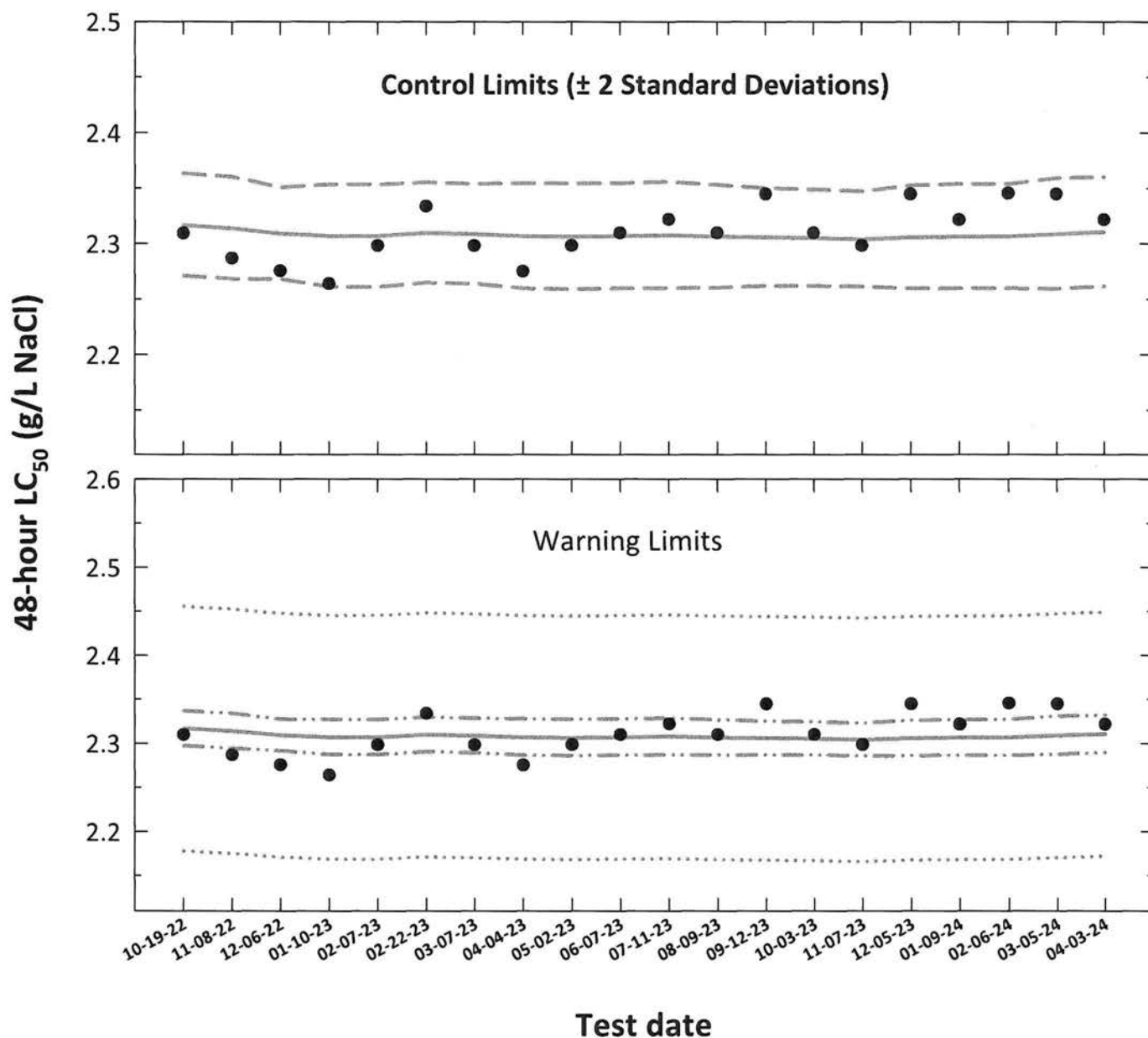


Ceriodaphnia dubia

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	10-19-22	2.3096	0.3635	0.3649	0.0043	2.3168	2.2712 2.3633	2.2971 2.3368	2.1778 2.4558
2	11-08-22	2.2868	0.3592	0.3643	0.0043	2.3139	2.2685 2.3602	2.2943 2.3339	2.1750 2.4527
3	12-06-22	2.2755	0.3571	0.3635	0.0039	2.3093	2.2682 2.3510	2.2915 2.3274	2.1707 2.4478
4	01-10-23	2.2639	0.3549	0.3630	0.0043	2.3069	2.2613 2.3536	2.2871 2.3271	2.1685 2.4454
5	02-07-23	2.2984	0.3614	0.3630	0.0043	2.3069	2.2612 2.3535	2.2871 2.3271	2.1685 2.4454
6	02-22-23	2.3339	0.3681	0.3636	0.0042	2.3099	2.2651 2.3555	2.2905 2.3296	2.1713 2.4485
7	03-07-23	2.2984	0.3614	0.3634	0.0042	2.3087	2.2641 2.3542	2.2894 2.3284	2.1702 2.4472
8	04-04-23	2.2753	0.3570	0.3630	0.0045	2.3070	2.2600 2.3549	2.2866 2.3278	2.1686 2.4454
9	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593 2.3545	2.2860 2.3272	2.1680 2.4448
10	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600 2.3549	2.2866 2.3278	2.1686 2.4454
11	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601 2.3560	2.2870 2.3286	2.1691 2.4460
12	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605 2.3534	2.2865 2.3268	2.1681 2.4448
13	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623 2.3503	2.2870 2.3251	2.1675 2.4442
14	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622 2.3492	2.2866 2.3243	2.1670 2.4436
15	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617 2.3474	2.2857 2.3229	2.1659 2.4424
16	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598 2.3529	2.2859 2.3263	2.1675 2.4442
17	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599 2.3540	2.2863 2.3271	2.1681 2.4449
18	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598 2.3542	2.2863 2.3272	2.1681 2.4449
19	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594 2.3593	2.2874 2.3307	2.1703 2.4474
20	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619 2.3603	2.2895 2.3321	2.1719 2.4492

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 Test

CdNaClAC # 407

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
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 #: 2264

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.92	8.13	7.68
	Dissolved oxygen (mg/L)	7.9	7.6	7.7
	Conductivity (µmhos/cm)	299		
	Alkalinity (mg/L CaCO ₃)	61		
	Hardness (mg/L CaCO ₃)	88		
	Temperature (°C)	24.7	25.0	24.9
1750 mg/L	pH (S.U.)	7.92	8.14	7.90
	Dissolved oxygen (mg/L)	7.9	7.6	7.7
	Conductivity (µmhos/cm)	3290		
	Temperature (°C)	24.6	25.2	25.2
2000 mg/L	pH (S.U.)	7.91	8.15	7.80
	Dissolved oxygen (mg/L)	8.0	7.5	7.7
	Conductivity (µmhos/cm)	3780		
	Temperature (°C)	24.6	25.2	25.1
2250 mg/L	pH (S.U.)	7.93	8.14	7.81
	Dissolved oxygen (mg/L)	8.0	7.5	7.8
	Conductivity (µmhos/cm)	4120		
	Temperature (°C)	24.6	25.3	24.8
2500 mg/L	pH (S.U.)	7.94	8.14	7.80
	Dissolved oxygen (mg/L)	8.0	7.6	8.0
	Conductivity (µmhos/cm)	4540		
	Temperature (°C)	24.6	25.1	25.3
2750 mg/L	pH (S.U.)	7.91	8.15	7.81
	Dissolved oxygen (mg/L)	8.0	7.6	8.0
	Conductivity (µmhos/cm)	5040		
	Temperature (°C)	24.6	24.9	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 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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1366468

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 # 401

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-03-24	0415	K	0700	K	2B2	RED	04-01-24A
24	04-04-24			0700	K			
48 Termination	04-05-24			0700	K			

*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):	03-26-24 D
Age:	< 24-hours old
Date and time organisms were born between:	04-02-24 0900 TO 04-03-24 0415
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.00
	Temperature (°C): 25.1

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 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	2250 mg/L				2500 mg/L				2750 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	5	5	5	4 ^{id}	5	4 ^{id}	3 ^{id}	3 ^{id}	4 ^{id}
48 Termination	4 ^{id}	2 ^{id}	4 ^{id}	2 ^{id}	0 ^{id}	2 ^{id}	2 ^{id}	0 ^{id}	0 ^{id}	0 ^{id}	0 ^{id}	0 ^{id}
Mean Survival	60%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	2249.4
Upper 95% confidence limit (mg NaCl/L)	2391.9
48-hour LC ₅₀ (mg NaCl/L)	2321.7

Comments:

Test
Reviewed by:
M

Acute Daphnid Test-48 Hr Survival

Start Date: 4/3/2024 Test ID: CdNaCIAC Sample ID: REF-Ref Toxicant
End Date: 4/5/2024 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:

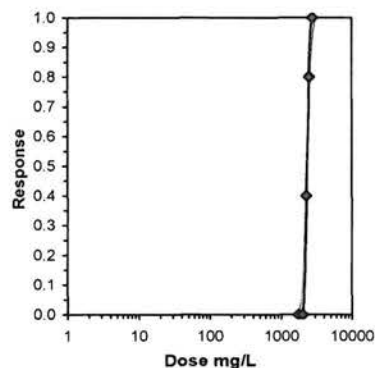
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.8000	0.4000	0.8000	0.4000
2500	0.0000	0.4000	0.4000	0.0000
2750	0.0000	0.0000	0.0000	0.0000

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
1750	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
*2250	0.6000	0.6000	0.8959	0.6847	1.1071	27.222	4	10.00	10.00	8	20
*2500	0.2000	0.2000	0.4551	0.2255	0.6847	58.254	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

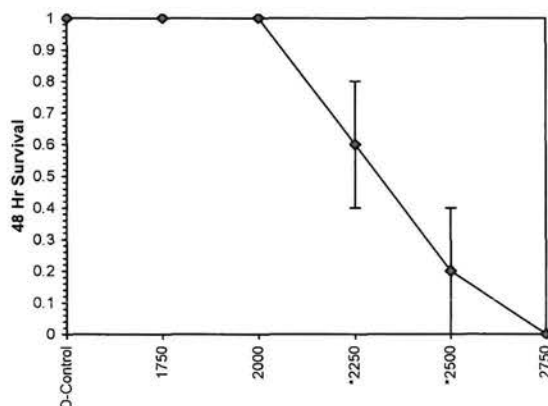
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.80917	0.868	9.3E-16	-0.2568
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	32.4604	6.07902	20.5455 44.3753	0	1.40785	7.81472	0.70369	3.3658	0.03081
Intercept	-104.26	20.4773	-144.39 -64.12						
TSCR									

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1968.48	1771.73 2073.73
EC05	3.355	2065.98	1908.25 2152.99
EC10	3.718	2119.91	1983.94 2197.95
EC15	3.964	2157.1	2035.78 2229.82
EC20	4.158	2187.11	2077.15 2256.34
EC25	4.326	2213.2	2112.56 2280.16
EC40	4.747	2280.31	2199.75 2346.33
EC50	5.000	2321.66	2249.35 2391.92
EC60	5.253	2363.76	2295.8 2442.92
EC75	5.674	2435.44	2366.08 2539.82
EC80	5.842	2464.48	2392.07 2582.02
EC85	6.036	2498.78	2421.44 2633.49
EC90	6.282	2542.61	2457.41 2701.36
EC95	6.645	2608.98	2509.57 2807.57
EC99	7.326	2738.2	2606.34 3022.92



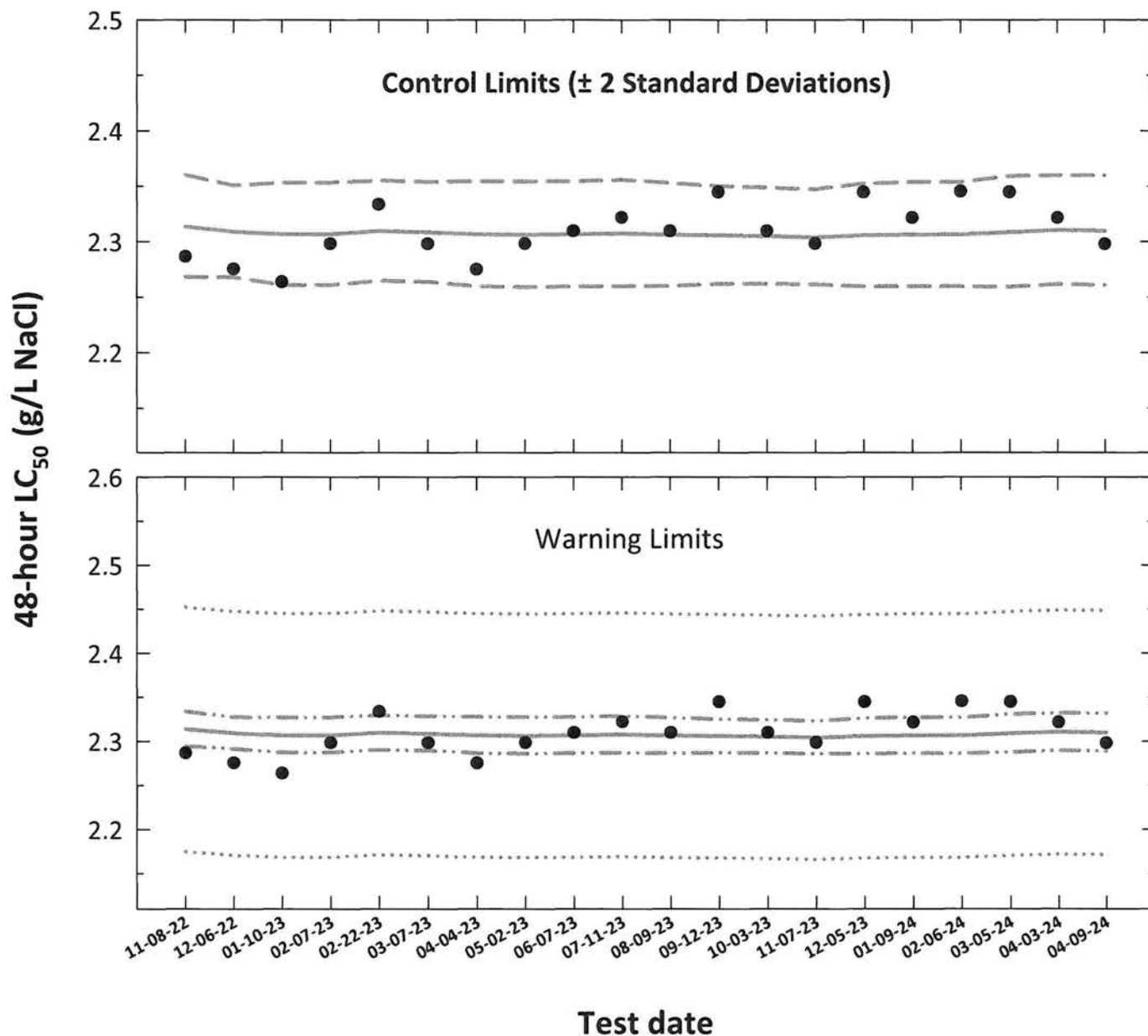
Dose-Response Plot



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		CT	Anti-logarithmic Values (g/L NaCl)		
			48-hour LC ₅₀	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	11-08-22	2.2868	0.3592	0.3643	0.0043	2.2685	2.2943	2.1750
2	12-06-22	2.2755	0.3571	0.3635	0.0039	2.2682	2.2915	2.1707
3	01-10-23	2.2639	0.3549	0.3630	0.0043	2.2613	2.2871	2.1685
4	02-07-23	2.2984	0.3614	0.3630	0.0043	2.2612	2.2871	2.1685
5	02-22-23	2.3339	0.3681	0.3636	0.0042	2.2651	2.2905	2.1713
6	03-07-23	2.2984	0.3614	0.3634	0.0042	2.2641	2.2894	2.1702
7	04-04-23	2.2753	0.3570	0.3630	0.0045	2.2600	2.2866	2.1686
8	05-02-23	2.2985	0.3614	0.3629	0.0045	2.2593	2.2860	2.1680
9	06-07-23	2.3099	0.3636	0.3630	0.0045	2.2600	2.2866	2.1686
10	07-11-23	2.3220	0.3659	0.3632	0.0045	2.2601	2.2870	2.1691
11	08-09-23	2.3099	0.3636	0.3629	0.0044	2.2605	2.2865	2.1681
12	09-12-23	2.3449	0.3701	0.3628	0.0041	2.2623	2.2870	2.1675
13	10-03-23	2.3099	0.3636	0.3627	0.0041	2.2622	2.2866	2.1670
14	11-07-23	2.2985	0.3614	0.3625	0.0040	2.2617	2.2857	2.1659
15	12-05-23	2.3449	0.3701	0.3628	0.0044	2.2598	2.2859	2.1675
16	01-09-24	2.3217	0.3658	0.3630	0.0044	2.2599	2.2863	2.1681
17	02-06-24	2.3455	0.3702	0.3630	0.0044	2.2598	2.2863	2.1681
18	03-05-24	2.3449	0.3701	0.3634	0.0047	2.2594	2.2874	2.1703
19	04-03-24	2.3217	0.3658	0.3637	0.0046	2.2619	2.2895	2.1719
20	04-09-24	2.2980	0.3613	0.3636	0.0047	2.2610	2.2888	2.1714

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 Test

CdNaClAC # 408

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
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 NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2261

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.70	7.69	7.69
	Dissolved oxygen (mg/L)	7.0	7.7	7.0
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L CaCO ₃)	61		
	Hardness (mg/L CaCO ₃)	90		
	Temperature (°C)	24.8	25.3	25.4
1750 mg/L	pH (S.U.)	7.77	7.67	7.66
	Dissolved oxygen (mg/L)	8.0	7.7	7.6
	Conductivity (µmhos/cm)	3290		
	Temperature (°C)	24.9	25.1	25.2
2000 mg/L	pH (S.U.)	7.75	7.67	7.72
	Dissolved oxygen (mg/L)	8.0	7.7	7.6
	Conductivity (µmhos/cm)	3730		
	Temperature (°C)	24.9	25.3	25.2
2250 mg/L	pH (S.U.)	7.74	7.68	7.73
	Dissolved oxygen (mg/L)	8.0	7.8	7.6
	Conductivity (µmhos/cm)	4190		
	Temperature (°C)	25.0	25.0	25.2
2500 mg/L	pH (S.U.)	7.74	7.71	7.72
	Dissolved oxygen (mg/L)	8.0	7.8	7.6
	Conductivity (µmhos/cm)	4540		
	Temperature (°C)	25.0	25.0	24.9
2750 mg/L	pH (S.U.)	7.71	7.72	7.74
	Dissolved oxygen (mg/L)	9.1	7.9	7.8
	Conductivity (µmhos/cm)	5850		
	Temperature (°C)	25.0	25.2	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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	154461

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 408

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-09-24	0520	HL	0720	HL	283	RED	04-09-24 B
24	04-10-24			0715	HL			
48 Termination	04-11-24			0718	HL			

*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):	04-02-24 D
Age:	< 24-hours old
Date and time organisms were born between:	04-08-24 1100 to 04-09-24 0520
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				1750 mg/L				2000 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%			

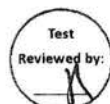
Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	4 ^u	4 ^u	5	4 ^u	3 ^u
48 Termination	3 ^u	2 ^u	2 ^u	2 ^u	2 ^u	1 ^u	1 ^u	1 ^u	0 ^u	0 ^u	0 ^u	0 ^u
Mean Survival	57%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2221.6
Upper 95% confidence limit (mg NaCl/L)	2372.6
48-hour LC ₅₀ (mg NaCl/L)	2298.0

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 4/9/2024 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 4/11/2024 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
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.4000	0.4000	0.4000
2500	0.4000	0.2000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

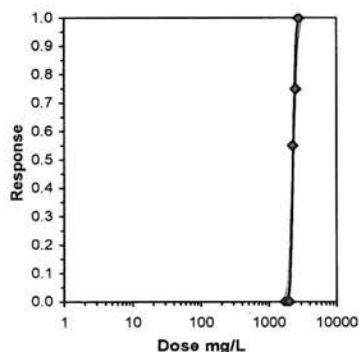
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.4500	0.4500	0.7351	0.6847	0.8861	13.697	4	10.00	10.00	11	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	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 ($p \leq 0.01$)	0.66241	0.868	1.98367	4.13297
Equality of variance cannot be confirmed				

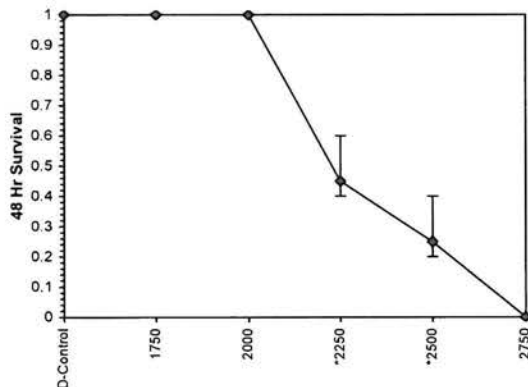
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	28.2327	5.00204	18.4287 38.0367	0	4.68438	7.81472	0.19642	3.36135	0.03542	7
Intercept	-89.9	16.8293	-122.89 -56.914							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1900.85	1700.87 2012.91
EC05	3.355	2009.49	1847.79 2102.51
EC10	3.718	2069.92	1929.85 2153.44
EC15	3.964	2111.72	1986.3 2189.56
EC20	4.158	2145.54	2031.51 2219.6
EC25	4.326	2174.99	2070.32 2246.57
EC40	4.747	2250.99	2166.47 2321.29
EC50	5.000	2297.98	2221.6 2372.6
EC60	5.253	2345.96	2273.62 2429.86
EC75	5.674	2427.94	2353.13 2538.5
EC80	5.842	2461.26	2382.8 2585.82
EC85	6.036	2500.67	2416.48 2643.6
EC90	6.282	2551.17	2457.92 2719.92
EC95	6.645	2627.89	2518.36 2839.69
EC99	7.326	2778.09	2631.39 3083.9



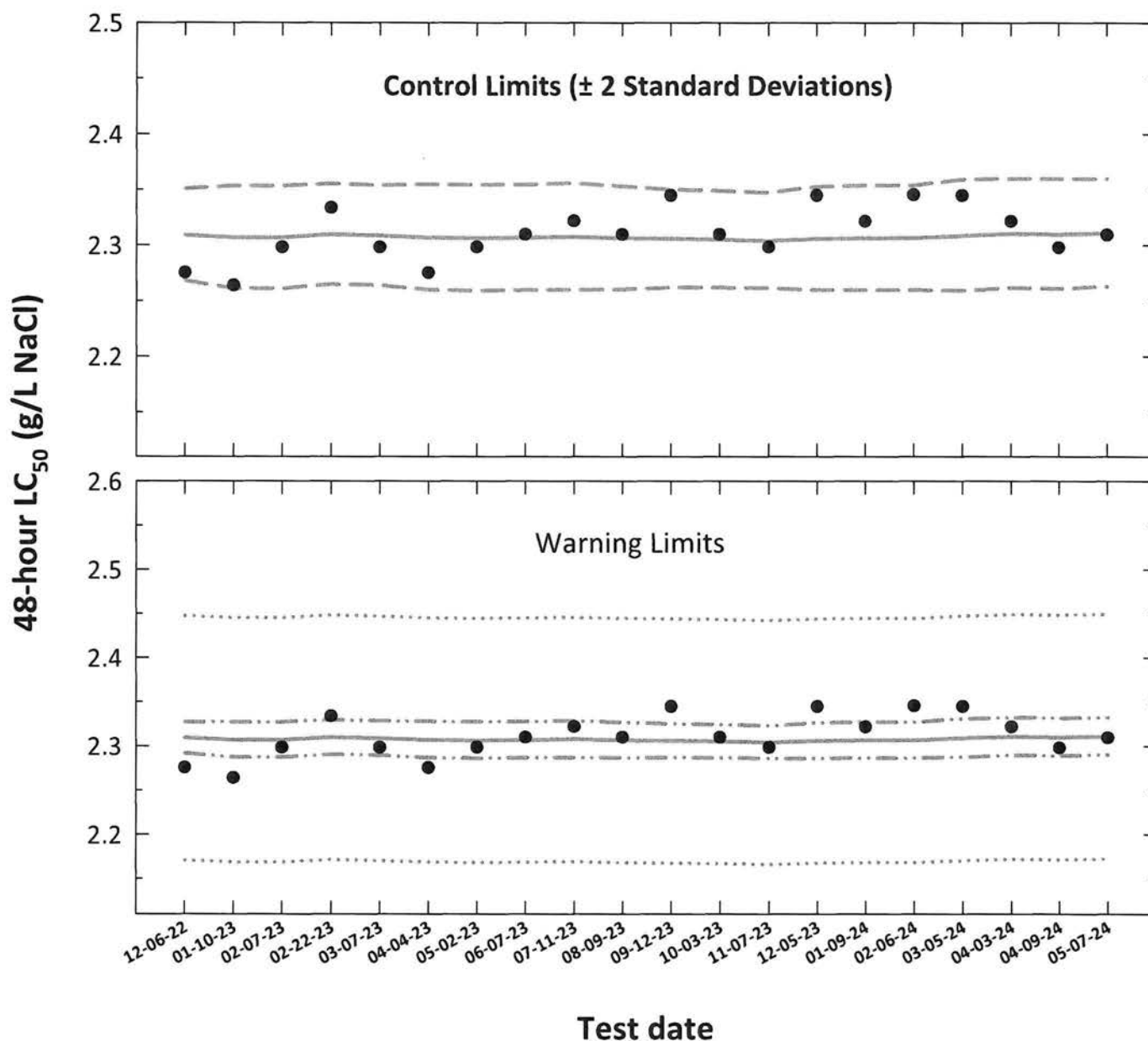
Dose-Response Plot



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		Laboratory Calculated CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	12-06-22	2.2755	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274
2	01-10-23	2.2639	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271
3	02-07-23	2.2984	0.3614	0.3630	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271
4	02-22-23	2.3339	0.3681	0.3636	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296
5	03-07-23	2.2984	0.3614	0.3634	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284
6	04-04-23	2.2753	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278
7	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272
8	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278
9	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286
10	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268
11	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251
12	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243
13	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229
14	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263
15	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271
16	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272
17	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307
18	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321
19	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317
20	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323

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 Test

CdNaClAC # 409

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
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 #: 2283

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	8.11	7.50	7.54
	Dissolved oxygen (mg/L)	7.8	7.9	7.9
	Conductivity (µmhos/cm)	289		
	Alkalinity (mg/L CaCO ₃)	59		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.9	24.9	25.2
1750 mg/L	pH (S.U.)	7.87	7.37	7.41
	Dissolved oxygen (mg/L)	8.0	7.9	8.0
	Conductivity (µmhos/cm)	3410		
	Temperature (°C)	25.1	25.2	25.4
2000 mg/L	pH (S.U.)	7.85	7.38	7.40
	Dissolved oxygen (mg/L)	8.0	7.9	8.0
	Conductivity (µmhos/cm)	3880		
	Temperature (°C)	25.0	25.3	25.1
2250 mg/L	pH (S.U.)	7.85	7.38	7.41
	Dissolved oxygen (mg/L)	8.0	8.1	8.0
	Conductivity (µmhos/cm)	4260		
	Temperature (°C)	25.0	25.0	25.1
2500 mg/L	pH (S.U.)	7.84	7.38	7.42
	Dissolved oxygen (mg/L)	8.0	8.1	8.1
	Conductivity (µmhos/cm)	4730		
	Temperature (°C)	25.2	25.2	25.2
2750 mg/L	pH (S.U.)	7.84	7.38	7.41
	Dissolved oxygen (mg/L)	8.1	8.1	8.1
	Conductivity (µmhos/cm)	6260		
	Temperature (°C)	25.0	25.2	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 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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130661685

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 # 409

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-07-24	0530	yl	0804	yl	2B3	BLACK	05-07-24A
24	05-08-24			0800	yl			
48 Termination	05-09-24			0800	yl			

*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):	04-30-24 D
Age:	< 24-hours old
Date and time organisms were born between:	05-06-24 1155 TO 05-07-24 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.02
	Temperature (°C): 24.9

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 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%			

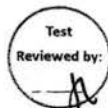
Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	3 ^{2d}	4 ^{4d}	5	5
48 Termination	3 ^{2d}	2 ^{3d}	2 ^{3d}	3 ^{2d}	2 ^{3d}	1 ^{4d}	1 ^{4d}	1 ^{4d}	0 ^{3d}	0 ^{4d}	0 ^{5d}	0 ^{5d}
Mean Survival	50%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2233.8
Upper 95% confidence limit (mg NaCl/L)	2383.6
48-hour LC ₅₀ (mg NaCl/L)	2309.6

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 5/7/2024 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 5/9/2024 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
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.4000	0.4000	0.6000
2500	0.4000	0.2000	0.2000	0.2000
2750	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
1750	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
*2250	0.5000	0.5000	0.7854	0.6847	0.8861	14.802	4	10.00	10.00	10	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$) Statistic: 0.82032 Critical: 0.868 Skew: 0.91483 Kurt: 1.68855

Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)

Steel's Many-One Rank Test NOEC: 2000 LOEC: 2250 ChV: 2121.32

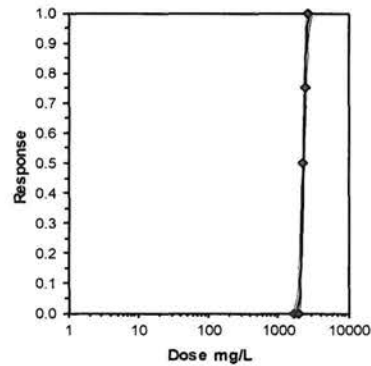
Treatments vs D-Control

Maximum Likelihood-Probit

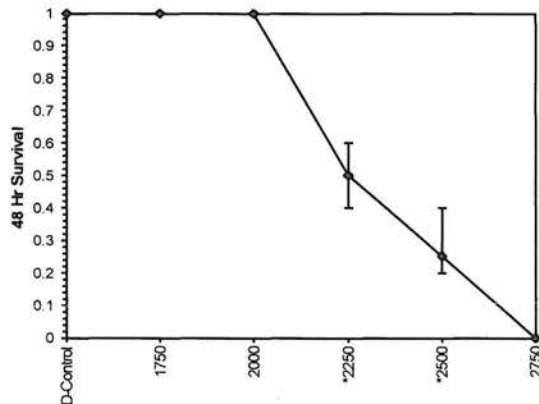
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	28.8917	5.16275	18.7727 39.0106	0	3.64582	7.81472	0.30234	3.36353	0.03461	6
Intercept	-92.178	17.3806	-126.24 -58.112							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1918.7	1718.88 2029.87
EC05	3.355	2025.8	1864.5 2117.99
EC10	3.718	2085.31	1945.73 2168.05
EC15	3.964	2126.45	2001.56 2203.56
EC20	4.158	2159.72	2046.25 2233.09
EC25	4.326	2188.68	2084.59 2259.6
EC40	4.747	2263.39	2179.46 2333.09
EC50	5.000	2309.55	2233.77 2383.59
EC60	5.253	2356.66	2284.96 2439.95
EC75	5.674	2437.1	2363.07 2546.91
EC80	5.842	2469.78	2392.17 2593.49
EC85	6.036	2508.42	2425.18 2650.35
EC90	6.282	2557.91	2465.79 2725.42
EC95	6.645	2633.05	2524.95 2843.16
EC99	7.326	2780.02	2635.45 3082.98



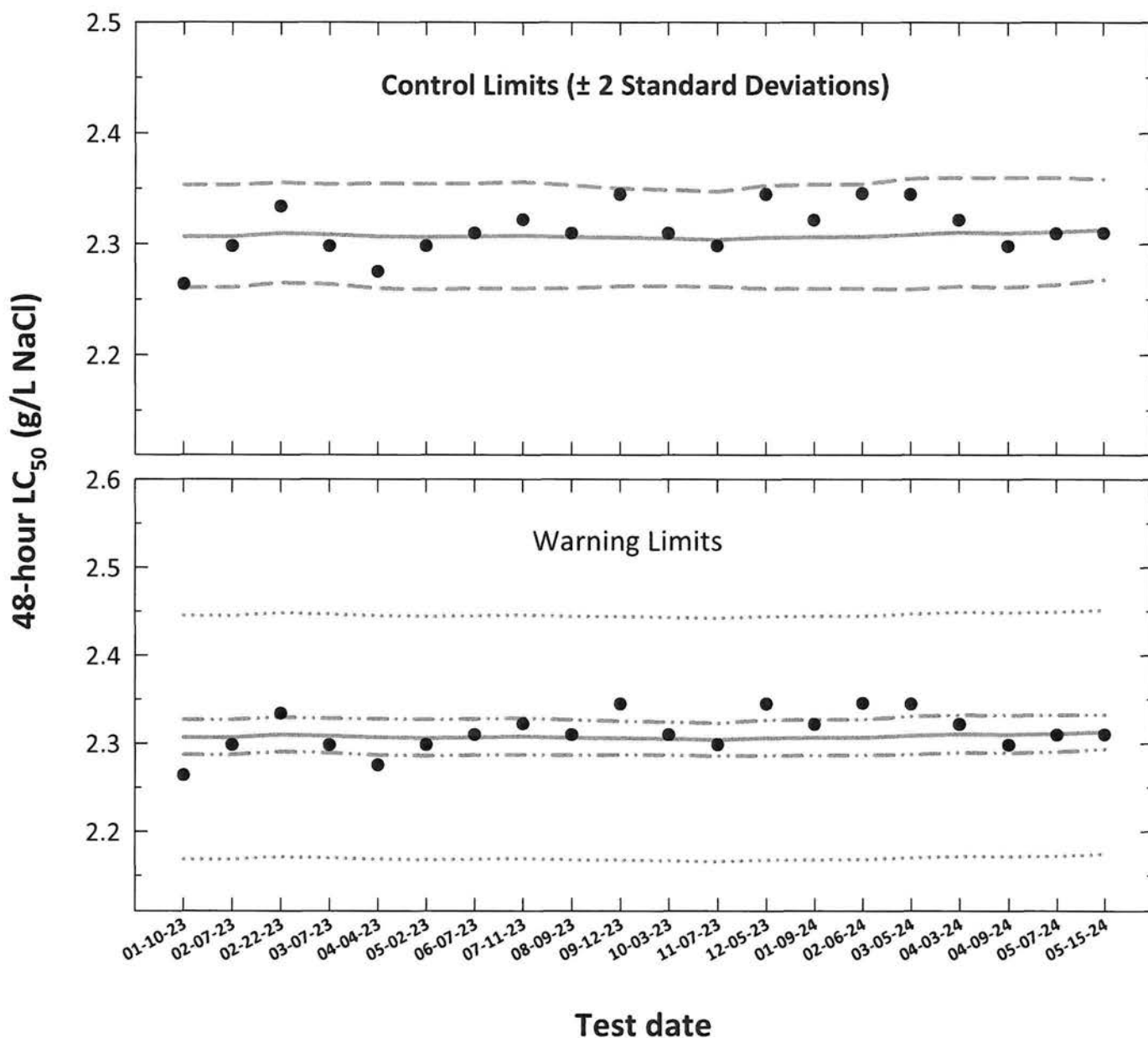
Dose-Response Plot



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,
 $S_{A.10} = 10^{\text{th}}$ 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		CT	Anti-logarithmic Values (g/L NaCl)			10th Percentile CV Warning Limits	
			48-hour LC ₅₀	S		CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV		
1	01-10-23	2.2639	0.3549	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685
2	02-07-23	2.2984	0.3614	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271	2.1685
3	02-22-23	2.3339	0.3681	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296	2.1713
4	03-07-23	2.2984	0.3614	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284	2.1702
5	04-04-23	2.2753	0.3570	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686
6	05-02-23	2.2985	0.3614	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272	2.1680
7	06-07-23	2.3099	0.3636	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686
8	07-11-23	2.3220	0.3659	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691
9	08-09-23	2.3099	0.3636	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681
10	09-12-23	2.3449	0.3701	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675
11	10-03-23	2.3099	0.3636	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670
12	11-07-23	2.2985	0.3614	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659
13	12-05-23	2.3449	0.3701	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675
14	01-09-24	2.3217	0.3658	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681
15	02-06-24	2.3455	0.3702	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681
16	03-05-24	2.3449	0.3701	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703
17	04-03-24	2.3217	0.3658	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719
18	04-09-24	2.2980	0.3613	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714
19	05-07-24	2.3096	0.3635	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725
20	05-15-24	2.3099	0.3636	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741

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 Test

CdNaClAC # 410

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
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 NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2283

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.45	7.55	7.49
	Dissolved oxygen (mg/L)	7.9	7.9	7.6
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L CaCO ₃)	57		
	Hardness (mg/L CaCO ₃)	88		
	Temperature (°C)	24.7	25.0	25.2
1750 mg/L	pH (S.U.)	7.62	7.56	7.57
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	3260		
	Temperature (°C)	24.8	25.2	24.9
2000 mg/L	pH (S.U.)	7.62	7.61	7.58
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	3700		
	Temperature (°C)	24.8	25.2	24.9
2250 mg/L	pH (S.U.)	7.62	7.60	7.58
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	4090		
	Temperature (°C)	24.8	25.2	24.9
2500 mg/L	pH (S.U.)	7.62	7.61	7.58
	Dissolved oxygen (mg/L)	8.0	7.8	7.9
	Conductivity (µmhos/cm)	4500		
	Temperature (°C)	24.7	24.9	25.1
2750 mg/L	pH (S.U.)	7.63	7.62	7.62
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	4920		
	Temperature (°C)	24.7	25.3	24.9

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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130661689

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 # 410

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-16-24	0500 0640	JL	1135	JL	2B3	RED	05-09-24C
24	05-16-24			1138	JL			
48 Termination	05-17-24			1130	JL			

*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):	05-07-24 D
Age:	< 24-hours old
Date and time organisms were born between:	05-14-24 1427 To 05-15-24 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.92
	Temperature (°C): 24.8

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 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%			

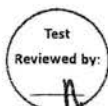
Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	4 ^d	4 ^d	4 ^d	4 ^d
48 Termination	3 ^u	2 ^u	3 ^u	3 ^u	1 ^u	0 ^d	2 ^u	1 ^u	0 ^u	0 ^u	0 ^u	0 ^u
Mean Survival	55%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2226.9
Upper 95% confidence limit (mg NaCl/L)	2381.0
48-hour LC ₅₀ (mg NaCl/L)	2309.9

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 5/15/2024 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 5/17/2024 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
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.4000	0.6000	0.6000
2500	0.2000	0.0000	0.4000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

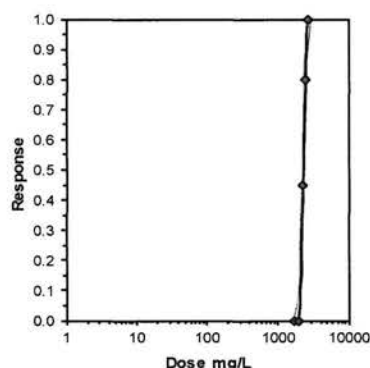
Transform: Arcsin Square Root										Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical			Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4					0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00			0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00			0	20
*2250	0.5500	0.5500	0.8357	0.6847	0.8861	12.047	4	10.00	10.00			9	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00			16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4					20	20

Auxiliary Tests
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)
Equality of variance cannot be confirmed

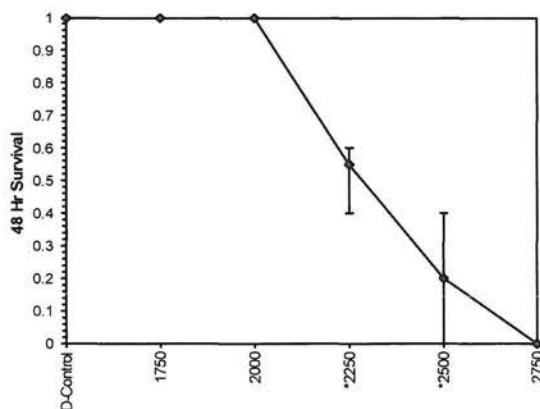
Hypothesis Test (1-tail, 0.05)
Steel's Many-One Rank Test
Treatments vs D-Control

Maximum Likelihood-Probit
Parameter Value SE 95% Fiducial Limits Control Chi-Sq Critical P-value Mu Sigma Iter
Slope 31.4761 5.81124 20.0861 42.8661 0 1.98377 7.81472 0.57578 3.3636 0.03177 5
Intercept -100.87 19.5631 -139.22 -62.529

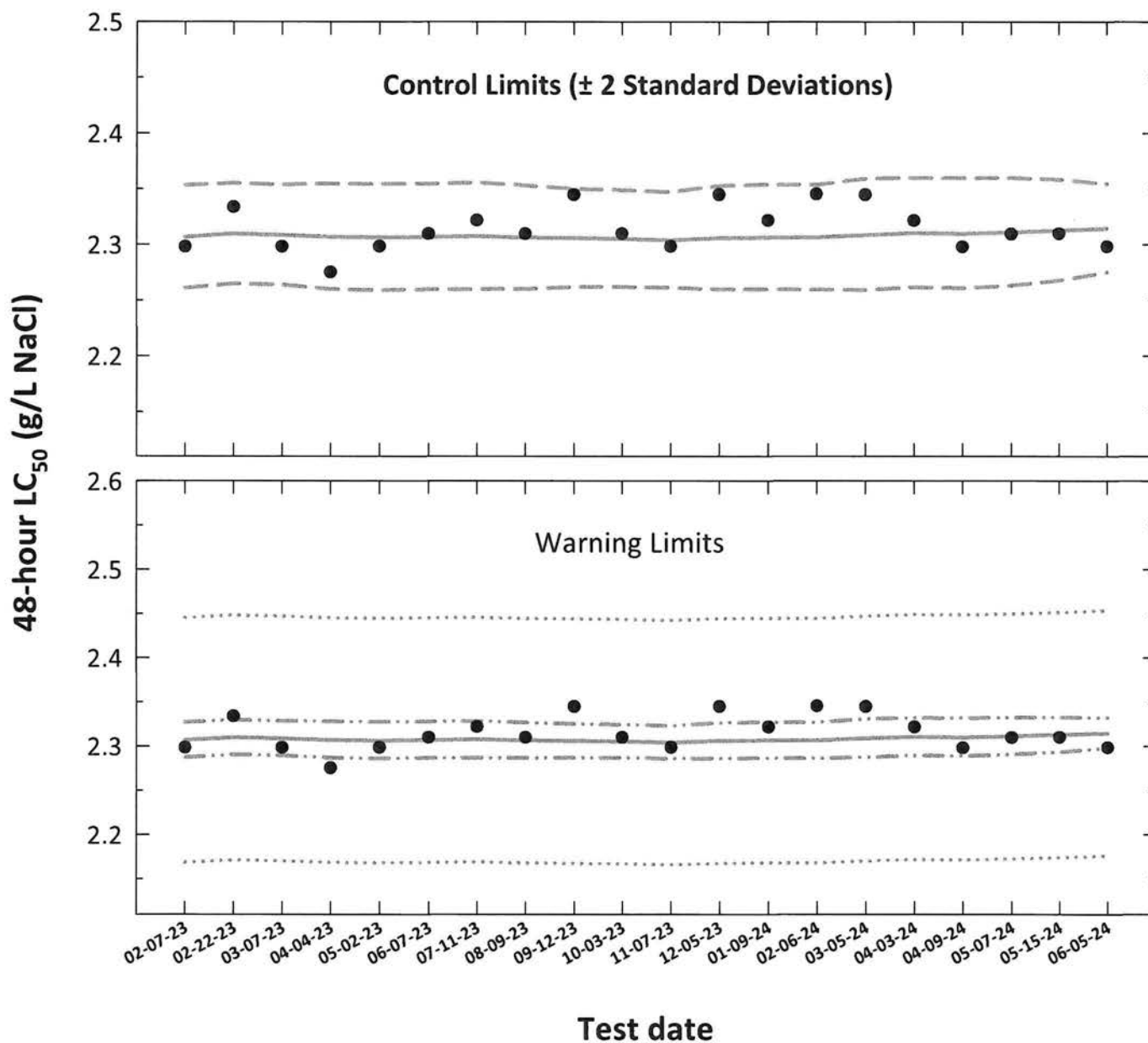
Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1948.47	1752 2054.85
EC05	3.355	2048.07	1890.23 2136.13
EC10	3.718	2103.23	1966.98 2182.25
EC15	3.964	2141.28	2019.59 2214.95
EC20	4.158	2172.02	2061.61 2242.15
EC25	4.326	2198.74	2097.59 2266.57
EC40	4.747	2267.53	2186.32 2334.36
EC50	5.000	2309.94	2236.9 2381
EC60	5.253	2353.15	2284.37 2433.11
EC75	5.674	2426.78	2356.4 2532.04
EC80	5.842	2456.63	2383.1 2575.12
EC85	6.036	2491.89	2413.3 2627.67
EC90	6.282	2536.98	2450.33 2696.99
EC95	6.645	2605.31	2504.1 2805.53
EC99	7.326	2738.48	2604.02 3025.86



Dose-Response Plot



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



- **48-hour LC_{50}** = 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 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 $LC_{50} \pm S_{A.10}$ converted to anti-logarithmic values,
 $S_{A.10} = 10^{\text{th}}$ 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		CT	Anti-logarithmic Values (g/L NaCl)			10th Percentile CV Warning Limits
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	
1	02-07-23	2.2984	0.3614	0.3630	0.0043	2.3069	2.2612 2.3535	2.2871 2.3271	2.1685 2.4454
2	02-22-23	2.3339	0.3681	0.3636	0.0042	2.3099	2.2651 2.3555	2.2905 2.3296	2.1713 2.4485
3	03-07-23	2.2984	0.3614	0.3634	0.0042	2.3087	2.2641 2.3542	2.2894 2.3284	2.1702 2.4472
4	04-04-23	2.2753	0.3570	0.3630	0.0045	2.3070	2.2600 2.3549	2.2866 2.3278	2.1686 2.4454
5	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593 2.3545	2.2860 2.3272	2.1680 2.4448
6	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600 2.3549	2.2866 2.3278	2.1686 2.4454
7	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601 2.3560	2.2870 2.3286	2.1691 2.4460
8	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605 2.3534	2.2865 2.3268	2.1681 2.4448
9	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623 2.3503	2.2870 2.3251	2.1675 2.4442
10	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622 2.3492	2.2866 2.3243	2.1670 2.4436
11	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617 2.3474	2.2857 2.3229	2.1659 2.4424
12	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598 2.3529	2.2859 2.3263	2.1675 2.4442
13	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599 2.3540	2.2863 2.3271	2.1681 2.4449
14	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598 2.3542	2.2863 2.3272	2.1681 2.4449
15	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594 2.3593	2.2874 2.3307	2.1703 2.4474
16	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619 2.3603	2.2895 2.3321	2.1719 2.4492
17	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610 2.3600	2.2888 2.3317	2.1714 2.4486
18	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633 2.3600	2.2905 2.3323	2.1725 2.4498
19	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680 2.3586	2.2935 2.3327	2.1741 2.4516
20	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753 2.3546	2.2976 2.3319	2.1757 2.4535

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 Test

CdNaClAC # 411

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
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 NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2283

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.49	7.39	7.67
	Dissolved oxygen (mg/L)	7.6	7.7	8.0
	Conductivity (µmhos/cm)	308		
	Alkalinity (mg/L CaCO ₃)	57		
	Hardness (mg/L CaCO ₃)	85		
	Temperature (°C)	24.8	25.1	25.1
1750 mg/L	pH (S.U.)	7.43	7.35	7.57
	Dissolved oxygen (mg/L)	7.9	7.7	8.0
	Conductivity (µmhos/cm)	3440		
	Temperature (°C)	24.9	25.0	25.3
2000 mg/L	pH (S.U.)	7.43	7.36	7.58
	Dissolved oxygen (mg/L)	8.0	7.9	8.0
	Conductivity (µmhos/cm)	3800		
	Temperature (°C)	25.0	25.1	25.0
2250 mg/L	pH (S.U.)	7.42	7.38	7.59
	Dissolved oxygen (mg/L)	7.9	7.9	8.1
	Conductivity (µmhos/cm)	4240		
	Temperature (°C)	24.9	25.1	25.0
2500 mg/L	pH (S.U.)	7.42	7.37	7.59
	Dissolved oxygen (mg/L)	8.0	7.9	8.1
	Conductivity (µmhos/cm)	4680		
	Temperature (°C)	24.9	25.1	25.2
2750 mg/L	pH (S.U.)	7.42	7.38	7.60
	Dissolved oxygen (mg/L)	8.0	8.0	8.1
	Conductivity (µmhos/cm)	5040		
	Temperature (°C)	24.9	25.1	25.2

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	130664685

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 # 411

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	06-06-24	0405	JL	0605	JL	2B3	RED	05-29-24C
24	06-06-24			0600	JL			
48 Termination	06-07-24			0600	JL			

*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):	05-18-24 C + D
Age:	< 24-hours old 24 hours
Date and time organisms were born between:	06-04-24 1645 to 06-05-24 0405
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.67
	Temperature (°C): 24.8

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 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%			

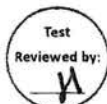
Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 ^u	5	4 ^u	5	3 ^u	3 ^u	4 ^u	3 ^u
48 Termination	2 ^u	4 ^u	2 ^u	2 ^u	3 ^u	1 ^u	0 ^u	0 ^u	0 ^u	0 ^u	0 ^u	0 ^u
Mean Survival	50%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	2224.7
Upper 95% confidence limit (mg NaCl/L)	2370.1
48-hour LC ₅₀ (mg NaCl/L)	2298.4

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 6/5/2024 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 6/7/2024 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
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.8000	0.4000	0.4000
2500	0.6000	0.2000	0.0000	0.0000
2750	0.0000	0.0000	0.0000	0.0000

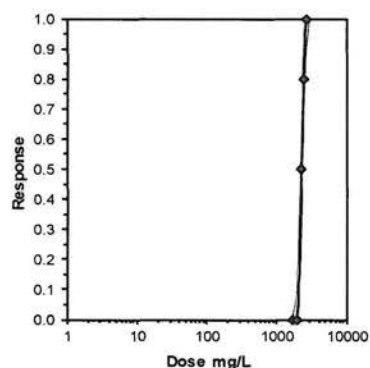
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7903	0.6847	1.1071	26.725	4	10.00	10.00	10	20
*2500	0.2000	0.2000	0.4502	0.2255	0.8861	69.198	4	10.00	10.00	16	20
2750	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 ($p \leq 0.01$)	0.72992	0.868	1.54553	3.91812
Equality of variance cannot be confirmed				

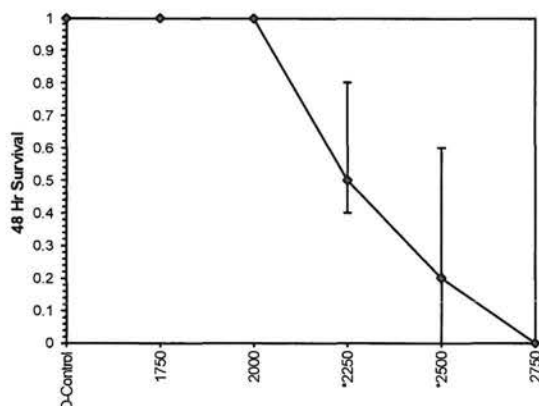
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	30.6709	5.60227	19.6905 41.6514	0	2.7074	7.81472	0.43897	3.36142	0.0326
Intercept	-98.098	18.848	-135.04 -61.156						
TSCR									

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1930.04	1733.59 2037.4
EC05	3.355	2031.36	1873.25 2120.31
EC10	3.718	2087.52	1950.89 2167.38
EC15	3.964	2126.29	2004.15 2200.75
EC20	4.158	2157.62	2046.72 2228.51
EC25	4.326	2184.86	2083.19 2253.43
EC40	4.747	2255.05	2173.24 2322.55
EC50	5.000	2298.35	2224.66 2370.05
EC60	5.253	2342.48	2273 2423.1
EC75	5.674	2417.72	2346.49 2523.77
EC80	5.842	2448.25	2373.78 2567.61
EC85	6.036	2484.32	2404.68 2621.1
EC90	6.282	2530.46	2442.6 2691.68
EC95	6.645	2600.43	2497.7 2802.26
EC99	7.326	2736.93	2600.25 3026.95



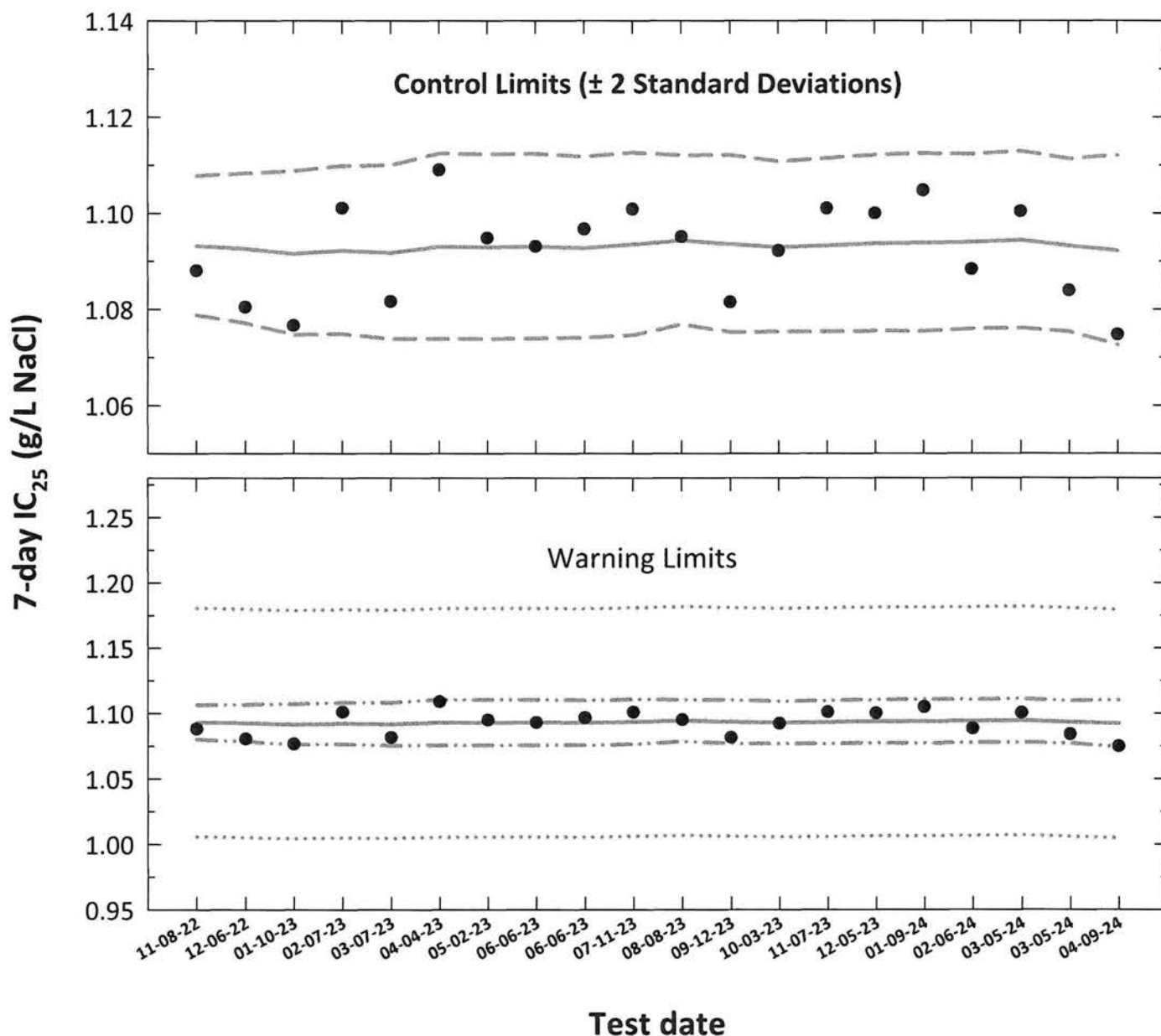
Dose-Response Plot



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,
 $S_{A.10} = 10^{\text{th}}$ 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			Anti-logarithmic Values (g/L NaCl)							
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits		
1	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
2	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772		1.1083	1.0785	1.1068	1.0052	1.1800
3	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748		1.1088	1.0762	1.1071	1.0043	1.1790
4	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749		1.1098	1.0764	1.1081	1.0049	1.1796
5	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739		1.1100	1.0754	1.1082	1.0045	1.1791
6	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739		1.1125	1.0756	1.1105	1.0056	1.1805
7	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739		1.1123	1.0755	1.1104	1.0055	1.1804
8	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740		1.1124	1.0756	1.1105	1.0056	1.1805
9	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741		1.1118	1.0757	1.1099	1.0054	1.1802
10	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747		1.1126	1.0763	1.1107	1.0060	1.1810
11	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769		1.1121	1.0784	1.1103	1.0068	1.1819
12	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753		1.1121	1.0768	1.1103	1.0061	1.1810
13	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754		1.1108	1.0769	1.1090	1.0055	1.1804
14	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753		1.1115	1.0769	1.1097	1.0058	1.1807
15	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756		1.1122	1.0772	1.1103	1.0062	1.1812
16	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755		1.1125	1.0771	1.1106	1.0063	1.1813
17	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760		1.1123	1.0775	1.1105	1.0065	1.1815
18	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761		1.1129	1.0777	1.1110	1.0068	1.1819
19	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754		1.1113	1.0769	1.1095	1.0058	1.1807
20	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

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.

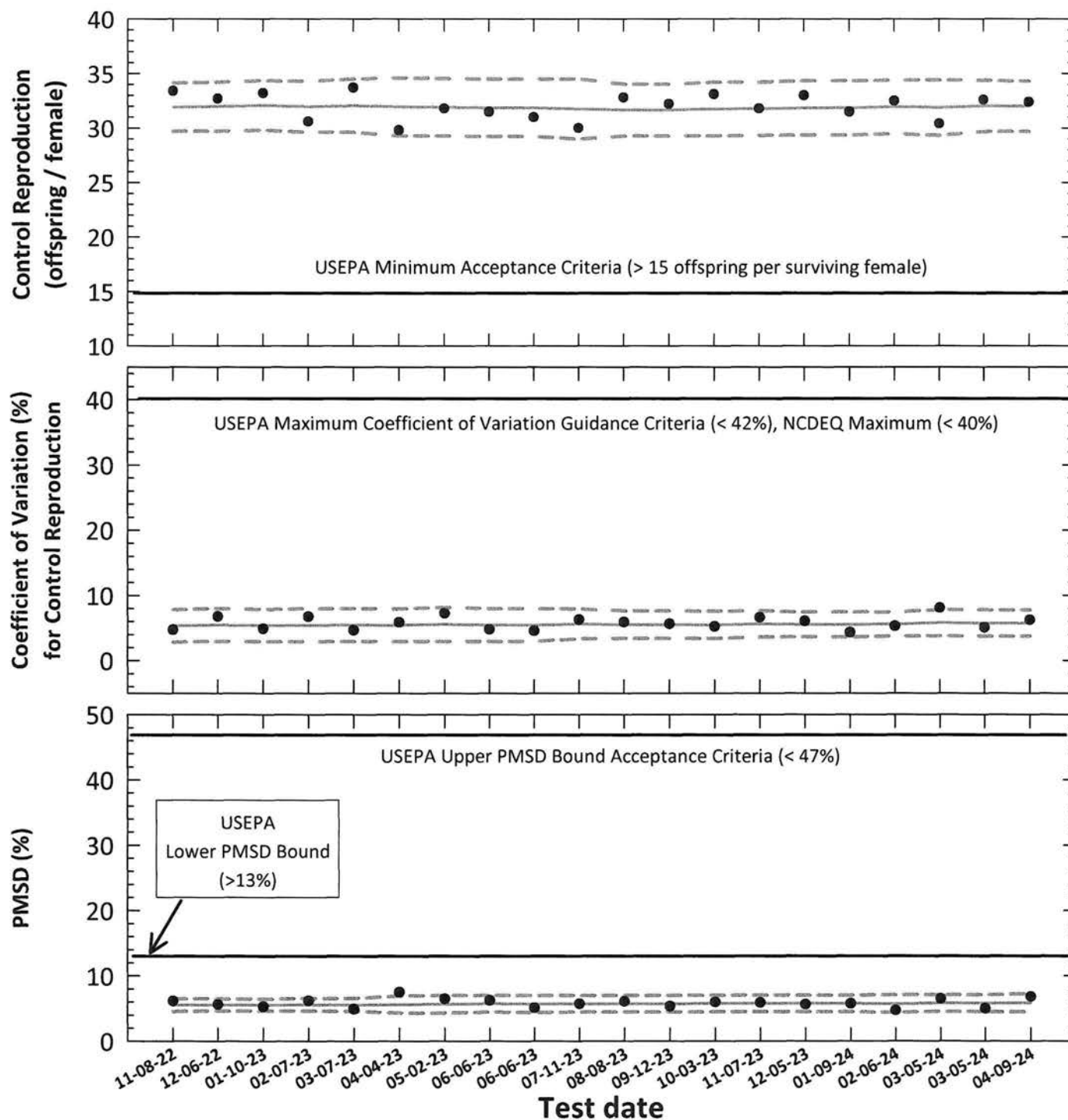
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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	PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
			Mean (offspring/female)	CV (%)											
1	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	4.5	6.5
2	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	4.6	6.5
3	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	4.6	6.4
4	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5
5	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
6	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9
7	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0
8	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0
9	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0
10	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
11	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
12	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
13	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
14	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
15	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
16	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
17	02-06-24	100	32.5	5.3	1.526	4.7	31.9	29.5	34.4	5.6	3.7	7.4	5.7	4.4	7.0
18	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	5.8	4.5	7.1
19	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
20	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

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 #: 297

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>764</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>01-04-24 0510 TO 0850</u>									
Culture board:	<u>04-02-24 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>6</u>	<u>7</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>17</u>	<u>22</u>	<u>23</u>
Transfer vessel information:	pH (S.U.): <u>7.92</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>2B1</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	04-09-24	<u>0900</u>	<u>03-28-24</u>	<u>03-28-24</u>	<u>04-01-24 B</u>	<u>JK</u>
1	04-10-24	<u>0800</u>	↓	↓	↓	<u>JK</u>
2	04-11-24	<u>0831</u>	↓	↓	<u>04-01-24 D</u>	<u>JK</u>
3	04-12-24	<u>0600</u>	↓	↓	↓	<u>JK</u>
4	04-13-24	<u>0846</u>	↓	↓	<u>04-08-24 A</u>	<u>JK</u>
5	04-14-24	<u>0830</u>	↓	↓	↓	<u>JK</u>
6	04-15-24	<u>0800</u>	↓	↓	↓	<u>JK</u>
7	04-16-24	<u>0800</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-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	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>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>71400</u>
% Adults having 3 rd Broods:	<u>100.</u>	≥ 80%	NOEC (mg/L NaCl)	<u>1000</u>
% Mortality:	<u>07.</u>	≤ 20%	LOEC (mg/L NaCl)	<u>1200</u>
Mean Offspring/Female:	<u>32.4</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>6.27.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1074.8</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 297

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	4	6	4	5	5	4	4	4	6	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	13	12	10	12	12	12	11	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	14	16	17	12	17	19	19	18	14	14
Total young produced		31	32	34	29	32	35	35	34	31	31
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: 32.4

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	4	5	5	4	6	4	6	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	13	12	12	13	10	10	10	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	16	18	19	15	18	15	19	19	18
Total young produced		35	31	36	36	31	37	29	35	34	36
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: 34.0

% Reduction from Control: -41.97.

Species: Ceriodaphnia dubia

CdNaClCR #: 297

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	4	5	4	5	5	6	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	14	10	10	11	10	12	12	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	17	15	16	19	13	16	16	15	18	14
Total young produced		33	33	30	34	28	31	33	33	35	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:	07.
Mean Offspring/Female:	32.1
% Reduction from Control:	0.97.

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	4	4	4	5	4	4	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	10	10	11	10	12	11	11
	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	15	16	12	15	15	17	13	16	13
Total young produced		29	29	30	27	29	30	33	29	31	28
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:	29.5
% Reduction from Control:	9.07.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 297

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	5	4	4	3	4	4	4	2	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	5	9	6	10	8	11	7	6	9	9
	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	6	3	4	4	5	6	13	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		13	18	16	17	15	19	16	16	24	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:	17.2
% Reduction from Control:	46.97.

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	2	0	0	0	3	1	1	2	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	2	0	1	1	3	0	0	1	0	2
	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	3	1	0	0	3	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		2	2	4	2	3	3	4	2	2	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:	2.6
% Reduction from Control:	92.07.

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	4	6	4	5	5	4	4	4	6	5	47
5	13	10	13	12	10	12	12	12	11	12	117
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	17	12	17	19	19	18	14	14	160
Total	31	32	34	29	32	35	35	34	31	31	324

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	4	5	5	4	6	4	6	5	5	49
5	12	11	13	12	12	13	10	10	10	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	18	16	18	19	15	18	15	19	19	18	175
Total	35	31	36	36	31	37	29	35	34	36	340

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	4	5	4	5	5	6	5	5	47
5	12	14	10	10	11	10	12	12	12	12	115
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	16	19	13	16	16	15	18	14	159
Total	33	33	30	34	28	31	33	33	35	31	321

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	4	4	4	5	4	4	6	4	4	4	43
5	12	10	10	10	10	11	10	12	11	11	107
6	0	0	0	0	0	0	0	0	0	0	0
7	13	15	16	12	15	15	17	13	16	13	145
Total	29	29	30	27	29	30	33	29	31	28	295

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	5	4	4	3	4	4	4	2	4	37
5	5	9	6	10	8	11	7	6	9	9	80
6	0	0	0	0	0	0	0	0	0	0	0
7	5	4	6	3	4	4	5	6	13	5	55
Total	13	18	16	17	15	19	16	16	24	18	172

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	2	0	0	0	0	3	1	1	2	9
5	2	0	1	1	3	0	0	1	0	2	10
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	3	1	0	0	3	0	0	0	7
Total	2	2	4	2	3	3	4	2	2	2	26

Checked and
Reviewed by
Jim Anderson

***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: CdNaClCR #297
Test dates: April 09-16, 2024

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	31	32	34	29	32	35	35	34	31	31	100	32.4	6.2	Not applicable
600	35	31	36	36	31	37	29	35	34	36	100	34.0	8.0	-4.9
800	33	33	30	34	28	31	33	33	35	31	100	32.1	6.5	0.9
1000	29	29	30	27	29	30	33	29	31	28	100	29.5	5.6	9.0
1200	13	18	16	17	15	19	16	16	24	18	100	17.2	17.1	46.9
1400	2	2	4	2	3	3	4	2	2	2	100	2.6	32.4	92.0

Dunnett's MSD value: 2.199 MSD = Minimum Significant Difference
PMSD: 6.8 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.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 4/9/2024 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
End Date: 4/16/2024 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:

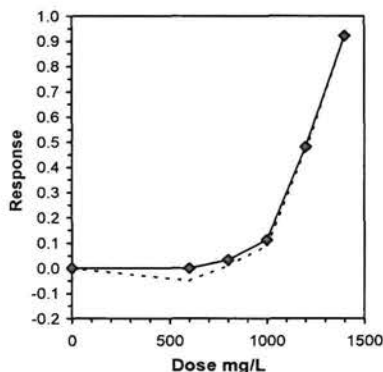
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	31.000	32.000	34.000	29.000	32.000	35.000	35.000	34.000	31.000	31.000
600	35.000	31.000	36.000	36.000	31.000	37.000	29.000	35.000	34.000	36.000
800	33.000	33.000	30.000	34.000	28.000	31.000	33.000	33.000	35.000	31.000
1000	29.000	29.000	30.000	27.000	29.000	30.000	33.000	29.000	31.000	28.000
1200	13.000	18.000	16.000	17.000	15.000	19.000	16.000	16.000	24.000	18.000
1400	2.000	2.000	4.000	2.000	3.000	3.000	4.000	2.000	2.000	2.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	32.400	1.0000	32.400	29.000	35.000	6.207	10				33.200	1.0000
600	34.000	1.0494	34.000	29.000	37.000	7.965	10	-1.663	2.287	2.199	33.200	1.0000
800	32.100	0.9907	32.100	28.000	35.000	6.477	10	0.312	2.287	2.199	32.100	0.9669
*1000	29.500	0.9105	29.500	27.000	33.000	5.593	10	3.015	2.287	2.199	29.500	0.8886
*1200	17.200	0.5309	17.200	13.000	24.000	17.072	10	15.803	2.287	2.199	17.200	0.5181
*1400	2.600	0.0802	2.600	2.000	4.000	32.434	10	30.981	2.287	2.199	2.600	0.0783

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution ($p > 0.01$)					0.66922	1.035	0.17244	1.29772						
Bartlett's Test indicates equal variances ($p = 0.02$)					13.1276	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					800	1000	894.427		2.19947	0.06788	1536.43	4.62593	3.1E-39	5, 54
Treatments vs D-Control														

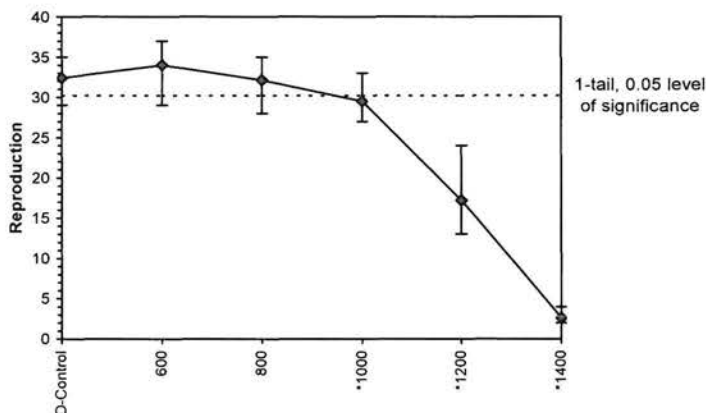
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	843.077	49.9359	748.57 932.733	-0.2581
IC10	970.769	36.8265	887.781 1014.09	-0.8050
IC15	1020.81	10.9764	1001.34 1039.84	-0.5549
IC20	1047.8	9.81287	1029.34 1066.79	0.0050
IC25	1074.8	9.80957	1057.25 1095.49	0.1373
IC40	1155.77	12.7301	1136.91 1185.93	0.6623
IC50	1208.22	11.6254	1186.18 1231.7	0.0876



While hypothesis test results indicate a significant difference in reproduction for the 1000 mg/L concentration, the PMSD was below the lower bound (13%) established by EPA. Guidance in EPA 833-R-00-003 (June 2000) Section 6.4.2 for determining the NOEC was followed, and it was concluded that the hypothesis test yielded a Type I Error. The ChV was recalculated to be 1095.4 mg/L.

Dose-Response Plot



Entered and
Reviewed by
Zion Sumner
JK

Species: *Ceriodaphnia dubia*

CdNaClCR #: 297

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		N		N		N	
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.70	7.70	7.48	7.81	7.75	7.90
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.8	7.8	7.6
	Conductivity (µmhos/cm)	295		310		295	
	Alkalinity (mg CaCO ₃ /L)	61				59	
	Hardness (mg CaCO ₃ /L)	90				86	
	Temperature (°C)	24.9	25.3	24.8	25.3	24.9	25.1
600 mg NaCl/L	pH (S.U.)	7.74	7.60	7.54	7.75	7.78	7.87
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.8	7.8	7.4
	Conductivity (µmhos/cm)	1370		1440		1390	
	Temperature (°C)	25.0	25.0	24.9	25.0	25.0	25.0
800 mg NaCl/L	pH (S.U.)	7.73	7.59	7.50	7.75	7.78	7.88
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.8	7.6
	Conductivity (µmhos/cm)	1730		1810		1750	
	Temperature (°C)	25.0	25.2	25.0	25.2	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.74	7.60	7.50	7.75	7.79	7.88
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.7	7.9	7.6
	Conductivity (µmhos/cm)	2100		2170		2130	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.9	25.2
1200 mg NaCl/L	pH (S.U.)	7.74	7.60	7.57	7.75	7.80	7.88
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.7	7.9	7.7
	Conductivity (µmhos/cm)	2470		2530		2490	
	Temperature (°C)	25.0	25.4	24.9	25.2	25.1	25.2
1400 mg NaCl/L	pH (S.U.)	7.74	7.60	7.60	7.74	7.80	7.86
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	7.7	7.9	7.7
	Conductivity (µmhos/cm)	2860		2920		2860	
	Temperature (°C)	25.0	25.1	24.9	25.2	25.1	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

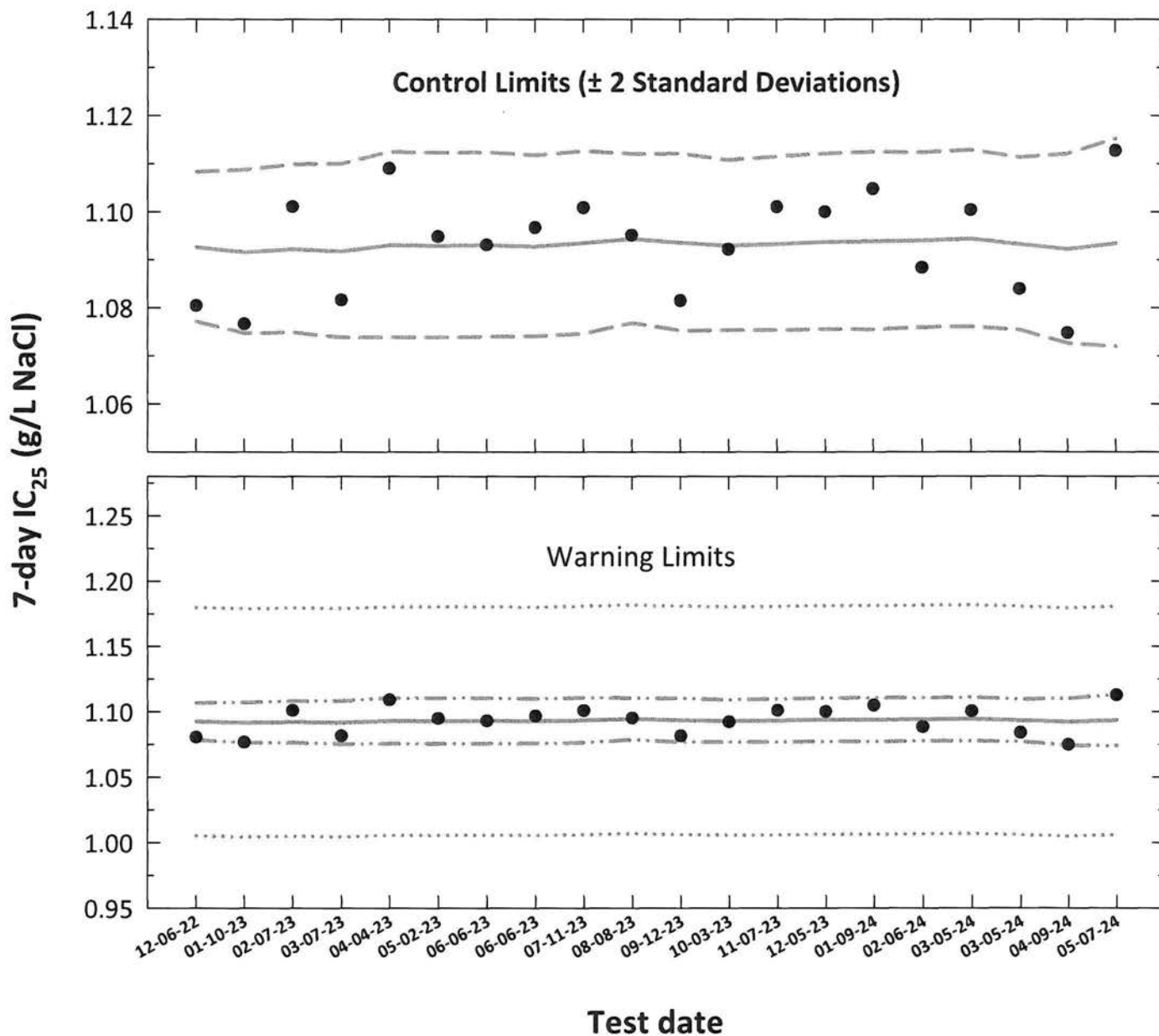
CdNaClCR #: 297

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		Analyst							
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.92	7.76	7.41	7.76	7.33	7.78	7.57	7.57
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.7	7.7	7.7	7.8	7.6
	Conductivity (µmhos/cm)	295		290		304		311	
	Alkalinity (mg CaCO ₃ /L)			59					
	Hardness (mg CaCO ₃ /L)			88					
	Temperature (°C)	24.8	25.0	24.8	25.1	24.9	25.3	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.92	7.83	7.74	7.83	7.74	7.72	7.62	7.63
	Dissolved oxygen (mg/L)	7.6	7.7	7.7	7.8	7.8	7.7	7.8	7.6
	Conductivity (µmhos/cm)	1370		1360		1420		1440	
	Temperature (°C)	24.9	25.2	24.9	24.8	24.9	25.0	25.0	24.8
800 mg NaCl/L	pH (S.U.)	7.92	7.82	7.79	7.83	7.79	7.71	7.65	7.65
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.8	7.8	7.7	7.8	7.6
	Conductivity (µmhos/cm)	1750		1740		1800		1830	
	Temperature (°C)	24.9	25.2	24.9	24.9	25.0	25.0	25.0	24.9
1000 mg NaCl/L	pH (S.U.)	7.92	7.82	7.77	7.82	7.78	7.71	7.69	7.68
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.8	7.8	7.7	7.8	7.6
	Conductivity (µmhos/cm)	2120		2100		2170		2170	
	Temperature (°C)	25.0	24.9	25.0	25.1	25.0	25.0	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.92	7.80	7.81	7.81	7.81	7.71	7.71	7.70
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.7	7.8	7.6	7.8	7.7
	Conductivity (µmhos/cm)	2480		2460		2570		2600	
	Temperature (°C)	25.0	24.9	25.0	25.2	25.0	25.2	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	7.93	7.90	7.80	7.80	7.81	7.73	7.73	7.71
	Dissolved oxygen (mg/L)	7.9	7.6	7.8	7.7	7.8	7.6	7.8	7.7
	Conductivity (µmhos/cm)	2890		2860		2940		3030	
	Temperature (°C)	24.9	24.9	25.0	25.2	25.0	24.9	24.8	25.0
		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		Anti-logarithmic Values (g/L NaCl)							
			7-day IC ₂₅	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	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
2	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
3	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
4	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
5	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
6	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
7	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
8	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
9	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
10	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
11	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
12	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
13	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
14	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
15	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
16	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760	1.1123	1.0775	1.1105	1.0065	1.1815
17	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
18	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
19	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
20	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

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 ToxCal).

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.

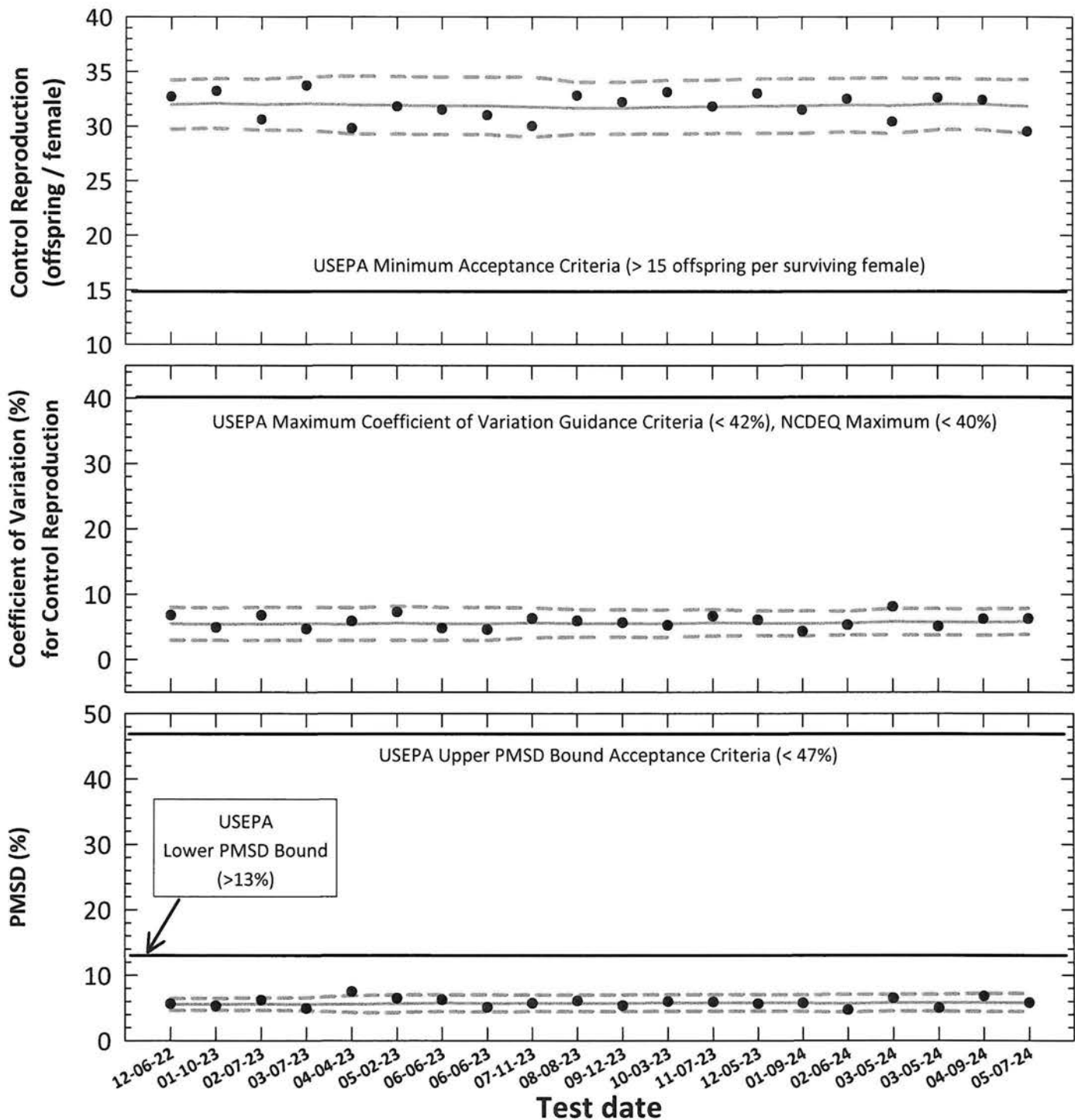
S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

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

Source: In-house Culture

Ceriodaphnia dubia

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				
			Mean (offspring/female)	CV (%)			MSD	PMSD (%)		CT - 2S	CT + 2S	CT - 2S	CT + 2S	
1	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	4.6	6.5
2	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	4.6	6.4
3	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	4.6	6.5
4	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	4.5	6.5
5	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	4.3	6.9
6	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	4.3	7.0
7	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	4.5	7.0
8	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	4.4	7.0
9	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	4.4	7.0
10	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	4.4	7.0
11	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	4.4	7.0
12	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	4.5	7.0
13	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	4.5	7.0
14	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	4.5	7.0
15	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	4.5	7.0
16	02-06-24	100	32.5	5.3	1.526	4.7	31.9	29.5	34.4	5.6	3.7	7.4	4.4	7.0
17	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	4.5	7.1
18	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	4.5	7.1
19	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	4.4	7.2
20	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	4.4	7.2

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 #: 298

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS 2283					
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:	05-07-24 0530 TO 0845									
Culture board:	04-30-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	5	7	10	11	12	14	15	17	21	27
Transfer vessel information:	pH (S.U.): 8.02 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	05-07-24	0900	04-26-24	05-03-24	05-01-24 A	JL
1	05-08-24	0802	↓	↓	↓	JL
2	05-09-24	0806	↓	↓	05-01-24 E	JL
3	05-10-24	0800	↓	↓	↓	JL
4	05-11-24	0900	↓	↓	05-06-24 A	JL
5	05-12-24	0841	↓	↓	↓	JL
6	05-13-24	0752	↓	↓	↓	JL
7	05-14-24	0700				JL

*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-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	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:	07.	≤ 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:	29.5	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.5
% CV:	6.27.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1112.7

Species: Ceriodaphnia dubia

CdNaClCR #: 298

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	4	5	4	5	5	4	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	9	12	9	11	10	12	10	10	9
	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	13	14	17	15	14	14	17	13	15
Total young produced		30	27	30	31	31	28	32	31	27	28
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: 29.5

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	6	4	5	5	4	6	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	12	11	11	10	9	9	11	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	14	18	16	15	12	15	17	14	15	15
Total young produced		31	34	32	31	29	29	32	33	30	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: 07.

Mean Offspring/Female: 31.1

% Reduction from Control: -5.47.

Species: Ceriodaphnia dubia

CdNaClCR #: 298

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	3	5	5	4	4	6	4	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	10	11	9	12	10	10	10	11
	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	15	13	15	16	14	14	15	15	15
Total young produced		34	30	28	31	29	30	35	29	31	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:	30.9
% Reduction from Control:	-4.77.

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	4	6	4	6	5	3	4	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	10	10	10	11	11	9	11	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	13	13	16	14	17	16	14	14	17
Total young produced		30	29	27	32	29	31	31	32	29	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:	30.2
% Reduction from Control:	-2.47.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 298

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	3	4	4	4	3	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	7	9	9	6	4	5	10	7	6	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	5	7	6	7	8	10	4	5	5	6
Total young produced		17	19	18	17	16	19	17	16	15	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:	17.2
% Reduction from Control:	41.77.

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	3	2	2	2	2	2	1
	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	0	0	0	0	0	0	0	0	1	0
Total young produced		2	1	1	3	2	2	2	2	3	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:	07.
Mean Offspring/Female:	1.4
% Reduction from Control:	93.67.

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	4	5	4	5	5	4	6	4	4	4	45
5	9	9	12	9	11	10	12	10	10	9	101
6	0	0	0	0	0	0	0	0	0	0	0
7	17	13	14	17	15	14	14	17	13	15	149
Total	30	27	30	31	28	32	31	27	28		295

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	6	4	5	5	4	6	5	4	4	47
5	13	10	12	11	11	10	9	9	11	12	108
6	0	0	0	0	0	0	0	0	0	0	0
7	14	18	16	15	12	15	17	19	15	15	156
Total	31	34	32	31	28	29	32	33	30	31	311

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	3	5	5	4	4	6	4	6	6	48
5	12	12	10	11	9	12	10	10	10	11	107
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	13	15	16	14	19	15	15	15	154
Total	34	30	28	31	29	30	35	29	31	32	309

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	4	6	4	6	5	3	4	4	4	5	45
5	11	10	10	10	10	11	11	9	11	10	103
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	13	16	14	17	16	19	14	17	154
Total	30	29	27	32	29	31	31	32	29	32	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	5	3	3	4	4	4	3	4	4	4	38
5	7	9	9	6	4	5	10	7	6	8	71
6	0	0	0	0	0	0	0	0	0	0	0
7	5	7	6	7	8	10	4	5	5	6	63
Total	17	19	18	17	16	19	17	16	15	18	172

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	2	2	2	2	2	1	18
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	0	0	0	0	0	0	0	0	1	0	1
Total	2	1	1	3	2	2	2	2	3	1	19

Reviewed and
Approved by
John A. Sauer
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***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: **CdNaClCR #298**
Test dates: **May 07-14, 2024**

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	27	30	31	31	28	32	31	27	28	100	29.5	6.2	Not applicable
600	31	34	32	31	28	29	32	33	30	31	100	31.1	5.8	-5.4
800	34	30	28	31	29	30	35	29	31	32	100	30.9	7.2	-4.7
1000	30	29	27	32	29	31	31	32	29	32	100	30.2	5.6	-2.4
1200	17	19	18	17	16	19	17	16	15	18	100	17.2	7.7	41.7
1400	2	1	1	3	2	2	2	2	3	1	100	1.9	38.8	93.6

Dunnett's MSD value: 1.707
PMSD: 5.8

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%.
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.

Start Date:	5/7/2024	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant													
End Date:	5/14/2024	Lab ID:	ETS-Envr. 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	27.000	30.000	31.000	31.000	28.000	32.000	31.000	27.000	28.000								
600	31.000	34.000	32.000	31.000	28.000	29.000	32.000	33.000	30.000	31.000								
800	34.000	30.000	28.000	31.000	29.000	30.000	35.000	29.000	31.000	32.000								
1000	30.000	29.000	27.000	32.000	29.000	31.000	31.000	32.000	29.000	32.000								
1200	17.000	19.000	18.000	17.000	16.000	19.000	17.000	16.000	15.000	18.000								
1400	2.000	1.000	1.000	3.000	2.000	2.000	2.000	2.000	3.000	1.000								
Transform: Untransformed																		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Isotonic Mean	Isotonic N-Mean						
D-Control	29.500	1.0000	29.500	27.000	32.000	6.240	10				30.500	1.0000						
600	31.100	1.0542	31.100	28.000	34.000	5.762	10	-2.144	2.287	1.707	30.500	1.0000						
800	30.900	1.0475	30.900	28.000	35.000	7.228	10	-1.876	2.287	1.707	30.500	1.0000						
1000	30.200	1.0237	30.200	27.000	32.000	5.585	10	-0.938	2.287	1.707	30.200	0.9902						
*1200	17.200	0.5831	17.200	15.000	19.000	7.654	10	16.480	2.287	1.707	17.200	0.5639						
*1400	1.900	0.0644	1.900	1.000	3.000	38.835	10	36.980	2.287	1.707	1.900	0.0623						
Auxiliary Tests																		
Kolmogorov D Test indicates normal distribution ($p > 0.01$)						Statistic		Critical		Skew Kurt								
						0.65504		1.035		0.08788 -0.2333								
Bartlett's Test indicates equal variances ($p = 0.07$)						10.0432		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		1.70665	0.05785	1399.31	2.78519	5.7E-44	5, 54						
Treatments vs D-Control																		
Linear Interpolation (200 Resamples)																		
Point	mg/L	SD	95%CL		Skew													
IC05	1018.85	8.3868	1000.5	1023.99	-2.1980													
IC10	1042.31	6.30485	1025.26	1047.98	-0.8366													
IC15	1065.77	5.8238	1049.91	1071.97	-0.7413													
IC20	1089.23	5.50419	1074.64	1095.99	-0.6141													
IC25	1112.69	5.37488	1099.58	1120.27	-0.4643													
IC40	1183.08	6.16052	1169.69	1194.15	-0.0351													

Species: Ceriodaphnia dubia

CdNaClCR #: 298

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.)	0.11	7.69	7.62	7.71	7.62	7.99
	Dissolved oxygen (mg/L)	7.8	7.0	7.8	7.9	7.8	7.8
	Conductivity (µmhos/cm)	209		309		312	
	Alkalinity (mg CaCO ₃ /L)	59				59	
	Hardness (mg CaCO ₃ /L)	86				86	
	Temperature (°C)	24.2	25.2	24.8	25.2	24.7	25.1
600 mg NaCl/L	pH (S.U.)	8.06	7.56	7.60	7.61	7.60	7.92
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.8	7.8	7.8
	Conductivity (µmhos/cm)	1400		1480		1420	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.8	24.9
800 mg NaCl/L	pH (S.U.)	7.98	7.54	7.59	7.60	7.61	7.88
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1780		1870		1850	
	Temperature (°C)	25.0	24.9	24.9	24.8	24.7	25.0
1000 mg NaCl/L	pH (S.U.)	7.97	7.52	7.58	7.58	7.60	7.87
	Dissolved oxygen (mg/L)	8.0	7.8	7.8	7.9	7.8	7.9
	Conductivity (µmhos/cm)	2100		2230		2210	
	Temperature (°C)	25.0	24.9	25.0	25.2	24.7	25.2
1200 mg NaCl/L	pH (S.U.)	7.95	7.52	7.58	7.58	7.58	7.87
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	7.9	7.7	7.9
	Conductivity (µmhos/cm)	2500		2020		2550	
	Temperature (°C)	25.0	24.9	25.0	25.0	24.7	25.2
1400 mg NaCl/L	pH (S.U.)	7.95	7.50	7.58	7.59	7.58	7.87
	Dissolved oxygen (mg/L)	8.1	7.9	8.0	7.8	7.7	7.9
	Conductivity (µmhos/cm)	2910		3010		2960	
	Temperature (°C)	25.0	24.9	25.0	24.8	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

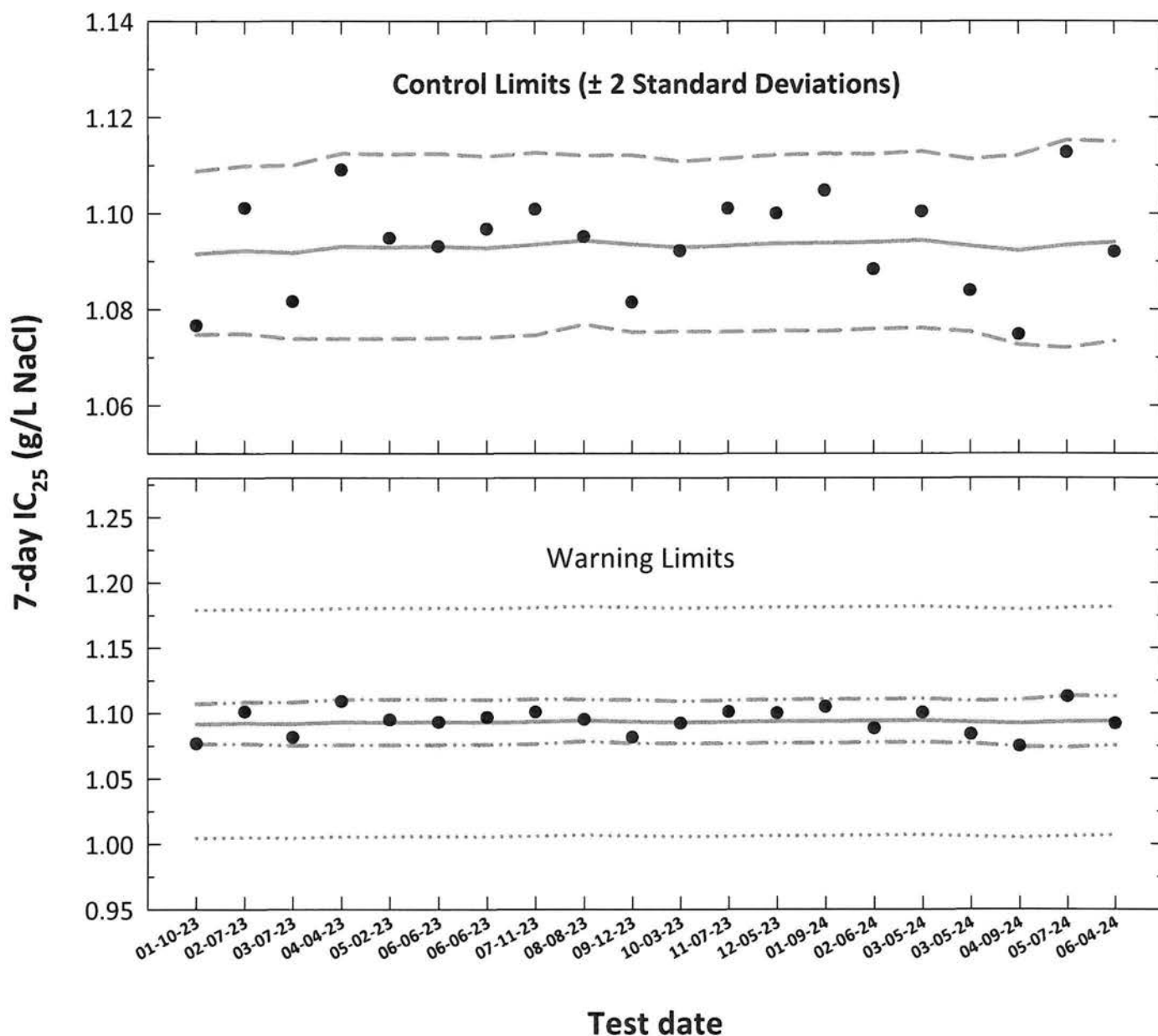
CdNaClCR #: 298

Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	IL	BSC	BSC	BSC	BSC	IL	IL	IL
CONTROL, MHSW	pH (S.U.)	8.41	7.72	7.63	7.70	7.69	7.68	7.62	7.46
	Dissolved oxygen (mg/L)	7.9	8.0	7.7	7.8	7.7	7.8	7.9	7.7
	Conductivity (µmhos/cm)	299		293		300		312	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			88					
	Temperature (°C)	24.9	25.0	24.9	25.1	24.9	25.2	24.8	25.1
600 mg NaCl/L	pH (S.U.)	8.01	7.63	7.42 (7.50)	7.65	7.57	7.73	7.77	7.56
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.8	7.7	7.8	7.9	7.7
	Conductivity (µmhos/cm)	1420		1410		1410		1430	
	Temperature (°C)	24.8	25.3	25.0	24.9	25.0	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.98	7.61	7.54	7.63	7.60	7.73	7.79	7.58
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.8	7.7	7.7	7.8	7.8
	Conductivity (µmhos/cm)	1810		1800		1820		1870	
	Temperature (°C)	24.8	25.0	24.9	24.9	24.9	25.2	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.98	7.59	7.54	7.61	7.59	7.74	7.79	7.60
	Dissolved oxygen (mg/L)	7.9	8.0	7.9	7.7	7.8	7.7	7.8	7.7
	Conductivity (µmhos/cm)	2160		2130		2170		2220	
	Temperature (°C)	24.9	25.0	24.9	25.1	24.9	24.8	25.0	24.8
1200 mg NaCl/L	pH (S.U.)	7.98	7.57	7.55	7.59	7.59	7.74	7.80	7.61
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	7.8	7.8	7.7	7.9	7.6
	Conductivity (µmhos/cm)	2530		2470		2530		2630	
	Temperature (°C)	24.9	25.0	25.0	25.1	24.9	24.8	25.0	24.8
1400 mg NaCl/L	pH (S.U.)	7.98	7.54	7.56	7.58	7.59	7.76	7.80	7.62
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	7.9	7.9	7.7	7.9	7.6
	Conductivity (µmhos/cm)	2920		2920		2900		3010	
	Temperature (°C)	24.8	25.1	25.0	25.0	24.9	24.9	25.0	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,
 $S_{A.10} = 10^{th}$ 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		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
2	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
3	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
4	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
5	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
6	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
7	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
8	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
9	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
10	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
11	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
12	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
13	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
14	01-09-24	1.1048	0.0433	0.0390	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
15	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760	1.1123	1.0775	1.1105	1.0065	1.1815
16	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
17	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
18	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
19	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
20	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

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 ToxCal).

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.

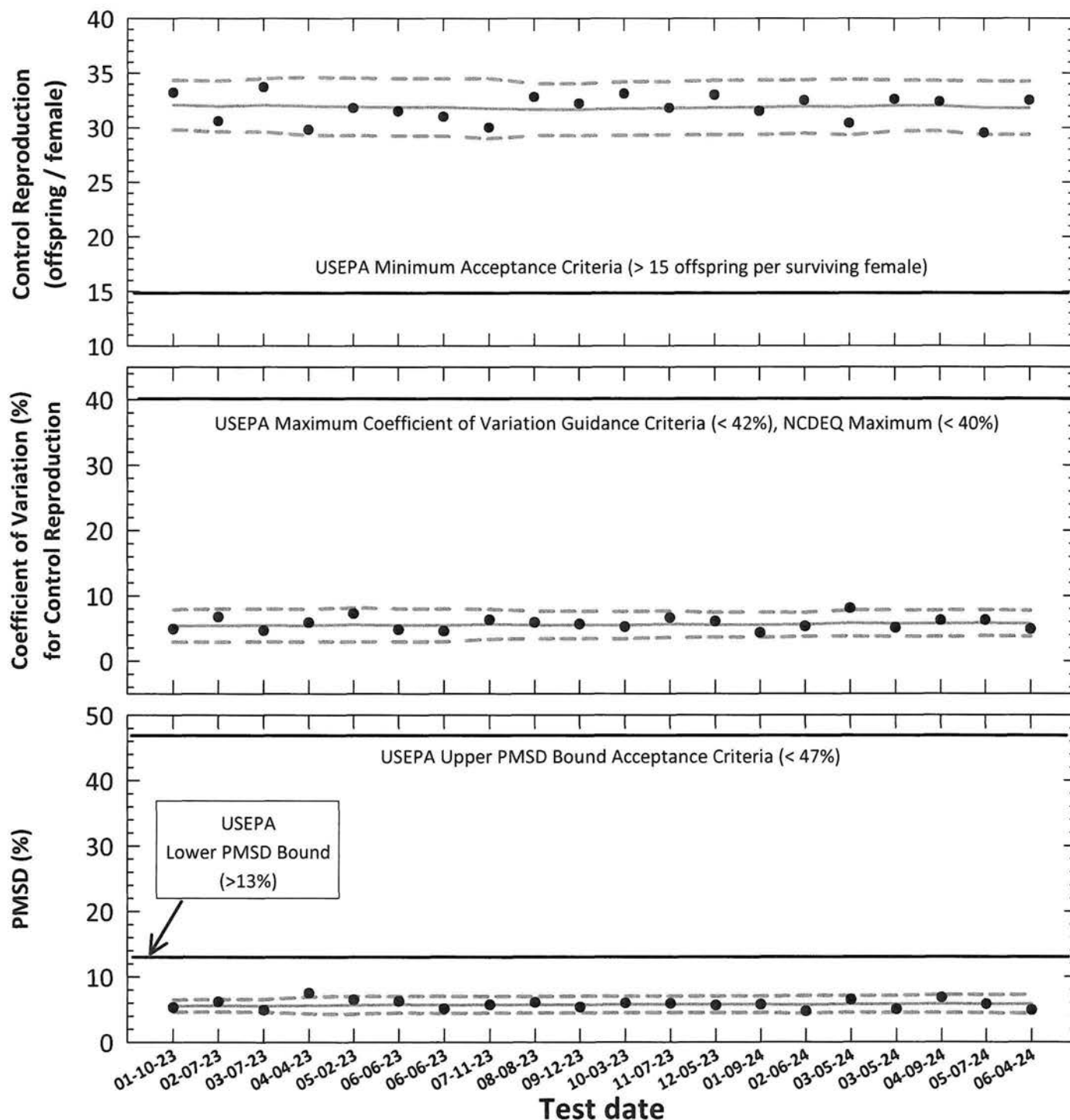
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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	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	CT + 2S
1	01-10-23	100	33.2	4.9	1.756	5.3	29.8	34.3	32.1	29.8	34.3	5.4	29.8	34.3
2	02-07-23	100	30.6	6.8	1.891	6.2	29.6	34.3	32.0	29.6	34.3	5.4	29.6	34.3
3	03-07-23	100	33.7	4.7	1.648	4.9	29.6	34.5	32.1	29.6	34.5	5.4	29.6	34.5
4	04-04-23	100	29.8	5.9	2.241	7.5	29.3	34.6	32.0	29.3	34.6	5.4	29.3	34.6
5	05-02-23	100	31.8	7.2	2.054	6.5	29.3	34.5	31.9	29.3	34.5	5.5	29.3	34.5
6	06-06-23	100	31.5	4.8	1.965	6.2	29.2	34.5	31.9	29.2	34.5	5.4	29.2	34.5
7	06-06-23	100	31.0	4.6	1.573	5.1	29.2	34.5	31.9	29.2	34.5	5.5	29.2	34.5
8	07-11-23	100	30.0	6.3	1.710	5.7	29.0	34.5	31.8	29.0	34.5	5.6	29.0	34.5
9	08-08-23	100	32.8	5.9	1.990	6.1	29.3	34.0	31.7	29.3	34.0	5.5	29.3	34.0
10	09-12-23	100	32.2	5.6	1.717	5.3	29.3	34.0	31.7	29.3	34.0	5.5	29.3	34.0
11	10-03-23	100	33.1	5.2	1.970	6.0	29.3	34.2	31.8	29.3	34.2	5.5	29.3	34.2
12	11-07-23	100	31.8	6.6	1.867	5.9	29.3	34.2	31.8	29.3	34.2	5.6	29.3	34.2
13	12-05-23	100	33.0	6.1	1.849	5.6	29.4	34.4	31.9	29.4	34.4	5.5	29.4	34.4
14	01-09-24	100	31.5	4.3	1.810	5.7	29.4	34.4	31.9	29.4	34.4	5.5	29.4	34.4
15	02-06-24	100	32.5	5.3	1.526	4.7	29.5	34.4	31.9	29.5	34.4	5.6	29.5	34.4
16	03-05-24	100	30.4	8.1	1.973	6.5	29.3	34.4	31.9	29.3	34.4	5.8	29.3	34.4
17	03-05-24	100	32.6	5.1	1.629	5.0	29.7	34.4	32.0	29.7	34.4	5.7	29.7	34.4
18	04-09-24	100	32.4	6.2	2.199	6.8	29.7	34.3	32.0	29.7	34.3	5.7	29.7	34.3
19	05-07-24	100	29.5	6.2	1.707	5.8	29.3	34.3	31.8	29.3	34.3	5.8	29.3	34.3
20	06-04-24	100	32.5	4.9	1.587	4.9	29.3	34.2	31.8	29.3	34.2	5.7	29.3	34.2

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.

PMMSD = Percent minimum significant difference.

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

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

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

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

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

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

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

Species: *Ceriodaphnia dubia*

CdNaClCR #: 299

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS 2283					
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:	06-04-24 0540 TO 0800									
Culture board:	05-28-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	7	8	11	12	13	17	20	23
Transfer vessel information:	pH (S.U.): 7.67 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	ORANGE
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	06-04-24	0810	05-31-24	05-31-24	05-29-24C	JK
1	06-05-24	0730	↓	↓	↓	JK
2	06-06-24	0745			05-29-24D	JK
3	06-07-24	0727			↓	JK
4	06-08-24	0833			06-05-24B	JK
5	06-09-24	0830			↓	JK
6	06-10-24	0726			↓	JK
7	06-11-24	0610				JK

*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	1506646ES

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	07.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	71400
% Adults having 3 rd Broods:	1007.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	07.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	32.5	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.4
% CV:	4.97.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1092.0

Species: *Ceriodaphnia dubia*

CdNaClCR #: 299

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	5	5	4	5	3	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	12	10	13	12	10	10	13	11
	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	18	18	17	14	17	19	18	14	18
Total young produced		33	31	35	32	32	33	31	31	30	34
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: 32.5

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	3	5	4	5	5	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	12	13	10	12	12	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	15	19	16	17	16	16	19	17
Total young produced		33	34	32	35	34	32	32	33	36	34
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: 33.5

% Reduction from Control: -3.17.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 299

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	4	4	4	4	5	3	3	3	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	11	10	12	13	12	11	11	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	14	19	14	16	17	13	18	17	17	15
Total young produced		30	33	29	30	33	31	33	31	31	34
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:	31.5
% Reduction from Control:	3.17.

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	4	5	4	4	4	4	3	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	10	13	11	10	12	12	11	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	16	14	15	14	16	13	18	15	15	15
Total young produced		30	31	29	31	31	27	33	30	31	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:	30.5
% Reduction from Control:	6.27.

Species: Ceriodaphnia dubia

CdNaClCR #: 299

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	4	3	4	4	5	4	3	4	4	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	7	9	6	6	5	10	8	7	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	4	9	5	9	4	11	5	9	4	4
Total young produced		18	19	18	19	15	20	18	21	15	17
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:	18.0
% Reduction from Control:	44.67.

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	3	2	1	1	3	1	1	1	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	2	0	2	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	0	0	0	0	0	0	0	0
Total young produced		3	2	1	1	3	1	3	1	3	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:	2.0
% Reduction from Control:	93.87.

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	5	5	4	5	3	3	5	43
5	10	10	12	10	13	12	10	10	13	11	111
6	0	0	0	0	0	0	0	0	0	0	0
7	18	18	18	17	14	17	19	18	14	18	171
Total	33	31	35	32	32	33	34	31	30	34	325

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	4	5	4	4	4	4	3	3	5	5	41
5	10	12	10	13	11	10	12	12	11	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	15	14	16	13	18	15	15	15	151
Total	30	31	29	31	31	27	33	30	31	32	305

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	3	5	4	5	5	4	5	5	5	45
5	11	12	12	13	10	12	12	12	12	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	18	19	15	19	16	17	16	16	19	17	172
Total	33	34	32	35	34	32	32	33	36	34	335

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	4	3	4	4	5	4	3	4	4	3	38
5	10	7	9	6	6	5	10	8	7	10	78
6	0	0	0	0	0	0	0	0	0	0	0
7	4	9	5	9	4	11	5	9	4	4	64
Total	18	19	18	19	15	20	18	21	15	17	180

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	4	4	4	4	5	3	3	3	6	41
5	11	10	11	10	12	13	12	11	11	13	114
6	0	0	0	0	0	0	0	0	0	0	0
7	14	19	14	16	17	13	18	17	17	15	160
Total	30	33	29	30	33	31	33	31	31	34	315

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	3	2	1	1	3	1	1	1	1	2	16
5	0	0	0	0	0	0	2	0	2	0	4
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	3	2	1	1	3	1	3	1	3	2	20

Reviewed and
Approved by
Jon Sumner



***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: **CdNaClCR #299**
Test dates: **June 04-11, 2024**

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	31	35	32	32	33	34	31	30	34	100	32.5	4.9	Not applicable
600	33	34	32	35	34	32	32	33	36	34	100	33.5	4.0	-3.1
800	30	33	29	30	33	31	33	31	31	34	100	31.5	5.2	3.1
1000	30	31	29	31	31	27	33	30	31	32	100	30.5	5.4	6.2
1200	18	19	18	19	15	20	18	21	15	17	100	18.0	10.8	44.6
1400	3	2	1	1	3	1	3	1	3	2	100	2.0	47.1	93.8

Dunnett's MSD value: 1.587
PMSD: 4.9
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%.
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: 6/4/2024 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
End Date: 6/11/2024 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	31.000	35.000	32.000	32.000	33.000	34.000	31.000	30.000	34.000
600	33.000	34.000	32.000	35.000	34.000	32.000	32.000	33.000	36.000	34.000
800	30.000	33.000	29.000	30.000	33.000	31.000	33.000	31.000	31.000	34.000
1000	30.000	31.000	29.000	31.000	31.000	27.000	33.000	30.000	31.000	32.000
1200	18.000	19.000	18.000	19.000	15.000	20.000	18.000	21.000	15.000	17.000
1400	3.000	2.000	1.000	1.000	3.000	1.000	3.000	1.000	3.000	2.000

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	32.500	1.0000	32.500	30.000	35.000	4.865	10				33.000	1.0000
600	33.500	1.0308	33.500	32.000	36.000	4.042	10	-1.441	2.287	1.587	33.000	1.0000
800	31.500	0.9692	31.500	29.000	34.000	5.238	10	1.441	2.287	1.587	31.500	0.9545
*1000	30.500	0.9385	30.500	27.000	33.000	5.410	10	2.882	2.287	1.587	30.500	0.9242
*1200	18.000	0.5538	18.000	15.000	21.000	10.798	10	20.897	2.287	1.587	18.000	0.5455
*1400	2.000	0.0615	2.000	1.000	3.000	47.140	10	43.955	2.287	1.587	2.000	0.0606

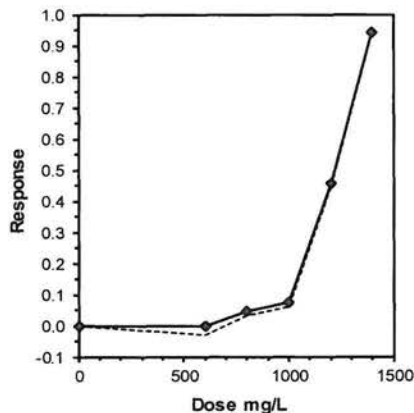
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ($p > 0.01$)	0.77318	1.035	-0.1769	-0.3316
Bartlett's Test indicates equal variances ($p = 0.46$)	4.61233	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		1.58669	0.04882	1556.67	2.40741	0.0E+00	5, 54
Treatments vs D-Control										

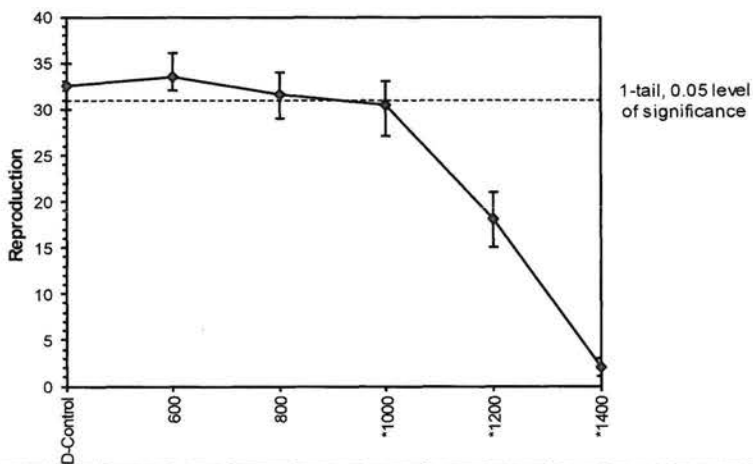
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	830	82.5198	722.327	1003.55
IC10	1012.8	20.3288	934.569	1028.37
IC15	1039.2	8.42269	1021.2	1053.87
IC20	1065.6	7.84031	1050.05	1079.36
IC25	1092	7.54315	1077.37	1105.38
IC40	1171.2	8.51961	1155.23	1189.47
IC50	1218.75	6.64728	1205.99	1230.82

While hypothesis test results indicate a significant difference in reproduction for the 1000 mg/L concentration, the PMSD was below the lower bound (13%) established by EPA. Guidance in EPA 833-R-00-003 (June 2000) Section 6.4.2 for determining the NOEC was followed, and it was concluded that the hypothesis test yielded a Type I Error. The ChV was recalculated to be 1095.4 mg/L.



Dose-Response Plot



Species: Ceriodaphnia dubiaCdNaClCR #: 299**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	Analyst	Analyst	Analyst	Analyst	Analyst
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.01	7.75	7.49	7.53	7.41	7.92
	Dissolved oxygen (mg/L)	7.8	7.8	7.6	7.9	8.0	7.8
	Conductivity (µmhos/cm)	294		308		315	
	Alkalinity (mg CaCO ₃ /L)	57				58	
	Hardness (mg CaCO ₃ /L)	85				85	
	Temperature (°C)	24.9	25.1	24.8	25.2	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.79	7.40	7.50	7.46	7.43	7.06
	Dissolved oxygen (mg/L)	7.8	7.8	7.6	7.9	7.9	7.8
	Conductivity (µmhos/cm)	1380		1480		1450	
	Temperature (°C)	25.0	24.9	24.8	24.9	24.9	25.3
800 mg NaCl/L	pH (S.U.)	7.79	7.46	7.50	7.44	7.43	7.04
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.9	7.9	7.7
	Conductivity (µmhos/cm)	1720		1840		1820	
	Temperature (°C)	25.0	24.9	24.9	25.2	24.8	25.1
1000 mg NaCl/L	pH (S.U.)	7.78	7.45	7.49	7.43	7.45	7.03
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.9	8.0	7.7
	Conductivity (µmhos/cm)	2070		2210		2190	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.8	25.1
1200 mg NaCl/L	pH (S.U.)	7.78	7.45	7.49	7.42	7.44	7.02
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.9	8.0	7.7
	Conductivity (µmhos/cm)	2420		2570		2590	
	Temperature (°C)	25.0	25.0	25.0	25.0	24.8	25.0
1400 mg NaCl/L	pH (S.U.)	7.78	7.43	7.40	7.43	7.44	7.03
	Dissolved oxygen (mg/L)	7.0	7.7	7.7	7.9	8.1	7.6
	Conductivity (µmhos/cm)	2750		2940		2870	
	Temperature (°C)	25.1	25.0	25.0	25.2	24.8	25.0
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

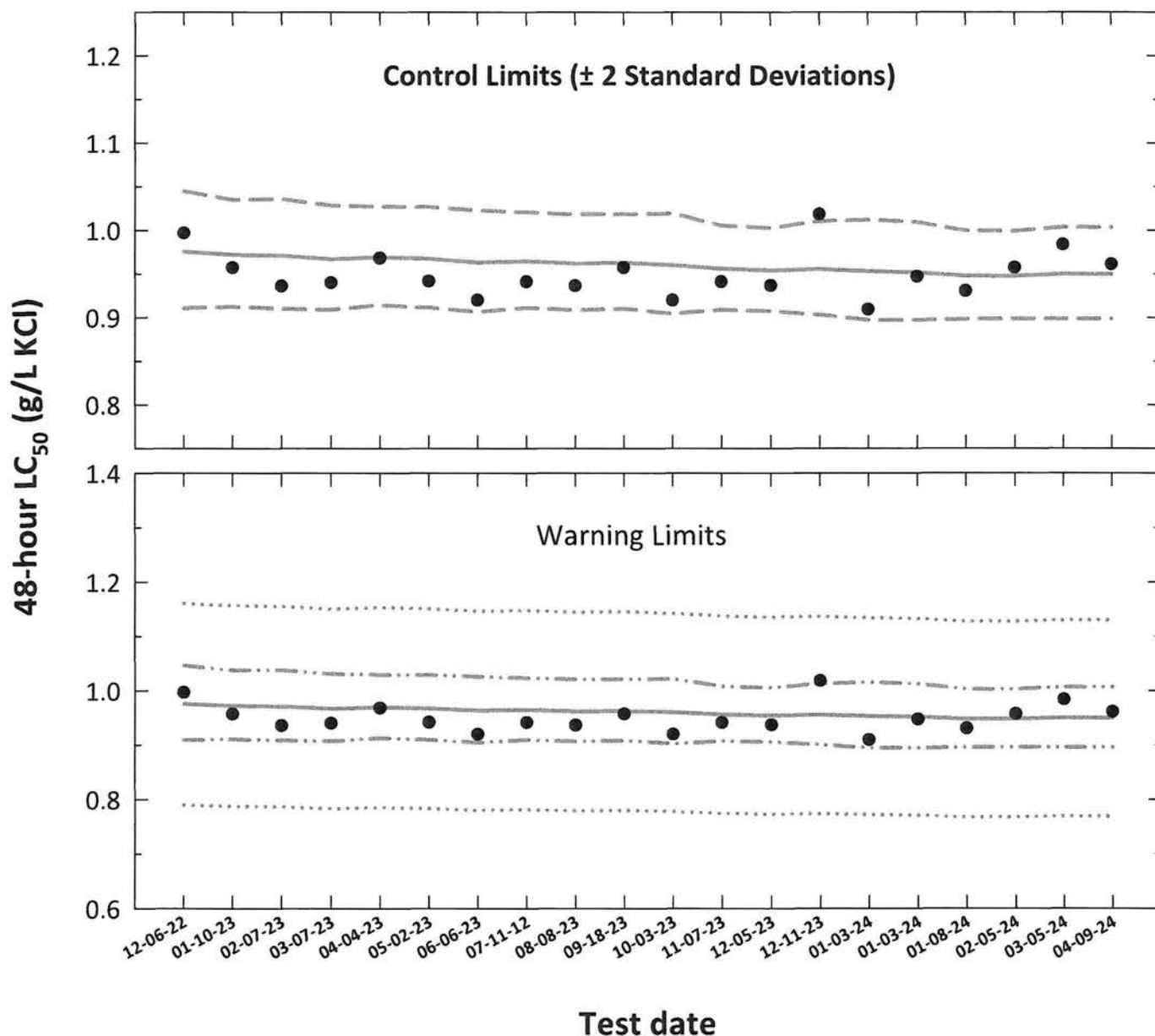
CdNaClCR #: 299

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		Analyst	BSL	BSL	BSL	BSL	KD N	KD N	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.94	7.60	7.70	7.44	7.71	7.76	7.73	8.11
	Dissolved oxygen (mg/L)	7.7	7.9	7.7	7.9	7.7	7.9	8.0	7.9
	Conductivity (µmhos/cm)	302		301		302		304	
	Alkalinity (mg CaCO ₃ /L)			58					
	Hardness (mg CaCO ₃ /L)			87					
	Temperature (°C)	24.7	25.1	24.8	25.2	24.9	24.9	24.7	24.9
600 mg NaCl/L	pH (S.U.)	7.90	7.53	7.43	7.55	7.43	7.82	7.80	8.03
	Dissolved oxygen (mg/L)	7.7	7.9	7.7	7.8	7.8	7.9	7.7	7.9
	Conductivity (µmhos/cm)	1390		1400		1390		1400	
	Temperature (°C)	24.8	24.9	24.9	24.8	25.0	25.2	24.7	25.2
800 mg NaCl/L	pH (S.U.)	7.91	7.50	7.45	7.52	7.43	7.82	7.83	8.02
	Dissolved oxygen (mg/L)	7.6	7.9	7.8	7.8	7.8	7.9	7.7	8.0
	Conductivity (µmhos/cm)	1760		1800		1780		1830	
	Temperature (°C)	24.9	24.9	24.7	25.2	24.9	24.9	24.8	25.0
1000 mg NaCl/L	pH (S.U.)	7.89	7.48	7.44	7.49	7.43	7.83	7.85	8.00
	Dissolved oxygen (mg/L)	7.7	7.8	7.8	7.8	7.9	7.9	7.7	8.0
	Conductivity (µmhos/cm)	2110		2150		2130		2170	
	Temperature (°C)	24.9	24.9	24.7	25.1	24.9	24.9	24.7	25.0
1200 mg NaCl/L	pH (S.U.)	7.88	7.46	7.44	7.47	7.44	7.83	7.84	7.99
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.8	7.8	7.8	7.7	8.1
	Conductivity (µmhos/cm)	2430		2500		2500		2530	
	Temperature (°C)	24.7	25.2	24.8	24.9	24.9	25.1	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.88	7.45	7.43	7.45	7.44	7.84	7.84	7.98
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.8	7.9	7.9	7.7	8.1
	Conductivity (µmhos/cm)	2810		2880		2880		2890	
	Temperature (°C)	24.7	25.0	24.8	24.9	24.9	24.9	24.8	25.0
		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		CT	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	12-06-22	0.9971	-0.0013	-0.0106	0.0149	0.9758	0.9111 1.0450	0.9095 1.0468	0.7904 1.1612
2	01-10-23	0.9572	-0.0190	-0.0123	0.0137	0.9720	0.9126 1.0352	0.9109 1.0370	0.7873 1.1566
3	02-07-23	0.9362	-0.0286	-0.0127	0.0140	0.9711	0.9103 1.0360	0.9085 1.0379	0.7866 1.1557
4	03-07-23	0.9402	-0.0268	-0.0145	0.0134	0.9672	0.9093 1.0287	0.9073 1.0308	0.7834 1.1509
5	04-04-23	0.9683	-0.0140	-0.0135	0.0126	0.9693	0.9145 1.0275	0.9127 1.0293	0.7851 1.1535
6	05-02-23	0.9420	-0.0260	-0.0143	0.0129	0.9677	0.9117 1.0270	0.9098 1.0290	0.7838 1.1515
7	06-06-23	0.9201	-0.0362	-0.0162	0.0131	0.9633	0.9068 1.0233	0.9046 1.0256	0.7803 1.1463
8	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116 1.0208	0.9096 1.0229	0.7814 1.1479
9	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088 1.0185	0.9067 1.0208	0.7793 1.1449
10	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103 1.0185	0.9083 1.0207	0.7800 1.1459
11	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049 1.0194	0.9026 1.0219	0.7780 1.1429
12	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092 1.0055	0.9071 1.0078	0.7745 1.1378
13	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077 1.0028	0.9054 1.0052	0.7728 1.1353
14	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035 1.0107	0.9011 1.0133	0.7740 1.1372
15	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974 1.0124	0.8947 1.0153	0.7721 1.1343
16	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971 1.0093	0.8943 1.0123	0.7708 1.1323
17	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985 0.9999	0.8958 1.0027	0.7678 1.1279
18	02-05-24	0.9572	-0.0190	-0.0234	0.0115	0.9475	0.8986 0.9992	0.8958 1.0020	0.7675 1.1276
19	03-05-24	0.9839	-0.0070	-0.0223	0.0120	0.9499	0.8988 1.0039	0.8961 1.0067	0.7694 1.1304
20	04-09-24	0.9556	-0.0197	-0.0225	0.0119	0.9495	0.8987 1.0031	0.8961 1.0060	0.7691 1.1299

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 TestPpKCIAC # 163

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 #: 2275

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.70	7.04	7.00
	Dissolved oxygen (mg/L)	7.0	7.9	7.5
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L CaCO ₃)	61		
	Hardness (mg/L CaCO ₃)	90		
	Temperature (°C)	24.8	24.9	25.2
500 mg/L	pH (S.U.)	7.04	7.77	7.79
	Dissolved oxygen (mg/L)	0.0	7.9	7.3
	Conductivity (µmhos/cm)	1170		
	Temperature (°C)	24.9	25.2	24.8
750 mg/L	pH (S.U.)	7.02	7.77	7.70
	Dissolved oxygen (mg/L)	0.0	7.9	7.3
	Conductivity (µmhos/cm)	1550		
	Temperature (°C)	24.9	25.0	25.1
1000 mg/L	pH (S.U.)	7.04	7.76	7.70
	Dissolved oxygen (mg/L)	0.0	7.9	7.3
	Conductivity (µmhos/cm)	1980		
	Temperature (°C)	24.8	25.0	25.1
1250 mg/L	pH (S.U.)	7.04	7.74	7.70
	Dissolved oxygen (mg/L)	0.1	7.9	7.3
	Conductivity (µmhos/cm)	2440		
	Temperature (°C)	24.7	25.3	25.2
1500 mg/L	pH (S.U.)	7.66	7.74	
	Dissolved oxygen (mg/L)	0.1	7.9	
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	24.9	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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130604164

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 # 163

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-09-24	0505	J	0705	J	1C	WHITE	04-01-14B
24	04-10-24			0700	J			
48 Termination	04-11-24			0703	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
Spawning date:	03-27-24
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	04-02-24 1139 TO 04-03-24 0814
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.75
	Temperature (°C): 24.2

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*.

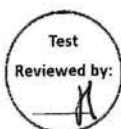
Survival Data (number of living organisms):

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	5 ^{sd}	5 ^{sd}	2 ^{sd}	1 ^{sd}	0	0
48 Termination	10	10	10	10	9 ^{sd}	9 ^{sd}	5	5	0 ^{sd}	0 ^{sd}	0	0
Mean Survival	100%		100%		90%		50%		0%		0%	

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

Statistics:

Method	SVK	120615	Comments:
Lower 95% confidence limit (mg KCl/L)	845.9	885.8	
Upper 95% confidence limit (mg KCl/L)	1031.4	1122.0	
48-hour LC ₅₀ (mg KCl/L)	961.3	955.6	



Acute Fathead Minnow Test-24 Hr Survival

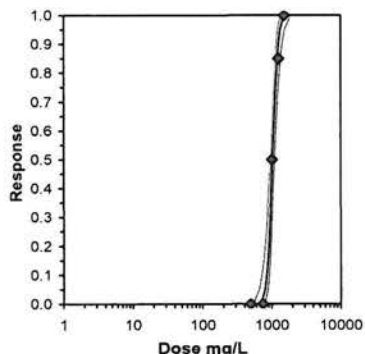
Start Date: 4/9/2024 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/11/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas
Comments:

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	0.2000	0.1000
1500	0.0000	0.0000

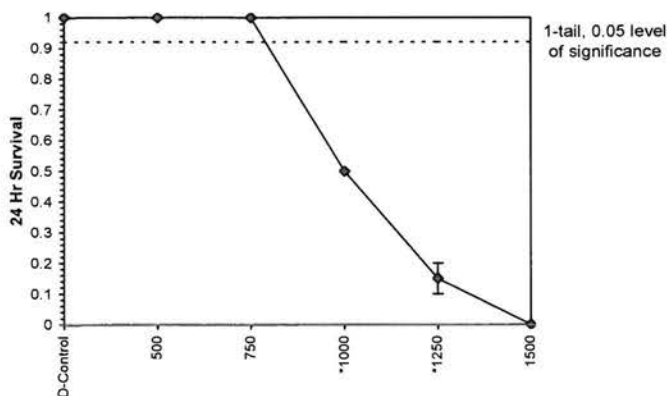
Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	13.965	2.850	0.1279	10	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	22.716	2.850	0.1279	17	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		0.05495	0.05636	0.44492	0.00201	8.3E-06	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	15.1232	2.89765	9.44383	20.8026	0	1.50888	7.81472	0.68022	3.0127	0.06612	5
Intercept	-40.562	8.76793	-57.747	-23.377							
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	722.56	566.603	812.668							
EC05	3.355	801.562	666.136	880.141							
EC10	3.718	847.149	725.218	919.56							
EC15	3.964	879.362	767.35	947.968							
EC20	4.158	905.836	801.996	971.87							
EC25	4.326	929.182	832.395	993.534							
EC40	4.747	990.714	910.448	1054.62							
EC50	5.000	1029.68	956.986	1097.62							
EC60	5.253	1070.17	1001.92	1146.9							
EC75	5.674	1141.04	1071.75	1244.82							
EC80	5.842	1170.45	1097.97	1289.27							
EC85	6.036	1205.68	1127.8	1344.91							
EC90	6.282	1251.53	1164.67	1420.56							
EC95	6.645	1322.71	1218.88	1543.96							
EC99	7.326	1467.33	1322.25	1812.21							



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 4/9/2024 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/11/2024 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

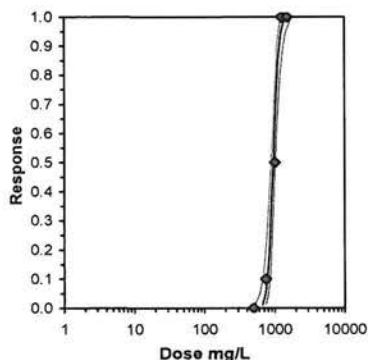
Comments:

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	0.0000	0.0000
1500	0.0000	0.0000

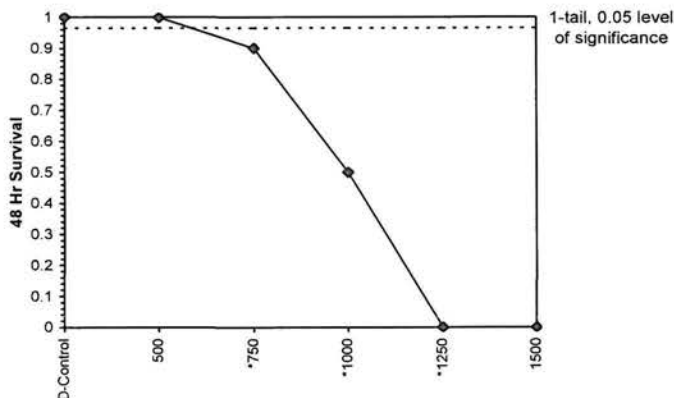
Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	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	500	750	612.372		0.00967	0.00991	0.57759	0.0001	2.4E-09	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	14.7254	2.78615	9.26455 20.1862	0	2.58099	7.81472	0.46083	2.98027	0.06791	5
Intercept	-38.886	8.33932	-55.231 -22.541							
TSCR										
Point	Probits	mg/L	95% Fiducial Limits							
EC01	2.674	664.186	519.996 748.873							
EC05	3.355	738.873	613.091 813.21							
EC10	3.718	782.063	668.425 850.917							
EC15	3.964	812.62	707.901 878.148							
EC20	4.158	837.756	740.369 901.095							
EC25	4.326	859.938	768.861 921.914							
EC40	4.747	918.474	842.044 980.672							
EC50	5.000	955.59	885.755 1021.98							
EC60	5.253	994.206	928.11 1069.18							
EC75	5.674	1061.88	994.374 1162.58							
EC80	5.842	1090	1019.4 1204.89							
EC85	6.036	1123.71	1047.95 1257.85							
EC90	6.282	1167.62	1083.31 1329.89							
EC95	6.645	1235.87	1135.41 1447.53							
EC99	7.326	1374.85	1234.97 1703.91							



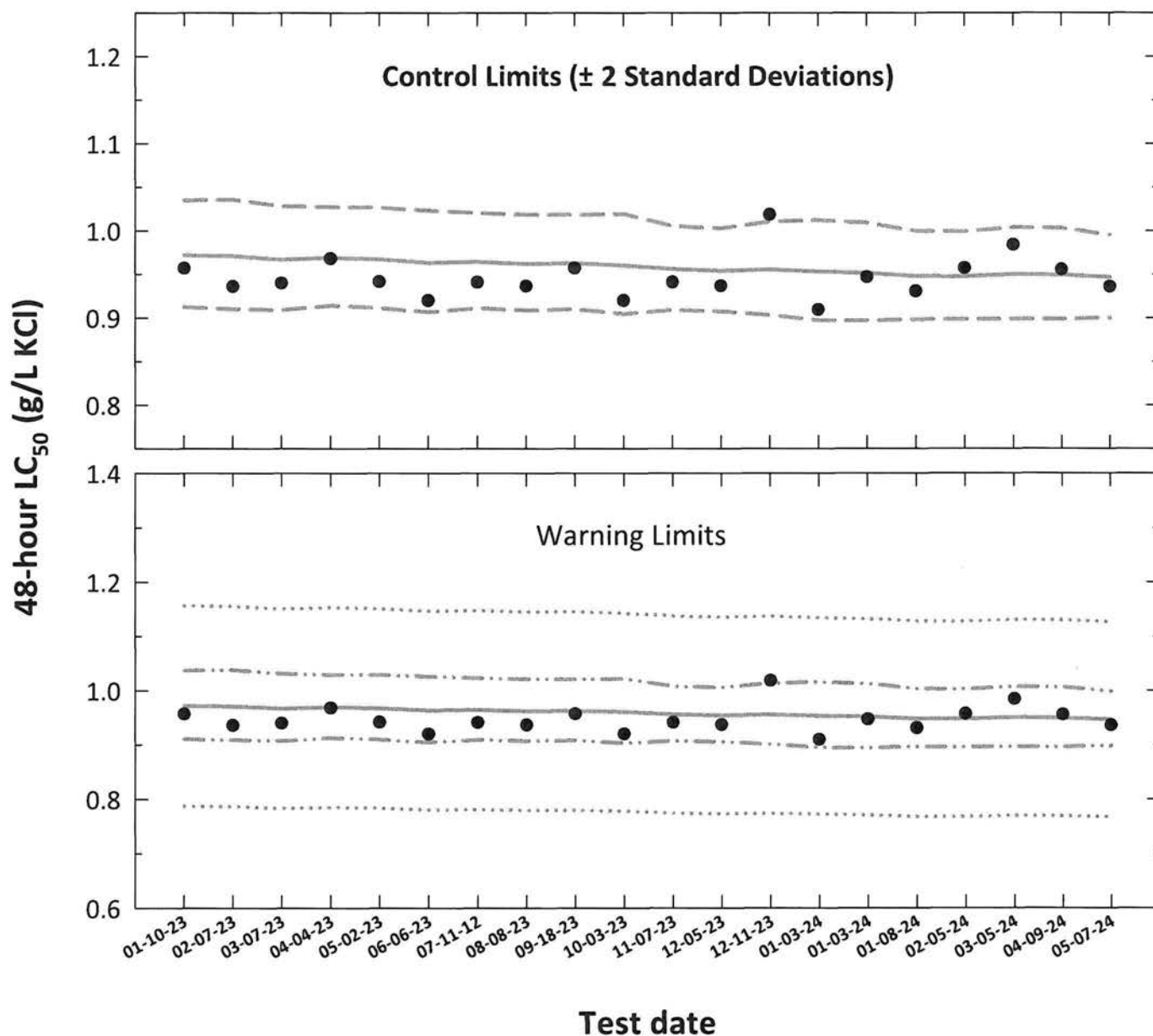
Dose-Response Plot



Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = 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 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,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		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	01-10-23	0.9572	-0.0190	-0.0123	0.0137	0.9720	0.9126	1.0352	0.9109	1.0370	0.7873	1.1566
2	02-07-23	0.9362	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379	0.7866	1.1557
3	03-07-23	0.9402	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308	0.7834	1.1509
4	04-04-23	0.9683	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293	0.7851	1.1535
5	05-02-23	0.9420	-0.0260	-0.0143	0.0129	0.9677	0.9117	1.0270	0.9098	1.0290	0.7838	1.1515
6	06-06-23	0.9201	-0.0362	-0.0162	0.0131	0.9633	0.9068	1.0233	0.9046	1.0256	0.7803	1.1463
7	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
8	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
9	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
10	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
11	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
12	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
13	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
14	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
15	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
16	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
17	02-05-24	0.9572	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
18	03-05-24	0.9839	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
19	04-09-24	0.9556	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
20	05-07-24	0.9358	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263

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 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,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 # 16-1

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 #: 2275

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	8.11	7.08	7.46
	Dissolved oxygen (mg/L)	7.8	7.7	7.7
	Conductivity (µmhos/cm)	289		
	Alkalinity (mg/L CaCO ₃)	59		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.8	24.5	24.2
500 mg/L	pH (S.U.)	7.96	7.74	7.57
	Dissolved oxygen (mg/L)	8.1	7.7	7.7
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	24.7	24.3	24.2
750 mg/L	pH (S.U.)	7.94	7.75	7.57
	Dissolved oxygen (mg/L)	8.1	7.8	7.7
	Conductivity (µmhos/cm)	1540		
	Temperature (°C)	24.7	24.3	24.1
1000 mg/L	pH (S.U.)	7.92	7.72	7.57
	Dissolved oxygen (mg/L)	8.1	7.8	7.7
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	24.7	24.6	24.4
1250 mg/L	pH (S.U.)	7.92	7.70	7.40
	Dissolved oxygen (mg/L)	8.1	7.8	7.7
	Conductivity (µmhos/cm)	2370		
	Temperature (°C)	24.7	24.5	24.2
1500 mg/L	pH (S.U.)	7.91	7.70	
	Dissolved oxygen (mg/L)	8.1	7.8	
	Conductivity (µmhos/cm)	2800		
	Temperature (°C)	24.9	24.6	

*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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066485

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 # 164

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-07-24	0505	J	0705	J	1E	PINK	05-01-24A
24	05-08-24			0700	J			
48 Termination	05-09-24			0700	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
Spawning date:	04-25-24
Age (1 to 14 days old):	5 to 6 DAYS
Hatch date and times:	05-01-24 1144 TO 05-02-24 0620
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.2-7.4 05-07-24 J Temperature (°C): 24.2°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	8 ^{2d}	10	5 ^{sd}	5 ^{sd}	1 ^{2d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	8	10	4 ^{1d}	3 ^{2d}	1	0	0	0
Mean Survival	100%		100%		90%		35%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	863.7
Upper 95% confidence limit (mg KCl/L)	1004.0
48-hour LC ₅₀ (mg KCl/L)	935.8

Comments:

Start Date:	5/7/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2024	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	0.8000	1.0000
1000	0.5000	0.5000
1250	0.1000	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	0.3116	0	20
750	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.394	2.850	0.3116	2	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	5.732	2.850	0.3116	10	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	10.719	2.850	0.3116	19	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

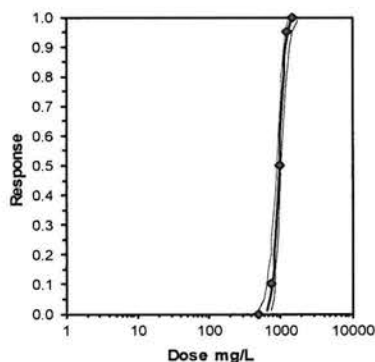
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	750	1000	866.025		0.18038	0.185	0.51388	0.01195	4.6E-04	4.5

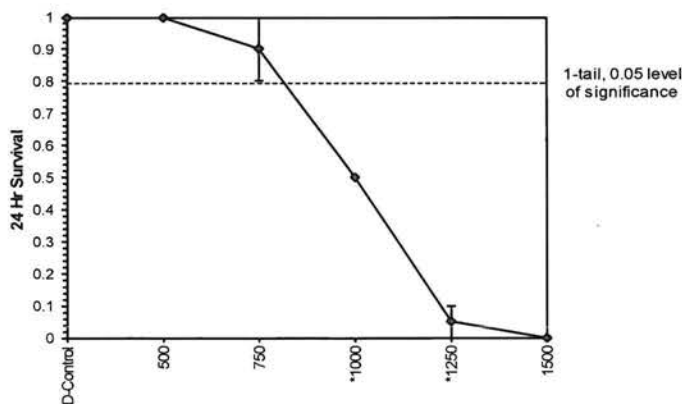
Treatments vs D-Control

Treatments vs D-control					Maximum Likelihood-Probbit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2148	2.40897	8.4932	17.9364	0	0.94914	7.81472	0.81355	2.98587	0.07567	3

Intercept	-34.458	7.23006	-48.629	-20.287
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	645.405	498.603	734.866
EC05	3.355	726.78	596.936	805.88
EC10	3.718	774.274	656.126	847.698
EC15	3.964	808.06	698.686	877.957
EC20	4.158	835.96	733.899	903.478
EC25	4.326	860.663	764.958	926.643
EC40	4.747	926.194	845.444	992.045
EC50	5.000	967.996	894.019	1038.06
EC60	5.253	1011.68	941.438	1090.77
EC75	5.674	1088.71	1016.28	1195.58
EC80	5.842	1120.89	1044.74	1243.31
EC85	6.036	1159.59	1077.35	1303.25
EC90	6.282	1210.19	1117.94	1385.13
EC95	6.645	1289.27	1178.14	1519.06
EC99	7.326	1451.83	1294.34	1816.66



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date:	5/7/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2024	Lab ID:	ETS-Envr. 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	0.8000	1.0000
1000	0.4000	0.3000
1250	0.1000	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	0.3256	0	20
750	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.334	2.850	0.3256	2	20
*1000	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	6.825	2.850	0.3256	13	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	10.255	2.850	0.3256	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
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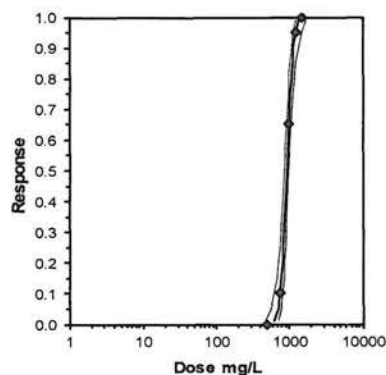
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

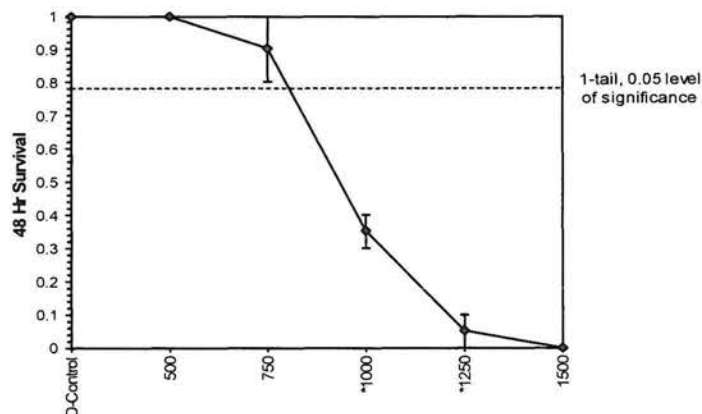
[illegible]

				Maximum Likelihood-ProbIt							
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	13.4854	2.50568	8.57425	18.3965	0	0.07313	7.81472	0.99485	2.97116	0.07415	3
Intercept	-35.067	7.48222	-49.732	-20.402							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	629.008	484.83	715.724
EC05	3.355	706.629	579.4	783.219
EC10	3.718	751.852	636.228	822.94
EC15	3.964	783.987	677.043	851.681
EC20	4.158	810.503	710.78	875.927
EC25	4.326	833.966	740.511	897.943
EC40	4.747	896.144	817.422	960.16
EC50	5.000	935.76	863.729	1003.99
EC60	5.253	977.128	908.839	1054.24
EC75	5.674	1049.98	979.82	1154.23
EC80	5.842	1080.38	1006.74	1199.76
EC85	6.036	1116.92	1037.55	1256.95
EC90	6.282	1164.65	1075.82	1335.04
EC95	6.645	1239.19	1132.47	1463.29
EC99	7.326	1392.11	1241.51	1745.55



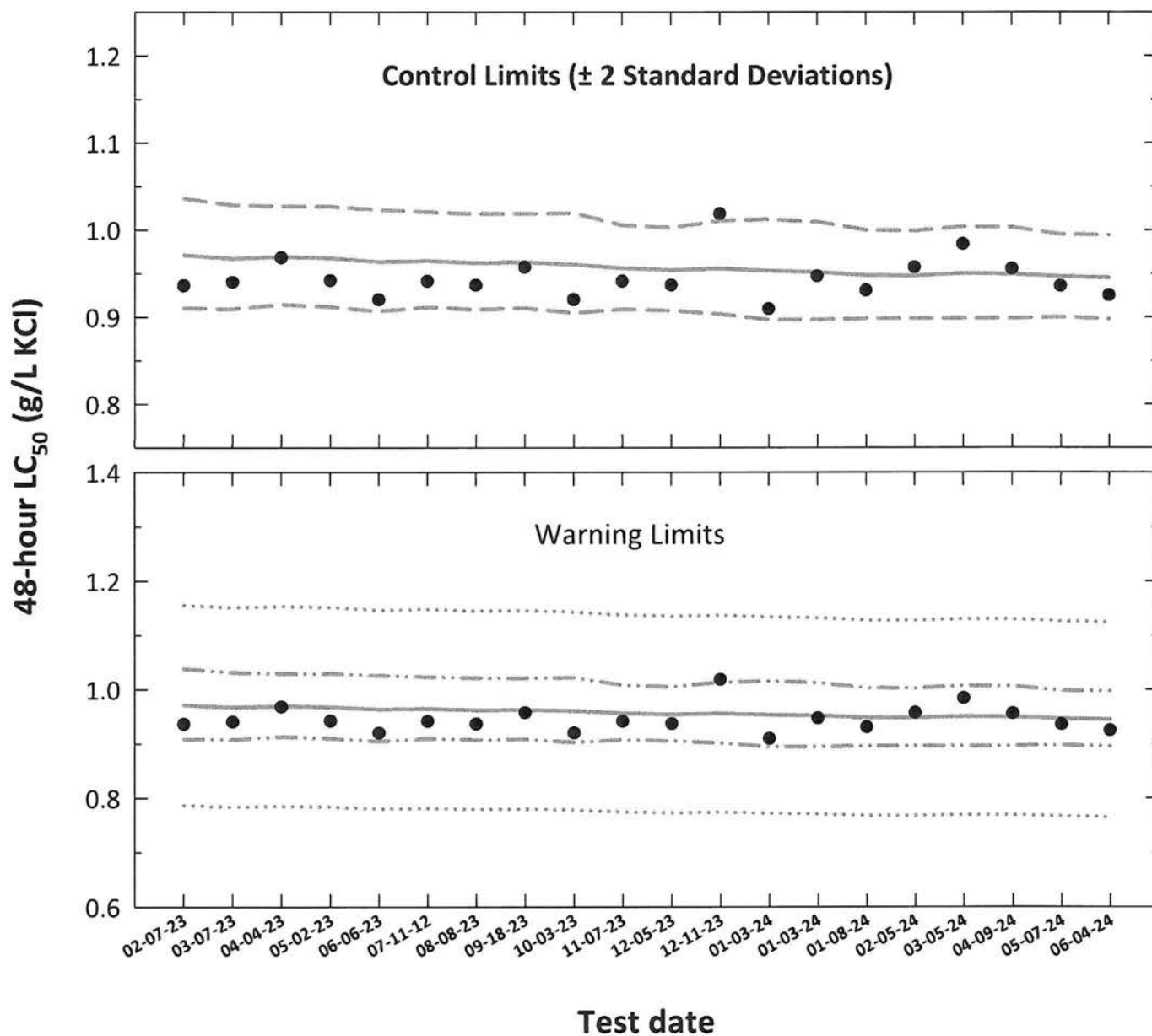
Dose-Response Plot



Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = 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 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.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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	02-07-23	0.9362	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379
2	03-07-23	0.9402	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308
3	04-04-23	0.9683	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293
4	05-02-23	0.9420	-0.0260	-0.0143	0.0129	0.9677	0.9117	1.0270	0.9098	1.0290
5	06-06-23	0.9201	-0.0362	-0.0162	0.0131	0.9633	0.9068	1.0233	0.9046	1.0256
6	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229
7	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208
8	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207
9	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219
10	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078
11	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052
12	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133
13	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153
14	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123
15	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027
16	02-05-24	0.9572	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020
17	03-05-24	0.9839	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067
18	04-09-24	0.9556	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060
19	05-07-24	0.9358	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979
20	06-04-24	0.9251	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970
									CT - S _{A,75}	CT + S _{A,75}
									0.7866	1.1557
									0.7834	1.1509
									0.7851	1.1535
									0.7838	1.1515
									0.7803	1.1463
									0.7814	1.1479
									0.7793	1.1449
									0.7800	1.1459
									0.7780	1.1429
									0.7745	1.1378
									0.7728	1.1353
									0.7740	1.1372
									0.7721	1.1343
									0.7708	1.1323
									0.7678	1.1279
									0.7675	1.1276
									0.7694	1.1304
									0.7691	1.1299
									0.7667	1.1263
									0.7653	1.1244

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 # 165

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 #: 2288

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.81	7.35	7.57
	Dissolved oxygen (mg/L)	7.8	7.7	7.7
	Conductivity (µmhos/cm)	294		
	Alkalinity (mg/L CaCO ₃)	57		
	Hardness (mg/L CaCO ₃)	85		
	Temperature (°C)	24.2	25.0	25.1
500 mg/L	pH (S.U.)	7.77	7.38	7.53
	Dissolved oxygen (mg/L)	7.8	7.7	7.8
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	24.6	25.1	25.0
750 mg/L	pH (S.U.)	7.75	7.38	7.51
	Dissolved oxygen (mg/L)	7.7	7.8	7.8
	Conductivity (µmhos/cm)	1830		
	Temperature (°C)	24.6	25.1	25.0
1000 mg/L	pH (S.U.)	7.74	7.37	7.52
	Dissolved oxygen (mg/L)	7.6	7.8	7.8
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	24.7	24.9	25.2
1250 mg/L	pH (S.U.)	7.74	7.38	7.52
	Dissolved oxygen (mg/L)	7.7	7.9	7.8
	Conductivity (µmhos/cm)	2370		
	Temperature (°C)	24.7	25.2	25.0
1500 mg/L	pH (S.U.)	7.74	7.37	
	Dissolved oxygen (mg/L)	7.8	7.9	
	Conductivity (µmhos/cm)	1800		
	Temperature (°C)	24.7	24.9	

*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	00664685

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

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant TestPpKCIAC # 165

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	06-04-24	* 0505	JH	0705	JH	1E-1B	ANK ORANGE 10-24-24	05-29-24C
24	06-05-24			0655	JH			
48 Termination	06-06-24			0700	JH			

*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:	05-22-24
Age (1 to 14 days old):	6 to 7 DAYS
Hatch date and times:	05-28-24 1147 to 05-29-24 0839
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.77
	Temperature (°C): 24.6

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):

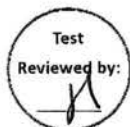
Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^{id}	5 ^{sa}	7 ^{sa}	1 ^{sa}	2 ^{sa}	0 ^{sa}	0 ^{sa}
48 Termination	10	10	10	10	9 ^{id}	9	3 ^{sa}	3 ^{sa}	0 ^{id}	1 ^{id}	0	0
Mean Survival	1007.		1007.		907.		307.		57.		07.	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	853.9
Upper 95% confidence limit (mg KCl/L)	992.5
48-hour LC ₅₀ (mg KCl/L)	925.1

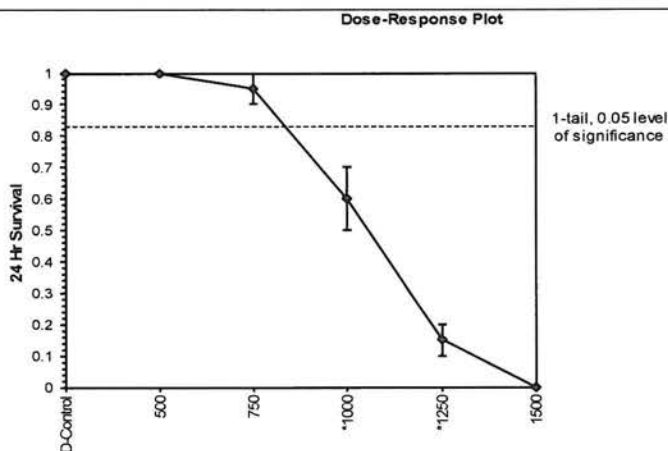
Comments:



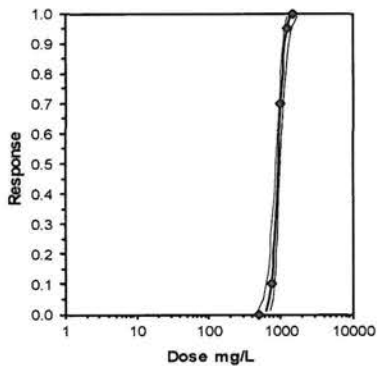
Start Date:	6/4/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/6/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	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	0.2689	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.864	2.850	0.2689	1	20
*1000	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	5.551	2.850	0.2689	8	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	10.803	2.850	0.2689	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

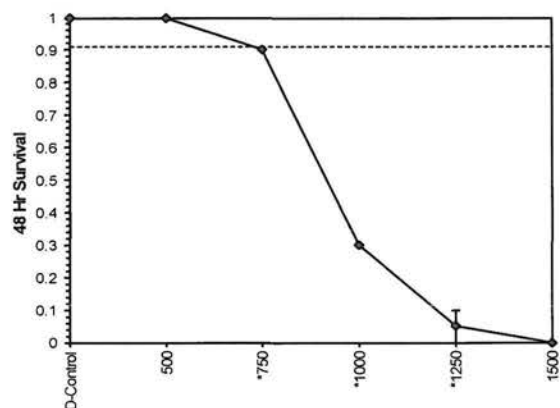
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.1768	2.38936	8.49362 17.8599	0	0.5866	7.81472	0.89949	3.01327	0.07589	3
Intercept	-34.705	7.23492	-48.886 -20.525							
TSCR					1.0					



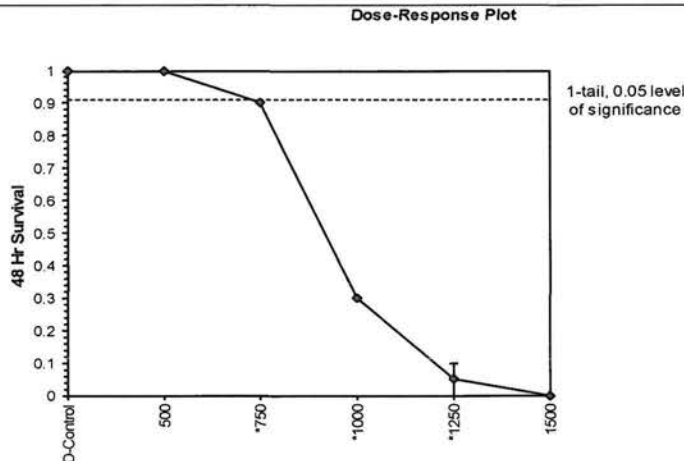
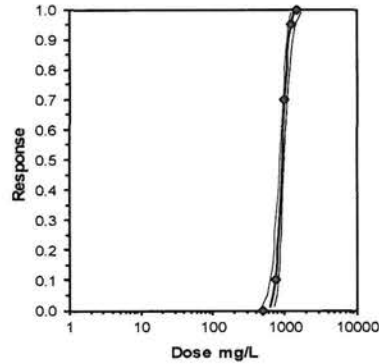
Acute Fathead Minnow Test-48 Hr Survival														
Start Date:	6/4/2024		Test ID:	PpKCIAC		Sample ID:	REF-Ref Toxicant							
End Date:	6/6/2024		Lab ID:	ETS-Envir. Testing Sol.		Sample Type:	KCL-Potassium chloride							
Sample Date:			Protocol:	ACUTE-EPA-821-R-02-012		Test Species:	PP-Pimephales promelas							
Comments:														
Conc-mg/L	1	2												
D-Control	1.0000	1.0000												
500	1.0000	1.0000												
750	0.9000	0.9000												
1000	0.3000	0.3000												
1250	0.0000	0.1000												
1500	0.0000	0.0000												
Transform: Arcsin Square Root														
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total 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	0.1469	0	20		
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	3.162	2.850	0.1469	2	20		
*1000	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	2	16.151	2.850	0.1469	14	20		
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	22.737	2.850	0.1469	19	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			500	750	612.372		0.06555	0.06723	0.57657	0.00266	8.6E-06	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	13.6684	2.56554	8.63993 18.6969		0	0.19203	7.81472	0.97887	2.96618	0.07316	3			
Intercept	-35.543	7.64704	-50.531 -20.555											
TSCR														
Point	Probits	mg/L	95% Fiducial Limits											
EC01	2.674	625.147	481.698 710.752											
EC05	3.355	701.199	574.848 776.724											
EC10	3.718	745.454	630.754 815.531											
EC15	3.964	776.88	670.872 843.608											
EC20	4.158	802.799	704.012 867.297											
EC25	4.326	825.723	733.198 888.812											
EC40	4.747	886.433	808.608 949.646											
EC50	5.000	925.084	853.933 992.542											
EC60	5.253	965.42	898.019 1041.74											
EC75	5.674	1036.4	967.253 1139.66											
EC80	5.842	1066	993.473 1184.24											
EC85	6.036	1101.56	1023.45 1240.22											
EC90	6.282	1148	1060.66 1316.65											
EC95	6.645	1220.45	1115.67 1442.08											
EC99	7.326	1368.93	1221.4 1717.89											



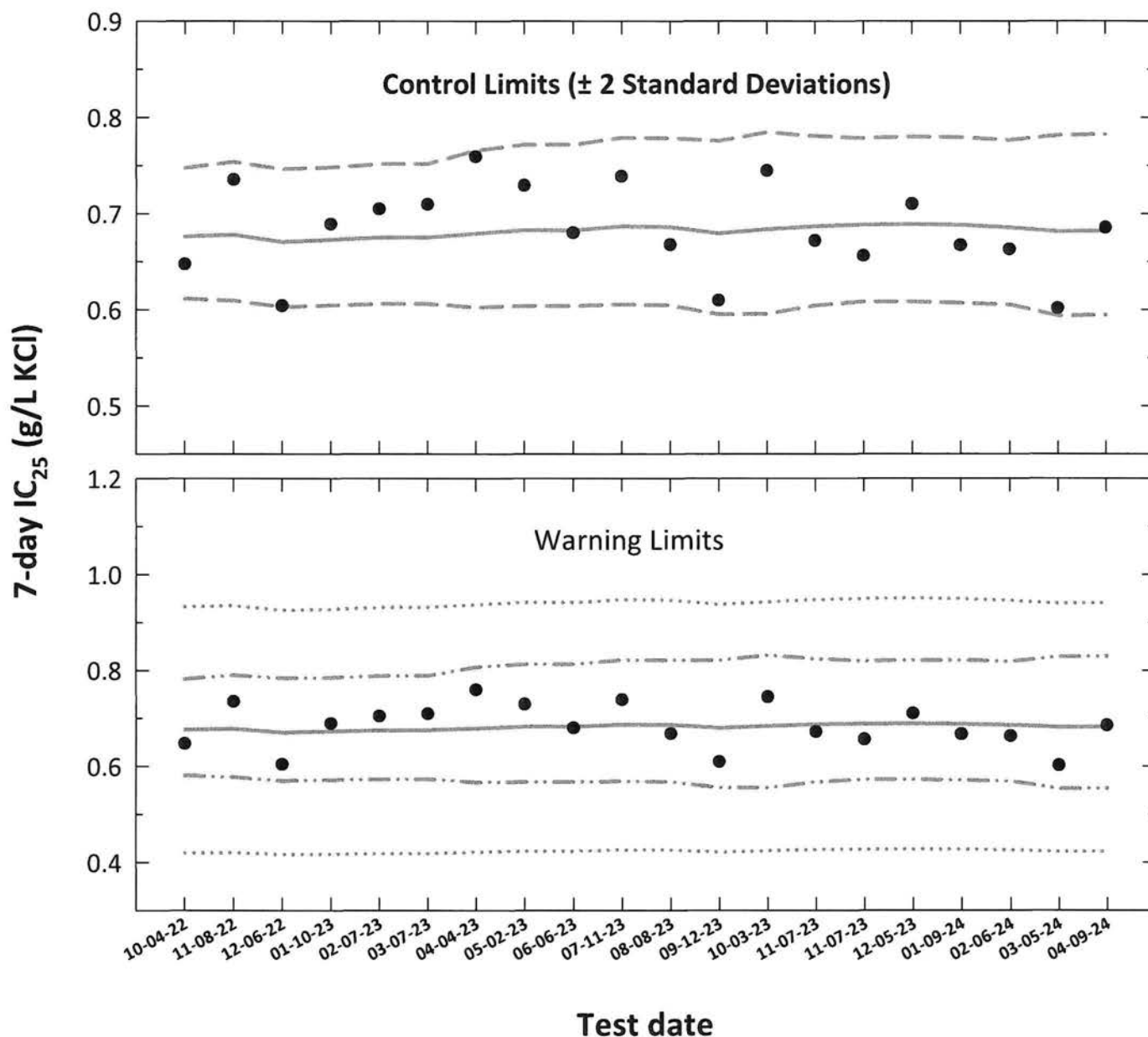
Dose-Response Plot



1-tail, 0.05 level of significance



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		Laboratory Calculated CV		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		
1	10-04-22	0.6477	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
2	11-08-22	0.7354	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
3	12-06-22	0.6041	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
4	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
5	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
6	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318
7	04-04-23	0.7592	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373
8	05-02-23	0.7295	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424
9	06-06-23	0.6800	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422
10	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
11	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
12	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
13	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
14	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
15	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
16	12-05-23	0.7102	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510
17	01-09-24	0.6671	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
18	02-06-24	0.6628	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
19	03-05-24	0.6019	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
20	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

Note: 7-day IC_{25} = 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 IC_{25} values.

S = Standard deviation of the IC_{25} values.

Control Limits = Mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic $IC_{25} \pm 2CV$ or $S_{A,75}$ converted to anti-logarithmic values.

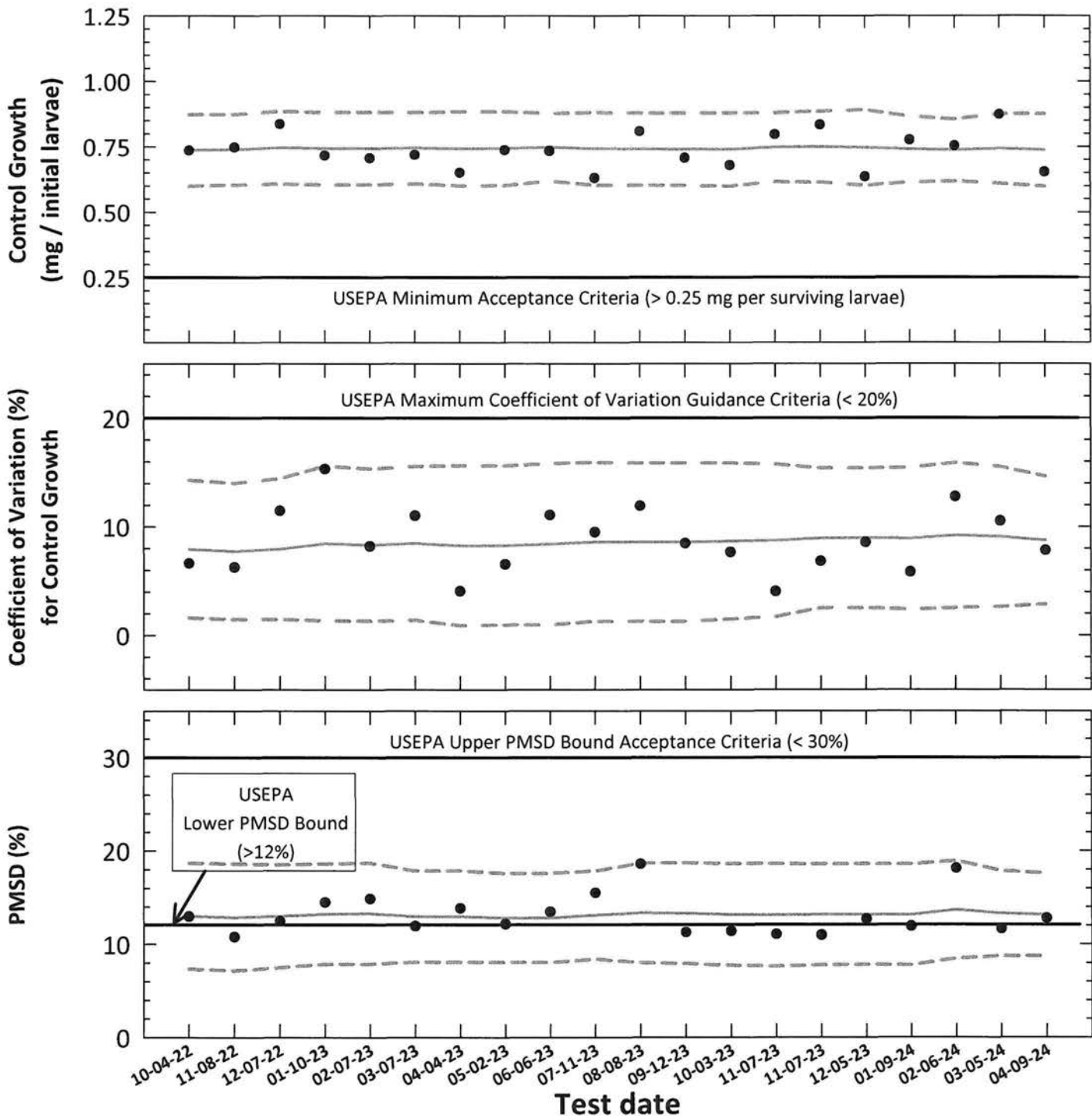
$S_{A,75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A,75} = 0.38$).

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		Control Growth CV		Test PMSD	
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S
			Mean (mg/initial larvae)	CV (%)									
1	10-04-22	100	0.736	6.6	0.0955	0.736	0.600	0.873	0.600	7.9	1.6	13.0	7.4
2	11-08-22	100	0.747	6.3	0.0804	0.739	0.604	0.874	0.604	7.7	1.5	12.9	7.1
3	12-07-22	100	0.837	11.5	0.1043	0.747	0.608	0.886	0.608	8.0	1.5	13.0	7.5
4	01-10-23	100	0.716	15.3	0.1037	0.744	0.605	0.882	0.605	8.5	1.3	13.2	7.9
5	02-07-23	100	0.707	8.2	0.1049	0.743	0.604	0.882	0.604	8.3	1.3	13.3	7.9
6	03-07-23	100	0.719	11.0	0.0858	0.745	0.609	0.881	0.609	8.5	1.4	13.0	8.1
7	04-04-23	100	0.651	4.1	0.0900	0.742	0.601	0.884	0.601	8.3	0.9	13.0	8.1
8	05-02-23	100	0.737	6.5	0.0895	0.743	0.602	0.884	0.602	8.3	1.0	12.8	8.0
9	06-06-23	100	0.734	11.1	0.0988	0.749	0.619	0.878	0.619	8.4	1.0	12.8	8.0
10	07-11-23	100	0.630	9.5	0.0976	0.742	0.603	0.882	0.603	8.6	1.3	13.1	8.4
11	08-08-23	100	0.809	11.9	0.1504	0.741	0.603	0.879	0.603	8.6	1.3	13.4	8.0
12	09-12-23	100	0.707	8.5	0.0796	0.740	0.602	0.879	0.602	8.6	1.3	13.3	7.9
13	10-03-23	100	0.678	7.7	0.0772	0.739	0.599	0.880	0.599	8.7	1.5	13.2	7.7
14	11-07-23	100	0.797	4.0	0.0880	0.748	0.616	0.880	0.616	8.7	1.7	13.1	7.6
15	11-07-23	100	0.833	6.8	0.0913	0.750	0.614	0.886	0.614	8.9	2.5	13.2	7.8
16	12-05-23	100	0.635	8.6	0.0805	0.746	0.602	0.890	0.602	9.0	2.5	13.2	7.8
17	01-09-24	100	0.776	5.8	0.0923	0.740	0.614	0.866	0.614	8.9	2.4	13.1	7.7
18	02-06-24	100	0.753	12.8	0.1364	0.736	0.618	0.855	0.618	9.2	2.5	13.7	8.4
19	03-05-24	100	0.873	10.5	0.1016	0.742	0.609	0.876	0.609	9.1	2.6	13.3	8.7
20	04-09-24	100	0.653	7.8	0.0834	0.736	0.598	0.874	0.598	8.7	2.8	13.1	8.7

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*

PpKClCR Test Number: 114

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2275</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>04-02-24</u>	Artemia CHM number:	CHM1294
Hatch dates and times:	<u>04-08-24 1303 to 04-09-24 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.75</u> S.U. Temperature = <u>24.2</u> °C	Date / Time in oven:	<u>04-16-24 0740</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>04-17-24 0740</u>
		*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24-Hours</u>

*60°C Oven, Thermometer SN: 14-985B5

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	04-09-24	0505	<u>JK</u>	1110	<u>JK</u>	0811	<u>JK</u>	<u>04-01-24 B</u>
1	04-10-24	0500	<u>JK</u>	1100	<u>JK</u>	0726	<u>JK</u>	<u>↓</u>
2	04-11-24	0500	<u>JK</u>	1300	<u>JK</u>	0800	<u>JK</u>	<u>04-01-24 D</u>
3	04-12-24	0500	<u>JK</u>	1100	<u>JK</u>	0713	<u>JK</u>	<u>↓</u>
4	04-13-24	0615	<u>JK</u>	1215	<u>JK</u>	0815	<u>JK</u>	<u>04-01-24 A</u>
5	04-14-24	0600	<u>JK</u>	1200	<u>JK</u>	0800	<u>JK</u>	<u>↓</u>
6	04-15-24	0500	<u>JK</u>	1100	<u>JK</u>	0712	<u>JK</u>	<u>↓</u>
7	04-16-24					0711	<u>JK</u>	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	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>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>826.5</u>
Average weight per initial larvae:	<u>0.653</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.653</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>620.8</u>
			IC ₂₅ (mg/L KCl)	<u>685.5</u>

Species: *Pimephales promelas*

PpKCICR Test Number: 114

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>light pink</u> Analyst: <u>JP</u> Date: <u>03-19-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>04-10-24</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 (%)												
0.653 0.874 -33.8% 0.739 -13.1%												

*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: 114

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	9 rd	10	6 rd	5 rd	6 rd	6 rd	
2	10	10	10	10	9 rd	10	9	10	5 rd	5	6	6	
3	10	10	10	10	9	10	9	10	5	5	5 rd	6	
4	10	10	10	10	9	10	9	9 rd	4 rd	4 rd	4 rd	4 rd	
5	10	10	10	10	8 rd	10	8 rd	9	3 rd	3 rd	4	3 rd	
6	10	10	10	10	8	9 rd	8	8 rd	3	2 rd	3 rd	3	
7	9 rd	10	10	9 rd	7 rd	7 rd	8	8	2 rd	2	3	3	
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>JP</u> Date: <u>03-19-24</u>													
13.75 14.43 13.69 13.31 14.58 14.20 12.76 13.92 13.77 15.21 12.79 14.35													
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>04-18-24</u>													
19.66 21.25 20.43 20.81 20.05 18.65 17.69 18.92 15.64 16.57 15.61 16.32													
C = Larvae weight (mg) = B - A													
Analyst: <u>JP</u>													
5.91 6.82 6.74 7.50 5.47 4.45 4.93 5.00 1.87 1.36 2.82 1.97													
Weight per initial number of larvae (mg) = C / Initial number of larvae													
Analyst: <u>JP</u>													
0.591 0.682 0.674 0.750 0.547 0.445 0.493 0.500 0.187 0.136 0.282 0.197													
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:

Species: *Pimephales promelas*

PpKICR Test Number: 114

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 ^{7d}	3 ^{7d}	4 ^{6d}	4 ^{6d}
2	3	2 ^{1d}	3 ^{1d}	3 ^{1d}
3	3	2	3	3
4	3	2	3	3
5	2 ^{1d} 1	1 ^{1d} 1	2 ^{1d} 2	2 ^{1d} 2
6	1	1	1 ^{1d}	2
7	1	1	1	2
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>JP</u> Date: <u>03-19-24</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>03-18-24</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 (%)				
0.074 88.6%				

*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: 114
Test dates: April 09-16, 2024

Concentration (mg/L KC)	Replicate	Initial number of larvae	Final number of larvae	A = Pen weight (mg)	B = Pen + 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	14.39	20.78	6.39	0.639							
	B	10	10	12.19	19.48	7.29	0.729							
	C	10	10	13.86	20.07	6.21	0.621	0.653	7.8		100.0	0.653	7.8	Not applicable
	D	10	10	14.36	20.60	6.24	0.624							
300	E	10	10	12.29	21.08	8.79	0.879							
	F	10	10	13.57	21.80	8.23	0.823	0.874	5.4		100.0	0.874	5.4	-33.8
	G	10	10	13.31	22.67	9.36	0.936							
	H	10	10	13.89	22.48	8.59	0.859							
450	I	10	10	14.95	22.61	7.66	0.766							
	J	10	10	14.25	21.09	6.84	0.684	0.739	5.3		100.0	0.739	5.3	-13.1
	K	10	10	15.47	22.86	7.39	0.739							
	L	10	10	12.31	19.98	7.67	0.767							
600	M	10	9	13.75	19.66	5.91	0.657							
	N	10	10	14.43	21.25	6.82	0.682	0.712	11.5		95.0	0.674	9.7	-3.2
	O	10	10	13.69	20.43	6.74	0.674							
	P	10	9	13.31	20.81	7.50	0.833							
750	Q	10	7	14.58	20.05	5.47	0.781							
	R	10	7	14.20	18.65	4.45	0.636	0.665	11.8		75.0	0.496	8.4	24.0
	S	10	8	12.76	17.69	4.93	0.616							
	T	10	8	13.92	18.92	5.00	0.625							
900	U	10	2	13.77	15.64	1.87	0.935							
	V	10	2	15.21	16.57	1.36	0.680	0.803	19.4		25.0	0.201	30.2	69.3
	W	10	3	12.79	15.61	2.82	0.940							
	X	10	3	14.35	16.32	1.97	0.657							
1050	Y	10	1	14.26	14.94	0.68	0.680							
	Z	10	1	11.99	12.89	0.90	0.900	0.619	35.7		12.5	0.074	35.4	88.6
	AA	10	1	14.59	14.99	0.40	0.400							
	BB	10	2	14.33	15.32	0.99	0.495							

Dunnett's MSD value: 0.0834
PMSD: 12.8

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.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival					
Start Date:	4/9/2024	Test ID:	PpKICR	Sample ID:	REF-Ref Toxicant
End Date:	4/16/2024	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:					

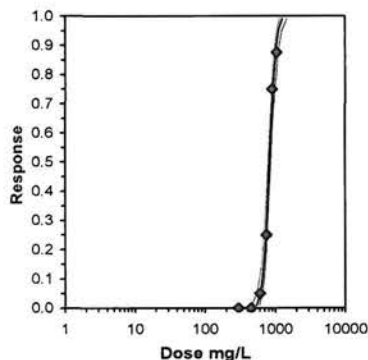
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	1.0000	1.0000	0.9000
750	0.7000	0.7000	0.8000	0.8000
900	0.2000	0.2000	0.3000	0.3000
1050	0.1000	0.1000	0.1000	0.2000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%					
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	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	40
*900	0.2500	0.2500	0.5216	0.4636	0.5796	12.838	4	10.00	10.00	30	40
*1050	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

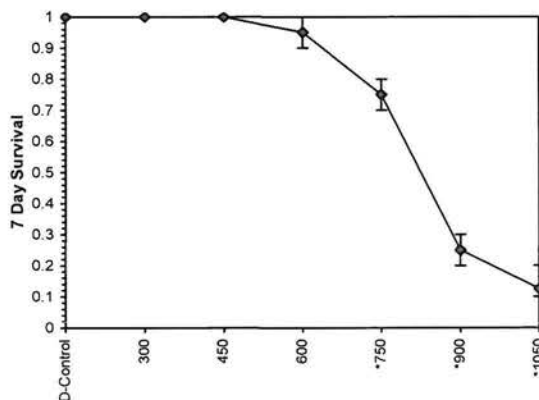
Auxiliary Tests		Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)		0.91534	0.896	0.33391	-0.4959
Equality of variance cannot be confirmed					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test		600	750	670.82	
Treatments vs D-Control					

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit					
				Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	12.4738	1.60309	9.33176 15.6159	0	1.86226	9.48773	0.76107	2.91723	0.08017
Intercept	-31.389	4.68135	-40.564 -22.214						

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	537.94	460.881 592.023
EC05	3.355	610.053	543.599 656.628
EC10	3.718	652.368	592.989 694.622
EC15	3.964	682.564	628.363 722.018
EC20	4.158	707.557	657.558 745.034
EC25	4.326	729.726	683.258 765.84
EC40	4.747	788.718	749.647 824.071
EC50	5.000	826.48	789.643 864.497
EC60	5.253	866.049	828.928 910.018
EC75	5.674	936.062	892.677 997.633
EC80	5.842	965.391	917.796 1036.41
EC85	6.036	1000.74	947.223 1084.37
EC90	6.282	1047.06	984.738 1148.88
EC95	6.645	1119.69	1041.87 1253.07
EC99	7.326	1269.79	1155.73 1477.77



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS

Larval Fish Growth and Survival Test-7 Day Growth

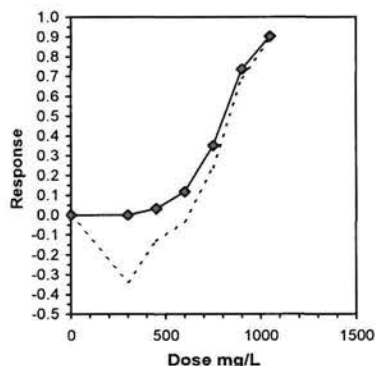
Start Date: 4/9/2024 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
End Date: 4/16/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.6390	0.7290	0.6210	0.6240
300	0.8790	0.8230	0.9360	0.8590
450	0.7660	0.6840	0.7390	0.7670
600	0.5910	0.6820	0.6740	0.7500
750	0.5470	0.4450	0.4930	0.5000
900	0.1870	0.1360	0.2820	0.1970
1050	0.0680	0.0900	0.0400	0.0990

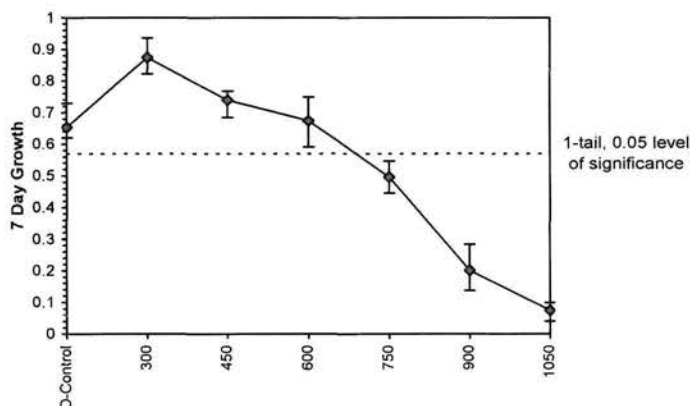
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.6533	1.0000	0.6533	0.6210	0.7290	7.824	4				0.7638	1.0000
300	0.8743	1.3383	0.8743	0.8230	0.9360	5.403	4	-6.072	2.290	0.0834	0.7638	1.0000
450	0.7390	1.1313	0.7390	0.6840	0.7670	5.263	4	-2.356	2.290	0.0834	0.7390	0.9676
600	0.6743	1.0321	0.6743	0.5910	0.7500	9.661	4	-0.577	2.290	0.0834	0.6743	0.8828
750	0.4963	0.7597	0.4963	0.4450	0.5470	8.411	4				0.4963	0.6498
900	0.2005	0.3069	0.2005	0.1360	0.2820	30.197	4				0.2005	0.2625
1050	0.0743	0.1137	0.0743	0.0400	0.0990	35.401	4				0.0743	0.0972

Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.96486	0.844	0.15926	-0.4379			
Bartlett's Test indicates equal variances (p = 0.87)					0.72695	11.3449					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		600	>600			0.08335	0.1276	0.03971	0.00265	2.3E-04	3, 12
Treatments vs D-Control											

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	481.13	54.48	328.26	683.29	0.2458
IC10	569.59	43.82	424.53	674.71	-0.4962
IC15	621.12	26.34	528.17	684.54	-0.9695
IC20	653.30	19.83	585.44	710.67	-0.3281
IC25	685.48	17.28	632.67	746.57	0.1834
IC40	769.27	9.74	737.12	801.57	-0.2153
IC50	808.01	8.49	783.28	834.19	0.1152



Dose-Response Plot



Reviewed and
Reviewed by
Jim Sumner

Species: *Pimephales promelas*

PpKCICR Test Number: 114

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	Analyst	Analyst	Analyst	Analyst	Analyst
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.70	7.64	7.48	7.45	7.75	7.66
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.8	7.8	7.0
	Conductivity (µmhos/cm)	295		310		295	
	Alkalinity (mg CaCO ₃ /L)	61				59	
	Hardness (mg CaCO ₃ /L)	90				86	
	Temperature (°C)	24.7	24.5	24.8	25.1	24.7	25.2
300 mg KCl/L	pH (S.U.)	7.82	7.62	7.73	7.63	7.80	7.67
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.8	7.7	7.0
	Conductivity (µmhos/cm)	822		802		820	
	Temperature (°C)	24.8	24.7	24.9	24.9	24.9	25.0
450 mg KCl/L	pH (S.U.)	7.79	7.62	7.72	7.64	7.80	7.66
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.8	7.7	7.0
	Conductivity (µmhos/cm)	1060		1120		1090	
	Temperature (°C)	24.8	24.6	24.8	24.9	24.9	25.0
600 mg KCl/L	pH (S.U.)	7.79	7.61	7.72	7.65	7.80	7.66
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.7	7.0
	Conductivity (µmhos/cm)	1320		1370		1320	
	Temperature (°C)	24.9	24.6	24.7	25.2	24.8	25.0
750 mg KCl/L	pH (S.U.)	7.79	7.61	7.72	7.65	7.82	7.66
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.7	7.8	6.9
	Conductivity (µmhos/cm)	1570		1630		1590	
	Temperature (°C)	24.9	24.6	24.8	25.2	24.8	25.3
900 mg KCl/L	pH (S.U.)	7.80	7.63	7.72	7.65	7.83	7.69
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.7	7.9	6.9
	Conductivity (µmhos/cm)	1820		1880		1840	
	Temperature (°C)	24.8	24.8	24.8	24.8	24.9	25.1
1050 mg KCl/L	pH (S.U.)	7.82	7.63	7.74	7.65	7.83	7.71
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.6	7.9	7.5
	Conductivity (µmhos/cm)	2100		2190		2140	
	Temperature (°C)	24.8	24.6	24.6	24.7	24.8	25.3
		Initial	Final	Initial	Final	Initial	Final

Species: Pimephales promelas

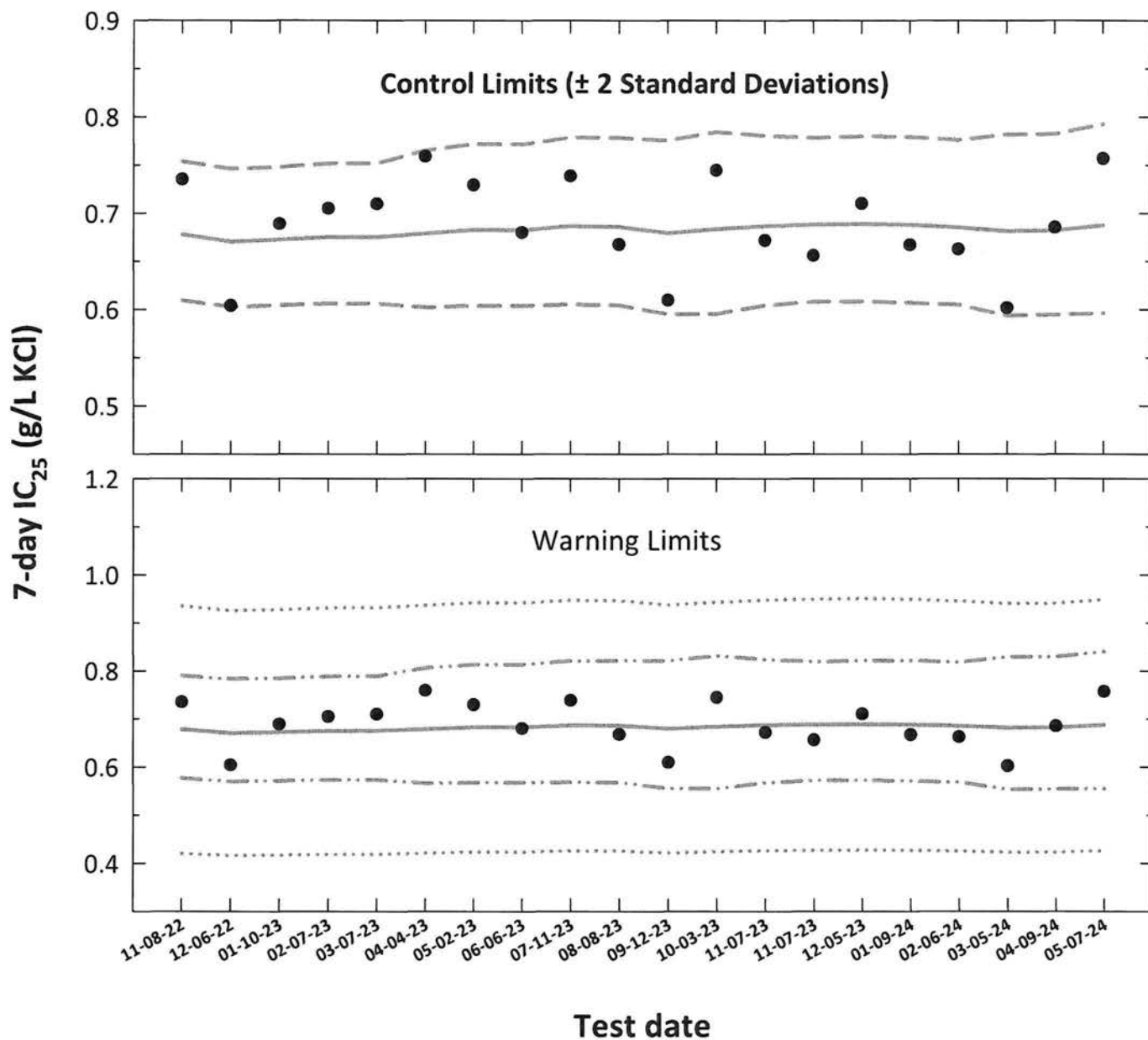
PpKICR Test Number: 114

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		<i>✓</i>	<i>BSC</i>	<i>BSC</i>	<i>BSC</i>	<i>BSC</i>	<i>N</i>	<i>N</i>	<i>N</i>
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.92	7.52	7.41	7.40	7.33	7.49	7.51	7.00
	Dissolved oxygen (mg/L)	7.7	7.4	7.7	7.4	7.7	7.2	7.0	6.8
	Conductivity (µmhos/cm)	295		290		304		311	
	Alkalinity (mg CaCO ₃ /L)			59					
	Hardness (mg CaCO ₃ /L)			88					
	Temperature (°C)	24.8	24.9	24.7	24.6	24.8	25.1	24.7	25.0
300 mg KCl/L	pH (S.U.)	7.91	7.61	7.85	7.47	7.84	7.50	7.81	7.20
	Dissolved oxygen (mg/L)	7.6	7.4	7.7	7.4	7.8	7.2	7.8	6.7
	Conductivity (µmhos/cm)	834		833		845		863	
	Temperature (°C)	24.8	25.1	24.9	24.9	24.9	24.8	24.7	24.8
450 mg KCl/L	pH (S.U.)	7.91	7.65	7.88	7.49	7.85	7.50	7.80	7.99
	Dissolved oxygen (mg/L)	7.7	7.4	7.6	7.0	7.8	7.2	7.8	6.7
	Conductivity (µmhos/cm)	1080		1070		1100		1120	
	Temperature (°C)	24.9	24.8	24.9	25.0	24.9	24.7	24.8	24.8
600 mg KCl/L	pH (S.U.)	7.92	7.65	7.90	7.49	7.85	7.50	7.81	7.22
	Dissolved oxygen (mg/L)	7.7	7.1	7.7	6.7	7.9	7.2	7.9	6.6
	Conductivity (µmhos/cm)	1330		1310		1370		1390	
	Temperature (°C)	24.9	24.8	24.8	24.8	25.0	24.7	24.9	24.9
750 mg KCl/L	pH (S.U.)	7.92	7.69	7.91	7.48	7.86	7.51	7.82	7.24
	Dissolved oxygen (mg/L)	7.8	7.1	7.7	6.7	7.9	7.0	7.9	6.6
	Conductivity (µmhos/cm)	1580		1580		1620		1640	
	Temperature (°C)	24.8	25.0	24.8	24.7	25.0	24.6	24.8	25.1
900 mg KCl/L	pH (S.U.)	7.93	7.70	7.93	7.52	7.86	7.53	7.82	7.33
	Dissolved oxygen (mg/L)	7.9	7.1	7.8	6.7	8.0	7.1	8.0	6.5
	Conductivity (µmhos/cm)	1840		1840		1880		1890	
	Temperature (°C)	24.8	25.0	24.8	24.7	25.0	24.9	24.8	24.9
1050 mg KCl/L	pH (S.U.)	7.96	7.75	7.93	7.62	7.91	7.80	7.83	7.33
	Dissolved oxygen (mg/L)	8.0	7.1	7.8	7.0	8.0	7.1	8.0	6.8
	Conductivity (µmhos/cm)	2120		2040		2150		2200	
	Temperature (°C)	24.8	25.0	24.9	24.7	24.9	24.6	24.8	24.9
		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		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	11-08-22	0.7354	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
2	12-06-22	0.6041	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
3	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
4	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
5	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318
6	04-04-23	0.7592	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373
7	05-02-23	0.7295	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424
8	06-06-23	0.6800	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422
9	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
10	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
11	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
12	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
13	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
14	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
15	12-05-23	0.7102	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510
16	01-09-24	0.6671	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
17	02-06-24	0.6628	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
18	03-05-24	0.6019	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
19	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
20	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

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.

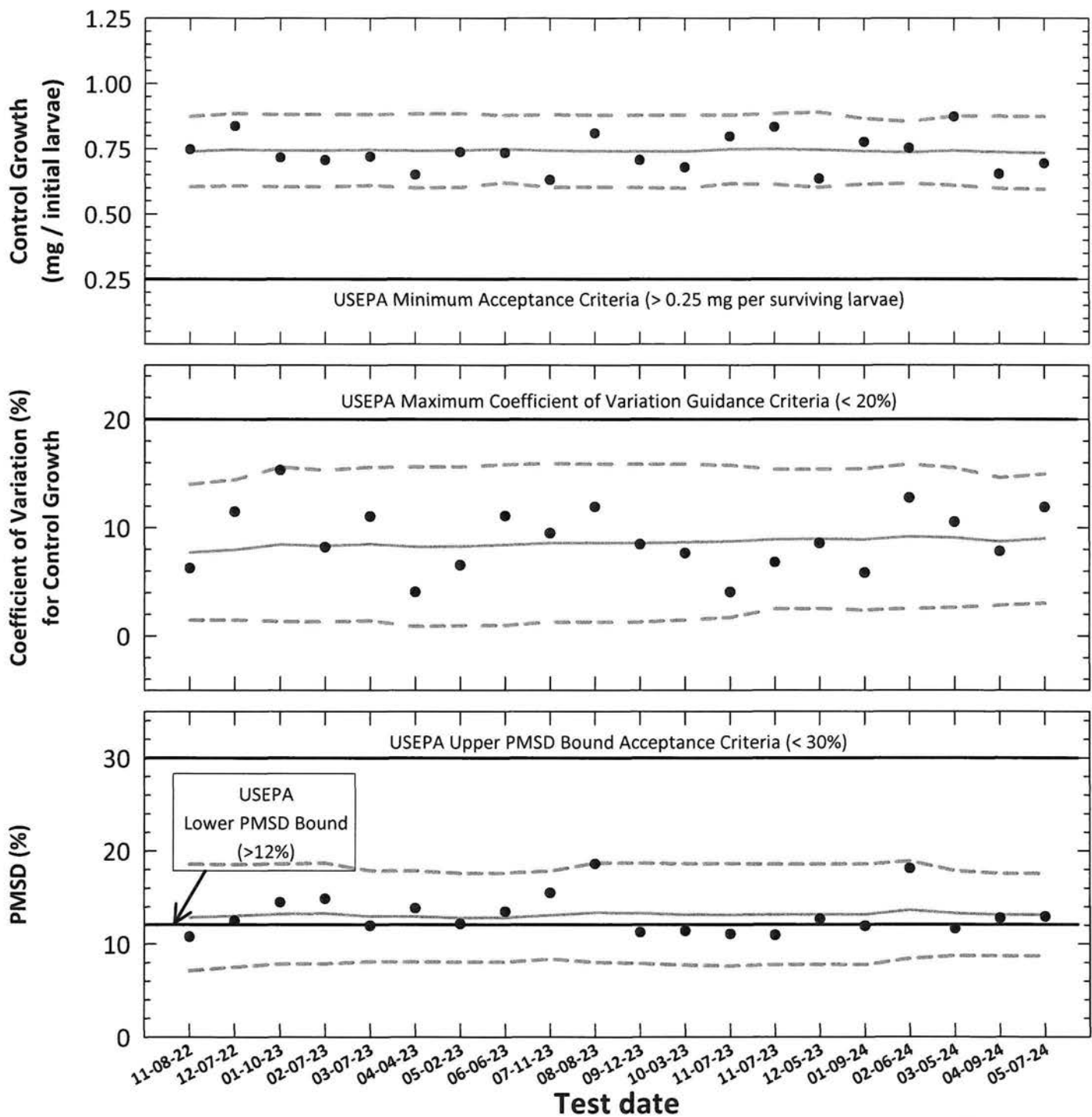
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

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	ToxCat Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	14.0	12.9	7.1	18.6
2	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	14.4	13.0	7.5	18.5
3	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	15.6	13.2	7.9	18.6
4	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3	7.9	18.7
5	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8
6	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9
7	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6
8	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6
9	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
10	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
11	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
12	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
13	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
14	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
15	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6
16	01-09-24	100	0.776	5.8	0.0923	11.9	0.740	0.614	0.866	8.9	2.4	15.4	13.1	7.7	18.6
17	02-06-24	100	0.753	12.8	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
18	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
19	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
20	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 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*

PpKClCR Test Number: **115**

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS 2275				
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:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	04-30-14	Artemia CHM number:	CHM1294
Hatch dates and times:	05-06-14 1420 to 05-07-14 0500	Drying information for weight determination:	
Transfer vessel information:	pH = 8.10 S.U. Temperature = 21.2 °C	Date / Time in oven:	05-14-14 0900
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	60°C
		Date / Time out of oven:	05-15-14 0900
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

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	05-07-14	0505	JL	1300	JL	0715	JL	05-01-14 A
1	05-08-14	0500	JL	1230	JL	0710	JL	↓
2	05-09-14	0500	JL	1330	JL	0710	JL	05-01-14 E
3	05-10-14	0500	JL	1100	JL	0700	JL	↓
4	05-11-14	0600	JL	1240	JL	0810	JL	05-06-14 A
5	05-12-14	0600	JL	1200	JL	0800	JL	↓
6	05-13-14	0500	JL	1100	JL	0700	JL	↓
7	05-14-14					0525	JL	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	0%	≤ 20%	7-day LC ₅₀ (mg/L KCl)	802.8
Average weight per initial larvae:	0.693		NOEC (mg/L KCl)	620
Average weight per surviving larvae:	0.693	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC ₂₅ (mg/L KCl)	756.9

Species: *Pimephales promelas*

PpKCICR Test Number: 115

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>Forest Green</u> Analyst: <u>JP</u> Date: <u>04-24-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>05-16-24</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 (%)												
0.693 0.774 -11.7% 0.636 8.3%												

*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: 115

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 ^{id}	9 ^{id}	9 ^{id}	9 ^{id}	6 ^{id}	7 ^{id}	6 ^{id}	6 ^{id}
2	10	10	10	10	9	9	9	9	6	7	5 ^{id}	6
3	10	10	10	10	9	9	9	9	6	7	5	6
4	10	10	10	10	8 ^{id}	9	8 ^{id}	9	4 ²⁰	5 ²⁰	5	5 ¹⁰
5	10	9 ^{id}	10	10	8	8 ^{id}	8	8 ^{id}	3 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}
6	10	9	10	10	8	7 ^{id}	8	8	3	3 ^{id}	3 ^{id}	3 ^{id}
7	9 ^{id}	9 ^{id}	10	10	7 ^{id}	7	7 ^{id}	8	3	2 ^{id}	2 ^{id}	2 ^{id}
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>JP</u> Date: <u>01-24-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>05-16-24</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 (%)												
0.657 5.2% 0.569 18.0% 0.173 75.0%												

*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: 115

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 rd	2 ^{sd}	2 ^{sd}	2 ^{sd}
2	2 rd	2	2	1
3	2	2	2	1
4	2	1 rd	1 rd	0 rd
5	2	D rd	D rd	0
6	2	0	0	0
7	1 rd	0	0	0
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>JP</u> Date: <u>04-24-24</u>				
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
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 (%)				
0.016 97.7%				

*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: 115
Test dates: May 07-14, 2024

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	12.67	20.06	7.39	0.739							
	B	10	10	12.74	20.38	7.64	0.764							
	C	10	10	13.88	20.80	6.92	0.692	0.693	11.9		100.0	0.693	11.9	Not applicable
	D	10	10	14.10	19.88	5.78	0.578							
300	E	10	10	13.69	21.51	7.82	0.782							
	F	10	10	14.71	22.87	8.16	0.816	0.774	4.6		100.0	0.774	4.6	-11.7
	G	10	10	14.84	22.53	7.69	0.769							
	H	10	10	13.52	20.82	7.30	0.730							
450	I	10	10	13.96	20.66	6.70	0.670							
	J	10	10	13.56	19.74	6.18	0.618	0.636	3.9		100.0	0.636	3.9	8.3
	K	10	10	13.86	20.24	6.38	0.638							
	L	10	10	16.15	22.31	6.16	0.616							
600	M	10	9	13.04	19.28	6.24	0.693							
	N	10	9	11.70	17.62	5.92	0.658	0.691	3.8		95.0	0.657	9.1	5.2
	O	10	10	12.49	19.40	6.91	0.691							
	P	10	10	12.96	20.18	7.22	0.722							
750	Q	10	7	11.94	17.27	5.33	0.761							
	R	10	7	13.83	18.64	4.81	0.687	0.782	9.4		72.5	0.569	14.8	18.0
	S	10	7	14.73	20.54	5.81	0.830							
	T	10	8	13.07	19.86	6.79	0.849							
900	U	10	3	14.03	15.41	1.38	0.460							
	V	10	2	14.50	16.03	1.53	0.765	0.808	33.4		22.5	0.173	20.8	75.0
	W	10	2	13.27	15.47	2.20	1.100							
	X	10	2	14.47	16.28	1.81	0.905							
1050	Y	10	1	13.46	14.10	0.64	0.640							
	Z	10	0	0.00	0.00	0.00	0.000	0.640	0.0		2.5	0.016	200.0	97.7
	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: 0.0896
PMSD: 12.9

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.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 5/7/2024 Test ID: PpKICIR Sample ID: REF-Ref Toxicant
End Date: 5/14/2024 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	0.9000	0.9000	1.0000	1.0000
750	0.7000	0.7000	0.7000	0.8000
900	0.3000	0.2000	0.2000	0.2000
1050	0.1000	0.0000	0.0000	0.0000

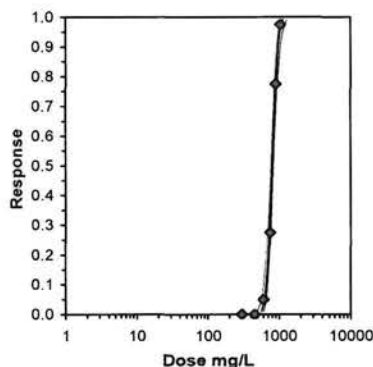
Conc-mg/L	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4		0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11
*900	0.2250	0.2250	0.4926	0.4636	0.5796	11.772	4	10.00	10.00	31
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.85264	0.896	0.9083	0.63258
Equality of variance cannot be confirmed				

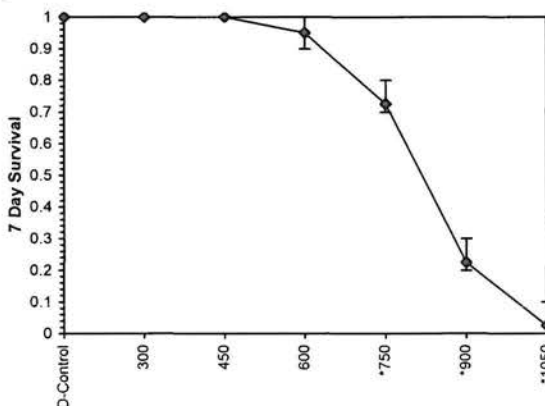
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.095	1.91552	11.3406 18.8494	0	1.42364	9.48773	0.84008	2.90461	0.06625	3
Intercept	-38.845	5.57975	-49.781 -27.909							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	562.986	493.773 611.158
EC05	3.355	624.661	565.501 666.033
EC10	3.718	660.255	607.39 697.864
EC15	3.964	685.41	637.025 720.61
EC20	4.158	706.084	661.287 739.572
EC25	4.326	724.316	682.522 756.584
EC40	4.747	772.374	737.064 803.428
EC50	5.000	802.807	769.884 835.233
EC60	5.253	834.439	802.151 870.477
EC75	5.674	889.803	854.262 937.347
EC80	5.842	912.78	874.594 966.695
EC85	6.036	940.311	898.233 1002.81
EC90	6.282	976.135	928.097 1051.08
EC95	6.645	1031.76	973.045 1128.25
EC99	7.326	1144.79	1061.04 1291.38



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Growth

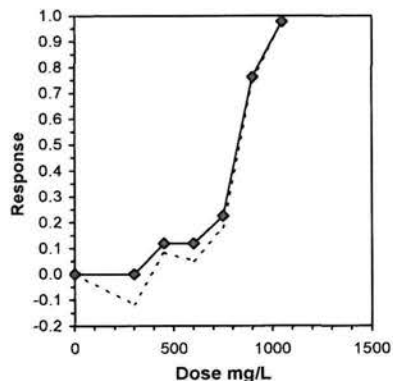
Start Date: 5/7/2024 Test ID: PpKClCR Sample ID: REF-Ref Toxicant
End Date: 5/14/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.7390	0.7640	0.6920	0.5780
300	0.7820	0.8160	0.7690	0.7301
450	0.6700	0.6180	0.6380	0.6160
600	0.6240	0.5920	0.6910	0.7220
750	0.5330	0.4810	0.5810	0.6790
900	0.1380	0.1530	0.2200	0.1810
1050	0.0640	0.0000	0.0000	0.0000

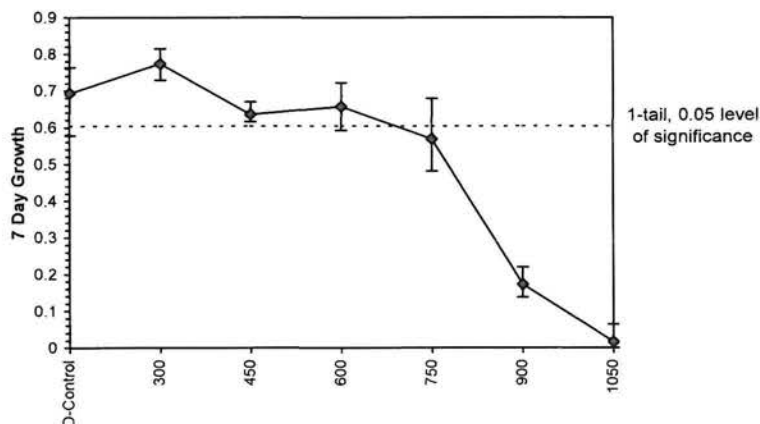
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.6933	1.0000	0.6933	0.5780	0.7640	11.890	4				0.7338	1.0000
300	0.7743	1.1169	0.7743	0.7301	0.8160	4.584	4	-2.071	2.290	0.0896	0.7338	1.0000
450	0.6355	0.9167	0.6355	0.6160	0.6700	3.942	4	1.476	2.290	0.0896	0.6464	0.8809
600	0.6573	0.9481	0.6573	0.5920	0.7220	9.084	4	0.920	2.290	0.0896	0.6464	0.8809
750	0.5685	0.8201	0.5685	0.4810	0.6790	14.816	4				0.5685	0.7748
900	0.1730	0.2495	0.1730	0.1380	0.2200	20.836	4				0.1730	0.2358
1050	0.0160	0.0231	0.0160	0.0000	0.0640	200.000	4				0.0160	0.0218

Auxiliary Tests					Statistic		Critical		Skew		Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.96023		0.844		-0.6728		0.48599			
Bartlett's Test indicates equal variances (p = 0.26)					4.01266		11.3449							
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					600	>600			0.0896	0.12924	0.01487	0.00306	0.01945	3, 12
Treatments vs D-Control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	362.97	48.53	331.66	745.90	3.9220
IC10	425.95	100.71	366.82	848.09	1.1366
IC15	643.68	92.82	307.77	826.35	-0.8807
IC20	714.35	46.43	579.79	803.39	-1.1793
IC25	756.89	26.60	638.87	797.29	-1.3546
IC40	798.64	10.89	763.20	828.53	-0.2208
IC50	826.47	8.47	799.92	852.70	-0.0532



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS

Species: *Pimephales promelas*

PpKCICR Test Number: **115**

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	Analyst	Analyst	Analyst	Analyst	Analyst
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.11	7.64	7.62	7.65	7.62	7.66
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.6	7.8	6.5
	Conductivity (µmhos/cm)	289		309		312	
	Alkalinity (mg CaCO ₃ /L)	59				59	
	Hardness (mg CaCO ₃ /L)	86				86	
	Temperature (°C)	24.8	24.6	24.8	24.5	24.7	24.7
300 mg KCl/L	pH (S.U.)	7.99	7.50	7.60	7.57	7.60	7.50
	Dissolved oxygen (mg/L)	8.1	7.6	7.7	7.6	7.9	6.7
	Conductivity (µmhos/cm)	800		840		820	
	Temperature (°C)	24.9	24.8	24.8	24.2	24.7	24.5
450 mg KCl/L	pH (S.U.)	7.97	7.57	7.60	7.55	7.60	7.50
	Dissolved oxygen (mg/L)	8.1	7.6	7.8	7.6	7.9	6.6
	Conductivity (µmhos/cm)	1040		1040		1080	
	Temperature (°C)	24.9	24.5	24.8	24.2	24.7	24.7
600 mg KCl/L	pH (S.U.)	7.95	7.54	7.67	7.55	7.58	7.54
	Dissolved oxygen (mg/L)	8.1	7.6	7.8	7.7	7.9	6.6
	Conductivity (µmhos/cm)	1310		1330		1300	
	Temperature (°C)	24.7	24.5	24.9	24.2	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.95	7.55	7.67	7.53	7.58	7.53
	Dissolved oxygen (mg/L)	8.1	7.6	7.8	7.7	8.0	6.6
	Conductivity (µmhos/cm)	1570		1590		1570	
	Temperature (°C)	24.7	24.5	24.9	24.6	24.6	24.6
900 mg KCl/L	pH (S.U.)	7.95	7.54	7.60	7.53	7.63	7.52
	Dissolved oxygen (mg/L)	8.2	7.6	7.9	7.6	8.0	6.6
	Conductivity (µmhos/cm)	1790		1870		1830	
	Temperature (°C)	24.9	24.7	25.0	24.6	24.6	24.6
1050 mg KCl/L	pH (S.U.)	7.95	7.67	7.67	7.59	7.67	7.54
	Dissolved oxygen (mg/L)	8.2	7.6	7.9	7.5	8.1	6.6
	Conductivity (µmhos/cm)	2080		2110		2090	
	Temperature (°C)	25.0	24.7	25.0	24.3	24.6	24.6
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

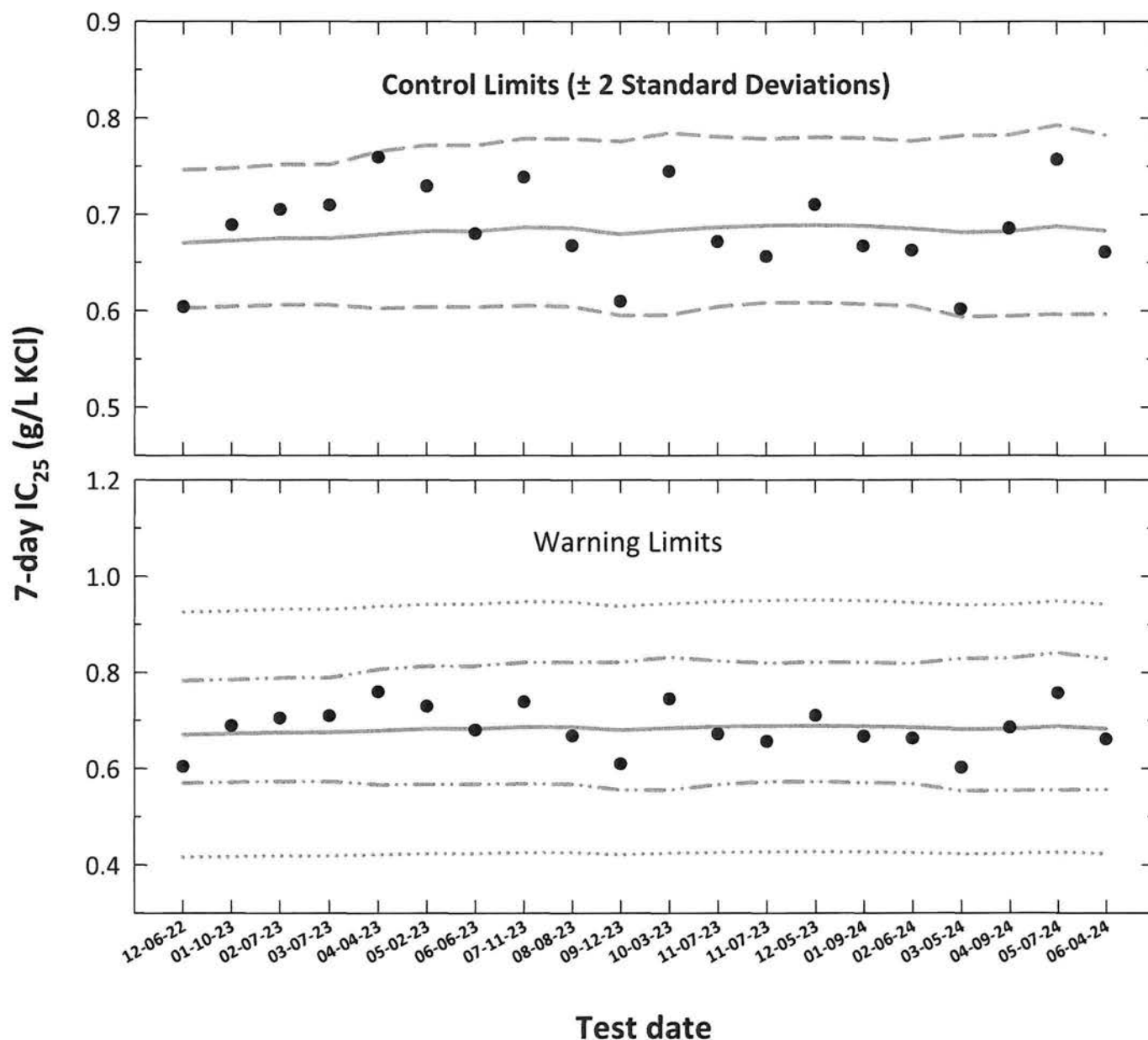
PpKICR Test Number: 115

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		K	BSL	BSL	BSL	BSL	N	K	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.11	7.45	7.43	7.32	7.44	7.35	7.62	7.10
	Dissolved oxygen (mg/L)	7.7	7.4	7.7	7.2	7.7	7.4	7.9	5.2
	Conductivity (µmhos/cm)	299		293		300		312	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			88					
	Temperature (°C)	24.7	24.6	24.7	24.7	24.7	24.5	24.8	24.6
300 mg KCl/L	pH (S.U.)	8.01	7.45	7.71	7.40	7.70	7.30	7.80	7.11
	Dissolved oxygen (mg/L)	7.7	7.4	7.9	7.1	8.0	6.2	7.7	5.1
	Conductivity (µmhos/cm)	817		813		804		850	
	Temperature (°C)	24.7	24.5	24.8	24.8	24.8	24.4	24.7	24.4
450 mg KCl/L	pH (S.U.)	7.98	7.46	7.71	7.35	7.70	7.37	7.78	7.11
	Dissolved oxygen (mg/L)	7.7	7.4	7.9	7.2	8.0	7.0	7.7	5.4
	Conductivity (µmhos/cm)	1070		1030		1040		1030	
	Temperature (°C)	24.8	24.7	24.7	24.8	24.8	24.5	24.7	24.4
600 mg KCl/L	pH (S.U.)	7.98	7.45	7.71	7.40	7.49	7.37	7.78	7.13
	Dissolved oxygen (mg/L)	7.8	7.7	8.0	7.3	8.0	7.3	7.8	5.8
	Conductivity (µmhos/cm)	1330		1290		1310		1350	
	Temperature (°C)	24.7	24.5	24.7	24.7	24.9	24.7	24.7	24.5
750 mg KCl/L	pH (S.U.)	7.97	7.48	7.70	7.39	7.49	7.42	7.79	7.11
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.3	8.0	7.2	7.7	5.8
	Conductivity (µmhos/cm)	1570		1520		1560		1610	
	Temperature (°C)	24.6	24.5	24.7	24.7	24.8	24.4	24.6	24.7
900 mg KCl/L	pH (S.U.)	7.98	7.48	7.70	7.47	7.70	7.47	7.81	7.20
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.3	8.0	7.3	7.9	5.6
	Conductivity (µmhos/cm)	1840		1770		1740		1860	
	Temperature (°C)	24.6	24.5	24.8	24.6	24.8	24.4	24.7	24.3
1050 mg KCl/L	pH (S.U.)	7.98	7.51	7.70	7.44	7.70	7.44	7.82	7.23
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	7.3	8.0	7.5	8.0	5.5
	Conductivity (µmhos/cm)	2070		2050		2080		2150	
	Temperature (°C)	24.6	24.8	24.8	24.6	24.8	24.4	24.6	24.3
		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		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	12-06-22	0.6041	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
2	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
3	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
4	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318
5	04-04-23	0.7592	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373
6	05-02-23	0.7295	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424
7	06-06-23	0.6800	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422
8	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
9	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
10	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
11	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
12	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
13	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
14	12-05-23	0.7102	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510
15	01-09-24	0.6671	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
16	02-06-24	0.6628	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
17	03-05-24	0.6019	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
18	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
19	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
20	06-04-24	0.6610	-0.1798	-0.1656	0.0295	0.6830	0.5963	0.7823	0.5561	0.8283	0.4234	0.9425

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

CT = Central tendency of the IC_{25} values.

S = Standard deviation of the IC_{25} values.

Control Limits = Mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

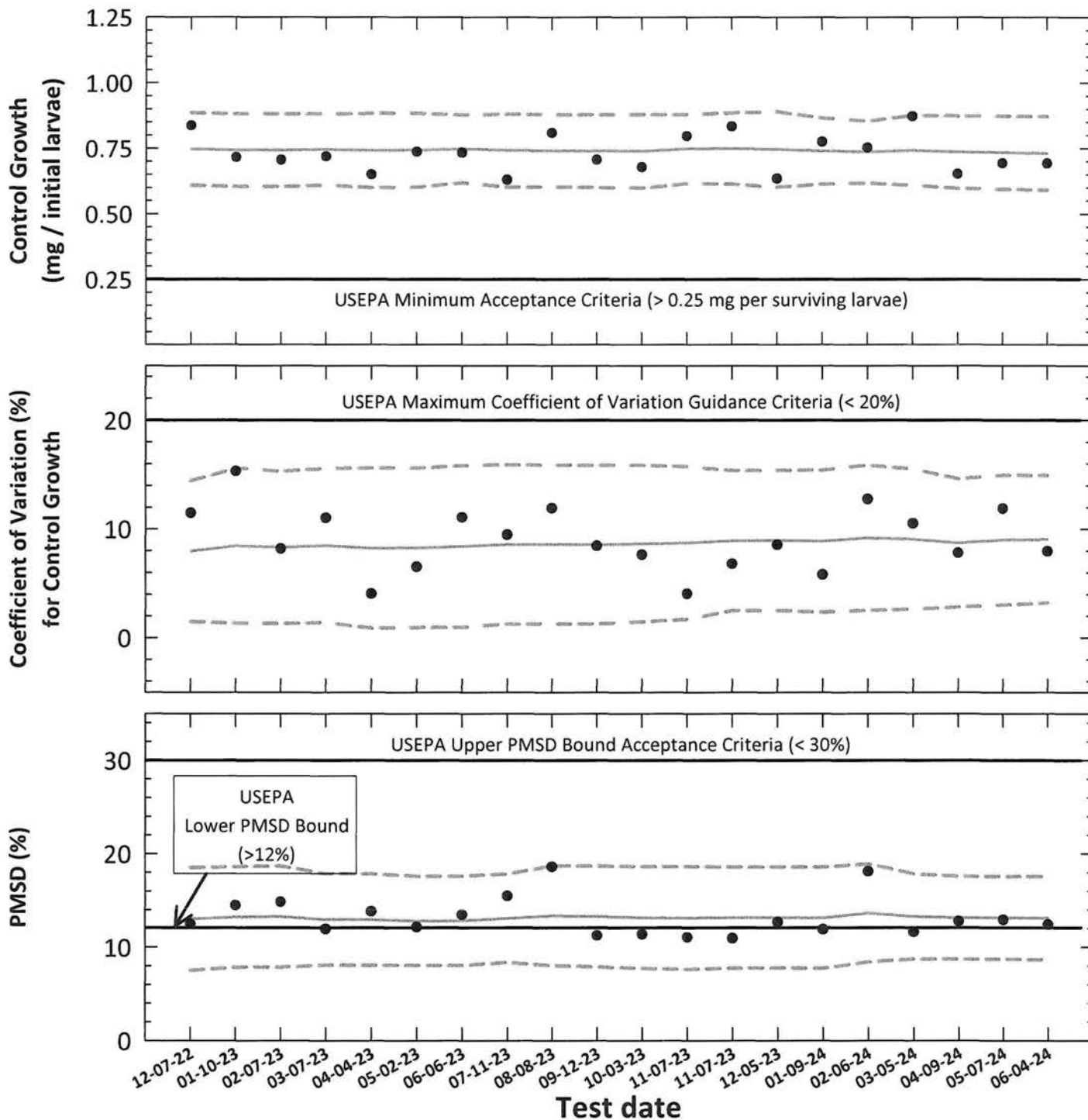
Warning Limits = Mean logarithmic $IC_{25} \pm 2CV$ or $S_{A,75}$ converted to anti-logarithmic values.

$S_{A,75}$ = 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		Test		Control Growth (mg/initial larvae)		Control Growth CV (%)		Test PMSD (%)	
		Control Survival (%)	Control Growth		CV (%)	Mean (mg/initial larvae)	MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT
1	12-07-22	100			11.5	0.837	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	14.4	13.0
2	01-10-23	100			15.3	0.716	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	15.6	13.2
3	02-07-23	100			8.2	0.707	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3
4	03-07-23	100			11.0	0.719	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0
5	04-04-23	100			4.1	0.651	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0
6	05-02-23	100			6.5	0.737	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8
7	06-06-23	100			11.1	0.734	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8
8	07-11-23	100			9.5	0.630	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1
9	08-08-23	100			11.9	0.809	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4
10	09-12-23	100			8.5	0.707	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3
11	10-03-23	100			7.7	0.678	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2
12	11-07-23	100			4.0	0.797	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1
13	11-07-23	100			6.8	0.833	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2
14	12-05-23	100			8.6	0.635	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2
15	01-09-24	100			5.8	0.776	0.0923	11.9	0.740	0.614	0.866	8.9	2.4	15.4	13.1
16	02-06-24	100			12.8	0.753	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7
17	03-05-24	100			10.5	0.873	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3
18	04-09-24	100			7.8	0.653	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1
19	05-07-24	100			11.9	0.693	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1
20	06-04-24	100			8.0	0.693	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.1

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.

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: 116

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2288</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>7 B</u>
Spawn date:	<u>05-18-24</u>	Artemia CHM number:	CHM1294
Hatch dates and times:	<u>06-03-24 1210 to 06-04-24 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.71</u> S.U. Temperature = <u>24.6</u> °C	Date / Time in oven:	<u>06-11-24 0500</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>06-12-24 0515</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	06-04-24	0505	<u>J</u>	1300	<u>K</u>	0717	<u>H</u>	05-29-24 C
1	06-05-24	0500	<u>H</u>	1100	<u>K</u>	0704	<u>H</u>	↓
2	06-06-24	0500	<u>J</u>	1200	<u>H</u>	0715	<u>H</u>	05-29-24 D
3	06-07-24	0500	<u>J</u>	1200	<u>H</u>	0700	<u>H</u>	↓
4	06-08-24	0610	<u>J</u>	1210	<u>H</u>	0810	<u>H</u>	06-03-24 B
5	06-09-24	0600	<u>J</u>	1150	<u>H</u>	0800	<u>H</u>	↓
6	06-10-24	0500	<u>H</u>	1100	<u>H</u>	0700	<u>H</u>	↓
7	06-11-24					0518	<u>H</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>150664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>790.3</u>
Average weight per initial larvae:	<u>0.693</u>		NOEC (mg/L KCl)	<u>450</u>
Average weight per surviving larvae:	<u>0.693</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>600</u>
			ChV (mg/L KCl)	<u>519.6</u>
			IC ₂₅ (mg/L KCl)	<u>661.0</u>

Species: Pimephales promelas

PpKICR Test Number: 116

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 ^{lg}	10	10	10 ^{lg}	10	10	10 ^{lg}	10
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>JP EM</u> Date: <u>05-24-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>06-24-24</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)	0.693				0.964				0.804			
Percent reduction from control (%)					-39.07				-16.07			

*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: 116

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	9 ^{id}	9 ^{id}	6 ^{id}	6 ^{id}	7 ^{id}	6 ^{id}
2	10	10	10	10	10	10	9	9	6	6	7	5 ^{id}
3	10	10	10	10	9 ^{id}	9 ^{id}	9	8 ^{id}	6	5 ^{id}	6	5
4	10	9 ^{id}	10	10	7 ^{id}	7 ^{id}	8 ^{id}	8	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}
5	9 ^{id}	9	10	10	7	7	8	8	3 ^{id}	3 ^{id}	2 ^{id}	2 ^{id}
6	9	9	10	10	6 ^{id}	7	7 ^{id}	7 ^{id}	3	2 ^{id}	1 ^{id}	2
7	9	9	9 ^{id}	9 ^{id}	6	7	7	7	2 ^{id}	2 ^{id}	1	2
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>EM</u> Date: <u>05-24-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>06-14-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JK</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JK</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.683 1.47 0.531 23.47 0.102 85.37												

*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: 116

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 rd	3 rd	4 th	4 th
2	2 nd	3	3 rd	3 rd
3	2	2 nd	2 nd	2 nd
4	2	2	2	1 st
5	2	2	1 st	0 th
6	2	2	1	0
7	2	1 st	1	0
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>EM</u> Date: <u>05-24-24</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>06-14-24</u>				
C = Larvae weight (mg) = B - A Analyst: <u>Y</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>N</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: 116
Test dates: June 04-11, 2024

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	13.72	20.08	6.36	0.636							
	B	10	10	15.27	22.48	7.21	0.721							
	C	10	10	13.12	19.71	6.59	0.659	0.693	8.0		100.0	0.693	8.0	Not applicable
	D	10	10	14.65	22.21	7.56	0.756							
300	E	10	10	12.87	23.18	10.31	1.031							
	F	10	10	14.55	23.82	9.27	0.927	0.964	6.9		100.0	0.964	6.9	-39.0
	G	10	10	12.19	21.09	8.90	0.890							
	H	10	10	14.32	24.38	10.06	1.006							
450	I	10	10	15.19	23.30	8.11	0.811							
	J	10	10	14.18	22.05	7.87	0.787	0.804	5.5		100.0	0.804	5.5	-16.0
	K	10	10	13.19	21.80	8.61	0.861							
	L	10	10	13.53	21.09	7.56	0.756							
600	M	10	9	13.16	20.47	7.31	0.812							
	N	10	9	15.04	22.06	7.02	0.780	0.759	8.0		90.0	0.683	8.0	1.4
	O	10	9	12.52	19.47	6.95	0.772							
	P	10	9	13.13	19.18	6.05	0.672							
750	Q	10	6	11.60	16.41	4.81	0.802							
	R	10	7	13.02	18.38	5.36	0.766	0.786	2.0		67.5	0.531	6.4	23.4
	S	10	7	15.25	20.74	5.49	0.784							
	T	10	7	12.31	17.87	5.56	0.794							
900	U	10	2	13.90	14.99	1.09	0.545							
	V	10	2	12.68	14.08	1.40	0.700	0.598	21.0		17.5	0.102	29.3	85.3
	W	10	1	13.88	14.58	0.70	0.700							
	X	10	2	13.77	14.66	0.89	0.445							
1050	Y	10	2	12.36	13.66	1.30	0.650							
	Z	10	1	12.67	13.46	0.79	0.790	0.797	25.1		10.0	0.076	72.3	89.0
	AA	10	1	14.09	15.04	0.95	0.950							
	BB	10	0	0.00	0.00	0.00	0.000							

Dunnett's MSD value: 0.0862
PMSD: 12.4

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: 6/4/2024 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
End Date: 6/11/2024 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	0.9000	0.9000	0.9000	0.9000
750	0.6000	0.7000	0.7000	0.7000
900	0.2000	0.2000	0.1000	0.2000
1050	0.2000	0.1000	0.1000	0.0000

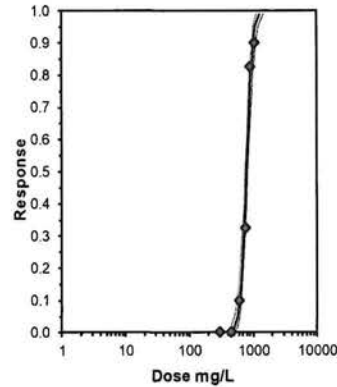
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*600	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	4	10.00	10.00	4	40
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	40
*1050	0.1000	0.1000	0.3165	0.1588	0.4636	39.374	4	10.00	10.00	36	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.72053	0.896	-0.6776	5.32131
Equality of variance cannot be confirmed				

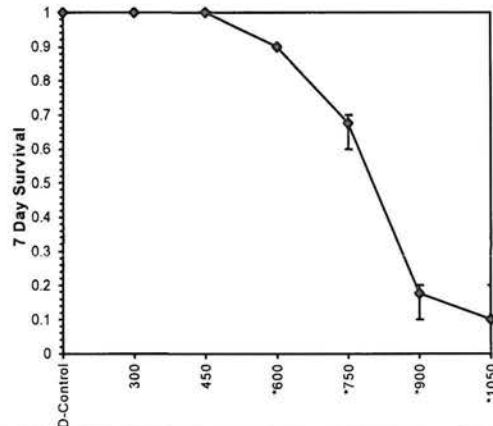
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	450	600	519.615	
Treatments vs D-Control				

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.7569	1.45387	8.90736 14.6065	0	2.8325	9.48773	0.58623	2.89777	0.08506	3
Intercept	-29.069	4.22356	-37.347 -20.791							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	501.066	427.362 554.026
EC05	3.355	572.612	508.096 618.795
EC10	3.718	614.839	556.628 657.032
EC15	3.964	645.075	591.546 684.641
EC20	4.158	670.163	620.475 707.84
EC25	4.326	692.462	646.037 728.8
EC40	4.747	751.998	712.594 787.292
EC50	5.000	790.252	753.151 827.72
EC60	5.253	830.451	793.331 873.168
EC75	5.674	901.851	859.023 960.862
EC80	5.842	931.859	885 999.834
EC85	6.036	968.1	915.478 1048.17
EC90	6.282	1015.71	954.401 1113.39
EC95	6.645	1090.61	1013.83 1219.2
EC99	7.326	1246.34	1132.83 1448.9



Dose-Response Plot



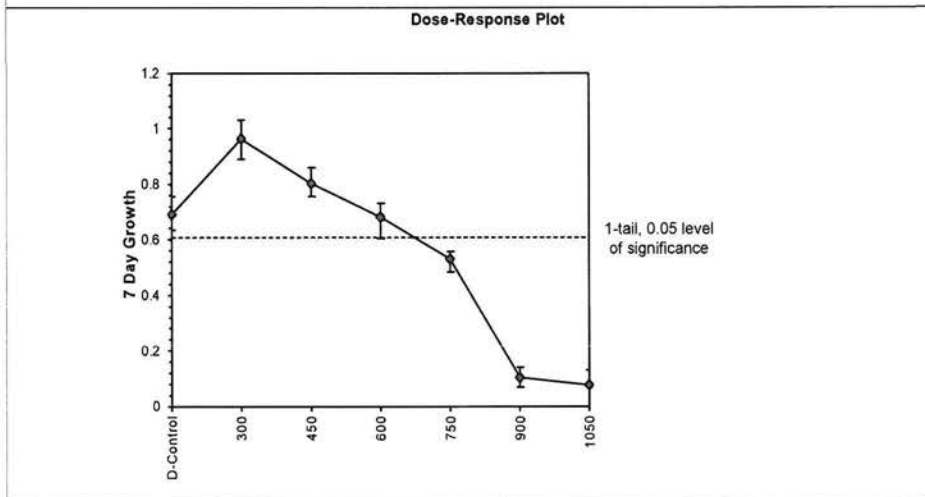
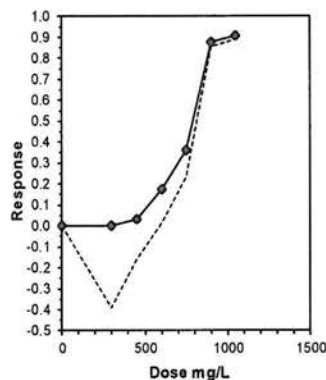
Larval Fish Growth and Survival Test-7 Day Growth			
Start Date:	6/4/2024	Test ID:	PpKCICR
End Date:	6/11/2024	Lab ID:	ETS-Envir. Testing Sol.
Sample Date:		Sample Type:	KCL-Potassium chloride
Comments:		Protocol:	FWCHR-EPA-821-R-02-013
		Test Species:	PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	0.6360	0.7210	0.6590	0.7560
300	1.0310	0.9270	0.8900	1.0060
450	0.8110	0.7870	0.8610	0.7560
600	0.7310	0.7020	0.6950	0.6050
750	0.4810	0.5360	0.5490	0.5560
900	0.1090	0.1400	0.0700	0.0890
1050	0.1300	0.0790	0.0950	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed			Isotonic	
			Mean	Min	Max	CV%			Critical	MSD	Mean	N-Mean	
D-Control	0.6930	1.0000	0.6930	0.6360	0.7560	7.973	4				0.8283	1.0000	
300	0.9635	1.3903	0.9635	0.8900	1.0310	6.858	4	-6.841	2.180	0.0862	0.8283	1.0000	
450	0.8038	1.1598	0.8038	0.7560	0.8610	5.513	4	-2.801	2.180	0.0862	0.8038	0.9704	
600	0.6833	0.9859	0.6833	0.6050	0.7310	7.969	4				0.6833	0.8249	
750	0.5305	0.7655	0.5305	0.4810	0.5560	6.414	4				0.5305	0.6405	
900	0.1020	0.1472	0.1020	0.0700	0.1400	29.335	4				0.1020	0.1232	
1050	0.0760	0.1097	0.0760	0.0000	0.1300	72.317	4				0.0760	0.0918	

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)				0.91769	0.805	0.05105	-1.6442
Bartlett's Test indicates equal variances ($p = 0.82$)				0.4075	9.21035		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnnett's Test				450	>450		
Treatments vs D-Control				0.0862	0.12439	0.07397	0.00313
							2.6E-04
							2, 9

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	471.05	34.09	333.48	537.97	-0.9322
IC10	522.60	28.08	433.85	610.30	-0.0480
IC15	574.15	27.90	498.23	652.01	-0.1059
IC20	620.28	24.32	536.14	681.72	-0.3577
IC25	660.95	21.42	583.37	714.33	-0.5126
IC40	761.74	6.53	733.91	777.06	-0.8313
IC50	790.74	4.94	774.95	803.97	-0.2063



Species: *Pimephales promelas*

PpKCICR Test Number: 116

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	
		Initial	Final	Initial	Final	Initial	Final
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.81	7.55	7.49	7.36	7.41	7.58
	Dissolved oxygen (mg/L)	7.0	7.5	7.6	7.6	8.0	7.2
	Conductivity (µmhos/cm)	294		308		315	
	Alkalinity (mg CaCO ₃ /L)	57				58	
	Hardness (mg CaCO ₃ /L)	85				85	
	Temperature (°C)	24.9	24.7	24.7	24.8	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.81	7.48	7.54	7.34	7.56	7.48
	Dissolved oxygen (mg/L)	8.0	7.6	8.0	7.7	7.7	7.0
	Conductivity (µmhos/cm)	816		867		848	
	Temperature (°C)	25.0	24.8	24.7	24.6	24.7	25.1
450 mg KCl/L	pH (S.U.)	7.78	7.44	7.54	7.37	7.56	7.48
	Dissolved oxygen (mg/L)	8.0	7.6	8.0	7.7	7.8	6.8
	Conductivity (µmhos/cm)	1200		1120		1090	
	Temperature (°C)	25.0	24.8	24.7	24.5	24.8	24.8
600 mg KCl/L	pH (S.U.)	7.78	7.45	7.58	7.33	7.57	7.48
	Dissolved oxygen (mg/L)	8.0	7.6	8.0	7.7	7.8	6.8
	Conductivity (µmhos/cm)	1310		1370		1360	
	Temperature (°C)	24.9	24.9	24.7	24.7	24.7	24.8
750 mg KCl/L	pH (S.U.)	7.77	7.45	7.58	7.37	7.56	7.52
	Dissolved oxygen (mg/L)	8.0	7.6	8.1	7.7	7.8	6.8
	Conductivity (µmhos/cm)	1540		1620		1610	
	Temperature (°C)	25.1	24.7	24.8	24.5	24.6	24.7
900 mg KCl/L	pH (S.U.)	7.77	7.44	7.58	7.41	7.53	7.57
	Dissolved oxygen (mg/L)	8.1	7.7	8.1	7.8	7.9	6.9
	Conductivity (µmhos/cm)	1790		1880		1890	
	Temperature (°C)	25.1	24.7	24.8	24.5	24.6	24.8
1050 mg KCl/L	pH (S.U.)	7.77	7.43	7.59	7.41	7.58	7.57
	Dissolved oxygen (mg/L)	8.1	7.7	8.1	7.9	7.9	6.8
	Conductivity (µmhos/cm)	2060		2120		2090	
	Temperature (°C)	25.1	24.6	24.8	24.6	24.6	25.0
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

PpKICR Test Number: 116

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		Analyst							
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.94	7.42	7.70	7.27	7.71	7.49	7.73	7.36
	Dissolved oxygen (mg/L)	7.7	7.4	7.7	7.4	7.7	7.4	8.0	7.3
	Conductivity (µmhos/cm)	302		301		302		306	
	Alkalinity (mg CaCO ₃ /L)			58					
	Hardness (mg CaCO ₃ /L)			87					
	Temperature (°C)	24.7	24.6	24.8	25.0	24.8	24.6	24.7	24.7
300 mg KCl/L	pH (S.U.)	7.92	7.43	7.66	7.33	7.69	7.59	7.86	7.39
	Dissolved oxygen (mg/L)	7.8	7.4	7.7	7.4	7.8	7.4	8.0	7.3
	Conductivity (µmhos/cm)	835		820		836		836	
	Temperature (°C)	24.8	24.5	24.8	24.7	24.8	25.0	24.7	24.9
450 mg KCl/L	pH (S.U.)	7.90	7.47	7.70	7.32	7.73	7.61	7.91	7.40
	Dissolved oxygen (mg/L)	7.9	7.4	7.8	7.3	7.8	7.4	8.0	7.3
	Conductivity (µmhos/cm)	1000		1070		1100		1090	
	Temperature (°C)	24.8	24.9	24.7	24.7	24.9	25.1	24.7	24.6
600 mg KCl/L	pH (S.U.)	7.90	7.48	7.71	7.31	7.72	7.59	7.94	7.41
	Dissolved oxygen (mg/L)	7.9	7.4	7.9	7.3	7.9	7.4	8.6	7.3
	Conductivity (µmhos/cm)	1300		1340		1360		1350	
	Temperature (°C)	24.7	24.7	24.7	24.9	24.9	24.9	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.90	7.48	7.71	7.33	7.74	7.58	7.90	7.45
	Dissolved oxygen (mg/L)	8.0	7.3	7.9	7.2	7.9	7.5	8.6	7.3
	Conductivity (µmhos/cm)	1580		1570		1600		1610	
	Temperature (°C)	24.7	24.6	24.7	24.9	25.0	24.8	24.7	24.6
900 mg KCl/L	pH (S.U.)	7.90	7.44	7.71	7.42	7.74	7.73	7.92	7.57
	Dissolved oxygen (mg/L)	8.0	7.2	7.8	7.2	7.9	7.5	8.0	7.5
	Conductivity (µmhos/cm)	1830		1840		1870		1860	
	Temperature (°C)	24.9	24.6	24.9	25.1	25.0	24.8	24.7	24.9
1050 mg KCl/L	pH (S.U.)	7.90	7.51	7.71	7.39	7.75	7.74	7.92	7.57
	Dissolved oxygen (mg/L)	8.1	7.5	7.9	7.2	7.9	7.5	7.9	7.5
	Conductivity (µmhos/cm)	2090		2080		2080		2140	
	Temperature (°C)	24.7	24.6	24.7	24.8	25.0	25.0	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final