/13/2025	Test ID:	OR0525	Sample ID:	ORAM052025
End Date:	5/15/2025	Lab ID:	0044: Beckmar Lab	Sample Type:	EFF1-POTW
Sample Date:	5/12/2025	Protocol:	EPA 821 R 02 012	Test Species:	CD-Ceriodaphnia dubia
Comments:	Ohio River Cerio Acute AM May 2025				

Conc-%	1	2	3	4
B-Control	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000

Transform: Arcsin Square Root							
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N
B-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
20	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
40	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
60	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
80	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
100	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.884		
Equality of variance cannot be confirmed				

Grab # A

Toxicity Test Results

Results of Ceriodaphnia dubia 48 HR. ACUTE Toxicity Test
(Genus) (Species) (Type / Duration)

Conducted: 05/13/25 -- 05/15/25 Using Effluent from Outfall # 1
(mm/dd/yy) (mm/dd/yy)

Test Solution	Percent Survival (time intervals used:- DAY)								# of Young		Dry Weight	
	1	2	3	4	5	6	7	8	Total	Mean	Total	Mean
Control	100	100										
20% Effluent	100	100										
40% Effluent	100	100										
60% Effluent	100	100										
80% Effluent	100	100										
100% Effluent	100	100										
LC ₅₀ / IC ₂₅ Value: <u>>100%</u>					Calculated TU Estimate * <u>< 1.00 Tua</u> (indicate Acute/Chronic)							
95% Confidence Limits					Permit Limits: <u>1.00 Tua</u> (Indicate TU _a / TU _c)							
UL: <u>na</u>					If acute test, method used to determine LC50 and Confidence Limit Valued:							
LL: <u>na</u>												
UL = Upper Limit												
LL = Lower Limit												

Note: TU_a = 100/LC₅₀; TU_c = 100/IC₂₅

Reference Toxicant Test Results					
Species	Date	Time	Duration	Toxicant	Results (LC ₅₀ / IC ₂₅)
<u>Ceriodaphnia</u>	<u>05/10/25</u>	<u>2:30 PM</u>	<u>48 hrs.</u>	<u>NaCl</u>	<u>LC50=2.3023 g/l</u>
<u>dubia</u>					

Client: OHIO RIVER

Beckmar Sample Number 250512022.01

Client Contact:

NPDES Permit No:

Sample Collector:

Grab Sample Collected: (1) 830 am/pm; 5 / 12 / 25 (Date).

(2) _____ am/pm; _____ / / (Date)

Persons Conducting Test:

Ed: Barker

Test Period: Beginning

Date: 5-12-2025

Time:

8.30

Ending

Date: 5-15-2025

Time:

9:30

Test Species:

C. dubia

Age / Source: <24 hrs. / lab culture

Dilution Water Used: MHSW

[illegible]

Ceriodaphnia Survival and Reproduction Test-48 Hr Survival					
Start Date:	5/13/2025	Test ID:	OR0525	Sample ID:	ORPM052025
End Date:	5/15/2025	Lab ID:	0044: Beckmar Lab	Sample Type:	EFF1-POTW
Sample Date:	5/12/2025	Protocol:	EPA 821 R 02 012	Test Species:	CD-Ceriodaphnia dubia
Comments:	Ohio River Cerio Acute PM May 2025				

Conc-%	1	2	3	4
B-Control	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000

Transform: Arcsin Square Root							
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N
B-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
20	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
40	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
60	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
80	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4
100	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.884		
Equality of variance cannot be confirmed				

Grab # B

Toxicity Test Results

Results of *Ceriodaphnia* *dubia* 48 HR. ACUTE Toxicity Test
(Genus) (Species) (Type / Duration)

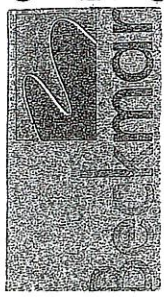
Conducted: 05/13/25 -- 05/15/25 Using Effluent from Outfall # 1
(mm/dd/yy) (mm/dd/yy)

Test Solution	Percent Survival (time intervals used:- DAY)								# of Young		Dry Weight	
	1	2	3	4	5	6	7	8	Total	Mean	Total	Mean
Control	100	100										
20% Effluent	100	100										
40% Effluent	100	100										
60% Effluent	100	100										
80% Effluent	100	100										
100% Effluent	100	100										
LC ₅₀ / IC ₂₅ Value: <u>>100%</u> 95% Confidence Limits UL: <u>na</u> LL: <u>na</u> UL = Upper Limit LL = Lower Limit					Calculated TU Estimate * <u>< 1.00 Tua</u> (indicate Acute/Chronic) Permit Limits: <u>1.00 Tua</u> (Indicate TU _a / TU _c)							
					If acute test, method used to determine LC50 and Confidence Limit Valued:							

Note: TU_a = 100/LC₅₀; TU_c = 100/IC₂₅

Reference Toxicant Test Results

Species	Date	Time	Duration	Toxicant	Results (LC ₅₀ / IC ₂₅)
<u><i>Ceriodaphnia</i></u>	<u>05/10/25</u>	<u>2:30 PM</u>	<u>48 hrs.</u>	<u>NaCl</u>	<u>LC50=2.3023 g/l</u>
<u><i>dubia</i></u>					



Client: OHIO RIVER
Beckmar Sample Number 250513014.01
Client Contact: _____
NPDES Permit No: _____
Sample Collector: _____

Persons Conducting Test: El Baker
Test Period: Beginning Date: 5-13-2025 Time: 3:30
Ending Date: 5-15-2025 Time: 3:30
Test Species: C. dubia

Grab Sample Collected: (1) 830 am/pm; 5/12/25 (Date)
(2) _____ am/pm; _____ (Date)

Age / Source: 24 hrs. / lab culture
Dilution Water Used: MHSW

Sample ID	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l) / Temp C		pH		Total Alkalinity (mg/l as CaCO ₃)	Total Hardness (mg/l as CaCO ₃)	Conductivity (umhos/cm)	Total Residual Chlorine (mg/L)
		0	24	48	0	48	0	48				
C	1	5	5	5	8.1/24.0	8.0/24.0	7.83	8.06	81.6	114.6	404	20.01
	2	5	5	5								
	3	5	5	5								
	4	5	5	5								
20%	1	5	5	5	8.1/24.0	7.9/24.0	7.73	7.98				
	2	5	5	5								
	3	5	5	5								
	4	5	5	5								
40%	1	5	5	5	8.1/24.0	7.8/24.0	7.57	7.87				
	2	5	5	5								
	3	5	5	5								
	4	5	5	5								
60%	1	5	5	5	8.2/24.0	7.7/24.0	7.40	7.81				
	2	5	5	5								
	3	5	5	5								
	4	5	5	5								
80%	1	5	5	5	8.2/24.0	7.6/24.0	7.24	7.75				
	2	5	5	5								
	3	5	5	5								
	4	5	5	5								
100%	1	5	5	5	8.2/24.0	7.5/24.0	7.03	7.57	124.0	343.1	932	20.01
	2	5	5	5								
	3	5	5	5								
	4	5	5	5								

Appendix II

Chain of Custody Data Sheets

Metropolitan Sewer District - OC

Month: May Year: 2025



Beckmar

Beckmar Environmental Laboratory
3251 Ruckriegel Parkway
Louisville, KY 40299
Phone: (502) 266-6533
Fax: (502) 266-6446
www.beckmarlab.com

[illegible]

*Job ID:250513014



Metropolitan Sewer District - OC

CHAIN OF CUSTODY AND ANALYTICAL REQUEST

Month: MAY Year: 2025



Beckmar

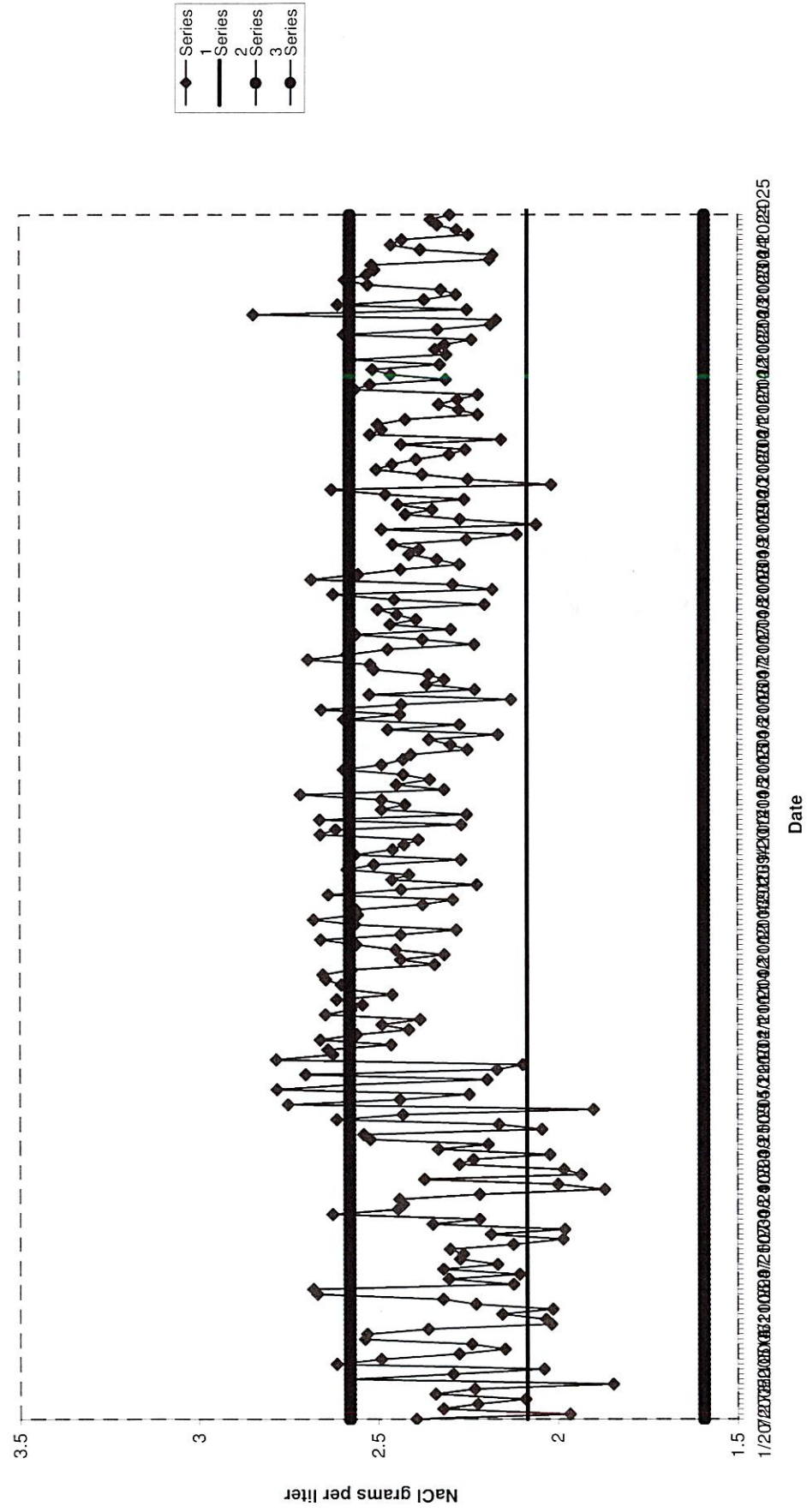
Beckmar Environmental Laboratory
3251 Ruckriegel Parkway
Louisville, KY 40299
Phone: (502) 266-6533
Fax: (502) 266-6446
www.beckmarlab.com

Special Instructions:				Calibration ID: _____			
Facility Location & Contact Information Client Name: <u>LOUISVILLE MSD</u> Address: <u>Ohio River WWTP</u> City, St, ZIP: _____ Phone: _____ Contact: _____				Send Results To: (same as client info ☐ yes ☐ no) Client Name: <u>Metropolitan Sewer District</u> Address: <u>P.O. Box 740011</u> City, State, ZIP: <u>Louisville, KY 40201</u> Phone / Fax: <u>502-540-6735</u> E-mail: <u>david.radke@louisvillemisd.org</u>			
Billing Information Client Name: _____ Address: _____ City, State, ZIP: _____ Contact Name: _____ A.P. Email: _____				Permit ID (if applicable): <u>KY0060577</u> Compliance (Y/N): _____ Samples chlorinated (Y/N): <u>N</u> Initial Laboratory Location: _____ Preservative Code: _____ Type of Container: _____ Number of Containers: _____ Collection Type: _____ (See Bottom Right)			
Analysis Requested <u>Bio-Monitoring</u>				Field Data pH (S.U.): _____ DO (Mg/L): _____ Cl ₂ (mg/L): _____ Temp (°C): _____ Free: _____ Total: _____			
Sample Point / Description <u>Final Effluent</u>				Sample rejection : Reason: Temp. At Receipt: <u>5</u> °C Check Applicable Field: Wet Ice ☒ Blue Ice _____ Sample Integrity: Yes _____ No ☒ N/A _____ Broken Containers: _____ Custody Seals Intact: _____ COC / Sample Label Agreement: _____ Proper Containers: _____ Samples Within Holding Times: _____ All Samples on COC Received: _____ W1 & D1 Filled to 100ml mark: _____ Headspace acceptable: _____ Preservative Added (Date/Time): _____			
Type of Container G10 = 1000ml Glass G5 = 500ml Glass P10 = 1000ml Plastic H40c = 40ml Headspace Clear H40a = 40ml Headspace Amber SG = 16 Oz Glass - Soil W1 = 120ml Plastic Sterile D1 = 120ml Plastic Sterile P25 = 250ml Plastic P1 = 100ml Plastic 4LC = 4 Liter Cube 1LC = 1 Liter Cube G2 = 250ml Glass VA = 1000ml Virgin Amber Glass AB = 8oz Glass Amber				Type of Container G10 = 1000ml Glass G5 = 500ml Glass P10 = 1000ml Plastic H40c = 40ml Headspace Clear H40a = 40ml Headspace Amber SG = 16 Oz Glass - Soil W1 = 120ml Plastic Sterile D1 = 120ml Plastic Sterile P25 = 250ml Plastic P1 = 100ml Plastic 4LC = 4 Liter Cube 1LC = 1 Liter Cube G2 = 250ml Glass VA = 1000ml Virgin Amber Glass AB = 8oz Glass Amber			
Date: <u>5-12-25</u> Time: <u>0945</u> Date: <u>13MAY25</u> Time: <u>0935</u> Date: <u>13MAY25</u> Time: <u>1455</u> Date: _____ Time: _____ Date: _____ Time: _____ Date: _____ Time: _____				Date: <u>5-12-25</u> Time: <u>0945</u> Date: <u>13MAY25</u> Time: <u>0935</u> Date: <u>13MAY25</u> Time: <u>1455</u> Date: _____ Time: _____ Date: _____ Time: _____ Date: _____ Time: _____			
Received by: <u>FRIDGE</u> Relinquished by: <u>Lab</u> Received by: <u>Lab</u> Relinquished by: <u>Lab</u> Received by: _____ Relinquished by: _____ Received by: _____ Relinquished by: _____				Received by: <u>FRIDGE</u> Relinquished by: <u>Lab</u> Received by: <u>Lab</u> Relinquished by: <u>Lab</u> Received by: _____ Relinquished by: _____ Received by: _____ Relinquished by: _____			
Comments: <u>TEMP @ 10:50C</u> <u>TEMP @ 01:50C</u> Client Collects E-coli Separately				Initial: <u>JMK</u>			

Appendix III

NaCl Reference Data Sheets

C. dubia NaCl Reference Test Acceptability



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

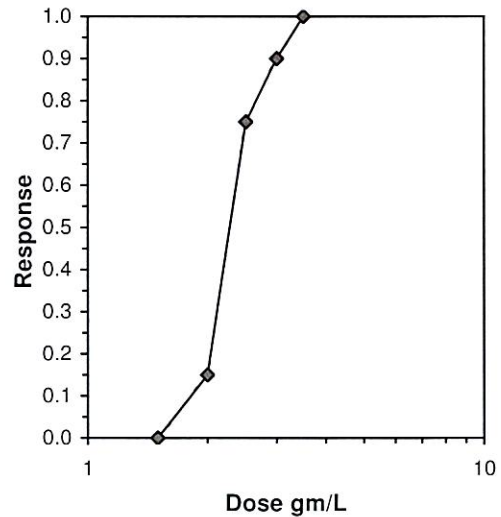
Start Date: 5/10/2025	Test ID: NACL0525	Sample ID: NACL0525
End Date: 5/12/2025	Lab ID: 0044: Beckmar Lab	Sample Type: NACL-Sodium chloride
Sample Date:	Protocol: EPA-821-R-02-012	Test Species: CD-Ceriodaphnia dubia
Comments: NaCl Cerio Acute May 2025		

Conc-gm/L	1	2	3	4
B-Control	1.0000	1.0000	1.0000	1.0000
1.5	1.0000	1.0000	1.0000	1.0000
2	0.8000	1.0000	0.8000	0.8000
2.5	0.4000	0.2000	0.0000	0.4000
3	0.2000	0.0000	0.0000	0.2000
3.5	0.0000	0.0000	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
B-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	0	20
1.5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	0	20
2	0.8500	0.8500	1.1667	1.1071	1.3453	10.206	4	3	20
2.5	0.2500	0.2500	0.5146	0.2255	0.6847	42.578	4	15	20
3	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	18	20
3.5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4	20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9037	0.868	-0.3795	1.0994
Equality of variance cannot be confirmed				

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	2.3023	2.1662	2.4470
5.0%	2.3023	2.1578	2.4565
10.0%	2.2997	2.1570	2.4518
20.0%	2.2820	2.1529	2.4190
Auto-0.0%	2.3023	2.1662	2.4470



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Permit

Permit #:

KY0106143

Major:

Yes

Permittee:

Louisville and Jefferson County MSD

Permittee Address:

700 W Liberty St
Louisville, KY 40203

Facility:

OHIO RIVER WWTP

Facility Location:

1901 LANDING RD
GOSHEN, KY 40026

Permitted Feature:

001
External Outfall

Discharge:

001-2
Quarterly Biomonitoring

Report Dates & Status

Monitoring Period:

From 04/01/25 to 06/30/25

DMR Due Date:

07/28/25

Status:

NetDMR Validated

Considerations for Form Completion

Principal Executive Officer

First Name:

Tony

Last Name:

Parrott

Title:

Executive Director

Telephone:

502-540-6533

No Data Indicator (NODI)

Form NODI:

--

Parameter		Monitoring Location	Season #	Param. NODI		Quantity or Loading					Quality or Concentration						# of Ex.	Frequency of Analysis	Sample Type	
Code	Name					Qualifier 1	Value 1	Qualifier 2	Value 2	Units	Qualifier 1	Value 1	Qualifier 2	Value 2	Qualifier 3	Value 3	Units			
61406	Toxicity, final conc toxicity units	1 - Effluent Gross	0	--	Sample										<	1.0	2F - tox acute		01/90 - Quarterly	24 - 24 Hour Composite
					Permit Req.										<=	1.0 MAXIMUM	2F - tox acute		01/90 - Quarterly	24 - 24 Hour Composite
					Value NODI															

Submission Note

If a parameter row does not contain any values for the Sample nor Effluent Trading, then none of the following fields will be submitted for that row: Units, Number of Excursions, Frequency of Analysis, and Sample Type.

Edit Check Errors

No errors.

Comments

Attachments

Name	Type	Size
ohio_river_bio_rpt_may_2025.pdf	pdf	6078618.0

Report Last Saved By

Louisville and Jefferson County MSD

User:

ALEX.SMITHER@LOUISVILLEMSD.ORG

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Date/Time:

2025-07-21 07:59 (Time Zone: -04:00)

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Date/Time:

2025-07-21 08:02 (Time Zone: -04:00)