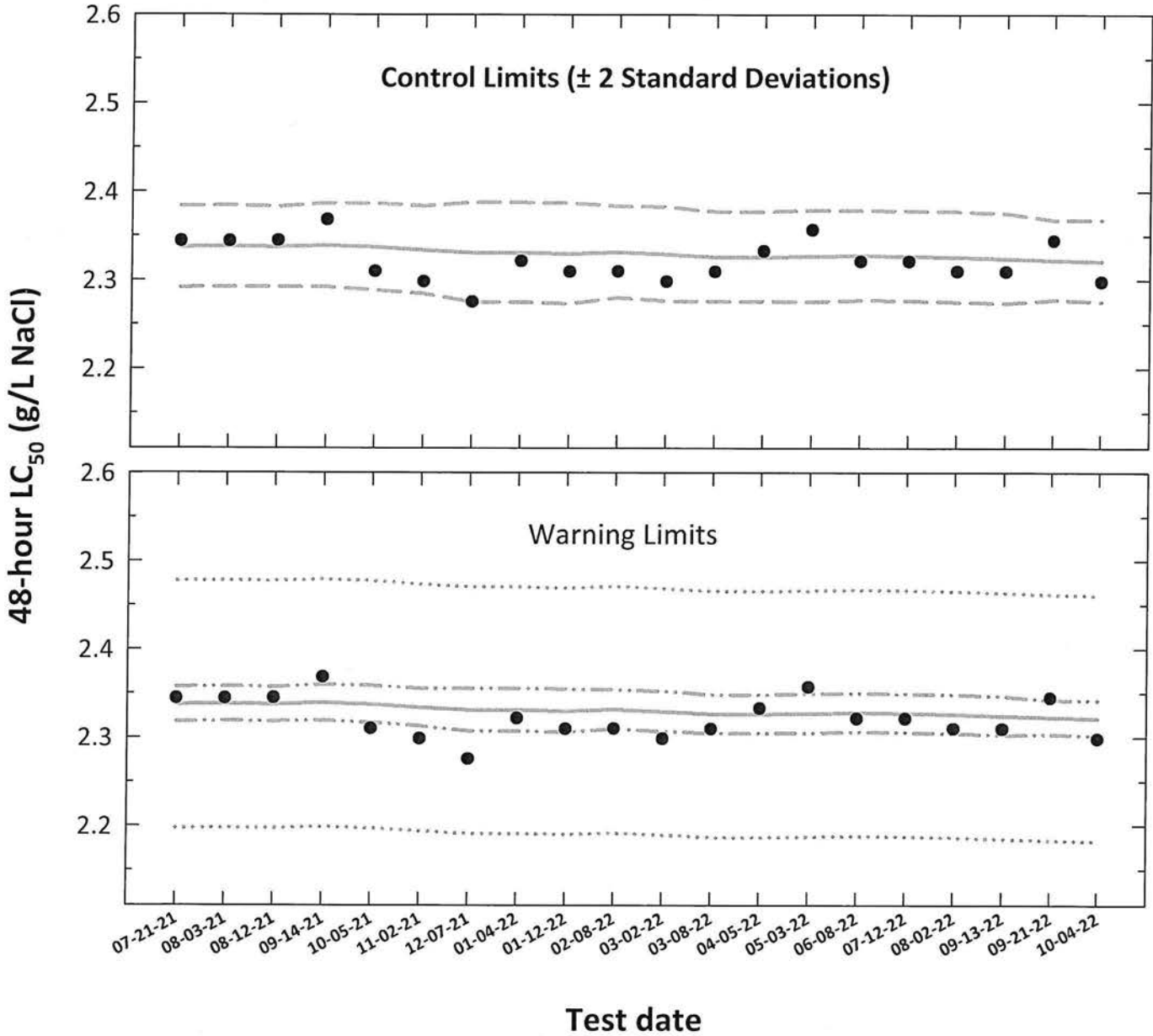


Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits 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	07-21-21	2.3442	0.3700	0.3687	0.0043	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
2	08-03-21	2.3442	0.3700	0.3688	0.0043	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
3	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
4	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
5	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
6	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
7	12-07-21	2.2755	0.3571	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
8	01-04-22	2.3217	0.3658	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708
9	01-12-22	2.3096	0.3635	0.3673	0.0053	2.3297	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695
10	02-08-22	2.3099	0.3636	0.3676	0.0048	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
11	03-02-22	2.2985	0.3614	0.3672	0.0050	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
12	03-08-22	2.3096	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
13	04-05-22	2.3330	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
14	05-03-22	2.3569	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
15	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
16	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
17	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
18	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
19	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
20	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607

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 # 386

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 #: 2130

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	7.51	7.20	7.53
	Dissolved oxygen (mg/L)	7.6	8.1	7.8
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	85		
	Temperature (°C)	24.9	25.0	25.2
1750 mg/L	pH (S.U.)	7.72	7.54	7.63
	Dissolved oxygen (mg/L)	7.8	8.1	7.8
	Conductivity (µmhos/cm)	3290		
	Temperature (°C)	25.0	25.2	25.0
2000 mg/L	pH (S.U.)	7.70	7.58	7.62
	Dissolved oxygen (mg/L)	7.8	8.1	7.7
	Conductivity (µmhos/cm)	3820		
	Temperature (°C)	25.0	25.1	24.9
2250 mg/L	pH (S.U.)	7.69	7.61	7.62
	Dissolved oxygen (mg/L)	7.8	8.1	7.7
	Conductivity (µmhos/cm)	4150		
	Temperature (°C)	25.1	25.1	25.0
2500 mg/L	pH (S.U.)	7.69	7.62	7.63
	Dissolved oxygen (mg/L)	7.8	8.2	7.7
	Conductivity (µmhos/cm)	4660		
	Temperature (°C)	25.1	25.0	24.9
2750 mg/L	pH (S.U.)	7.69	7.62	7.63
	Dissolved oxygen (mg/L)	7.8	8.2	7.7
	Conductivity (µmhos/cm)	5160		
	Temperature (°C)	24.9	25.2	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	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 # 386

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-04-22	0520	J	0745	J	2B3	RED	09-28-22A
24	10-05-22			0740	J			
48 Termination	11-06-22			0743	J			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	09-27-22 D
Age:	< 24-hours old
Date and time organisms were born between:	10-03-22 1456 TO 10-04-22 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.30 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	S	S	S	S	S	S	S	S	S	S	S	S
48 Termination	S	S	S	S	S	S	S	S	S	S	S	S
Mean Survival												

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	S	S	S	S	S	S	S	S	^{2d} 3	^{4d} 4	^{4d} 4	^{2d} 3
48 Termination	^{2d} 3	^{2d} 3	^{4d} 1	^{2d} 3	^{5d} 0	^{4d} 1	^{4d} 1	^{2d} 2	^{3d} 0	^{4d} 0	^{4d} 0	^{3d} 0
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:

Test Reviewed by: J

Acute Daphnid Test-48 Hr Survival

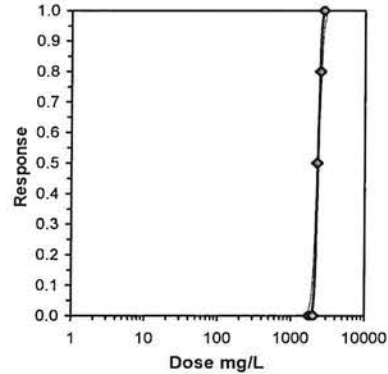
Start Date:	10/4/2022	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/6/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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	0.6000	0.2000	0.6000
2500	0.0000	0.2000	0.2000	0.4000
2750	0.0000	0.0000	0.0000	0.0000

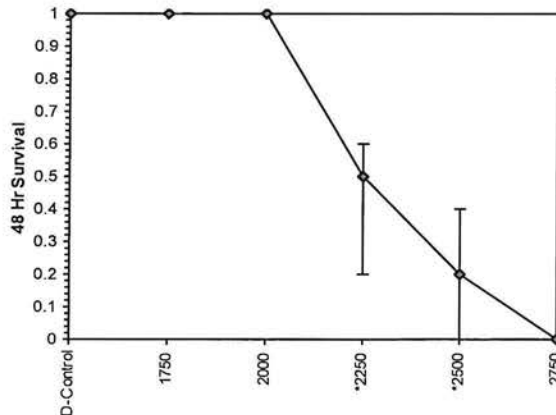
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7805	0.4636	0.8861	27.063	4	10.00	10.00	10	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	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.72325	0.868	-1.225	3.75298
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				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	30.6709	5.60227	19.6905	41.6514	0	2.7074	7.81472	0.43897	3.36142	0.0326	6
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							



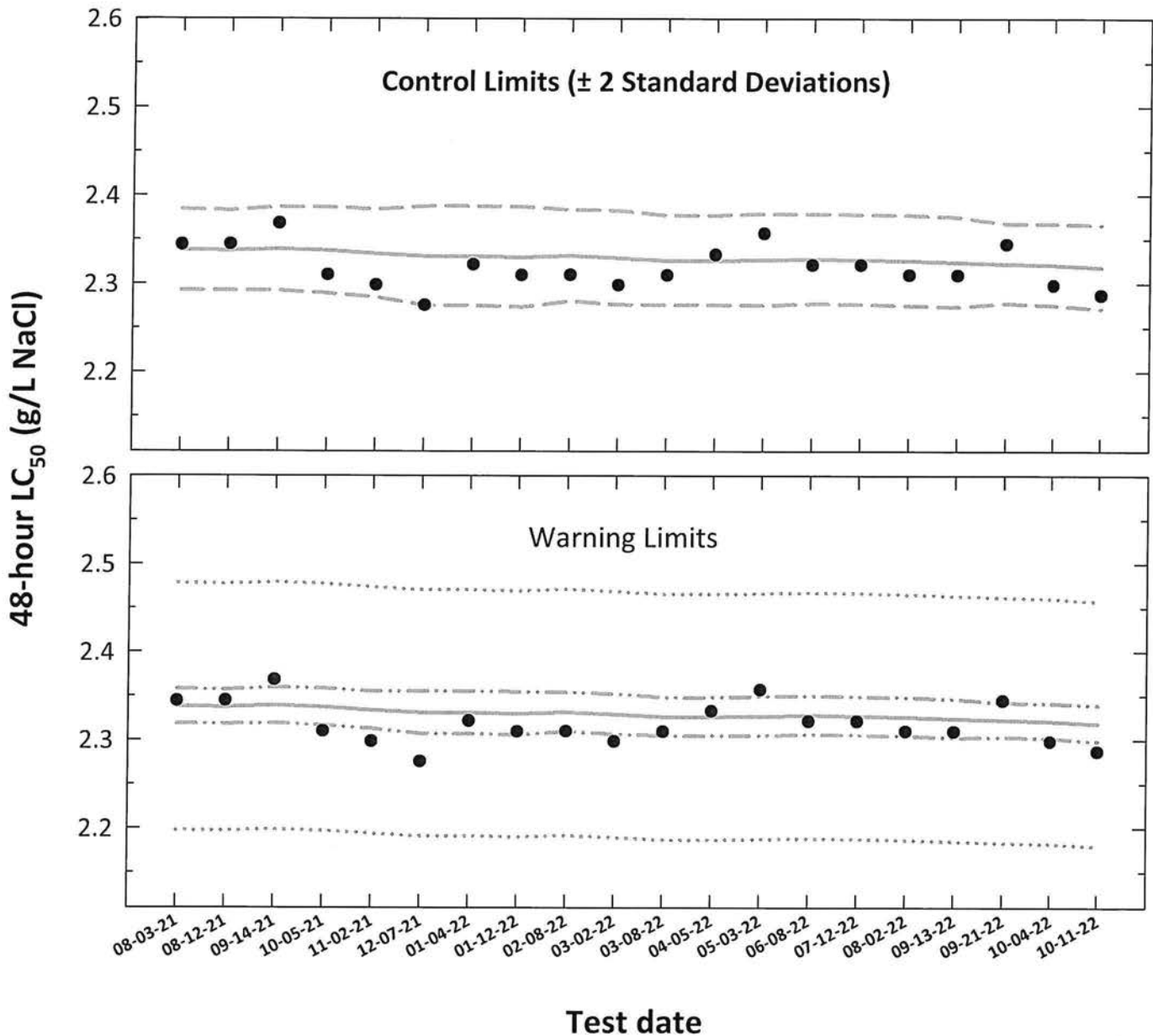
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits 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	08-03-21	2.3442	0.3700	0.3688	0.0043	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
2	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
3	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
4	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
5	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
6	12-07-21	2.2755	0.3571	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
7	01-04-22	2.3217	0.3658	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708
8	01-12-22	2.3096	0.3635	0.3673	0.0053	2.3297	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695
9	02-08-22	2.3099	0.3636	0.3676	0.0048	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
10	03-02-22	2.2985	0.3614	0.3672	0.0050	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
11	03-08-22	2.3096	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
12	04-05-22	2.3330	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
13	05-03-22	2.3569	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
14	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
15	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
16	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
17	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
18	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
19	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
20	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576

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 # 387

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 #: 2147

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	7.60	7.31	7.39
	Dissolved oxygen (mg/L)	7.7	7.7	7.8
	Conductivity (µmhos/cm)	312		
	Alkalinity (mg/L CaCO ₃)	58		
	Hardness (mg/L CaCO ₃)	87		
	Temperature (°C)	24.8	25.2	25.1
1750 mg/L	pH (S.U.)	7.02	7.49	7.65
	Dissolved oxygen (mg/L)	7.6	7.7	7.7
	Conductivity (µmhos/cm)	3190		
	Temperature (°C)	24.8	25.0	24.9
2000 mg/L	pH (S.U.)	7.02	7.49	7.65
	Dissolved oxygen (mg/L)	7.7	7.8	7.8
	Conductivity (µmhos/cm)	3780		
	Temperature (°C)	24.9	25.0	24.9
2250 mg/L	pH (S.U.)	7.03	7.49	7.64
	Dissolved oxygen (mg/L)	7.7	7.9	7.8
	Conductivity (µmhos/cm)	4210		
	Temperature (°C)	24.9	25.3	24.9
2500 mg/L	pH (S.U.)	7.05	7.50	7.68
	Dissolved oxygen (mg/L)	7.7	7.9	7.8
	Conductivity (µmhos/cm)	4090		
	Temperature (°C)	25.0	25.3	25.0
2750 mg/L	pH (S.U.)	7.04	7.52	7.68
	Dissolved oxygen (mg/L)	7.7	7.8	7.8
	Conductivity (µmhos/cm)	5130		
	Temperature (°C)	25.0	25.1	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	130664665

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 # 387

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-11-22	0654	JL	1100	JL	2F	RED	10-06-22D
24	10-12-22			1100	JL			
48 Termination	11-13-22			1100	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):	10-04-22 D
Age:	< 24-hours old
Date and time organisms were born between:	10-10-22 1455 TO 10-11-22 0654
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.66
	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	5	3 ^d	4 ^u	3 ^d
48 Termination	4 ^{id}	2 ^{sd}	2 ^{sd}	2 ^{sd}	0 ^{sd}	0 ^{sd}	1 ^{id}	2 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
Mean Survival	50%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2215.7
Upper 95% confidence limit (mg NaCl/L)	2356.1
48-hour LC ₅₀ (mg NaCl/L)	2287.0

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 10/11/2022 Test ID: CdNaCIAC Sample ID: REF-Ref Toxicant
 End Date: 10/13/2022 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	0.4000	0.4000
2500	0.0000	0.0000	0.2000	0.4000
2750	0.0000	0.0000	0.0000	0.0000

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	1.3453	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	0.1500	0.1500	0.3998	0.2255	0.6847	55.174	4	10.00	10.00	17	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)	0.76684	0.868	1.34295	2.75401

Equality of variance cannot be confirmed

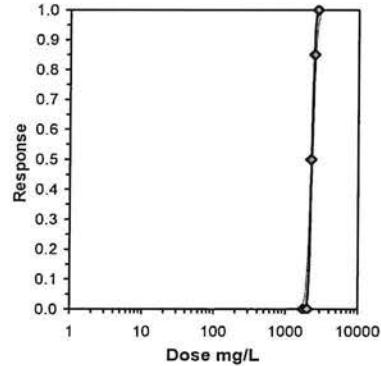
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	

Treatments vs D-Control

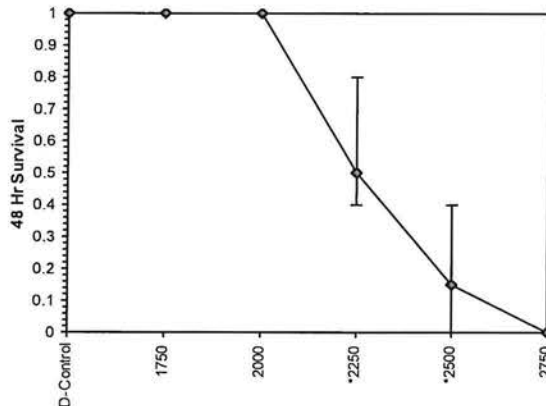
Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	33.0214	6.23087	20.8089	45.234	0	1.88467	7.81472	0.59669	3.35926	0.03028	5
Intercept	-105.93	20.9481	-146.99	-64.869							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1944.51	1750.86	2047.54
EC05	3.355	2039.15	1883.94	2124.39
EC10	3.718	2091.47	1957.66	2167.98
EC15	3.964	2127.52	2008.11	2198.89
EC20	4.158	2156.62	2048.35	2224.61
EC25	4.326	2181.9	2082.78	2247.71
EC40	4.747	2246.93	2167.53	2311.9
EC50	5.000	2286.97	2215.7	2356.12
EC60	5.253	2327.73	2260.82	2405.58
EC75	5.674	2397.1	2329.02	2499.54
EC80	5.842	2425.2	2354.23	2540.46
EC85	6.036	2458.37	2382.69	2590.37
EC90	6.282	2500.75	2417.51	2656.19
EC95	6.645	2564.91	2467.96	2759.18
EC99	7.326	2689.74	2561.45	2967.89



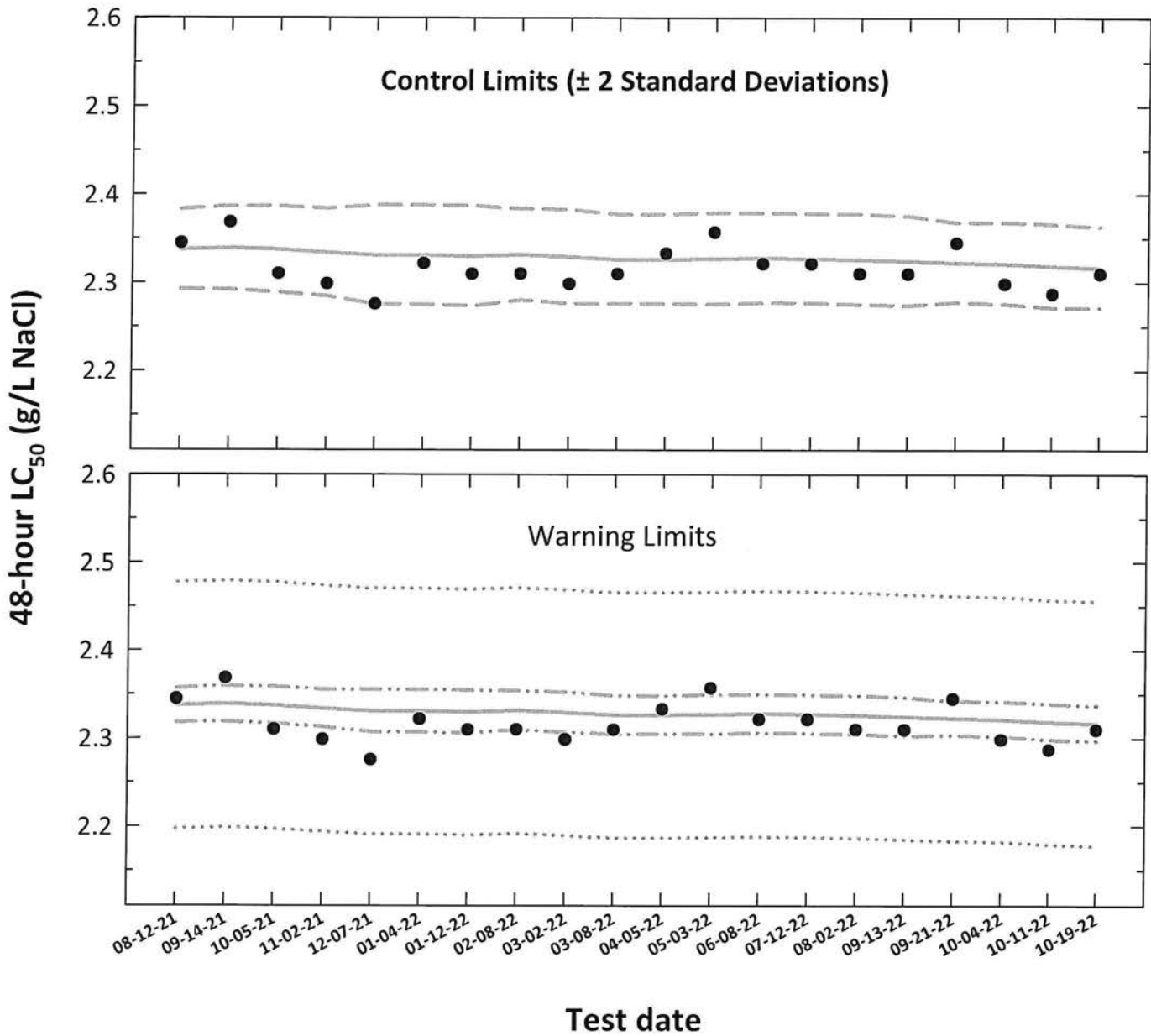
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)				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	CT - S _{A,10}	CT + S _{A,10}		
1	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
2	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
3	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
4	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
5	12-07-21	2.2755	0.3571	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
6	01-04-22	2.3217	0.3658	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708
7	01-12-22	2.3096	0.3635	0.3673	0.0053	2.3297	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695
8	02-08-22	2.3099	0.3636	0.3676	0.0048	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
9	03-02-22	2.2985	0.3614	0.3672	0.0050	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
10	03-08-22	2.3096	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
11	04-05-22	2.3330	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
12	05-03-22	2.3569	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
13	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
14	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
15	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
16	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
17	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
18	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
19	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
20	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

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

CdNaCIAC # 388

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 #: 2147

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Analyst	<u>JH</u>	<u>JH</u>	<u>JH</u>
	pH (S.U.)	<u>7.72</u>	<u>7.99</u>	<u>8.00</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>318</u>		
	Alkalinity (mg/L CaCO ₃)	<u>57</u>		
	Hardness (mg/L CaCO ₃)	<u>86</u>		
Temperature (°C)	<u>24.9</u>	<u>25.0</u>	<u>25.1</u>	
1750 mg/L	pH (S.U.)	<u>7.71</u>	<u>8.00</u>	<u>8.01</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>3490</u>		
	Temperature (°C)	<u>25.0</u>	<u>24.9</u>	<u>25.0</u>
2000 mg/L	pH (S.U.)	<u>7.72</u>	<u>7.99</u>	<u>8.04</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.8</u>	<u>7.8</u>
	Conductivity (µmhos/cm)	<u>3910</u>		
	Temperature (°C)	<u>25.0</u>	<u>25.1</u>	<u>25.0</u>
2250 mg/L	pH (S.U.)	<u>7.73</u>	<u>7.98</u>	<u>8.05</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>
	Conductivity (µmhos/cm)	<u>4340</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.1</u>	<u>25.2</u>
2500 mg/L	pH (S.U.)	<u>7.74</u>	<u>7.98</u>	<u>8.05</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>
	Conductivity (µmhos/cm)	<u>4760</u>		
	Temperature (°C)	<u>24.9</u>	<u>24.8</u>	<u>24.9</u>
2750 mg/L	pH (S.U.)	<u>7.74</u>	<u>7.98</u>	<u>8.05</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>
	Conductivity (µmhos/cm)	<u>5250</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.0</u>	<u>24.9</u>

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	<u>130664685</u>

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 # 388

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-19-22	0504	JL	0830	JL	2D3	GOLD	10-13-22
24	10-20-22			0832	JL			
48 Termination	10-21-22			0830	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):	10-11-22 A-D
Age:	< 24-hours old
Date and time organisms were born between:	10-18-22 1100 TO 10-19-22 0504
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.80
	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	4 ^{ud}	5	3 ^{ud}	5
48 Termination	4 ^{ud}	2 ^{ud}	2 ^{ud}	2 ^{ud}	2	1 ^{ud}	1 ^{ud}	1 ^{ud}	0 ^{ud}	0 ^{ud}	0 ^{ud}	0 ^{ud}
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:	10/19/2022	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/21/2022	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	0.4000	0.4000
2500	0.4000	0.2000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

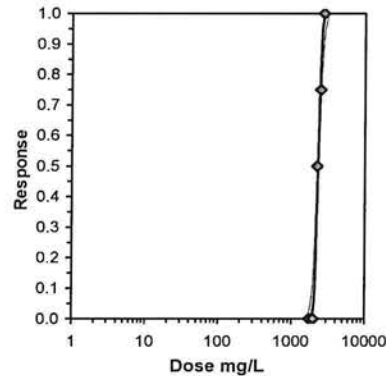
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	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	0.2500	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 <= 0.01) Equality of variance cannot be confirmed	0.68778	0.868	2.22358	6.53833

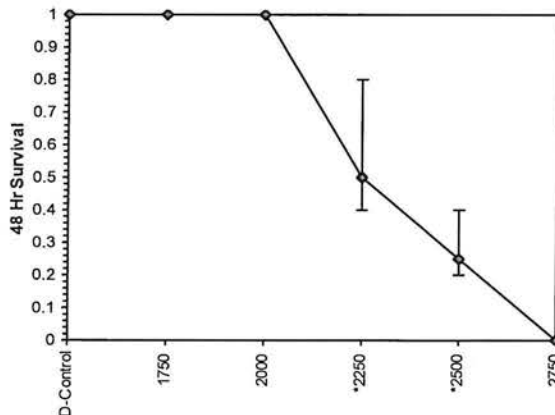
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs D-Control	2000	2250	2121.32	

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							

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



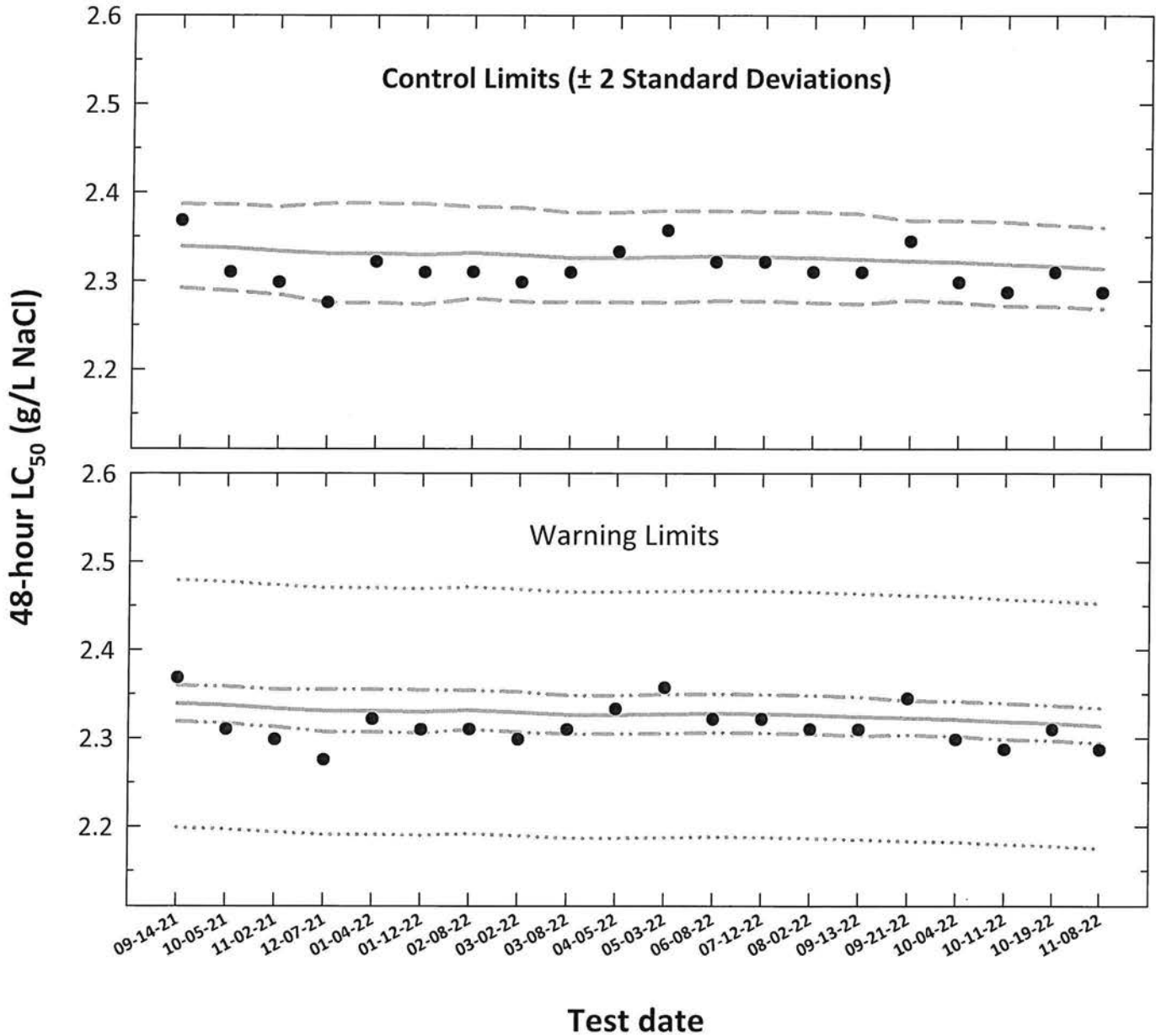
Dose-Response Plot



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 \pm 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic LC₅₀ \pm 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ \pm S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
2	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
3	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
4	12-07-21	2.2755	0.3571	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
5	01-04-22	2.3217	0.3658	0.3675	0.0052	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708
6	01-12-22	2.3096	0.3635	0.3673	0.0053	2.3297	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695
7	02-08-22	2.3099	0.3636	0.3676	0.0048	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
8	03-02-22	2.2985	0.3614	0.3672	0.0050	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
9	03-08-22	2.3096	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
10	04-05-22	2.3330	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
11	05-03-22	2.3569	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
12	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
13	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
14	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
15	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
16	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
17	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
18	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
19	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
20	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

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 # 389

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 #: 2147

Chemical Analyses:

Concentration		Analyst	Hours		
			0	24	48
Control, MHSW	pH (S.U.)	EC	7.30	7.30	7.30
	Dissolved oxygen (mg/L)	EC	7.7	7.8	7.7
	Conductivity (µmhos/cm)	EC	813		
	Alkalinity (mg/L CaCO ₃)	EC	58		
	Hardness (mg/L CaCO ₃)	EC	85		
	Temperature (°C)	EC	24.9	25.2	25.1
1750 mg/L	pH (S.U.)	EC	7.47	7.62	7.61
	Dissolved oxygen (mg/L)	EC	7.7	7.7	7.7
	Conductivity (µmhos/cm)	EC	3430		
	Temperature (°C)	EC	25.0	24.9	25.0
2000 mg/L	pH (S.U.)	EC	7.48	7.63	7.63
	Dissolved oxygen (mg/L)	EC	7.7	7.7	7.7
	Conductivity (µmhos/cm)	EC	3930		
	Temperature (°C)	EC	25.0	25.1	25.0
2250 mg/L	pH (S.U.)	EC	7.48	7.64	7.63
	Dissolved oxygen (mg/L)	EC	7.8	7.8	7.7
	Conductivity (µmhos/cm)	EC	41340		
	Temperature (°C)	EC	25.0	25.1	25.2
2500 mg/L	pH (S.U.)	EC	7.49	7.64	7.64
	Dissolved oxygen (mg/L)	EC	7.8	7.8	7.8
	Conductivity (µmhos/cm)	EC	41880		
	Temperature (°C)	EC	25.0	24.8	25.2
2750 mg/L	pH (S.U.)	EC	7.49	7.66	7.66
	Dissolved oxygen (mg/L)	EC	7.9	7.8	7.8
	Conductivity (µmhos/cm)	EC	5330		
	Temperature (°C)	EC	25.0	24.8	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	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

CdNaCIAC # 389

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-08-22	0530	JL	0807	JL	2B4	ORANGE	11-03-23B
24	11-09-22			0800	JL			
48 Termination	11-10-22			0903	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):	11-01-22 D
Age:	< 24-hours old
Date and time organisms were born between:	11-07-22 1453 TO 11-08-22 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.36
	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	S	S	S	S	S	S	S	S	S	S	S	S
48 Termination	S	S	S	S	S	S	S	S	S	S	S	S
Mean Survival	100%				100%				100%			

Hours	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	S	S	S	S	S	4 ^u	4 ^u	S	3 ^{2d}	3 ^{2d}	2 ^{3d}	2 ^{3d}
48 Termination	3 ^{2d}	2 ^{3d}	2 ^{3d}	2 ^{3d}	1 ^{4d}	1 ^{3d}	1 ^{2d}	1 ^{4d}	0 ^{3d}	0 ^{3d}	0 ^{2d}	0 ^{2d}
Mean Survival	45%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT	Comments:
Lower 95% confidence limit (mg NaCl/L)	2212.7	
Upper 95% confidence limit (mg NaCl/L)	2359.1	
48-hour LC ₅₀ (mg NaCl/L)	2286.8	

Test Reviewed by: JL

Acute Daphnid Test-48 Hr Survival

Start Date: 11/8/2022 Test ID: CdNaCIAC Sample ID: REF-Ref Toxicant
 End Date: 11/10/2022 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	0.2000	0.2000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

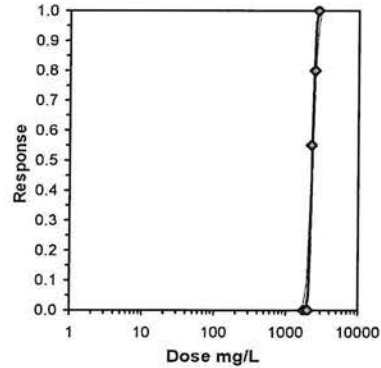
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	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	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	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)	0.5089	0.868	2.79623	11.6732
Equality of variance cannot be confirmed				

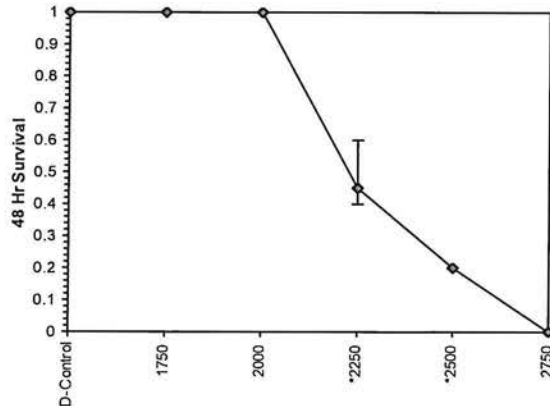
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	30.009	5.43229	19.3617 40.6563	0	3.59118	7.81472	0.30913	3.35923	0.03332	6
Intercept	-95.807	18.2649	-131.61 -60.008							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1912.99	1716.7 2021.08
EC05	3.355	2015.69	1857.45 2105.33
EC10	3.718	2072.67	1935.8 2153.18
EC15	3.964	2112.02	1989.57 2187.1
EC20	4.158	2143.82	2032.58 2215.31
EC25	4.326	2171.49	2069.44 2240.63
EC40	4.747	2242.81	2160.55 2310.84
EC50	5.000	2286.83	2212.65 2359.06
EC60	5.253	2331.72	2261.69 2412.87
EC75	5.674	2408.3	2336.4 2514.95
EC80	5.842	2439.39	2364.18 2559.41
EC85	6.036	2476.12	2395.65 2613.67
EC90	6.282	2523.13	2434.3 2685.27
EC95	6.645	2594.46	2490.51 2797.52
EC99	7.326	2733.73	2595.25 3025.82



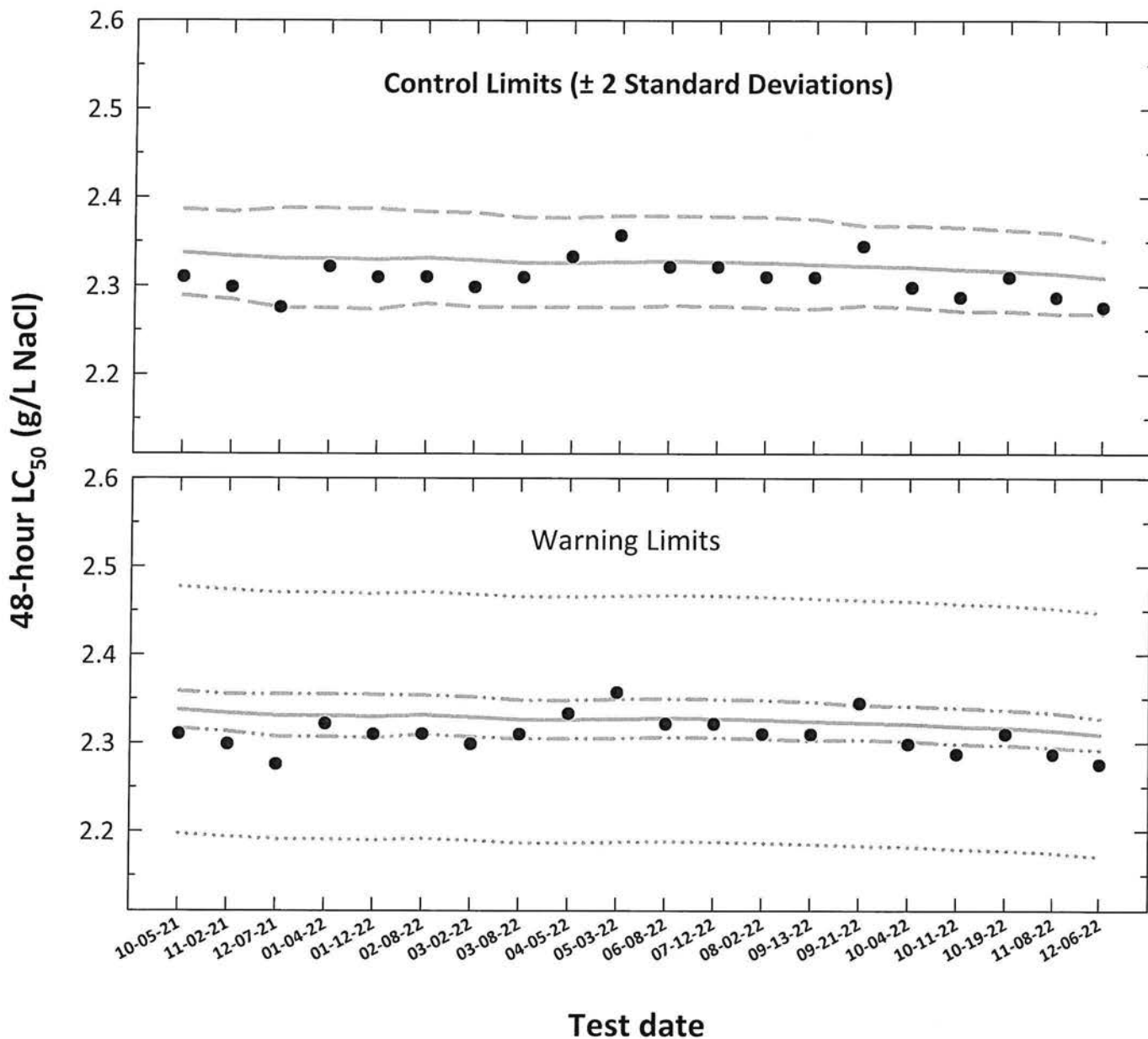
Dose-Response Plot



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 ± S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV		
						CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}		
1	10-05-21	2.3099	0.3636	0.3687	0.0046	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775		
2	11-02-21	2.2985	0.3614	0.3681	0.0046	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738		
3	12-07-21	2.2755	0.3571	0.3675	0.0052	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707		
4	01-04-22	2.3217	0.3658	0.3675	0.0052	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708		
5	01-12-22	2.3096	0.3635	0.3673	0.0053	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695		
6	02-08-22	2.3099	0.3636	0.3676	0.0048	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714		
7	03-02-22	2.2985	0.3614	0.3672	0.0050	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689		
8	03-08-22	2.3096	0.3635	0.3667	0.0047	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658		
9	04-05-22	2.3330	0.3679	0.3667	0.0047	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658		
10	05-03-22	2.3569	0.3723	0.3668	0.0048	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665		
11	06-08-22	2.3212	0.3657	0.3669	0.0047	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675		
12	07-12-22	2.3212	0.3657	0.3668	0.0047	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668		
13	08-02-22	2.3099	0.3636	0.3666	0.0048	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656		
14	09-13-22	2.3096	0.3635	0.3663	0.0048	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637		
15	09-21-22	2.3449	0.3701	0.3660	0.0042	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619		
16	10-04-22	2.2984	0.3614	0.3657	0.0043	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607		
17	10-11-22	2.2870	0.3593	0.3652	0.0044	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576		
18	10-19-22	2.3096	0.3635	0.3649	0.0043	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558		
19	11-08-22	2.2868	0.3592	0.3643	0.0043	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527		
20	12-06-22	2.2755	0.3571	0.3635	0.0039	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478		

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 TestCdNaCIAC # 390Dilution 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 #: 2149Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	7.29	7.58	7.36
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	304		
	Alkalinity (mg/L CaCO ₃)	62		
	Hardness (mg/L CaCO ₃)	84		
	Temperature (°C)	24.9	25.1	25.2
1750 mg/L	pH (S.U.)	7.67	7.77	7.61
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	3550		
	Temperature (°C)	25.0	24.9	24.9
2000 mg/L	pH (S.U.)	7.70	7.78	7.63
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	3930		
	Temperature (°C)	25.0	25.2	24.9
2250 mg/L	pH (S.U.)	7.73	7.78	7.64
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	4290		
	Temperature (°C)	24.9	25.2	25.1
2500 mg/L	pH (S.U.)	7.70	7.79	7.64
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	4740		
	Temperature (°C)	24.9	25.0	25.1
2750 mg/L	pH (S.U.)	7.76	7.80	7.60
	Dissolved oxygen (mg/L)	8.0	7.9	8.0
	Conductivity (µmhos/cm)	5220		
	Temperature (°C)	24.9	25.2	25.0

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	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 TestCdNaClAC # 390Dilution 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 #: 2149Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	7.29	7.58	7.30
	Dissolved oxygen (mg/L)	7.8	7.0	7.0
	Conductivity (µmhos/cm)	304		
	Alkalinity (mg/L CaCO ₃)	62		
	Hardness (mg/L CaCO ₃)	84		
	Temperature (°C)	24.9	25.1	25.2
1750 mg/L	pH (S.U.)	7.07	7.77	7.01
	Dissolved oxygen (mg/L)	7.8	7.0	7.0
	Conductivity (µmhos/cm)	8550		
	Temperature (°C)	25.0	24.9	24.9
2000 mg/L	pH (S.U.)	7.70	7.78	7.03
	Dissolved oxygen (mg/L)	7.9	7.0	7.0
	Conductivity (µmhos/cm)	3930		
	Temperature (°C)	25.0	25.2	24.9
2250 mg/L	pH (S.U.)	7.73	7.78	7.04
	Dissolved oxygen (mg/L)	7.9	7.9	7.0
	Conductivity (µmhos/cm)	4290		
	Temperature (°C)	24.9	25.2	25.1
2500 mg/L	pH (S.U.)	7.70	7.79	7.04
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	4740		
	Temperature (°C)	24.9	25.0	25.1
2750 mg/L	pH (S.U.)	7.70	7.80	7.00
	Dissolved oxygen (mg/L)	8.0	7.9	0.0
	Conductivity (µmhos/cm)	5220		
	Temperature (°C)	24.9	25.2	25.0

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	130664685

Acute Daphnid Test-48 Hr Survival

Start Date: 12/6/2022 Test ID: CdNaCIAC Sample ID: REF-Ref Toxicant
End Date: 12/8/2022 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	0.2000	0.0000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

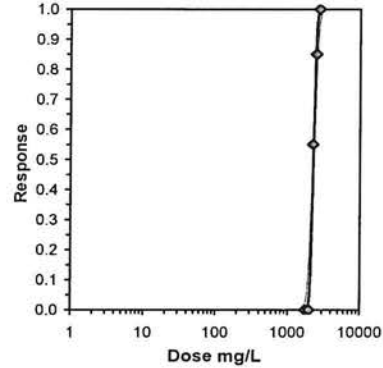
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	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	0.1500	0.1500	0.4041	0.2255	0.4636	29.464	4	10.00	10.00	17	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) Equality of variance cannot be confirmed	0.80565	0.868	-0.4923	4.27708

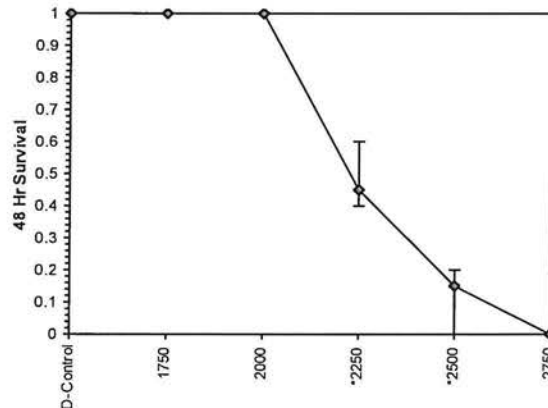
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	32.3602	6.05281	20.4967 44.2237	0	2.60823	7.81472	0.45605	3.35708	0.0309	6
Intercept	-103.64	20.3367	-143.5 -63.776							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1928.38	1735.07	2032.02
EC05	3.355	2024.2	1869.04	2110.02
EC10	3.718	2077.21	1943.32	2154.27
EC15	3.964	2113.75	1994.19	2185.65
EC20	4.158	2143.26	2034.79	2211.76
EC25	4.326	2168.9	2069.53	2235.21
EC40	4.747	2234.88	2155.12	2300.32
EC50	5.000	2275.53	2203.84	2345.14
EC60	5.253	2316.92	2249.53	2395.23
EC75	5.674	2387.4	2318.73	2490.34
EC80	5.842	2415.96	2344.34	2531.76
EC85	6.036	2449.69	2373.27	2582.28
EC90	6.282	2492.79	2408.71	2648.92
EC95	6.645	2558.07	2460.08	2753.24
EC99	7.326	2685.17	2555.38	2964.81



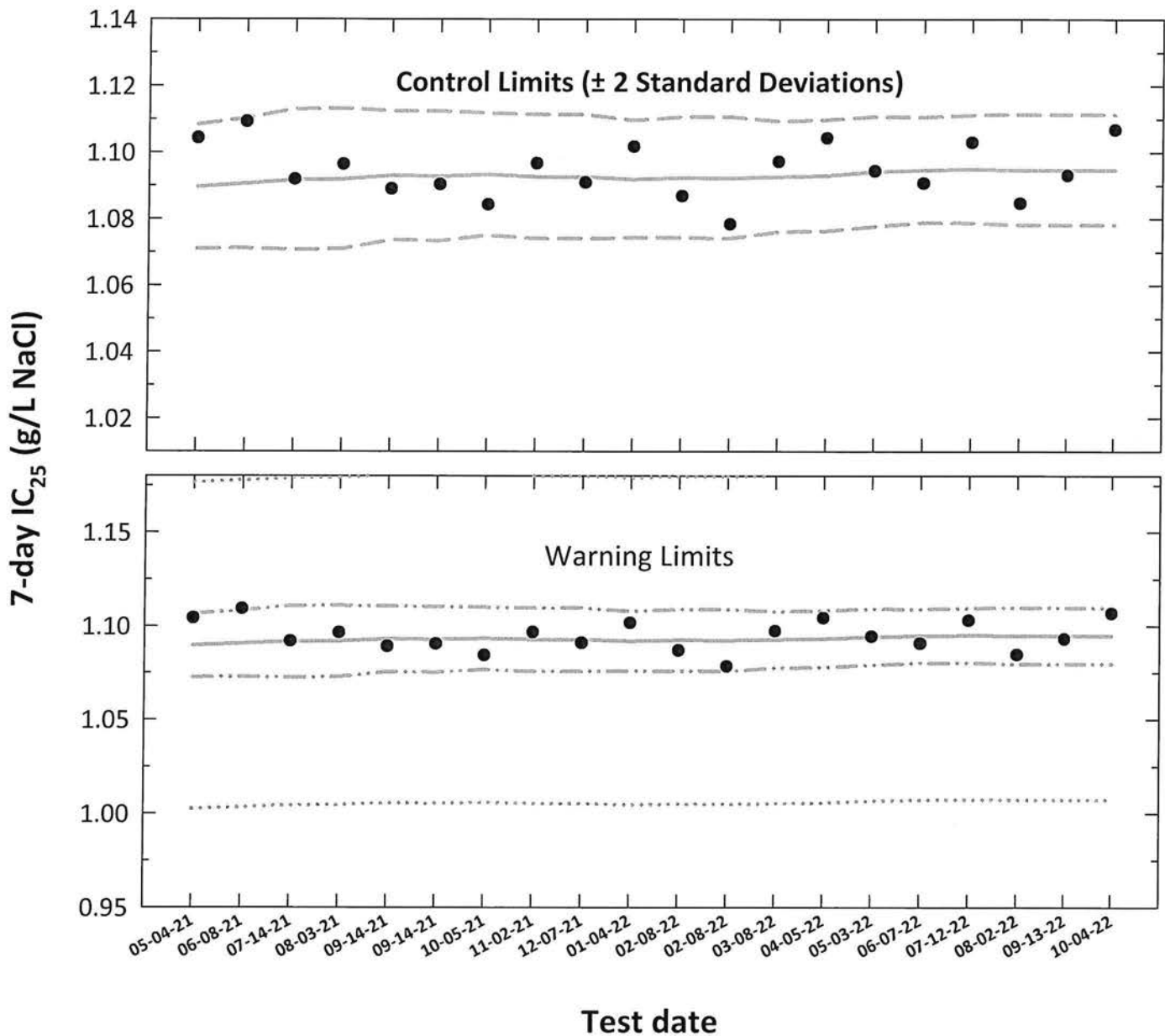
Dose-Response Plot



Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

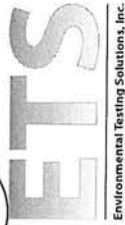
Source: In-house Culture



- **7-day IC₂₅** = 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 IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ ± S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

Independent Review by
Kelley E. Keenan:

Entered and Reviewed by
Jim Sumner



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)			10th Percentile CV				
			7-day IC ₂₅	CT	S	CT	Control Limits		Warning Limits			
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	05-04-21	1.1043	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
2	06-08-21	1.1092	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
3	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
4	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
5	09-14-21	1.0890	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
6	09-14-21	1.0904	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
7	10-05-21	1.0843	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
8	11-02-21	1.0967	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
9	12-07-21	1.0909	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
10	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
11	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
12	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
13	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
14	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
15	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
16	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
17	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
18	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
19	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
20	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823

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

S = Standard deviation of the IC₂₅ values.

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

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

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

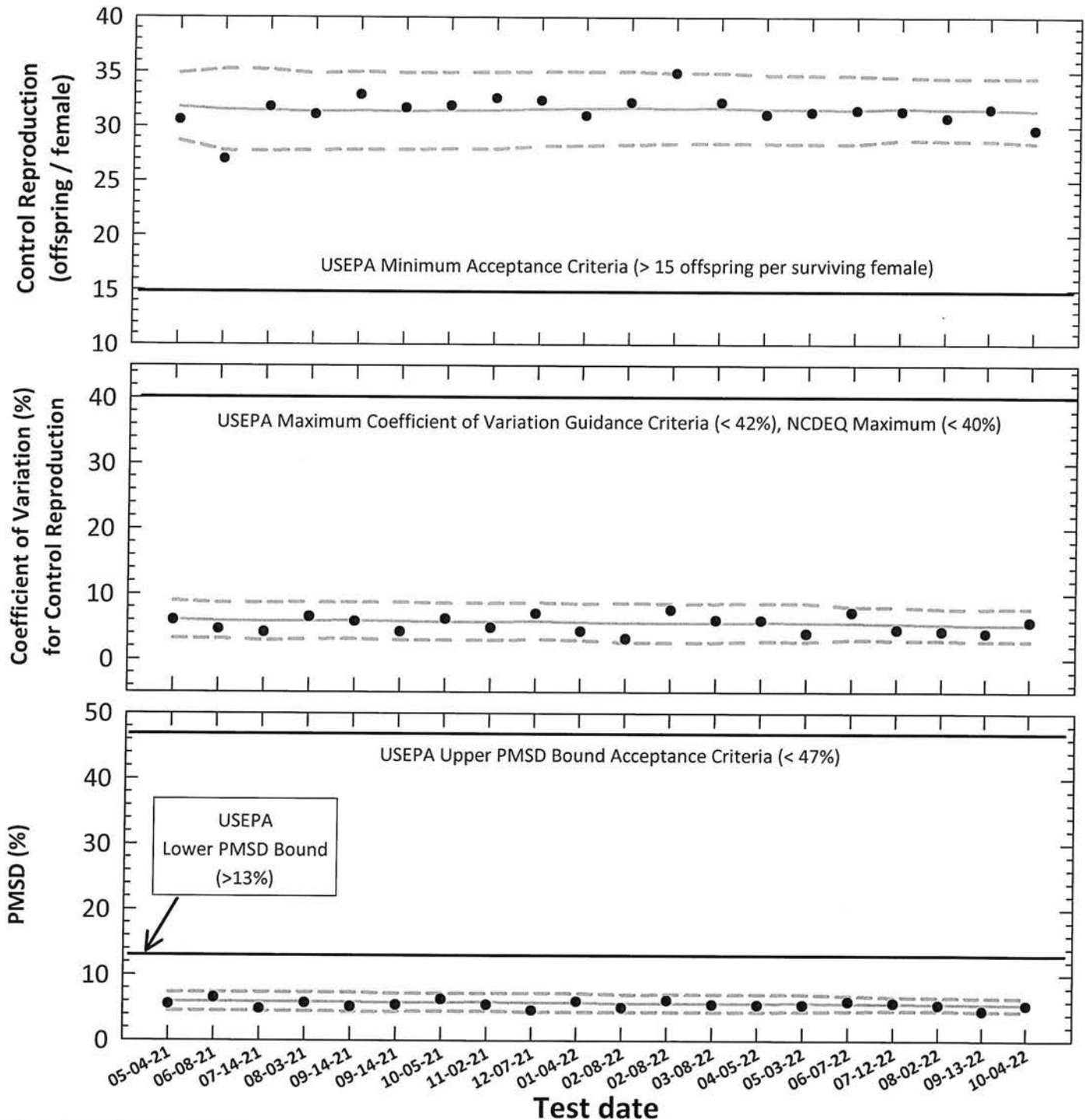
CV = Coefficient of variation.



Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture

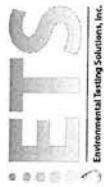


- Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
 PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Central Tendency (mean Control Reproduction, CV or PMSD)

95% Confidence Interval (mean Control Reproduction, CV or PMSD \pm 2 Standard Deviations)





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

Ceriodaphnia dubia

Test number	Test date	ToxCal Determination				Control Reproduction			Control Reproduction CV			Test PMSD (%)			
		Control Survival (%)	Control Reproduction		PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval		
			Mean (offspring/female)	CV (%)			MSD	CT - 2S		CT + 2S	CT - 2S			CT + 2S	
1	05-04-21	100	30.6	6.0	1.690	5.5	31.8	28.7	34.9	6.1	3.2	8.9	5.9	4.5	7.3
2	06-08-21	100	27.0	4.6	1.762	6.5	31.5	27.8	35.2	5.9	3.1	8.7	5.9	4.5	7.4
3	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.9	4.5	7.4
4	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	8.7	5.9	4.5	7.4
5	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	8.7	5.9	4.4	7.3
6	09-14-21	100	31.7	4.2	1.729	5.5	31.4	27.9	34.9	5.8	2.9	8.7	5.8	4.4	7.2
7	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	2.9	8.6	5.8	4.5	7.2
8	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	8.6	5.9	4.5	7.2
9	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	3.0	8.7	5.8	4.4	7.3
10	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	8.5	5.8	4.4	7.2
11	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0
12	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1
13	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1
14	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
15	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
16	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
17	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
18	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
19	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
20	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

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

CV = Coefficient of variation for control reproduction.

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

PMSD = 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 #: 276

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2130</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:

Test randomization and location:

Organism age:	< 24-hours old										Randomizing template color:	<u>Green</u>
Date and times organisms were born between:	<u>10-04-22 0520 to 0800</u>										Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>09-21-22 A</u>											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	<u>7</u>	<u>8</u>	<u>11</u>	<u>13</u>	<u>17</u>	<u>18</u>	<u>23</u>	<u>30</u>	<u>32</u>	<u>36</u>		
Transfer vessel information:	pH (S.U.): <u>7.30</u> Temperature (°C): <u>24.9</u>											
Average transfer volume (mL):	< 0.25 mL											

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches		MHSW batch used	Analyst
			<i>Selenastrum</i>	YWT		
0	10-04-22	<u>0811</u>	<u>10-03-22</u>	<u>09-01-22</u>	<u>09-29-22 A</u>	<u>H</u>
1	10-05-22	<u>0742</u>	↓	↓	↓	<u>H</u>
2	10-06-22	<u>0740</u>	↓	↓	<u>09-29-22 C</u>	<u>H</u>
3	10-07-22	<u>0732</u>	↓	↓	↓	<u>H</u>
4	10-08-22	<u>0833</u>	↓	↓	<u>10-06-22 A</u>	<u>H</u>
5	10-09-22	<u>0832</u>	↓	↓	↓	<u>H</u>
6	10-10-22	<u>0733</u>	↓	↓	↓	<u>H</u>
7	10-11-22	<u>0712</u>	↓	↓	↓	<u>H</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>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>>1400</u>
% Adults having 3 rd Broods:	<u>100%</u>	≥ 80%	NOEC (mg/L NaCl)	<u>1000</u>
% Mortality:	<u>0%</u>	≤ 20%	LOEC (mg/L NaCl)	<u>1200</u>
Mean Offspring/Female:	<u>29.7</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>5.7%</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1106.8</u>



Species: Ceriodaphnia dubia

CdNaClCR #: 276

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	4	3	3	5	4	3	3	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	9	9	11	10	12	10	12	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	16	15	15	18	15	16	13	14	16
Total young produced		30	29	27	29	33	31	29	28	31	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	29.7

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	5	3	4	4	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	10	9	12	10	11	11	12	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	15	18	17	17	14	15	13	16	16
Total young produced		32	32	33	29	33	28	30	27	31	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:	30.6
% Reduction from Control:	-3.07.



Species: *Ceriodaphnia dubia*
800 mg NaCl/L

CdNaClCR #: 276

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	5	4	5	3	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	9	12	11	10	12	10	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	15	15	17	12	17	16	13	13	14	13
Total young produced		31	29	31	28	33	29	29	27	29	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:	29.4
% Reduction from Control:	1.07.

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	3	5	3	3	4	4	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	9	10	10	10	10	10	11	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	15	13	16	15	13	16	12	14	11
Total young produced		27	29	26	29	29	27	30	27	28	27
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:	27.9
% Reduction from Control:	6.17.



Species: Ceriodaphnia dubia

CdNaClCR #: 276

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	2	4	4	3	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	10	5	9	7	6	11	9	9	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	3	10	4	9	7	3	9	7	8
Total young produced		17	16	19	17	18	17	18	21	19	18
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	18.0
% Reduction from Control:	34.7.

1400 mg NaCl/L

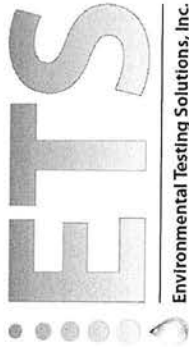
Survival and Reproduction Data

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	3	1	1	2	2	3	1	1	3
	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	1	0	0	0	0	0
Total young produced		2	3	1	1	3	2	3	1	1	3
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:	93.37.





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

Environmental Testing Solutions, Inc.

Quality Control

Verification of Data Entry, Calculations, and Statistical Analyses

Test number: **CdNaClCR #275**
 Test dates: **October 04-11, 2022**

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	29	27	29	33	31	29	28	31	30	100	29.7	5.7	Not applicable
600	32	32	33	29	33	28	30	27	31	31	100	30.6	6.8	-3.0
800	31	29	31	28	33	29	29	27	29	28	100	29.4	6.0	1.0
1000	27	29	26	29	29	27	30	27	28	27	100	27.9	4.6	6.1
1200	17	16	19	17	18	17	18	21	19	18	100	18.0	7.9	39.4
1400	2	3	1	1	3	2	3	1	1	3	100	2.0	47.1	93.3

Dunnnett's MSD value: 1.610
 PMSD: 5.4

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) = 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: 10/4/2022 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
 End Date: 10/11/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia

Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	29.000	27.000	29.000	33.000	31.000	29.000	28.000	31.000	30.000
600	32.000	32.000	33.000	29.000	33.000	28.000	30.000	27.000	31.000	31.000
800	31.000	29.000	31.000	28.000	33.000	29.000	29.000	27.000	29.000	28.000
1000	27.000	29.000	26.000	29.000	29.000	27.000	30.000	27.000	28.000	27.000
1200	17.000	16.000	19.000	17.000	18.000	17.000	18.000	21.000	19.000	18.000
1400	2.000	3.000	1.000	1.000	3.000	2.000	3.000	1.000	1.000	3.000

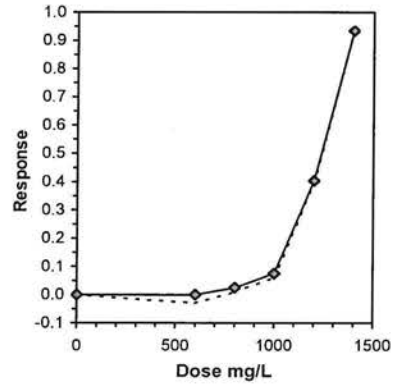
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	29.700	1.0000	29.700	27.000	33.000	5.734	10				30.150	1.0000	
600	30.600	1.0303	30.600	27.000	33.000	6.750	10	-1.278	2.287	1.610	30.150	1.0000	
800	29.400	0.9899	29.400	27.000	33.000	6.042	10	0.426	2.287	1.610	29.400	0.9751	
*1000	27.900	0.9394	27.900	26.000	30.000	4.612	10	2.557	2.287	1.610	27.900	0.9254	
*1200	18.000	0.6061	18.000	16.000	21.000	7.857	10	16.620	2.287	1.610	18.000	0.5970	
*1400	2.000	0.0673	2.000	1.000	3.000	47.140	10	39.349	2.287	1.610	2.000	0.0663	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.75844	1.035	0.18606	-0.0484						
Bartlett's Test indicates equal variances (p = 0.30)	6.03874	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		1.60972	0.0542	1267.19	2.47778	3.6E-44	5, 54

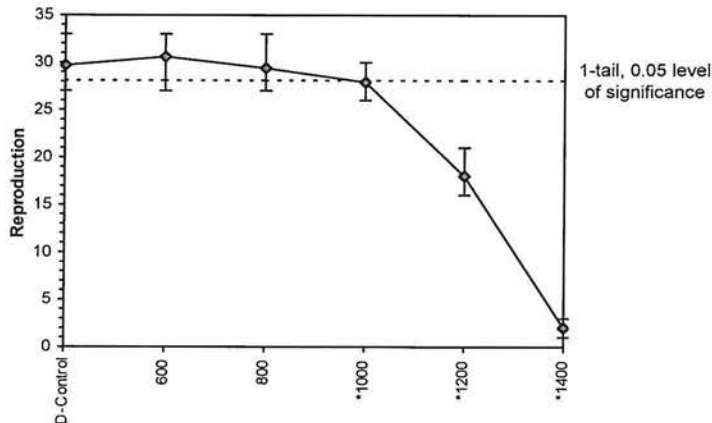
Treatments vs D-Control

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	901	66.0296	761.495	1007.54	-0.3354
IC10	1015.45	13.0363	974.548	1038.03	-1.1346
IC15	1045.91	9.30013	1026.7	1065.81	0.2392
IC20	1076.36	8.6995	1059.15	1093.99	0.3917
IC25	1106.82	8.4691	1091.72	1123.86	0.5606
IC40	1198.18	7.65544	1183.46	1211.27	0.0726
IC50	1236.56	4.99994	1227.92	1246.03	0.4829

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 Jim Sumner

Species: Ceriodaphnia dubia

CdNaClCR #: 276

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.

Concentration		Parameter	Day					
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
			0		1		2	
Analyst		N	EC N	EC N	N	N	EC N	
CONTROL, MHSW	pH (S.U.)	7.57	7.12	7.18	7.40	7.50	7.14	
	Dissolved oxygen (mg/L)	7.6	7.0	7.7	7.9	7.0	7.6	
	Conductivity (µmhos/cm)	300		310		302		
	Alkalinity (mg CaCO ₃ /L)	60				60		
	Hardness (mg CaCO ₃ /L)	85				89		
	Temperature (°C)	24.8	25.1	24.8	24.9	24.7	25.2	
					10 ^{-2.2}			
600 mg NaCl/L	pH (S.U.)	7.68	7.50	7.58	7.62	7.73	7.32	
	Dissolved oxygen (mg/L)	7.5	7.0	7.0	7.0	7.0	7.6	
	Conductivity (µmhos/cm)	1400		1420		1380		
	Temperature (°C)	24.9	25.3	24.9	25.2	24.7	25.0	
800 mg NaCl/L	pH (S.U.)	7.70	7.55	7.60	7.62	7.74	7.35	
	Dissolved oxygen (mg/L)	7.5	7.7	7.0	7.0	7.9	7.6	
	Conductivity (µmhos/cm)	1750		1780		1740		
	Temperature (°C)	24.9	25.2	24.9	25.0	24.8	25.0	
1000 mg NaCl/L	pH (S.U.)	7.71	7.55	7.60	7.63	7.76	7.37	
	Dissolved oxygen (mg/L)	7.4	7.0	7.0	7.7	7.0	7.6	
	Conductivity (µmhos/cm)	2100		2120		2090		
	Temperature (°C)	25.0	25.0	24.8	25.0	24.8	24.9	
1200 mg NaCl/L	pH (S.U.)	7.71	7.57	7.61	7.63	7.77	7.39	
	Dissolved oxygen (mg/L)	7.7	7.0	7.0	7.0	7.0	7.7	
	Conductivity (µmhos/cm)	2440		2470		2440		
	Temperature (°C)	25.0	25.0	24.8	24.9	24.8	24.9	
1400 mg NaCl/L	pH (S.U.)	7.72	7.57	7.62	7.63	7.79	7.41	
	Dissolved oxygen (mg/L)	7.7	7.0	7.0	7.0	7.0	7.0	
	Conductivity (µmhos/cm)	2020		2810		2800		
	Temperature (°C)	25.0	25.1	24.8	24.9	24.9	25.1	
		Initial	Final	Initial	Final	Initial	Final	



Species: Ceriodaphnia dubia

CdNaClCR #: 276

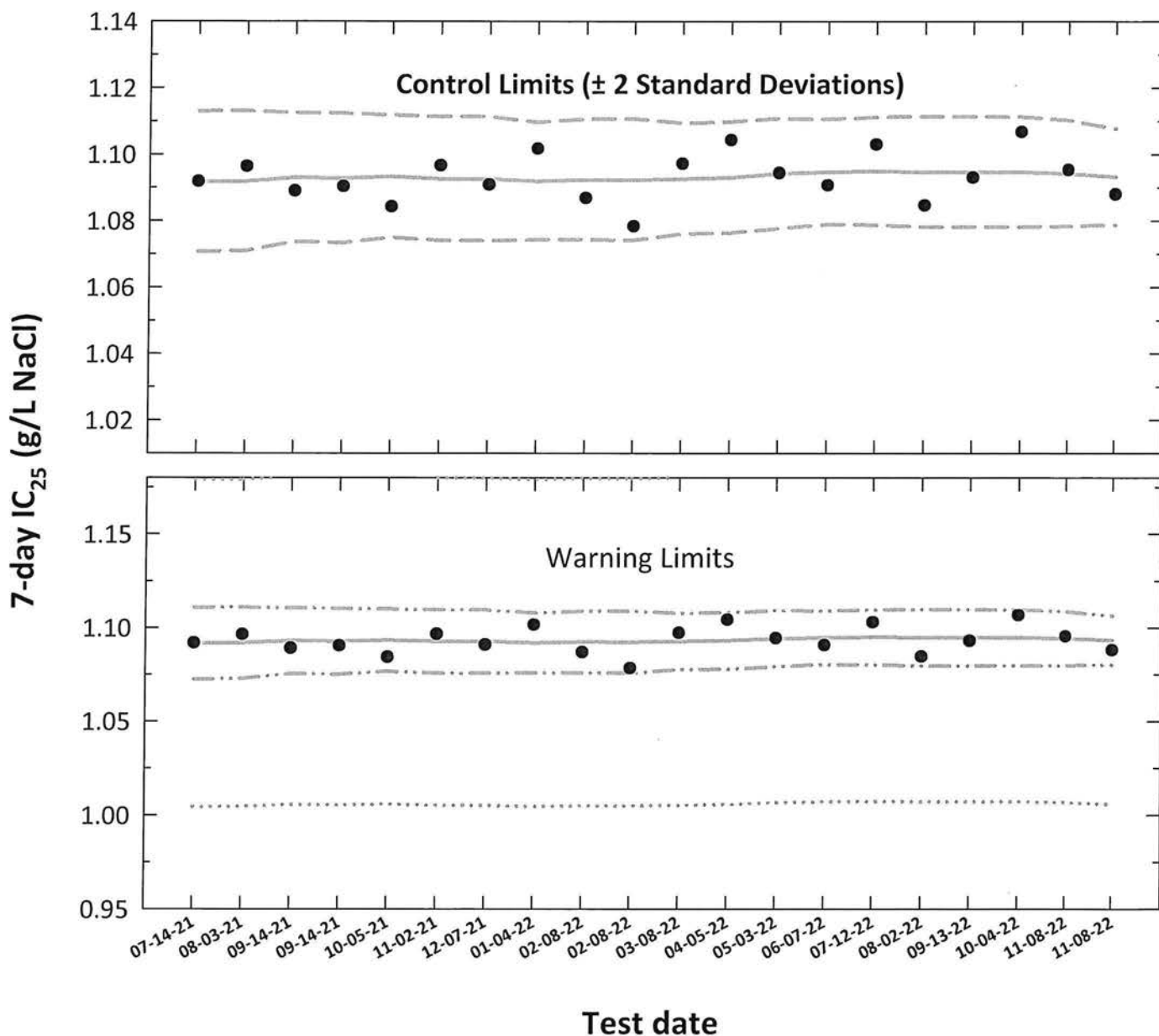
Concentration		Analyst		Day								
				(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)								
				3		4		5		6		
Parameter	EC	N	BSC	BSC	N	N	N	N	N			
CONTROL, MHSW	pH (S.U.)	7.33		7.45	7.64	7.71	7.61	7.50	7.61	7.64		
	Dissolved oxygen (mg/L)	7.6		7.5	7.7	7.6	7.7	7.6	7.7	7.9		
	Conductivity (µmhos/cm)	314			307		302			317		
	Alkalinity (mg CaCO ₃ /L)				61							
	Hardness (mg CaCO ₃ /L)				87							
	Temperature (°C)	24.8		25.2	24.8	25.2	24.9	25.2	24.8	25.3		
600 mg NaCl/L	pH (S.U.)	7.29		7.61	7.84	7.43	7.45	7.61	7.72	7.71		
	Dissolved oxygen (mg/L)	7.6		7.4	7.5	7.6	7.7	7.6	7.7	7.9		
	Conductivity (µmhos/cm)	1470			1440		1480		1460			
	Temperature (°C)	24.9		25.0	24.7	25.2	24.9	25.0	24.9	25.0		
800 mg NaCl/L	pH (S.U.)	7.34		7.64	7.61	7.43	7.46	7.65	7.72	7.69		
	Dissolved oxygen (mg/L)	7.6		7.4	7.5	7.7	7.7	7.5	7.6	7.9		
	Conductivity (µmhos/cm)	1780			1750		1800		1820			
	Temperature (°C)	24.9		25.0	24.7	25.0	25.0	25.0	24.9	25.2		
1000 mg NaCl/L	pH (S.U.)	7.38		7.64	7.62	7.45	7.50	7.65	7.74	7.69		
	Dissolved oxygen (mg/L)	7.6		7.3	7.5	7.7	7.6	7.6	7.6	8.0		
	Conductivity (µmhos/cm)	2120			2040		2170		2140			
	Temperature (°C)	25.0		25.2	24.7	25.3	24.9	25.0	24.9	25.2		
1200 mg NaCl/L	pH (S.U.)	7.41		7.64	7.62	7.51	7.51	7.65	7.75	7.69		
	Dissolved oxygen (mg/L)	7.6		7.4	7.5	7.8	7.7	7.6	7.7	8.0		
	Conductivity (µmhos/cm)	2470			2430		2510		2470			
	Temperature (°C)	24.8		25.2	24.8	25.0	25.0	25.1	24.9	25.2		
1400 mg NaCl/L	pH (S.U.)	7.44		7.64	7.63	7.57	7.54	7.67	7.76	7.68		
	Dissolved oxygen (mg/L)	7.6		7.5	7.6	7.8	7.7	7.7	7.6	8.0		
	Conductivity (µmhos/cm)	2860			2790		2850		2830			
	Temperature (°C)	24.8		24.9	24.8	25.3	25.0	25.1	24.9	25.3		
		Initial		Final		Initial		Final		Initial		Final



Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- 7-day IC₂₅ = 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 IC₂₅ converted to anti-logarithmic values)
- - - Control Limits (mean logarithmic IC₂₅ \pm 2 standard deviations converted to anti-logarithmic values)
- . . . Laboratory Warning Limits (mean logarithmic IC₂₅ \pm 2 coefficient of variations converted to anti-logarithmic values)
- USEPA Warning Limits (mean logarithmic IC₂₅ \pm S_{A.10} converted to anti-logarithmic values, S_{A.10} = 10th 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			
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	10th Percentile CV Warning Limits CT + S _{A,10}
1	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
2	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
3	09-14-21	1.0890	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
4	09-14-21	1.0904	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
5	10-05-21	1.0843	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
6	11-02-21	1.0967	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
7	12-07-21	1.0909	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
8	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
9	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
10	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
11	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
12	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
13	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
14	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
15	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
16	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
17	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
18	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
19	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
20	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807

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.

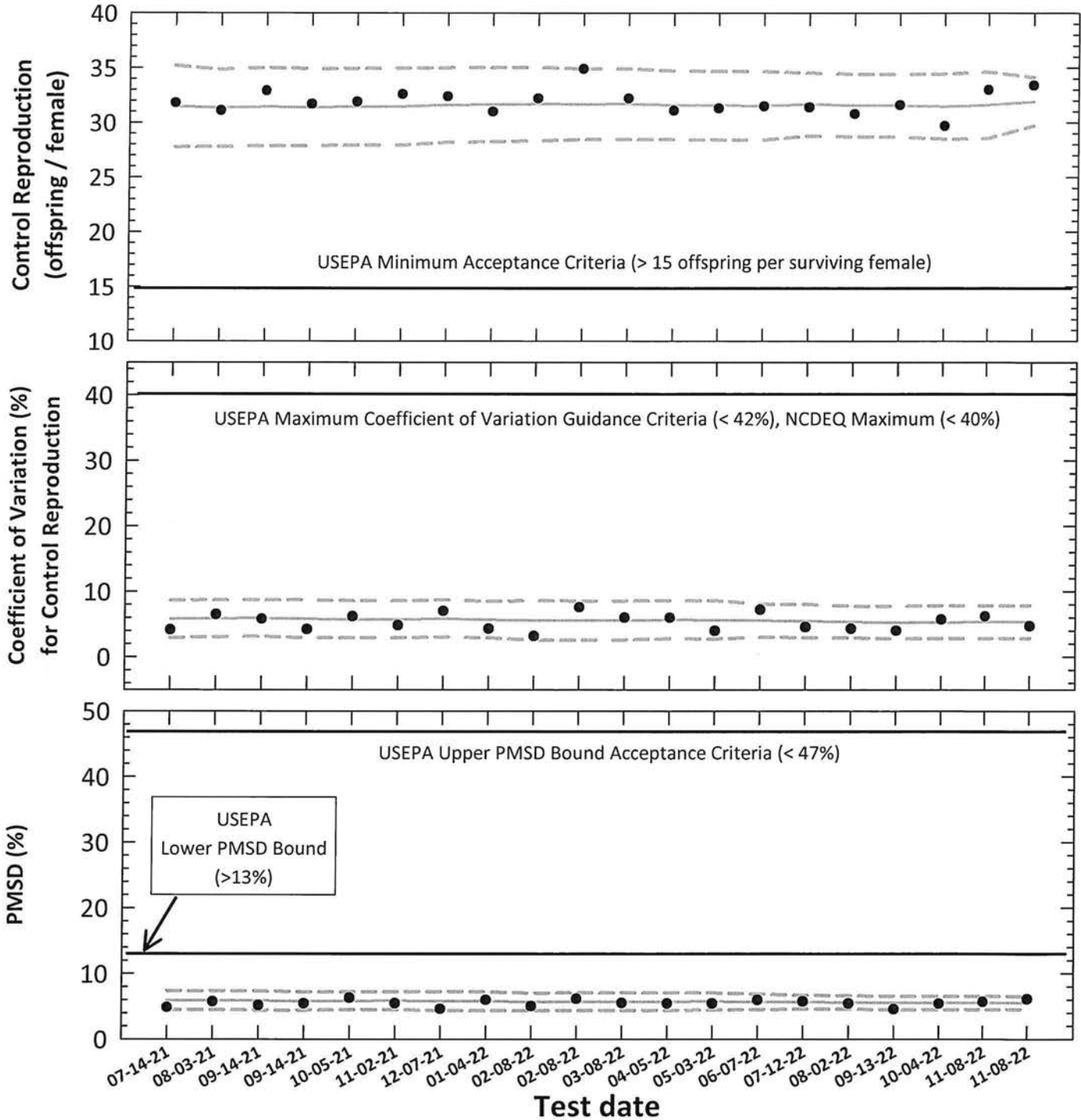
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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)

Entered and Reviewed by
 Jim Sumner

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		CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT + 2S				
			Mean (offspring/female)	CV (%)								MSD	PMSD (%)		
1	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.9	4.5	7.4
2	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	8.7	5.9	4.5	7.4
3	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	8.7	5.9	4.4	7.3
4	09-14-21	100	31.7	4.2	1.729	5.5	31.4	27.9	34.9	5.8	2.9	8.7	5.8	4.4	7.2
5	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	2.9	8.6	5.8	4.5	7.2
6	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	8.6	5.9	4.5	7.2
7	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	3.0	8.7	5.8	4.4	7.3
8	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	8.5	5.8	4.4	7.2
9	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0
10	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1
11	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1
12	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.1
13	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
14	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
15	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
16	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
17	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
18	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6
19	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6
20	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

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.

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

PMSD = Minimum significant difference.

PMMSD = Percent minimum significant difference.

CT = 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%.

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

CV = Standard deviation 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 #: 278 - New YWT

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS <u>2147</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>11-08-22 0530 TO 0810</u>									
Culture board:	<u>11-01-22 A</u>									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>11</u>	<u>12</u>	<u>18</u>	<u>19</u>	<u>22</u>	<u>23</u>
Transfer vessel information:	pH (S.U.): <u>7.36</u> Temperature (°C): <u>25.1</u>									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	<u>BLACK</u>
Incubator number and shelf location:	<u>2B2</u>

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches		MHSW batch used	Analyst
			<i>Selenastrum</i>	YWT		
0	11-08-22	<u>0834</u>	<u>10-27-22</u>	<u>11-05-22</u>	<u>11-05-22 B</u>	<u>JL</u>
1	11-09-22	<u>0802</u>	↓	↓	↓	<u>JL</u>
2	11-10-22	<u>0806</u>	↓	↓	<u>11-05-22 C</u>	<u>JL</u>
3	11-11-22	<u>0805</u>	↓	↓	↓	<u>JL</u>
4	11-12-22	<u>0912</u>	↓	↓	<u>11-10-22 A</u>	<u>JL</u>
5	11-13-22	<u>0902</u>	↓	↓	↓	<u>JL</u>
6	11-14-22	<u>0900</u>	↓	↓	↓	<u>JL</u>
7	11-15-22	<u>0756</u>				<u>JL</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>>1400</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>33.4</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>4.77.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1088.1</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 278 - New YWT

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	4	6	6	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	11	14	12	13	12	13	13	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	15	19	18	18	15	14	16	13	15	17
Total young produced		34	33	37	34	34	32	33	31	33	33
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: 33.4

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	3	6	4	6	5	5	5	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	14	12	14	10	12	12	13	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	15	17	19	16	18	16	18	18	17
Total young produced		35	32	35	37	32	35	33	36	33	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality: 07.

Mean Offspring/Female: 34.3

% Reduction from Control: -2.77.

Species: Ceriodaphnia dubia
800 mg NaCl/L

CdNaClCR #: 278 - New YWT

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	4	4	4	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	14	10	12	11	12	13	14
	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	15	16	18	17	17	17	13	19	15
Total young produced		33	29	31	36	31	33	33	30	37	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:	32.7
% Reduction from Control:	2.17.

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	5	4	5	5	3	5	6	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	13	10	10	11	10	13	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	15	14	19	16	14	14	14	17	16
Total young produced		29	32	32	34	29	30	30	31	33	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:	31.2
% Reduction from Control:	6.67.

Species: *Ceriodaphnia dubia*
1200 mg NaCl/L

CdNaClCR #: 278 - New YWT

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	3	4	4	5	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	11	8	7	12	10	9	9	8	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	5	8	4	7	3	6	5	7	4
Total young produced		17	19	19	15	23	18	19	18	19	13
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	0%
Mean Offspring/Female:	18.0
% Reduction from Control:	46.1%

1400 mg NaCl/L

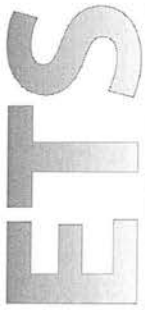
Survival and Reproduction Data

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	3	1	4	1	1	3	3	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	3	0	0	2	0	0	0	0	0	0
Total young produced		6	2	3	3	4	1	1	3	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:	0%
Mean Offspring/Female:	2.8
% Reduction from Control:	91.6%



Environmental Testing Solutions, Inc.

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	4	6	6	4	5	5	5	48
5	14	11	14	12	13	12	13	13	13	11	126
6	0	0	0	0	0	0	0	0	0	0	0
7	15	19	18	18	15	14	16	13	15	17	160
Total	34	33	37	34	34	32	33	31	33	33	334

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	3	6	4	6	5	5	5	3	5	47
5	13	14	12	14	10	12	12	13	12	13	125
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	17	19	16	18	16	18	18	17	171
Total	35	32	35	37	32	35	33	36	33	35	343

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	5	4	4	4	5	5	5	5	46
5	12	10	10	14	10	12	11	12	13	14	118
6	0	0	0	0	0	0	0	0	0	0	0
7	16	15	16	18	17	17	17	13	19	15	163
Total	33	29	31	36	31	33	33	30	37	34	327

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	5	3	5	6	4	4	6	47
5	10	13	13	10	10	11	10	13	12	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	14	15	14	14	19	16	14	14	17	16	153
Total	29	32	32	34	29	30	30	31	33	32	312

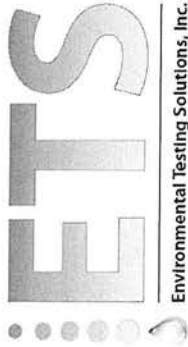
1200 mg NaCl/L

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	3	4	4	5	4	4	4	4	39
5	8	11	8	7	12	10	9	9	8	5	87
6	0	0	0	0	0	0	0	0	0	0	0
7	5	5	8	4	7	3	6	5	7	4	54
Total	17	19	19	15	23	18	19	18	19	13	180

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	3	1	4	1	1	3	3	2	23
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	3	0	0	2	0	0	0	0	0	0	5
Total	6	2	3	3	4	1	1	3	3	2	28

Checked and
approved by
Jim Sumner



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

Environmental Testing Solutions, Inc.

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

Test number: CdNaClCR #278, New YWT
 Test dates: November 08-15, 2022

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	34	33	37	34	34	32	33	31	33	33	100	33.4	4.7	Not applicable
600	35	32	35	37	32	35	33	36	33	33	100	34.3	5.0	-2.7
800	33	29	31	36	31	33	30	30	37	34	100	32.7	7.8	2.1
1000	29	32	32	34	29	30	31	31	33	32	100	31.2	5.4	6.6
1200	17	19	19	15	23	18	19	18	19	13	100	18.0	14.8	46.1
1400	6	2	3	3	4	1	3	3	3	2	100	2.8	52.7	91.6

Dunnett's MSD value: 2.044

PMSD: 6.1

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:	11/8/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2022	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:	New YWT				

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	33.000	37.000	34.000	34.000	32.000	33.000	31.000	33.000	33.000
600	35.000	32.000	35.000	37.000	32.000	35.000	33.000	36.000	33.000	35.000
800	33.000	29.000	31.000	36.000	31.000	33.000	33.000	30.000	37.000	34.000
1000	29.000	32.000	32.000	34.000	29.000	30.000	30.000	31.000	33.000	32.000
1200	17.000	19.000	19.000	15.000	23.000	18.000	19.000	18.000	19.000	13.000
1400	6.000	2.000	3.000	3.000	4.000	1.000	1.000	3.000	3.000	2.000

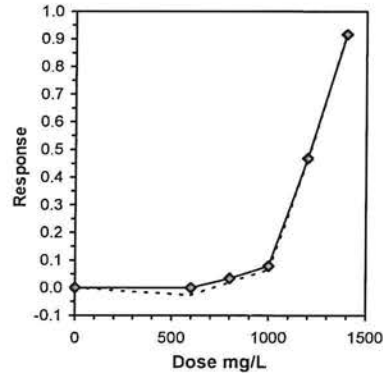
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	33.400	1.0000	33.400	31.000	37.000	4.723	10				33.850	1.0000
600	34.300	1.0269	34.300	32.000	37.000	4.965	10	-1.007	2.287	2.044	33.850	1.0000
800	32.700	0.9790	32.700	29.000	37.000	7.770	10	0.783	2.287	2.044	32.700	0.9660
*1000	31.200	0.9341	31.200	29.000	34.000	5.406	10	2.461	2.287	2.044	31.200	0.9217
*1200	18.000	0.5389	18.000	13.000	23.000	14.815	10	17.226	2.287	2.044	18.000	0.5318
*1400	2.800	0.0838	2.800	1.000	6.000	52.705	10	34.228	2.287	2.044	2.800	0.0827

Auxiliary Tests				Statistic	Critical	Skew	Kurt			
Kolmogorov D Test indicates normal distribution (p > 0.01)				0.91949	1.035	0.16964	0.54284			
Bartlett's Test indicates equal variances (p = 0.31)				5.913	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		2.04431	0.06121	1591.32	3.9963	2.6E-41	5, 54

Treatments vs D-Control

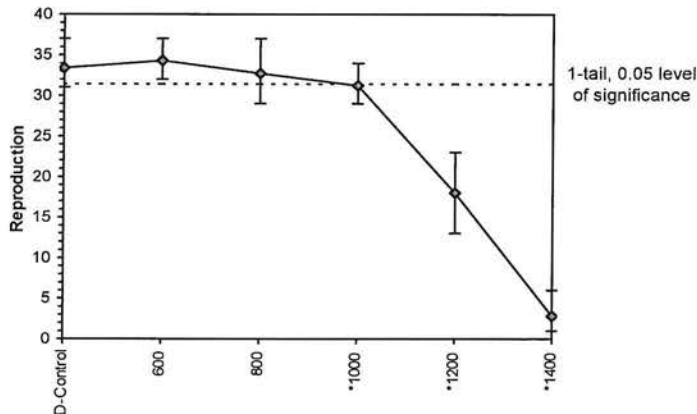
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	872.333	74.2252	718.931	1000.18	-0.1335
IC10	1011.14	24.4715	941.067	1024.37	-4.5140
IC15	1036.78	8.78252	1016.47	1050.16	-0.4348
IC20	1062.42	8.50302	1044.9	1076.26	-0.2855
IC25	1088.07	8.58158	1071.26	1103.38	-0.1187
IC40	1165	10.7103	1146.61	1183.26	0.1018
IC50	1214.14	9.94958	1192.93	1228.94	-0.3282



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 Jim Sumner
JK

Species: Ceriodaphnia dubia

CdNaClCR #: 278 - New YWT

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.)	J	ZC N	SC N	ZC	ZC	W
	Dissolved oxygen (mg/L)	7.43	7.22	7.25	7.08	7.13	7.33
	Conductivity (µmhos/cm)	7.7	7.0	7.6	7.9	7.8	7.9
	Alkalinity (mg CaCO ₃ /L)	313		312		317	
	Hardness (mg CaCO ₃ /L)	58				59	
	Temperature (°C)	85				85	
	Temperature (°C)	24.9	25.0	24.8	24.9	24.7	25.1
600 mg NaCl/L	pH (S.U.)	7.47	7.43	7.60	7.38	7.36	7.50
	Dissolved oxygen (mg/L)	7.7	7.0	7.7	7.9	7.8	7.9
	Conductivity (µmhos/cm)	1480		1400		1400	
	Temperature (°C)	24.9	25.1	24.9	25.0	24.8	24.9
800 mg NaCl/L	pH (S.U.)	7.48	7.47	7.57	7.44	7.44	7.51
	Dissolved oxygen (mg/L)	7.7	7.0	7.7	7.9	7.8	8.0
	Conductivity (µmhos/cm)	1820		1780		1790	
	Temperature (°C)	24.9	25.1	24.9	25.0	24.8	24.9
1000 mg NaCl/L	pH (S.U.)	7.50	7.50	7.56	7.48	7.50	7.52
	Dissolved oxygen (mg/L)	7.8	7.9	7.7	7.9	7.8	8.0
	Conductivity (µmhos/cm)	2190		2140		2160	
	Temperature (°C)	25.0	24.9	24.8	24.8	24.8	25.2
1200 mg NaCl/L	pH (S.U.)	7.50	7.53	7.58	7.52	7.52	7.53
	Dissolved oxygen (mg/L)	7.8	7.9	7.0	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2550		2520		2530	
	Temperature (°C)	25.0	24.9	24.8	24.8	24.7	25.2
1400 mg NaCl/L	pH (S.U.)	7.53	7.56	7.58	7.54	7.55	7.53
	Dissolved oxygen (mg/L)	7.9	7.9	7.0	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2950		2860		2890	
	Temperature (°C)	24.9	25.2	24.8	25.1	24.7	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

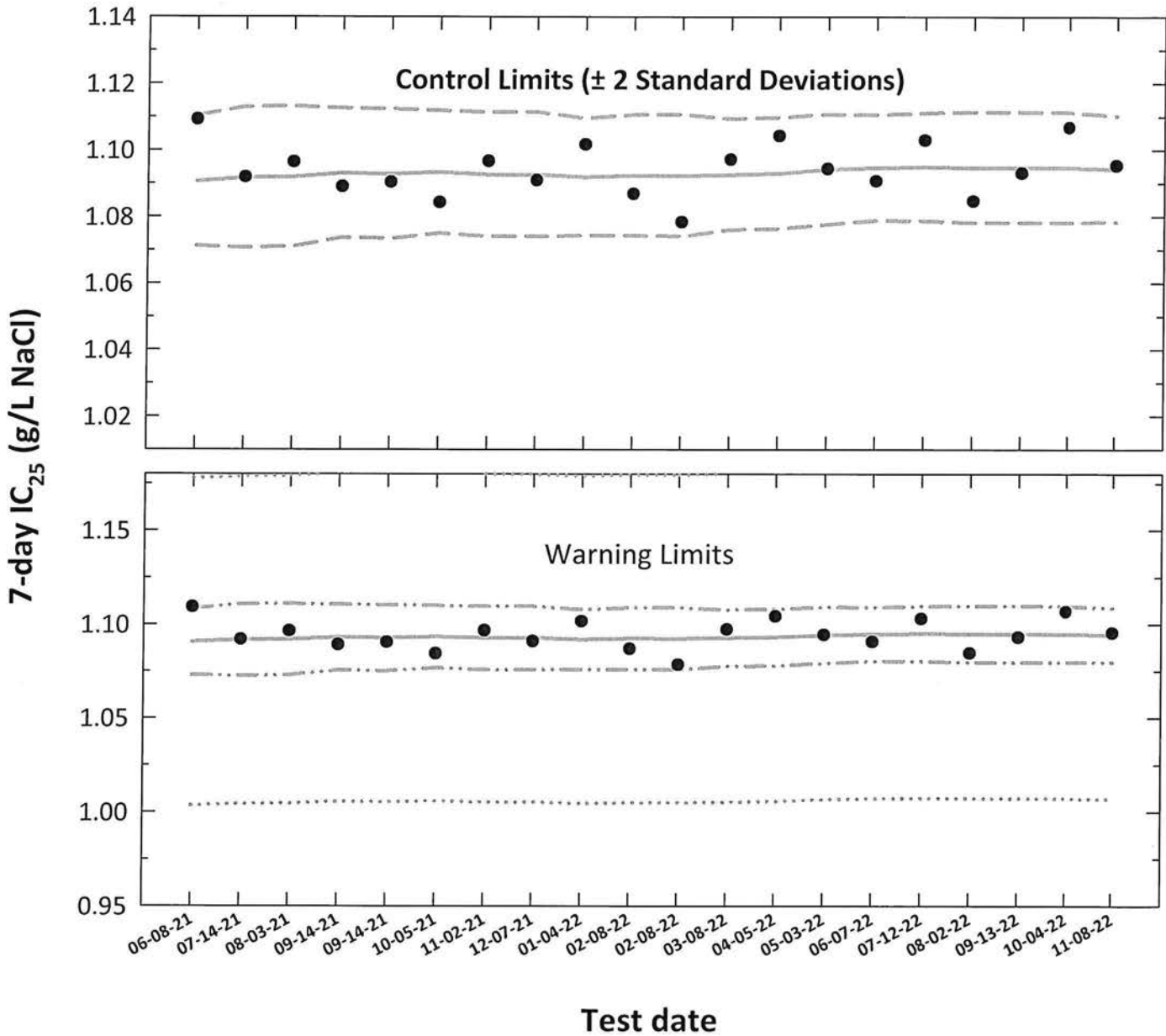
CdNaClCR #: 278 - New YWT

Concentration		Parameter	Day								
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)								
			3		4		5		6		
Analyst		U	BSL	BSL	U	U	EC U	EC U	EC		
CONTROL, MHSW	pH (S.U.)	7.43	7.38	7.15	7.40	7.44	7.40	7.10	7.57		
	Dissolved oxygen (mg/L)	7.9	7.7	7.7	7.8	7.6	7.6	7.6	7.9		
	Conductivity (µmhos/cm)	307		300		313		804			
	Alkalinity (mg CaCO ₃ /L)			58							
	Hardness (mg CaCO ₃ /L)			83							
	Temperature (°C)	24.9	25.2	24.9	25.1	24.9	24.9	24.9	25.2		
600 mg NaCl/L	pH (S.U.)	7.60	7.62	7.56	7.59	7.90	7.55	7.57	7.09		
	Dissolved oxygen (mg/L)	8.0	7.7	7.6	7.8	7.7	7.6	7.7	7.9		
	Conductivity (µmhos/cm)	1400		1370		1420		1380			
	Temperature (°C)	24.8	25.0	24.8	24.9	25.0	25.1	25.0	25.0		
800 mg NaCl/L	pH (S.U.)	7.69	7.67	7.65	7.61	7.61	7.57	7.60	7.68		
	Dissolved oxygen (mg/L)	8.0	7.7	7.7	7.8	7.7	7.6	7.6	7.9		
	Conductivity (µmhos/cm)	1790		1730		1790		1790			
	Temperature (°C)	24.8	25.0	24.8	24.9	25.0	25.0	25.0	25.0		
1000 mg NaCl/L	pH (S.U.)	7.77	7.70	7.70	7.61	7.67	7.58	7.62	7.69		
	Dissolved oxygen (mg/L)	8.0	7.7	7.7	7.9	7.7	7.6	7.7	7.8		
	Conductivity (µmhos/cm)	2120		2080		2150		2130			
	Temperature (°C)	24.8	24.9	24.9	25.0	25.0	25.2	24.9	24.9		
1200 mg NaCl/L	pH (S.U.)	7.73	7.72	7.75	7.65	7.70	7.60	7.65	7.70		
	Dissolved oxygen (mg/L)	8.0	7.7	7.7	8.0	7.8	7.7	7.6	7.8		
	Conductivity (µmhos/cm)	2520		2460		2540		2480			
	Temperature (°C)	24.9	24.9	24.9	24.8	25.0	25.2	24.9	24.9		
1400 mg NaCl/L	pH (S.U.)	7.75	7.75	7.77	7.45	7.73	7.61	7.67	7.72		
	Dissolved oxygen (mg/L)	8.0	7.7	7.8	8.1	7.9	7.8	7.6	7.8		
	Conductivity (µmhos/cm)	2840		2760		2800		2880			
	Temperature (°C)	24.9	24.9	24.9	24.8	25.0	25.0	24.9	25.1		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final		

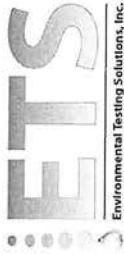
Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC₂₅** = 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 IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ \pm 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic IC₂₅ \pm 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ \pm S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th 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	06-08-21	1.1092	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
2	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
3	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
4	09-14-21	1.0890	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
5	09-14-21	1.0904	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
6	10-05-21	1.0843	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
7	11-02-21	1.0967	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
8	12-07-21	1.0909	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
9	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
10	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
11	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
12	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
13	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
14	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
15	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
16	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
17	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
18	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
19	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
20	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818

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.

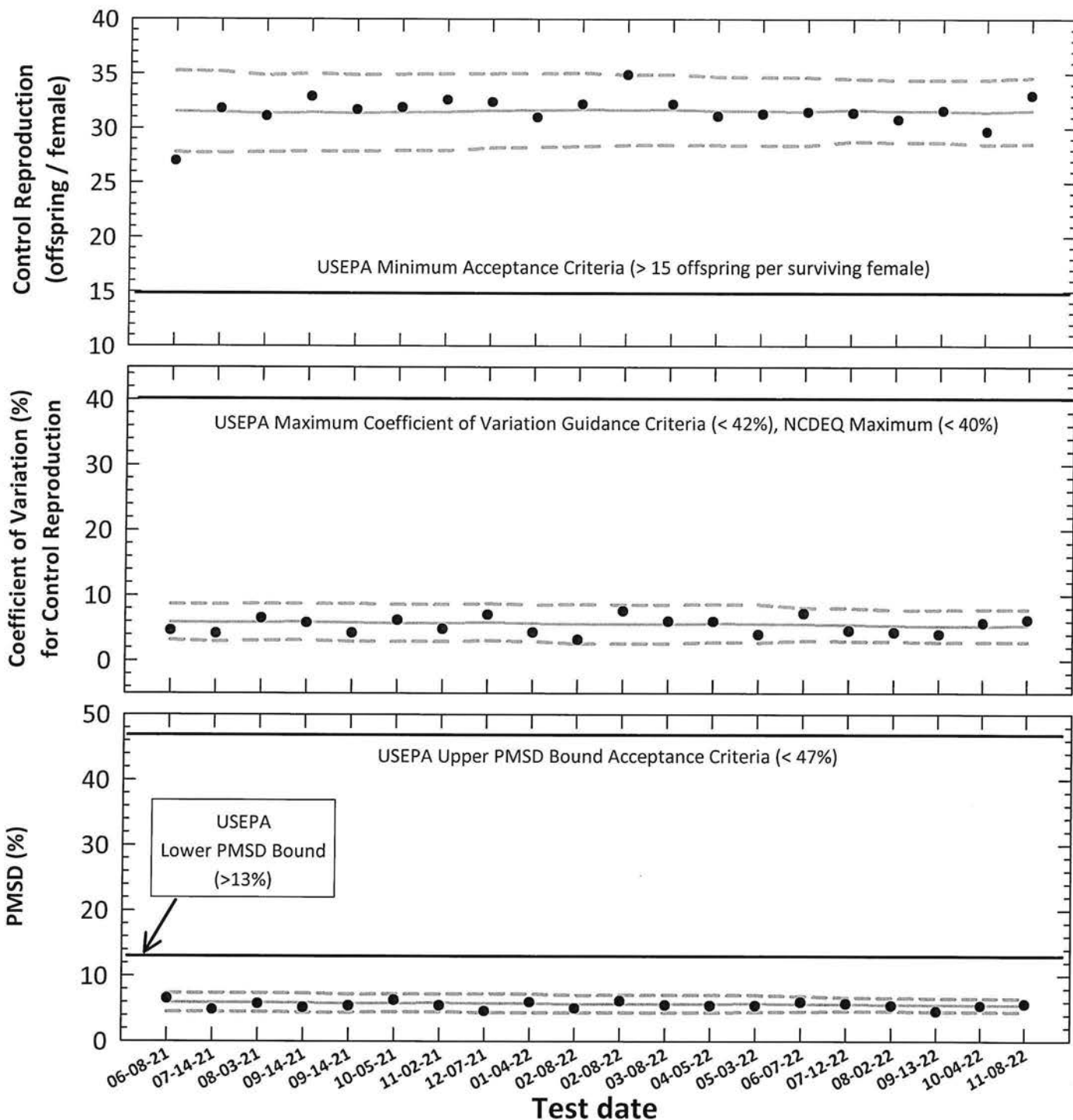
S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

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)

Entered and Reviewed by
 Jim Sumner

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

Test number	Test date	ToxCal Determination				Control Reproduction		Test		Control Reproduction (offspring/female)			Control Reproduction CV (%)		Test PMSD (%)	
		Control Survival (%)	Control Reproduction (offspring/female)		CV (%)	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	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean	CV												
1	06-08-21	100	27.0	4.6	1.762	6.5	31.5	27.8	35.2	5.9	3.1	8.7	5.9	4.5	7.4	
2	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.9	4.5	7.4	
3	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	8.7	5.9	4.5	7.4	
4	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	8.7	5.9	4.4	7.3	
5	09-14-21	100	31.7	4.2	1.729	5.5	31.4	27.9	34.9	5.8	2.9	8.7	5.8	4.4	7.2	
6	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	2.9	8.6	5.8	4.5	7.2	
7	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	8.6	5.9	4.5	7.2	
8	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	3.0	8.7	5.8	4.4	7.3	
9	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	8.5	5.8	4.4	7.2	
10	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0	
11	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1	
12	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1	
13	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.6	2.6	8.6	5.7	4.4	7.1	
14	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0	
15	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9	
16	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7	
17	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6	
18	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6	
19	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6	
20	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6	

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 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 #: 277

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS <u>2147</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>11-08-22 0530 to 0810</u>									
Culture board:	<u>11-01-22 A</u>									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>11</u>	<u>12</u>	<u>18</u>	<u>19</u>	<u>22</u>	<u>23</u>
Transfer vessel information:	pH (S.U.): <u>7.36</u> Temperature (°C): <u>25.1</u>									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	<u>Bwe</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		MHSW batch used	Analyst
			<i>Selenastrum</i>	YWT		
0	11-08-22	<u>0824</u>	<u>10-27-22</u>	<u>10-27-22</u>	<u>11-03-22B</u>	<u>J</u>
1	11-09-22	<u>0750</u>	↓	↓	↓	<u>J</u>
2	11-10-22	<u>0755</u>	↓	↓	<u>11-03-22C</u>	<u>J</u>
3	11-11-22	<u>0754</u>	↓	↓	↓	<u>J</u>
4	11-12-22	<u>0901</u>	↓	↓	<u>11-10-22 A</u>	<u>J</u>
5	11-13-22	<u>0851</u>	↓	↓	↓	<u>J</u>
6	11-14-22	<u>0850</u>	↓	↓	↓	<u>J</u>
7	11-15-22	<u>0741</u>	↓	↓	↓	<u>J</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>>1400</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>33.0</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>1095.4</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 277

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	4	5	5	5	3	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	12	14	12	11	12	12	12	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	15	15	17	18	14	19	14	17	18	16
Total young produced		30	32	34	37	31	33	31	34	34	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:	33.6

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	5	4	6	4	4	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	14	12	10	12	11	11	13	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	17	18	18	15	19	15	19	16	14
Total young produced		35	34	37	34	31	35	30	35	34	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:	33.6
% Reduction from Control:	-1.87.

Species: Ceriodaphnia dubia

CdNaClCR #: 277

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	6	4	5	5	5	4	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	10	12	13	13	12	12	12	14
	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	17	17	18	14	18	16	15	16
Total young produced		30	31	31	34	36	32	34	33	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:	32.6
% Reduction from Control:	1.27.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	5	5	3	5	3	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	12	10	13	10	10	12	12	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	13	14	16	16	14	15	15	15	17
Total young produced		33	27	31	31	32	29	28	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:	30.7
% Reduction from Control:	7.07.

Species: Ceriodaphnia dubia

CdNaClCR #: 277

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	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	8	10	9	11	10	7	10	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	3	5	5	6	3	7	8	8	5	4
Total young produced		18	16	18	19	18	21	19	21	19	18
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	16.7
% Reduction from Control:	43.37.

1400 mg NaCl/L

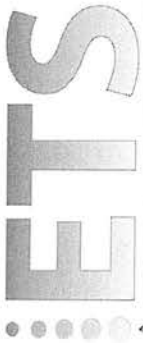
Survival and Reproduction Data

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	3	2	2	1	2	1	1	1	3
	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	0	0
Total young produced		2	3	2	2	1	2	1	1	1	3
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:	1.8
% Reduction from Control:	94.57.



Environmental Testing Solutions, Inc.

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	4	5	5	5	3	5	5	4	5	45
5	11	13	12	14	12	11	12	12	12	13	122
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	17	18	14	19	14	17	18	16	163
Total	30	32	34	37	31	33	31	34	34	34	330

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	5	3	5	3	4	4	4	42
5	10	10	12	10	13	10	10	12	12	13	112
6	0	0	0	0	0	0	0	0	0	0	0
7	18	13	14	16	16	14	15	15	15	17	153
Total	33	27	31	31	32	29	28	31	31	34	307

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	5	4	6	4	4	5	5	4	47
5	12	12	14	12	10	12	11	11	13	13	120
6	0	0	0	0	0	0	0	0	0	0	0
7	18	17	18	18	15	19	15	19	16	14	169
Total	35	34	37	34	31	35	30	35	34	31	336

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	4	3	3	4	37
5	10	8	10	9	11	10	7	10	11	10	96
6	0	0	0	0	0	0	0	0	0	0	0
7	3	5	5	6	3	7	8	8	5	4	54
Total	18	16	18	19	18	21	19	21	19	18	187

800 mg NaCl/L

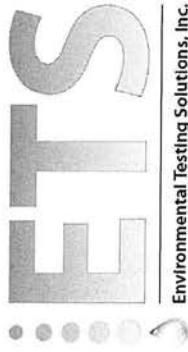
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	5	5	5	4	5	4	4	46
5	11	12	10	12	13	13	12	12	12	14	121
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	17	17	18	14	18	16	15	16	159
Total	30	31	31	34	36	32	34	33	31	34	326

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

Reviewed and
Approved By
Jim Sumner

JS



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

Quality Control

Verification of Data Entry, Calculations, and Statistical Analyses

Test number: **CdNaClCR #277**
 Test dates: **November 08-15, 2022**

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	32	34	37	31	33	31	34	34	34	100	33.0	6.2	Not applicable
600	35	34	37	34	31	35	30	35	34	31	100	33.6	6.6	-1.8
800	30	31	31	34	36	32	34	33	31	34	100	32.6	5.8	1.2
1000	33	27	31	31	32	29	28	31	31	34	100	30.7	7.0	7.0
1200	18	16	18	19	18	21	19	21	19	18	100	18.7	8.0	43.3
1400	2	3	2	2	1	2	1	1	1	3	100	1.8	43.8	94.5

Dunnett's MSD value: **1.880**
 PMSD: **5.7**

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:	11/8/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

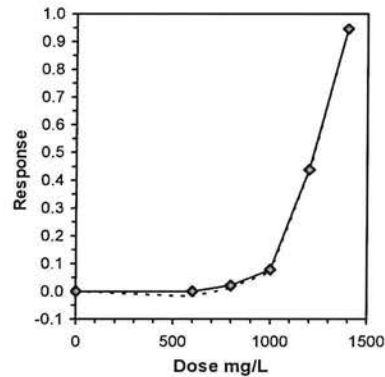
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	32.000	34.000	37.000	31.000	33.000	31.000	34.000	34.000	34.000
600	35.000	34.000	37.000	34.000	31.000	35.000	30.000	35.000	34.000	31.000
800	30.000	31.000	31.000	34.000	36.000	32.000	34.000	33.000	31.000	34.000
1000	33.000	27.000	31.000	31.000	32.000	29.000	28.000	31.000	31.000	34.000
1200	18.000	16.000	18.000	19.000	18.000	21.000	19.000	21.000	19.000	18.000
1400	2.000	3.000	2.000	2.000	1.000	2.000	1.000	1.000	1.000	3.000

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	33.000	1.0000	33.000	30.000	37.000	6.227	10				33.300	1.0000	
600	33.600	1.0182	33.600	30.000	37.000	6.610	10	-0.730	2.287	1.880	33.300	1.0000	
800	32.600	0.9879	32.600	30.000	36.000	5.820	10	0.486	2.287	1.880	32.600	0.9790	
*1000	30.700	0.9303	30.700	27.000	34.000	7.045	10	2.797	2.287	1.880	30.700	0.9219	
*1200	18.700	0.5667	18.700	16.000	21.000	7.992	10	17.389	2.287	1.880	18.700	0.5616	
*1400	1.800	0.0545	1.800	1.000	3.000	43.823	10	37.939	2.287	1.880	1.800	0.0541	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)	1.00888	1.035	-0.0406	-0.2134						
Bartlett's Test indicates equal variances (p = 0.09)	9.66863	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		1.88049	0.05698	1612.23	3.38148	2.3E-43	5, 54

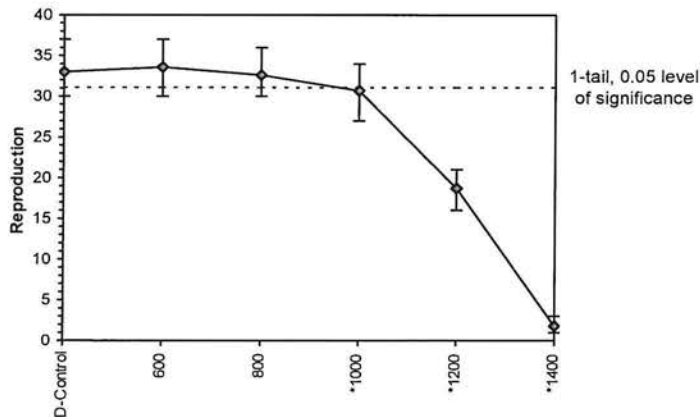
Treatments vs D-Control

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	901.579	71.4643	718.206	1007.49	-0.4581
IC10	1012.17	25.0827	935.258	1032.58	-2.3237
IC15	1039.92	11.2246	1012.93	1057.71	-0.4772
IC20	1067.67	9.84383	1045.77	1083.41	-0.3662
IC25	1095.42	8.70425	1076.87	1108.81	-0.2210
IC40	1178.67	7.751	1163.54	1191.1	0.1358
IC50	1224.26	5.22789	1213.68	1232.19	-0.0500



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
Jim Sumner

Species: Ceriodaphnia dubia

CdNaCICR #: 277

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	
		EC	EC μ	EC μ	EC	EC	μ
CONTROL, MHSW	pH (S.U.)	7.43	7.24	7.25	7.00	7.13	7.37
	Dissolved oxygen (mg/L)	7.7	7.0	7.8	7.9	7.8	8.0
	Conductivity (μ mhos/cm)	1100	312	312		317	
	Alkalinity (mg CaCO ₃ /L)	58				59	
	Hardness (mg CaCO ₃ /L)	85				85	
	Temperature (°C)	25.0	25.2	24.8	24.9	24.7	25.3
600 mg NaCl/L	pH (S.U.)	7.47	7.40	7.00	7.30	7.30	7.50
	Dissolved oxygen (mg/L)	7.7	7.0	7.1	7.9	7.8	8.0
	Conductivity (μ mhos/cm)	1480		1460		1400	
	Temperature (°C)	24.9	24.8	24.8	24.4	24.7	25.1
800 mg NaCl/L	pH (S.U.)	7.48	7.43	7.57	7.44	7.44	7.51
	Dissolved oxygen (mg/L)	7.7	7.0	7.1	7.9	7.8	8.0
	Conductivity (μ mhos/cm)	1820		1780		1790	
	Temperature (°C)	24.9	24.8	24.8	25.0	24.8	25.1
1000 mg NaCl/L	pH (S.U.)	7.50	7.40	7.50	7.48	7.50	7.52
	Dissolved oxygen (mg/L)	7.8	7.0	7.1	7.9	7.8	8.0
	Conductivity (μ mhos/cm)	2190		2140		2160	
	Temperature (°C)	24.9	24.9	24.9	24.8	24.8	24.9
1200 mg NaCl/L	pH (S.U.)	7.50	7.48	7.58	7.52	7.52	7.52
	Dissolved oxygen (mg/L)	7.8	7.0	7.0	7.9	7.9	8.0
	Conductivity (μ mhos/cm)	2550		2520		2530	
	Temperature (°C)	25.0	24.9	24.9	25.0	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.53	7.51	7.58	7.55	7.55	7.53
	Dissolved oxygen (mg/L)	7.9	7.0	7.0	7.9	7.9	8.0
	Conductivity (μ mhos/cm)	2950		2860		2890	
	Temperature (°C)	25.0	25.1	25.0	25.0	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

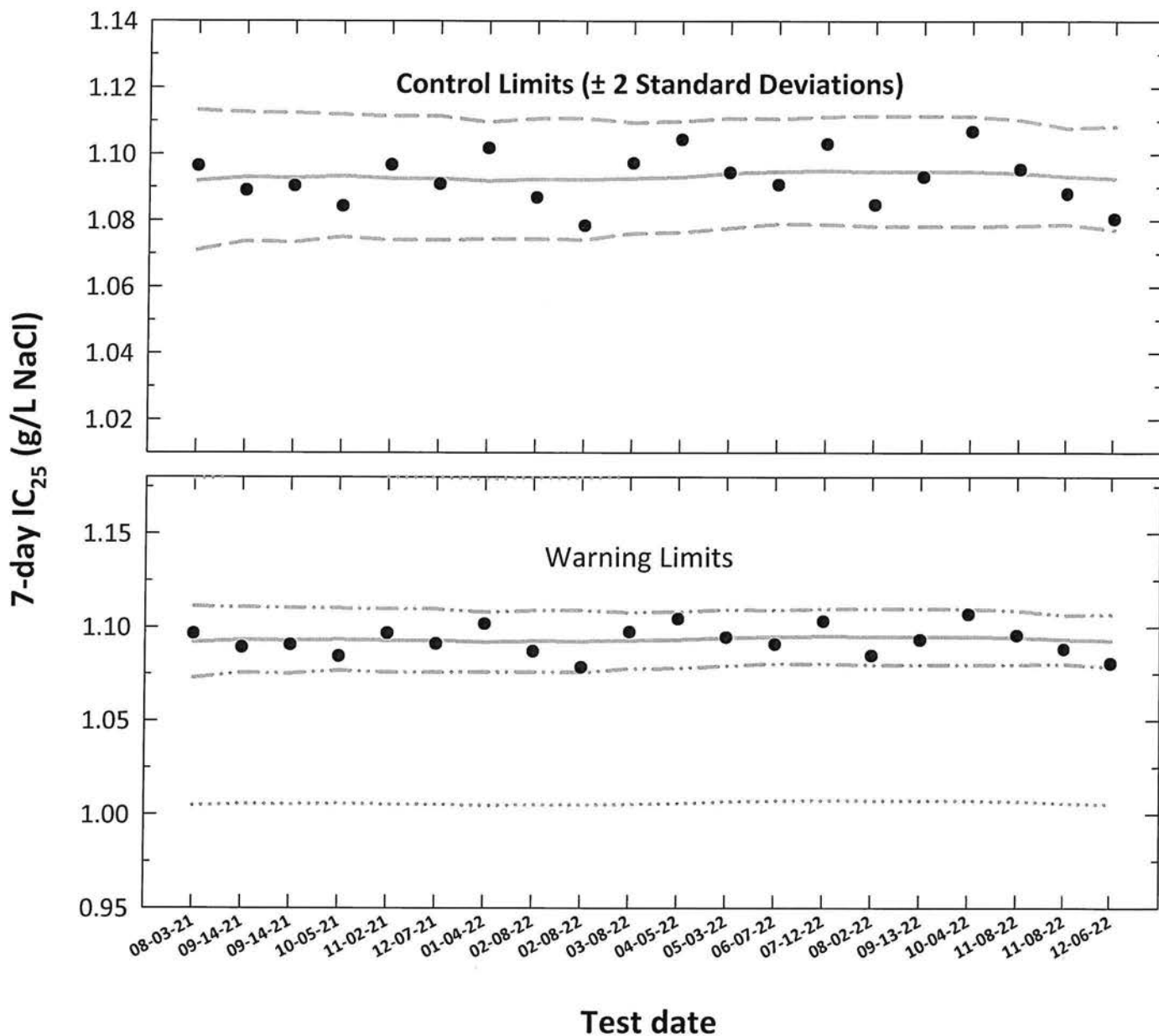
CdNaCICR #: 277

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		W	BSL	BSL	N	N	EC N	EC N	EC
Concentration	Parameter	Analyst							
CONTROL, MHSW	pH (S.U.)	7.43	7.37	7.15	7.42	7.44	7.39	7.10	7.50
	Dissolved oxygen (mg/L)	7.9	7.5	7.7	7.3	7.6	7.7	7.6	7.9
	Conductivity (µmhos/cm)	307		300		313		304	
	Alkalinity (mg CaCO ₃ /L)			58					
	Hardness (mg CaCO ₃ /L)			83					
	Temperature (°C)	24.8	25.2	24.7	25.0	24.9	25.2	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.66	7.63	7.56	7.59	7.56	7.57	7.57	7.60
	Dissolved oxygen (mg/L)	8.0	7.5	7.6	7.7	7.7	7.6	7.7	7.9
	Conductivity (µmhos/cm)	1400		1370		1420		1380	
	Temperature (°C)	24.9	25.0	24.9	24.9	25.0	25.0	24.8	24.9
800 mg NaCl/L	pH (S.U.)	7.69	7.67	7.65	7.66	7.61	7.59	7.60	7.69
	Dissolved oxygen (mg/L)	8.0	7.6	7.7	7.8	7.7	7.6	7.6	7.9
	Conductivity (µmhos/cm)	1790		1730		1790		1790	
	Temperature (°C)	24.9	25.0	24.9	24.9	25.0	25.0	24.9	25.1
1000 mg NaCl/L	pH (S.U.)	7.71	7.71	7.70	7.63	7.67	7.59	7.62	7.69
	Dissolved oxygen (mg/L)	8.0	7.6	7.7	7.9	7.7	7.6	7.7	7.8
	Conductivity (µmhos/cm)	2120		2080		2150		2130	
	Temperature (°C)	24.9	25.1	24.8	25.1	25.1	24.9	24.9	25.1
1200 mg NaCl/L	pH (S.U.)	7.73	7.74	7.75	7.64	7.70	7.60	7.65	7.69
	Dissolved oxygen (mg/L)	8.0	7.6	24.605	8.0	7.8	7.6	7.6	7.8
	Conductivity (µmhos/cm)	2520		7.7L 1022		2540		2480	
	Temperature (°C)	25.0	25.1	24.9	25.1	24.9	24.9	24.9	24.8
1400 mg NaCl/L	pH (S.U.)	7.75	7.76	7.77	7.67	7.73	7.62	7.67	7.72
	Dissolved oxygen (mg/L)	8.0	7.6	7.8	8.0	7.9	7.6	7.6	7.9
	Conductivity (µmhos/cm)	2840		2760		2860		2880	
	Temperature (°C)	25.0	25.2	24.9	25.1	24.9	24.9	24.9	24.7
		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		S	CT	Anti-logarithmic Values (g/L NaCl)					
			7-day IC ₂₅	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	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
2	09-14-21	1.0890	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
3	09-14-21	1.0904	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
4	10-05-21	1.0843	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
5	11-02-21	1.0967	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
6	12-07-21	1.0909	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
7	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
8	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
9	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
10	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
11	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
12	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
13	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
14	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
15	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
16	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
17	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
18	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
19	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
20	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

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.

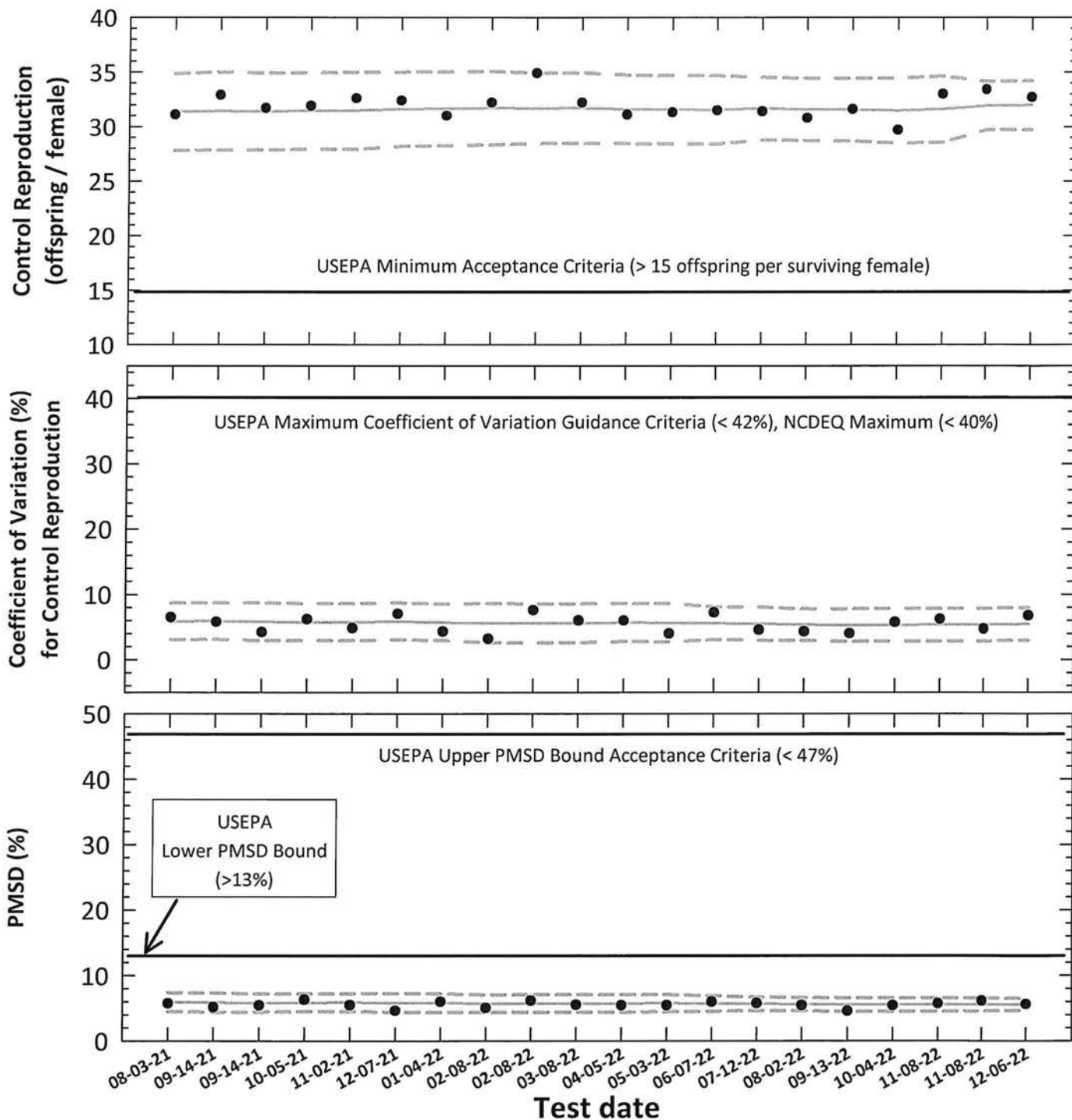
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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 ± 2 Standard Deviations)

Entered and Reviewed by
Jim Sumner

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

Ceriodaphnia dubia

Test number	Test date	ToxCal Determination				Control Reproduction		Test		Control Reproduction (offspring/female)			Control Reproduction CV		Test PMSD (%)	
		Control Survival (%)	Control Reproduction		PMSD (%)	MSD	CV (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (offspring/female)	CV (%)												
1	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	31.3	27.8	34.8	5.9	31.1	34.8
2	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	31.4	27.9	35.0	5.9	31.1	35.0
3	09-14-21	100	31.7	4.2	1.729	5.5	31.4	27.9	34.9	5.8	31.4	27.9	34.9	5.8	2.9	34.9
4	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	31.5	27.9	35.0	5.8	2.9	35.0
5	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	31.5	27.9	35.0	5.9	2.9	35.0
6	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	31.6	28.2	35.0	5.8	3.0	35.0
7	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	31.6	28.3	35.0	5.8	2.9	35.0
8	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	31.7	28.3	35.1	5.7	2.6	35.1
9	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	31.7	28.5	34.9	5.7	2.6	34.9
10	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	31.7	28.5	34.9	5.7	2.6	34.9
11	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	31.6	28.5	34.7	5.7	2.8	34.7
12	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	31.6	28.4	34.7	5.7	2.7	34.7
13	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	31.5	28.4	34.7	5.7	3.0	34.7
14	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	31.7	28.8	34.5	5.6	2.9	34.5
15	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	31.6	28.7	34.4	5.6	2.9	34.4
16	09-13-22	100	31.6	4.0	1.437	4.5	31.5	28.5	34.5	5.3	31.5	28.5	34.5	5.5	2.8	34.5
17	10-04-22	100	29.7	5.7	1.610	5.4	31.6	28.6	34.6	5.3	31.6	28.6	34.6	5.5	2.8	34.6
18	11-08-22	100	33.0	6.2	1.880	5.7	31.6	29.7	34.1	5.3	31.6	29.7	34.1	5.5	2.8	34.1
19	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	31.9	29.7	34.1	5.5	2.8	34.1
20	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	32.0	29.7	34.2	5.5	2.9	34.2

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

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

CV = Coefficient of variation for control reproduction.

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

PMSD = Minimum significant difference.

PMDS = Percent minimum significant difference.

PMDS 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 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.

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

Reviewed and
Reviewed by
Jim Sumner



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

CdNaClCR #: 279

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2149</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:

Test randomization and location:

Organism age:	< 24-hours old										Randomizing template color:	<u>BWE</u>
Date and times organisms were born between:	<u>11-06-22 0545 TO 0800</u>										Incubator number and shelf location:	<u>281</u>
Culture board:	<u>11-29-22 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>5</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>18</u>	<u>22</u>	<u>23</u>	<u>27</u>		
Transfer vessel information:	pH (S.U.): <u>7.44</u> Temperature (°C): <u>24.9</u>											
Average transfer volume (mL):	< 0.25 mL											

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches		MHSW batch used	Analyst
			<i>Selenastrum</i>	YWT		
0	12-06-22	<u>0811</u>	<u>11-28-22</u>	<u>11-28-22</u>	<u>11-30-22 A</u>	<u>JL</u>
1	12-07-22	<u>0742</u>	↓	↓	↓	<u>JL</u>
2	12-08-22	<u>0740</u>	↓	↓	<u>11-30-22 D</u>	<u>JL</u>
3	12-09-22	<u>0741</u>	↓	↓	↓	<u>JL</u>
4	12-10-22	<u>0843</u>	↓	↓	<u>12-06-22 B</u>	<u>JL</u>
5	12-11-22	<u>0842</u>	↓	↓	↓	<u>JL</u>
6	12-12-22	<u>0733</u>	↓	↓	↓	<u>JL</u>
7	12-13-22	<u>0741</u>				<u>JL</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>32.7</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>6.87.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1080.5</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 279

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	6	4	5	4	5	5	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	10	11	13	10	13	12	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	18	17	18	16	18	15	17	17
Total young produced		34	29	33	32	31	31	36	31	32	33
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:	32.7

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	6	5	4	5	6	4	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	12	10	12	10	12	12	10	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	17	18	14	19	15	15	18	18	17
Total young produced		38	35	33	30	34	33	31	32	31	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	33.3
% Reduction from Control:	-1.87.

Species: *Ceriodaphnia dubia*
800 mg NaCl/L

CdNaClCR #: 279

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	3	5	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	12	11	13	11	13	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	15	19	16	15	15	17	13	16	17
Total young produced		32	31	36	32	31	31	35	28	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:	31.9
% Reduction from Control:	2.47.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	4	4	5	4	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	9	12	10	13	9	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	14	16	16	13	16	15	18	17	13	13
Total young produced		29	31	29	29	30	33	31	30	28	29
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:	29.9
% Reduction from Control:	8.67.

Species: *Ceriodaphnia dubia*
 1200 mg NaCl/L

CdNaClCR #: 279

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	4	5	3	3	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	5	7	8	4	6	5	5	4	7	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	10	6	6	5	9	9	8	10	5	5
Total young produced		18	17	18	14	18	17	17	18	16	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:	17.1
% Reduction from Control:	47.67.

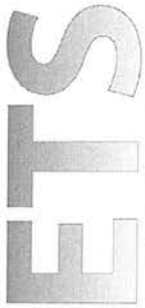
1400 mg NaCl/L

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	2	3	1	1	1	3	1	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	0	0
Total young produced		2	3	1	1	1	3	1	2	2	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:	1.7
% Reduction from Control:	94.87.



Environmental Testing Solutions, Inc.

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	6	4	5	4	5	5	4	5	5	5	48
5	11	10	10	11	13	10	13	12	10	11	111
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	18	17	18	16	18	15	17	17	168
Total	34	29	33	32	36	31	36	31	32	33	327

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	6	5	4	5	6	4	4	4	6	49
5	14	12	10	12	10	12	12	10	12	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	19	17	18	14	19	15	15	18	18	17	170
Total	38	35	33	30	34	33	31	32	34	33	333

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	5	5	3	5	5	5	5	5	47
5	10	12	12	11	13	11	13	10	10	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	19	16	15	15	17	13	16	17	160
Total	32	31	36	32	31	31	35	28	31	32	319

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	4	4	5	4	3	5	5	43
5	10	11	9	12	10	13	9	10	10	11	105
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	16	13	16	15	18	17	13	13	151
Total	29	31	29	29	30	33	31	30	28	29	299

1200 mg NaCl/L

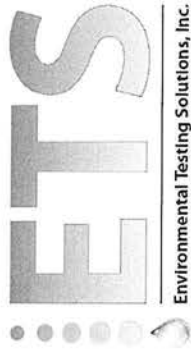
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	4	4	5	3	4	4	4	4	38
5	5	7	8	4	6	5	5	4	7	9	60
6	0	0	0	0	0	0	0	0	0	0	0
7	10	6	6	5	9	9	8	10	5	5	73
Total	18	17	18	14	18	17	17	18	16	18	171

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	3	1	1	1	3	1	2	2	1	17
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	0	0	0
Total	2	3	1	1	1	3	1	2	2	1	17

Prepared and
Reviewed by
Jim Spitzer

18



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

Environmental Testing Solutions, Inc.

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

Test number: **CdNaClCR #279**
 Test dates: **December 06-13, 2022**

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	34	29	33	32	36	31	36	31	32	33	100	32.7	6.8	Not applicable
600	38	35	33	30	34	33	31	32	34	33	100	33.3	6.6	-1.8
800	32	31	36	32	31	31	35	28	31	32	100	31.9	7.0	2.4
1000	29	31	29	29	30	33	31	30	28	29	100	29.9	4.8	8.6
1200	18	17	18	14	18	17	17	18	16	18	100	17.1	7.5	47.7
1400	2	3	1	1	1	3	1	2	2	1	100	1.7	48.4	94.8

Dunnett's MSD value: 1.830
 PMSD: 5.6

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

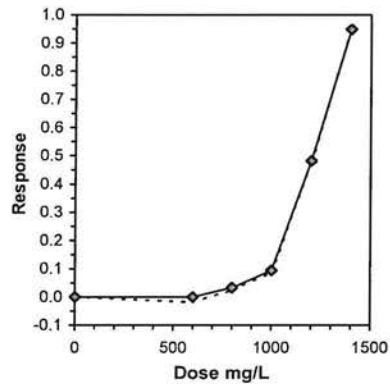
Start Date:	12/6/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	12/13/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	29.000	33.000	32.000	36.000	31.000	36.000	31.000	32.000	33.000
600	38.000	35.000	33.000	30.000	34.000	33.000	31.000	32.000	34.000	33.000
800	32.000	31.000	36.000	32.000	31.000	31.000	35.000	28.000	31.000	32.000
1000	29.000	31.000	29.000	29.000	30.000	33.000	31.000	30.000	28.000	29.000
1200	18.000	17.000	18.000	14.000	18.000	17.000	17.000	18.000	16.000	18.000
1400	2.000	3.000	1.000	1.000	1.000	3.000	1.000	2.000	2.000	1.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
D-Control	32.700	1.0000	32.700	29.000	36.000	6.769	10			33.000	1.0000
600	33.300	1.0183	33.300	30.000	38.000	6.647	10	113.00	75.00	33.000	1.0000
800	31.900	0.9755	31.900	28.000	36.000	7.002	10	92.00	75.00	31.900	0.9667
*1000	29.900	0.9144	29.900	28.000	33.000	4.847	10	69.00	75.00	29.900	0.9061
*1200	17.100	0.5229	17.100	14.000	18.000	7.524	10	55.00	75.00	17.100	0.5182
*1400	1.700	0.0520	1.700	1.000	3.000	48.428	10	55.00	75.00	1.700	0.0515

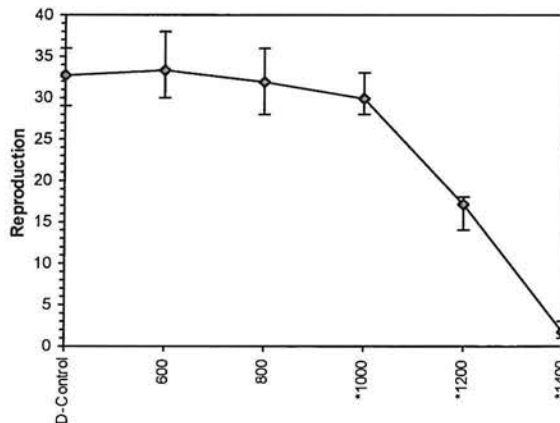
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.04285	1.035	0.37169	1.02005
Bartlett's Test indicates equal variances (p = 0.04)	11.5866	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	800	1000	894.427	
Treatments vs D-Control				

Point	mg/L	SD	Linear Interpolation (200 Resamples)		Skew
			95% CL	Skew	
IC05	855	67.9592	708.018	950.072	-0.0938
IC10	1003.13	34.1474	911.439	1019.86	-1.4339
IC15	1028.91	10.1359	1010.41	1044.01	-1.2345
IC20	1054.69	8.60525	1037.69	1070.59	-0.0596
IC25	1080.47	8.01402	1065.36	1095.77	-0.0604
IC40	1157.81	7.3228	1143.43	1170.92	-0.0686
IC50	1207.79	6.50197	1193.33	1217.95	-0.4311



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
Jen Summer
js



Statistical Analyses

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 12/6/2022	Test ID: CdNaCICR	Sample ID: REF-Ref Toxicant
End Date: 12/13/2022	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:

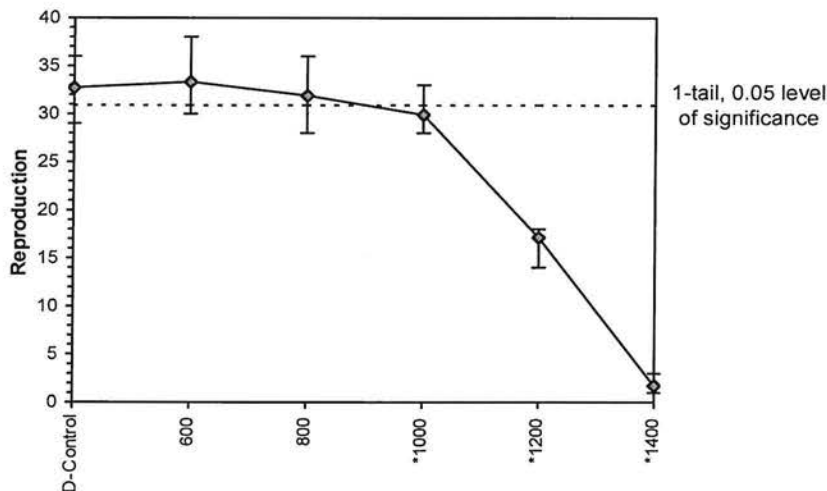
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	29.000	33.000	32.000	36.000	31.000	36.000	31.000	32.000	33.000
600	38.000	35.000	33.000	30.000	34.000	33.000	31.000	32.000	34.000	33.000
800	32.000	31.000	36.000	32.000	31.000	31.000	35.000	28.000	31.000	32.000
1000	29.000	31.000	29.000	29.000	30.000	33.000	31.000	30.000	28.000	29.000
1200	18.000	17.000	18.000	14.000	18.000	17.000	17.000	18.000	16.000	18.000
1400	2.000	3.000	1.000	1.000	1.000	3.000	1.000	2.000	2.000	1.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed	
			Mean	Min	Max	CV%			Critical	MSD
D-Control	32.700	1.0000	32.700	29.000	36.000	6.769	10			
600	33.300	1.0183	33.300	30.000	38.000	6.647	10	-0.750	2.287	1.830
800	31.900	0.9755	31.900	28.000	36.000	7.002	10	0.999	2.287	1.830
*1000	29.900	0.9144	29.900	28.000	33.000	4.847	10	3.498	2.287	1.830
*1200	17.100	0.5229	17.100	14.000	18.000	7.524	10	19.489	2.287	1.830
*1400	1.700	0.0520	1.700	1.000	3.000	48.428	10	38.728	2.287	1.830

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.04285	1.035	0.37169	1.02005
Bartlett's Test indicates equal variances (p = 0.04)	11.5866	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	800	1000	894.427		1.83039	0.05598	1606.35	3.2037	6.2E-44	5, 54

Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Species: Ceriodaphnia dubia

CdNaClCR #: 279

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		EC	EC N	EC N	N	N	EC
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.29	7.23	7.27	7.42	7.44	7.33
	Dissolved oxygen (mg/L)	7.8	7.7	7.7	7.6	7.7	7.9
	Conductivity (µmhos/cm)	304		311		300	
	Alkalinity (mg CaCO ₃ /L)	62				63	
	Hardness (mg CaCO ₃ /L)	84				88	
	Temperature (°C)	24.8	25.2	24.8	25.0	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.62	7.64	7.50	7.59	7.64	7.52
	Dissolved oxygen (mg/L)	7.8	7.0	7.7	7.7	7.7	7.9
	Conductivity (µmhos/cm)	1550		1440		1300	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.9	24.8
800 mg NaCl/L	pH (S.U.)	7.68	7.65	7.62	7.61	7.65	7.55
	Dissolved oxygen (mg/L)	7.8	7.7	7.7	7.6	7.7	8.0
	Conductivity (µmhos/cm)	1920		1820		1760	
	Temperature (°C)	24.9	25.0	24.8	24.9	25.0	25.1
1000 mg NaCl/L	pH (S.U.)	7.71	7.65	7.65	7.62	7.60	7.57
	Dissolved oxygen (mg/L)	7.8	7.7	7.7	7.6	7.6	7.9
	Conductivity (µmhos/cm)	2290		2190		2130	
	Temperature (°C)	24.9	24.8	24.8	25.1	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.74	7.67	7.68	7.64	7.69	7.59
	Dissolved oxygen (mg/L)	7.8	7.7	7.0	7.7	7.9	7.9
	Conductivity (µmhos/cm)	2050		2500		2470	
	Temperature (°C)	24.9	25.1	24.8	25.1	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.75	7.68	7.69	7.65	7.71	7.61
	Dissolved oxygen (mg/L)	7.8	7.0	7.0	7.7	8.0	7.9
	Conductivity (µmhos/cm)	3090		2870		2810	
	Temperature (°C)	24.9	24.8	24.9	25.1	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

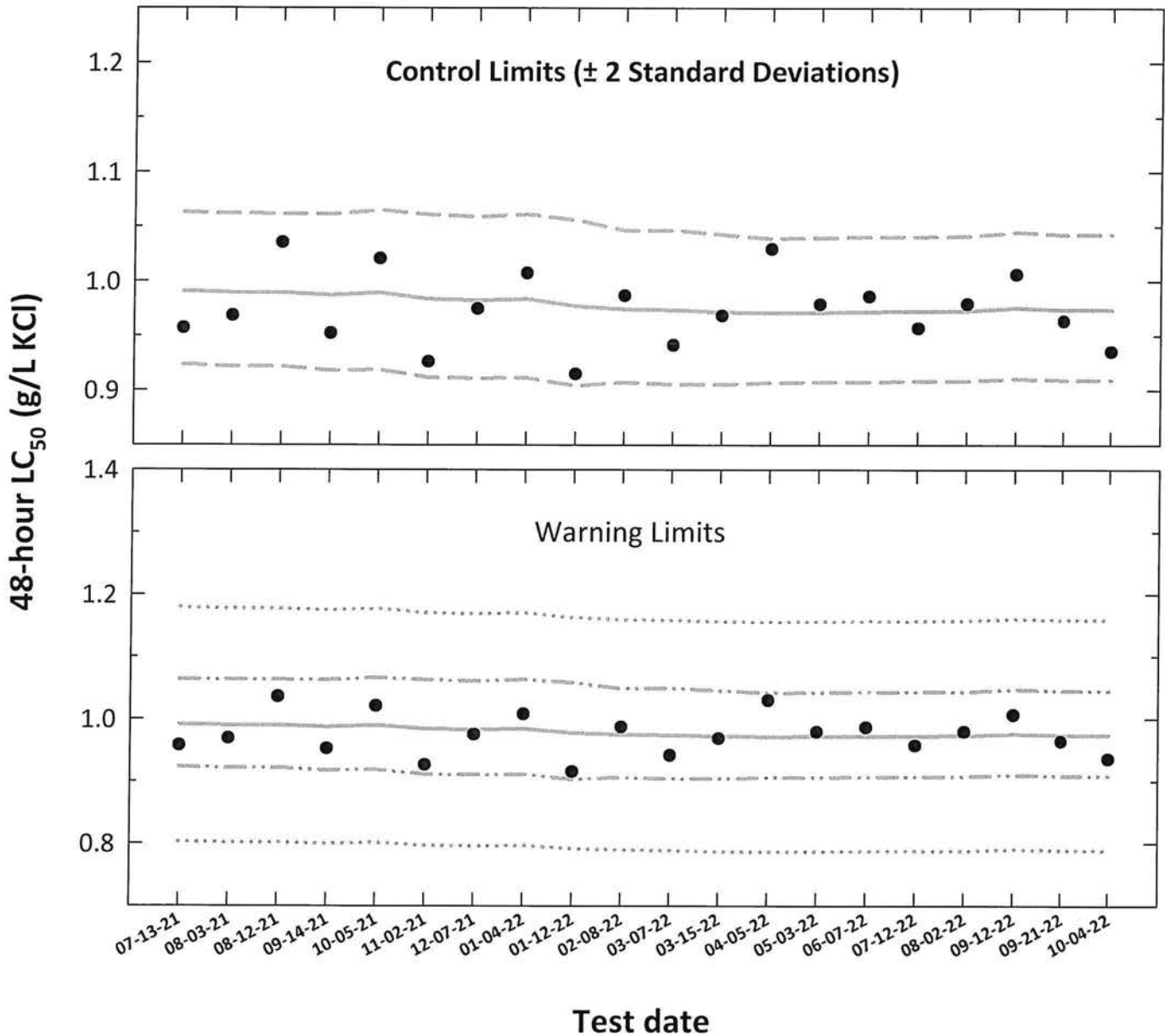
CdNaClCR #: 279

Concentration		Parameter	Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
			EC	BSL	BSL	BSL	BSL	EC	EC	EC
CONTROL, MHSW	pH (S.U.)	7.20	7.50	7.12	7.44	7.15	7.30	7.20	7.74	
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.7	7.9	7.8	7.8	
	Conductivity (µmhos/cm)	304		301		310		300		
	Alkalinity (mg CaCO ₃ /L)			62						
	Hardness (mg CaCO ₃ /L)		12-11-22	86					12-11-22	
	Temperature (°C)	24.8	25.2	24.8	25.1	24.7	25.2	24.8	25.2	
600 mg NaCl/L	pH (S.U.)	7.43	7.69	7.46	7.66	7.58	7.49	7.40	7.81	
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.7	7.8	8.0	7.8	7.9	
	Conductivity (µmhos/cm)	1430		1460		1470		1420		
	Temperature (°C)	24.9	25.0	24.9	24.9	24.7	25.0	24.9	25.1	
800 mg NaCl/L	pH (S.U.)	7.53	7.72	7.53	7.70	7.66	7.53	7.55	7.80	
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.8	8.0	7.8	7.9	
	Conductivity (µmhos/cm)	1830		1770		1820		1790		
	Temperature (°C)	24.9	25.0	24.9	24.9	24.8	25.2	24.9	25.1	
1000 mg NaCl/L	pH (S.U.)	7.58	7.74	7.59	7.72	7.70	7.57	7.60	7.81	
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.7	7.9	8.0	7.8	7.9	
	Conductivity (µmhos/cm)	2200		2150		2190		2180		
	Temperature (°C)	24.9	25.0	24.9	25.1	24.9	25.1	24.9	25.3	
1200 mg NaCl/L	pH (S.U.)	7.61	7.76	7.63	7.74	7.73	7.61	7.63	7.81	
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.7	7.9	8.0	7.9	7.8	
	Conductivity (µmhos/cm)	2550		2480		2520	121222 EC 2475	2420 2475		
	Temperature (°C)	24.8	25.2	25.0	25.1	24.8	25.1	24.9	25.3	
1400 mg NaCl/L	pH (S.U.)	7.64	7.76	7.65	7.75	7.77	7.65	7.65	7.80	
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.7	7.9	8.0	7.9	7.9	
	Conductivity (µmhos/cm)	2900		2760		2740 11-22-22 7.9 (2910)		2820 11-22-22		
	Temperature (°C)	24.9	25.2	25.0	24.8	24.8	25.2	24.9	25.3	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	

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 ± 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 $\pm S_{A.75}$ 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	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9909	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791
2	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9894	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774
3	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
4	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
5	10-05-21	1.0207	0.0089	-0.0047	0.0160	0.9893	0.9190	1.0650	0.9182	1.0659	0.8014	1.1773
6	11-02-21	0.9262	-0.0333	-0.0071	0.0164	0.9837	0.9120	1.0610	0.9108	1.0623	0.7968	1.1706
7	12-07-21	0.9747	-0.0111	-0.0078	0.0163	0.9823	0.9111	1.0590	0.9099	1.0604	0.7957	1.1689
8	01-04-22	1.0075	0.0033	-0.0072	0.0165	0.9836	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705
9	01-12-22	0.9151	-0.0385	-0.0100	0.0168	0.9773	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630
10	02-08-22	0.9869	-0.0057	-0.0112	0.0155	0.9745	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597
11	03-07-22	0.9416	-0.0261	-0.0116	0.0157	0.9737	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587
12	03-15-22	0.9685	-0.0139	-0.0124	0.0153	0.9718	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564
13	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
14	05-03-22	0.9788	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562
15	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
16	07-12-22	0.9572	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
17	08-02-22	0.9794	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
18	09-12-22	1.0063	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
19	09-21-22	0.9638	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
20	10-04-22	0.9358	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591

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 # 142

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 #: 2128

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	K	ECN	K
	Dissolved oxygen (mg/L)	7.51	7.10	7.15
	Conductivity (µmhos/cm)	7.0	7.9	7.9
	Alkalinity (mg/L CaCO ₃)	300		
	Hardness (mg/L CaCO ₃)	60		
	Temperature (°C)	85		
500 mg/L	pH (S.U.)	24.9	24.6	24.8
	Dissolved oxygen (mg/L)	7.65	7.61	7.23
	Conductivity (µmhos/cm)	7.9	7.9	7.9
	Alkalinity (mg/L CaCO ₃)	1160		
	Temperature (°C)	24.9	24.4	24.7
750 mg/L	pH (S.U.)	7.65	7.61	7.30
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	1590		
	Alkalinity (mg/L CaCO ₃)	75.0	24.4	24.8
	Temperature (°C)	25.0	24.4	24.8
1000 mg/L	pH (S.U.)	7.67	7.50	7.32
	Dissolved oxygen (mg/L)	7.9	8.0	7.9
	Conductivity (µmhos/cm)	2040		
	Alkalinity (mg/L CaCO ₃)	75.0	24.3	24.8
	Temperature (°C)	25.0	24.3	24.8
1250 mg/L	pH (S.U.)	7.68	7.55	7.34
	Dissolved oxygen (mg/L)	7.9	8.0	7.8
	Conductivity (µmhos/cm)	2450		
	Alkalinity (mg/L CaCO ₃)	75.0	24.6	24.8
	Temperature (°C)	25.0	24.6	24.8
1500 mg/L	pH (S.U.)	7.70	7.55	
	Dissolved oxygen (mg/L)	7.9	8.0	
	Conductivity (µmhos/cm)	1800		
	Alkalinity (mg/L CaCO ₃)	75.0	24.6	
	Temperature (°C)	25.0	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	13066465

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 # 142

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-04-22	0505	J	0705	J	1D	WHITE	09-28-22A
24	10-05-22			0700	J			
48 Termination	10-06-22			0704	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:	09-22-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	09-28-22 1210 TO 09-29-22 0750
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.68
	Temperature (°C): 24.3

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ^d	9 ^d	4 ^{ud}	4 ^{ud}	1 ^{ud}	2 ^{sd}	0 ^{ud}	0 ^{ud}
48 Termination	10	10	10	10	9	9	4	3 ^{ud}	0 ^{ud}	1 ^{ud}	0	0
Mean Survival	100%		100%		90%		35%		5%		0%	

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

Statistics:

Method	PROB15
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:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 10/4/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 10/6/2022 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

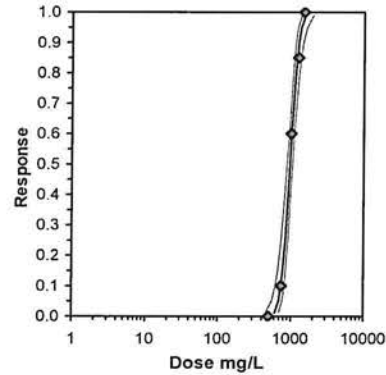
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.4000	0.4000
1250	0.1000	0.2000
1500	0.0000	0.0000

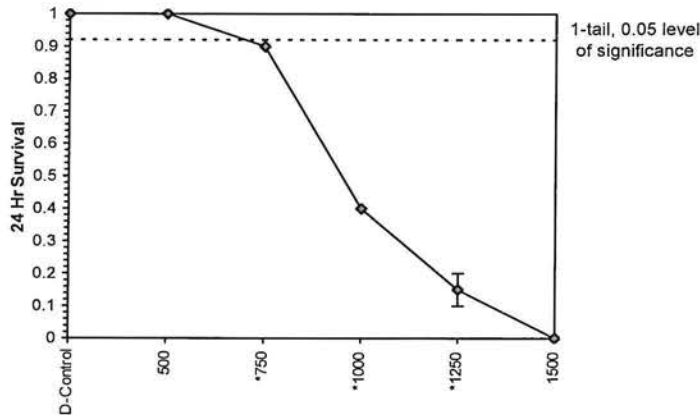
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	3.632	2.850	0.1279	2	20
*1000	0.4000	0.4000	0.6847	0.6847	0.6847	0.000	2	16.208	2.850	0.1279	12	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	500	750	612.372		0.05495	0.05636	0.43261	0.00201	8.9E-06	4, 5
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	11.3411	2.00231	7.41661	15.2657	0	0.89	7.81472	0.82784	2.986	0.08817	3
Intercept	-28.865	6.01764	-40.659	-17.07							
TSCR	Point	Probits	mg/L	95% Fiducial Limits							
	EC01	2.674	603.77	453.278	699.353						
	EC05	3.355	693.366	557.331	778.894						
	EC10	3.718	746.443	621.322	826.149						
	EC15	3.964	784.53	667.936	860.492						
	EC20	4.158	816.182	706.88	889.538						
	EC25	4.326	844.353	741.511	915.958						
	EC40	4.747	919.725	832.472	990.82						
	EC50	5.000	968.271	888.184	1043.8						
	EC60	5.253	1019.38	943.086	1104.9						
	EC75	5.674	1110.37	1030.61	1227.87						
	EC80	5.842	1148.7	1064.17	1284.46						
	EC85	6.036	1195.04	1102.82	1355.98						
	EC90	6.282	1256.02	1151.25	1454.43						
	EC95	6.645	1352.17	1223.74	1617.93						
	EC99	7.326	1552.82	1365.76	1985.2						



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 10/4/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 10/6/2022	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	0.4000	0.3000
1250	0.0000	0.1000
1500	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1748	0	20	
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.658	2.850	0.1748	2	20	
*1000	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	12.718	2.850	0.1748	13	20	
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.109	2.850	0.1748	19	20	
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

Auxiliary Tests

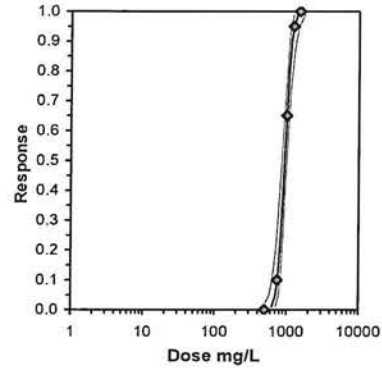
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08218	0.08429	0.55672	0.00376	2.2E-05	4, 5

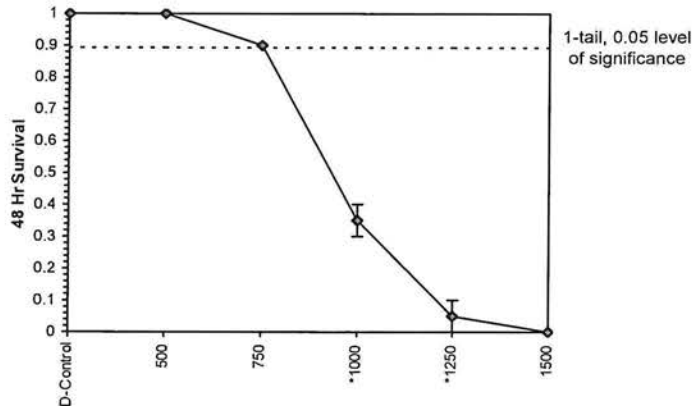
Maximum Likelihood-Probit

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



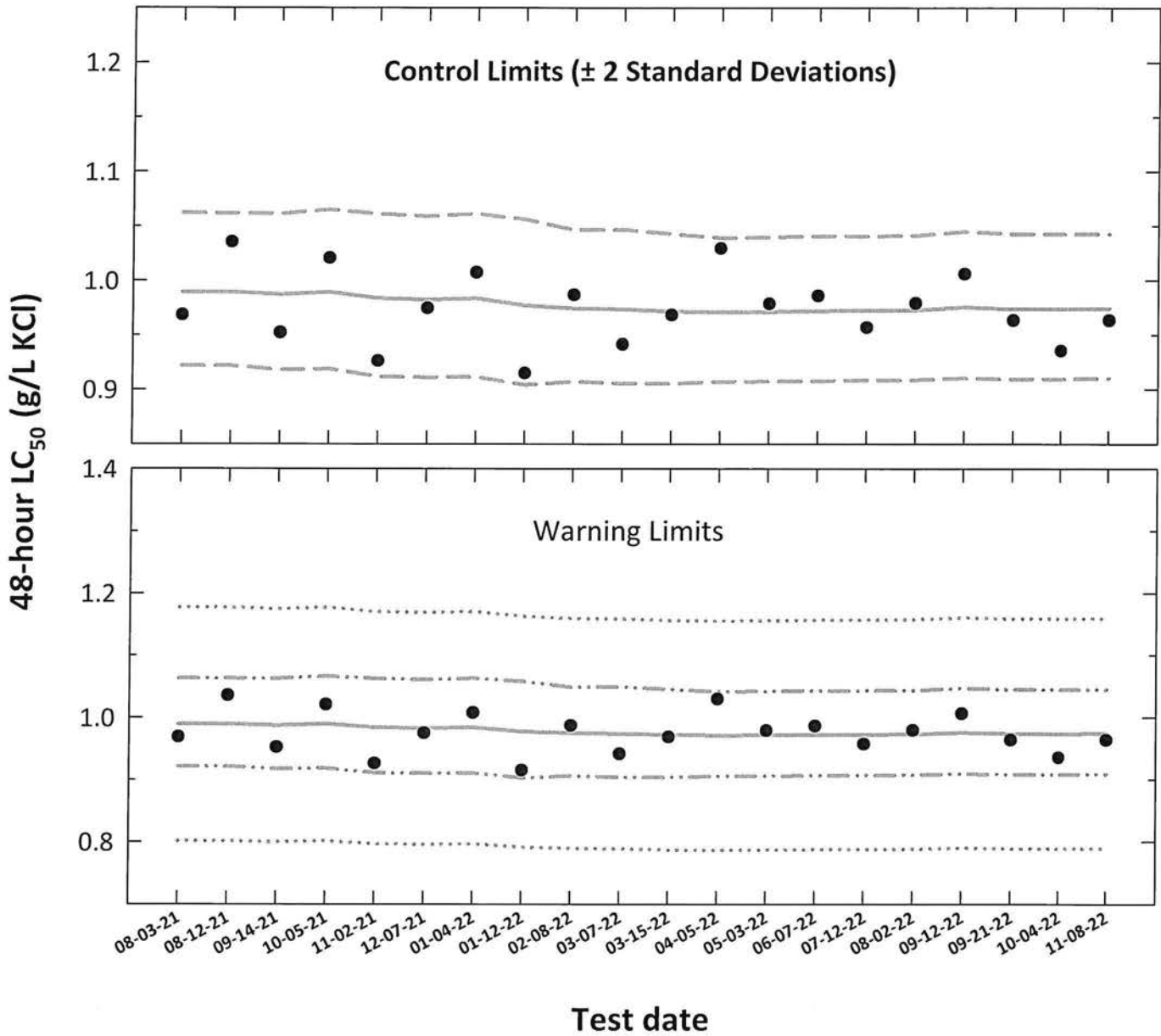
Dose-Response Plot



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		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9894	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774
2	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
3	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
4	10-05-21	1.0207	0.0089	-0.0047	0.0160	0.9893	0.9190	1.0650	0.9182	1.0659	0.8014	1.1773
5	11-02-21	0.9262	-0.0333	-0.0071	0.0164	0.9837	0.9120	1.0610	0.9108	1.0623	0.7968	1.1706
6	12-07-21	0.9747	-0.0111	-0.0078	0.0163	0.9823	0.9111	1.0590	0.9099	1.0604	0.7957	1.1689
7	01-04-22	1.0075	0.0033	-0.0072	0.0165	0.9836	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705
8	01-12-22	0.9151	-0.0385	-0.0100	0.0168	0.9773	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630
9	02-08-22	0.9869	-0.0057	-0.0112	0.0155	0.9745	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597
10	03-07-22	0.9416	-0.0261	-0.0116	0.0157	0.9737	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587
11	03-15-22	0.9685	-0.0139	-0.0124	0.0153	0.9718	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564
12	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
13	05-03-22	0.9788	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562
14	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
15	07-12-22	0.9572	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
16	08-02-22	0.9794	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
17	09-12-22	1.0063	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
18	09-21-22	0.9638	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
19	10-04-22	0.9358	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
20	11-08-22	0.9637	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595

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 # 143

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 #: 2150

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	EC 6.94 ^{7.43} EC ✓	7.48	7.40
	Dissolved oxygen (mg/L)	7.7	7.0	7.7
	Conductivity (µmhos/cm)	313		
	Alkalinity (mg/L CaCO ₃)	58		
	Hardness (mg/L CaCO ₃)	85		
	Temperature (°C)	24.8	24.7	24.9
500 mg/L	pH (S.U.)	7.22	7.58	7.52
	Dissolved oxygen (mg/L)	7.8	7.5	7.6
	Conductivity (µmhos/cm)	1220		
	Temperature (°C)	24.8	24.6	24.7
750 mg/L	pH (S.U.)	7.32	7.59	7.50
	Dissolved oxygen (mg/L)	7.8	7.5	7.7
	Conductivity (µmhos/cm)	1050		
	Temperature (°C)	24.9	24.7	24.7
1000 mg/L	pH (S.U.)	7.30	7.01	7.59
	Dissolved oxygen (mg/L)	7.8	7.0	7.6
	Conductivity (µmhos/cm)	2110		
	Temperature (°C)	24.7	24.5	24.9
1250 mg/L	pH (S.U.)	7.41	7.03	7.02
	Dissolved oxygen (mg/L)	7.8	7.0	7.6
	Conductivity (µmhos/cm)	2510		
	Temperature (°C)	24.9	24.7	24.9
1500 mg/L	pH (S.U.)	7.40	7.04	7.03
	Dissolved oxygen (mg/L)	7.8	7.0	7.5
	Conductivity (µmhos/cm)	2980		
	Temperature (°C)	24.9	24.7	24.8

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 143

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-08-22	0505	J	0705	J	1C	WHTE	11-05-22B
24	11-09-22			0700	J			
48 Termination	11-10-22			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:	10-26-22
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	11-01-22 1450 TO 11-02-22 0650
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.51
	Temperature (°C): 25.0

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	4 ^d 6	5 ^{sd} 5	1 ^{sd} 1	1 ^{sd} 1	0 ^{sd} 0	1 ^{sd} 1
48 Termination	10	10	10	10	10	9 ^{sd} 9	5 ^{sd} 5	3 ^{sd} 3	1 ^{sd} 1	0 ^{sd} 0	0 ^{sd} 0	0 ^{sd} 0
Mean Survival	100%		100%		95%		40%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	894.1
Upper 95% confidence limit (mg KCl/L)	1029.8
48-hour LC ₅₀ (mg KCl/L)	963.7

Comments:

Test Reviewed by:

Acute Fathead Minnow Test-24 Hr Survival

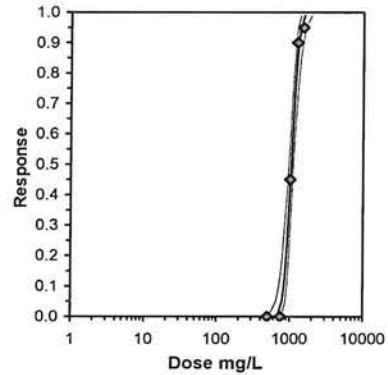
Start Date: 11/8/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 11/10/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.5000
1250	0.1000	0.1000
1500	0.0000	0.1000

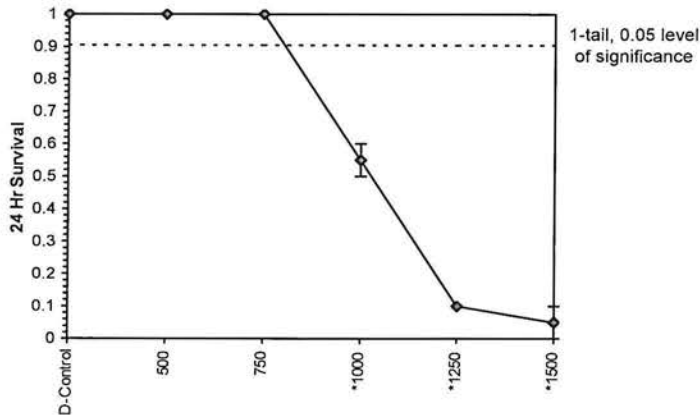
Conc-mg/L	Transform: Arcsin Square Root							1-Tailed		Number		Total
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1565	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1565	0	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	10.421	2.830	0.1565	9	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	19.716	2.830	0.1565	18	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	21.189	2.830	0.1565	19	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		0.07115	0.07297	0.62045	0.00306	1.3E-06	5, 6

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.5671	2.48675	8.69304	18.4411	0	2.48867	7.81472	0.47734	3.0178	0.07371	5
Intercept	-35.943	7.53967	-50.72	-21.165							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	701.983	545.682	796.177							
EC05	3.355	788.058	650.688	870.818							
EC10	3.718	838.179	713.72	914.671							
EC15	3.964	873.783	758.97	946.363							
EC20	4.158	903.156	796.362	973.073							
EC25	4.326	929.141	829.307	997.309							
EC40	4.747	997.983	914.478	1065.75							
EC50	5.000	1041.83	965.681	1113.98							
EC60	5.253	1087.6	1015.46	1169.31							
EC75	5.674	1168.19	1093.61	1279.42							
EC80	5.842	1201.8	1123.23	1329.52							
EC85	6.036	1242.2	1157.14	1392.36							
EC90	6.282	1294.96	1199.32	1478.06							
EC95	6.645	1377.32	1261.82	1618.53							
EC99	7.326	1546.21	1382.36	1926.85							



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 11/8/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 11/10/2022 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:

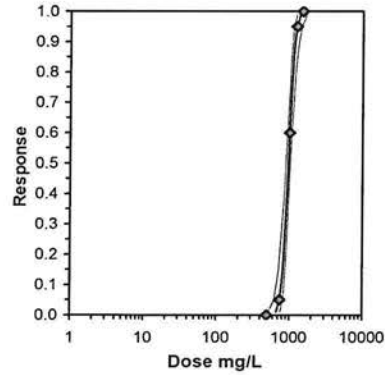
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.5000	0.3000
1250	0.1000	0.0000
1500	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2784	0	20	
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.834	2.850	0.2784	1	20	
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	7.467	2.850	0.2784	12	20	
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.993	2.850	0.2784	19	20	
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

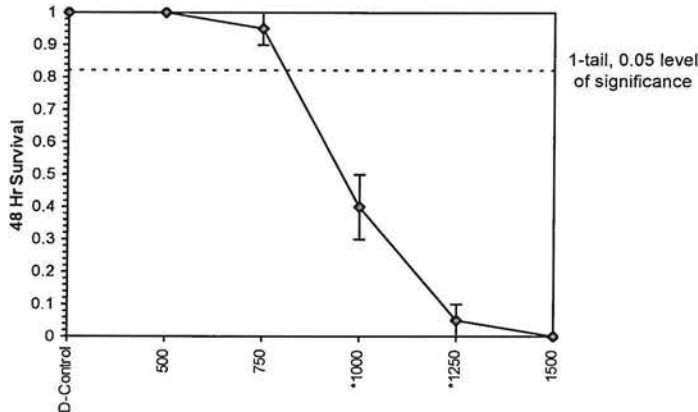
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	750	1000	866.025	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	15.0656	2.87343	9.43368	20.6975	0	0.05472	7.81472	0.99665	2.98396	0.06638	3
Intercept	-39.955	8.61041	-56.832	-23.079							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	675.376	530.048	760.01
EC05	3.355	749.516	623.088	823.667
EC10	3.718	792.311	678.261	860.931
EC15	3.964	822.556	717.57	887.825
EC20	4.158	847.416	749.867	910.479
EC25	4.326	869.341	778.185	931.029
EC40	4.747	927.137	850.816	989.008
EC50	5.000	963.741	894.12	1029.76
EC60	5.253	1001.79	936.015	1076.33
EC75	5.674	1068.39	1001.41	1168.44
EC80	5.842	1096.03	1026.06	1210.14
EC85	6.036	1129.16	1054.15	1262.32
EC90	6.282	1172.26	1088.91	1333.24
EC95	6.645	1239.2	1140.03	1448.93
EC99	7.326	1375.23	1237.51	1700.51



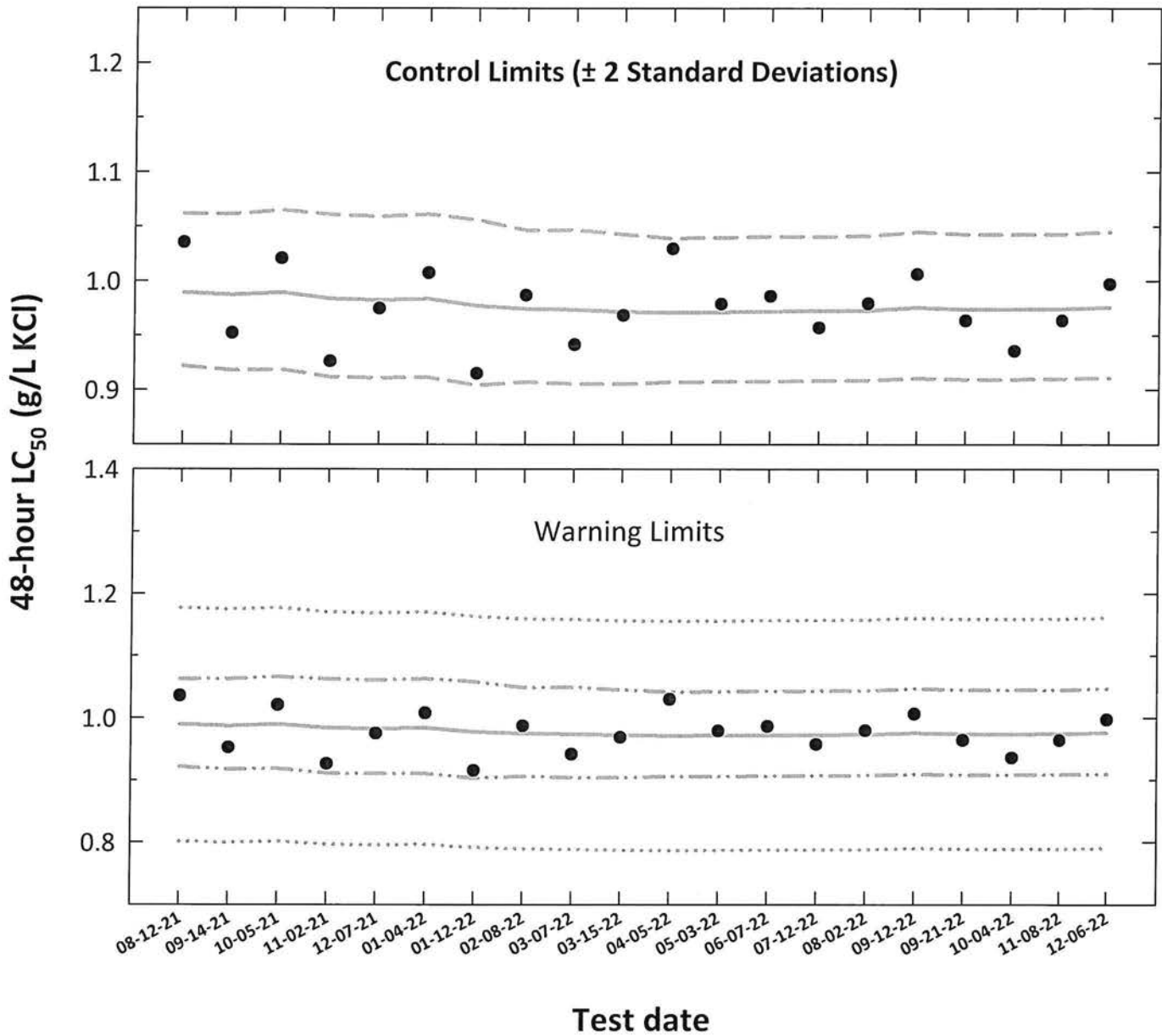
Dose-Response Plot



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 ± 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 $\pm S_{A.75}$ 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	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	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
2	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
3	10-05-21	1.0207	0.0089	-0.0047	0.0160	0.9893	0.9190	1.0650	0.9182	1.0659	0.8014	1.1773
4	11-02-21	0.9262	-0.0333	-0.0071	0.0164	0.9837	0.9120	1.0610	0.9108	1.0623	0.7968	1.1706
5	12-07-21	0.9747	-0.0111	-0.0078	0.0163	0.9823	0.9111	1.0590	0.9099	1.0604	0.7957	1.1689
6	01-04-22	1.0075	0.0033	-0.0072	0.0165	0.9836	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705
7	01-12-22	0.9151	-0.0385	-0.0100	0.0168	0.9773	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630
8	02-08-22	0.9869	-0.0057	-0.0112	0.0155	0.9745	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597
9	03-07-22	0.9416	-0.0261	-0.0116	0.0157	0.9737	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587
10	03-15-22	0.9685	-0.0139	-0.0124	0.0153	0.9718	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564
11	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
12	05-03-22	0.9788	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562
13	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
14	07-12-22	0.9572	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
15	08-02-22	0.9794	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
16	09-12-22	1.0063	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
17	09-21-22	0.9638	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
18	10-04-22	0.9358	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
19	11-08-22	0.9637	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595
20	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

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 # 144

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 #: 2128

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Analyst	EC	EC N	N
	pH (S.U.)	7.29	7.57	7.49
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	804		
	Alkalinity (mg/L CaCO ₃)	62		
	Hardness (mg/L CaCO ₃)	54		
Temperature (°C)	24.9	24.8	25.0	
500 mg/L	pH (S.U.)	7.71	7.70	7.50
	Dissolved oxygen (mg/L)	7.8	7.7	7.7
	Conductivity (µmhos/cm)	1230		
	Temperature (°C)	24.7	24.6	24.8
750 mg/L	pH (S.U.)	7.73	7.78	7.60
	Dissolved oxygen (mg/L)	7.8	7.8	7.6
	Conductivity (µmhos/cm)	1030		
	Temperature (°C)	24.7	24.6	24.7
1000 mg/L	pH (S.U.)	7.75	7.78	7.62
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	2050		
	Temperature (°C)	24.9	24.9	24.7
1250 mg/L	pH (S.U.)	7.76	7.78	7.62
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2470		
	Temperature (°C)	24.9	24.9	24.8
1500 mg/L	pH (S.U.)	7.79	7.78	
	Dissolved oxygen (mg/L)	7.9	7.8	
	Conductivity (µmhos/cm)	2910		
	Temperature (°C)	24.8	24.6	24.7

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified 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	130664685

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 # 144

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-06-22	0505	JL	0705	JL	7F	GREEN	11-30-22A
24	12-01-22			0700	JL			
48 Termination	12-06-22			0700	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
Spawning date:	11-25-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	11-30-22 1050 TO 12-01-22 0705
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.78
	Temperature (°C): 25.2

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}	6 ^{ud}	2 ^{sd} 0 ^{ud} 11-27-22	0 ^{sd}	0 ^{sd}	0 ^{sd}
48 Termination	10	10	10	10	10	10	4 ^{id}	5 ^{id}	1 ^{id}	0	0	0
Mean Survival	100%		100%		100%		45%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	928.8
Upper 95% confidence limit (mg KCl/L)	1058.0
48-hour LC ₅₀ (mg KCl/L)	997.1

Comments:

Test Reviewed by: JL



Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date: 12/6/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 12/8/2022	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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.5000	0.6000
1250	0.2000	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	0.2894	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	5.676	2.850	0.2894	9	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	10.842	2.850	0.2894	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

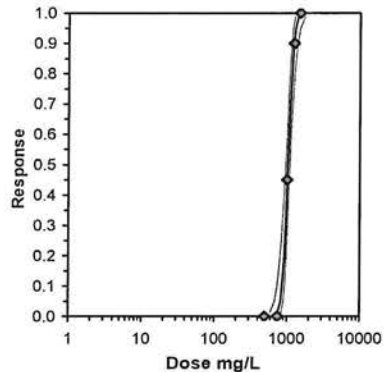
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.16274	0.16691	0.49067	0.01031	3.6E-04	4, 5

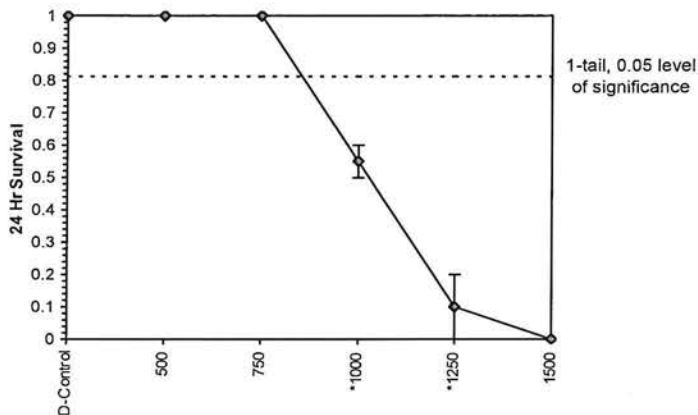
Treatments vs D-Control

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	17.071	3.53267	10.1469	23.995	0	0.52223	7.81472	0.91398	3.01329	0.05858	4
Intercept	-46.44	10.6862	-67.385	-25.495							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	753.374	590.534	840.959
EC05	3.355	825.909	686.525	901.42
EC10	3.718	867.389	743.014	936.551
EC15	3.964	896.546	783.087	961.813
EC20	4.158	920.417	815.908	983.054
EC25	4.326	941.402	844.604	1002.31
EC40	4.747	996.426	917.761	1056.77
EC50	5.000	1031.06	960.893	1095.37
EC60	5.253	1066.91	1002.03	1139.94
EC75	5.674	1129.27	1064.71	1229.1
EC80	5.842	1155.01	1087.85	1269.65
EC85	6.036	1185.77	1113.96	1320.39
EC90	6.282	1225.63	1145.95	1389.25
EC95	6.645	1287.18	1192.54	1501.13
EC99	7.326	1411.11	1280.28	1742.41



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 12/6/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 12/8/2022 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:

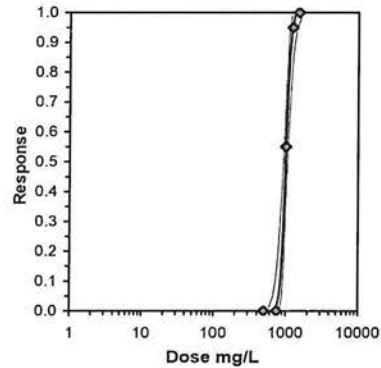
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.4000	0.5000
1250	0.1000	0.0000
1500	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20	
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20	
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	11.175	2.850	0.1726	11	20	
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.343	2.850	0.1726	19	20	
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

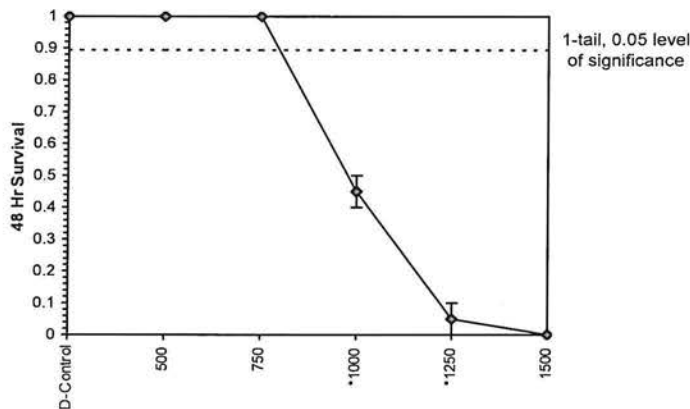
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnnett's Test	750	1000	866.025	0.08088
Treatments vs D-Control				0.08295
				0.57386
				0.00367
				1.9E-05
				4, 5

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	18.7019	4.18191	10.5054	26.8985	0	0.52579	7.81472	0.91319	2.99875	0.05347	4
Intercept	-51.082	12.5873	-75.753	-26.411							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	748.783	580.681	832.841
EC05	3.355	814.321	671.623	886.301
EC10	3.718	851.572	724.934	917.27
EC15	3.964	877.663	762.664	939.524
EC20	4.158	898.969	793.51	958.242
EC25	4.326	917.659	820.433	975.229
EC40	4.747	966.496	888.809	1023.5
EC50	5.000	997.118	928.824	1058.03
EC60	5.253	1028.71	966.612	1098.28
EC75	5.674	1083.46	1023.12	1179.73
EC80	5.842	1105.98	1043.62	1216.99
EC85	6.036	1132.83	1066.55	1263.68
EC90	6.282	1167.54	1094.39	1327.06
EC95	6.645	1220.95	1134.56	1429.96
EC99	7.326	1327.81	1209.35	1651.23



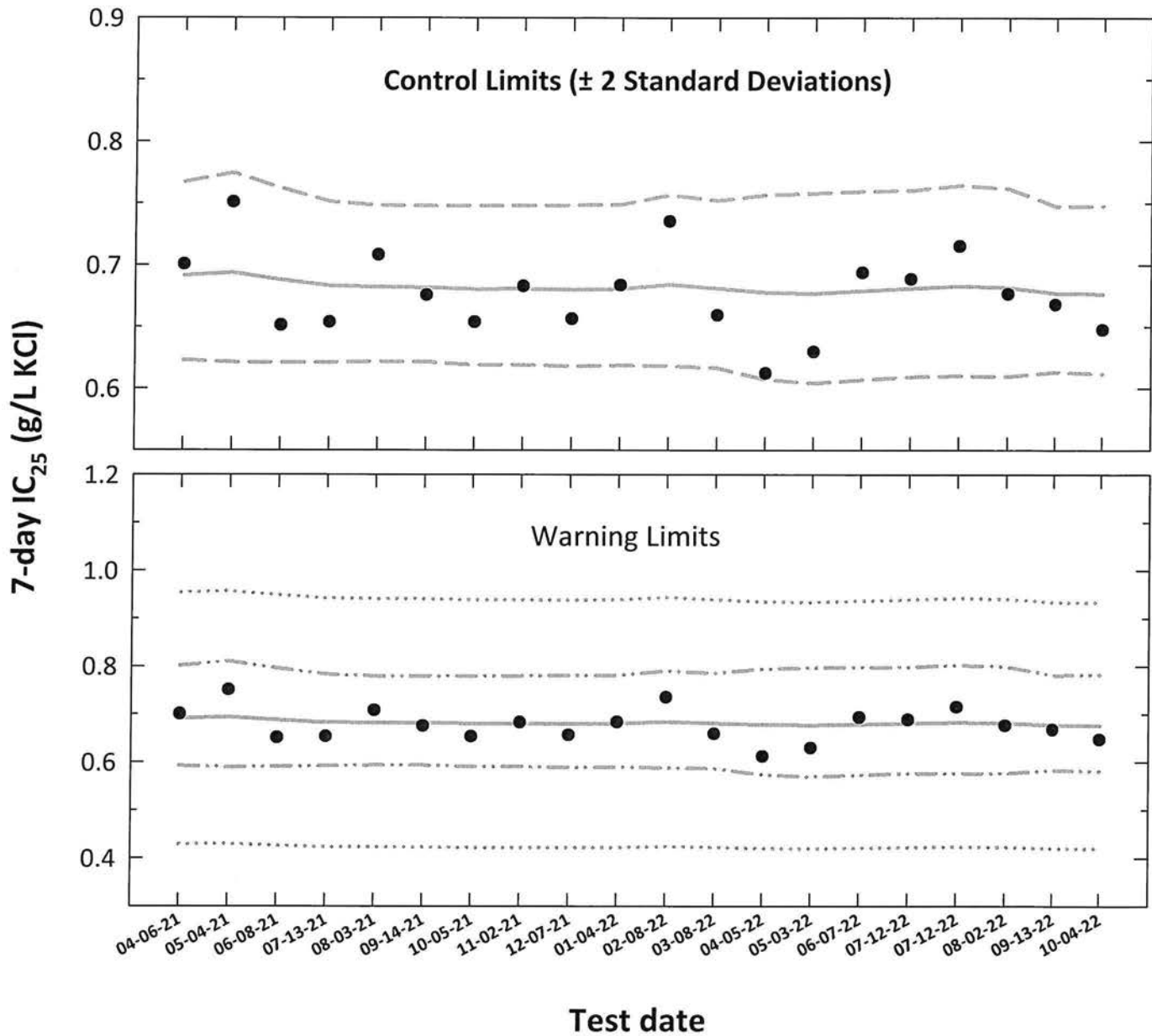
Dose-Response Plot



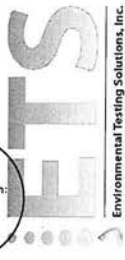
Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ ± 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	04-06-21	0.7006	-0.1545	-0.1603	0.0226	0.6914	0.6230	0.7672	0.5925	0.8011	0.4287	0.9541
2	05-04-21	0.7509	-0.1244	-0.1589	0.0240	0.6937	0.6212	0.7746	0.5892	0.8103	0.4301	0.9573
3	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
4	07-13-21	0.6537	-0.1846	-0.1655	0.0207	0.6831	0.6210	0.7513	0.5922	0.7830	0.4235	0.9426
5	08-03-21	0.7083	-0.1498	-0.1661	0.0201	0.6822	0.6219	0.7484	0.5938	0.7792	0.4230	0.9415
6	09-14-21	0.6756	-0.1703	-0.1663	0.0201	0.6818	0.6215	0.7481	0.5933	0.7790	0.4227	0.9410
7	10-05-21	0.6538	-0.1846	-0.1672	0.0205	0.6805	0.6190	0.7480	0.5902	0.7797	0.4219	0.9390
8	11-02-21	0.6829	-0.1656	-0.1670	0.0205	0.6808	0.6193	0.7483	0.5905	0.7799	0.4221	0.9395
9	12-07-21	0.6565	-0.1828	-0.1675	0.0208	0.6800	0.6180	0.7484	0.5887	0.7805	0.4216	0.9385
10	01-04-22	0.6838	-0.1651	-0.1670	0.0207	0.6808	0.6188	0.7490	0.5897	0.7810	0.4221	0.9395
11	02-08-22	0.7354	-0.1335	-0.1649	0.0219	0.6841	0.6184	0.7567	0.5881	0.7903	0.4241	0.9440
12	03-08-22	0.6594	-0.1808	-0.1668	0.0216	0.6811	0.6167	0.7522	0.5866	0.7855	0.4223	0.9399
13	04-05-22	0.6124	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
14	05-03-22	0.6299	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
15	06-07-22	0.6939	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372
16	07-12-22	0.6887	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
17	07-12-22	0.7153	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
18	08-02-22	0.6766	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
19	09-13-22	0.6682	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
20	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

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).

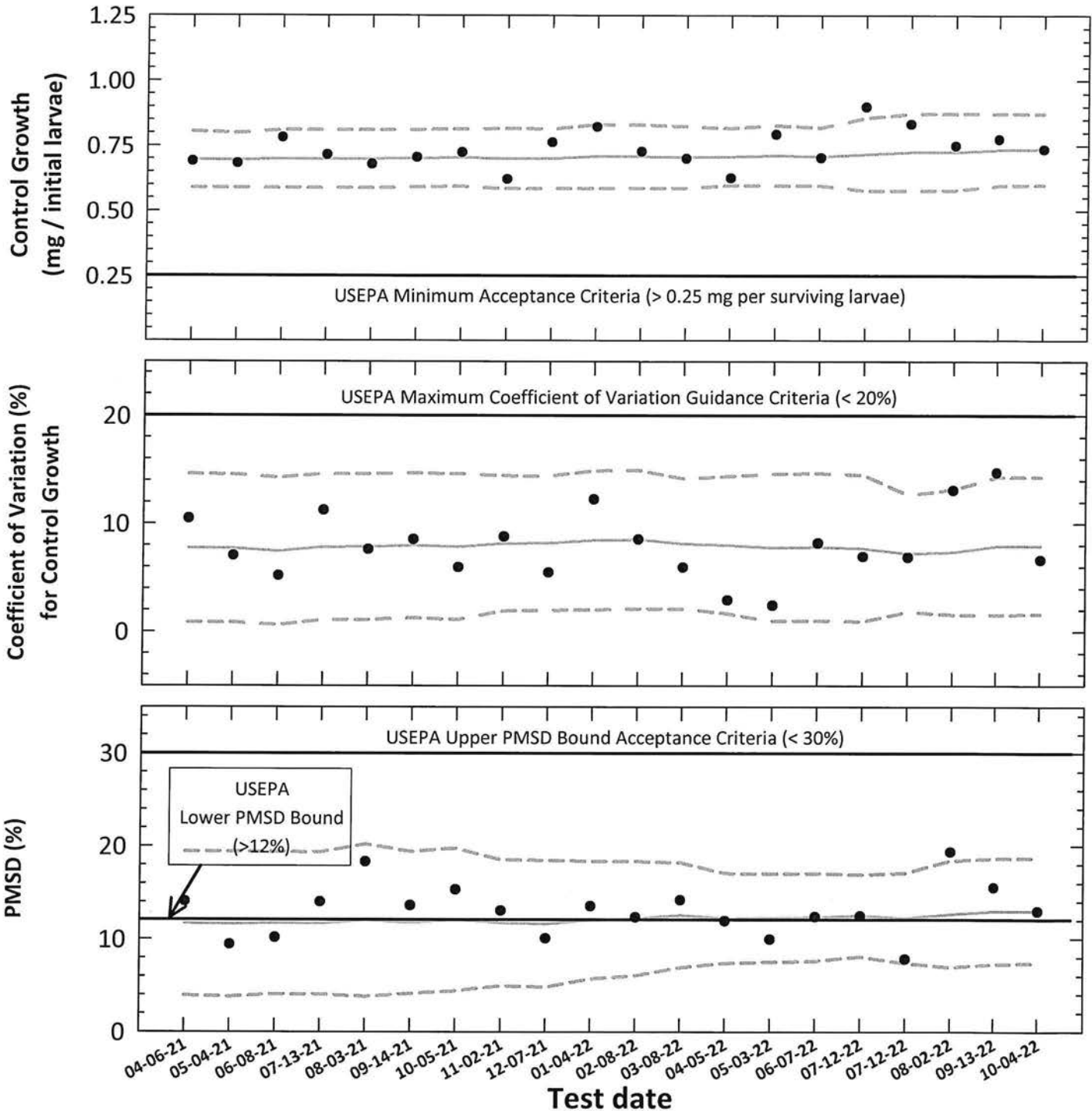
CV = Coefficient of variation.



Entered and
Reviewed by
Jim Sumner

Pimephales promelas

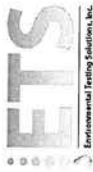
Chronic Reference Toxicant Testing, Test Acceptability Criteria Organism Source: In-house Culture



- **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)

Independent Review by
Kelley E. Keenan

Entered and Reviewed by
Jim Sumner



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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		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	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial larvae)	CV (%)											
1	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4
2	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.8	14.6	11.6	3.8	19.4
3	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
4	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
5	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
6	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
7	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
8	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
9	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
10	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
11	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
12	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
13	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
14	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
15	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
16	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
17	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
18	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4
19	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	14.3	13.0	7.3	18.7
20	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	14.3	13.0	7.4	18.7

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*

PpKCICR Test Number: 95

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2128</u>					
Stock preparation:							
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>09-27-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>10-03-22 1445 to 10-04-22 0505</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.68</u> S.U. Temperature = <u>24.3</u> °C	Date / Time in oven:	<u>10-11-22 0922</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>10-12-22 0925</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	10-04-22	0505	JL	1200	JL	0713	JL	09-29-22 A
1	10-05-22	0500	JL	1100	JL	0710	JL	↓
2	10-06-22	0500	JL	1130	JL	0703	JL	09-29-22 C
3	10-07-22	0500	JL	1100	JL	0700	JL	↓
4	10-08-22	0600	JL	1200	JL	0800	JL	10-06-22 A
5	10-09-22	0600	JL	1200	JL	0800	JL	↓
6	10-10-22	0500	JL	1100	JL	0700	JL	↓
7	10-11-22					0613	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	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>839.7</u>
Average weight per initial larvae:	<u>0.736</u>		NOEC (mg/L KCl)	<u>450</u>
Average weight per surviving larvae:	<u>0.736</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>647.7</u>



Species: Pimephales promelas

PpKICR Test Number: 95

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>Grey</u> Analyst: <u>EC</u> Date: <u>092722</u>	14.73	14.03	12.87	14.87	14.85	14.90	14.37	13.00	15.15	14.39	14.24	13.10
*B = Pan + Larvae weight (mg) Analyst: <u>AJ</u> Date: <u>101622</u>	22.70	22.03	20.15	21.65	20.97	21.91	19.96	19.98	21.90	20.35	21.12	20.19
C = Larvae weight (mg) = B - A Analyst: <u>J</u>	7.97	7.40	7.28	6.78	6.12	7.01	5.59	6.98	6.75	5.96	6.88	7.03
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>	0.797	0.740	0.728	0.678	0.612	0.701	0.559	0.698	0.675	0.596	0.688	0.703
Average weight per initial number of larvae (mg)	0.736				0.643		12.77		0.666		9.57	
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

PpKCICR Test Number: 95

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}	5 ^{sd}	6 ^{id}	6 ^{id}	5 ^{sd}				
2	10	10	10	9 ^{id}	9	9	9	9	4 ^{id}	5 ^{id}	5 ^{id}	5				
3	10	10	10	9	8 ^{id}	8 ^{id}	7 ^{2d}	7 ^{2d}	4	5	5	5				
4	10	10	10	9	8	8	7	7	4	5	5	5				
5	10	10	10	9	8	8	7	7	4	5	4 ^{id}	4 ^{id}				
6	10	10	10	9	8	8	7	7	4	3 ^{2d}	3 ^{id}	3 ^{id}				
7	10	10	10	9	8	8	7	7	3 ^{id}	3	2 ^{id}	3				
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>EC</u> Date: <u>092722</u>	10.08	13.87	14.22	13.02	13.30	10.30	14.89	15.33	14.87	14.02	14.17	13.02				
*B = Pan + Larvae weight (mg) Analyst: <u>AJ</u> Date: <u>101622</u>	22.21	20.44	19.70	18.69	18.82	21.71	19.41	19.49	17.13	16.08	16.13	16.16				
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	6.13	6.57	5.48	5.67	5.46	5.41	4.52	4.16	2.26	2.06	1.96	2.54				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0.613	0.657	0.548	0.567	0.546	0.541	0.452	0.416	0.226	0.206	0.196	0.254				
Average weight per initial number of larvae (mg)	0.581			21.07.			0.489			33.67.			0.221		70.07.	
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

PpKCICR Test Number: 95

Survival and Growth Data

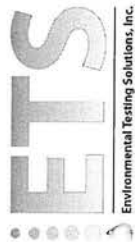
Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10	10	10	10	
1	4 ^{wd}	4 ^{wd}	4 ^{wd}	4 ^{wd}	
2	4	4	4	4	
3	4	4	4	4	
4	4	4	4	3 ^{id}	
5	3 ^{id}	3 ^{id}	3 ^{id}	2 ^{id}	
6	3	3	2 ^{id}	2	
7	2 ^{id}	2 ^{id}	1 ^{id}	1 ^{id}	
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>EC</u> Date: <u>092722</u>		15.24	14.44	13.04	15.25
*B = Pan + Larvae weight (mg) Analyst: <u>AT</u> Date: <u>101622</u>		16.97	16.37	13.81	15.75
C = Larvae weight (mg) = B - A Analyst: <u>J</u>		1.73	1.93	0.77	0.50
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>M</u>		0.173	0.193	0.077	0.050
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.123	83.27.

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

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

Comments:





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

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

Test number: 95
 Test dates: October 04-11, 2022

Concentration (mg/L KO)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / initial number of larvae (mg)	Mean survival (%)	Mean weight / initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.73	22.70	7.97	0.797			0.797				
	B	10	10	14.63	22.03	7.40	0.740	0.736	6.6	0.740	100.0	0.736	6.6	Not applicable
	C	10	10	12.87	20.15	7.28	0.728			0.728				
	D	10	10	14.87	21.65	6.78	0.678			0.678				
300	E	10	10	14.85	20.97	6.12	0.612	0.643	10.8	0.612	100.0	0.643	10.8	12.7
	F	10	10	14.90	21.91	7.01	0.701			0.701				
	G	10	10	14.37	19.96	5.59	0.559			0.559				
	H	10	10	13.00	19.98	6.98	0.698			0.698				
450	I	10	10	15.15	21.90	6.75	0.675	0.666	7.2	0.675	100.0	0.666	7.2	9.5
	J	10	10	14.39	20.35	5.96	0.596			0.596				
	K	10	10	14.24	21.12	6.88	0.688			0.688				
	L	10	10	13.16	20.19	7.03	0.703			0.703				
600	M	10	10	16.08	22.21	6.13	0.613	0.595	8.3	0.613	97.5	0.581	11.5	21.0
	N	10	10	13.87	20.44	6.57	0.657			0.657				
	O	10	10	14.22	19.70	5.48	0.548			0.548				
	P	10	9	13.62	18.69	5.07	0.563			0.507				
750	Q	10	8	13.36	18.82	5.46	0.683	0.650	6.2	0.546	75.0	0.489	13.3	33.6
	R	10	8	16.30	21.71	5.41	0.676			0.541				
	S	10	7	14.89	19.41	4.52	0.646			0.452				
	T	10	7	15.33	19.49	4.16	0.594			0.416				
900	U	10	3	14.87	17.13	2.26	0.753	0.817	15.6	0.226	27.5	0.221	11.6	70.0
	V	10	3	14.02	16.08	2.06	0.687			0.206				
	W	10	2	14.17	16.13	1.96	0.980			0.196				
	X	10	3	13.62	16.16	2.54	0.847			0.254				
1050	Y	10	2	15.24	16.97	1.73	0.865	0.775	25.8	0.173	15.0	0.123	57.1	83.2
	Z	10	2	14.44	16.37	1.93	0.965			0.193				
	AA	10	1	13.04	13.81	0.77	0.770			0.077				
	BB	10	1	15.25	15.75	0.50	0.500			0.050				

Dunnett's MSD value: 0.0955
 PMSD: 13.0

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: 10/4/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 10/11/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas
Comments:		

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

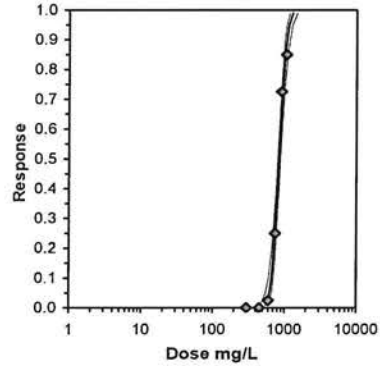
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	40
*900	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40
*1050	0.1500	0.1500	0.3927	0.3218	0.4636	20.862	4	10.00	10.00	34	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.89906	0.896	-0.7705	0.28329
Equality of variance cannot be confirmed				

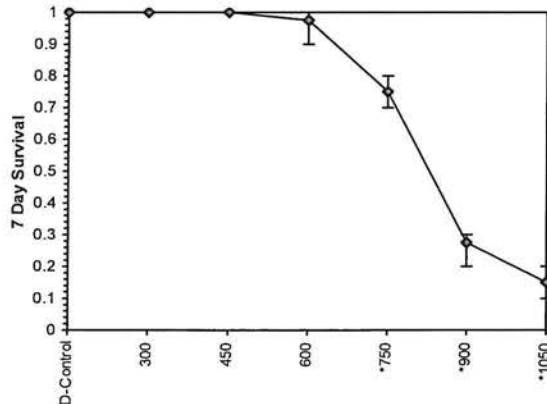
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	12.7033	1.6594	9.45086	15.9557	0	1.84365	9.48773	0.76449	2.92414	0.07872	3
Intercept	-32.146	4.85451	-41.661	-22.631							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	550.822	472.309	605.41
EC05	3.355	623.245	555.901	670.037
EC10	3.718	665.668	605.717	708.006
EC15	3.964	695.91	641.347	735.38
EC20	4.158	720.923	670.717	758.384
EC25	4.326	743.097	696.536	779.19
EC40	4.747	802.043	763.032	837.529
EC50	5.000	839.733	802.91	878.133
EC60	5.253	879.193	841.948	923.905
EC75	5.674	948.935	905.132	1011.94
EC80	5.842	978.122	930.002	1050.86
EC85	6.036	1013.28	959.127	1098.94
EC90	6.282	1059.31	996.242	1163.55
EC95	6.645	1131.42	1052.73	1267.78
EC99	7.326	1280.18	1165.14	1492.12



Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 10/4/2022	Test ID: PpKClCR	Sample ID: REF-Ref Toxicant
End Date: 10/11/2022	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

Conc-mg/L	1	2	3	4
D-Control	0.7970	0.7400	0.7280	0.6780
300	0.6120	0.7010	0.5590	0.6980
450	0.6750	0.5960	0.6880	0.7030
600	0.6130	0.6570	0.5480	0.5070
750	0.5460	0.5410	0.4520	0.4160
900	0.2260	0.2060	0.1960	0.2540
1050	0.1730	0.1930	0.0770	0.0500

Conc-mg/L	Transform: Untransformed							t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N				Mean	N-Mean
D-Control	0.7358	1.0000	0.7358	0.6780	0.7970	6.642	4				0.7358	1.0000
300	0.6425	0.8733	0.6425	0.5590	0.7010	10.785	4	2.235	2.290	0.0955	0.6540	0.8889
450	0.6655	0.9045	0.6655	0.5960	0.7030	7.171	4	1.684	2.290	0.0955	0.6540	0.8889
*600	0.5813	0.7900	0.5813	0.5070	0.6570	11.483	4	3.704	2.290	0.0955	0.5813	0.7900
750	0.4888	0.6643	0.4888	0.4160	0.5460	13.287	4				0.4888	0.6643
900	0.2205	0.2997	0.2205	0.1960	0.2540	11.601	4				0.2205	0.2997
1050	0.1233	0.1675	0.1233	0.0500	0.1930	57.074	4				0.1233	0.1675

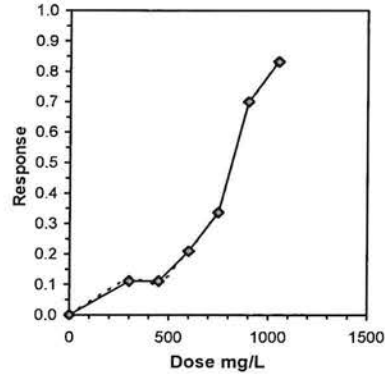
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93592	0.844	-0.2348	-1.3057
Bartlett's Test indicates equal variances (p = 0.89)	0.61155	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.09553	0.12984	0.01629	0.00348	0.02181	3, 12

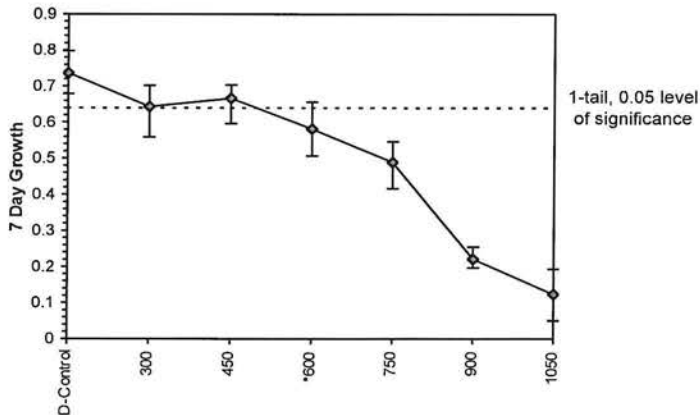
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew	
IC05*	135.00	89.46	49.46	666.69	2.2021
IC10*	270.00	120.77	98.93	683.79	0.5518
IC15	508.99	99.97	85.99	691.68	-1.1098
IC20	584.85	50.41	426.19	724.07	-0.1055
IC25	647.74	45.61	494.08	784.41	-0.0284
IC40	776.45	15.73	714.39	815.22	-0.9420
IC50	817.59	10.64	778.19	846.36	-0.5844

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Entered and Reviewed by Jim Sumner
JS

Species: Pimephales promelas

PpKICR Test Number: 95

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	
		U	EC U	EC U	U	U	EC U
Concentration	Parameter	Analyst					
CONTROL, MHSW	pH (S.U.)	7.51	7.09	7.18	7.19	7.50	7.00
	Dissolved oxygen (mg/L)	7.6	7.7	7.7	6.9	7.0	7.5
	Conductivity (µmhos/cm)	300		310		302	
	Alkalinity (mg CaCO ₃ /L)	60				60	
	Hardness (mg CaCO ₃ /L)	85				89	
	Temperature (°C)	24.8	24.5	24.7	24.7	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.64	7.33	7.59 7.21	7.21	7.59	7.10
	Dissolved oxygen (mg/L)	7.0	7.6	7.9	6.6	7.7	7.5
	Conductivity (µmhos/cm)	837		850		835	
	Temperature (°C)	24.9	24.7	24.7	24.8	24.8	24.8
450 mg KCl/L	pH (S.U.)	7.65	7.33	7.60 7.21	7.21	7.61	7.12
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	6.4	7.7	7.6
	Conductivity (µmhos/cm)	1080		1110		1080	
	Temperature (°C)	24.9	24.6	24.8	24.6	24.7	24.6
600 mg KCl/L	pH (S.U.)	7.67	7.34	7.60 7.21	7.21	7.63	7.14
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	6.7	7.0	7.6
	Conductivity (µmhos/cm)	1350		1360		1340	
	Temperature (°C)	25.0	24.6	24.7	24.6	24.8	24.8
750 mg KCl/L	pH (S.U.)	7.68	7.38	7.60 7.21	7.21	7.60	7.17
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.0	7.9	7.6
	Conductivity (µmhos/cm)	1600		1600		1590	
	Temperature (°C)	24.9	24.6	24.7	24.5	24.7	24.5
900 mg KCl/L	pH (S.U.)	7.69	7.39	7.60 7.22	7.22	7.67	7.30
	Dissolved oxygen (mg/L)	7.8	7.9	8.0	6.9	8.0	7.7
	Conductivity (µmhos/cm)	1860		1850		1840	
	Temperature (°C)	24.9	24.5	24.8	24.5	24.7	24.7
1050 mg KCl/L	pH (S.U.)	7.71	7.43	7.60 7.24	7.24	7.69	7.31
	Dissolved oxygen (mg/L)	7.0	7.8	8.0	7.0	8.1	7.0
	Conductivity (µmhos/cm)	2130		2110		2110	
	Temperature (°C)	24.9	24.5	24.8	24.8	24.7	24.7
		Initial	Final	Initial	Final	Initial	Final



* 7.60 ^{10-05-21 K}
* CONFIRMED pH

Species: *Pimephales promelas*

PpKICR Test Number: 95

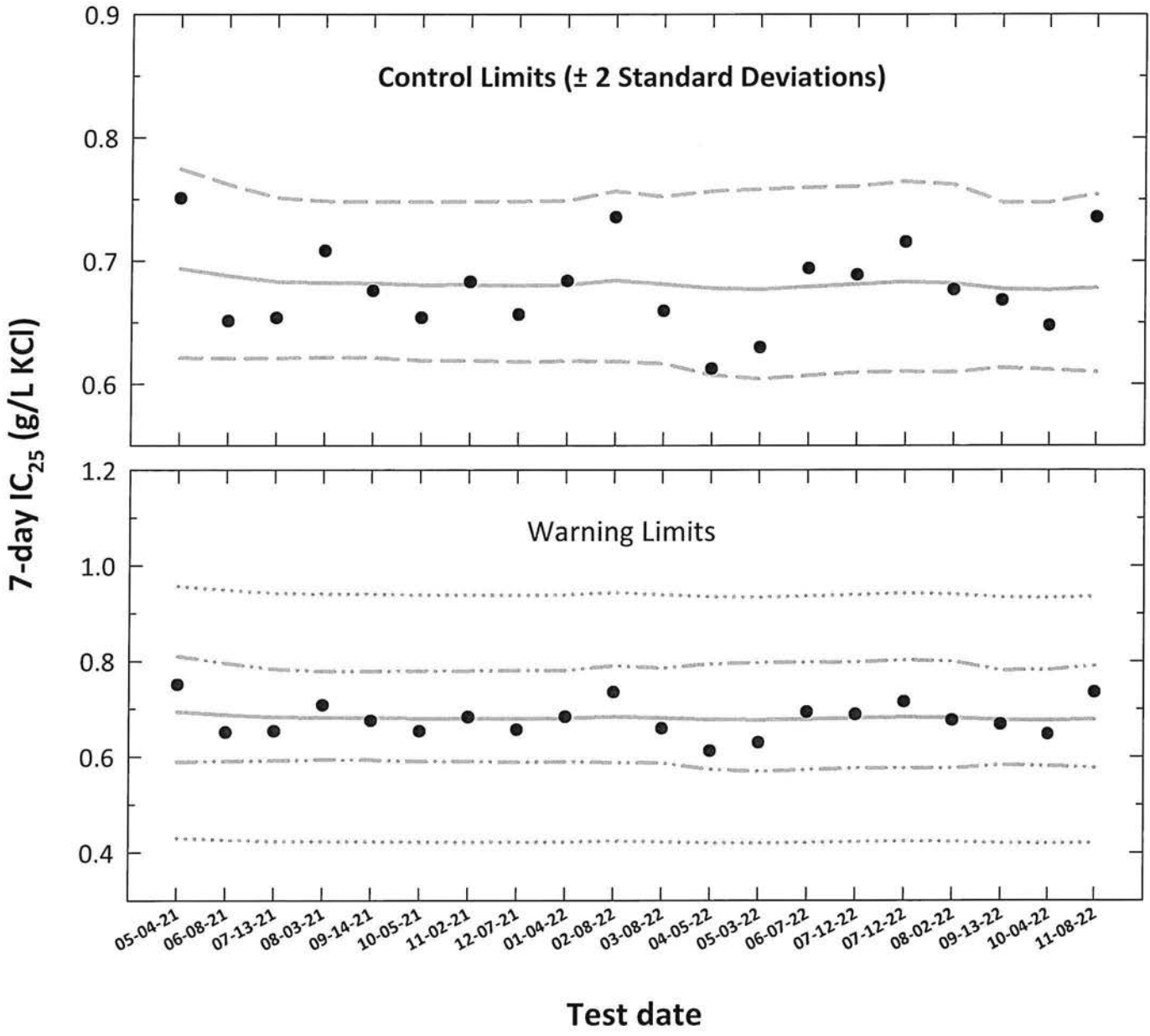
Concentration		Parameter	Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
			EC	u	EC	u	EC	u	EC	u
CONTROL, MHSW	pH (S.U.)	7.33	7.15	7.44	7.32	7.61	7.27	7.61	7.28	
	Dissolved oxygen (mg/L)	7.6	7.3	7.7	6.7	7.7	7.5	7.7	6.4	
	Conductivity (µmhos/cm)	314		307		302		317		
	Alkalinity (mg CaCO ₃ /L)			61						
	Hardness (mg CaCO ₃ /L)			87						
	Temperature (°C)	24.8	24.9	24.7	25.1	24.8	24.7	24.7	24.7	
300 mg KCl/L	pH (S.U.)	7.30	7.21	7.45	7.35	7.75	7.30	7.67	7.27	
	Dissolved oxygen (mg/L)	7.7	7.2	7.6	6.4	7.7	7.3	7.8	6.0	
	Conductivity (µmhos/cm)	860		847		803		864		
	Temperature (°C)	24.8	24.8	24.8	24.8	24.9	24.8	24.7	24.9	
450 mg KCl/L	pH (S.U.)	7.42	7.22	7.53	7.35	7.76	7.27	7.67	7.27	
	Dissolved oxygen (mg/L)	7.8	7.2	7.6	6.9	7.7	7.3	7.8	6.2	
	Conductivity (µmhos/cm)	1090		1090		1120		1090		
	Temperature (°C)	24.9	24.8	24.8	24.8	24.9	24.7	24.8	24.9	
600 mg KCl/L	pH (S.U.)	7.38	7.19	7.51	7.32	7.78	7.27	7.68	7.29	
	Dissolved oxygen (mg/L)	7.9	7.1	7.4	6.7	7.7	7.2	7.8	6.5	
	Conductivity (µmhos/cm)	1360		1420		1590		1350		
	Temperature (°C)	24.9	25.0	24.7	24.7	24.9	24.7	24.8	24.8	
750 mg KCl/L	pH (S.U.)	7.47	7.25	7.60	7.31	7.82	7.28	7.69	7.30	
	Dissolved oxygen (mg/L)	8.0	7.1	7.7	6.2	7.7	7.2	7.8	6.5	
	Conductivity (µmhos/cm)	1580		1580		1630		1610		
	Temperature (°C)	24.9	24.7	24.7	24.8	24.8	24.7	24.8	24.9	
900 mg KCl/L	pH (S.U.)	7.49	7.30	7.63	7.34	7.84	7.30	7.69	7.30	
	Dissolved oxygen (mg/L)	8.0	7.1	7.7	6.9	7.8	7.2	7.9	5.9	
	Conductivity (µmhos/cm)	1860		1890		1910		1870		
	Temperature (°C)	25.0	24.7	24.7	24.8	24.9	24.8	24.7	24.9	
1050 mg KCl/L	pH (S.U.)	7.52	7.31	7.65	7.35	7.86	7.30	7.70	7.28	
	Dissolved oxygen (mg/L)	8.0	7.2	7.7	6.5	7.8	7.4	8.0	5.5	
	Conductivity (µmhos/cm)	2120		2100		2120		2110		
	Temperature (°C)	24.8	24.7	24.7	24.7	24.9	24.8	24.8	24.7	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	

Independent Review by
Kelley E. Keenan:


Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ ± 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	05-04-21	0.7509	-0.1244	-0.1589	0.0240	0.6937	0.6212	0.7746	0.5892	0.8103	0.4301	0.9573
2	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
3	07-13-21	0.6537	-0.1846	-0.1655	0.0207	0.6831	0.6210	0.7513	0.5922	0.7830	0.4235	0.9426
4	08-03-21	0.7083	-0.1498	-0.1661	0.0201	0.6822	0.6219	0.7484	0.5938	0.7792	0.4230	0.9415
5	09-14-21	0.6756	-0.1703	-0.1663	0.0201	0.6818	0.6215	0.7481	0.5933	0.7790	0.4227	0.9410
6	10-05-21	0.6538	-0.1846	-0.1672	0.0205	0.6805	0.6190	0.7480	0.5902	0.7797	0.4219	0.9390
7	11-02-21	0.6829	-0.1656	-0.1670	0.0205	0.6808	0.6193	0.7483	0.5905	0.7799	0.4221	0.9395
8	12-07-21	0.6565	-0.1828	-0.1675	0.0208	0.6800	0.6180	0.7484	0.5887	0.7805	0.4216	0.9385
9	01-04-22	0.6838	-0.1651	-0.1670	0.0207	0.6808	0.6188	0.7490	0.5897	0.7810	0.4221	0.9395
10	02-08-22	0.7354	-0.1335	-0.1649	0.0219	0.6841	0.6184	0.7567	0.5881	0.7903	0.4241	0.9440
11	03-08-22	0.6594	-0.1808	-0.1668	0.0216	0.6811	0.6167	0.7522	0.5866	0.7855	0.4223	0.9399
12	04-05-22	0.6124	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
13	05-03-22	0.6299	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
14	06-07-22	0.6939	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372
15	07-12-22	0.6887	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
16	07-12-22	0.7153	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
17	08-02-22	0.6766	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
18	09-13-22	0.6682	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
19	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
20	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

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.

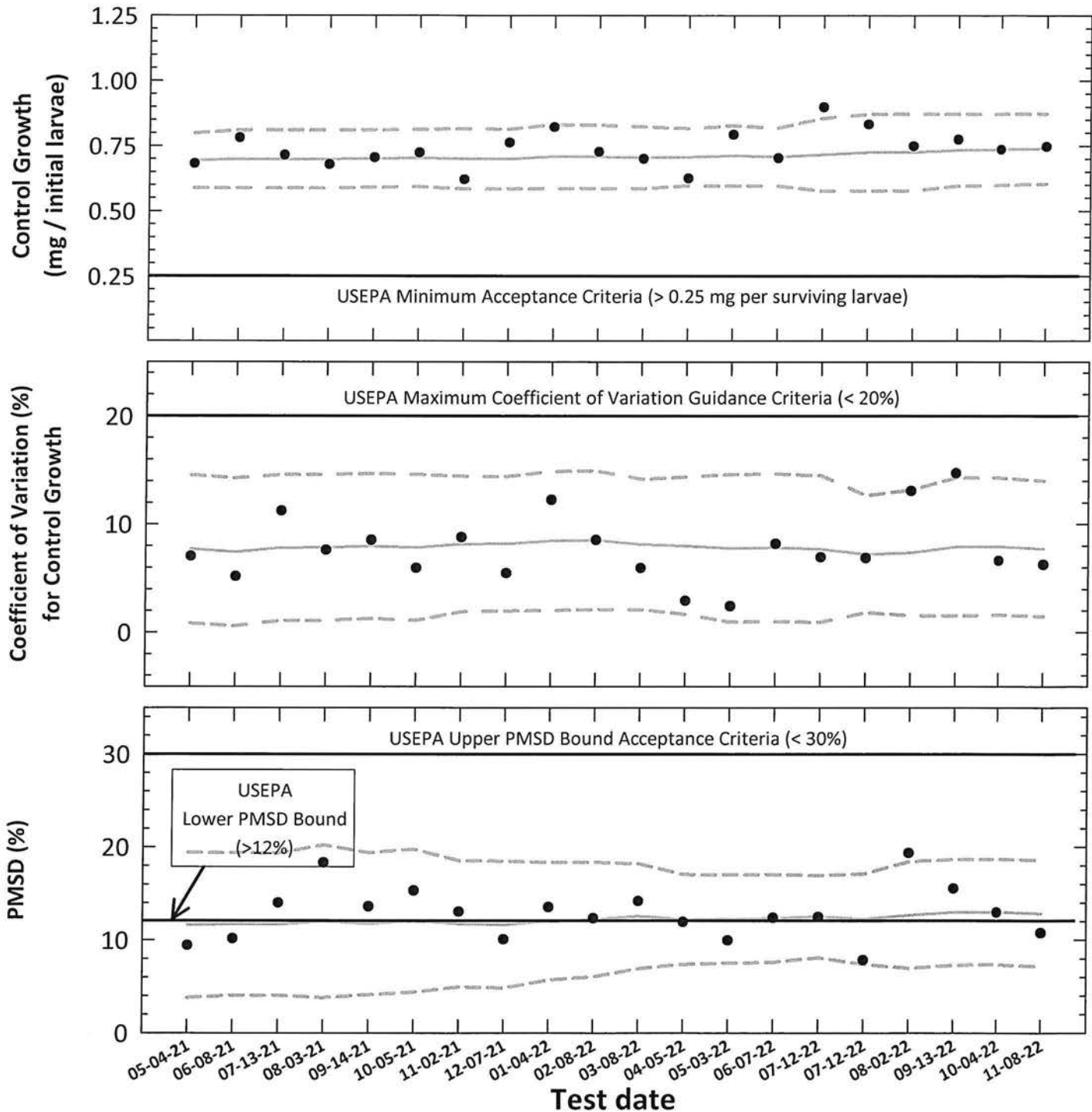
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

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 ± 2 Standard Deviations)

Entered and
Reviewed by
Jim Sumner

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

Test number	Test date	ToxCal Determination				Control Growth			Control Growth CV			Test PMSD			
		Control Survival (%)	Control Growth		PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval		
			Mean (mg/initial larvae)	CV (%)			CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S	
1	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.8	11.6	11.6	3.8	19.4
2	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	11.7	11.7	4.0	19.4
3	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	11.7	11.7	4.0	19.3
4	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	12.0	12.0	3.8	20.2
5	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	11.7	11.7	4.1	19.4
6	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	12.1	12.1	4.4	19.7
7	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	11.7	11.7	4.9	18.5
8	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	11.6	11.6	4.8	18.5
9	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	12.0	12.0	5.7	18.3
10	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	12.2	12.2	6.0	18.3
11	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	12.6	12.6	6.9	18.2
12	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	12.2	12.2	7.4	17.0
13	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	12.3	12.3	7.5	17.0
14	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	12.3	12.3	7.6	17.0
15	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	12.5	12.5	8.1	16.9
16	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.2	12.2	7.4	17.1
17	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	12.7	12.7	6.9	18.4
18	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	13.0	13.0	7.3	18.4
19	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	13.0	13.0	7.4	18.7
20	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	12.9	12.9	7.1	18.6

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%
PMSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



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

Species: *Pimephales promelas*

PpKCICR Test Number: 96

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2150</u>					
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.					
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>11-01-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>11-07-22 1502 to 11-08-22 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.51</u> S.U. Temperature = <u>25.0</u> °C	Date / Time in oven:	<u>11-15-22 0730</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>11-16-22 0730</u>
		*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24-1400RS</u>

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	11-08-22	0505	JL	1105	JL	0715	JL	11-03-22 B
1	11-09-22	0500	JL	1100	JL	0700	JL	↓
2	11-10-22	0500	JL	1115	JL	0700	JL	11-03-22 C
3	11-11-22	0500	JL	1100	JL	0700	JL	↓
4	11-12-22	0610	JL	1210	JL	0810	JL	11-10-22 A
5	11-13-22	0600	JL	1200	JL	0800	JL	↓
6	11-14-22	0600	JL	1200	JL	0800	JL	↓
7	11-15-22					0620	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	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>825.3</u>
Average weight per initial larvae:	<u>0.747</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.747</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>670.8</u>
			IC ₂₅ (mg/L KCl)	<u>735.4</u>

Species: Pimephales promelas

PpKICR Test Number: 96

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 ²⁶	10	10	10	
*A = Pan weight (mg) Tray color code: <u>G104</u> Analyst: <u>AJ</u> Date: <u>10-26-22</u>	15.22	14.55	14.43	13.54	13.43	14.68	13.19	15.97	12.47	12.65	15.38	14.08	
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>11-18-22</u>	22.91	22.25	22.10	20.31	21.05	22.32	20.92	22.64	21.97	21.21	23.41	22.15	
C = Larvae weight (mg) = B - A Analyst: <u>A</u>	7.69	7.70	7.73	6.77	7.62	7.64	7.73	6.67	9.50	8.56	8.03	8.07	
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>A</u>	0.769	0.770	0.773	0.677	0.762	0.764	0.773	0.667	0.950	0.856	0.803	0.807	
Average weight per initial number of larvae (mg)	0.747				0.742				0.87		0.854		-14.37
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

PpKCICR Test Number: 96

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}	5 ^{sd}	6 ^{id}	6 ^{id}
2	10	10	10	10	10	10	9	9	6	4 ^{id}	5 ^{id}	5 ^{id}
3	10	10	10	10	9 ^{id}	10	9	9	6	4	4 ^{id}	4 ^{id}
4	10	10	10	10	9	10	9	9	4 ^{2d}	3 ^{id}	3 ^{id}	3 ^{id}
5	10	10	10	9 ^{id}	9	9 ^{id}	8 ^{id}	8 ^{id}	4	3	2 ^{id}	3
6	10	10	10	9	9	9	8	8	4	3	2	3
7	10	10	10	9	9	8 ^{id}	8	8	2 ^{2d}	2 ^{id}	1 ^{id}	2 ^{id}
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>JAT</u> Date: <u>10-26-22</u>	14.78	13.44	13.98	14.38	14.67	15.16	13.60	13.94	13.46	12.29	15.07	14.42
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>11-18-22</u>	22.00	20.40	21.21	21.14	20.39	20.28	19.47	20.14	15.80	13.98	16.17	16.00
C = Larvae weight (mg) = B - A Analyst: <u>JH</u>	7.22	7.02	7.23	6.76	5.72	5.12	5.87	6.20	2.40	1.69	1.10	1.58
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JH</u>	0.722	0.702	0.723	0.676	0.572	0.512	0.587	0.620	0.240	0.169	0.110	0.158
Average weight per initial number of larvae (mg)	0.706				0.573				0.169			
Percent reduction from control (%)	5.67				23.47				77.47			

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

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

Comments:

Species: *Pimephales promelas*

PpKCICR Test Number: 96

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 rd	3 rd	3 rd	3 rd
2	3	3	3	3
3	3	3	3	1 rd
4	1 rd	2 rd	2 rd	1
5	1	2	2	1
6	1	2	2	1
7	1	1 rd	0 rd	1
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>AJ</u> Date: <u>10-26-22</u>	13.90	15.33	12.29	14.68
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>11-18-22</u>	14.60	15.90	→	15.02
C = Larvae weight (mg) = B - A Analyst: <u>jl</u>	0.76	0.57	→	0.94
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>jl</u>	0.076	0.057	0	0.094
Average weight per initial number of larvae (mg)	0.057		92.42	
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: 96
Test dates: November 08-15, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pin weight (mg)	B = Pin + 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	15.22	22.91	7.69	0.769	0.747	6.3	0.769	100.0	0.747	6.3	Not applicable
	B	10	10	14.55	22.25	7.70	0.770			0.770				
	C	10	10	14.43	22.16	7.73	0.773			0.773				
	D	10	10	13.54	20.31	6.77	0.677			0.677				
300	E	10	10	13.43	21.05	7.62	0.762	0.742	6.7	0.762	100.0	0.742	6.7	0.8
	F	10	10	14.68	22.32	7.64	0.764			0.764				
	G	10	10	13.19	20.92	7.73	0.773			0.773				
	H	10	10	15.97	22.64	6.67	0.667			0.667				
450	I	10	10	12.47	21.97	9.50	0.950	0.854	8.0	0.950	100.0	0.854	8.0	-14.3
	J	10	10	12.65	21.21	8.56	0.856			0.856				
	K	10	10	15.38	23.41	8.03	0.803			0.803				
	L	10	10	14.08	22.15	8.07	0.807			0.807				
600	M	10	10	14.78	22.00	7.22	0.722	0.725	2.8	0.722	97.5	0.706	3.1	5.6
	N	10	10	13.44	20.46	7.02	0.702			0.702				
	O	10	10	13.98	21.21	7.23	0.723			0.723				
	P	10	9	14.38	21.14	6.76	0.751			0.676				
750	Q	10	9	14.67	20.39	5.72	0.636	0.696	10.0	0.572	82.5	0.573	7.9	23.4
	R	10	8	15.16	20.28	5.12	0.640			0.512				
	S	10	8	13.60	19.47	5.87	0.734			0.587				
	T	10	8	13.94	20.14	6.20	0.775			0.620				
900	U	10	2	13.46	15.86	2.40	1.200	0.984	20.1	0.240	17.5	0.169	31.7	77.4
	V	10	2	12.29	13.98	1.69	0.845			0.169				
	W	10	1	15.07	16.17	1.10	1.100			0.110				
	X	10	2	14.42	16.00	1.58	0.790			0.158				
1050	Y	10	1	13.90	14.66	0.76	0.760	0.757	24.5	0.076	7.5	0.057	71.8	92.4
	Z	10	1	15.33	15.90	0.57	0.570			0.057				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	1	14.68	15.62	0.94	0.940			0.094				

Dunnett's MSD value: 0.0804
PMSD: 10.8

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: 11/8/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 11/15/2022	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

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	0.9000
750	0.9000	0.8000	0.8000	0.8000
900	0.2000	0.2000	0.1000	0.2000
1050	0.1000	0.1000	0.0000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.8250	0.8250	1.1426	1.1071	1.2490	6.209	4	10.00	10.00	7	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	40
*1050	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84517	0.896	-0.98	1.64735

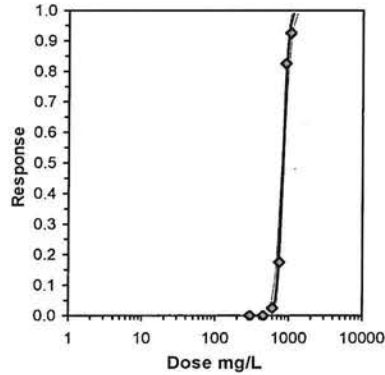
Equality of variance cannot be confirmed

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

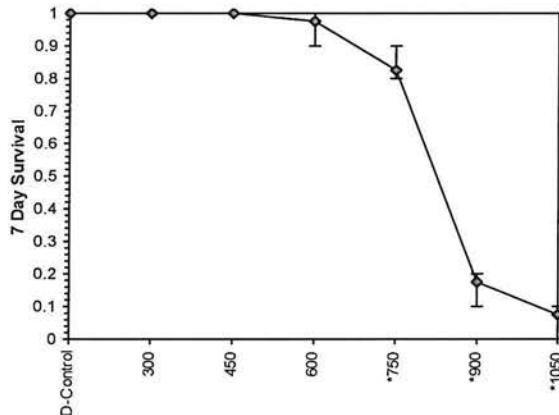
Treatments vs D-Control

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.1885	2.11383	12.0454 20.3316	0	4.41952	9.48773	0.3522	2.9166	0.06177	3
Intercept	-42.215	6.17979	-54.328 -30.103							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	592.778	522.418 640.839
EC05	3.355	653.115	593.547 694.079
EC10	3.718	687.752	634.821 724.841
EC15	3.964	712.153	663.906 746.78
EC20	4.158	732.162	687.646 765.049
EC25	4.326	749.776	708.369 781.429
EC40	4.747	796.061	761.334 826.516
EC50	5.000	825.27	792.997 857.133
EC60	5.253	855.551	823.963 891.057
EC75	5.674	908.366	873.658 955.319
EC80	5.842	930.219	892.959 983.454
EC85	6.036	956.355	915.349 1018.01
EC90	6.282	990.286	943.563 1064.08
EC95	6.645	1042.8	985.892 1137.49
EC99	7.326	1148.95	1068.33 1291.71



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS



Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 11/8/2022	Test ID: PpKICR	Sample ID: REF-Ref Toxicant
End Date: 11/15/2022	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

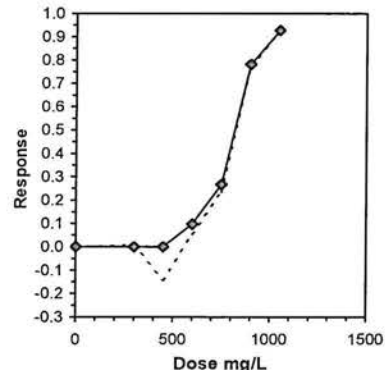
Conc-mg/L	1	2	3	4
D-Control	0.7690	0.7700	0.7730	0.6770
300	0.7620	0.7640	0.7730	0.6670
450	0.9500	0.8560	0.8030	0.8070
600	0.7220	0.7020	0.7230	0.6760
750	0.5720	0.5120	0.5870	0.6200
900	0.2400	0.1690	0.1100	0.1580
1050	0.0760	0.0570	0.0000	0.0940

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	0.7473	1.0000	0.7473	0.6770	0.7730	6.272	4				0.7809	1.0000
300	0.7415	0.9923	0.7415	0.6670	0.7730	6.729	4	0.164	2.290	0.0804	0.7809	1.0000
450	0.8540	1.1429	0.8540	0.8030	0.9500	8.008	4	-3.042	2.290	0.0804	0.7809	1.0000
600	0.7058	0.9445	0.7058	0.6760	0.7230	3.127	4	1.183	2.290	0.0804	0.7058	0.9037
750	0.5728	0.7665	0.5728	0.5120	0.6200	7.890	4				0.5728	0.7334
900	0.1693	0.2265	0.1693	0.1100	0.2400	31.713	4				0.1693	0.2167
1050	0.0568	0.0759	0.0568	0.0000	0.0940	71.785	4				0.0568	0.0727

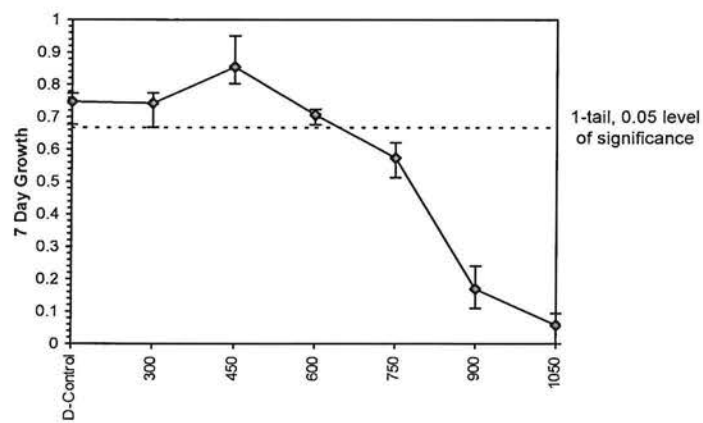
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90794	0.844	-0.0335	0.27168
Bartlett's Test indicates equal variances (p = 0.42)	2.85194	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.08035	0.10753	0.01635	0.00246	0.00681	3, 12

Point	mg/L	SD	Linear Interpolation (200 Resamples)		Skew
			95% CL(Exp)		
IC05	527.92	18.87	486.62	608.46	1.3184
IC10	603.30	20.83	524.76	652.58	-0.1720
IC15	647.34	18.08	583.23	704.19	0.0767
IC20	691.37	19.25	632.75	752.69	0.3541
IC25	735.41	17.46	678.86	771.46	-0.3915
IC40	788.74	6.41	767.94	806.60	-0.0052
IC50	817.77	6.21	797.43	837.83	0.2106



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Species: Pimephales promelas

PpKCICR Test Number: 96

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	
		ΣC	ΣC U	ΣC U	ΣC	ΣC	U
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.18	7.25	7.00	7.13	7.01	
	Dissolved oxygen (mg/L)	7.7	7.6	7.6	7.8	7.2	
	Conductivity (µmhos/cm)	813		312		317	
	Alkalinity (mg CaCO ₃ /L)	58			59		
	Hardness (mg CaCO ₃ /L)	85			85		
	Temperature (°C)	24.8	24.6	24.7	24.6	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.31	7.29	7.41	7.15	7.38	7.11
	Dissolved oxygen (mg/L)	7.7	7.6	7.7	7.6	7.8	7.2
	Conductivity (µmhos/cm)	878		850		832	
	Temperature (°C)	24.8	24.8	24.8	24.7	24.8	24.8
450 mg KCl/L	pH (S.U.)	7.40	7.33	7.47	7.22	7.46	7.14
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.6	7.8	7.2
	Conductivity (µmhos/cm)	1150		1130		1110	
	Temperature (°C)	24.9	24.5	24.8	24.7	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.45	7.30	7.51	7.25	7.50	7.17
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.6	7.9	7.3
	Conductivity (µmhos/cm)	1410		1370		1350	
	Temperature (°C)	24.9	24.5	24.8	24.7	24.8	24.5
750 mg KCl/L	pH (S.U.)	7.49	7.38	7.54	7.28	7.52	7.20
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.6	7.9	7.5
	Conductivity (µmhos/cm)	1670		1650		1620	
	Temperature (°C)	24.8	24.7	24.8	24.5	24.8	24.8
900 mg KCl/L	pH (S.U.)	7.52	7.41	7.57	7.30	7.54	7.21
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.7	7.9	7.5
	Conductivity (µmhos/cm)	1890		1900		1880	
	Temperature (°C)	24.8	24.7	24.9	24.5	24.7	24.8
1050 mg KCl/L	pH (S.U.)	7.53	7.43	7.59	7.38	7.54	7.24
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.7	7.9	7.5
	Conductivity (µmhos/cm)	2190		2220		2150	
	Temperature (°C)	24.8	24.6	24.9	24.5	24.7	24.8
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

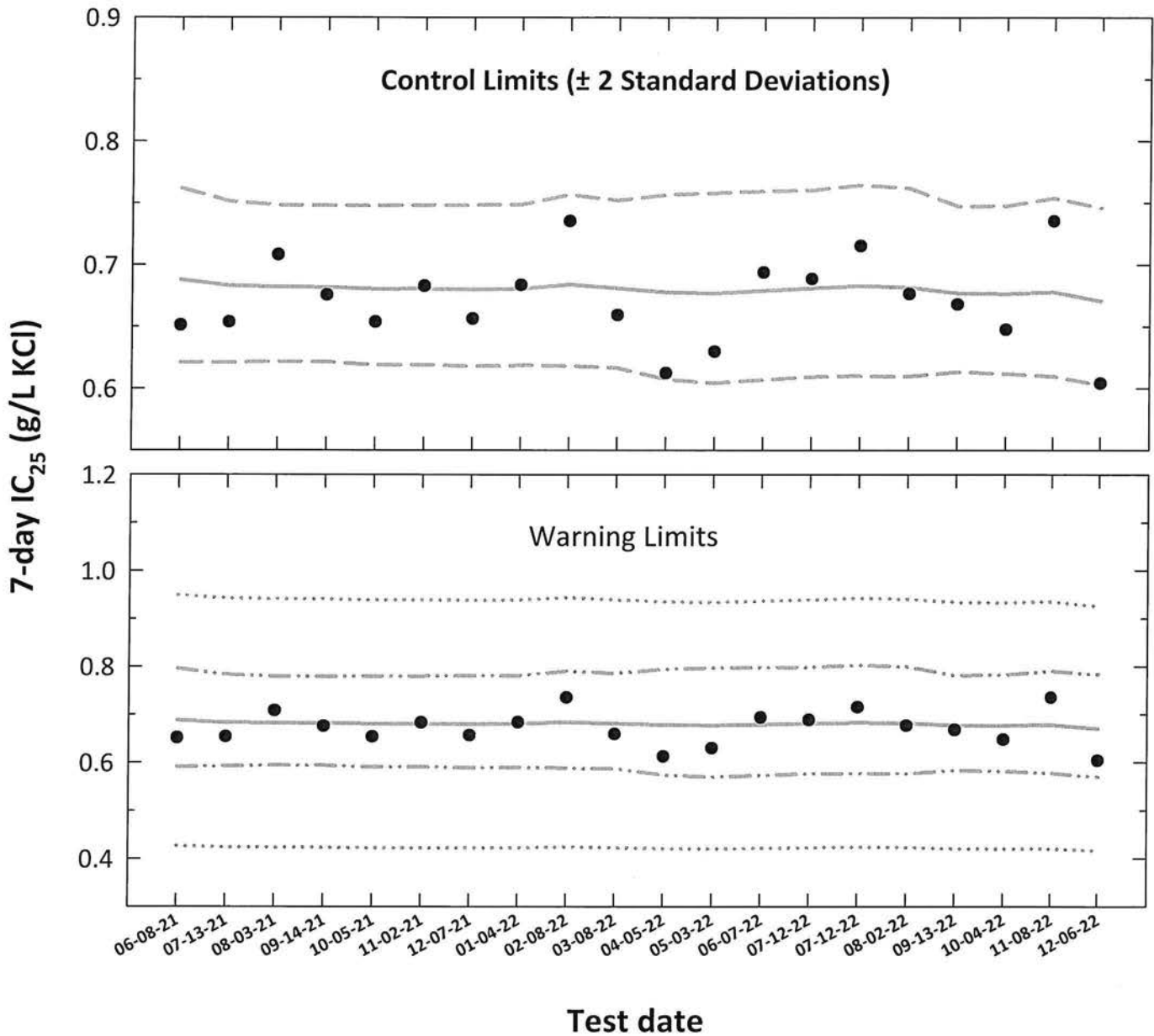
PpKCICR Test Number: 96

Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	u	BSL	BSL	u	u	EC u	EC u	EC
CONTROL, MHSW	pH (S.U.)	7.43	7.21	7.15	7.03	7.44	7.20	7.10	7.05
	Dissolved oxygen (mg/L)	7.9	7.3	7.7	6.7	7.6	7.5	7.6	7.5
	Conductivity (µmhos/cm)	307		300		313		304	
	Alkalinity (mg CaCO ₃ /L)			58					
	Hardness (mg CaCO ₃ /L)			83					
	Temperature (°C)	24.7	24.5	24.7	24.8	24.7	24.8	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.45	7.36	7.42	7.17	7.50	7.39	7.57	7.19
	Dissolved oxygen (mg/L)	8.0	7.3	7.8	6.7	7.0	7.3	7.9	7.5
	Conductivity (µmhos/cm)	835		823		844		852	
	Temperature (°C)	24.7	24.9	24.8	24.6	24.7	24.5	24.9	24.4
450 mg KCl/L	pH (S.U.)	7.47	7.39	7.49	7.19	7.59	7.36	7.58	7.23
	Dissolved oxygen (mg/L)	8.0	7.2	7.8	6.7	7.7	7.4	7.8	7.4
	Conductivity (µmhos/cm)	1090		1070		1110		1110	
	Temperature (°C)	24.7	24.9	24.7	24.6	24.8	24.8	24.9	24.5
600 mg KCl/L	pH (S.U.)	7.50	7.41	7.73	7.21	7.62	7.40	7.61	7.27
	Dissolved oxygen (mg/L)	8.0	7.2	7.8	6.5	7.7	7.5	7.8	7.4
	Conductivity (µmhos/cm)	1340		1330		1360		1360	
	Temperature (°C)	24.7	24.7	24.7	24.6	24.8	24.7	24.9	24.7
750 mg KCl/L	pH (S.U.)	7.53	7.43	7.75	7.23	7.64	7.43	7.63	7.30
	Dissolved oxygen (mg/L)	8.0	7.2	7.8	6.4	7.8	7.5	7.8	7.5
	Conductivity (µmhos/cm)	1600		1590		1590		1600	
	Temperature (°C)	24.8	24.7	24.7	24.7	24.9	24.7	25.0	24.4
900 mg KCl/L	pH (S.U.)	7.50	7.48	7.78	7.24	7.66	7.47	7.63	7.34
	Dissolved oxygen (mg/L)	8.0	7.2	7.9	6.6	7.8	7.6	7.9	7.5
	Conductivity (µmhos/cm)	1870		1840		1890		1870	
	Temperature (°C)	24.8	24.7	24.7	24.5	24.9	24.6	24.9	24.4
1050 mg KCl/L	pH (S.U.)	7.59	7.49	7.78	7.25	7.67	7.48	7.64	7.41
	Dissolved oxygen (mg/L)	8.0	7.2	7.9	6.9	7.8	7.6	7.9	7.5
	Conductivity (µmhos/cm)	2130		2610		2120		2130	
	Temperature (°C)	24.8	24.6	24.8	24.5	24.9	24.6	24.9	24.6
		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	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
2	07-13-21	0.6537	-0.1846	-0.1655	0.0207	0.6831	0.6210	0.7513	0.5922	0.7830	0.4235	0.9426
3	08-03-21	0.7083	-0.1498	-0.1661	0.0201	0.6822	0.6219	0.7484	0.5938	0.7792	0.4230	0.9415
4	09-14-21	0.6756	-0.1703	-0.1663	0.0201	0.6818	0.6215	0.7481	0.5933	0.7790	0.4227	0.9410
5	10-05-21	0.6538	-0.1846	-0.1672	0.0205	0.6805	0.6190	0.7480	0.5902	0.7797	0.4219	0.9390
6	11-02-21	0.6829	-0.1656	-0.1670	0.0205	0.6808	0.6193	0.7483	0.5905	0.7799	0.4221	0.9395
7	12-07-21	0.6565	-0.1828	-0.1675	0.0208	0.6800	0.6180	0.7484	0.5887	0.7805	0.4216	0.9385
8	01-04-22	0.6838	-0.1651	-0.1670	0.0207	0.6808	0.6188	0.7490	0.5897	0.7810	0.4221	0.9395
9	02-08-22	0.7354	-0.1335	-0.1649	0.0219	0.6841	0.6184	0.7567	0.5881	0.7903	0.4241	0.9440
10	03-08-22	0.6594	-0.1808	-0.1668	0.0216	0.6811	0.6167	0.7522	0.5866	0.7855	0.4223	0.9399
11	04-05-22	0.6124	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
12	05-03-22	0.6299	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
13	06-07-22	0.6939	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372
14	07-12-22	0.6887	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
15	07-12-22	0.7153	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
16	08-02-22	0.6766	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
17	09-13-22	0.6682	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
18	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
19	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
20	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

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.

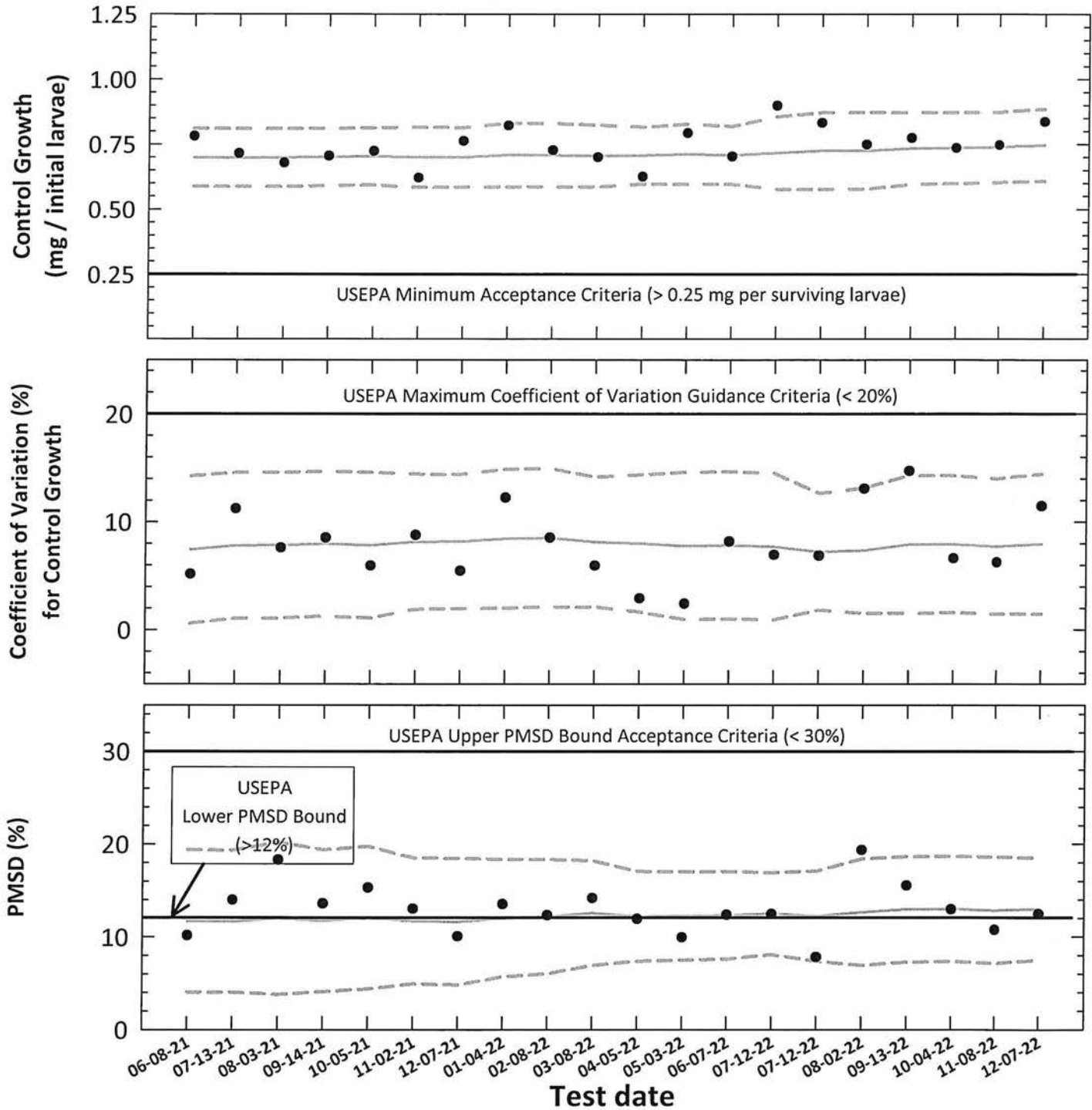
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

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 ± 2 Standard Deviations)

Entered and Reviewed by
Jim Sumner

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

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD (%)					
		Control Survival (%)	Control Growth		CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S				
			Mean (mg/initial larvae)	CV (%)								MSD	PMSD (%)		
1	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
2	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
3	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
4	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
5	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
6	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
7	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
8	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
9	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
10	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
11	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
12	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
13	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
14	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
15	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
16	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4
17	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	14.3	13.0	7.3	18.7
18	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	14.3	13.0	7.4	18.7
19	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
20	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

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%.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



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

Species: *Pimephales promelas*

PpKCICR Test Number: 97

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2128</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>11-30-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>12-05-22 1455 to 12-06-22 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.76</u> S.U. Temperature = <u>25.2</u> °C	Date / Time in oven:	<u>12-13-22 0730</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>12-14-22 0730</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	12-06-22	0505	JL	1230	JL	0715	JL	11-30-22 A
1	12-07-22	0500	JL	1100	JL	0700	JL	↓
2	12-08-22	0500	JL	1100	JL	0700	JL	11-30-22 D
3	12-09-22	0500	JL	1100	JL	0700	JL	↓
4	12-10-22	0600	JL	1200	JL	0800	JL	12-06-22 B
5	12-11-22	0600	JL	1200	JL	0800	JL	↓
6	12-12-22	0555	JL	1200	JL	0755	JL	↓
7	12-13-22					0642	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	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>01.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>754.3</u>
Average weight per initial larvae:	<u>0.837</u>		NOEC (mg/L KCl)	<u>300</u>
Average weight per surviving larvae:	<u>0.837</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>450</u>
			ChV (mg/L KCl)	<u>367.4</u>
			IC ₂₅ (mg/L KCl)	<u>604.1</u>

Species: Pimephales promelas

PpKICR Test Number: 97

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>Turquoise</u> Analyst: <u>EC</u> Date: <u>11-29-22</u>	13.62	15.43	13.07	14.82	15.50	15.40	12.35	14.36	15.33	11.63	16.09	15.04
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>12-15-22</u>	26.04	23.76	22.10	23.90	23.62	24.36	21.18	23.74	22.04	18.64	23.26	22.68
C = Larvae weight (mg) = B - A Analyst: <u>J</u>	7.02	8.33	9.03	9.08	8.12	8.96	8.83	9.38	6.71	7.01	7.17	7.64
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>	0.702	0.833	0.903	0.908	0.812	0.896	0.883	0.938	0.671	0.701	0.717	0.764
Average weight per initial number of larvae (mg)	0.837				0.882				0.713			
Percent reduction from control (%)									-5.57			

*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: 97

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L							
	M	N	O	P	Q	R	S	T	U	V	W	X				
0	10	10	10	10	10	10	10	10	10	10	10	10				
1	10	10	10	10	9 ^{1d}	8 ^{2d}	8 ^{2d}	8 ^{2d}	5 ^{5d}	5 ^{5d}	5 ^{5d}	5 ^{5d}				
2	10	10	10	10	9	8	8	8	5	5	4 ^{1d}	5				
3	10	10	10	10	9	8	8	8	5	4 ^{1d}	4	5				
4	10	10	10	10	8 ^{1d}	8	7 ^{1d}	7 ^{1d}	4 ^{1d}	3 ^{1d}	3 ^{1d}	4 ^{1d}				
5	10	9 ^{1d}	10	9 ^{1d}	7 ^{1d}	7 ^{1d}	6 ^{1d}	6 ^{1d}	2 ^{2d}	3	3	2 ^{2d}				
6	10	9	10	9	6 ^{1d}	4 ^{2d}	5 ^{1d}	5 ^{1d}	2	1 ^{2d}	1 ^{2d}	1 ^{1d}				
7	10	9	10	9	5 ^{1d}	4	5	5	2	1	1	1				
*A = Pan weight (mg) Tray color code: <u>Turquoise</u> Analyst: <u>EC</u> Date: <u>11-29-22</u>	13.68	15.50	15.19	15.70	13.29	14.85	10.21	14.78	15.88	14.24	13.44	14.49 14.39				
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>12-15-22</u>	19.81	22.78	21.20	22.34	10.87	18.83	20.90	18.50	17.80	15.45	14.71	15.20				
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	6.13	7.28	6.01	6.58	3.58	3.98	4.69	3.72	1.92	1.21	1.27	0.87				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0.613	0.728	0.601	0.658	0.358	0.398	0.469	0.372	0.192	0.121	0.127	0.087				
Average weight per initial number of larvae (mg)	0.652			22.17			0.399			52.37			0.132		84.27	
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

PpKCICR Test Number: 97

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 ^{sd}	1 ^{sd}	2 ^{sd}	2 ^{sd}
2	1 ^{id}	1	2	1 ^{id}
3	1	1	2	1
4	0 ^{id}	0 ^{id}	0 ^{sd}	1
5	0	0	0	1
6	0	0	0	1
7	0	0	0	1
*A = Pan weight (mg) Tray color code: <u>Turquoise</u> Analyst: <u>EC</u> Date: <u>11-29-22</u>	14.67	15.20	14.43	15.27
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>12-15-22</u>	—————→ 12-20-22			15.70
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	—————→ 12-20-22			0.49
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0	0	0	0.012
Average weight per initial number of larvae (mg)	0.012		48.57.	
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: 97
Test dates: December 07-14, 2022

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 (less 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	13.62	20.64	7.02	0.702	0.837	11.5	0.702	100.0	0.837	11.5	Not applicable
	B	10	10	15.43	23.76	8.33	0.833			0.833				
	C	10	10	13.07	22.10	9.03	0.903			0.903				
300	D	10	10	14.82	23.90	9.08	0.908	0.882	5.9	0.908	100.0	0.882	5.9	-5.5
	E	10	10	15.50	23.62	8.12	0.812			0.812				
	F	10	10	15.40	24.36	8.96	0.896			0.896				
450	G	10	10	12.35	21.18	8.83	0.883	0.713	5.4	0.883	100.0	0.713	5.4	14.7
	H	10	10	14.36	23.74	9.38	0.938			0.938				
	I	10	10	15.33	22.04	6.71	0.671			0.671				
600	J	10	10	11.63	18.64	7.01	0.701	0.690	14.2	0.701	95.0	0.652	8.6	22.1
	K	10	10	16.09	23.26	7.17	0.717			0.717				
	L	10	10	15.04	22.68	7.64	0.764			0.764				
750	M	10	10	13.68	19.81	6.13	0.613	0.848	16.4	0.613	47.5	0.399	12.4	52.3
	N	10	9	15.50	22.78	7.28	0.809			0.728				
	O	10	10	15.19	21.76	6.07	0.607	0.848	16.4	0.607	100.0	0.652	8.6	22.1
900	P	10	9	15.76	22.34	6.58	0.731	0.848	16.4	0.658	47.5	0.399	12.4	52.3
	Q	10	5	13.29	16.87	3.58	0.716			0.358				
	R	10	4	14.85	18.83	3.98	0.995			0.398				
1050	S	10	5	16.21	20.90	4.69	0.938	0.490	0.0	0.469	2.5	0.012	200.0	98.5
	T	10	5	14.78	18.50	3.72	0.744			0.372				
	U	10	2	15.88	17.80	1.92	0.960	1.078	17.9	0.192	12.5	0.132	33.3	84.2
Control	V	10	1	14.24	15.45	1.21	1.210			0.121				
	W	10	1	13.44	14.71	1.27	1.270			0.127				
	X	10	1	14.39	15.26	0.87	0.870			0.087				
Control	Y	10	0	0.00	0.00	0.00	0.000	0.490	0.0	0.000				
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
Control	BB	10	1	15.27	15.76	0.49	0.490			0.049				

Dunnett's MSD value: 0.1043
PMSD: 12.5
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: 12/6/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 12/13/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas
Comments:		

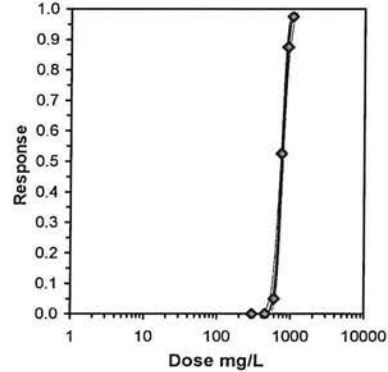
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	0.9000	1.0000	0.9000
750	0.5000	0.4000	0.5000	0.5000
900	0.2000	0.1000	0.1000	0.1000
*1050	0.0000	0.0000	0.0000	0.1000

Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.4750	0.4750	0.7602	0.6847	0.7854	6.622	4	10.00	10.00	21	40
*900	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

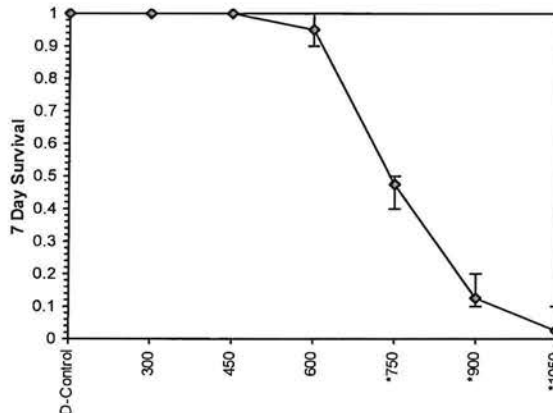
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.89436	0.896	0.71095	0.68435
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.0561	1.88017	11.371 18.7413	0	0.71123	9.48773	0.94994	2.87755	0.06642	3
Intercept	-38.325	5.43106	-48.97 -27.68							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	528.487	463.497	574.158
EC05	3.355	586.54	530.608	626.037
EC10	3.718	620.051	569.795	656.13
EC15	3.964	643.736	597.525	677.623
EC20	4.158	663.203	620.237	695.526
EC25	4.326	680.373	640.132	711.57
EC40	4.747	725.636	691.355	755.604
EC50	5.000	754.303	722.325	785.346
EC60	5.253	784.102	752.91	818.18
EC75	5.674	836.265	802.503	880.346
EC80	5.842	857.915	821.871	907.637
EC85	6.036	883.86	844.382	941.248
EC90	6.282	917.622	872.799	986.198
EC95	6.645	970.049	915.527	1058.14
EC99	7.326	1076.61	999.083	1210.34



Dose-Response Plot



Entered and Reviewed by
 Jon Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 12/6/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 12/13/2022	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

Conc-mg/L	1	2	3	4
D-Control	0.7020	0.8330	0.9030	0.9080
300	0.8120	0.8960	0.8830	0.9380
450	0.6710	0.7010	0.7170	0.7640
600	0.6130	0.7280	0.6070	0.6580
750	0.3580	0.3980	0.4690	0.3720
900	0.1920	0.1210	0.1270	0.0870
1050	0.0000	0.0000	0.0000	0.0490

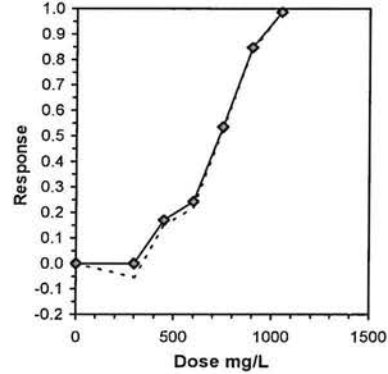
Conc-mg/L	Transform: Untransformed						N	t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.8365	1.0000	0.8365	0.7020	0.9080	11.474	4				0.8594	1.0000
300	0.8823	1.0547	0.8823	0.8120	0.9380	5.938	4	-1.005	2.290	0.1043	0.8594	1.0000
*450	0.7133	0.8527	0.7133	0.6710	0.7640	5.445	4	2.707	2.290	0.1043	0.7133	0.8300
*600	0.6515	0.7788	0.6515	0.6070	0.7280	8.572	4	4.063	2.290	0.1043	0.6515	0.7581
750	0.3993	0.4773	0.3993	0.3580	0.4690	12.364	4				0.3993	0.4646
900	0.1318	0.1575	0.1318	0.0870	0.1920	33.289	4				0.1318	0.1533
1050	0.0123	0.0146	0.0123	0.0000	0.0490	200.000	4				0.0123	0.0143

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94197	0.844	-0.6334	0.43209
Bartlett's Test indicates equal variances (p = 0.49)	2.39713	11.3449		

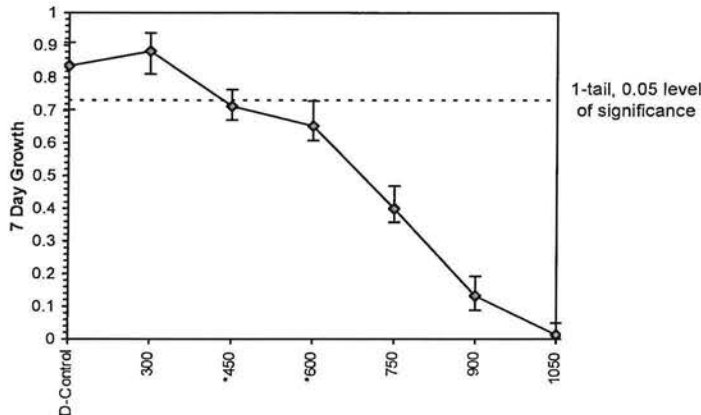
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	300	450	367.423		0.10426	0.12464	0.04571	0.00415	9.2E-04	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	344.11	23.82	273.70	382.79	-2.8392
IC10	388.22	22.67	326.05	465.57	1.3645
IC15	432.32	36.71	368.85	596.67	1.9277
IC20	512.55	55.72	380.97	678.29	0.3536
IC25	604.14	33.95	466.42	658.25	-0.8476
IC40	680.80	13.93	639.81	723.44	0.2063
IC50	731.90	13.79	694.10	778.09	0.2488



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Pimephales promelas

PpKCICR Test Number: 97

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.

Concentration		Parameter	Day					
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
			0		1		2	
Analyst		EC	EC N	EC N	U	U	EC	
CONTROL, MHSW	pH (S.U.)	7.29	7.23	7.27	7.28	7.44	7.31	
	Dissolved oxygen (mg/L)	7.8	7.9	7.7	7.2	7.7	7.2	
	Conductivity (µmhos/cm)	304		311		300		
	Alkalinity (mg CaCO ₃ /L)	62			211.2	63		
	Hardness (mg CaCO ₃ /L)	84				88		
	Temperature (°C)	24.8	24.7	24.9	24.7	24.8	25.0	
	300 mg KCl/L	pH (S.U.)	7.04	7.53	7.03	7.30	7.59	7.41
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.4	7.9	7.1	
	Conductivity (µmhos/cm)	1200 ²⁷ EC 1550 (898)		850		923		
	Temperature (°C)	24.7	24.5	24.8	24.6	24.8	24.8	
450 mg KCl/L	pH (S.U.)	7.07	7.54	7.06	7.38	7.61	7.45	
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.4	7.9	7.1	
	Conductivity (µmhos/cm)	1200 ²⁷ EC 1425 (1100)		1100		1080		
	Temperature (°C)	24.7	24.8	24.8	24.6	24.8	24.8	
600 mg KCl/L	pH (S.U.)	7.70	7.55	7.08	7.40	7.63	7.48	
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.5	7.9	7.3	
	Conductivity (µmhos/cm)	1200 ²⁷ EC 1220 (1110)		1300		1330		
	Temperature (°C)	24.7	24.9	24.9	24.6	24.9	24.7	
750 mg KCl/L	pH (S.U.)	7.72	7.59	7.70	7.41	7.64	7.51	
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.5	7.9	7.2	
	Conductivity (µmhos/cm)	1070		1010		1500		
	Temperature (°C)	24.8	24.9	24.9	24.8	24.9	24.7	
900 mg KCl/L	pH (S.U.)	7.74	7.01	7.73	7.43	7.66	7.52	
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.6	8.0	7.2	
	Conductivity (µmhos/cm)	1930		1880		1840		
	Temperature (°C)	24.9	24.7	24.9	24.5	24.9	24.9	
1050 mg KCl/L	pH (S.U.)	7.75	7.02	7.74	7.47	7.66	7.55	
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.7	8.0	7.2	
	Conductivity (µmhos/cm)	2170		2140		2100		
	Temperature (°C)	24.9	24.7	24.9	24.7	24.9	24.7	
		Initial	Final	Initial	Final	Initial	Final	

Species: *Pimephales promelas*

PpKCICR Test Number: 97

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		EC	BSL	BSL	BSL	BSL	EC	EC	EC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.20	7.23	7.12	*7.16 (7.2)	7.15	7.18	7.20	7.10
	Dissolved oxygen (mg/L)	7.8	7.9	7.8	7.4	7.7	7.5	7.8	7.4
	Conductivity (µmhos/cm)	304		301		310		300	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)		12-11-22	86					12-11-22
	Temperature (°C)	24.8	25.0	24.7	24.7	24.8	24.6	24.8	24.4
300 mg KCl/L	pH (S.U.)	7.05	7.37	7.61	7.40	7.70	7.43	7.04	7.24
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.4	7.9	7.5	7.8	7.3
	Conductivity (µmhos/cm)	848		854		860		830	
	Temperature (°C)	24.9	24.7	24.7	24.6	24.7	24.6	24.8	24.4
450 mg KCl/L	pH (S.U.)	7.00	7.41	7.64	7.43	7.75	7.44	7.07	7.29
	Dissolved oxygen (mg/L)	7.8	7.7	8.0	7.4	8.0	7.4	7.9	7.3
	Conductivity (µmhos/cm)	1080		1090		1110		1070	
	Temperature (°C)	24.8	24.7	24.8	24.6	24.7	24.7	24.9	24.4
600 mg KCl/L	pH (S.U.)	7.08	7.49	7.67	*7.43 (7.50)	7.79	7.40	7.09	7.39
	Dissolved oxygen (mg/L)	7.8	7.5	8.0	7.3	8.0	7.4	7.9	7.3
	Conductivity (µmhos/cm)	1330		1340		1350		1320	
	Temperature (°C)	24.8	24.7	24.8	24.6	24.7	24.5	24.9	24.6
750 mg KCl/L	pH (S.U.)	7.09	7.51	7.69	*7.50 (7.50)	7.81	7.47	7.71	7.42
	Dissolved oxygen (mg/L)	7.8	7.5	8.0	7.3	8.0	7.4	8.0	7.4
	Conductivity (µmhos/cm)	1560		1580		1630		1570	
	Temperature (°C)	24.9	24.6	24.8	24.6	24.7	24.7	24.9	24.4
900 mg KCl/L	pH (S.U.)	7.71	7.44	7.71	*7.50 (7.50)	7.83	7.50	7.73	7.44
	Dissolved oxygen (mg/L)	7.8	7.6	8.0	7.3	8.0	7.3	8.0	7.4
	Conductivity (µmhos/cm)	1830		1850		1920		1830	
	Temperature (°C)	24.9	24.7	24.8	24.7	24.8	24.7	24.9	24.4
1050 mg KCl/L	pH (S.U.)	7.72	7.55	7.72	*7.00 (7.50)	7.85	7.55	7.74	7.50
	Dissolved oxygen (mg/L)	7.9	7.4	8.0	7.3	8.0	7.4	8.1	7.4
	Conductivity (µmhos/cm)	2100		2090		2140		2120	
	Temperature (°C)	25.0	24.8	24.8	24.6	24.8	24.6	24.9	24.3
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 12-11-22 wrote in wrong place
VALUES CONFIRMED SOP AT21-Revision 5-Exhibit AT21.1