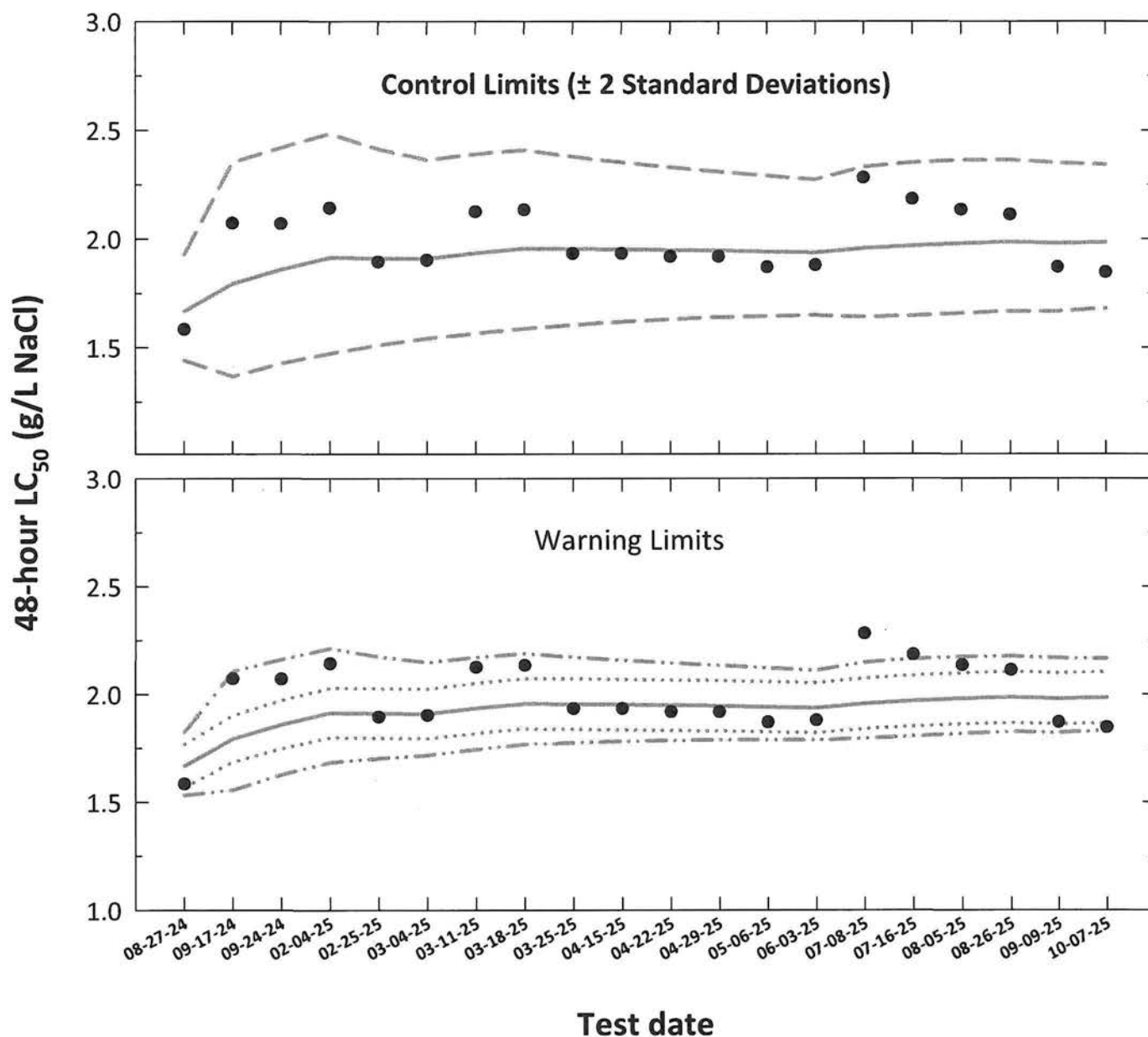


Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A,10}$ converted to anti-logarithmic values, $S_{A,10}$ = 10th percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	08-27-24	1.5847	0.2000	0.2224	0.0317	1.6686	1.4422	1.9306	1.5329	1.8256	1.5685	1.7687
2	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
3	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
4	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
5	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
6	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
7	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
8	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
9	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
10	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
11	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
12	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
13	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
14	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
15	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
16	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
17	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
18	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.8658	2.1040
19	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.8603	2.0977
20	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.8649	2.1030

Note: 48-hour LC_{50} = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic $LC_{50} \pm 2CV$ or $S_{A,10}$ converted to anti-logarithmic values.

$S_{A,10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. ($S_{A,10} = 0.06$).

CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Ceriodaphnia dubia

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 23

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2411

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.79	7.77	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.0
	Conductivity (µmhos/cm)	299		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.9	25.3	25.2
1000 mg/L	pH (S.U.)	7.95	7.81	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	2080		
	Temperature (°C)	24.9	25.1	25.0
1500 mg/L	pH (S.U.)	7.94	7.81	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	2870		
	Temperature (°C)	24.8	25.2	24.9
2000 mg/L	pH (S.U.)	7.94	7.83	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	3730		
	Temperature (°C)	24.7	25.2	24.9
2500 mg/L	pH (S.U.)	7.92	7.83	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	4530		
	Temperature (°C)	25.1	25.2	24.8
3000 mg/L	pH (S.U.)	7.92	7.83	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	5460		
	Temperature (°C)	25.0	25.0	25.2

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurement only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306647

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

***Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test**

CdNaClAC # 23

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-07-25	0530*	JP	1000	JP	2B3	RED	09-24-25A
24	10-08-25			1712	JP			
48 Termination	10-09-25			0952	JP			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	09-30-25 D
Age:	< 24-hours old
Date and time organisms were born between:	10-06-25 1245 TO 10-07-25 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.87
	Temperature (°C): 24.6°C

Survival Data (number of living organisms):

Hours	Control				1000 mg/L				1500 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

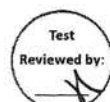
Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	5	5 ^{5d}	3 ^{2d}	2 ^{3d}	4 ^{1d}
48 Termination	0 ^{5d}	4 ^{1d}	0 ^{5d}	1 ^{3d}	0 ^{4d}	0 ^{4d}	0 ^{4d}	0 ^{5d}	0	0 ^{3d}	0 ^{2d}	0 ^{4d}
Mean Survival	25%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1757.2
Upper 95% confidence limit (mg NaCl/L)	1939.9
48-hour LC ₅₀ (mg NaCl/L)	1846.3

Comments:



Statistical Analyses

Acute Daphnid Test-24 Hr Survival					
Start Date:	10/7/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

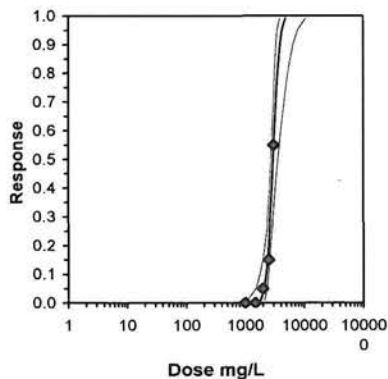
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	0.8000
2500	0.8000	0.8000	0.8000	1.0000
3000	0.0000	0.6000	0.4000	0.8000

Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
2500	0.8500	0.8500	1.1667	1.1071	1.3453	10.206	4	12.00	10.00	3	20
*3000	0.4500	0.4500	0.7259	0.2255	1.1071	51.737	4	10.00	10.00	11	20

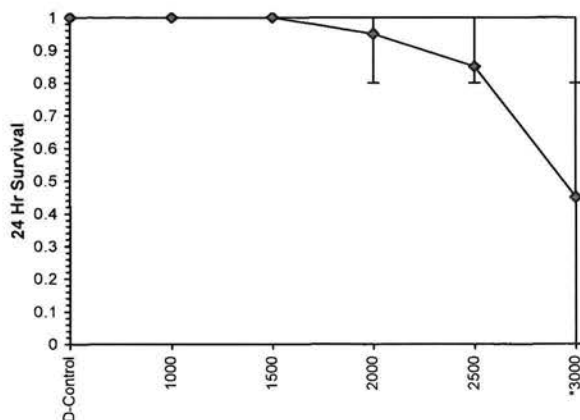
Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)				0.76916	0.884	-0.9502	6.58061
Equality of variance cannot be confirmed							
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test				2500	3000	2738.61	
Treatments vs D-Control							

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	10.938	3.17125	4.72234	17.1536	0	0.75185	7.81472	0.86095	3.4731	0.09142	3
Intercept	-32.989	10.8591	-54.273	-11.705							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1821.43	1093.98 2118.77
EC05	3.355	2102.41	1512.38 2341.38
EC10	3.718	2269.51	1788.51 2481.73
EC15	3.964	2389.7	1992.94 2593.86
EC20	4.158	2489.74	2159.93 2701.55
EC25	4.326	2578.89	2299.49 2815.49
EC40	4.747	2817.97	2596.39 3240.03
EC50	5.000	2972.34	2735.95 3599.38
EC60	5.253	3135.16	2860.62 4029.9
EC75	5.674	3425.81	3056.19 4901.25
EC80	5.842	3548.49	3133.04 5304.65
EC85	6.036	3697.04	3223.18 5820.33
EC90	6.282	3892.81	3338.21 6545.07
EC95	6.645	4202.21	3513.33 7795.09
EC99	7.326	4850.47	3860.96 10836.4



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival					
Start Date:	10/7/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

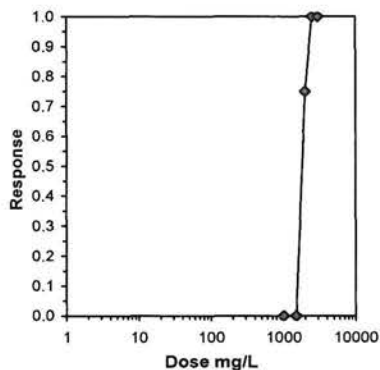
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.0000	0.8000	0.0000	0.2000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.2500	0.2500	0.5055	0.2255	1.1071	82.409	4	10.00	10.00	15	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

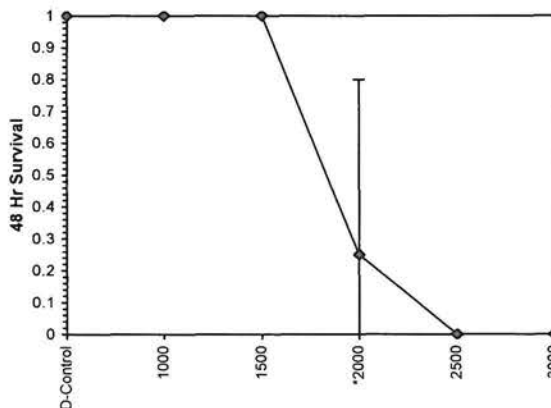
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.57777	0.844	2.04948	8.15246
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

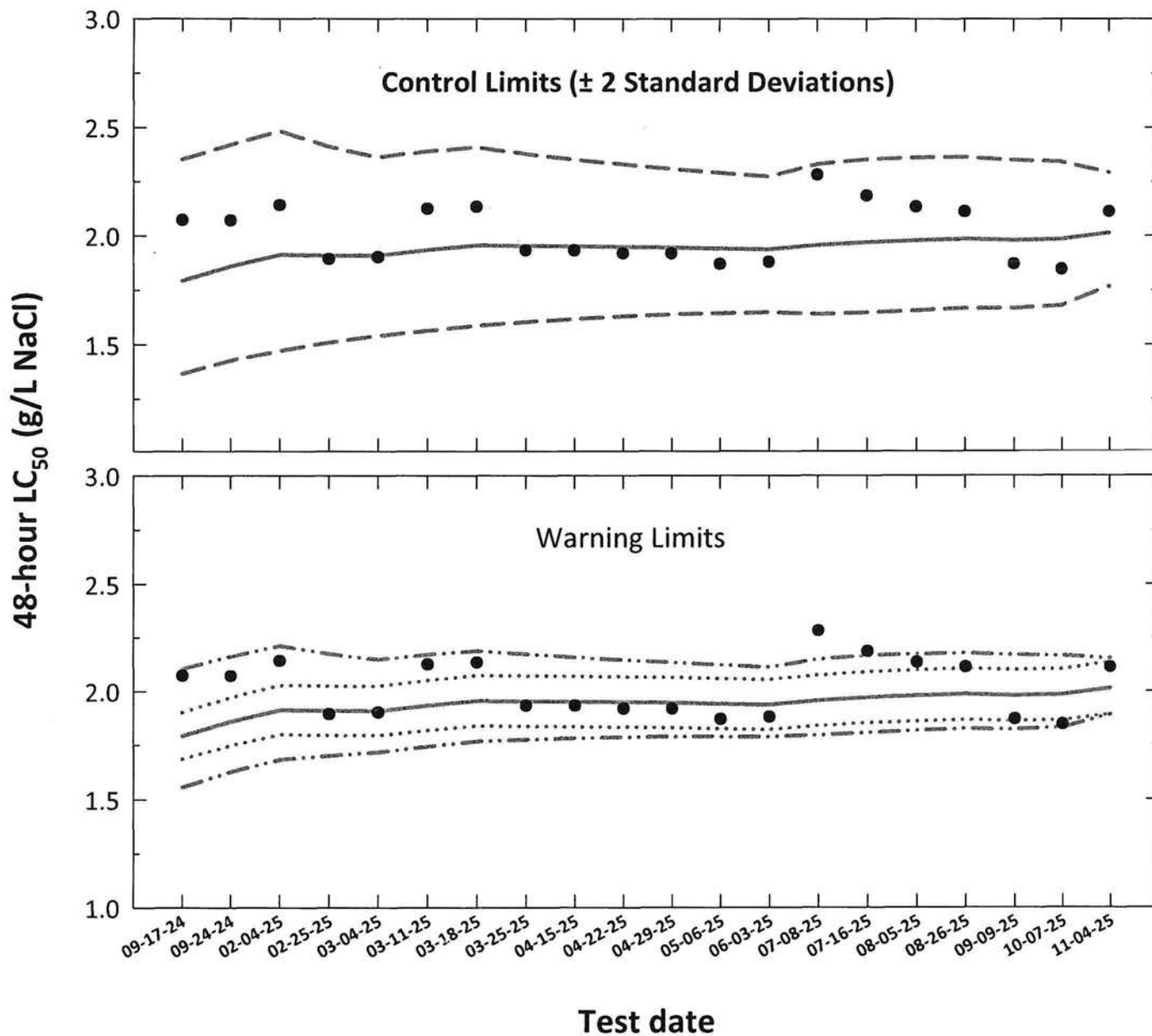
Trim Level	EC50	95% CL
0.0%	1846.26	1757.16 1939.87
5.0%	1837.79	1740.95 1940.02
10.0%	1830.17	1726.83 1939.7
20.0%	1819.05	1708.51 1936.73
Auto-0.0%	1846.26	1757.16 1939.87



Dose-Response Plot



Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A,10}$ converted to anti-logarithmic values, $S_{A,10}$ = 10th percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S		Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV		10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}	
1	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
2	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
3	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
4	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
5	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
6	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
7	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
8	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
9	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
10	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
11	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
12	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
13	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
14	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
15	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
16	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
17	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.8658	2.1040
18	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.8603	2.0977
19	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.8649	2.1030
20	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.8918	2.1333

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCal).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.06).

CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant TestCdNaClAC # 24Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2411Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	En XL	XL	XL
Control, MHSW	pH (S.U.)	7.80	7.76	7.80
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	299		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	91		
	Temperature (°C)	24.6	25.3	25.2
1000 mg/L	pH (S.U.)	7.96	7.83	7.86
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	2030		
	Temperature (°C)	24.4	25.0	25.0
1500 mg/L	pH (S.U.)	7.95	7.83	7.87
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	2860		
	Temperature (°C)	24.5	25.3	24.7
2000 mg/L	pH (S.U.)	7.93	7.84	7.88
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	3670		
	Temperature (°C)	24.5	24.9	25.2
2500 mg/L	pH (S.U.)	7.92	7.84	7.87
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	4450		
	Temperature (°C)	24.8	24.9	24.7
3000 mg/L	pH (S.U.)	7.92	7.84	7.87
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	5280		
	Temperature (°C)	24.6	25.1	24.7

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified or test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numb
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicab
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130660

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

***Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test**

CdNaClAC # 21

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-04-25	0515	gp	0826	gp	2B3	62404	10-26-25B
24	11-05-25			0813	gp			
48 Termination	11-06-25			0801	gp			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	10-28-25 D
Age:	< 24-hours old
Date and time organisms were born between:	11-03-25 1230 to 11-04-25 0515
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.85
	Temperature (°C): 24.1°C

Survival Data (number of living organisms):

Hours	Control				1000 mg/L				1500 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	4 ^{1d}	3 ^{2d}	4 ^{1d}	3 ^{2d}	4 ^{1d}	5 ^{1d}	4 ^{1d}	5 ^{1d}	4 ^{1d}
48 Termination	3 ^{2d}	4 ^{1d}	4 ^{1d}	3 ^{1d}	5 ^{1d}	1	5 ^{3d}	0 ^{4d}	0	0 ^{1d}	0	0 ^{1d}
Mean Survival	70%				5%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	1991.0
Upper 95% confidence limit (mg NaCl/L)	2229.7
48-hour LC ₅₀ (mg NaCl/L)	2111.1

Comments:



Statistical Analyses

Acute Daphnid Test-24 Hr Survival					
Start Date:	11/4/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia

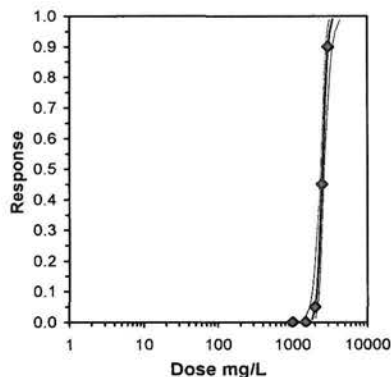
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	0.8000
2500	0.6000	0.2000	0.6000	0.8000
3000	0.0000	0.2000	0.0000	0.2000

Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2500	0.5500	0.5500	0.8357	0.4636	1.1071	32.195	4	10.00	10.00	9	20
*3000	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20

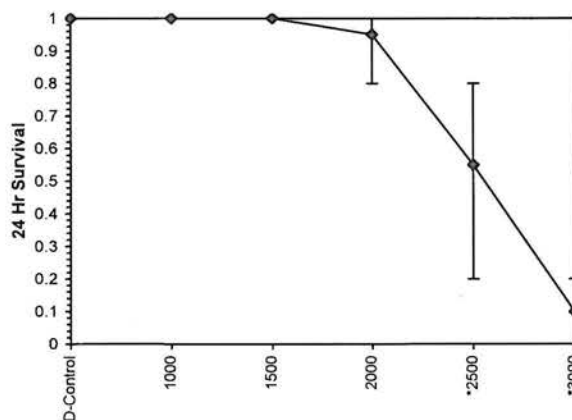
Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.8269	0.884	-1.0679	4.46097
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					2000	2500	2236.07	
Treatments vs D-Control								

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.7095	3.45552	9.93666	23.4823	0	0.05125	7.81472	0.99696	3.40293	0.05985	3
Intercept	-51.861	11.7662	-74.923	-28.799							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1835.3	1455.02	2037.86
EC05	3.355	2016	1697.01	2187.58
EC10	3.718	2119.5	1839.53	2274.96
EC15	3.964	2192.31	1940.5	2338.15
EC20	4.158	2251.96	2022.98	2391.64
EC25	4.326	2304.43	2094.79	2440.54
EC40	4.747	2442.12	2275.33	2581.65
EC50	5.000	2528.89	2379.08	2684.23
EC60	5.253	2618.73	2476	2803.9
EC75	5.674	2775.2	2622.24	3041.98
EC80	5.842	2839.86	2676.65	3149.02
EC85	6.036	2917.13	2738.6	3282.01
EC90	6.282	3017.35	2815.31	3461.38
EC95	6.645	3172.25	2928.38	3751.29
EC99	7.326	3484.6	3144.16	4374.31



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival					
Start Date:	11/4/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

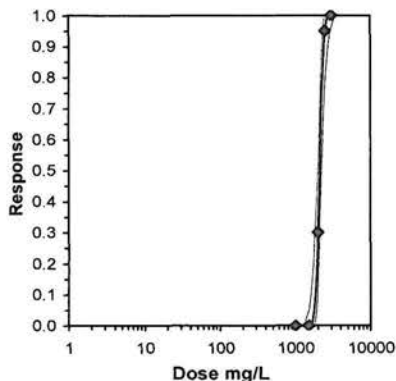
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.6000	0.8000	0.8000	0.6000
2500	0.0000	0.2000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.7000	0.7000	0.9966	0.8861	1.1071	12.807	4	10.00	10.00	6	20
*2500	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

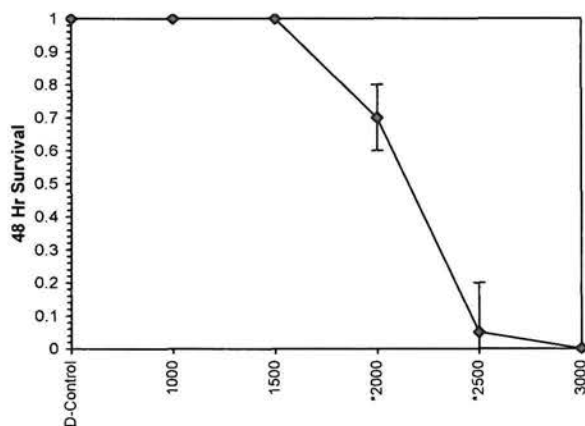
Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.82099	0.868	0.88754	1.61838
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					1500	2000	1732.05	
Treatments vs D-Control								

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	22.6291	5.43678	11.973 33.2852	0	0.0152	7.81472	0.9995	3.32452	0.04419
Intercept	-70.231	18.0928	-105.69 -34.769						
TSCR									
Iter									3

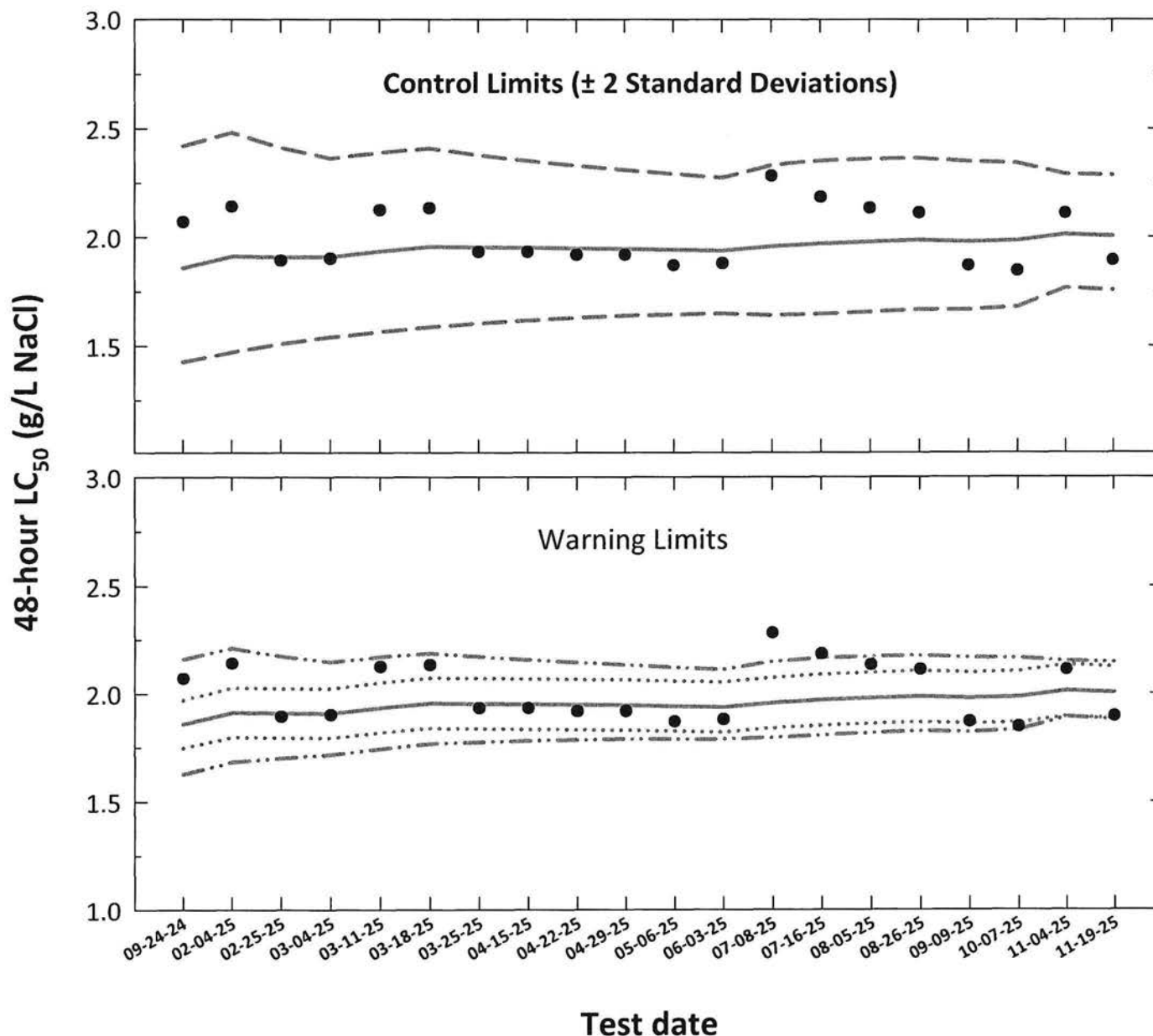
Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1666.15	1327.41 1820.22
EC05	3.355	1785.79	1507.68 1915.19
EC10	3.718	1853.04	1611.62 1970.24
EC15	3.964	1899.84	1684.32 2009.98
EC20	4.158	1937.87	1743.15 2043.65
EC25	4.326	1971.11	1793.96 2074.48
EC40	4.747	2057.41	1919.98 2163.97
EC50	5.000	2111.14	1991.03 2229.68
EC60	5.253	2166.27	2056.06 2307.04
EC75	5.674	2261.12	2150.64 2462.35
EC80	5.842	2299.9	2184.62 2532.34
EC85	6.036	2345.95	2222.57 2619.19
EC90	6.282	2405.2	2268.63 2735.86
EC95	6.645	2495.77	2335.01 2922.99
EC99	7.326	2674.98	2458.02 3318.36



Dose-Response Plot



Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ $\pm$ 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic LC₅₀ $\pm$ 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm$ S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia Acute Reference Toxicant Control Chart Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Warning Limits		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
2	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
3	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
4	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
5	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
6	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
7	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
8	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
9	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
10	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
11	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
12	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
13	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
14	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
15	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
16	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.8658	2.1040
17	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.8603	2.0977
18	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.8649	2.1030
19	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.8918	2.1333
20	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.8833	2.1237

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCat).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.06).

CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Ceriodaphnia dubia

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 75

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2433

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.74	7.88	8.02
	Dissolved oxygen (mg/L)	8.5	8.4	8.2
	Conductivity (µmhos/cm)	289		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	90		
	Temperature (°C)	25.1	24.7	24.7
1000 mg/L	pH (S.U.)	7.84	7.92	8.03
	Dissolved oxygen (mg/L)	8.5	8.4	8.2
	Conductivity (µmhos/cm)	1960		
	Temperature (°C)	24.9	24.6	24.6
1500 mg/L	pH (S.U.)	7.85	7.92	8.02
	Dissolved oxygen (mg/L)	8.5	8.4	8.2
	Conductivity (µmhos/cm)	2780		
	Temperature (°C)	24.9	24.6	24.6
2000 mg/L	pH (S.U.)	7.85	7.92	8.01
	Dissolved oxygen (mg/L)	8.5	8.3	8.2
	Conductivity (µmhos/cm)	3570		
	Temperature (°C)	24.7	24.7	24.6
2500 mg/L	pH (S.U.)	7.85	7.92	8.01
	Dissolved oxygen (mg/L)	8.5	8.3	8.2
	Conductivity (µmhos/cm)	4440		
	Temperature (°C)	25.0	24.5	24.7
3000 mg/L	pH (S.U.)	7.85	7.92	
	Dissolved oxygen (mg/L)	8.4	8.3	
	Conductivity (µmhos/cm)	5330		
	Temperature (°C)	24.9	24.5	

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13061465

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

***Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test**

CdNaCIAC # 25

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-19-25	0415	J	0615	J	203	ORANGE	11-17-25 B
24	11-20-25			0612	J			
48 Termination	11-21-25			0615	J			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	11-14-25 A-C
Age:	< 24-hours old
Date and time organisms were born between:	11-18-25 1440 TO 11-19-25 0415
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.92
	Temperature (°C): 25.0

Survival Data (number of living organisms):

Hours	Control				1000 mg/L				1500 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	S	S	S	S	S	S	S
48 Termination	S	S	S	S	S	S	S	S	S	S	S	S
Mean Survival	100%				100%				100%			

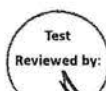
Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	4 ^d	1 ^u	4 ^d	1 ^u	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
48 Termination	3 ^u	1 ^u	2 ^u	1 ^u	0 ^u	0 ^u	0 ^u	0 ^u	0	0	0	0
Mean Survival	35%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1793.6
Upper 95% confidence limit (mg NaCl/L)	2000.1
48-hour LC ₅₀ (mg NaCl/L)	1894.0

Comments:





Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date: 11/19/2025 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
 End Date: 11/21/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia
 Comments:

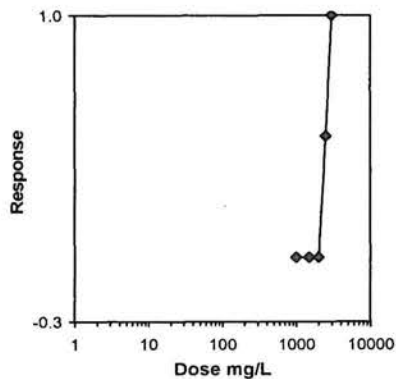
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2500	0.8000	0.2000	0.8000	0.2000
3000	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2500	0.5000	0.5000	0.7854	0.4636	1.1071	47.304	4	10.00	10.00	10	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

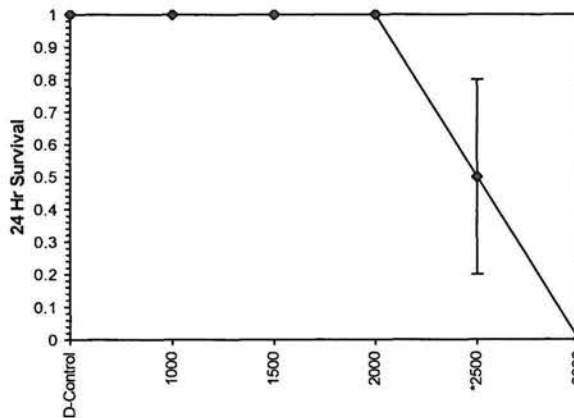
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.63123	0.868	-8E-16	2.98039
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	2474.62	2364.94	2589.38
5.0%	2477.14	2355.34	2605.24
10.0%	2479.67	2342.53	2624.84
20.0%	2484.74	2301.12	2683.01
Auto-0.0%	2474.62	2364.94	2589.38



Dose-Response Plot



Statistical Analyses

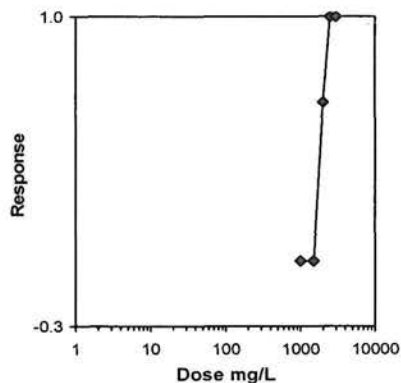
Acute Daphnid Test-48 Hr Survival					
Start Date:	11/19/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/21/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.6000	0.2000	0.4000	0.2000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

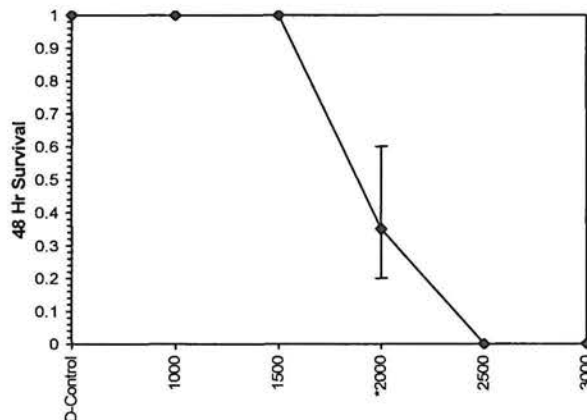
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.3500	0.3500	0.6245	0.4636	0.8861	32.527	4	10.00	10.00	13	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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 non-normal distribution (p <= 0.01)		0.66218	0.844	0.99425	5.11578
Equality of variance cannot be confirmed					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test		1500	2000	1732.05	
Treatments vs D-Control					

Trimmed Spearman-Kärber				
Trim Level	EC50	95% CL		
0.0%	1894.02	1793.59	2000.07	
5.0%	1889.87	1779.46	2007.13	
10.0%	1885.84	1764.37	2015.68	
20.0%	1878.39	1729.31	2040.32	
Auto-0.0%	1894.02	1793.59	2000.07	



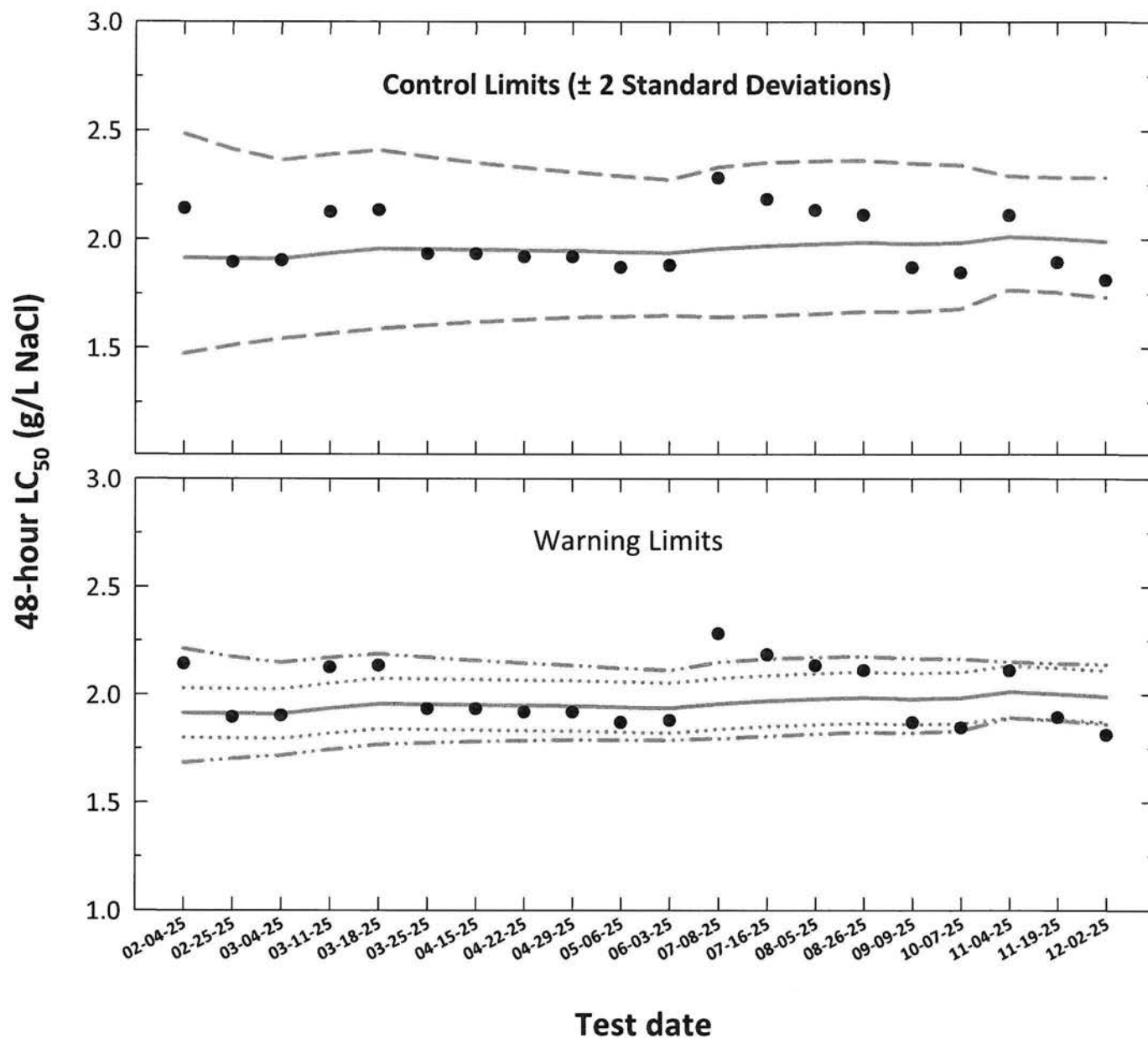
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ $\pm$ 2 standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ $\pm$ 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm$ S_{A.10} converted to anti-logarithmic values, S_{A.10} = 10th percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
1	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
2	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
3	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
4	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
5	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
6	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
7	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
8	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
9	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
10	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
11	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
12	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
13	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
14	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
15	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.8658	2.1040
16	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.8603	2.0977
17	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.8649	2.1030
18	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.8918	2.1333
19	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.8833	2.1237
20	12-02-25	1.8126	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.8708	2.1096

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.06).

CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant TestCdNaClAC # 26

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L Na stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2433

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.93	7.91	7.89
	Dissolved oxygen (mg/L)	8.4	8.2	8.3
	Conductivity (µmhos/cm)	290		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	88		
	Temperature (°C)	25.0	25.2	25.1
1000 mg/L	pH (S.U.)	7.99	7.93	7.91
	Dissolved oxygen (mg/L)	8.4	8.3	8.4
	Conductivity (µmhos/cm)	2020		
	Temperature (°C)	25.5	25.3	25.5
1500 mg/L	pH (S.U.)	8.00	7.95	7.93
	Dissolved oxygen (mg/L)	8.4	8.3	8.4
	Conductivity (µmhos/cm)	2800		
	Temperature (°C)	25.3	25.0	25.2
2000 mg/L	pH (S.U.)	8.00	7.95	7.93
	Dissolved oxygen (mg/L)	8.4	8.3	8.4
	Conductivity (µmhos/cm)	3590		
	Temperature (°C)	25.2	25.3	25.2
2500 mg/L	pH (S.U.)	7.99	7.95	7.93
	Dissolved oxygen (mg/L)	8.4	8.3	8.4
	Conductivity (µmhos/cm)	4320		
	Temperature (°C)	25.0	25.1	25.2
3000 mg/L	pH (S.U.)	7.99	7.95	
	Dissolved oxygen (mg/L)	8.4	8.2	
	Conductivity (µmhos/cm)	5370		
	Temperature (°C)	25.0	25.1	

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on the test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306647

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 26

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shell	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-02-25	0700	JP	0951	JP	2B2	Bine	1124-25A
24	12-02-25			0949	JP			
48 Termination	12-04-25			0951	JP			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	11-25-25
Age:	< 24-hours old
Date and time organisms were born between:	12-01-25 1245 to 12-02-25 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.18
	Temperature (°C): 25.0

Survival Data (number of living organisms):

Hours	Control				1000 mg/L				1500 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	4 ¹²	5	5
Mean Survival	100%				100%				97.5% (95%)			

Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	4 ¹²	4 ¹²	5	5	1 ⁴²	0 ⁵²	3 ²²	0 ⁵²	0 ⁵²	0 ⁵²	0 ⁵²	0 ⁵²
48 Termination	1 ³²	1 ³²	1 ⁴²	1 ⁴²	0 ¹²	0	0 ³²	0	0	0	0	0
Mean Survival	20%				0%				0%			

Comment codes: d = dead, u = unhealthy 12-04-25

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	1692.1
Upper 95% confidence limit (mg NaCl/L)	1926.7
48-hour LC ₅₀ (mg NaCl/L)	1812.6

Comments:





Statistical Analyses

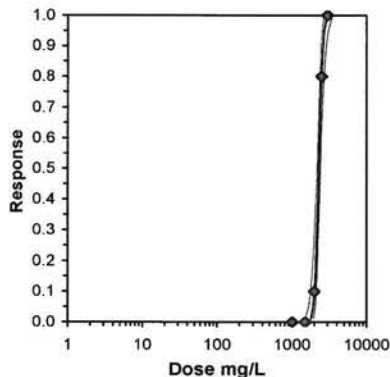
Acute Daphnid Test-24 Hr Survival					
Start Date:	12/2/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/4/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.8000	0.8000	1.0000	1.0000
2500	0.2000	0.0000	0.6000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

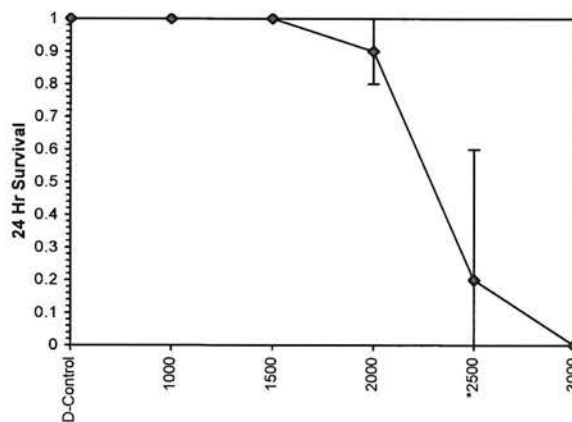
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9000	0.9000	1.2262	1.1071	1.3453	11.212	4	14.00	10.00	2	20
*2500	0.2000	0.2000	0.4502	0.2255	0.8861	69.198	4	10.00	10.00	16	20
3000	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 non-normal distribution (p <= 0.01)					0.75303	0.868	1.41974	5.51304
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					2000	2500	2236.07	
Treatments vs D-Control								

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	22.6262	4.65634	13.4997	31.7526	0	0.09798	7.81472	0.99208	3.35888	0.0442	3
Intercept	-70.999	15.6599	-101.69	-40.305							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	1803.3	1510.16	1957.31							
EC05	3.355	1932.8	1689.77	2064.44							
EC10	3.718	2005.6	1791.99	2126.41							
EC15	3.964	2056.26	1862.98	2170.95							
EC20	4.158	2097.43	1920.18	2208.43							
EC25	4.326	2133.41	1969.47	2242.43							
EC40	4.747	2226.83	2092.18	2338.46							
EC50	5.000	2284.99	2162.77	2405.83							
EC60	5.253	2344.67	2229.39	2482.18							
EC75	5.674	2447.34	2330.84	2630.07							
EC80	5.842	2489.33	2368.44	2695.63							
EC85	6.036	2539.17	2410.93	2776.54							
EC90	6.282	2603.31	2462.96	2884.75							
EC95	6.645	2701.36	2538.45	3057.4							
EC99	7.326	2895.35	2679.01	3418.93							



Dose-Response Plot





Statistical Analyses

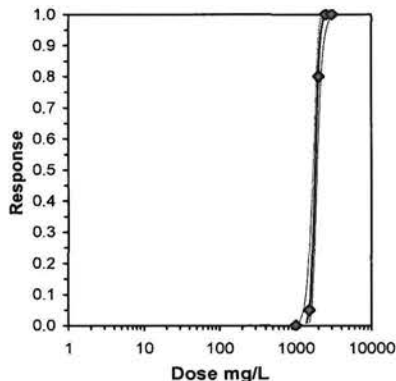
Acute Daphnid Test-48 Hr Survival					
Start Date:	12/2/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/4/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	0.8000	1.0000	1.0000
2000	0.2000	0.2000	0.2000	0.2000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

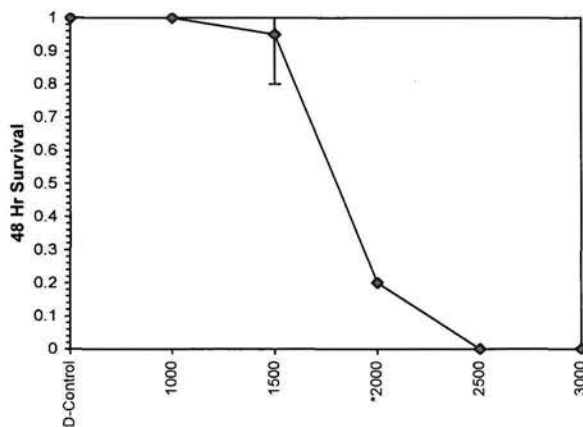
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2000	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	16	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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 non-normal distribution (p <= 0.01)					0.56485	0.844	-2.5555	9.36813
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					1500	2000	1732.05	
Treatments vs D-Control								

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	20.3134	4.27551	11.9335 28.6934	0	0.05546	7.81472	0.99658	3.25831	0.04923	3
Intercept	-61.187	13.9686	-88.566 -33.809							
TSCR										
Point	Probits	mg/L	95% Fiducial Limits							
EC01	2.674	1392.47	1125.92 1533.6							
EC05	3.355	1504.3	1278.33 1627.18							
EC10	3.718	1567.54	1366.07 1681.55							
EC15	3.964	1611.71	1427.45 1720.71							
EC20	4.158	1647.69	1477.18 1753.67							
EC25	4.326	1679.21	1520.28 1783.56							
EC40	4.747	1761.31	1628.74 1867.79							
EC50	5.000	1812.63	1692.08 1926.72							
EC60	5.253	1865.44	1752.55 1993.56							
EC75	5.674	1956.65	1845.42 2124.08							
EC80	5.842	1994.07	1879.89 2182.55							
EC85	6.036	2038.59	1918.76 2255.23							
EC90	6.282	2096.03	1966.22 2353.21							
EC95	6.645	2184.15	2034.82 2511.16							
EC99	7.326	2359.56	2162.1 2846.97							



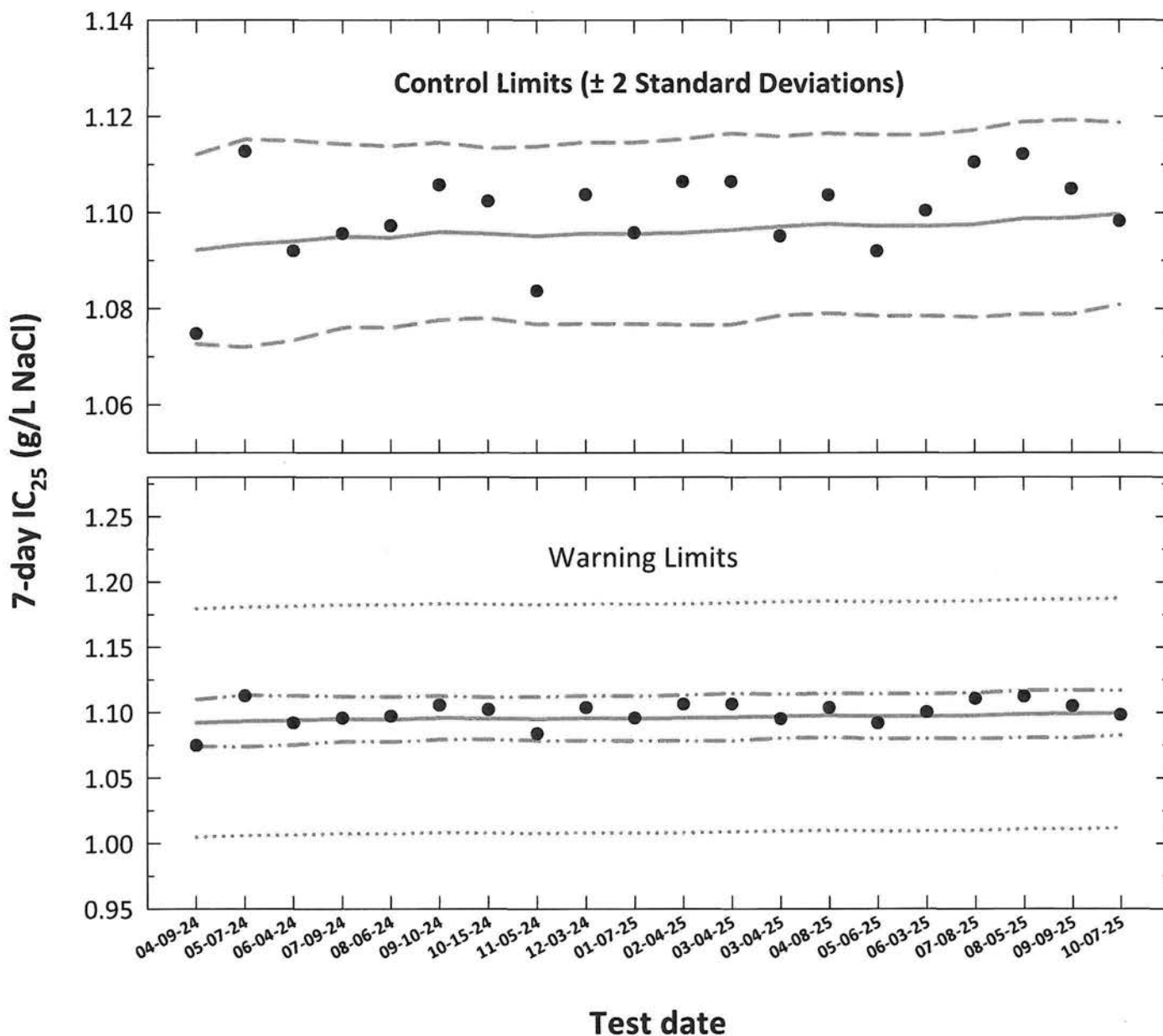
Dose-Response Plot



Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A,10}$ converted to anti-logarithmic values, $S_{A,10} = 10^{th}$ percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Warning Limits		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
2	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
3	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
4	07-09-24	1.0956	0.0396	0.0394	0.0038	1.0949	1.0760	1.1142	1.0777	1.1122	1.0073	1.1825
5	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
6	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
7	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
8	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
9	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
10	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
11	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
12	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
13	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
14	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
15	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
16	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
17	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
18	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
19	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
20	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

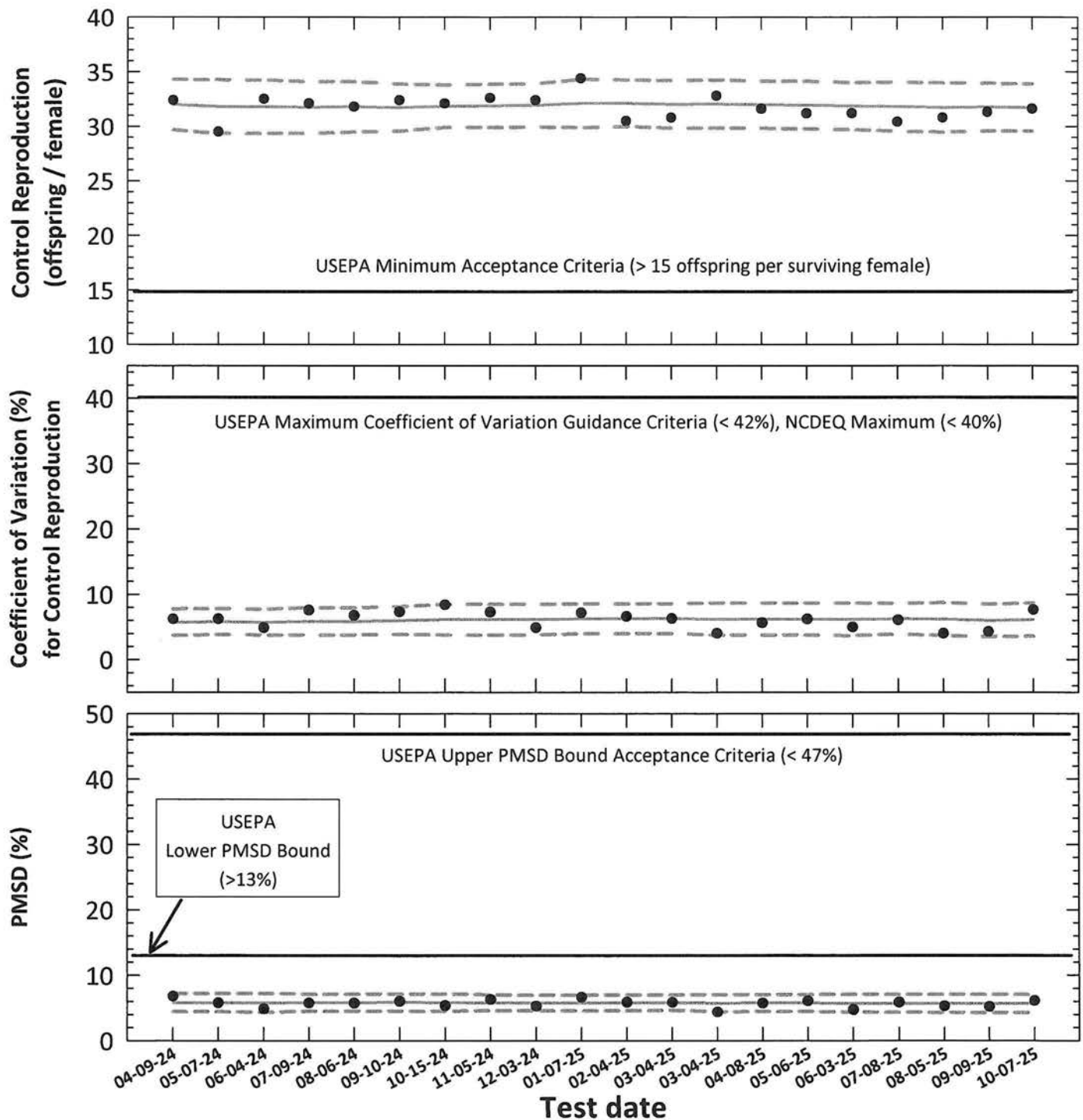
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

CV = Coefficient of variation.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria Organism Source: In-house Culture



- **Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Reproduction, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Reproduction, CV or PMSD $\pm$ 2 Standard Deviations)

Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction			Control Reproduction CV			Test PMSD		
		Control Survival (%)	Control Reproduction		Test		CT	(offspring/female)		CT	(%)		CT	(%)	
			Mean (offspring/female)	CV (%)	MSD	PMSD (%)		95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S		95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S			
1	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
2	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
3	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
4	07-09-24	100	32.1	7.6	1.846	5.8	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5	7.1
5	08-06-24	100	31.8	6.8	1.827	5.7	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
6	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
7	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
8	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
9	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
10	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
11	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
12	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
13	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
14	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
15	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
16	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
17	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
18	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
19	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
20	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.

USEPA maximum CV guidance criteria (90th percentile) $< 42\%$. NCDEQ maximum CV acceptance criteria $< 40\%$.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) $> 13\%$.

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) $< 47\%$.

CT = Central tendency of the reproduction, CV or PMSD values.

S = Standard deviation of the reproduction, CV or PMSD values.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)

Species: Ceriodaphnia dubia

CdNaClCR #: 316

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2911</u>				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	<u>10-07-25 0530 to 0710</u>									
Culture board:	<u>09-30-25 A</u>									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	<u>1</u>	<u>2</u>	<u>8</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>17</u>	<u>19</u>	<u>22</u>	<u>23</u>
Transfer vessel information:	pH (S.U.): <u>7.67</u> Temperature (°C): <u>25.0</u>									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	<u>RED</u>
Incubator number and shelf location:	<u>2 B1</u>

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches <i>Selenastrum</i>	YWT	MHSW batch used	Analyst
0	10-07-25	<u>0730</u>	<u>09-25-25</u>	<u>09-25-25</u>	<u>09-29-25 A</u>	<u>JK</u>
1	10-08-25	<u>0620</u>	↓	↓	↓	<u>JK</u>
2	10-09-25	<u>0700</u>	↓	↓	<u>10-06-25 A</u>	<u>JK</u>
3	10-10-25	<u>0600</u>	↓	↓	↓	<u>JK</u>
4	10-11-25	<u>0700</u>	↓	↓	<u>10-06-25 B</u>	<u>JK</u>
5	10-12-25	<u>0614</u>	↓	↓	↓	<u>JK</u>
6	10-13-25	<u>0710</u>	↓	↓	↓	<u>JK</u>
7	10-14-25	<u>0600</u>				<u>JK</u>

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	<u>01.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>71400</u>
% Adults having 3 rd Broods:	<u>1001.</u>	≥ 80%	NOEC (mg/L NaCl)	<u>1000</u>
% Mortality:	<u>01.</u>	≤ 20%	LOEC (mg/L NaCl)	<u>1200</u>
Mean Offspring/Female:	<u>31.6</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>7.67.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1098.2</u>

Species: *Ceriodaphnia dubia*

CdNaClCR #: 316

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	3	4	4	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	10	10	13	12	12	11	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	16	15	18	15	18	16	16	16
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		33	28	31	28	35	31	35	32	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality: 07

Mean Offspring/Female: 31.6

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	5	5	4	4	4	6	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	12	10	13	12	11	12	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	19	14	17	18	17	19	15	16	18
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		32	34	31	32	35	33	34	33	30	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality: 07

Mean Offspring/Female: 32.7

% Reduction from Control: -3.57

Species: *Ceriodaphnia dubia*

CdNaClCR #: 316

800 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	5	6	4	4	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	10	13	11	13	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	14	16	17	19	16	16	16	15	19
Total young produced		31	31	33	33	36	31	33	29	28	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality: 0%

Mean Offspring/Female: 31.8

% Reduction from Control: -0.67%

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	4	4	4	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	10	10	10	11	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	14	12	16	15	19	15	13	17	17
Total young produced		32	31	26	30	29	33	30	26	32	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality: 0%

Mean Offspring/Female: 30.2

% Reduction from Control: 4.47%

Species: Ceriodaphnia dubia

CdNaClCR #: 316

1200 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	2	4	3	3	3	4	2	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	10	9	7	11	8	10	9	7	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	5	6	7	4	6	4	5	10	9
Total young produced		17	19	17	18	18	17	17	18	19	18
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	07.
Mean Offspring/Female:	17.8
% Reduction from Control:	43.77.

1400 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	1	1	1	3	1	2	2	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	1	0	0	0	0	0	0	0	0	0
Total young produced		3	1	1	1	3	1	2	2	1	2
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	07.
Mean Offspring/Female:	1.77
% Reduction from Control:	94.67

10-24-25 M
4.4-9

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	3	4	4	5	5	4	4	43
5	13	10	10	10	13	12	12	11	10	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	16	15	18	15	18	16	16	16	160
Total	33	28	31	28	35	31	35	32	31	32	316

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	5	5	4	4	4	6	4	5	46
5	13	10	12	10	13	12	11	12	10	10	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	19	14	17	18	17	19	15	16	18	168
Total	32	34	31	32	35	33	34	33	30	33	327

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	5	6	4	4	4	3	3	4	42
5	11	12	12	10	13	11	13	10	10	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	16	17	19	16	16	16	15	19	164
Total	31	31	33	33	36	31	33	29	28	33	318

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	4	4	4	4	3	3	4	39
5	12	13	10	10	10	10	11	10	12	12	110
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	12	16	15	19	15	13	17	17	153
Total	32	31	26	30	29	33	30	26	32	33	302

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	2	4	3	3	3	4	2	2	30
5	9	10	9	7	11	8	10	9	7	7	87
6	0	0	0	0	0	0	0	0	0	0	0
7	5	5	6	7	4	6	4	5	10	9	61
Total	17	19	17	18	18	17	17	18	19	18	178

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	1	1	3	1	2	2	1	2	16
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	1	0	0	0	0	0	0	0	0	0	1
Total	3	1	1	1	3	1	2	2	1	2	17

Reviewed and
Approved by
Jim Sumner

JS

***Ceriodaphnia dubia* Chronic Reference Toxicant Test**
EPA-821-R-02-013, Method 1002.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 316
Test dates: October 07-14, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	33	28	31	28	35	31	35	32	31	32	100	31.6	7.6	Not applicable
600	32	34	31	32	35	33	34	33	30	33	100	32.7	4.6	-3.5
800	31	31	33	33	36	31	33	29	28	33	100	31.8	7.2	-0.6
1000	32	31	26	30	29	33	30	26	32	33	100	30.2	8.5	4.4
1200	17	19	17	18	18	17	17	18	19	18	100	17.8	4.4	43.7
1400	3	1	1	1	3	1	2	2	1	2	100	1.7	48.4	94.6

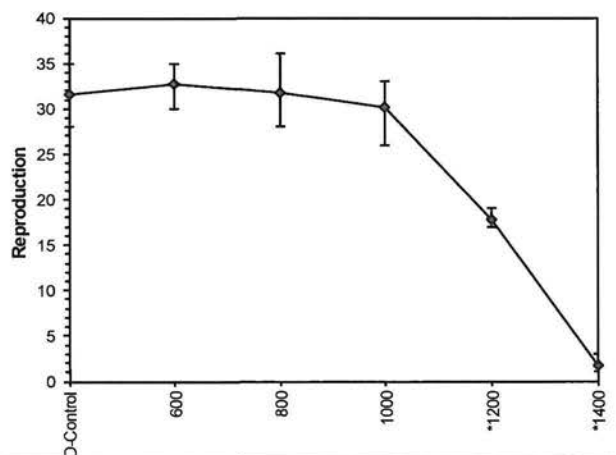
Dunnett's MSD value: 1.925
PMDS: 6.1

MSD = Minimum Significant Difference
PMDS = Percent Minimum Significant Difference

PMDS is a measure of test precision. The PMDS is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMDS bound determined by USEPA (10th percentile) = 13%.
Upper PMDS bound determined by USEPA (90th percentile) = 47%.
Lower and upper PMDS bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

USEPA, 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.





Statistical Analyses



Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction										
Start Date:	10/7/2025	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant					
End Date:	10/14/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride					
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia					
Comments:										
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	28.000	31.000	28.000	35.000	31.000	35.000	32.000	31.000	32.000
600	32.000	34.000	31.000	32.000	35.000	33.000	34.000	33.000	30.000	33.000
800	31.000	31.000	33.000	33.000	36.000	31.000	33.000	29.000	28.000	33.000
1000	32.000	31.000	26.000	30.000	29.000	33.000	30.000	26.000	32.000	33.000
1200	17.000	19.000	17.000	18.000	18.000	17.000	17.000	18.000	19.000	18.000
1400	3.000	1.000	1.000	1.000	3.000	1.000	2.000	2.000	1.000	2.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed	
			Mean	Min	Max	CV%			Critical	MSD
D-Control	31.600	1.0000	31.600	28.000	35.000	7.636	10			
600	32.700	1.0348	32.700	30.000	35.000	4.570	10	-1.306	2.287	1.925
800	31.800	1.0063	31.800	28.000	36.000	7.232	10	-0.238	2.287	1.925
1000	30.200	0.9557	30.200	26.000	33.000	8.521	10	1.663	2.287	1.925
*1200	17.800	0.5633	17.800	17.000	19.000	4.432	10	16.390	2.287	1.925
*1400	1.700	0.0538	1.700	1.000	3.000	48.428	10	35.513	2.287	1.925

Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)					1.40015	1.035	-0.3244	0.54145			
Bartlett's Test indicates unequal variances (p = 1.18E-03)					20.1399	15.0863					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		1000	1200	1095.45		1.92527	0.06093	1535.84	3.54444	2.9E-42	5, 54
Treatments vs D-Control											

Dose-Response Plot

1-tail, 0.05 level of significance

Conc-mg/L	Mean Reproduction	Min	Max
D-Control	31.600	28.000	35.000
600	32.700	30.000	35.000
800	31.800	28.000	36.000
1000	30.200	26.000	33.000
*1200	17.800	17.000	19.000
*1400	1.700	1.000	3.000

Species: *Ceriodaphnia dubia*

CdNaClCR #: 316

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.79	7.84	7.74	7.85	7.75	7.85
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.1	8.5	8.0
	Conductivity (µmhos/cm)	299		308		293	
	Alkalinity (mg CaCO ₃ /L)	63				62	
	Hardness (mg CaCO ₃ /L)	86				88	
	Temperature (°C)	24.8	25.2	24.8	25.0	24.8	24.9
600 mg NaCl/L	pH (S.U.)	7.89	7.87	7.85	7.87	7.89	7.90
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.2	8.4	8.0
	Conductivity (µmhos/cm)	1470		1420		1400	
	Temperature (°C)	24.9	25.3	24.9	24.9	24.9	25.1
800 mg NaCl/L	pH (S.U.)	7.91	7.88	7.86	7.88	7.91	7.90
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.1	8.4	8.0
	Conductivity (µmhos/cm)	1830		1790		1770	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.92	7.87	7.88	7.89	7.92	7.90
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.1	8.4	8.0
	Conductivity (µmhos/cm)	2180		2160		2140	
	Temperature (°C)	24.9	25.2	25.0	25.1	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.92	7.87	7.87	7.89	7.93	7.91
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.1	8.4	8.0
	Conductivity (µmhos/cm)	2430		2530		2490	
	Temperature (°C)	25.0	25.2	24.8	24.8	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.90	7.86	7.89	7.88	7.94	7.91
	Dissolved oxygen (mg/L)	8.5	8.0	8.3	8.1	8.4	8.0
	Conductivity (µmhos/cm)	3100		2870		2830	
	Temperature (°C)	24.9	25.0	24.8	24.8	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final

Species: *Ceriodaphnia dubia*

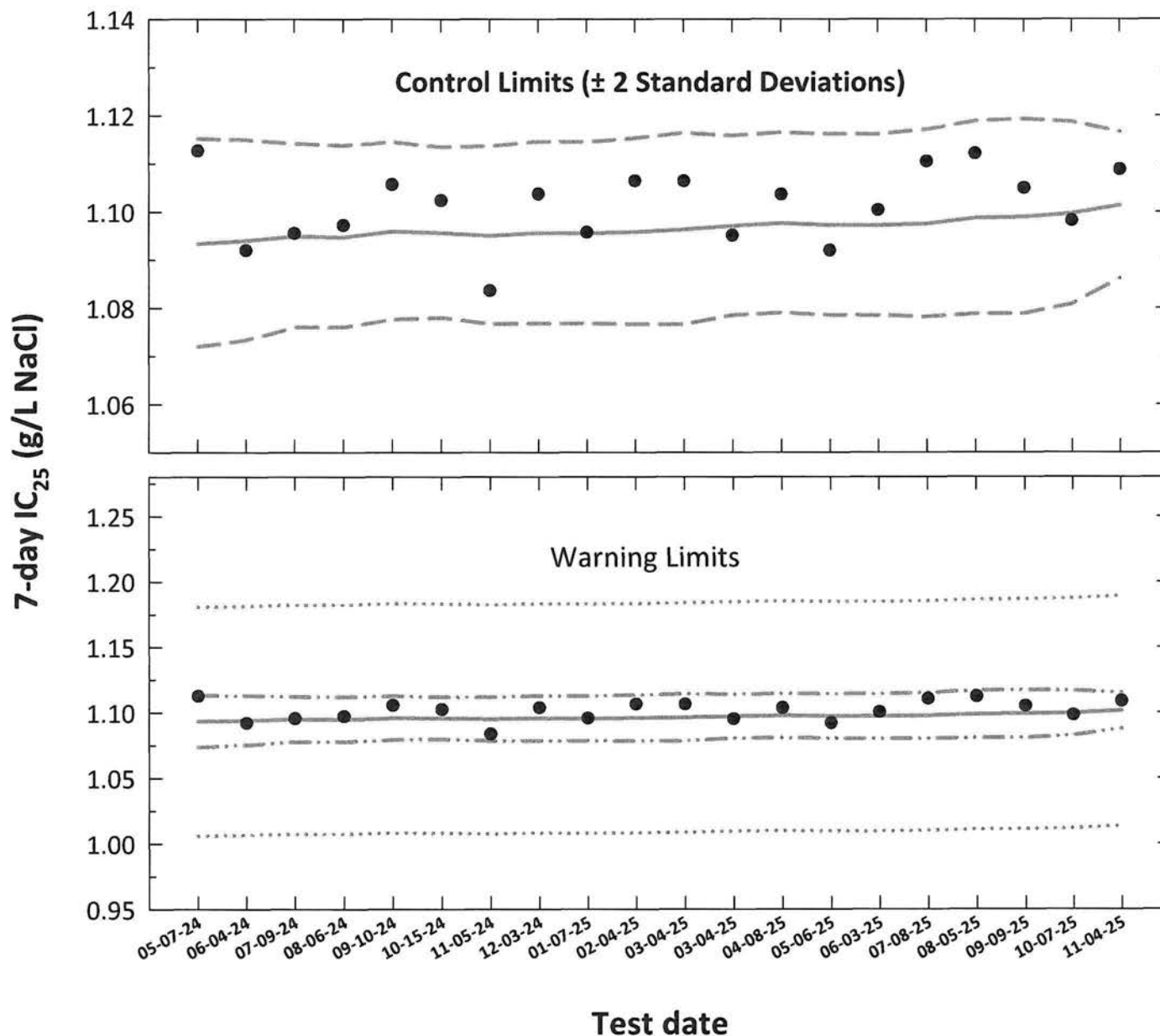
CdNaClCR #: 316

		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Analyst		XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.84	7.96	7.83	7.95	7.85	7.86	7.85	7.80
	Dissolved oxygen (mg/L)	8.4	8.2	8.6	8.0	8.4	8.1	8.3	7.9
	Conductivity (µmhos/cm)	297		288		292		306	
	Alkalinity (mg CaCO ₃ /L)			63					
	Hardness (mg CaCO ₃ /L)			86					
	Temperature (°C)	24.8	25.1	24.7	24.8	24.8	25.1	24.9	25.0
600 mg NaCl/L	pH (S.U.)	7.90	8.00	7.93	8.02	7.91	7.93	7.93	7.81
	Dissolved oxygen (mg/L)	8.4	8.2	8.4	8.1	8.3	8.1	8.4	7.9
	Conductivity (µmhos/cm)	1390		1360		1380		1420	
	Temperature (°C)	24.9	24.8	24.8	25.1	24.9	24.9	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.92	8.00	8.00	8.02	7.99	7.94	7.96	7.85
	Dissolved oxygen (mg/L)	8.4	8.2	8.4	8.1	8.4	8.1	8.4	7.9
	Conductivity (µmhos/cm)	1780		1710		1750		1830	
	Temperature (°C)	24.8	24.8	24.8	24.9	24.9	24.9	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.93	8.00	8.01	8.02	8.00	7.94	7.97	7.86
	Dissolved oxygen (mg/L)	8.4	8.2	8.4	8.1	8.4	8.1	8.4	7.9
	Conductivity (µmhos/cm)	2100		2040		2090		2170	
	Temperature (°C)	24.8	24.8	24.9	24.9	25.0	25.0	24.8	24.9
1200 mg NaCl/L	pH (S.U.)	7.94	8.00	8.01	8.02	8.02	7.95	7.98	7.80
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.4	8.1	8.4	7.9
	Conductivity (µmhos/cm)	2460		2400		2450		2570	
	Temperature (°C)	24.8	24.9	24.8	25.0	25.0	24.8	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.95	8.01	8.00	8.03	8.02	7.95	7.99	7.90
	Dissolved oxygen (mg/L)	8.4	8.2	8.4	8.1	8.4	8.1	8.4	7.9
	Conductivity (µmhos/cm)	2790		2740		2780		2930	
	Temperature (°C)	24.9	24.9	24.8	25.0	25.0	24.8	24.9	24.9
Initial			Final	Initial	Final	Initial	Final	Initial	Final

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10} = 10^{\text{th}}$ percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		CT	Anti-logarithmic Values (g/L NaCl)		10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}
			7-day IC ₂₅	CT		Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	
1	05-07-24	1.1127	0.0464	0.0388	1.0934	1.0720 1.1152	1.0738 1.1130	1.0059 1.1809
2	06-04-24	1.0920	0.0382	0.0390	1.0940	1.0734 1.1150	1.0752 1.1128	1.0065 1.1815
3	07-09-24	1.0956	0.0396	0.0394	1.0949	1.0760 1.1142	1.0777 1.1122	1.0073 1.1825
4	08-06-24	1.0972	0.0403	0.0393	1.0947	1.0760 1.1138	1.0776 1.1119	1.0072 1.1823
5	09-10-24	1.1057	0.0436	0.0398	1.0960	1.0777 1.1146	1.0793 1.1126	1.0083 1.1836
6	10-15-24	1.1024	0.0423	0.0397	1.0956	1.0781 1.1135	1.0796 1.1116	1.0080 1.1833
7	11-05-24	1.0837	0.0349	0.0394	1.0951	1.0767 1.1137	1.0783 1.1118	1.0075 1.1827
8	12-03-24	1.1037	0.0428	0.0396	1.0956	1.0769 1.1146	1.0785 1.1126	1.0079 1.1832
9	01-07-25	1.0958	0.0397	0.0396	1.0955	1.0769 1.1145	1.0785 1.1126	1.0079 1.1832
10	02-04-25	1.1064	0.0439	0.0397	1.0958	1.0767 1.1153	1.0783 1.1133	1.0082 1.1835
11	03-04-25	1.1064	0.0439	0.0400	1.0964	1.0767 1.1165	1.0784 1.1144	1.0087 1.1841
12	03-04-25	1.0951	0.0394	0.0402	1.0971	1.0786 1.1159	1.0802 1.1139	1.0093 1.1848
13	04-08-25	1.1036	0.0428	0.0405	1.0976	1.0791 1.1165	1.0807 1.1145	1.0098 1.1854
14	05-06-25	1.0920	0.0382	0.0403	1.0972	1.0785 1.1162	1.0802 1.1142	1.0094 1.1850
15	06-03-25	1.1004	0.0416	0.0403	1.0972	1.0785 1.1162	1.0802 1.1142	1.0094 1.1850
16	07-08-25	1.1104	0.0455	0.0404	1.0975	1.0782 1.1171	1.0799 1.1151	1.0097 1.1853
17	08-05-25	1.1121	0.0462	0.0409	1.0987	1.0788 1.1189	1.0806 1.1167	1.0108 1.1866
18	09-09-25	1.1049	0.0433	0.0410	1.0989	1.0789 1.1193	1.0807 1.1171	1.0110 1.1868
19	10-07-25	1.0982	0.0407	0.0412	1.0996	1.0808 1.1187	1.0825 1.1167	1.0116 1.1876
20	11-04-25	1.1088	0.0448	0.0419	1.1013	1.0862 1.1166	1.0876 1.1150	1.0132 1.1894

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

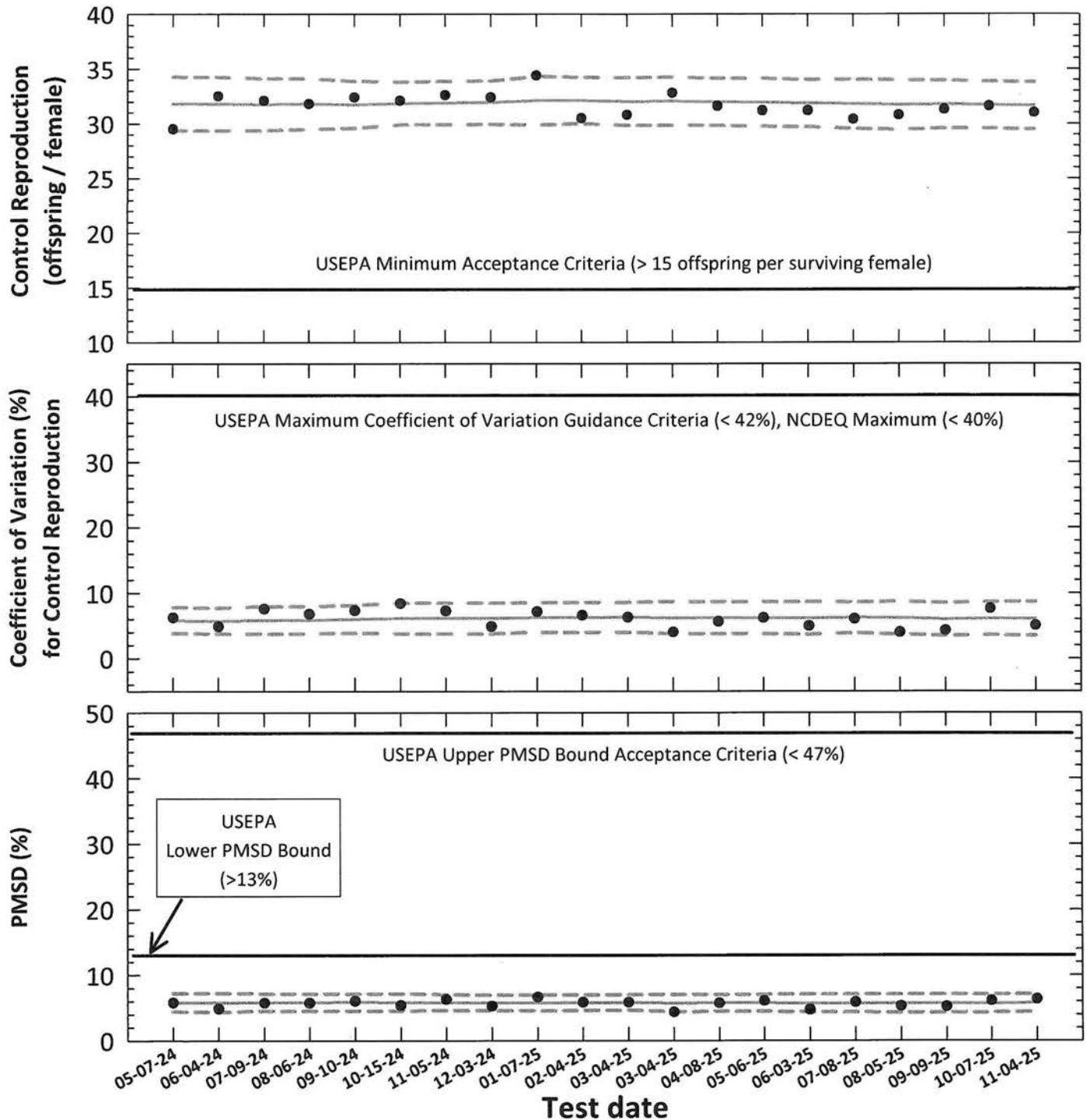
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

CV = Coefficient of variation.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Reproduction, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Reproduction, CV or PMSD $\pm$ 2 Standard Deviations)

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Ceriodaphnia dubia

Source: In-house Culture

Test number	Test date	ToxCal Determination				Control Reproduction			Control Reproduction CV			Test PMSD	
		Control Survival (%)	Control Reproduction		Test	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval
			Mean (offspring/female)	CV (%)			CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S
1	05-07-24	100	29.5	6.2	1.707	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4
2	06-04-24	100	32.5	4.9	1.587	31.8	29.3	34.2	5.8	3.8	7.7	5.8	4.3
3	07-09-24	100	32.1	7.6	1.846	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5
4	08-06-24	100	31.8	6.8	1.827	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5
5	09-10-24	100	32.4	7.3	1.947	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5
6	10-15-24	100	32.1	8.4	1.715	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5
7	11-05-24	100	32.6	7.3	2.050	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6
8	12-03-24	100	32.4	4.9	1.708	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5
9	01-07-25	100	34.4	7.1	2.280	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5
10	02-04-25	100	30.5	6.6	1.782	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6
11	03-04-25	100	30.8	6.3	1.797	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6
12	03-04-25	100	32.8	4.0	1.430	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4
13	04-08-25	100	31.6	5.6	1.806	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4
14	05-06-25	100	31.2	6.2	1.889	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4
15	06-03-25	100	31.2	5.0	1.470	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3
16	07-08-25	100	30.4	6.0	1.776	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3
17	08-05-25	100	30.8	4.0	1.623	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3
18	09-09-25	100	31.3	4.3	1.622	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3
19	10-07-25	100	31.6	7.6	1.925	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3
20	11-04-25	100	31.0	5.0	1.966	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4

Note: Control Survival = USEPA minimum test acceptability criteria $\geq$ 80% survival.

Control Mean Reproduction = USEPA minimum test acceptability criteria $\geq$ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.

USEPA maximum CV guidance criteria (90th percentile) $<$ 42%. NCDEQ maximum CV acceptance criteria $<$ 40%.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) $>$ 13%.

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Upper PMSD bound determined by USEPA (90th percentile) $<$ 47%.

CT = Central tendency of the reproduction, CV or PMSD values.

S = Standard deviation of the reproduction, CV or PMSD values.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)

Species: *Ceriodaphnia dubia*

CdNaClCR #: 317

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS 2411				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	11-04-25 0515 TO 0745									
Culture board:	10-28-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	3	5	11	12	13	18	22	23	30	33
Transfer vessel information:	pH (S.U.): 7.85 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	RED
Incubator number and shelf location:	2B1

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches <i>Selenastrum</i>	YWT	MHSW batch used	Analyst
0	11-04-25	0757	10-29-25	10-30-25	10-28-25B	J
1	11-05-25	0630	↓	↓	↓	J
2	11-06-25	0644	↓	↓	10-28-25 C	J
3	11-07-25	0610	↓	↓	↓	J
4	11-08-25	0740	↓	↓	11-06-25 A	J
5	11-09-25	0645	↓	↓	↓	J
6	11-10-25	0702	↓	↓	↓	J
7	11-11-25	0614				J

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	0%.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	> 1400
% Adults having 3 rd Broods:	100%.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	0%.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	31.0	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.4
% CV:	5.0%.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1180.3

Species: *Ceriodaphnia dubia*

CdNaClCR #: 317

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	5	5	4	4	4	3	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	13	12	12	9	12	11	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	16	14	16	15	18	13	18	14	14
Total young produced		30	31	32	32	31	31	28	34	31	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality: 0%

Mean Offspring/Female: 31.0

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	4	4	5	4	4	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	12	13	12	11	13	11	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	16	13	17	16	15	15	18	13	15
Total young produced		31	33	29	34	35	30	32	33	29	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality: 0%

Mean Offspring/Female: 31.7

% Reduction from Control: -2.3%

Species: *Ceriodaphnia dubia*

CdNaClCR #: 317

800 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	6	4	5	5	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	11	12	12	13	10	12	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	19	16	17	13	17	14	14	14	18
Total young produced		29	34	33	33	30	33	28	30	32	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	0%
Mean Offspring/Female:	31.6
% Reduction from Control:	-1.9%

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	5	4	4	4	5	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	9	12	12	11	12	11	13	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	13	15	14	16	13	15	14	15	15
Total young produced		31	26	29	31	32	26	31	35	31	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	0%
Mean Offspring/Female:	30.7
% Reduction from Control:	1.0%

Species: *Ceriodaphnia dubia*

CdNaClCR #: 317

1200 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	4	4	5	3	3	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	7	10	5	6	11	10	10	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	4	4	9	6	5	3	3	10	8
Total young produced		19	14	18	18	17	19	16	17	20	18
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	0%
Mean Offspring/Female:	17.6
% Reduction from Control:	43.2%

1400 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	0	0	2	1	2	1	3	1	1	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	2	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	1	0	0	0	0	0	0	0
Total young produced		2	0	3	1	2	1	3	1	1	1
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	0%
Mean Offspring/Female:	1.5
% Reduction from Control:	95.2%

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	5	5	4	4	4	3	5	5	4	42
5	12	10	13	12	12	9	12	11	12	12	115
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	14	16	15	18	13	18	14	14	153
Total	30	31	32	32	31	31	28	34	31	30	310

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	5	4	4	4	5	3	5	43
5	13	10	9	12	12	11	12	11	13	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	13	13	15	14	16	13	15	19	15	15	148
Total	31	26	29	31	32	28	31	35	31	33	307

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	4	5	4	4	4	4	6	45
5	12	12	13	12	11	13	11	12	10	118	118
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	13	17	18	15	15	18	13	15	154
Total	31	33	29	34	35	30	32	33	29	31	317

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	4	4	5	3	3	4	4	4	39
5	9	7	10	5	6	11	10	10	6	6	80
6	0	0	0	0	0	0	0	0	0	0	0
7	5	4	4	9	6	5	3	3	10	8	57
Total	19	14	18	18	17	19	16	17	20	18	176

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	6	4	5	5	4	4	6	4	46
5	12	11	11	12	12	13	10	12	12	10	115
6	0	0	0	0	0	0	0	0	0	0	0
7	13	19	16	17	13	17	14	14	14	18	155
Total	29	34	33	33	30	35	28	30	32	32	316

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	2	1	2	1	3	1	1	1	12
5	2	0	0	0	0	0	0	0	0	0	2
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	1	0	0	0	0	0	0	0	1
Total	2	0	3	1	2	1	3	1	1	1	15

***Ceriodaphnia dubia* Chronic Reference Toxicant Test**
EPA-821-R-02-013, Method 1002.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 317
Test dates: November 04-11, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	30	31	32	32	31	31	28	34	31	30	100	31.0	5.0	Not applicable
600	31	33	29	34	35	30	32	33	29	31	100	31.7	6.5	-2.3
800	29	34	33	33	30	35	28	30	32	32	100	31.6	7.2	-1.9
1000	31	26	29	31	32	28	31	35	31	33	100	30.7	8.3	1.0
1200	19	14	18	18	17	19	16	17	20	18	100	17.6	9.7	43.2
1400	2	0	3	1	2	1	3	1	1	1	100	1.5	64.8	95.2

Dunnett's MSD value: $\frac{1.966}{6.3}$ MSD = Minimum Significant Difference
 PMSD: PMSD = Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
 Lower PMSD bound determined by USEPA (10th percentile) = 13%.
 Upper PMSD bound determined by USEPA (90th percentile) = 47%.
 Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
 USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 11/4/2025 Test ID: CdNaCICR Sample ID: REF-Ref Toxicant
End Date: 11/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
Comments:

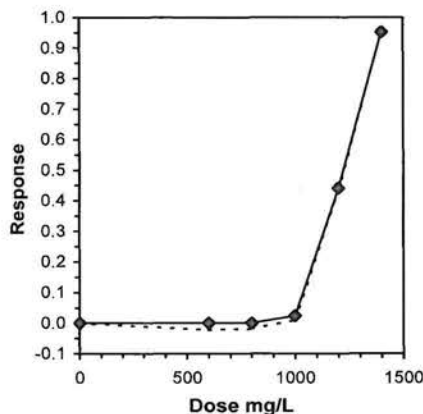
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	31.000	32.000	32.000	31.000	31.000	28.000	34.000	31.000	30.000
600	31.000	33.000	29.000	34.000	35.000	30.000	32.000	33.000	29.000	31.000
800	29.000	34.000	33.000	33.000	30.000	35.000	28.000	30.000	32.000	32.000
1000	31.000	26.000	29.000	31.000	32.000	28.000	31.000	35.000	31.000	33.000
1200	19.000	14.000	18.000	18.000	17.000	19.000	16.000	17.000	20.000	18.000
1400	2.000	0.000	3.000	1.000	2.000	1.000	3.000	1.000	1.000	1.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean
D-Control	31.000	1.0000	31.000	28.000	34.000	5.043	10				31.433	1.0000
600	31.700	1.0226	31.700	29.000	35.000	6.491	10	-0.814	2.287	1.966	31.433	1.0000
800	31.600	1.0194	31.600	28.000	35.000	7.185	10	-0.698	2.287	1.966	31.433	1.0000
1000	30.700	0.9903	30.700	26.000	35.000	8.276	10	0.349	2.287	1.966	30.700	0.9767
*1200	17.600	0.5677	17.600	14.000	20.000	9.731	10	15.589	2.287	1.966	17.600	0.5599
*1400	1.500	0.0484	1.500	0.000	3.000	64.788	10	34.319	2.287	1.966	1.500	0.0477

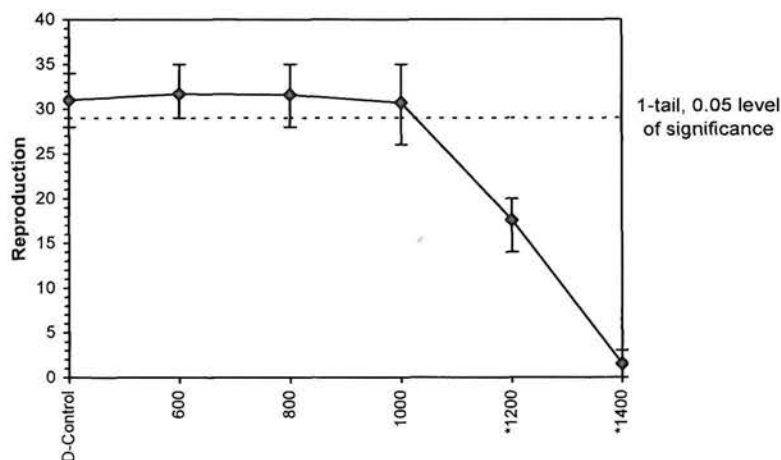
Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)					0.66733	1.035	-0.1953	0.10424						
Bartlett's Test indicates equal variances (p = 0.13)					8.4562	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					1000	1200	1095.45		1.96559	0.06341	1516.3	3.69444	1.2E-41	5, 54
Treatments vs D-Control														

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	1012.8	36.5257	899.258 1022.98	-3.8794
IC10	1036.79	9.04202	1012.86 1046.43	-0.6996
IC15	1060.79	8.08471	1038.68 1071.26	-0.5780
IC20	1084.78	7.35603	1065.69 1095.18	-0.4222
IC25	1108.78	6.92849	1091.45 1118.99	-0.2608
IC40	1180.76	7.7465	1165.67 1194.85	-0.0337
IC50	1223.4	6.08159	1211.02 1233.15	-0.3330



Dose-Response Plot



Species: *Ceriodaphnia dubia*

CdNaClCR #: 317

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		Analyst					
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.80	7.80	7.75	7.84	7.87	7.88
	Dissolved oxygen (mg/L)	8.5	8.1	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	299		300		299	
	Alkalinity (mg CaCO ₃ /L)	63				63	
	Hardness (mg CaCO ₃ /L)	91				89	
	Temperature (°C)	24.9	25.3	24.8	25.2	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.88	7.85	7.87	7.85	7.94	7.93
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	1390		1410		1400	
	Temperature (°C)	24.9	25.0	24.9	25.3	24.9	24.8
800 mg NaCl/L	pH (S.U.)	7.89	7.85	7.87	7.88	7.94	7.94
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	1730		1780		1780	
	Temperature (°C)	25.0	25.2	24.9	25.3	25.0	24.8
1000 mg NaCl/L	pH (S.U.)	7.90	7.84	7.88	7.87	7.95	7.96
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	2080		2130		2150	
	Temperature (°C)	24.9	25.2	25.0	25.0	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.90	7.84	7.89	7.87	7.96	7.96
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	2380		2470		2460	
	Temperature (°C)	25.0	25.2	24.8	25.0	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.90	7.84	7.89	7.88	7.96	7.95
	Dissolved oxygen (mg/L)	8.5	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	2780		2830		2850	
	Temperature (°C)	25.0	25.3	24.8	25.2	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

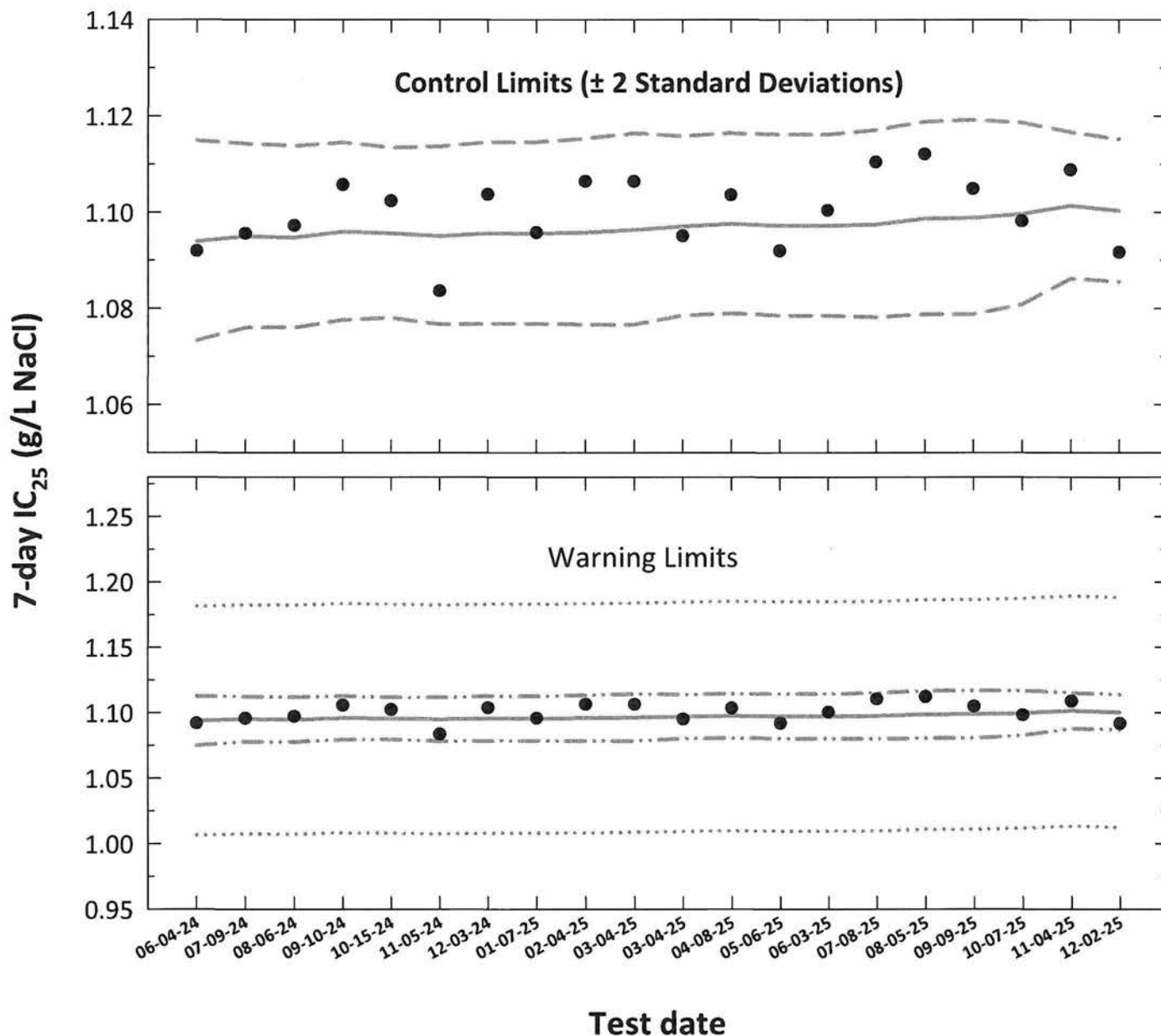
Species: *Ceriodaphnia dubia*

CdNaClCR #: 317

		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	XL	BSL	BSL	BSL	BSL	XL	XL	XL
CONTROL, MHSW	pH (S.U.)	7.85	7.96	7.85 (7.70)	8.00	7.78	7.90	7.73	8.13
	Dissolved oxygen (mg/L)	8.3	8.1	8.5	8.0	8.4	7.9	8.3	7.6
	Conductivity (µmhos/cm)	300		285		283		289	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			87					
	Temperature (°C)	24.9	25.5	24.7	25.0	24.8	25.3	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.95	8.00	7.94 (7.83)	8.02	7.98	7.95	7.93	8.10
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.0	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1390		1310		1300		1290	
	Temperature (°C)	25.0	25.0	24.9	25.1	24.9	25.1	24.8	25.0
800 mg NaCl/L	pH (S.U.)	7.95	8.01	7.94 (7.91)	8.02	8.00	7.96	7.95	8.10
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.1	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1780		1680		1680		1680	
	Temperature (°C)	24.9	25.0	24.9	25.1	24.9	25.1	24.8	25.0
1000 mg NaCl/L	pH (S.U.)	7.96	8.02	7.95 (7.94)	8.02	8.01	7.96	7.96	8.11
	Dissolved oxygen (mg/L)	8.2	8.1	8.3	8.0	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	2140		2020		2020		2050	
	Temperature (°C)	24.9	25.0	24.8	25.2	25.0	25.1	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.97	8.01	7.96	8.00	8.01	7.97	7.97	8.09
	Dissolved oxygen (mg/L)	8.2	8.1	8.3	8.0	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	2480		2370 BSL 2370-11.880		2370		2390	
	Temperature (°C)	25.1	25.2	24.8	25.2	25.0	24.9	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	7.97	8.03	7.97	8.00	8.00	7.97	7.98	8.03
	Dissolved oxygen (mg/L)	8.2	8.1	8.3	8.0	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	2840		2740		2710		2750	
	Temperature (°C)	24.8	24.9	24.9	25.2	25.0	24.9	24.9	25.3
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

+BSL 11.08.25 verified current

Ceriodaphnia dubia Chronic Reference Toxicant Control Chart Source: In-house Culture



- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A,10}$ converted to anti-logarithmic values, $S_{A,10} = 10^{th}$ percentile of CVs reported nationally by USEPA)

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Warning Limits		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
2	07-09-24	1.0956	0.0396	0.0394	0.0038	1.0949	1.0760	1.1142	1.0777	1.1122	1.0073	1.1825
3	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
4	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
5	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
6	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
7	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
8	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
9	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
10	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
11	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
12	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
13	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
14	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
15	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
16	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
17	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
18	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
19	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
20	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

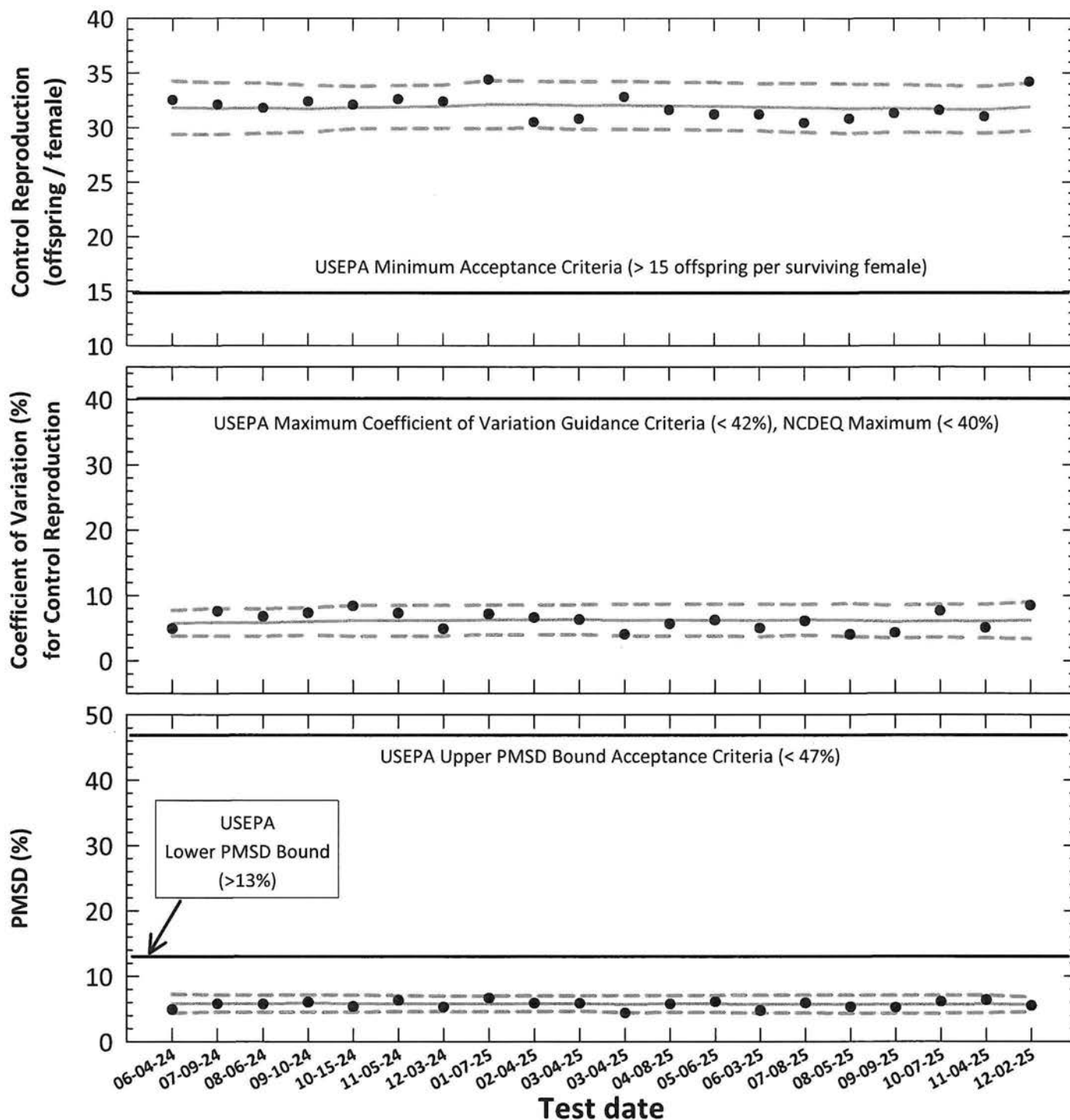
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

CV = Coefficient of variation.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Reproduction, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Reproduction, CV or PMSD $\pm$ 2 Standard Deviations)

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Ceriodaphnia dubia

Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction		Control Reproduction CV		Test PMSD			
		Control Survival (%)	Control Reproduction		Test	CT	(offspring/female)		CT	(%)		CT	(%)	
			Mean (offspring/female)	CV (%)			MSD	PMSD (%)		95% Confidence Interval CT - 2S	CT + 2S		95% Confidence Interval CT - 2S	CT + 2S
1	06-04-24	100	32.5	4.9	1.587	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
2	07-09-24	100	32.1	7.6	1.846	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5	7.1
3	08-06-24	100	31.8	6.8	1.827	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
4	09-10-24	100	32.4	7.3	1.947	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
5	10-15-24	100	32.1	8.4	1.715	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
6	11-05-24	100	32.6	7.3	2.050	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
7	12-03-24	100	32.4	4.9	1.708	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
8	01-07-25	100	34.4	7.1	2.280	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
9	02-04-25	100	30.5	6.6	1.782	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
10	03-04-25	100	30.8	6.3	1.797	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
11	03-04-25	100	32.8	4.0	1.430	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
12	04-08-25	100	31.6	5.6	1.806	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
13	05-06-25	100	31.2	6.2	1.889	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
14	06-03-25	100	31.2	5.0	1.470	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
15	07-08-25	100	30.4	6.0	1.776	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
16	08-05-25	100	30.8	4.0	1.623	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
17	09-09-25	100	31.3	4.3	1.622	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
18	10-07-25	100	31.6	7.6	1.925	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
19	11-04-25	100	31.0	5.0	1.966	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
20	12-02-25	100	34.2	8.5	1.866	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.

USEPA maximum CV guidance criteria (90th percentile) $< 42\%$. NCDEQ maximum CV acceptance criteria $< 40\%$.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) $> 13\%$.

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) $< 47\%$.

CT = Central tendency of the reproduction, CV or PMSD values.

S = Standard deviation of the reproduction, CV or PMSD values.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)
Species: Ceriodaphnia dubia

CdNaClCR #: **318**

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS 2433				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	12-02-25 0530 to 0630									
Culture board:	11-25-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	5	6	7	10	11	17	18	22
Transfer vessel information:	pH (S.U.): 8.18 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	Blue
Incubator number and shelf location:	2B1

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches <i>Selenastrum</i>	YWT	MHSW batch used	Analyst
0	12-02-25	0842	11-26-25	11-26-25	11-24-25A	HL
1	12-03-25	0645	↓	↓	↓	HL
2	12-04-25	0650	↓	↓	11-24-25B	HL
3	12-05-25	0645	↓	↓	↓	HL
4	12-06-25	0700	↓	↓	12-03-25	HL
5	12-07-25	0630	↓	↓	↓	HL
6	12-08-25	0741	↓	↓	↓	HL
7	12-09-25	0645				HL

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130641685

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	01.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	> 1400
% Adults having 3 rd Broods:	1007.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	07.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	31.2	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1045.4
% CV:	8.57.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1091.7

Species: *Ceriodaphnia dubia*

CdNaClCR #: 318

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	6	4	6	6	6	4	6	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	13	10	13	12	10	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	17	18	16	17	19	18	15	18	19
Total young produced		32	35	37	30	36	37	34	29	36	36
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	0%
Mean Offspring/Female:	34.2

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	7	5	5	6	4	7	6	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	13	13	10	12	12	12	13	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	15	15	18	19	19	14	16	20	18
Total young produced		34	35	33	36	35	35	33	34	39	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	0%
Mean Offspring/Female:	34.9
% Reduction from Control:	-2.0%

Species: Ceriodaphnia dubia

CdNaCICR #: 318

800 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	6	6	6	4	6	5	6	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	11	13	12	14	12	13	13	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	17	19	17	15	14	19	16	19	18
Total young produced		35	34	36	36	31	33	36	35	37	37
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	07.
Mean Offspring/Female:	35.1
% Reduction from Control:	-2.67.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	4	5	7	5	6	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	13	12	12	10	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	14	18	16	16	15	18	18	18	14
Total young produced		34	30	34	33	33	34	33	34	35	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	07.
Mean Offspring/Female:	33.2
% Reduction from Control:	2.97.

Species: Ceriodaphnia dubia

CdNaClCR #: 318

1200 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	3	5	4	4	4	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	6	9	7	5	7	6	8	4	9	8
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	8	6	6	7	9	6	7	10	4	4
Total young produced		19	19	16	17	20	16	19	17	17	16
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	07.
Mean Offspring/Female:	17.6
% Reduction from Control:	48.57.

1400 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	1	1	3	1	2	2	2	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	1	1	1	2	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	1	0	0	0	0	0	1	0	0	0
Total young produced		3	1	2	4	2	4	3	2	1	2
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:

% Mortality:	07.
Mean Offspring/Female:	2.4
% Reduction from Control:	93.07.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	6	4	6	6	6	4	6	5	53
5	12	13	13	10	13	12	10	10	12	12	117
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	18	16	17	19	18	15	18	19	172
Total	32	35	37	30	36	37	34	29	36	36	342

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	7	5	5	6	4	7	6	6	4	55
5	11	13	13	13	10	12	12	12	13	13	122
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	15	18	19	19	14	16	20	18	172
Total	34	35	33	36	35	35	33	34	39	35	349

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	6	6	6	4	6	5	6	5	6	55
5	13	11	11	13	12	14	12	13	13	13	125
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	19	17	15	14	19	16	19	18	171
Total	35	34	36	36	31	34	36	35	37	37	351

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	4	5	7	5	6	5	6	51
5	11	12	12	13	12	12	10	10	12	12	116
6	0	0	0	0	0	0	0	0	0	0	0
7	18	14	18	16	16	15	18	18	18	14	165
Total	34	30	34	33	33	34	33	34	35	32	332

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	3	5	4	4	4	3	4	4	40
5	6	9	7	5	7	6	8	4	9	8	69
6	0	0	0	0	0	0	0	0	0	0	0
7	8	6	6	7	9	6	7	10	4	4	67
Total	19	19	16	17	20	16	19	17	17	16	176

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	1	3	1	2	2	2	1	2	17
5	0	0	1	1	1	2	0	0	0	0	5
6	0	0	0	0	0	0	0	0	0	0	0
7	1	0	0	0	0	0	1	0	0	0	2
Total	3	1	2	4	2	4	3	2	1	2	24

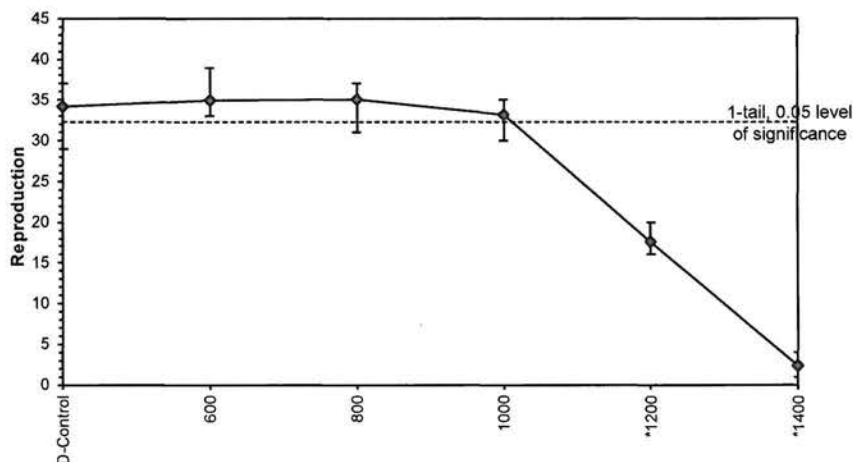
***Ceriodaphnia dubia* Chronic Reference Toxicant Test**
EPA-821-R-02-013, Method 1002.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 318
Test dates: December 02-09, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	32	35	37	30	36	37	34	29	36	36	100	34.2	8.5	Not applicable
600	34	35	33	36	35	35	33	34	39	35	100	34.9	5.0	-2.0
800	35	34	36	36	31	34	36	35	37	37	100	35.1	5.1	-2.6
1000	34	30	34	33	33	34	33	34	35	32	100	33.2	4.2	2.9
1200	19	19	16	17	20	16	19	17	17	16	100	17.6	8.6	48.5
1400	3	1	2	4	2	4	3	2	1	2	100	2.4	44.8	93.0

Dunnett's MSD value: $\frac{1.866}{5.5}$ MSD = Minimum Significant Difference
 PMSD: PMSD = Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
 Lower PMSD bound determined by USEPA (10th percentile) = 13%.
 Upper PMSD bound determined by USEPA (90th percentile) = 47%.
 Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
 USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.



Species: *Ceriodaphnia dubia*

CdNaClCR #: 318

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		Analyst	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.93	7.91	7.80	7.92	7.83	7.95
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.2	8.5	7.9
	Conductivity (µmhos/cm)	290		300		296	
	Alkalinity (mg CaCO ₃ /L)	63				63	
	Hardness (mg CaCO ₃ /L)	88				88	
	Temperature (°C)	24.9	25.2	24.8	25.1	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.99	7.90	7.93	7.94	7.93	7.95
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.2	8.5	8.0
	Conductivity (µmhos/cm)	1340		1390		1360	
	Temperature (°C)	25.0	25.0	24.7	24.9	24.9	25.0
800 mg NaCl/L	pH (S.U.)	8.00	7.90	7.94	7.94	7.94	7.96
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.2	8.5	8.0
	Conductivity (µmhos/cm)	1670		1750		1730	
	Temperature (°C)	25.0	25.0	24.7	24.9	24.9	25.3
1000 mg NaCl/L	pH (S.U.)	8.00	7.90	7.94	7.95	7.94	7.96
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.2	8.5	8.0
	Conductivity (µmhos/cm)	2010		2100		2080	
	Temperature (°C)	25.2	24.9	24.7	25.2	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	8.00	7.89	7.95	7.96	7.95	7.96
	Dissolved oxygen (mg/L)	8.4	8.0	8.3	8.2	8.4	8.0
	Conductivity (µmhos/cm)	2350		2450		2420	
	Temperature (°C)	25.0	24.9	24.9	25.2	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	8.01	7.89	7.96	7.94	7.96	7.97
	Dissolved oxygen (mg/L)	8.4	8.0	8.3	8.2	8.4	7.9
	Conductivity (µmhos/cm)	2740		2820		2800	
	Temperature (°C)	25.0	25.1	24.9	25.0	24.8	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: *Ceriodaphnia dubia*

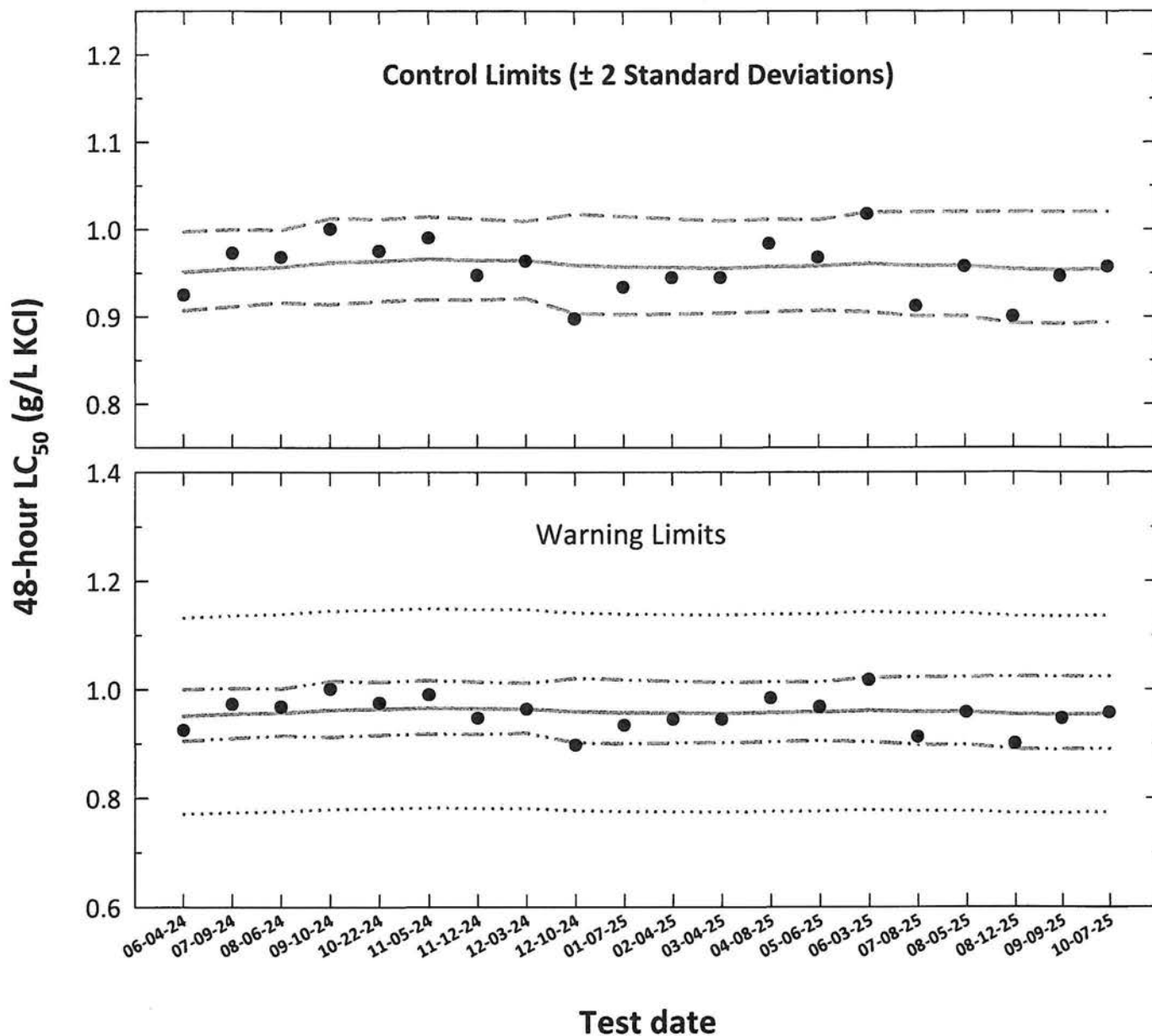
CdNaClCR #: 318

		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Analyst		XL	BSC	BSC	BSC	BSC	XL	XL	BSC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.89	7.94	7.82	7.99	7.85	7.95	7.87	7.89
	Dissolved oxygen (mg/L)	8.2	8.4	8.5	7.8	8.3	8.2	8.4	8.1
	Conductivity (µmhos/cm)	303		288		288		291	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			84					
	Temperature (°C)	24.9	25.1	24.8	25.2	24.9	25.1	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.96	7.97	7.85	8.01	7.74	7.95	7.96	7.93
	Dissolved oxygen (mg/L)	8.2	8.4	8.5	7.9	8.3	8.2	8.4	8.2
	Conductivity (µmhos/cm)	1400		1350		1370		1370	
	Temperature (°C)	25.0	25.0	24.9	25.0	25.0	24.9	24.9	25.3
800 mg NaCl/L	pH (S.U.)	7.97	7.97	7.98	8.00	8.02	7.96	7.98	7.95
	Dissolved oxygen (mg/L)	8.2	8.4	8.5	7.9	8.3	8.2	8.4	8.2
	Conductivity (µmhos/cm)	1760		1730		1770		1780	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.8	24.9	24.8	25.0
1000 mg NaCl/L	pH (S.U.)	7.98	7.98	7.99	8.00	8.03	7.97	7.99	7.96
	Dissolved oxygen (mg/L)	8.2	8.4	8.5	8.0	8.3	8.2	8.4	8.2
	Conductivity (µmhos/cm)	2080		2040		2110		2100	
	Temperature (°C)	24.9	25.2	25.0	25.2	24.8	24.9	24.8	25.0
1200 mg NaCl/L	pH (S.U.)	7.99	7.99	7.99	7.99	8.04	7.98	7.99	7.96
	Dissolved oxygen (mg/L)	8.2	8.4	8.5	8.0	8.3	8.2	8.4	8.2
	Conductivity (µmhos/cm)	2430		2400		2440		2470	
	Temperature (°C)	24.9	25.2	24.9	25.2	24.8	25.2	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.99	7.99	8.00	7.97	8.04	7.98	8.00	7.98
	Dissolved oxygen (mg/L)	8.2	8.4	8.5	8.0	8.3	8.1	8.3	8.1
	Conductivity (µmhos/cm)	2820		2750		2800		2820	
	Temperature (°C)	24.9	24.8	24.9	24.9	25.0	24.9	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- **Control Limits** (mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	06-04-24	0.9251	-0.0338	-0.0217	0.0103	0.9513	0.9073 0.9975	0.9050 0.9998	0.7705 1.1320
2	07-09-24	0.9729	-0.0119	-0.0201	0.0100	0.9549	0.9118 1.0000	0.9097 1.0021	0.7734 1.1363
3	08-06-24	0.9680	-0.0141	-0.0192	0.0094	0.9567	0.9161 0.9992	0.9142 1.0011	0.7749 1.1385
4	09-10-24	1.0007	0.0003	-0.0168	0.0111	0.9621	0.9141 1.0127	0.9122 1.0147	0.7793 1.1449
5	10-22-24	0.9751	-0.0109	-0.0161	0.0106	0.9635	0.9177 1.0117	0.9160 1.0135	0.7805 1.1466
6	11-05-24	0.9904	-0.0042	-0.0149	0.0107	0.9662	0.9199 1.0148	0.9183 1.0165	0.7826 1.1498
7	11-12-24	0.9473	-0.0235	-0.0157	0.0104	0.9645	0.9192 1.0120	0.9175 1.0137	0.7812 1.1477
8	12-03-24	0.9637	-0.0160	-0.0157	0.0100	0.9644	0.9212 1.0097	0.9196 1.0113	0.7812 1.1476
9	12-10-24	0.8977	-0.0469	-0.0181	0.0129	0.9591	0.9039 1.0176	0.9016 1.0201	0.7769 1.1413
10	01-07-25	0.9340	-0.0296	-0.0190	0.0127	0.9573	0.9028 1.0151	0.9003 1.0177	0.7754 1.1392
11	02-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035 1.0125	0.9011 1.0150	0.7747 1.1382
12	03-04-25	0.9448	-0.0247	-0.0197	0.0120	0.9557	0.9043 1.0101	0.9019 1.0126	0.7741 1.1373
13	04-08-25	0.9839	-0.0070	-0.0189	0.0120	0.9574	0.9058 1.0119	0.9035 1.0143	0.7755 1.1393
14	05-06-25	0.9680	-0.0141	-0.0187	0.0117	0.9579	0.9076 1.0111	0.9054 1.0134	0.7759 1.1400
15	06-03-25	1.0177	0.0076	-0.0173	0.0129	0.9610	0.9056 1.0198	0.9034 1.0222	0.7784 1.1436
16	07-08-25	0.9124	-0.0398	-0.0184	0.0135	0.9585	0.9006 1.0201	0.8981 1.0228	0.7764 1.1406
17	08-05-25	0.9576	-0.0188	-0.0184	0.0135	0.9585	0.9007 1.0201	0.8982 1.0228	0.7764 1.1406
18	08-12-25	0.9003	-0.0456	-0.0203	0.0145	0.9543	0.8925 1.0203	0.8896 1.0235	0.7730 1.1356
19	09-09-25	0.9465	-0.0239	-0.0205	0.0146	0.9538	0.8920 1.0199	0.8890 1.0231	0.7726 1.1351
20	10-07-25	0.9572	-0.0190	-0.0200	0.0144	0.9549	0.8935 1.0205	0.8907 1.0236	0.7735 1.1363

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. (S_{A,75} = 0.19).

CV = Coefficient of variation.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 184

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2412

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.79	7.83	7.90
	Dissolved oxygen (mg/L)	8.0	8.1	8.1
	Conductivity (µmhos/cm)	299		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.8	25.0	24.6
500 mg/L	pH (S.U.)	7.95	7.82	7.87
	Dissolved oxygen (mg/L)	8.5	8.1	8.1
	Conductivity (µmhos/cm)	1190		
	Temperature (°C)	24.9	24.7	24.7
750 mg/L	pH (S.U.)	7.95	7.82	7.87
	Dissolved oxygen (mg/L)	8.5	8.0	8.1
	Conductivity (µmhos/cm)	1570		
	Temperature (°C)	24.9	24.6	24.7
1000 mg/L	pH (S.U.)	7.95	7.83	7.87
	Dissolved oxygen (mg/L)	8.5	8.0	8.1
	Conductivity (µmhos/cm)	2030		
	Temperature (°C)	24.9	24.9	24.5
1250 mg/L	pH (S.U.)	7.95	7.83	7.88
	Dissolved oxygen (mg/L)	8.4	8.0	8.1
	Conductivity (µmhos/cm)	2450		
	Temperature (°C)	24.8	24.9	24.5
1500 mg/L	pH (S.U.)	7.96	7.82	7.88
	Dissolved oxygen (mg/L)	8.4	8.0	8.1
	Conductivity (µmhos/cm)	2840		
	Temperature (°C)	25.0	24.8	24.5

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on the test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13266470

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant TestPpKClAC # 184

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-07-25	* 0905	JP	0945	JP	6B	orange	09-29-25A
24	11-08-25			0950	JP			
48 Termination	10-09-25			0936	JP			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	09-25-25
Age (1 to 14 days old):	5 to 6 days old
Hatch date and times:	10-01-25 1205 to 10-02-25 0815
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.69
	Temperature (°C): 25.1 °C

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ^{1d}	9 ^{1d}	5 ^{5d}	5 ^{5d}	2 ^{4d}	0 ^{10d}	0 ^{10d}	1 ^{9d}
48 Termination	10	10	10	10	9	9	4 ^{1d}	5	1 ^{1d}	0	0	0 ^{1d}
Mean Survival	100%		100%		90%		45%		5%		0%	

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	883.8
Upper 95% confidence limit (mg KCl/L)	1026.7
48-hour LC ₅₀ (mg KCl/L)	951.2

Comments:

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	10/7/2025	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	10/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.5000	0.5000
1250	0.2000	0.0000
1500	0.0000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2824	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	1.633	2.830	0.2824	2	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	6.279	2.830	0.2824	10	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	11.031	2.830	0.2824	18	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.742	2.830	0.2824	19	20

Auxiliary Tests

Normality of the data set cannot be confirmed

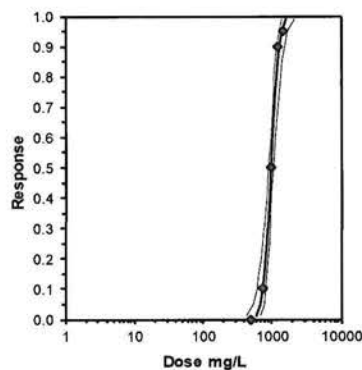
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.15735	0.16138	0.57648	0.00996	5.4E-05	5, 6

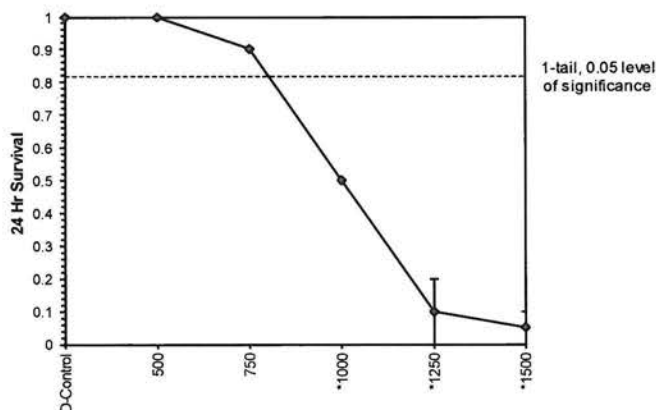
Treatments vs D-Control

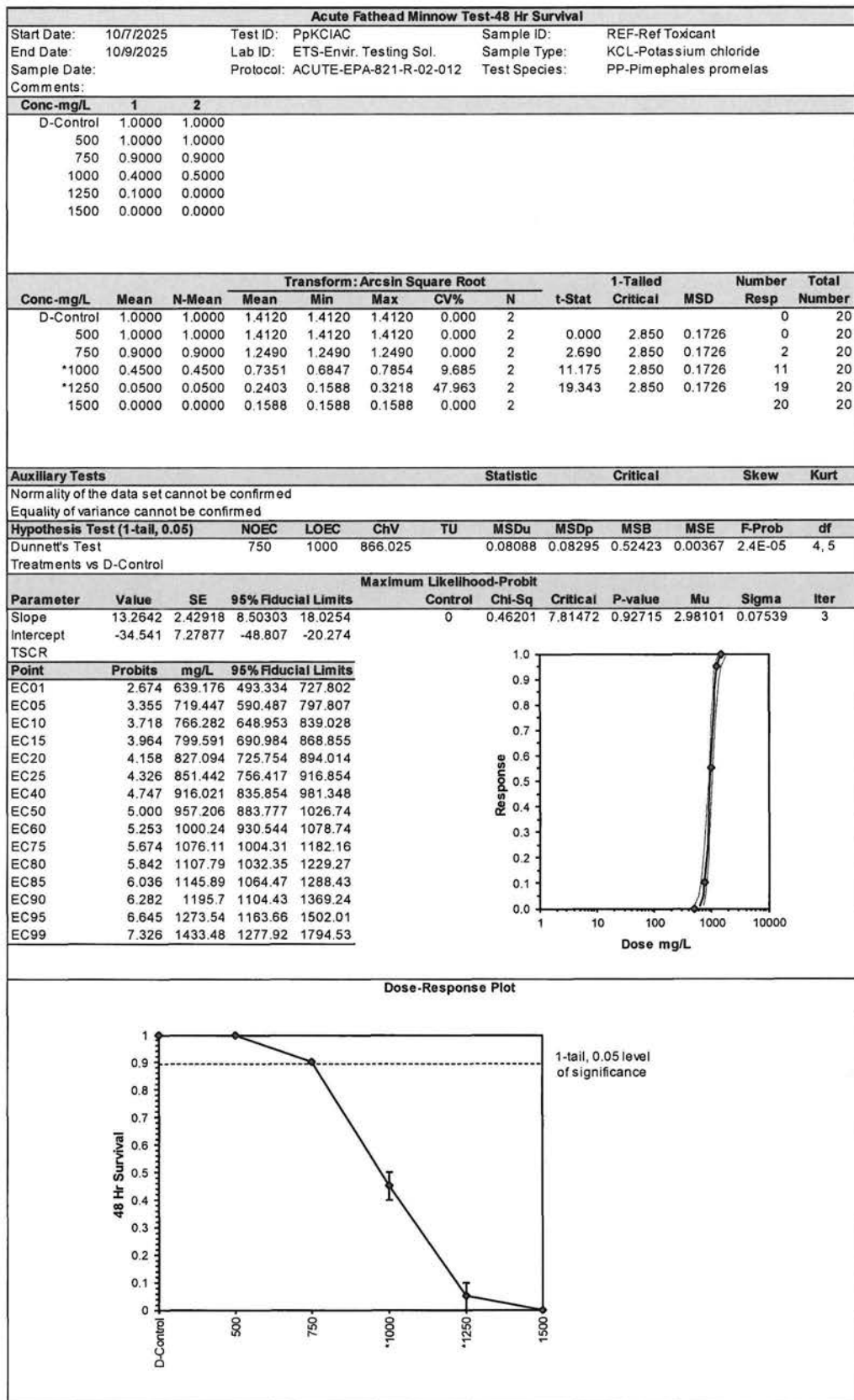
Parameter	Value	SE	95% Fiducial Limits			Maximum Likelihood-Probit						
						Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	10.557	1.84665	6.93758	14.1765		0	0.6732	7.81472	0.87949	2.99611	0.09472	3

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	596.696	441.551	696.762
EC05	3.355	692.319	550.846	782.238
EC10	3.718	749.411	618.826	833.287
EC15	3.964	790.566	668.676	870.503
EC20	4.158	824.882	710.52	902.053
EC25	4.326	855.506	747.87	930.813
EC40	4.747	937.811	846.51	1012.67
EC50	5.000	991.09	907.203	1070.99
EC60	5.253	1047.4	967.13	1138.66
EC75	5.674	1148.16	1062.91	1275.84
EC80	5.842	1190.79	1099.77	1339.27
EC85	6.036	1242.48	1142.37	1419.66
EC90	6.282	1310.71	1195.97	1530.72
EC95	6.645	1418.8	1276.63	1716.11
EC99	7.326	1646.17	1436.05	2136.71

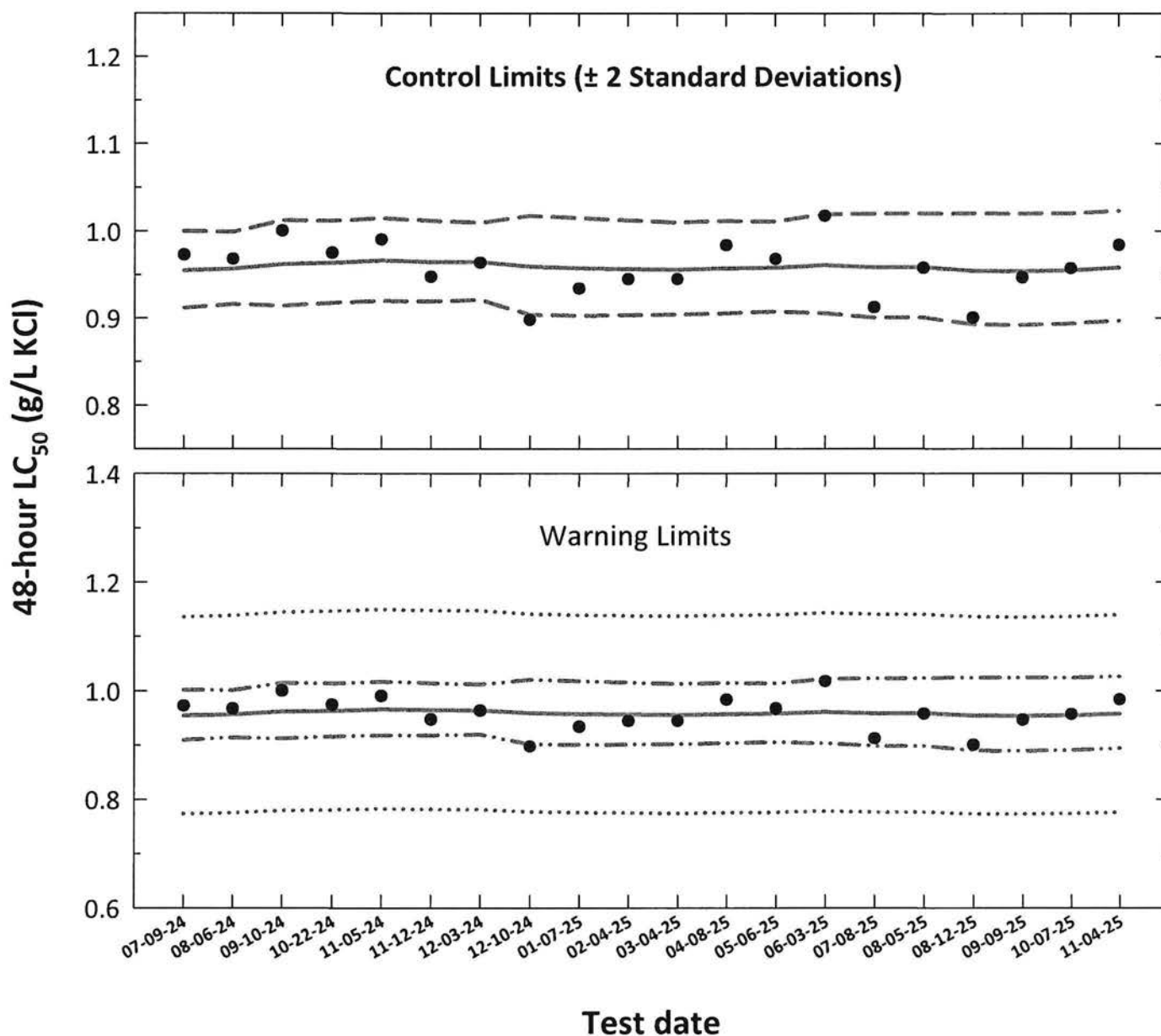


Dose-Response Plot





Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



- **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S		Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV		75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}	
1	07-09-24	0.9729	-0.0119	-0.0201	0.0100	0.9549	0.9118	1.0000	0.9097	1.0021	0.7734	1.1363
2	08-06-24	0.9680	-0.0141	-0.0192	0.0094	0.9567	0.9161	0.9992	0.9142	1.0011	0.7749	1.1385
3	09-10-24	1.0007	0.0003	-0.0168	0.0111	0.9621	0.9141	1.0127	0.9122	1.0147	0.7793	1.1449
4	10-22-24	0.9751	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
5	11-05-24	0.9904	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
6	11-12-24	0.9473	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
7	12-03-24	0.9637	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
8	12-10-24	0.8977	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
9	01-07-25	0.9340	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
10	02-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
11	03-04-25	0.9448	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
12	04-08-25	0.9839	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
13	05-06-25	0.9680	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
14	06-03-25	1.0177	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
15	07-08-25	0.9124	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
16	08-05-25	0.9576	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
17	08-12-25	0.9003	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
18	09-09-25	0.9465	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
19	10-07-25	0.9572	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
20	11-04-25	0.9840	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
CT = Central tendency of the LC₅₀ values.
S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A.75} converted to anti-logarithmic values.

S_{A.75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. (S_{A.75} = 0.19).

CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*

Page 1

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant TestPpKCIAC # 185

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2437

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.80	7.88	7.89
	Dissolved oxygen (mg/L)	8.5	8.1	8.1
	Conductivity (µmhos/cm)	299		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	91		
	Temperature (°C)	24.7	25.3	24.8
500 mg/L	pH (S.U.)	7.96	7.86	7.86
	Dissolved oxygen (mg/L)	8.5	8.1	8.1
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	24.4	25.2	25.1
750 mg/L	pH (S.U.)	7.97	7.87	7.86
	Dissolved oxygen (mg/L)	8.6	8.1	8.1
	Conductivity (µmhos/cm)	1530		
	Temperature (°C)	24.8	25.2	24.7
1000 mg/L	pH (S.U.)	7.97	7.86	7.88
	Dissolved oxygen (mg/L)	8.6	8.1	8.0
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	24.5	25.1	24.7
1250 mg/L	pH (S.U.)	7.96	7.85	
	Dissolved oxygen (mg/L)	8.6	8.0	
	Conductivity (µmhos/cm)	2350		
	Temperature (°C)	24.9	24.8	
1500 mg/L	pH (S.U.)	7.96	7.84	
	Dissolved oxygen (mg/L)	8.6	7.9	
	Conductivity (µmhos/cm)	2690		
	Temperature (°C)	24.6	25.3	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on the test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 185

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-04-25	* 0505 + 0400	JP	1115	JP	6E	right blue	10-28-25 B
24	11-05-25			1117	JP			
48 Termination	11-06-25			1115	JP			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	10-22-25
Age (1 to 14 days old):	5 to 6 days old
Hatch date and times:	10-29-25 1111 13 10-30-25 0800
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.23
	Temperature (°C): 25.8°C

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

Survival Data (number of living organisms):

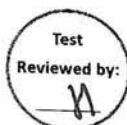
Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 ^{4d}	5 ^{2s}	0 ^{12d}	0 ^{12d}	0 ^{10d}	0 ^{12d}
48 Termination	10	10	10	10	10	10	5 ¹²	5	0	0	0	0
Mean Survival	100%		100%		100%		50%		0%		0%	

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	929.4
Upper 95% confidence limit (mg KCl/L)	1041.8
48-hour LC ₅₀ (mg KCl/L)	984.0

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 11/4/2025 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/6/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.5000
1250	0.0000	0.0000
1500	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.101	2.850	0.0907	9	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

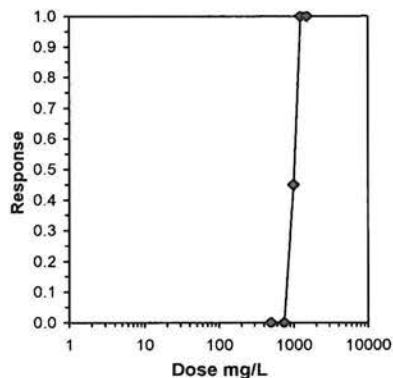
Auxiliary Tests Statistic Critical Skew Kurt

Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

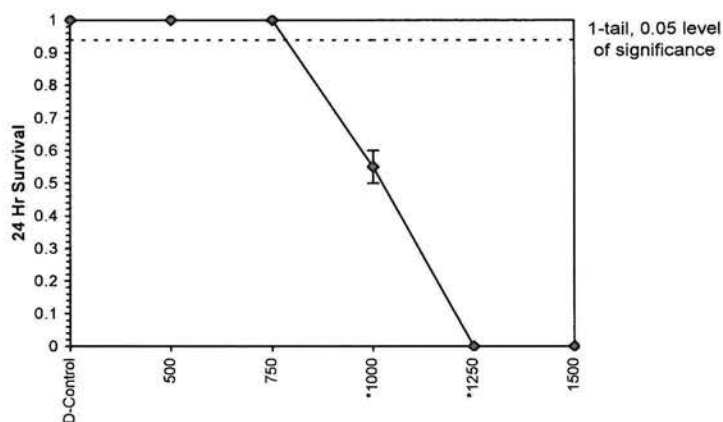
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.03598	0.0369	0.61664	0.00101	6.7E-07	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	996.642	941.586 1054.92
5.0%	999.523	938.167 1064.89
10.0%	1002.41	932.786 1077.22
20.0%	1008.15	912.42 1113.93
Auto-0.0%	996.642	941.586 1054.92



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 11/4/2025 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/6/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas
Comments:

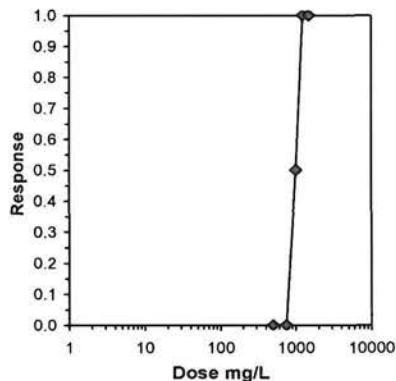
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.5000	0.5000
1250	0.0000	0.0000
1500	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	62.662	2.850	0.0285	10	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

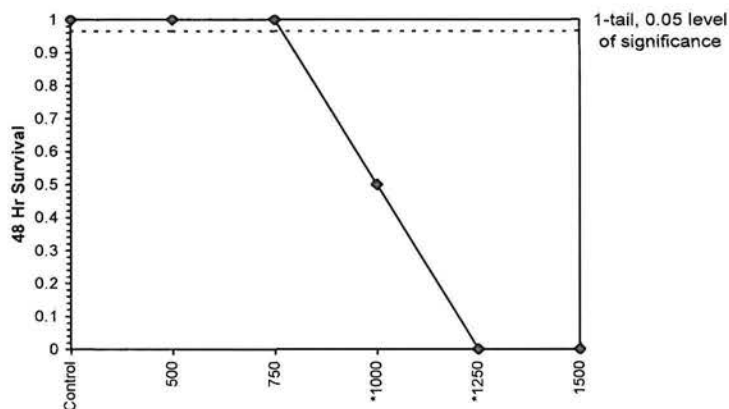
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.00967	0.00991	0.62824	0.0001	2.0E-09	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber

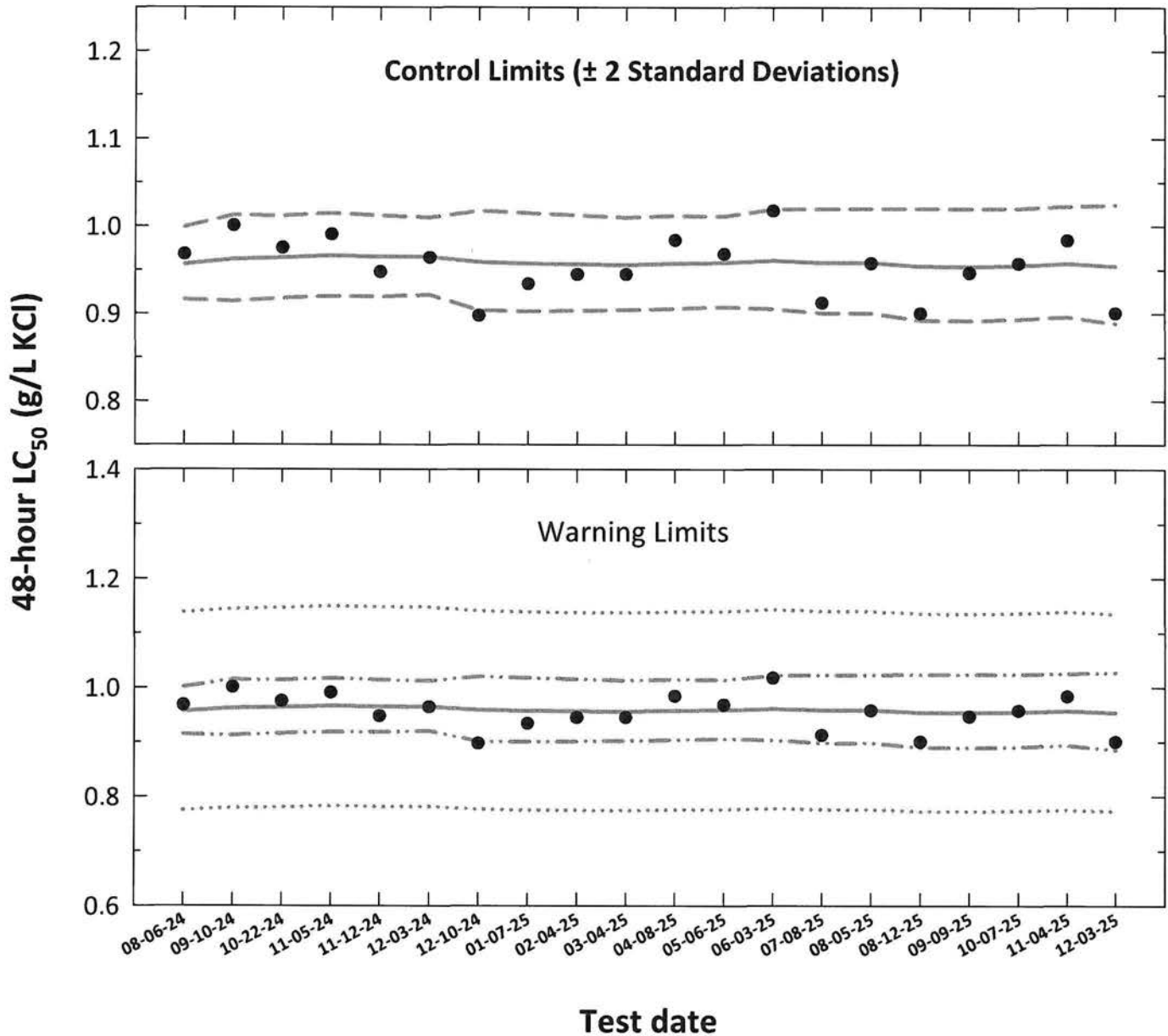
Trim Level	EC50	95% CL	
0.0%	983.99	929.37	1041.83
5.0%	985.58	924.91	1050.24
10.0%	987.18	918.83	1060.61
20.0%	990.37	898.71	1091.37
Auto-0.0%	983.99	929.37	1041.83



Dose-Response Plot



Pimephales promelas Acute Reference Toxicant Control Chart Source: In-house Culture



- **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ converted to anti-logarithmic values,
 $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
1	08-06-24	0.9680	-0.0141	-0.0192	0.0094	0.9567	0.9161	0.9992	0.9142	1.0011	0.7749	1.1385
2	09-10-24	1.0007	0.0003	-0.0168	0.0111	0.9621	0.9141	1.0127	0.9122	1.0147	0.7793	1.1449
3	10-22-24	0.9751	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
4	11-05-24	0.9904	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
5	11-12-24	0.9473	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
6	12-03-24	0.9637	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
7	12-10-24	0.8977	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
8	01-07-25	0.9340	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
9	02-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
10	03-04-25	0.9448	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
11	04-08-25	0.9839	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
12	05-06-25	0.9680	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
13	06-03-25	1.0177	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
14	07-08-25	0.9124	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
15	08-05-25	0.9576	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
16	08-12-25	0.9003	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
17	09-09-25	0.9465	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
18	10-07-25	0.9572	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
19	11-04-25	0.9840	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
20	12-03-25	0.9007	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A.75} converted to anti-logarithmic values.

S_{A.75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. (S_{A.75} = 0.19).

CV = Coefficient of variation.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: Pimephales promelas
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 186

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KC stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2437

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.93	7.95	7.95
	Dissolved oxygen (mg/L)	8.4	8.1	8.3
	Conductivity (µmhos/cm)	240		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	88		
	Temperature (°C)	25.0	24.9	24.9
500 mg/L	pH (S.U.)	7.99	7.92	7.93
	Dissolved oxygen (mg/L)	8.3	8.1	8.3
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	25.6	25.1	24.7
750 mg/L	pH (S.U.)	8.00	7.93	7.94
	Dissolved oxygen (mg/L)	8.3	8.2	8.3
	Conductivity (µmhos/cm)	1540		
	Temperature (°C)	25.7	24.6	24.6
1000 mg/L	pH (S.U.)	8.01	7.94	7.95
	Dissolved oxygen (mg/L)	8.3	8.2	8.3
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	25.0	24.8	24.9
1250 mg/L	pH (S.U.)	8.01	7.95	7.96
	Dissolved oxygen (mg/L)	8.3	8.2	8.2
	Conductivity (µmhos/cm)	2310		
	Temperature (°C)	25.2	25.0	24.8
1500 mg/L	pH (S.U.)	8.01	7.95	
	Dissolved oxygen (mg/L)	8.2	8.1	
	Conductivity (µmhos/cm)	2800		
	Temperature (°C)	25.4	24.8	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on the test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664701

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 186

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-02-25	* 0505	JP	0932	JP	7F	Green	11-24-25A
24	12-03-25			0922	JP			
48 Termination	12-04-25			0943	JP			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	11-20-25
Age (1 to 14 days old):	5 to 6 days old
Hatch date and times:	11-26-25 1146 to 11-27-25 0620
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.24
	Temperature (°C): 25.3

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	22 10	32 10	72 3	62 4	12-03-25 0320	12-03-25 460	0102	280	0102	0102
48 Termination	10	10	10	10	7	8	3	4	0	2	0	0
Mean Survival	100%		100%		75%		35%		10%		0%	

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

Statistics:

Method	probit
Lower 95% confidence limit (mg KCl/L)	817.1
Upper 95% confidence limit (mg KCl/L)	978.4
48-hour LC ₅₀ (mg KCl/L)	900.7

Comments:



Acute Fathead Minnow Test-24 & 48 Hr Survival

Start Date: 12/2/2025 Test ID: PpKCIAC Sample ID: PPKCLAC
End Date: 12/4/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.7000	0.8000
1000	0.3000	0.4000
1250	0.0000	0.2000
1500	0.0000	0.0000

Transform: Arcsin Square Root								1-Tailed		Number		Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.3089	0	20
*750	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	3.348	2.850	0.3089	5	20
*1000	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	7.196	2.850	0.3089	13	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	10.158	2.850	0.3089	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.1782	0.18277	0.47246	0.01174	5.4E-04	4, 5

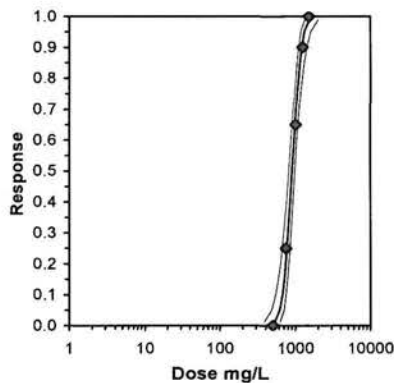
Treatments vs D-Control

Maximum Likelihood-Probit

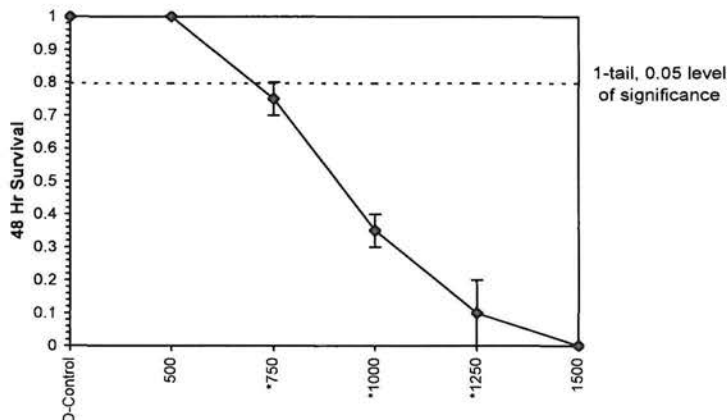
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.89164	1.70549	6.54889 13.2344	0	0.7057	7.81472	0.87186	2.95459	0.1011	4
Intercept	-24.226	5.08425	-34.191 -14.261							

TSCR

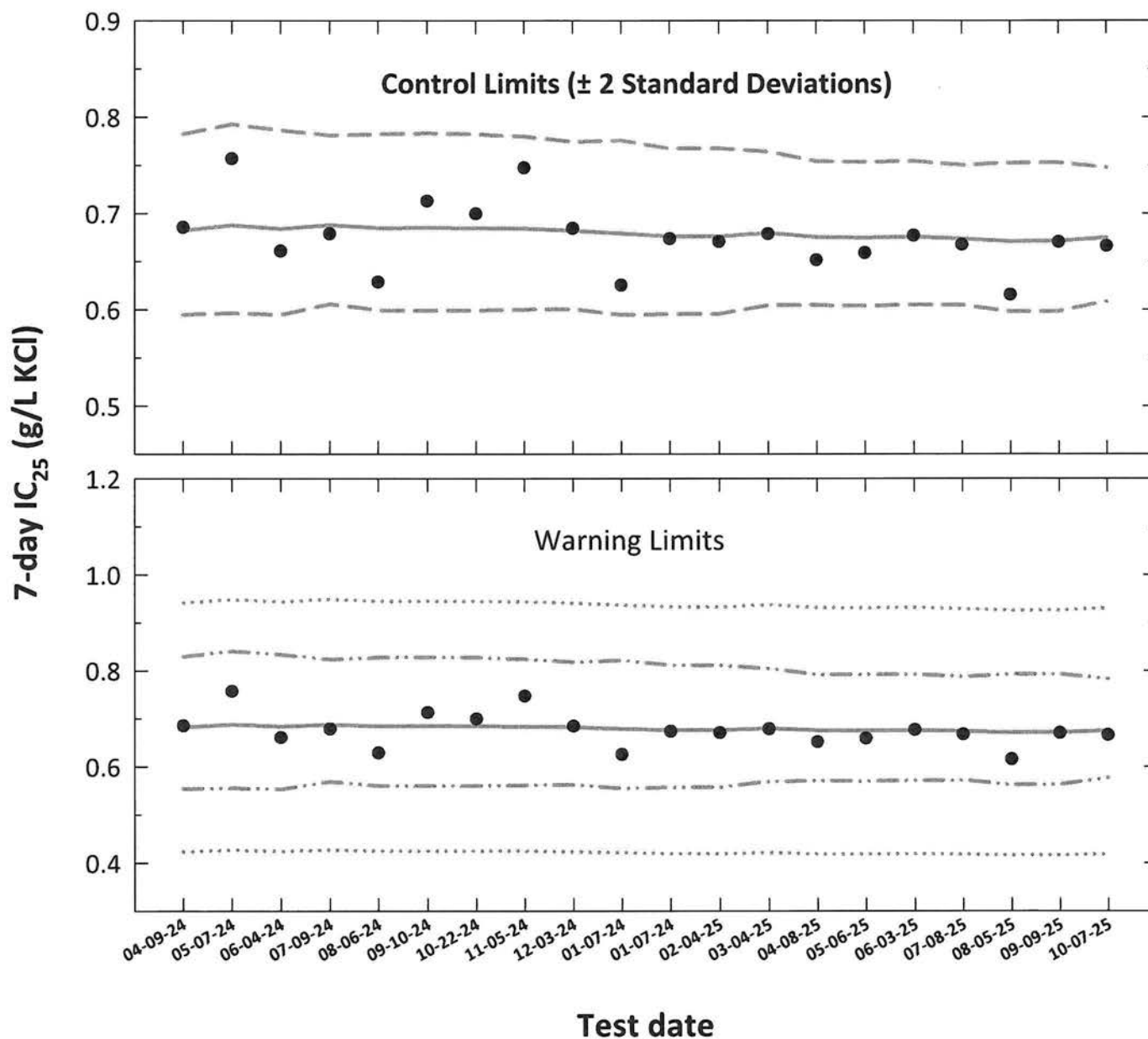
Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	524.087	379.974 619.519
EC05	3.355	614.185	480.328 701.176
EC10	3.718	668.386	543.409 750.179
EC15	3.964	707.633	589.975 785.974
EC20	4.158	740.462	629.272 816.34
EC25	4.326	769.837	664.518 844.018
EC40	4.747	849.13	758.507 922.631
EC50	5.000	900.713	817.137 978.412
EC60	5.253	955.43	875.699 1043.01
EC75	5.674	1053.84	970.392 1174.43
EC80	5.842	1095.65	1007.03 1235.6
EC85	6.036	1146.48	1049.43 1313.52
EC90	6.282	1213.8	1102.82 1421.79
EC95	6.645	1320.91	1183.3 1603.88
EC99	7.326	1548	1342.99 2021.86



Dose-Response Plot



Pimephales promelas Chronic Reference Toxicant Control Chart Source: In-house Culture



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ $\pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)			
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	04-09-24	0.6855	-0.1640	-0.1660	0.0298	0.6823	0.5949 0.7825	0.5542 0.8292	0.4230 0.9416
2	05-07-24	0.7569	-0.1210	-0.1627	0.0309	0.6876	0.5965 0.7927	0.5551 0.8404	0.4263 0.9489
3	06-04-24	0.6610	-0.1798	-0.1650	0.0303	0.6840	0.5949 0.7864	0.5537 0.8338	0.4241 0.9439
4	07-09-24	0.6789	-0.1682	-0.1624	0.0276	0.6880	0.6060 0.7811	0.5688 0.8233	0.4265 0.9494
5	08-06-24	0.6287	-0.2015	-0.1644	0.0289	0.6848	0.5994 0.7823	0.5602 0.8272	0.4246 0.9450
6	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994 0.7832	0.5600 0.8283	0.4248 0.9456
7	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994 0.7822	0.5601 0.8271	0.4245 0.9449
8	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004 0.7797	0.5617 0.8238	0.4242 0.9442
9	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006 0.7745	0.5626 0.8176	0.4228 0.9411
10	01-07-24	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946 0.7757	0.5547 0.8213	0.4211 0.9372
11	01-07-24	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955 0.7675	0.5569 0.8113	0.4191 0.9329
12	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956 0.7676	0.5571 0.8114	0.4192 0.9331
13	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047 0.7643	0.5693 0.8041	0.4215 0.9381
14	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048 0.7540	0.5708 0.7919	0.4187 0.9319
15	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039 0.7537	0.5697 0.7918	0.4183 0.9310
16	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053 0.7543	0.5715 0.7920	0.4189 0.9324
17	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048 0.7501	0.5715 0.7872	0.4176 0.9295
18	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981 0.7525	0.5623 0.7926	0.4159 0.9258
19	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985 0.7529	0.5628 0.7928	0.4162 0.9263
20	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087 0.7477	0.5769 0.7829	0.4183 0.9310

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalC).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

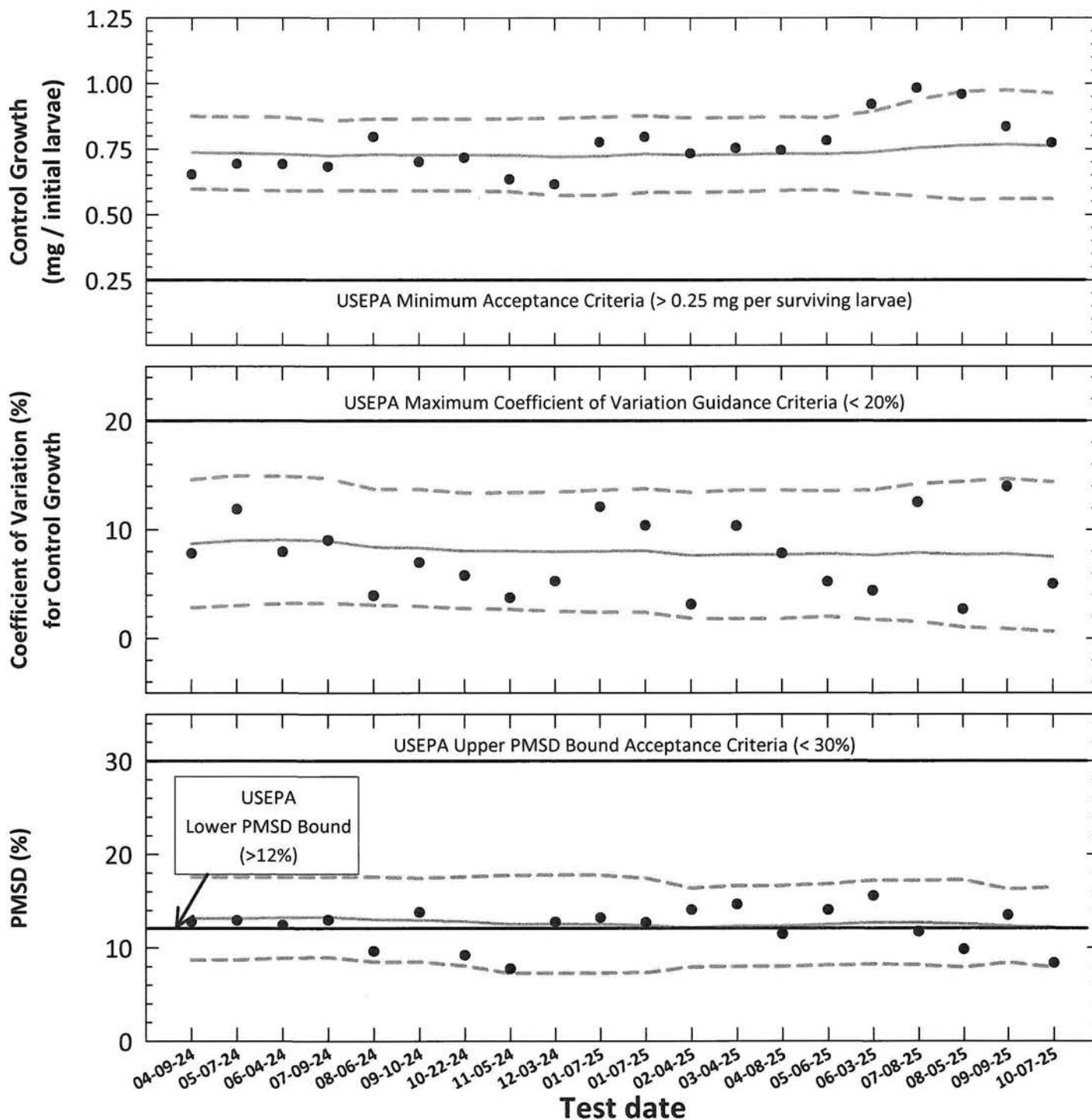
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

CV = Coefficient of variation.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Growth, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Growth, CV or PMSD $\pm$ 2 Standard Deviations)

Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD	
		Control Survival (%)	Control Growth		PMSD (%)	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S
			Mean (mg/initial larvae)	CV (%)							
1	04-09-24	100	0.653	7.8	12.8	0.736	0.598	8.7	2.8	13.1	8.7
2	05-07-24	100	0.693	11.9	12.9	0.734	0.595	9.0	3.0	13.1	8.7
3	06-04-24	100	0.693	8.0	12.4	0.732	0.591	9.1	3.2	13.2	8.9
4	07-09-24	100	0.683	9.0	12.9	0.724	0.591	9.0	3.2	13.2	8.9
5	08-06-24	100	0.796	3.9	9.6	0.728	0.591	8.4	3.0	13.0	8.4
6	09-10-24	100	0.701	7.0	13.8	0.728	0.591	8.3	2.9	12.9	8.5
7	10-22-24	100	0.717	5.8	9.2	0.727	0.590	8.1	2.7	12.8	8.0
8	11-05-24	100	0.635	3.7	7.7	0.727	0.588	8.0	2.7	12.5	7.3
9	12-03-24	100	0.615	5.3	12.8	0.721	0.573	8.0	2.5	12.5	7.3
10	01-07-25	100	0.776	12.1	13.2	0.721	0.573	8.0	2.4	12.5	7.3
11	01-07-25	100	0.796	10.4	12.7	0.731	0.585	8.1	2.4	12.4	7.3
12	02-04-25	100	0.733	3.1	14.1	0.727	0.585	7.6	1.8	12.2	7.9
13	03-04-25	100	0.754	10.4	14.6	0.729	0.588	7.7	1.8	12.3	8.0
14	04-08-25	100	0.746	7.8	11.5	0.733	0.593	7.7	1.8	12.3	8.0
15	05-06-25	100	0.783	5.2	14.0	0.732	0.593	7.8	2.0	12.5	8.1
16	06-03-25	100	0.920	4.4	15.5	0.736	0.580	7.7	1.7	12.7	8.2
17	07-08-25	100	0.983	12.5	11.7	0.754	0.570	7.9	1.5	12.7	8.1
18	08-05-25	100	0.959	2.7	9.8	0.763	0.558	7.7	1.0	12.6	7.9
19	09-09-25	100	0.835	14.0	13.5	0.767	0.559	7.8	0.9	12.3	8.4
20	10-07-25	100	0.774	5.0	8.3	0.762	0.560	7.5	0.6	12.2	7.9

Note: Control Survival = USEPA minimum test acceptability criteria $\geq$ 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria $\geq$ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) > 12%.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

CT = Central tendency of the growth, CV or PMSD values.

S = Standard deviation of the growth, CV or PMSD values.

Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1000.0)

Species: *Pimephales promelas*

PpKClCR Test Number: 133

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2412</u>				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>09-30-25</u>	Artemia CHM number:	CHM1385
Hatch dates and times:	<u>10-06-25 1430 to 10-07-25 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.69</u> S.U. Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>10-14-25 0815</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>10-15-25 0815</u>
		*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24-Hours</u>

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	10-07-25	0505	H	1500	A	0705	H	09-29-25A
1	10-08-25	0500	L	1400	H	0725	H	↓ 10-06-25A
2	10-09-25	0500	H	1330	H	0730	H	↓ 10-06-25A
3	10-10-25	0500	H	1100	H	0700	H	↓ 10-06-25B
4	10-11-25	0600	H	1200	H	0810	H	↓ 10-06-25B
5	10-12-25	0600	H	1200	H	0800	H	↓ 10-06-25B
6	10-13-25	0500	H	1100	H	0800	H	↓ 10-06-25B
7	10-14-25					0530	H	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>743.9</u>
Average weight per initial larvae:	<u>0.774</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.774</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>670.8</u>
			IC ₂₅ (mg/L KCl)	<u>666.1</u>

Species: *Pimephales promelas*

PpKICR Test Number: 133

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>gray</u> Analyst: <u>XL</u> Date: <u>09-19-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>10-16-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>XL</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>XL</u>												
Average weight per initial number of larvae (mg)	0.774				0.845				0.733			
Percent reduction from control (%)					-15.67				5.47			

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 133

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	9 ^{1d}	9 ^{1d}	9 ^{1d}	9 ^{1d}	7 ^{3d}	7 ^{3d}	7 ^{3d}	7 ^{3d}
2	10	10	10	9 ^{1d}	7 ^{2d}	8 ^{1d}	8 ^{1d}	8 ^{1d}	4 ^{3d}	5 ^{2d}	4 ^{3d}	5 ^{2d}
3	10	10	10	9	7	8	8	8	4	4 ^{1d}	4	4 ^{1d}
4	10	10	10	9	7	8	8	8	4	4	4	4
5	10	10	10	9	7	8	7 ^{1d}	7 ^{1d}	4	4	3 ^{1d}	3 ^{1d}
6	10	10	10	9	7	8	7	7	3 ^{1d}	3 ^{1d}	3	3
7	10	10	10	9	7	6 ^{2d}	6 ^{1d}	7	2 ^{1d}	2 ^{1d}	2 ^{1d}	3
*A = Pan weight (mg) Tray color code: <u>6v1w</u> Analyst: <u>XL</u> Date: <u>09-19-25</u>												
	11.54	13.95	10.35	10.60	10.21	10.58	10.24	10.60	10.06	10.22	10.68	10.88
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>10-16-25</u>												
	19.08	21.77	17.19	17.91	15.47	15.40	14.71	15.54	12.52	11.46	11.96	12.96
C = Larvae weight (mg) = B - A Analyst: <u>XL</u>												
	7.54	7.82	6.84	7.31	5.26	4.82	4.47	4.94	1.86	1.24	1.28	2.08
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>XL</u>												
	0.754	0.782	0.684	0.731	0.526	0.482	0.447	0.494	0.186	0.124	0.128	0.208
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
	0.738		4.77		0.487		37.07		0.162		79.17	

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: *Pimephales promelas*

PpKCICR Test Number: 133

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 rd	2 ^{sd}	3 rd	3 rd
2	2 ^d 1	1 ^d 1	2 ^d 1	2 ^d 2
3	1	1	1	1 ^d
4	1	1	0 ^{1d}	0 ^{1d}
5	0 ^{1d}	1	0	0
6	0	0 ^{1d}	0	0
7	0	0	0	0
*A = Pan weight (mg) Tray color code: <u>Gray</u> Analyst: <u>XZ</u> Date: <u>09-19-25</u>				
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B - A Analyst: _____				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: _____				
Average weight per initial number of larvae (mg) Percent reduction from control (%)				
0 1001.				

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Pimephales promelas Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1000.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 133
Test dates: October 07-14, 2025

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.56	22.43	7.87	0.787							
	B	10	10	13.67	21.15	7.48	0.748							
	C	10	10	13.26	20.64	7.38	0.738	0.774	5.0		100.0	0.774	5.0	Not applicable
	D	10	10	13.33	21.56	8.23	0.823							
300	E	10	10	14.27	23.56	9.29	0.929							
	F	10	10	15.18	24.52	9.44	0.944							
	G	10	10	10.38	18.71	8.33	0.833	0.895	5.7		100.0	0.895	5.7	-15.6
	H	10	10	10.63	19.37	8.74	0.874							
450	I	10	10	11.35	18.36	7.01	0.701							
	J	10	10	14.13	21.69	7.56	0.756							
	K	10	10	10.96	18.33	7.37	0.737	0.733	3.1		100.0	0.733	3.1	5.4
	L	10	10	13.81	21.17	7.36	0.736							
600	M	10	10	11.54	19.08	7.54	0.754							
	N	10	10	13.95	21.77	7.82	0.782							
	O	10	10	10.35	17.19	6.84	0.684	0.758	7.2		97.5	0.738	5.6	4.7
	P	10	9	10.60	17.91	7.31	0.812							
750	Q	10	7	10.21	15.47	5.26	0.751							
	R	10	6	10.58	15.40	4.82	0.803							
	S	10	6	10.24	14.71	4.47	0.745	0.751	5.3		65.0	0.487	6.7	37.0
	T	10	7	10.60	15.54	4.94	0.706							
900	U	10	2	10.66	12.52	1.86	0.930							
	V	10	2	10.22	11.46	1.24	0.620							
	W	10	2	10.68	11.96	1.28	0.640	0.721	19.8		22.5	0.162	26.0	79.1
	X	10	3	10.88	12.96	2.08	0.693							
1050	Y	10	0	0.00	0.00	0.00	0.000							
	Z	10	0	0.00	0.00	0.00	0.000							
	AA	10	0	0.00	0.00	0.00	0.000	0.000	0.0		0.0	0.000	0.0	100.0
	BB	10	0	0.00	0.00	0.00	0.000							

Dunnett's MSD value: 0.0646
PMSD: 8.3
MSD =
PMSD =

Minimum Significant Difference
Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 12%.

Upper PMSD bound determined by USEPA (90th percentile) = 30%.

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Larval Fish Growth and Survival Test-7 Day Growth

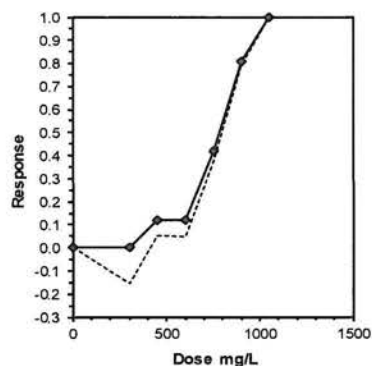
Start Date: 10/7/2025 Test ID: PpKICR Sample ID: REF-Ref Toxicant
End Date: 10/14/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.7870	0.7480	0.7380	0.8230
300	0.9290	0.9440	0.8330	0.8740
450	0.7010	0.7560	0.7370	0.7360
600	0.7540	0.7820	0.6840	0.7310
750	0.5260	0.4820	0.4470	0.4940
900	0.1860	0.1240	0.1280	0.2080
1050	0.0000	0.0000	0.0000	0.0000

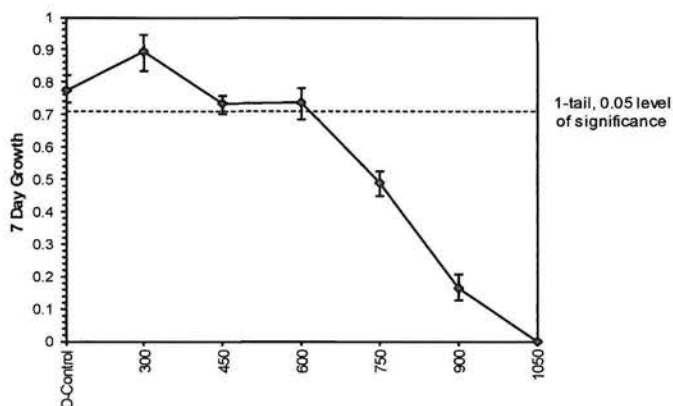
Conc-mg/L	Transform: Untransformed							1-Tailed		Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean N-Mean
D-Control	0.7740	1.0000	0.7740	0.7380	0.8230	5.027	4				0.8345 1.0000
300	0.8950	1.1563	0.8950	0.8330	0.9440	5.713	4	-4.287	2.290	0.0646	0.8345 1.0000
450	0.7325	0.9464	0.7325	0.7010	0.7560	3.130	4	1.470	2.290	0.0646	0.7351 0.8809
600	0.7378	0.9532	0.7378	0.6840	0.7820	5.620	4	1.284	2.290	0.0646	0.7351 0.8809
750	0.4873	0.6295	0.4873	0.4470	0.5260	6.697	4				0.4873 0.5839
900	0.1615	0.2087	0.1615	0.1240	0.2080	26.004	4				0.1615 0.1935
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000 0.0000

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)					0.95267	0.844	-0.1973	-1.0379						
Bartlett's Test indicates equal variances ($p = 0.67$)					1.55046	11.3449								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					600	>600			0.06463	0.0835	0.02295	0.00159	2.8E-04	3, 12
Treatments vs D-Control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95%CL(Exp)	Skew	
IC05	362.98	11.06	341.80	413.11	0.9073
IC10	425.96	60.05	383.59	716.54	1.9671
IC15	615.61	38.62	349.39	639.77	-3.0919
IC20	640.86	9.91	603.28	665.72	-0.4315
IC25	666.11	9.24	633.58	691.88	-0.1036
IC40	741.86	9.12	715.26	766.95	-0.0035
IC50	782.23	6.74	761.67	800.14	0.0170



Dose-Response Plot



Species: *Pimephales promelas*

PpKICR Test Number: 133

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.79	7.80	7.74	7.68	7.77 (7.75)	7.71
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	7.7	8.5	7.6
	Conductivity (µmhos/cm)	299		308		293	
	Alkalinity (mg CaCO ₃ /L)	63				62	
	Hardness (mg CaCO ₃ /L)	86				88	
	Temperature (°C)	24.8	24.5	24.7	24.5	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.94	7.78	7.89	7.68	7.93	7.72
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	7.7	8.4	7.6
	Conductivity (µmhos/cm)	863		854		832	
	Temperature (°C)	24.7	24.6	24.8	24.9	24.7	24.7
450 mg KCl/L	pH (S.U.)	7.95	7.80	7.89	7.70	7.94	7.72
	Dissolved oxygen (mg/L)	8.4	8.0	8.3	7.7	8.4	7.6
	Conductivity (µmhos/cm)	1110		1100		1100	
	Temperature (°C)	24.7	24.6	24.7	24.6	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.95	7.81	7.89	7.71	7.95	7.73
	Dissolved oxygen (mg/L)	8.6	8.0	8.4	7.7	8.4	7.6
	Conductivity (µmhos/cm)	1350		1370		1340	
	Temperature (°C)	24.8	24.7	24.7	24.6	24.7	24.5
750 mg KCl/L	pH (S.U.)	7.96	7.82	7.90	7.72	7.95	7.75
	Dissolved oxygen (mg/L)	8.6	8.0	8.4	7.7	8.4	7.7
	Conductivity (µmhos/cm)	1620		1646		1600	
	Temperature (°C)	24.9	24.7	24.7	24.6	24.6	24.8
900 mg KCl/L	pH (S.U.)	7.96	7.82	7.90	7.72	7.94	7.76
	Dissolved oxygen (mg/L)	8.6	8.0	8.4	7.7	8.4	7.6
	Conductivity (µmhos/cm)	1880		1900		1850	
	Temperature (°C)	24.7	24.7	24.9	24.7	24.6	24.8
1050 mg KCl/L	pH (S.U.)	7.97	7.83	7.91	7.78	7.97	7.77
	Dissolved oxygen (mg/L)	8.6	8.0	8.4	8.0	8.4	7.7
	Conductivity (µmhos/cm)	2130		2130		2090	
	Temperature (°C)	24.7	24.6	24.7	24.7	24.6	24.7
		Initial	Final	Initial	Final	Initial	Final

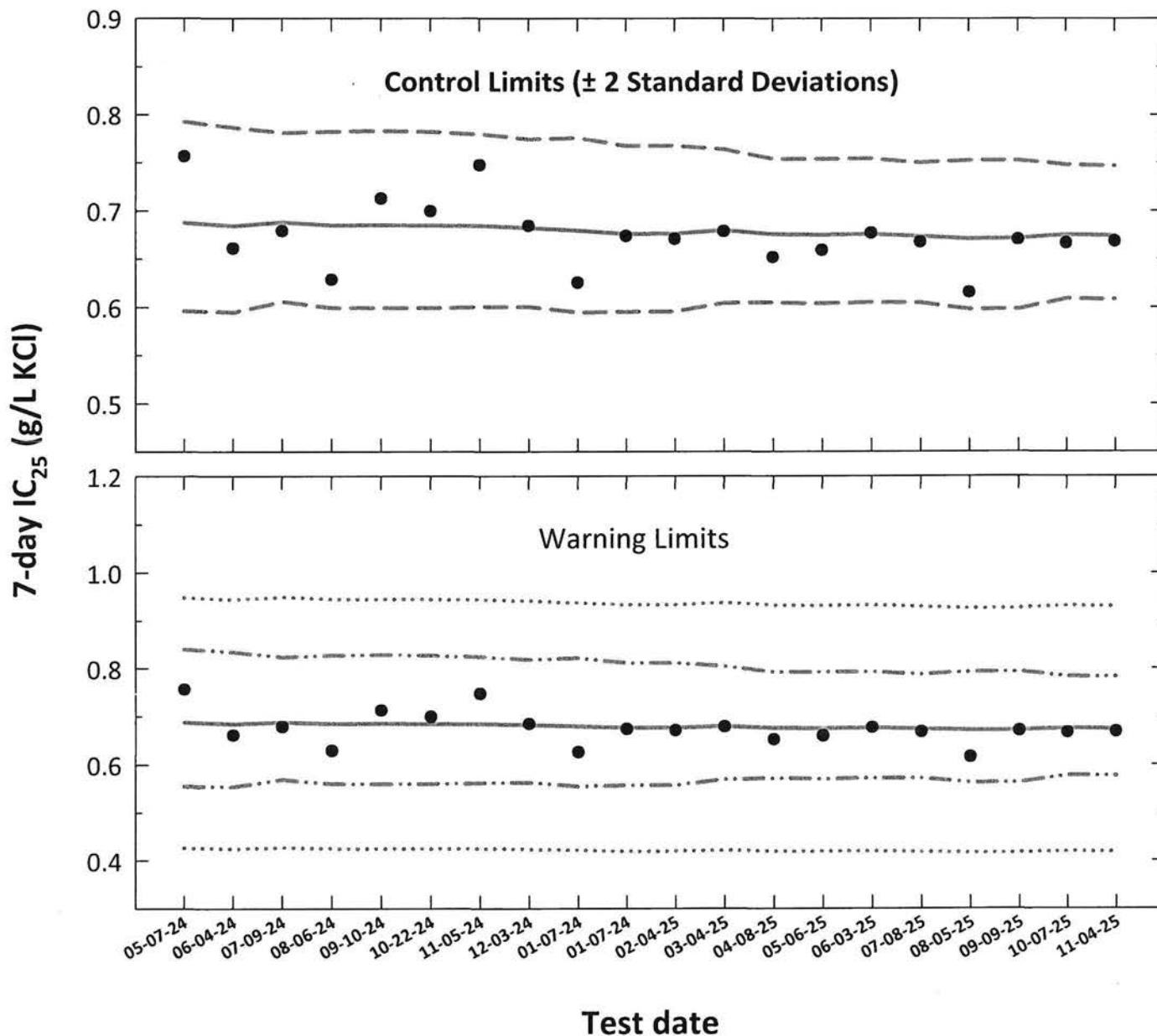
Species: *Pimephales promelas*

PpKICR Test Number: 133

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.84	7.46	7.83	7.72	7.85	7.60	7.85	7.40
	Dissolved oxygen (mg/L)	8.4	7.4	8.6	7.6	* 7.72 (8.4)	7.5	8.3	5.8
	Conductivity (µmhos/cm)	297		288		292		306	
	Alkalinity (mg CaCO ₃ /L)			63					
	Hardness (mg CaCO ₃ /L)			86					
	Temperature (°C)	24.8	24.8	24.7	24.5	24.7	24.8	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.93	7.74	7.92	7.72	7.84	7.65	7.93	7.39
	Dissolved oxygen (mg/L)	8.3	7.7	8.5	7.6	* 7.72 (8.4)	7.6	8.3	5.8
	Conductivity (µmhos/cm)	841		816		802		847	
	Temperature (°C)	24.7	24.5	24.7	24.5	24.8	24.6	24.7	24.5
450 mg KCl/L	pH (S.U.)	7.94	7.72	7.99	7.72	7.96	7.66	7.94	7.39
	Dissolved oxygen (mg/L)	8.4	7.5	8.4	7.2	* 7.72 (8.4)	7.6	8.4	5.9
	Conductivity (µmhos/cm)	1090		1080		1050		1090	
	Temperature (°C)	24.7	24.6	24.9	24.8	24.8	24.6	24.7	24.5
600 mg KCl/L	pH (S.U.)	7.95	7.72	8.00	7.72	7.98	7.69	7.94	7.40
	Dissolved oxygen (mg/L)	8.4	7.3	8.4	7.2	* 7.72 (8.4)	7.6	8.4	5.5
	Conductivity (µmhos/cm)	1330		1320		1290		1350	
	Temperature (°C)	24.9	24.6	24.9	24.6	24.7	24.9	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.95	7.76	8.00	7.70	8.00	7.70	7.95	7.41
	Dissolved oxygen (mg/L)	8.4	7.6	8.4	7.1	* 7.70 (8.5)	7.5	8.4	5.8
	Conductivity (µmhos/cm)	1580		1570		1540		1600	
	Temperature (°C)	24.9	24.6	24.7	24.6	24.7	24.7	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.97	7.76	8.02	7.74	8.00	7.70	7.96	7.42
	Dissolved oxygen (mg/L)	8.4	7.4	8.4	7.4	* 7.74 (8.5)	7.5	8.4	5.9
	Conductivity (µmhos/cm)	1810		1820		1780		1830	
	Temperature (°C)	24.7	24.6	24.7	24.6	24.7	24.7	24.8	24.7
1050 mg KCl/L	pH (S.U.)	7.97	7.76	8.03	7.75	8.02	7.70	7.96	
	Dissolved oxygen (mg/L)	8.4	7.6	8.4	7.5	* 7.75 (8.6)	7.4	8.4	
	Conductivity (µmhos/cm)	2070		2060		2030		2120	
	Temperature (°C)	24.7	24.5	24.8	24.7	24.9	24.7	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 10.12.25
verified correct.
SOP AT21-Revision 5-Exhibit AT21.1

Pimephales promelas Chronic Reference Toxicant Control Chart Source: In-house Culture



- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A,75}$ converted to anti-logarithmic values,
 $S_{A,75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas Chronic Reference Toxicant Control Chart Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)			
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	05-07-24	0.7569	-0.1210	-0.1627	0.0309	0.6876	0.5965 0.7927	0.5551 0.8404	0.4263 0.9489
2	06-04-24	0.6610	-0.1798	-0.1650	0.0303	0.6840	0.5949 0.7864	0.5537 0.8338	0.4241 0.9439
3	07-09-24	0.6789	-0.1682	-0.1624	0.0276	0.6880	0.6060 0.7811	0.5688 0.8233	0.4265 0.9494
4	08-06-24	0.6287	-0.2015	-0.1644	0.0289	0.6848	0.5994 0.7823	0.5602 0.8272	0.4246 0.9450
5	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994 0.7832	0.5600 0.8283	0.4248 0.9456
6	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994 0.7822	0.5601 0.8271	0.4245 0.9449
7	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004 0.7797	0.5617 0.8238	0.4242 0.9442
8	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006 0.7745	0.5626 0.8176	0.4228 0.9411
9	01-07-24	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946 0.7757	0.5547 0.8213	0.4211 0.9372
10	01-07-24	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955 0.7675	0.5569 0.8113	0.4191 0.9329
11	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956 0.7676	0.5571 0.8114	0.4192 0.9331
12	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047 0.7643	0.5693 0.8041	0.4215 0.9381
13	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048 0.7540	0.5708 0.7919	0.4187 0.9319
14	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039 0.7537	0.5697 0.7918	0.4183 0.9310
15	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053 0.7543	0.5715 0.7920	0.4189 0.9324
16	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048 0.7501	0.5715 0.7872	0.4176 0.9295
17	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981 0.7525	0.5623 0.7926	0.4159 0.9258
18	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985 0.7529	0.5628 0.7928	0.4162 0.9263
19	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087 0.7477	0.5769 0.7829	0.4183 0.9310
20	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081 0.7466	0.5763 0.7819	0.4177 0.9298

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

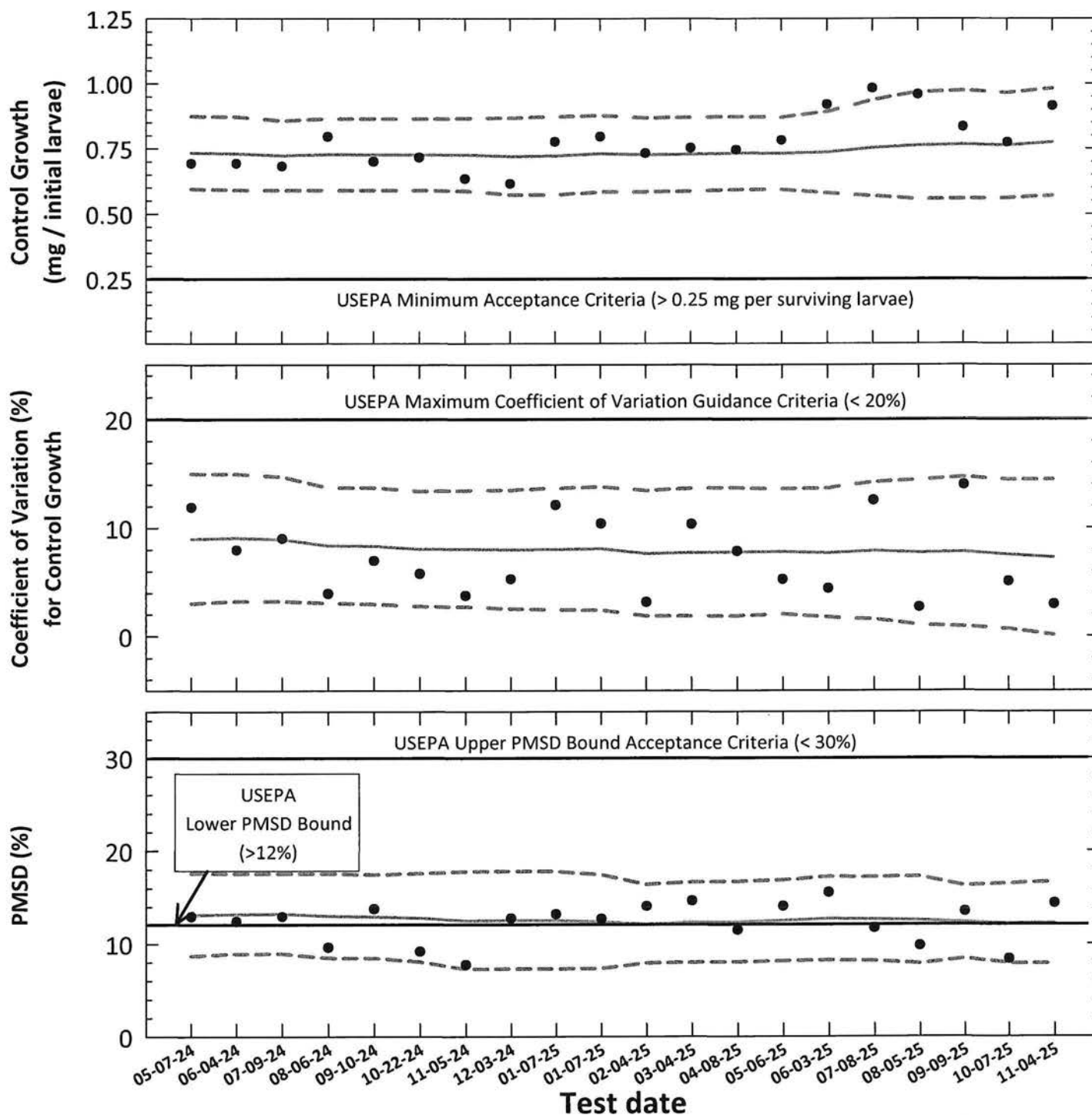
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

CV = Coefficient of variation.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Growth, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Growth, CV or PMSD $\pm$ 2 Standard Deviations)

Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination				Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial larvae)	CV (%)										
1	05-07-24	100	0.693	11.9	0.0896	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
2	06-04-24	100	0.693	8.0	0.0862	0.732	0.591	0.872	9.1	3.2	14.9	13.2	8.9	17.5
3	07-09-24	100	0.683	9.0	0.0883	0.724	0.591	0.857	9.0	3.2	14.7	13.2	8.9	17.5
4	08-06-24	100	0.796	3.9	0.0766	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
5	09-10-24	100	0.701	7.0	0.0964	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
6	10-22-24	100	0.717	5.8	0.0659	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
7	11-05-24	100	0.635	3.7	0.0491	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
8	12-03-24	100	0.615	5.3	0.0785	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
9	01-07-25	100	0.776	12.1	0.1024	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
10	01-07-25	100	0.796	10.4	0.1010	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
11	02-04-25	100	0.733	3.1	0.1030	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
12	03-04-25	100	0.754	10.4	0.1103	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
13	04-08-25	100	0.746	7.8	0.0855	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
14	05-06-25	100	0.783	5.2	0.1099	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
15	06-03-25	100	0.920	4.4	0.1429	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
16	07-08-25	100	0.983	12.5	0.1150	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
17	08-05-25	100	0.959	2.7	0.0941	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
18	09-09-25	100	0.835	14.0	0.1126	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
19	10-07-25	100	0.774	5.0	0.0646	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
20	11-04-25	100	0.914	2.9	0.1313	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.

Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1000.0)

Species: *Pimephales promelas*

PpKCICR Test Number: 134

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2437</u>				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:			Test information:	
Organism source:		In-house culture	Randomizing template:	<u>Yellow</u>
Age:		< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:		<u>10-27-25</u>	Artemia CHM number:	CHM1385
Hatch dates and times:		<u>11-03-25 1130 to 11-04-25 0500</u>	Drying information for weight determination:	
Transfer vessel information:		pH = <u>8.25</u> S.U. Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>11-11-25 0600</u>
Average transfer volume:		< 0.25 mL	*Initial oven temperature:	<u>60° C</u>
			Date / Time out of oven:	<u>11-12-25 0600</u>
			*Final oven temperature:	<u>60° C</u>
			Total drying time:	<u>24 Hours</u>

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	11-04-25	0505	<u>H</u>	1200	<u>HL</u>	0505	<u>H</u>	10-28-25 B
1	11-05-25	0500	<u>H</u>	1230	<u>HL</u>	0500	<u>H</u>	↓
2	11-06-25	0505	<u>H</u>	1120	<u>H</u>	0520	<u>H</u>	10-28-25 C
3	11-07-25	0500	<u>H</u>	1100	<u>H</u>	0500	<u>H</u>	↓
4	11-08-25	0600	<u>H</u>	1200	<u>H</u>	0600	<u>H</u>	11-06-25 A
5	11-09-25	0600	<u>H</u>	1100	<u>H</u>	0600	<u>H</u>	↓
6	11-10-25	0600	<u>HL</u>	1200	<u>H</u>	0600	<u>H</u>	↓
7	11-11-25					0530	<u>HL</u>	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	<u>1306646FS</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>742.1</u>
Average weight per initial larvae:	<u>0.914</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.914</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>670.9</u>
			IC ₂₅ (mg/L KCl)	<u>669.1</u>

Species: Pimephales promelas

PpKCICR Test Number: 134

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>EM</u> Date: <u>10-21-25</u>	10.36	13.59	12.74	10.37	12.20	10.17 <u>10.17</u> <u>10-21-25</u>	12.90	14.17	12.80	12.47	12.84	13.75
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>11-13-25</u>	19.34	22.53	22.26	19.48	23.30	21.82	23.35	24.16	20.79	22.88	22.87	23.51
C = Larvae weight (mg) = B - A Analyst: <u>JP</u>	8.98	8.94	9.52	9.11	11.10	11.65	10.45	9.99	7.99	10.41	10.03	9.76
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JP</u>	0.898	0.894	0.952	0.911	1.110	1.165 <u>1.165</u> <u>11-17-25</u>	1.045 <u>1.045</u> <u>11-17-25</u>	0.999	0.799	1.041	1.003	0.976
Average weight per initial number of larvae (mg)	0.914				1.080				0.955			
Percent reduction from control (%)					-18.2%				-4.5%			

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 134

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	7 ^{3d}	6 ^{4d}	7 ^{3d}	6 ^{4d}
2	10	10	10	10	10	10	9 ^{1d}	9 ^{1d}	7	6	6 ^{1d}	6
3	10	10	10	10	10	10	9	9	7	5 ^{1d}	5 ^{1d}	5 ^{1d}
4	10	10	10	10	9 ^{1d}	9 ^{1d}	9	8 ^{1d}	4 ^{3d}	4 ^{1d}	4 ^{1d}	4 ^{1d}
5	10	10	10	9 ^{1d}	5 ^{1d}	7 ^{2d}	7 ^{2d}	7 ^{1d}	1 ^{3d}	2 ^{2d}	2 ^{2d}	2 ^{2d}
6	10	10	10	9	5	7	7	7	1	2	1 ^{1d}	1 ^{1d}
7	10	10	10	9	5	7	7	7	1	2	1	1
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>EM</u> Date: <u>10-21-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>11-13-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JP</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JP</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.877 0.857 6.3% 0.948 0.617 32.5% 0.138 84.9%												

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 134

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	5 ^{sd}	5 ^{sd}	4 ^{wd}	3 rd
2	4 rd	4 rd	4	3
3	4	4	4	3
4	1 rd	2 rd	1 rd	2 rd
5	1	1 rd	1	1 rd
6	0 rd	1	1	1
7	0	1	1	1
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>EM</u> Date: <u>10-21-25</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>11-13-25</u>				
C = Larvae weight (mg) = B - A Analyst: <u>JP</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JP</u>				
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Pimephales promelas Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1000.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 134
Test dates: November 04-11, 2025

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mass weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	10.36	19.34	8.98	0.898	0.914	2.9	0.898	100.0	0.914	2.9	Not applicable
	B	10	10	13.59	22.53	8.94	0.894			0.894				
	C	10	10	12.74	22.26	9.52	0.952			0.952				
	D	10	10	10.37	19.48	9.11	0.911			0.911				
300	E	10	10	12.20	23.30	11.10	1.110	1.080	6.7	1.110	100.0	1.080	6.7	-18.2
	F	10	10	10.17	21.82	11.65	1.165			1.165				
	G	10	10	12.90	23.35	10.45	1.045			1.045				
	H	10	10	14.17	24.16	9.99	0.999			0.999				
450	I	10	10	12.80	20.79	7.99	0.799	0.955	11.2	0.799	100.0	0.955	11.2	-4.5
	J	10	10	12.47	22.88	10.41	1.041			1.041				
	K	10	10	12.84	22.87	10.03	1.003			1.003				
	L	10	10	13.75	23.51	9.76	0.976			0.976				
600	M	10	10	12.80	21.62	8.82	0.882	0.877	6.5	0.882	97.5	0.857	10.9	6.3
	N	10	10	13.60	23.00	9.40	0.940			0.940				
	O	10	10	12.69	21.51	8.82	0.882			0.882				
	P	10	9	14.10	21.32	7.22	0.802			0.722				
750	Q	10	5	15.06	19.75	4.69	0.938	0.948	5.6	0.469	65.0	0.617	17.1	32.5
	R	10	7	10.12	16.47	6.35	0.907			0.635				
	S	10	7	14.07	20.51	6.44	0.920			0.644				
	T	10	7	13.73	20.91	7.18	1.026			0.718				
900	U	10	1	10.27	11.68	1.41	1.410	1.116	17.8	0.141	12.5	0.138	38.7	84.9
	V	10	2	14.15	16.28	2.13	1.065			0.213				
	W	10	1	12.86	13.85	0.99	0.990			0.099				
	X	10	1	14.80	15.80	1.00	1.000			0.100				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.470	35.4	0.000	7.5	0.037	77.0	96.0
	Z	10	1	14.33	14.63	0.30	0.300			0.030				
	AA	10	1	12.79	13.33	0.54	0.540			0.054				
	BB	10	1	10.31	10.95	0.64	0.640			0.064				

Dunnett's MSD value: 0.1313
PMSD: 14.4

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. Lower PMSD bound determined by USEPA (10th percentile) = 12%. Upper PMSD bound determined by USEPA (90th percentile) = 30%. Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 11/4/2025 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
End Date: 11/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

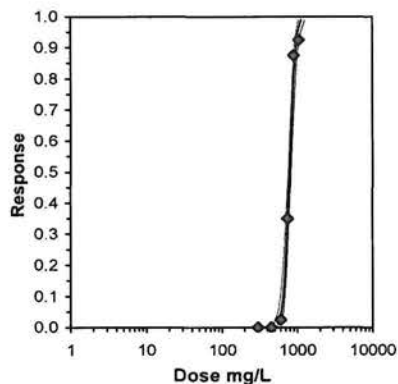
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	0.9000
750	0.5000	0.7000	0.7000	0.7000
900	0.1000	0.2000	0.1000	0.1000
1050	0.0000	0.1000	0.1000	0.1000

Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.6500	0.6500	0.9397	0.7854	0.9912	10.948	4	10.00	10.00	14	40
*900	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
*1050	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40

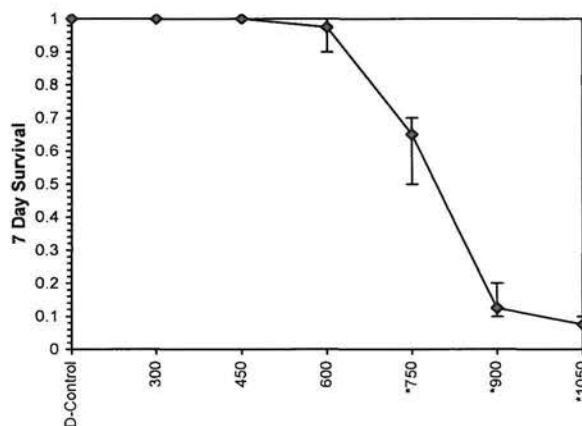
Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.84638	0.896	-1.1929	1.94937
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					600	750	670.82	
Treatments vs D-Control								

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit					
					Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	14.8748	1.8714	11.2069	18.5427	0	3.73489	9.48773	0.44307	2.8988	0.06723
Intercept	-38.119	5.44176	-48.785	-27.453						

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	552.593	484.188	600.458
EC05	3.355	614.074	555.426	655.289
EC10	3.718	649.598	597.085	687.121
EC15	3.964	674.719	626.584	709.875
EC20	4.158	695.376	650.751	728.846
EC25	4.326	713.602	671.919	745.865
EC40	4.747	761.673	726.365	792.708
EC50	5.000	792.137	759.196	824.486
EC60	5.253	823.82	791.533	859.682
EC75	5.674	879.316	843.855	926.467
EC80	5.842	902.362	864.29	955.795
EC85	6.036	929.988	888.06	991.908
EC90	6.282	965.953	918.101	1040.19
EC95	6.645	1021.83	963.34	1117.47
EC99	7.326	1135.52	1051.99	1281.05



Dose-Response Plot



Larval Fish Growth and Survival Test-7 Day Growth

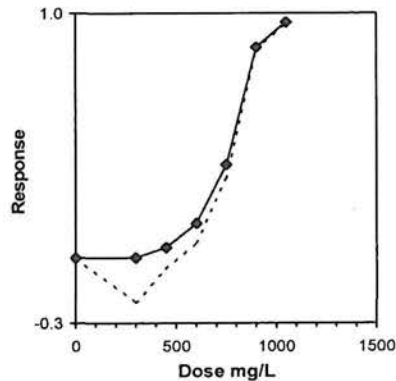
Start Date: 11/4/2025 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
End Date: 11/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.8980	0.8940	0.9520	0.9110
300	1.1100	1.1650	1.0450	0.9990
450	0.7990	1.0410	1.0030	0.9760
600	0.8820	0.9400	0.8820	0.7220
750	0.4690	0.6350	0.6440	0.7180
900	0.1410	0.2130	0.0990	0.1000
1050	0.0000	0.0300	0.0540	0.0640

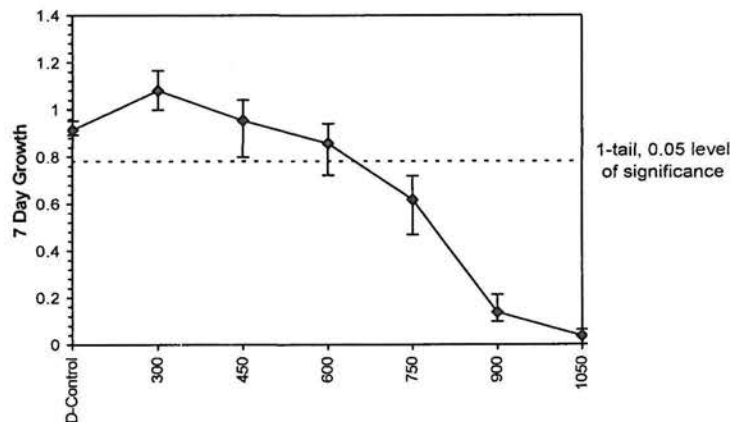
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed		MSD	Isotonic	
			Mean	Min	Max	CV%			Critical			Mean	N-Mean
D-Control	0.9138	1.0000	0.9138	0.8940	0.9520	2.902	4					0.9968	1.0000
300	1.0798	1.1817	1.0798	0.9990	1.1650	6.745	4	-2.896	2.290	0.1313		0.9968	1.0000
450	0.9548	1.0449	0.9548	0.7990	1.0410	11.228	4	-0.715	2.290	0.1313		0.9548	0.9579
600	0.8565	0.9373	0.8565	0.7220	0.9400	10.945	4	0.999	2.290	0.1313		0.8565	0.8593
750	0.6165	0.6747	0.6165	0.4690	0.7180	17.053	4					0.6165	0.6185
900	0.1383	0.1513	0.1383	0.0990	0.2130	38.725	4					0.1383	0.1387
1050	0.0370	0.0405	0.0370	0.0000	0.0640	77.015	4					0.0370	0.0371

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)				0.90352	0.844	-0.9413	0.35066
Bartlett's Test indicates equal variances ($p = 0.24$)				4.21501	11.3449		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnett's Test				600	>600		
Treatments vs D-Control				0.13127	0.14366	0.03588	0.00657
				0.01338	3, 12		

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL(Exp)	Skew	
IC05	461.97	57.15	292.94	593.39	-0.3837
IC10	538.05	56.87	337.40	659.47	-0.5225
IC15	605.79	34.38	491.44	666.01	-1.3217
IC20	636.94	23.09	557.73	694.39	-0.5940
IC25	668.09	21.07	605.23	734.47	-0.3203
IC40	755.79	17.28	682.10	788.73	-0.9244
IC50	787.05	12.25	736.99	814.31	-0.9172



Dose-Response Plot



Species: *Pimephales promelas*

PpKICR Test Number: 134

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

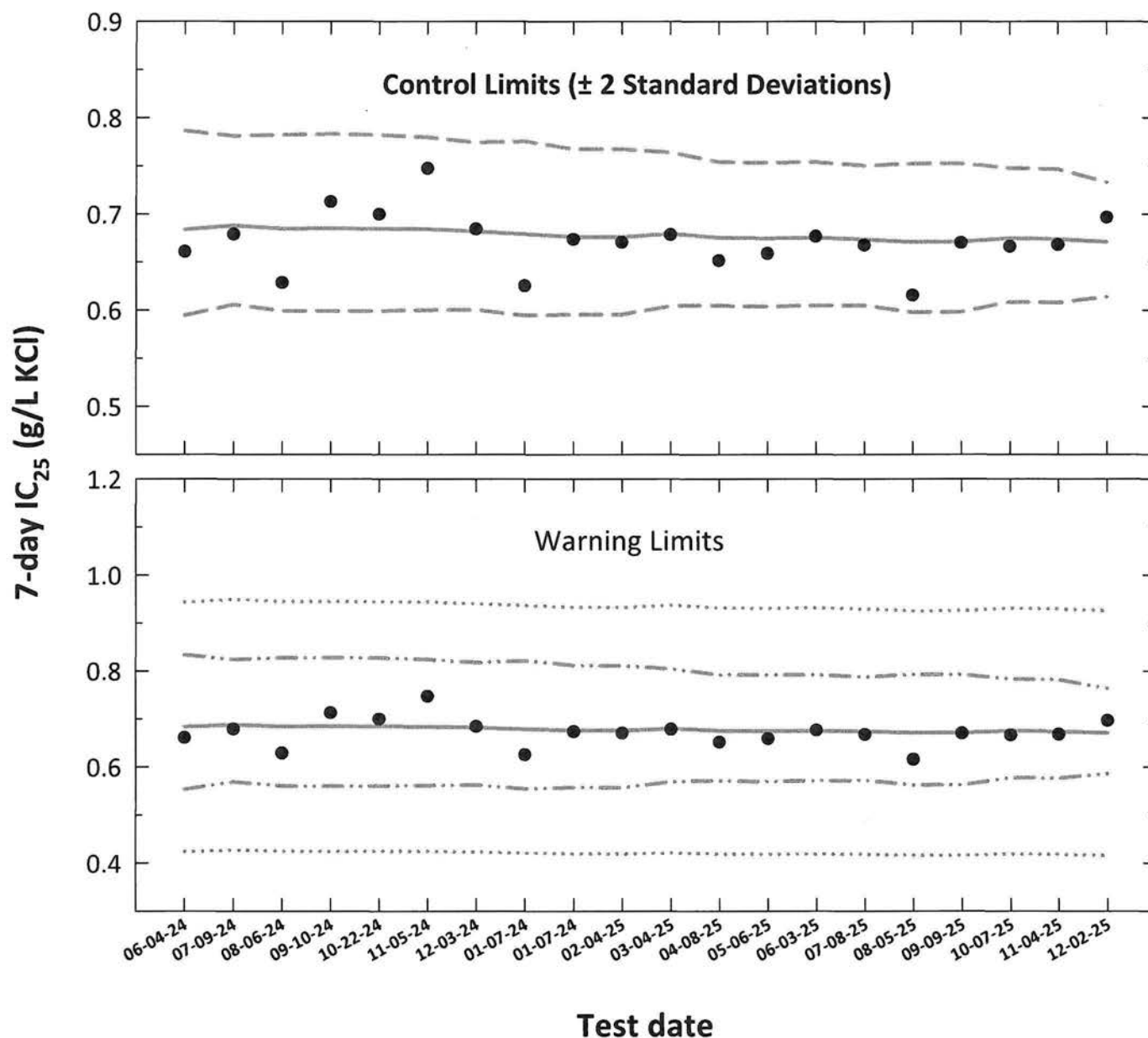
Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.80	7.85	7.75	7.70	7.87	7.67
	Dissolved oxygen (mg/L)	8.5	8.1	8.3	7.7	8.3	7.4
	Conductivity (µmhos/cm)	299		300		299	
	Alkalinity (mg CaCO ₃ /L)	63				63	
	Hardness (mg CaCO ₃ /L)	91				89	
	Temperature (°C)	24.8	24.7	24.8	24.7	24.7	25.1
300 mg KCl/L	pH (S.U.)	7.89	7.86	7.88	7.68	7.89	7.65
	Dissolved oxygen (mg/L)	8.6	7.9	8.2	7.5	8.2	7.4
	Conductivity (µmhos/cm)	809		814		816	
	Temperature (°C)	24.8	24.9	24.9	24.7	24.7	24.7
450 mg KCl/L	pH (S.U.)	7.90	7.86	7.89	7.68	7.91	7.66
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.4	8.3	7.4
	Conductivity (µmhos/cm)	1040		1060		1050	
	Temperature (°C)	24.9	24.9	24.9	24.6	24.7	24.7
600 mg KCl/L	pH (S.U.)	7.91	7.85	7.90	7.67	7.92	7.67
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.3	8.3	7.3
	Conductivity (µmhos/cm)	1280		1290		1290	
	Temperature (°C)	24.9	25.0	25.0	24.6	24.8	24.9
750 mg KCl/L	pH (S.U.)	7.91	7.86	7.90	7.67	7.92	7.67
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.3	8.3	7.3
	Conductivity (µmhos/cm)	1500		1520		1530	
	Temperature (°C)	25.0	24.8	25.0	24.6	24.7	24.9
900 mg KCl/L	pH (S.U.)	7.91	7.87	7.91	7.68	7.94	7.69
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.4	8.3	7.3
	Conductivity (µmhos/cm)	1760		1800		1790	
	Temperature (°C)	24.9	24.8	24.9	25.0	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.92	7.87	7.92	7.71	7.95	7.70
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	7.7	8.3	7.5
	Conductivity (µmhos/cm)	2000		2040		2020	
	Temperature (°C)	24.9	24.8	24.9	24.9	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

PpKCICR Test Number: 134

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.85	7.65	7.70	BSL 11-08-25 7.62 (4.62)	7.78	7.58	7.73	7.52
	Dissolved oxygen (mg/L)	8.3	7.5	8.5	7.2	8.4	7.3	8.3	6.3
	Conductivity (µmhos/cm)	800		BSL 11-08-25 782 (285)		285		289	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			87					
	Temperature (°C)	24.7	24.9	24.7	24.8	24.8	24.9	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.93	7.68	8.00	7.64	7.99	7.60	7.98	7.47
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	7.1	8.2	7.3	8.3	5.8
	Conductivity (µmhos/cm)	810		BSL 11-08-25 1010 (782)		778		782	
	Temperature (°C)	24.7	24.7	24.9	25.0	24.9	25.1	24.8	24.9
450 mg KCl/L	pH (S.U.)	7.94	7.69	8.01	7.64	8.01	7.61	7.99	7.49
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	7.1	8.3	7.3	8.3	5.0
	Conductivity (µmhos/cm)	1030		BSL 11-08-25 1240 (1010)		1010		1020	
	Temperature (°C)	24.7	24.7	24.7	25.1	24.9	26.1	24.9	24.9
600 mg KCl/L	pH (S.U.)	7.94	BSL 11-08-25 7.73 (7.73)	8.01	7.65	8.01	7.62	8.00	7.48
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	7.2	8.3	7.2	8.3	5.0
	Conductivity (µmhos/cm)	1290		BSL 11-08-25 1440 (1240)		1240		1260	
	Temperature (°C)	24.7	24.6	24.7	25.1	25.0	24.9	24.9	25.0
750 mg KCl/L	pH (S.U.)	7.96	BSL 11-08-25 7.73 (7.73)	8.01	7.64	8.03	7.62	8.00	7.50
	Dissolved oxygen (mg/L)	8.2	7.6	8.3	BSL 11-08-25 7.1 (6.8)	8.3	7.2	8.3	5.7
	Conductivity (µmhos/cm)	1520		BSL 11-08-25 1730 (1490)		1470		1480	
	Temperature (°C)	24.6	24.6	24.7	25.1	25.0	25.2	24.9	24.8
900 mg KCl/L	pH (S.U.)	7.96	BSL 11-08-25 7.73 (7.73)	8.01	7.65	8.03	7.62	8.01	7.49
	Dissolved oxygen (mg/L)	8.2	7.7	8.3	7.2	8.3	6.9	8.3	5.7
	Conductivity (µmhos/cm)	1770		1730		1750		1790	
	Temperature (°C)	24.6	24.8	24.8	24.7	25.0	25.2	25.0	24.8
1050 mg KCl/L	pH (S.U.)	7.97	BSL 11-08-25 7.82	8.01	7.71	8.04	7.64	8.01	7.48
	Dissolved oxygen (mg/L)	8.2	7.8	8.3	7.3	8.3	7.3	8.3	5.5
	Conductivity (µmhos/cm)	2020		1940		1970		2000	
	Temperature (°C)	24.6	24.7	24.8	24.7	25.0	25.0	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Pimephales promelas Chronic Reference Toxicant Control Chart Source: In-house Culture



- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas Chronic Reference Toxicant Control Chart Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)			
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	06-04-24	0.6610	-0.1798	-0.1650	0.0303	0.6840	0.5949 0.7864	0.5537 0.8338	0.4241 0.9439
2	07-09-24	0.6789	-0.1682	-0.1624	0.0276	0.6880	0.6060 0.7811	0.5688 0.8233	0.4265 0.9494
3	08-06-24	0.6287	-0.2015	-0.1644	0.0289	0.6848	0.5994 0.7823	0.5602 0.8272	0.4246 0.9450
4	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994 0.7832	0.5600 0.8283	0.4248 0.9456
5	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994 0.7822	0.5601 0.8271	0.4245 0.9449
6	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004 0.7797	0.5617 0.8238	0.4242 0.9442
7	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006 0.7745	0.5626 0.8176	0.4228 0.9411
8	01-07-24	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946 0.7757	0.5547 0.8213	0.4211 0.9372
9	01-07-24	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955 0.7675	0.5569 0.8113	0.4191 0.9329
10	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956 0.7676	0.5571 0.8114	0.4192 0.9331
11	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047 0.7643	0.5693 0.8041	0.4215 0.9381
12	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048 0.7540	0.5708 0.7919	0.4187 0.9319
13	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039 0.7537	0.5697 0.7918	0.4183 0.9310
14	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053 0.7543	0.5715 0.7920	0.4189 0.9324
15	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048 0.7501	0.5715 0.7872	0.4176 0.9295
16	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981 0.7525	0.5623 0.7926	0.4159 0.9258
17	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985 0.7529	0.5628 0.7928	0.4162 0.9263
18	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087 0.7477	0.5769 0.7829	0.4183 0.9310
19	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081 0.7466	0.5763 0.7819	0.4177 0.9298
20	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141 0.7331	0.5862 0.7636	0.4160 0.9260

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

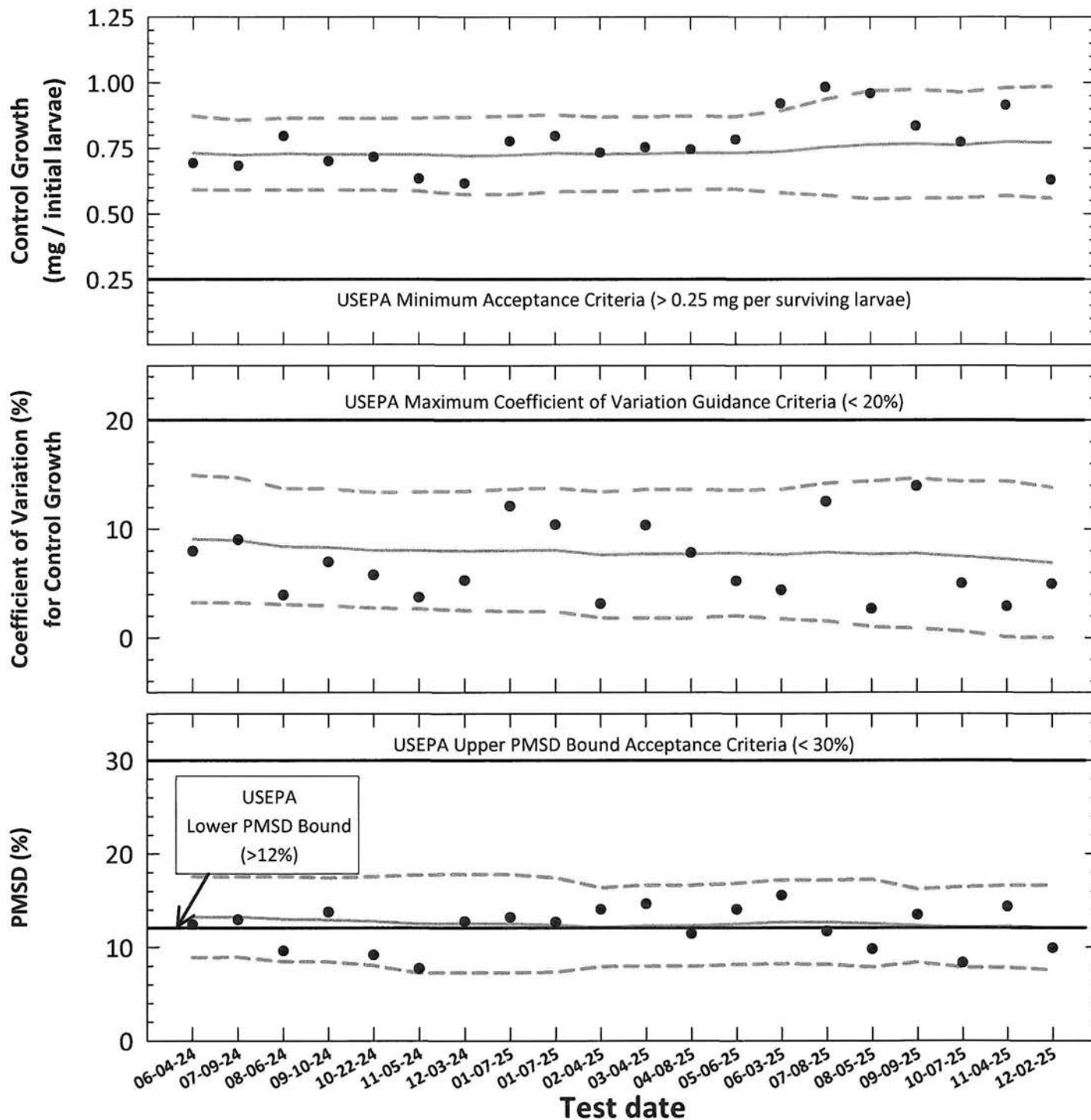
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

CV = Coefficient of variation.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Growth, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Growth, CV or PMSD $\pm$ 2 Standard Deviations)

Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Growth		Control Growth (mg/initial larvae)		Control Growth CV		Test PMSD		
		Control Survival (%)	Control Growth		Test		CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)									
1	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.2	8.9	17.5
2	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.2	8.9	17.5
3	08-06-24	100	0.796	3.9	0.0766	9.6	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
4	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
5	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
6	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
7	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
8	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
9	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
10	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
11	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
12	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
13	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
14	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
15	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
16	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
17	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
18	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
19	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
20	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.

USEPA maximum CV guidance criteria (90th percentile) < 20%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) > 12%.

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

CT = Central tendency of the growth, CV or PMSD values.

S = Standard deviation of the growth, CV or PMSD values.

Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1000.0)

Species: *Pimephales promelas*

PpKICR Test Number: 135

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2437</u>				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>11-25-25</u>	Artemia CHM number:	CHM1385
Hatch dates and times:	<u>12-01-25 1400 TO 12-02-25 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>8.24</u> S.U. Temperature = <u>24.1</u> °C	Date / Time in oven:	<u>12-09-25 0530</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>12-10-25 0530</u>
		*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24 hours</u>

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	12-02-25	0505	H	1430	H	0710	H	11-24-25 A
1	12-03-25	0500	H	1100	H	0700	H	↓
2	12-04-25	0500	H	1340	H	0700	H	11-24-25 B
3	12-05-25	0500	H	1100	H	0700	H	↓
4	12-06-25	0630	H	1230	H	0630	H	12-03-25
5	12-07-25	0600	H	1200	H	0600	H	↓
6	12-08-25	0500	H	1100	H	0700	H	↓
7	12-09-25					0530	H	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>814.8</u>
Average weight per initial larvae:	<u>0.629</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.629</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>670.8</u>
			IC ₂₅ (mg/L KCl)	<u>696.7</u>

Species: *Pimephales promelas*

PpKCICR Test Number: 135

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	11	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>magenta</u> Analyst: <u>BM</u> Date: <u>11-17-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>J</u> Date: <u>12-12-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>A</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.629 0.751 -14.37 0.687 -9.17												

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: *Pimephales promelas*

PpKCICR Test Number: 135

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	9 ^d	10	9 ^d	7 ^{sk}	7 ^{sk}	7 ^{sk}	7 ^{sk}
2	10	10	10	10	9 ^d	9	10	9	6 ^d	6 ^d	6 ^d	6 ^d
3	10	10	10	10	9	9	10	9	5 ^{sk}	5 ^{sk}	5 ^{sk}	4 ^{sk}
4	10	10	10	10	9	9	10	9	5	5	5	4
5	10	10	10	10	8 ^d	9	10	9	4 ^{sk}	4 ^{sk}	5	4
6	10	10	10	10	9	9	10	9	4	4	3 ^{sk}	4
7	10	10	10	10	6 ^{sk}	7 ^{sk}	7 ^{sk}	7 ^{sk}	3 ^{sk}	3 ^{sk}	2 ^{sk}	3 ^{sk}
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>jm</u> Date: <u>11-17-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>jm</u> Date: <u>12-13-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>jm</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>jm</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.737 -17.27 0.429 31.87 0.224 64.47												

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: Pimephales promelas

PpKCICR Test Number: 135

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	4 ^{ad}	3 ^{ad}	3 ^{ad}	3 ^{ad}
2	3 ^{ad}	1 ^{ad}	3	1 ^{ad}
3	3	1	1 ^{ad}	1
4	2 ^{ad}	1	0 ^{ad}	1
5	1 ^{ad}	0 ^{ad}	0	1
6	1	0	0	1
7	1	0	0	0 ^{ad}
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>gm</u> Date: <u>11-17-25</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>gm</u> Date: <u>12-13-25</u>				
C = Larvae weight (mg) = B - A Analyst: <u>gm</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>gm</u>				
Average weight per initial number of larvae (mg) Percent reduction from control (%)				
0.016 97.27				

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Pimephales promelas Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1000.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 135
Test dates: December 02-09, 2025

Concentration (mg/L KO)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	10.10	16.38	6.28	0.628							
	B	10	10	13.54	19.48	5.94	0.594	0.629	5.0		100.0	0.629	5.0	Not applicable
	C	10	10	11.87	18.57	6.70	0.670							
	D	10	10	12.39	18.64	6.25	0.625							
300	E	10	10	12.61	19.24	6.63	0.663							
	F	10	10	12.45	20.33	7.88	0.788	0.751	8.0		100.0	0.751	8.0	-19.3
	G	10	10	13.57	21.16	7.59	0.759							
	H	10	10	9.85	17.78	7.93	0.793							
450	I	10	10	10.67	17.61	6.94	0.694							
	J	10	10	9.75	16.58	6.83	0.683	0.687	1.3		100.0	0.687	1.3	-9.1
	K	10	10	13.65	20.41	6.76	0.676							
	L	10	10	13.35	20.28	6.93	0.693							
600	M	10	10	13.34	20.64	7.30	0.730							
	N	10	10	10.24	17.15	6.91	0.691	0.737	4.7		100.0	0.737	4.7	-17.2
	O	10	10	9.99	17.58	7.59	0.759							
	P	10	10	12.02	19.71	7.69	0.769							
750	Q	10	6	12.54	16.75	4.21	0.702							
	R	10	7	10.20	14.40	4.20	0.600	0.638	7.0		67.5	0.429	2.4	31.8
	S	10	7	10.73	15.15	4.42	0.631							
	T	10	7	15.11	19.44	4.33	0.618							
900	U	10	3	12.88	15.03	2.35	0.783							
	V	10	3	9.55	11.91	2.36	0.787	0.831	15.0		27.5	0.224	6.9	64.4
	W	10	2	13.89	15.92	2.03	1.015							
	X	10	3	14.26	16.48	2.22	0.740							
1050	Y	10	1	12.92	13.63	0.71	0.710							
	Z	10	0	0.00	0.00	0.00	0.000	0.710	0.0		2.5	0.018	200.0	97.2
	AA	10	0	0.00	0.00	0.00	0.000							
	BB	10	0	0.00	0.00	0.00	0.000							

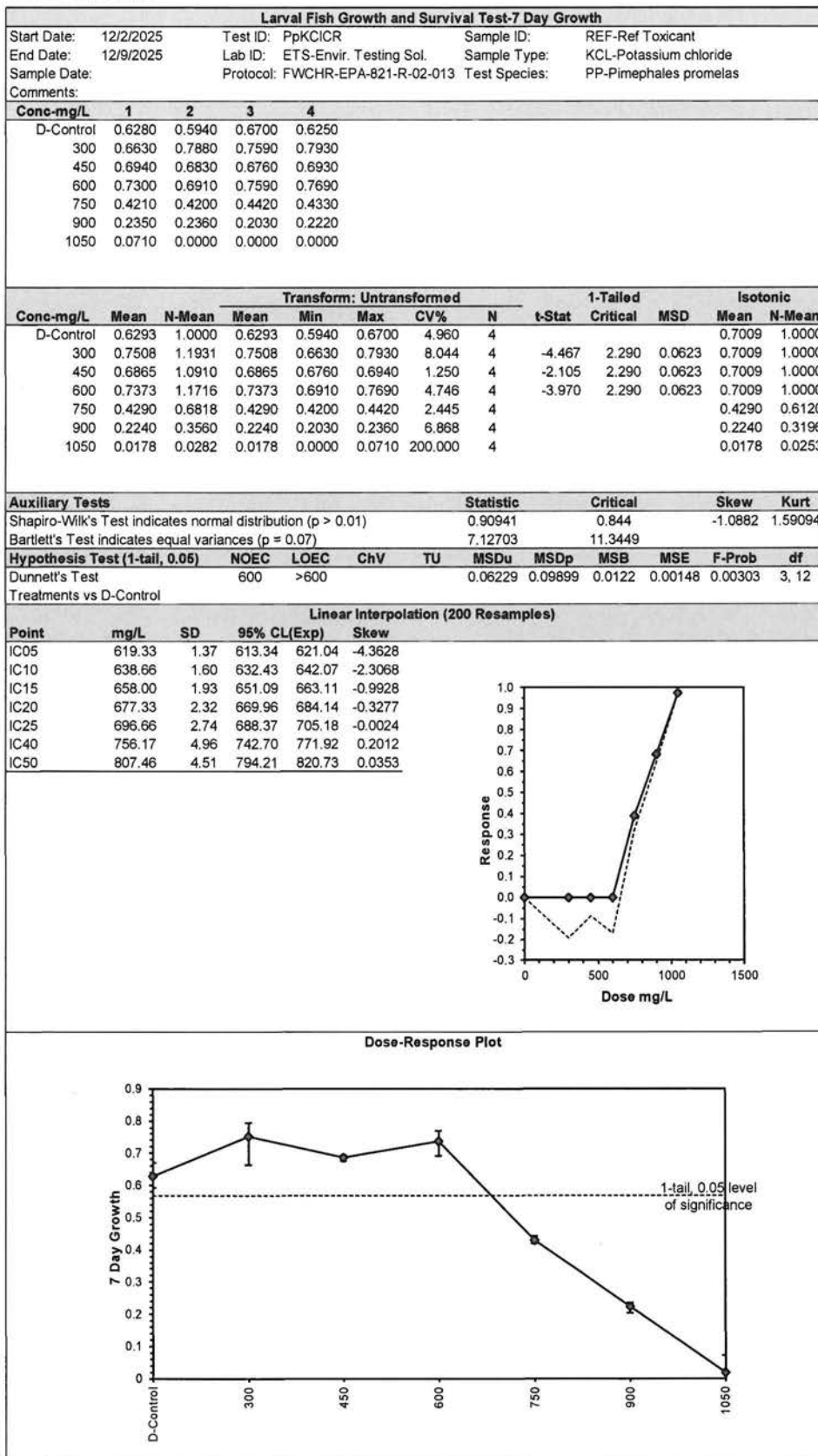
Dunnett's MSD value: 0.0623
PMDS: 9.9

MSD =
PMDS =

Minimum Significant Difference
Percent Minimum Significant Difference

PMDS is a measure of test precision. The PMDS is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. Lower PMDS bound determined by USEPA (10th percentile) = 12%. Upper PMDS bound determined by USEPA (90th percentile) = 30%. Lower and upper PMDS bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Larval Fish Growth and Survival Test-7 Day Survival												
Start Date:	12/2/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant							
End Date:	12/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride							
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas							
Comments:												
Conc-mg/L	1	2	3	4								
D-Control	1.0000	1.0000	1.0000	1.0000								
300	1.0000	1.0000	1.0000	1.0000								
450	1.0000	1.0000	1.0000	1.0000								
600	1.0000	1.0000	1.0000	1.0000								
750	0.6000	0.7000	0.7000	0.7000								
900	0.3000	0.3000	0.2000	0.3000								
1050	0.1000	0.0000	0.0000	0.0000								
Transform: Arcsin Square Root												
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total Number	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40	
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40	
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40	
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40	
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40	
*900	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40	
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40	
Auxiliary Tests								Statistic	Critical	Skew	Kurt	
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)								0.80498	0.896	0.45021	4.24647	
Equality of variance cannot be confirmed												
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU						
Steel's Many-One Rank Test			600	750	670.82							
Treatments vs D-Control												
Maximum Likelihood-Probit												
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	16.8172	2.19726	12.5106	21.1239	0	1.51206	9.48773	0.8245	2.91103	0.05946	3	
Intercept	-43.955	6.41338	-56.526	-31.385								
TSCR												
Point	Probits	mg/L	95% Fiducial Limits									
EC01	2.674	592.517	524.025	639.199								
EC05	3.355	650.466	592.524	690.266								
EC10	3.718	683.64	632.128	719.708								
EC15	3.964	706.973	659.98	740.674								
EC20	4.158	726.084	682.682	758.112								
EC25	4.326	742.891	702.483	773.727								
EC40	4.747	786.987	753.042	816.589								
EC50	5.000	814.765	783.266	845.586								
EC60	5.253	843.523	812.835	877.624								
EC75	5.674	893.592	860.261	938.167								
EC80	5.842	914.276	878.651	964.64								
EC85	6.036	938.991	899.954	997.139								
EC90	6.282	971.039	926.75	1040.42								
EC95	6.645	1020.56	966.862	1109.29								
EC99	7.326	1120.37	1044.72	1253.56								



Species: *Pimephales promelas*

PpKCICR Test Number: 135

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.93	7.91	7.80	7.81	7.83	7.79
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.5	7.7
	Conductivity (µmhos/cm)	290		300		290	
	Alkalinity (mg CaCO ₃ /L)	63				63	
	Hardness (mg CaCO ₃ /L)	88				88	
	Temperature (°C)	24.8	24.5	24.6	24.6	24.8	24.5
300 mg KCl/L	pH (S.U.)	7.99	7.91	7.95	7.81	7.96	7.76
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.4	7.4
	Conductivity (µmhos/cm)	800		825		820	
	Temperature (°C)	25.0	24.7	24.6	24.9	24.7	24.8
450 mg KCl/L	pH (S.U.)	8.00	7.90	7.96	7.81	7.97	7.76
	Dissolved oxygen (mg/L)	8.4	8.0	8.3	8.0	8.5	7.4
	Conductivity (µmhos/cm)	1050		1080		1060	
	Temperature (°C)	25.1	24.8	24.8	24.7	24.7	24.7
600 mg KCl/L	pH (S.U.)	8.01	7.90	7.96	7.83	7.97	7.77
	Dissolved oxygen (mg/L)	8.4	8.0	8.3	8.1	8.5	7.5
	Conductivity (µmhos/cm)	1300		1310		1300	
	Temperature (°C)	24.9	24.5	24.6	24.7	24.8	24.5
750 mg KCl/L	pH (S.U.)	8.01	7.90	7.97	7.83	7.98	7.77
	Dissolved oxygen (mg/L)	8.4	7.9	8.3	8.0	8.5	7.6
	Conductivity (µmhos/cm)	1530		1570		1570	
	Temperature (°C)	24.9	24.5	24.7	25.0	24.8	24.5
900 mg KCl/L	pH (S.U.)	8.01	7.88	7.97	7.84	7.98	7.79
	Dissolved oxygen (mg/L)	8.4	7.9	8.3	8.0	8.5	7.6
	Conductivity (µmhos/cm)	1760		1820		1800	
	Temperature (°C)	25.2	24.8	24.7	25.0	24.8	24.8
1050 mg KCl/L	pH (S.U.)	8.01	7.88	7.99	7.84	7.99	7.80
	Dissolved oxygen (mg/L)	8.4	7.9	8.3	8.0	8.5	7.6
	Conductivity (µmhos/cm)	2050		2090		2080	
	Temperature (°C)	25.2	24.5	25.0	24.9	24.7	24.7
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

PpKICR Test Number: 135

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.89	7.76	7.82	7.66	7.85	7.73	7.87	7.78
	Dissolved oxygen (mg/L)	8.2	8.0	8.5	7.4	8.3	7.8	8.4	8.1
	Conductivity (µmhos/cm)	303		288		288		291	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			81					
	Temperature (°C)	24.7	24.9	24.7	24.8	24.8	24.6	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.99	7.78	8.01	7.67	8.03	7.73	7.97	7.77
	Dissolved oxygen (mg/L)	8.2	8.0	8.5	7.4	8.3	7.8	8.3	7.8
	Conductivity (µmhos/cm)	819		795		817		818	
	Temperature (°C)	24.8	25.0	24.8	24.7	24.9	24.6	24.8	24.6
450 mg KCl/L	pH (S.U.)	7.98	7.81	8.02	7.68	8.05	7.74	7.98	7.78
	Dissolved oxygen (mg/L)	8.2	8.1	8.5	7.4	8.4	7.8	8.4	7.9
	Conductivity (µmhos/cm)	1070		1030		1060		1040	
	Temperature (°C)	24.7	24.7	24.7	24.7	24.7	25.0	24.8	24.6
600 mg KCl/L	pH (S.U.)	8.00	7.82	8.02	7.69	8.05	7.76	7.98	7.80
	Dissolved oxygen (mg/L)	8.2	8.1	8.5	7.4	8.4	7.8	8.4	8.0
	Conductivity (µmhos/cm)	1330		1270		1310		1300	
	Temperature (°C)	24.7	24.7	24.7	24.9	24.7	24.7	24.7	24.3
750 mg KCl/L	pH (S.U.)	8.01	7.83	8.02	7.70	8.06	7.77	7.99	7.80
	Dissolved oxygen (mg/L)	8.2	8.1	8.6	7.3	8.4	7.7	8.4	7.8
	Conductivity (µmhos/cm)	1570		1490		1560		1550	
	Temperature (°C)	24.9	24.8	24.7	24.9	24.7	24.7	24.7	24.8
900 mg KCl/L	pH (S.U.)	8.02	7.83	8.02	7.71	8.06	7.77	7.99	7.84
	Dissolved oxygen (mg/L)	8.3	8.1	8.6	7.4	8.4	7.7	8.4	8.0
	Conductivity (µmhos/cm)	1810		1730		1780		1800	
	Temperature (°C)	24.9	24.6	24.9	25.0	24.7	24.7	24.7	24.5
1050 mg KCl/L	pH (S.U.)	8.02	7.83	8.02	7.74	8.06	7.78	7.99	7.77
	Dissolved oxygen (mg/L)	8.2	8.1	8.5	7.4	8.4	7.8	8.4	7.8
	Conductivity (µmhos/cm)	2070		2000		2050		2050	
	Temperature (°C)	24.7	24.9	24.9	24.8	24.7	24.9	24.7	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final