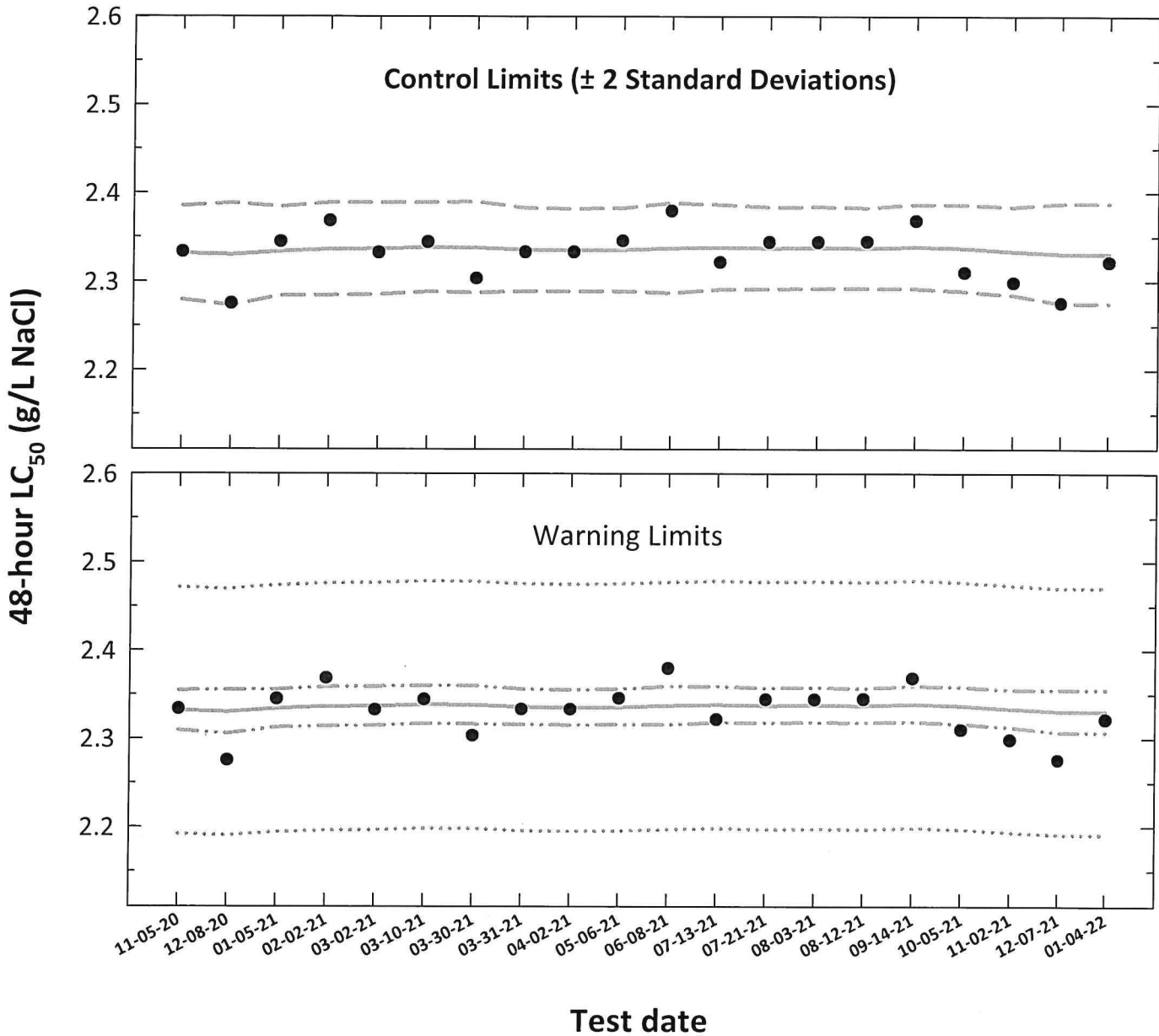


Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



11-05-20 12-08-20 01-05-21 02-02-21 03-02-21 03-10-21 03-30-21 03-31-21 04-02-21 05-06-21 06-08-21 07-13-21 07-21-21 08-03-21 08-12-21 09-14-21 10-05-21 11-02-21 12-07-21 01-04-22

- **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		CT	Anti-logarithmic Values (g/L NaCl)					
			48-hour LC ₅₀	CT		S	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}		
1	11-05-20	2.3335	0.3680	0.3677	2.3317	2.2795	2.3852	2.3093	2.3547	2.1918	2.4716
2	12-08-20	2.2753	0.3570	0.3674	2.3300	2.2727	2.3887	2.3054	2.3552	2.1902	2.4698
3	01-05-21	2.3449	0.3701	0.3681	2.3339	2.2843	2.3846	2.3126	2.3556	2.1938	2.4739
4	02-02-21	2.3682	0.3744	0.3685	2.3362	2.2847	2.3888	2.3141	2.3587	2.1960	2.4763
5	03-02-21	2.3324	0.3678	0.3686	2.3367	2.2856	2.3890	2.3148	2.3591	2.1965	2.4769
6	03-10-21	2.3442	0.3700	0.3689	2.3384	2.2887	2.3892	2.3171	2.3601	2.1981	2.4787
7	03-30-21	2.3032	0.3623	0.3689	2.3381	2.2874	2.3898	2.3164	2.3602	2.1978	2.4783
8	03-31-21	2.3330	0.3679	0.3684	2.3356	2.2893	2.3829	2.3158	2.3558	2.1955	2.4757
9	04-02-21	2.3330	0.3679	0.3683	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
10	05-06-21	2.3455	0.3702	0.3684	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
11	06-08-21	2.3795	0.3765	0.3687	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
12	07-13-21	2.3217	0.3658	0.3689	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
13	07-21-21	2.3442	0.3700	0.3687	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
14	08-03-21	2.3442	0.3700	0.3688	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
15	08-12-21	2.3449	0.3701	0.3687	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
16	09-14-21	2.3682	0.3744	0.3690	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
17	10-05-21	2.3099	0.3636	0.3687	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
18	11-02-21	2.2985	0.3614	0.3681	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
19	12-07-21	2.2755	0.3571	0.3675	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
20	01-04-22	2.3217	0.3658	0.3675	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708

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.

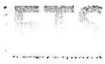
S = Standard deviation of the LC₅₀ values.

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 # 314

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 #: 2060

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	<u>W</u>	<u>W</u>	<u>W</u>
Control, MHSW	pH (S.U.)	<u>7.40</u>	<u>7.02</u>	<u>7.72</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.9</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>304</u>		
	Alkalinity (mg/L CaCO ₃)	<u>61</u>		
	Hardness (mg/L CaCO ₃)	<u>94</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.2</u>	<u>25.1</u>
1750 mg/L	pH (S.U.)	<u>7.01</u>	<u>7.07</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>3540</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.3</u>	<u>24.9</u>
2000 mg/L	pH (S.U.)	<u>7.01</u>	<u>7.91</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>8.0</u>	<u>7.8</u>
	Conductivity (µmhos/cm)	<u>3970</u>		
	Temperature (°C)	<u>25.0</u>	<u>25.0</u>	<u>25.1</u>
2250 mg/L	pH (S.U.)	<u>7.00</u>	<u>7.92</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>8.0</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>4440</u>		
	Temperature (°C)	<u>25.0</u>	<u>25.3</u>	<u>25.1</u>
2500 mg/L	pH (S.U.)	<u>7.00</u>	<u>7.91</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>8.1</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>4900</u>		
	Temperature (°C)	<u>25.0</u>	<u>25.1</u>	<u>25.2</u>
2750 mg/L	pH (S.U.)	<u>7.00</u>	<u>7.90</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>8.1</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>5320</u>		
	Temperature (°C)	<u>25.0</u>	<u>25.1</u>	<u>25.0</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

CdNaCIAC # 374

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-04-22	0630	J	0832	J	2B3	RES	12-19-21C
24	01-05-22			0829	J			
48 Termination	01-06-22			0830	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):	12-28-21 A
Age:	< 24-hours old
Date and time organisms were born between:	01-03-22 1411 TO 01-04-22 0630
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.5P
	Temperature (°C): 21.9°C

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	4 ^{1d}	S	S	4 ^{1d}	4 ^{1d}	2 ^{2d}	2 ^{2d}	2 ^{2d}
48 Termination	4 ^{1d}	2 ^{2d}	3 ^{2d}	3 ^{2d}	2 ^{2d}	2 ^{2d}	0 ^{5d}	1 ^{3d}	0 ^{4d}	0 ^{2d}	0 ^{2d}	0 ^{2d}
Mean Survival	60%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2391.4
Upper 95% confidence limit (mg NaCl/L)	2391.9
48-hour LC ₅₀ (mg NaCl/L)	2321.7

Comments:

Test Reviewed by: J

Acute Daphnid Test-24 Hr Survival

Start Date: 1/4/2022 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
 End Date: 1/6/2022 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	1.0000	1.0000	1.0000	1.0000
2500	0.8000	1.0000	1.0000	0.8000
2750	0.8000	0.4000	0.4000	0.4000

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	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2500	0.9000	0.9000	1.2262	1.1071	1.3453	11.212	4	14.00	10.00	2	20
*2750	0.5000	0.5000	0.7903	0.6847	1.1071	26.725	4	10.00	10.00	10	20

Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Statistic: 0.71358 Critical: 0.884 Skew: 1.7781 Kurt: 5.82258

Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU

Steel's Many-One Rank Test 2500 2750 2622.02

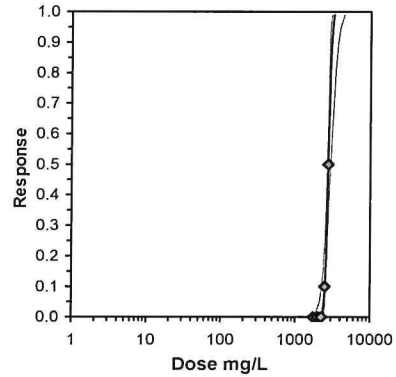
Treatments vs D-Control

Maximum Likelihood-Probit

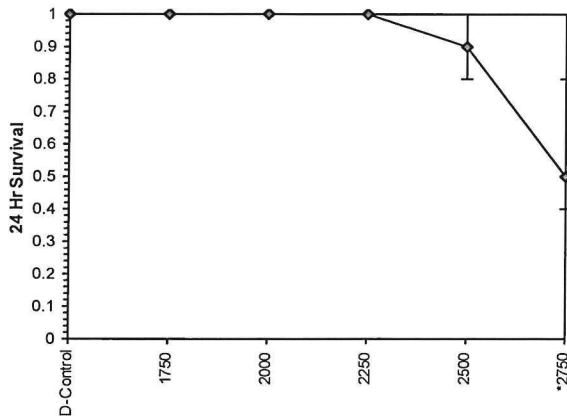
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	32.441	10.0555	12.7323	52.1497	0	0.06733	7.81472	0.99545	3.43892	0.03083	3
Intercept	-106.56	34.4261	-174.04	-39.087							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2329.23	1893.96	2461.17
EC05	3.355	2444.66	2135.34	2544.71
EC10	3.718	2508.52	2272.16	2595.18
EC15	3.964	2552.55	2365.21	2634.44
EC20	4.158	2588.09	2437.11	2671.27
EC25	4.326	2618.97	2494.94	2709.35
EC40	4.747	2698.44	2611.72	2845.45
EC50	5.000	2747.4	2662.43	2954.99
EC60	5.253	2797.25	2704.94	3079.16
EC75	5.674	2882.13	2767.34	3308.88
EC80	5.842	2916.52	2790.75	3406.85
EC85	6.036	2957.13	2817.56	3525.63
EC90	6.282	3009.03	2850.89	3681.98
EC95	6.645	3087.63	2899.93	3928.03
EC99	7.326	3240.65	2992.19	4437.78



Dose-Response Plot



Acute Daphnid Test-48 Hr Survival

Start Date: 1/4/2022 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
 End Date: 1/6/2022 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	0.8000	0.4000	0.6000	0.6000
2500	0.2000	0.4000	0.0000	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%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4		0	20	
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.6000	0.6000	0.8910	0.6847	1.1071	19.366	4	10.00	10.00	8	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Statistic: 0.65528 Critical: 0.868 Skew: -0.0017 Kurt: 3.03101

Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)

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

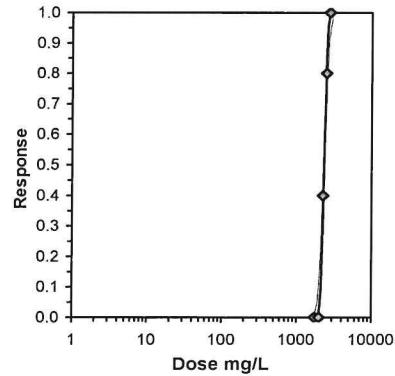
Treatments vs D-Control

Maximum Likelihood-Probit

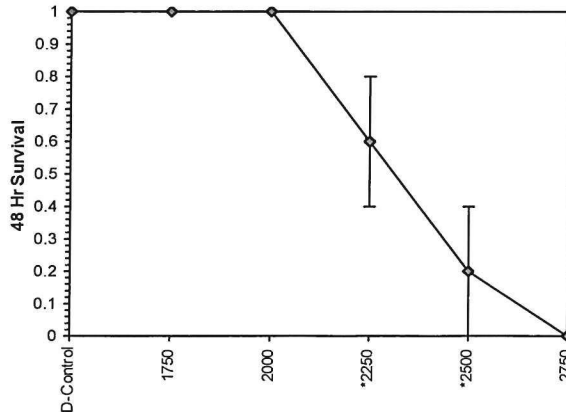
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	32.4604	6.07902	20.5455	44.3753	0	1.40785	7.81472	0.70369	3.3658	0.03081	5
Intercept	-104.26	20.4773	-144.39	-64.12							

TSCR

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



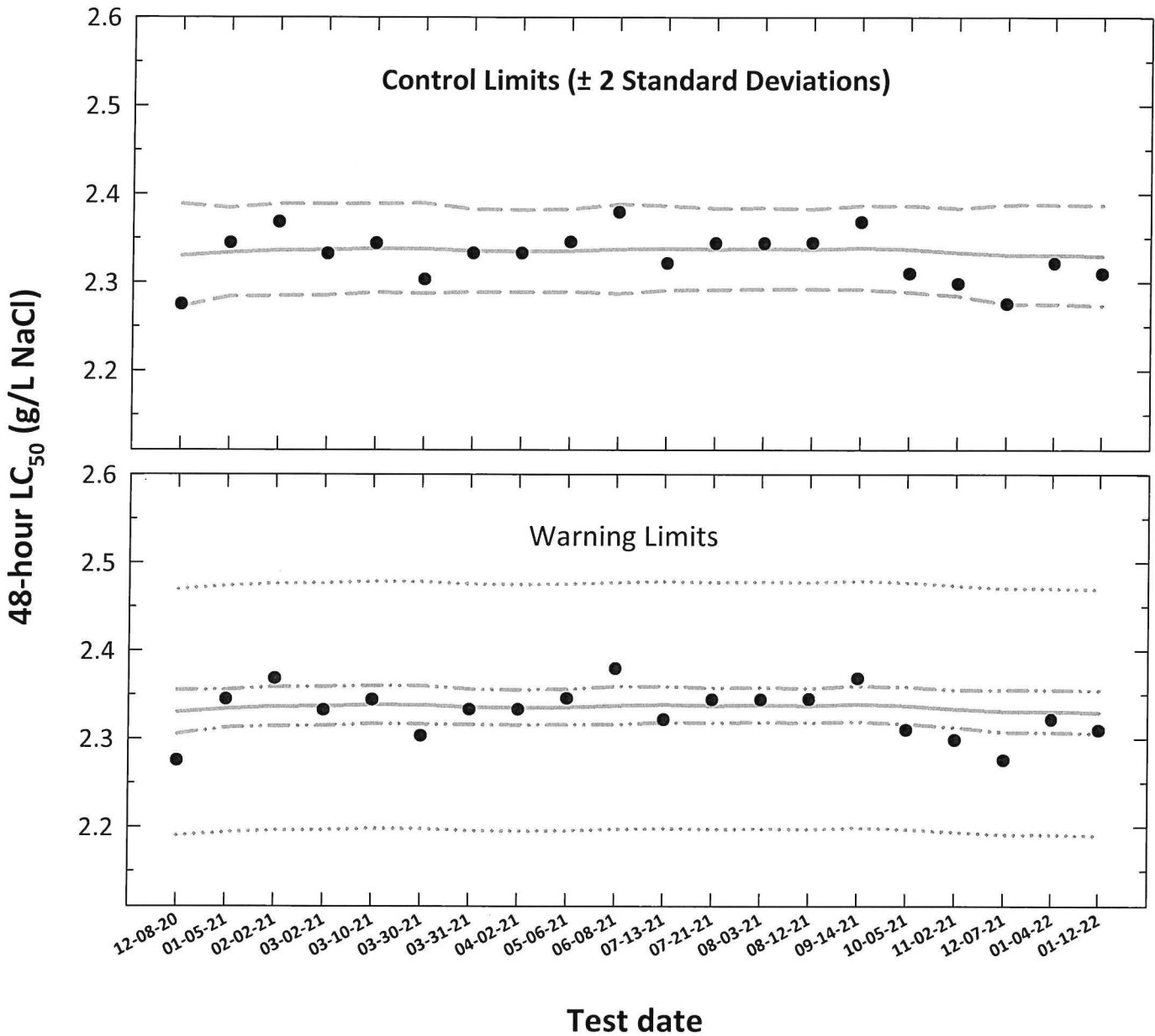
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	12-08-20	2.2753	0.3570	0.3674	0.0054	2.3300	2.2727	2.3887	2.3054	2.3552	2.1902	2.4698
2	01-05-21	2.3449	0.3701	0.3681	0.0047	2.3339	2.2843	2.3846	2.3126	2.3556	2.1938	2.4739
3	02-02-21	2.3682	0.3744	0.3685	0.0048	2.3362	2.2847	2.3888	2.3141	2.3587	2.1960	2.4763
4	03-02-21	2.3324	0.3678	0.3686	0.0048	2.3367	2.2856	2.3890	2.3148	2.3591	2.1965	2.4769
5	03-10-21	2.3442	0.3700	0.3689	0.0047	2.3384	2.2887	2.3892	2.3171	2.3601	2.1981	2.4787
6	03-30-21	2.3032	0.3623	0.3689	0.0048	2.3381	2.2874	2.3898	2.3164	2.3602	2.1978	2.4783
7	03-31-21	2.3330	0.3679	0.3684	0.0043	2.3356	2.2893	2.3829	2.3158	2.3558	2.1955	2.4757
8	04-02-21	2.3330	0.3679	0.3683	0.0043	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
9	05-06-21	2.3455	0.3702	0.3684	0.0044	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
10	06-08-21	2.3795	0.3765	0.3687	0.0047	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
11	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
12	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
13	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
14	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
15	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
16	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3338	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
17	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
18	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
19	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
20	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

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 # 315

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 #: 2060

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	DW 7.66	DW 7.48	K 7.70
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	312		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	82		
	Temperature (°C)	24.8	25.2	25.2
1750 mg/L	pH (S.U.)	2.79	7.78	7.07
	Dissolved oxygen (mg/L)	7.8	7.9	7.0
	Conductivity (µmhos/cm)	3630		
	Temperature (°C)	24.8	25.0	25.0
2000 mg/L	pH (S.U.)	7.82	7.79	7.90
	Dissolved oxygen (mg/L)	7.9	7.4	7.0
	Conductivity (µmhos/cm)	4040		
	Temperature (°C)	24.9	25.2	25.1
2250 mg/L	pH (S.U.)	7.83	7.80	7.90
	Dissolved oxygen (mg/L)	7.9	7.8	7.0
	Conductivity (µmhos/cm)	4510		
	Temperature (°C)	24.9	25.2	25.1
2500 mg/L	pH (S.U.)	7.84	7.82	7.90
	Dissolved oxygen (mg/L)	7.9	7.9	8.0
	Conductivity (µmhos/cm)	4840		
	Temperature (°C)	24.8	25.1	25.3
2750 mg/L	pH (S.U.)	7.84	7.82	7.93
	Dissolved oxygen (mg/L)	7.9	8.0	8.0
	Conductivity (µmhos/cm)	5400		
	Temperature (°C)	24.9	25.0	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 Test

CdNaClAC # 375

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-12-22	0600	J	0800	J	2F	RES	01-01-22
24	01-13-22			0755	J			
48 Termination	01-14-22			0800	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):	01-04-22 A
Age:	< 24-hours old
Date and time organisms were born between:	01-11-22 1449 TO 01-12-22 0600
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.89
	Temperature (°C): 25.0

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	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	S	S	S	3 ^u	4 ^u	S	S
48 Termination	2 ^u	4 ^u	2 ^u	2 ^u	2 ^u	1 ^u	2 ^u	0 ^u	0 ^u	0 ^u	0 ^u	0 ^u
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:

Test Reviewed by:

Acute Daphnid Test-48 Hr Survival

Start Date: 1/12/2022 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 1/14/2022 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:

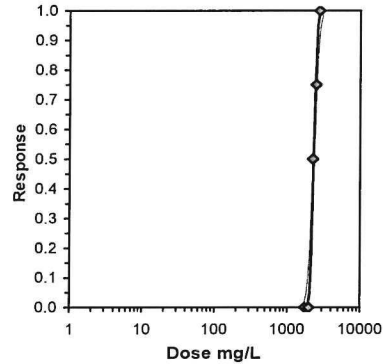
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.8000	0.4000	0.4000
2500	0.4000	0.2000	0.4000	0.0000
2750	0.0000	0.0000	0.0000	0.0000

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	0.2500	0.2500	0.5146	0.2255	0.6847	42.578	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

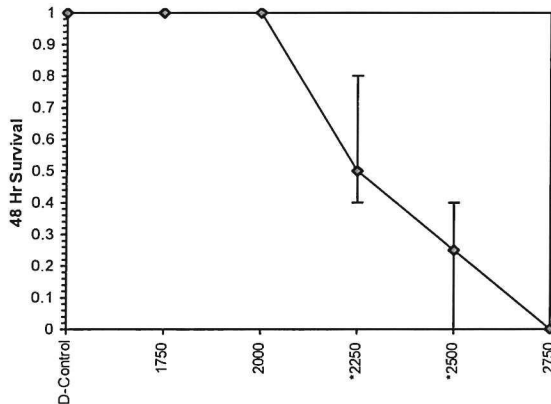
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8178	0.868	0.45632	2.91711
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

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

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



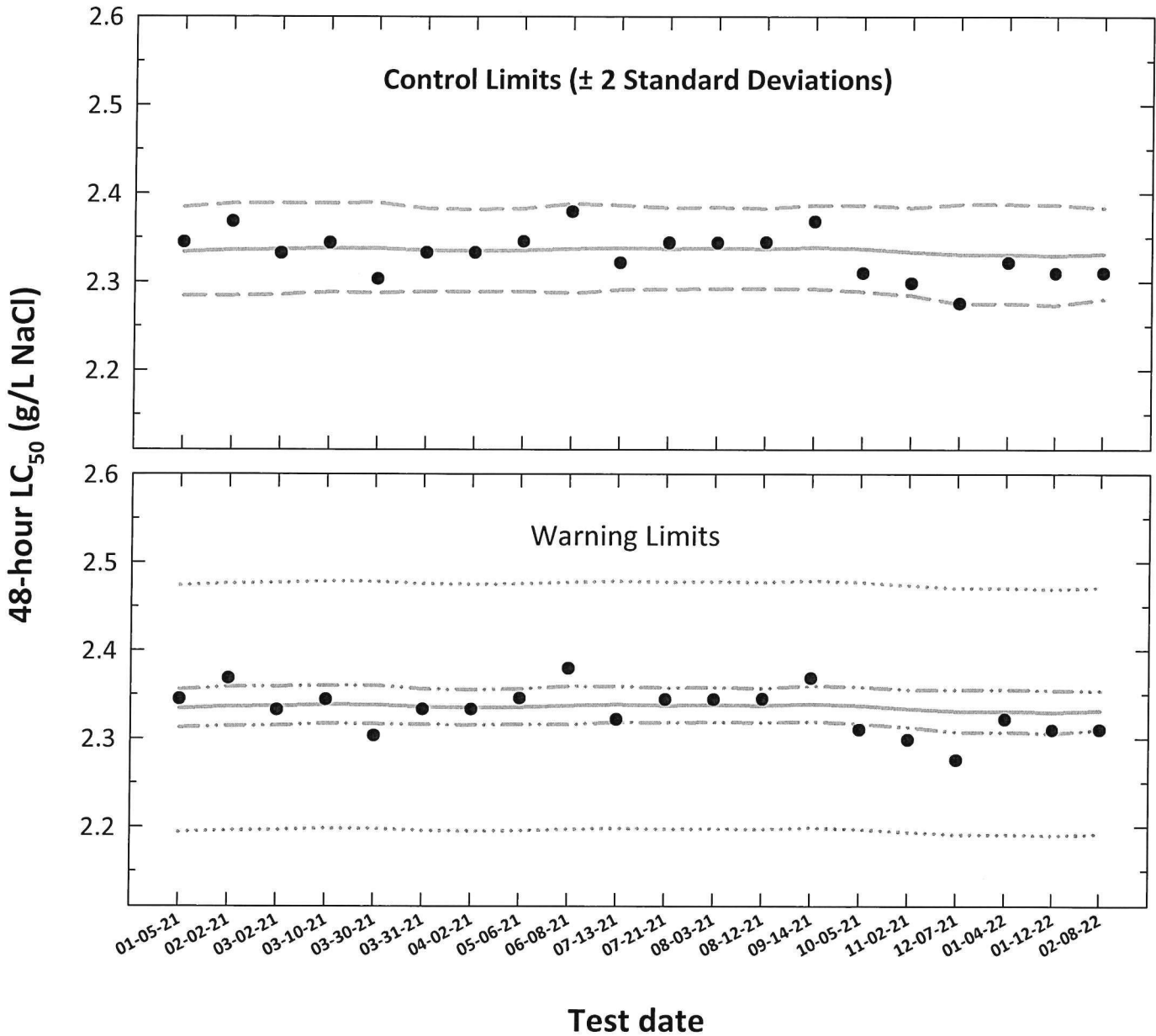
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

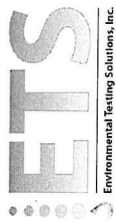
Source: In-house Culture



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

Independent Review by
Kelley E. Keenan:

Entered and Reviewed by
Jim Sumner



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	01-05-21	2.3449	0.3701	0.3681	0.0047	2.3339	2.2843	2.3846	2.3126	2.3556	2.1938	2.4739
2	02-02-21	2.3682	0.3744	0.3685	0.0048	2.3362	2.2847	2.3888	2.3141	2.3587	2.1960	2.4763
3	03-02-21	2.3324	0.3678	0.3686	0.0048	2.3367	2.2856	2.3890	2.3148	2.3591	2.1965	2.4769
4	03-10-21	2.3442	0.3700	0.3689	0.0047	2.3384	2.2887	2.3892	2.3171	2.3601	2.1981	2.4787
5	03-30-21	2.3032	0.3623	0.3689	0.0048	2.3381	2.2874	2.3898	2.3164	2.3602	2.1978	2.4783
6	03-31-21	2.3330	0.3679	0.3684	0.0043	2.3356	2.2893	2.3829	2.3158	2.3558	2.1955	2.4757
7	04-02-21	2.3330	0.3679	0.3683	0.0043	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
8	05-06-21	2.3455	0.3702	0.3684	0.0044	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
9	06-08-21	2.3795	0.3765	0.3687	0.0047	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
10	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
11	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
12	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
13	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
14	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
15	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3338	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
16	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
17	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
18	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
19	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
20	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

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 # 376

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 #: 2071

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Concentration			
	Analyst	JW	JW	N
	pH (S.U.)	7.91	7.85	7.01
	Dissolved oxygen (mg/L)	7.7	7.7	8.0
	Conductivity (µmhos/cm)	316		
	Alkalinity (mg/L CaCO ₃)	60		
1750 mg/L	Hardness (mg/L CaCO ₃)	87		
	Temperature (°C)	24.9	25.3	25.2
	pH (S.U.)	7.96	7.86	7.63
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	3420		
2000 mg/L	Temperature (°C)	25.1	25.2	25.0
	pH (S.U.)	7.96	7.88	7.69
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	3910		
2250 mg/L	Temperature (°C)	24.9	25.3	25.2
	pH (S.U.)	7.98	7.88	7.74
	Dissolved oxygen (mg/L)	8.0	7.9	7.9
	Conductivity (µmhos/cm)	4340		
2500 mg/L	Temperature (°C)	24.9	25.4	25.2
	pH (S.U.)	7.98	7.90	7.70
	Dissolved oxygen (mg/L)	8.0	7.9	8.0
	Conductivity (µmhos/cm)	4860		
2750 mg/L	Temperature (°C)	24.9	25.2	25.2
	pH (S.U.)	7.98	7.91	7.00
	Dissolved oxygen (mg/L)	8.0	8.0	8.1
	Conductivity (µmhos/cm)	5230		
2750 mg/L	Temperature (°C)	25.0	25.2	25.1

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	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 # 376

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-08-22	0620	J	0847	J	2C	RED	02-0228
24	02-09-22			0845	J			
48 Termination	02-10-22			0845	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):	02-01-22
Age:	< 24-hours old
Date and time organisms were born between:	02-07-22 1453 TO 02-08-22 0620
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.02
	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	4 ^{1d}	S	S	S	3 ^{2d}	4 ^{1d}	4 ^{1d}	3 ^{2d}
48 Termination	4 ^{1d}	3 ^{2d}	2 ^{3d}	2 ^{3d}	1 ^{3d}	1 ^{4d}	1 ^{4d}	1 ^{4d}	0 ^{3d}	0 ^{4d}	0 ^{4d}	0 ^{3d}
Mean Survival	55%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2236.9
Upper 95% confidence limit (mg NaCl/L)	2381.0
48-hour LC ₅₀ (mg NaCl/L)	2309.9

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 2/8/2022 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
 End Date: 2/10/2022 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	0.8000	0.6000	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%					
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	20	
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	20	
*2250	0.5500	0.5500	0.8407	0.6847	1.1071	23.960	4	10.00	10.00	9	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)	0.57538	0.868	1.30394	7.42774

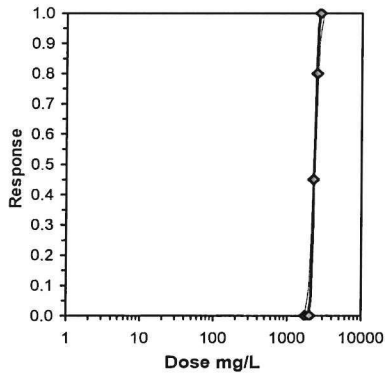
Equality of variance cannot be confirmed

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

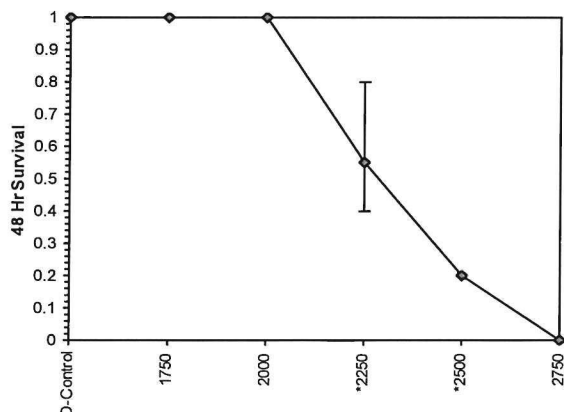
Treatments vs D-Control

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

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



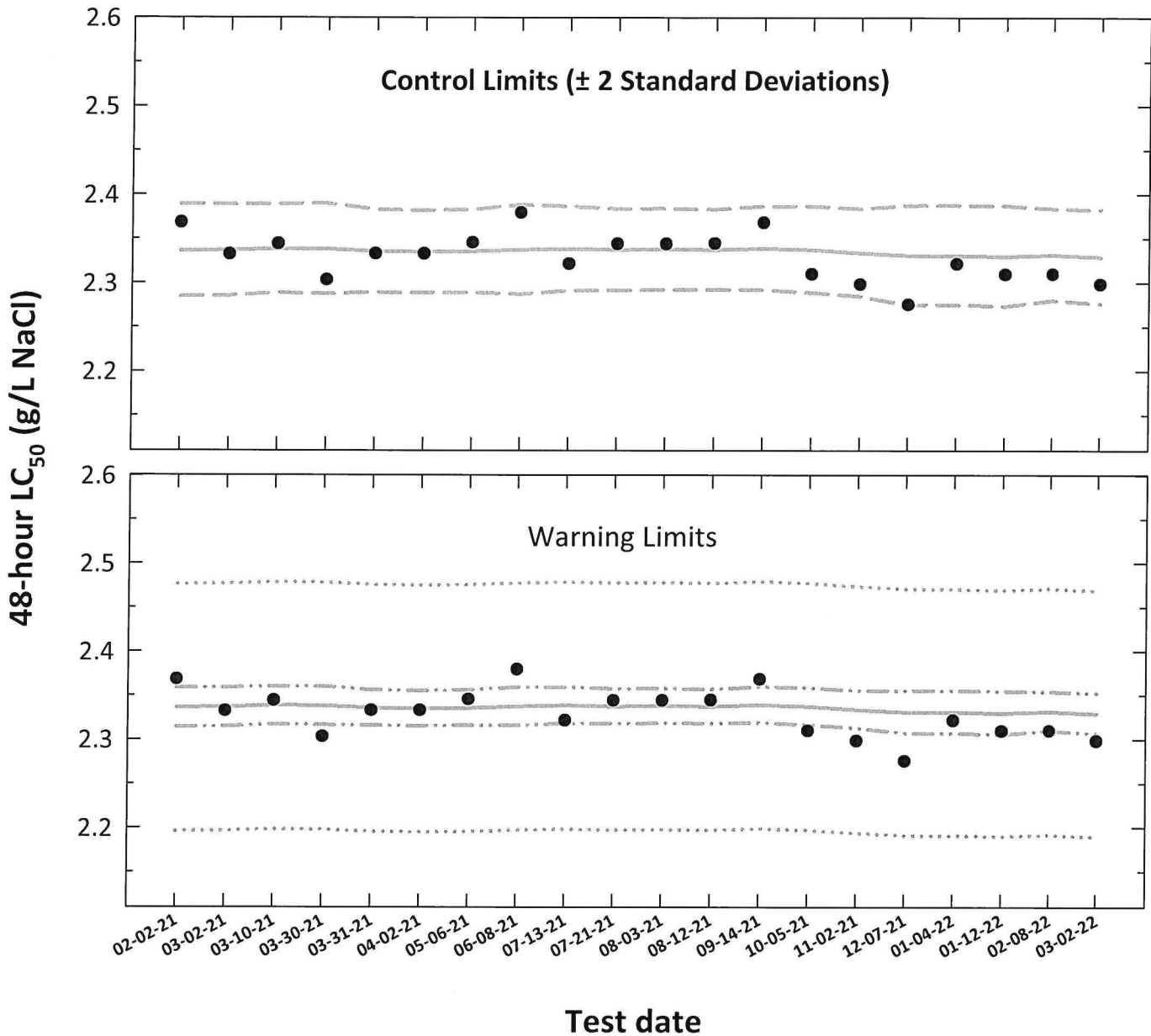
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour 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	02-02-21	2.3682	0.3744	0.3685	0.0048	2.3362	2.2847	2.3888	2.3141	2.3587	2.1960	2.4763
2	03-02-21	2.3324	0.3678	0.3686	0.0048	2.3367	2.2856	2.3890	2.3148	2.3591	2.1965	2.4769
3	03-10-21	2.3442	0.3700	0.3689	0.0047	2.3384	2.2887	2.3892	2.3171	2.3601	2.1981	2.4787
4	03-30-21	2.3032	0.3623	0.3689	0.0048	2.3381	2.2874	2.3898	2.3164	2.3602	2.1978	2.4783
5	03-31-21	2.3330	0.3679	0.3684	0.0043	2.3356	2.2893	2.3829	2.3158	2.3558	2.1955	2.4757
6	04-02-21	2.3330	0.3679	0.3683	0.0043	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
7	05-06-21	2.3455	0.3702	0.3684	0.0044	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
8	06-08-21	2.3795	0.3765	0.3687	0.0047	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
9	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 317

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 #: 2071

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Concentration			
	Analyst	JW	JW	K
	pH (S.U.)	7.75	7.65	7.92
	Dissolved oxygen (mg/L)	7.8	7.8	8.0
	Conductivity (µmhos/cm)	312		
	Alkalinity (mg/L CaCO ₃)	61		
	Hardness (mg/L CaCO ₃)	82		
Temperature (°C)	24.9	25.1	25.0	
1750 mg/L	pH (S.U.)	7.98	7.80	7.82
	Dissolved oxygen (mg/L)	8.0	7.9	8.0
	Conductivity (µmhos/cm)	3600		
	Temperature (°C)	25.0	25.0	24.8
2000 mg/L	pH (S.U.)	7.97	7.80	7.84
	Dissolved oxygen (mg/L)	8.0	8.0	8.0
	Conductivity (µmhos/cm)	4100		
	Temperature (°C)	24.8	25.2	24.9
2250 mg/L	pH (S.U.)	7.97	7.81	7.86
	Dissolved oxygen (mg/L)	8.0	8.0	8.1
	Conductivity (µmhos/cm)	4480		
	Temperature (°C)	24.9	25.2	24.9
2500 mg/L	pH (S.U.)	7.98	7.81	7.86
	Dissolved oxygen (mg/L)	8.0	8.0	8.2
	Conductivity (µmhos/cm)	4980		
	Temperature (°C)	24.9	25.2	25.1
2750 mg/L	pH (S.U.)	7.98	7.82	7.85
	Dissolved oxygen (mg/L)	8.0	8.0	8.2
	Conductivity (µmhos/cm)	5440		
	Temperature (°C)	25.0	25.0	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	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 # 377

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-01-22	0510	X	0713	X	2B3	RED	02-25-22
24	03-05-22			0715	X			
48 Termination	03-09-22			0713	X			

*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):	02-22-22 C
Age:	< 24-hours old
Date and time organisms were born between:	03-01-22 1503 TO 03-02-22 0510
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.81
	Temperature (°C): 25.0

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	3 ^d	4 ^d	4 ^d	5
48 Termination	3 ^{2d}	3 ^{2d}	3 ^{2d}	2 ^{3d}	1 ^{4d}	0 ^{5d}	2 ^{3d}	0 ^{5d}	0 ^{3d}	0 ^{4d}	0 ^{4d}	0 ^{5d}
Mean Survival	55%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2227.7
Upper 95% confidence limit (mg NaCl/L)	2367.1
48-hour LC ₅₀ (mg NaCl/L)	2298.5

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date:	3/2/2022	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/4/2022	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	0.6000	0.4000
2500	0.2000	0.0000	0.4000	0.0000
2750	0.0000	0.0000	0.0000	0.0000

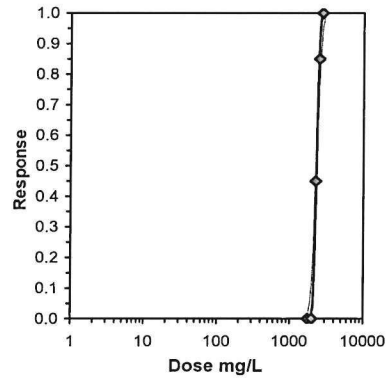
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8357	0.6847	0.8861	12.047	4	10.00	10.00	9	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)	0.75304	0.868	0.63524	3.99287
Equality of variance cannot be confirmed				

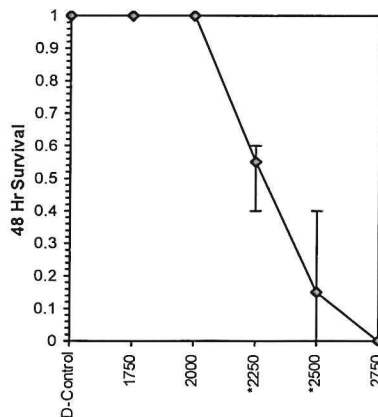
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	33.8498	6.46692	21.1747 46.525	0	1.31508	7.81472	0.72555	3.36145	0.02954	5
Intercept	-108.78	21.7554	-151.42 -66.144							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1962.1	1767.76	2064.38
EC05	3.355	2055.2	1899.71	2139.73
EC10	3.718	2106.62	1972.73	2182.45
EC15	3.964	2142.04	2022.67	2212.75
EC20	4.158	2170.62	2062.49	2237.96
EC25	4.326	2195.44	2096.53	2260.62
EC40	4.747	2259.24	2180.24	2323.62
EC50	5.000	2298.51	2227.74	2367.09
EC60	5.253	2338.47	2272.15	2415.76
EC75	5.674	2406.43	2339.1	2508.31
EC80	5.842	2433.94	2363.79	2548.63
EC85	6.036	2466.41	2391.64	2597.81
EC90	6.282	2507.88	2425.69	2662.64
EC95	6.645	2570.63	2474.96	2764.04
EC99	7.326	2692.6	2566.15	2969.37



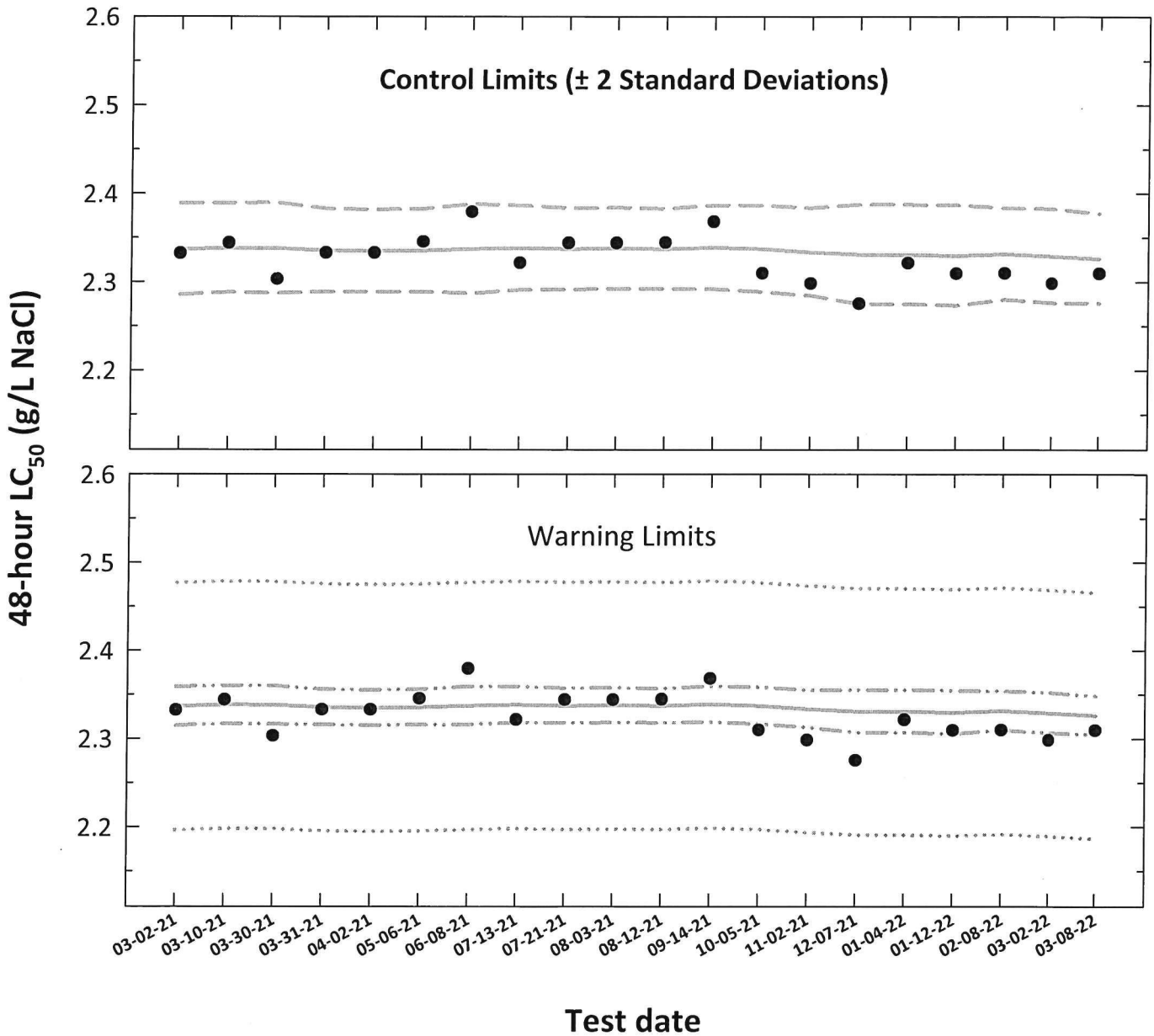
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

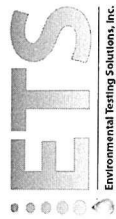
Source: In-house Culture



- **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)

Independent Review by
Kelley E. Keenan:

Entered and Reviewed by
Jim Sumner



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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		CT	Anti-logarithmic Values (g/L NaCl)					
			48-hour LC ₅₀	CT		S	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	03-02-21	2.3324	0.3678	0.3686	2.3367	2.2856	2.3890	2.3148	2.3591	2.1965	2.4769
2	03-10-21	2.3442	0.3700	0.3689	2.3384	2.2887	2.3892	2.3171	2.3601	2.1981	2.4787
3	03-30-21	2.3032	0.3623	0.3689	2.3381	2.2874	2.3898	2.3164	2.3602	2.1978	2.4783
4	03-31-21	2.3330	0.3679	0.3684	2.3356	2.2893	2.3829	2.3158	2.3558	2.1955	2.4757
5	04-02-21	2.3330	0.3679	0.3683	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
6	05-06-21	2.3455	0.3702	0.3684	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
7	06-08-21	2.3795	0.3765	0.3687	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
8	07-13-21	2.3217	0.3658	0.3689	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
9	07-21-21	2.3442	0.3700	0.3687	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
10	08-03-21	2.3442	0.3700	0.3688	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
11	08-12-21	2.3449	0.3701	0.3687	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
12	09-14-21	2.3682	0.3744	0.3690	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
13	10-05-21	2.3099	0.3636	0.3687	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
14	11-02-21	2.2985	0.3614	0.3681	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
15	12-07-21	2.2755	0.3571	0.3675	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
16	01-04-22	2.3217	0.3658	0.3675	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708
17	01-12-22	2.3096	0.3635	0.3673	2.3297	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695
18	02-08-22	2.3099	0.3636	0.3676	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
19	03-02-22	2.2985	0.3614	0.3672	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
20	03-08-22	2.3096	0.3635	0.3667	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658

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 # 318

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 #: 2511

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	<i>K</i>	<i>K</i>	<i>K</i>
Control, MHSW	pH (S.U.)	7.09	7.01	7.01
	Dissolved oxygen (mg/L)	7.4	7.9	7.7
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	80		
	Temperature (°C)	24.9	25.2	25.1
1750 mg/L	pH (S.U.)	7.96	7.08	7.70
	Dissolved oxygen (mg/L)	7.7	7.9	7.4
	Conductivity (µmhos/cm)	3470		
	Temperature (°C)	25.1	25.0	25.0
2000 mg/L	pH (S.U.)	7.94	7.07	7.03
	Dissolved oxygen (mg/L)	7.0	7.9	7.0
	Conductivity (µmhos/cm)	3950		
	Temperature (°C)	25.1	25.2	25.0
2250 mg/L	pH (S.U.)	7.93	7.07	7.05
	Dissolved oxygen (mg/L)	7.7	7.9	7.4
	Conductivity (µmhos/cm)	4320		
	Temperature (°C)	25.0	25.2	25.2
2500 mg/L	pH (S.U.)	7.93	7.04	7.05
	Dissolved oxygen (mg/L)	7.7	7.9	7.7
	Conductivity (µmhos/cm)	4790		
	Temperature (°C)	25.1	25.2	25.2
2750 mg/L	pH (S.U.)	7.99	7.06	7.90
	Dissolved oxygen (mg/L)	7.0	7.9	7.7
	Conductivity (µmhos/cm)	6170		
	Temperature (°C)	25.1	24.9	25.2

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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 # 378

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-08-22*	0545	J	0746	R	2C3	ORANGE	03-03-22B
24	03-08-22			0742	J			
48 Termination	03-10-22			0745	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):	03-01-22 D
Age:	< 24-hours old
Date and time organisms were born between:	03-07-22 1445 TO 03-08-22 0545
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.94
	Temperature (°C): 25.0

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

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

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROB
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: 3/8/2022 Test ID: CdNaCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/10/2022 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:

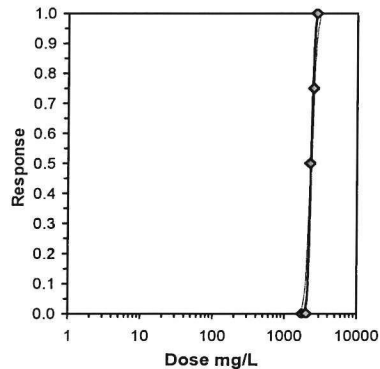
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.8000	0.4000	0.4000
2500	0.2000	0.2000	0.4000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%				
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	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	20
*2250	0.5000	0.5000	0.7903	0.6847	1.1071	26.725	4	10.00	10.00	10
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20

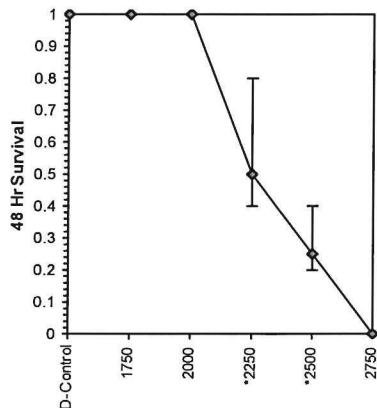
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.68778	0.868	2.22358	6.53833
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	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



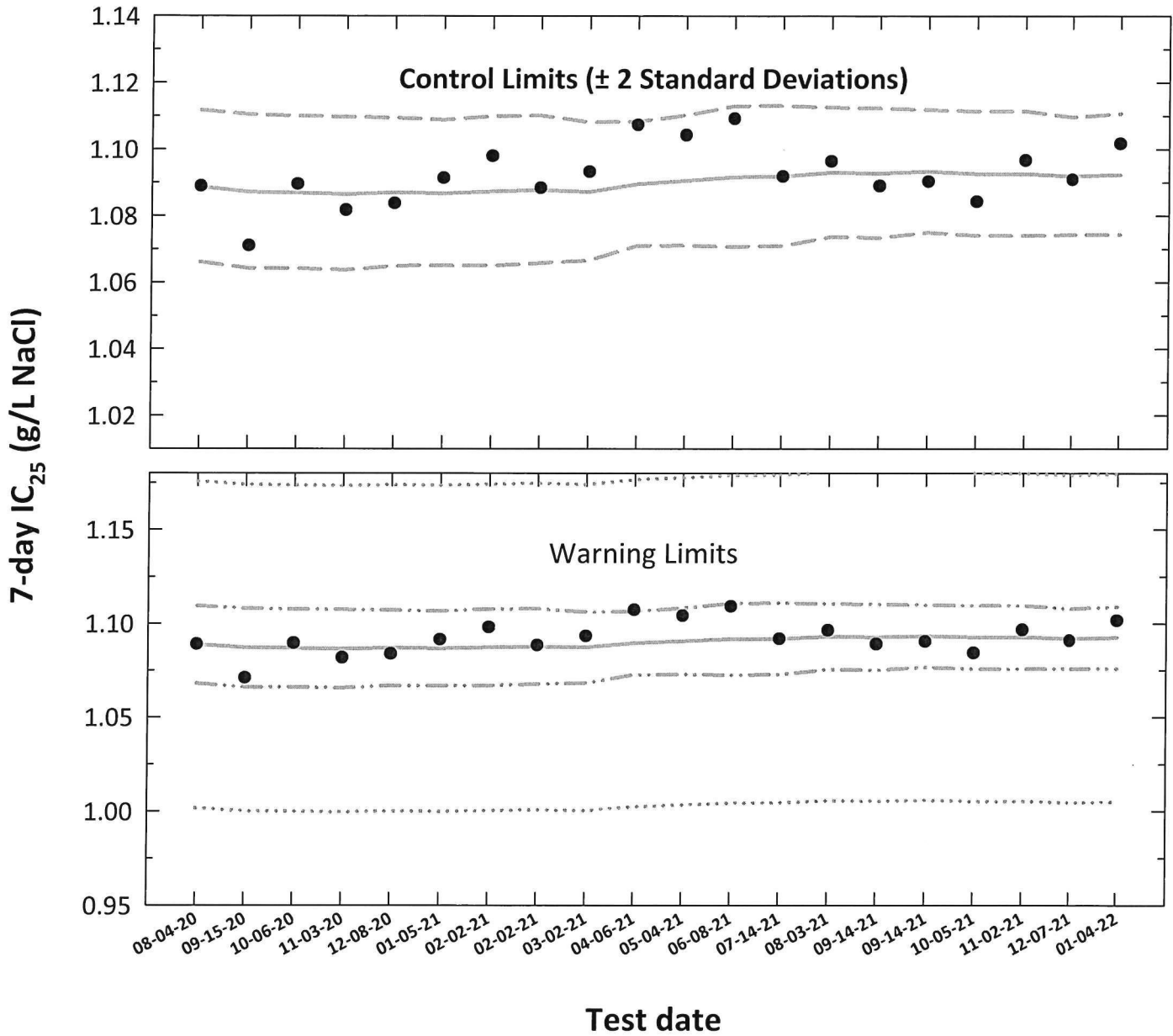
Dose-Response Plot



Ceriodaphnia dubia

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)

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	08-04-20	1.0890	0.0370	0.0369	0.0046	1.0887	1.0661	1.1117	1.0679	1.1094	1.0016	1.1758
2	09-15-20	1.0710	0.0298	0.0363	0.0046	1.0871	1.0642	1.1104	1.0661	1.1081	1.0001	1.1741
3	10-06-20	1.0896	0.0373	0.0362	0.0046	1.0869	1.0642	1.1100	1.0660	1.1077	0.9999	1.1738
4	11-03-20	1.0818	0.0341	0.0360	0.0046	1.0865	1.0638	1.1097	1.0656	1.1074	0.9996	1.1734
5	12-08-20	1.0838	0.0349	0.0362	0.0044	1.0870	1.0650	1.1095	1.0668	1.1073	1.0001	1.1740
6	01-05-21	1.0914	0.0380	0.0361	0.0044	1.0867	1.0651	1.1088	1.0668	1.1067	0.9998	1.1737
7	02-02-21	1.0980	0.0406	0.0364	0.0045	1.0873	1.0651	1.1100	1.0669	1.1077	1.0003	1.1743
8	02-02-21	1.0884	0.0368	0.0365	0.0044	1.0878	1.0659	1.1101	1.0677	1.1079	1.0008	1.1748
9	03-02-21	1.0933	0.0387	0.0363	0.0041	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
10	04-06-21	1.1074	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
11	05-04-21	1.1043	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
12	06-08-21	1.1092	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
13	07-14-21	1.0919	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
14	08-03-21	1.0964	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
15	09-14-21	1.0890	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
16	09-14-21	1.0904	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
17	10-05-21	1.0843	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
18	11-02-21	1.0967	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
19	12-07-21	1.0909	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
20	01-04-22	1.1017	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798

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.

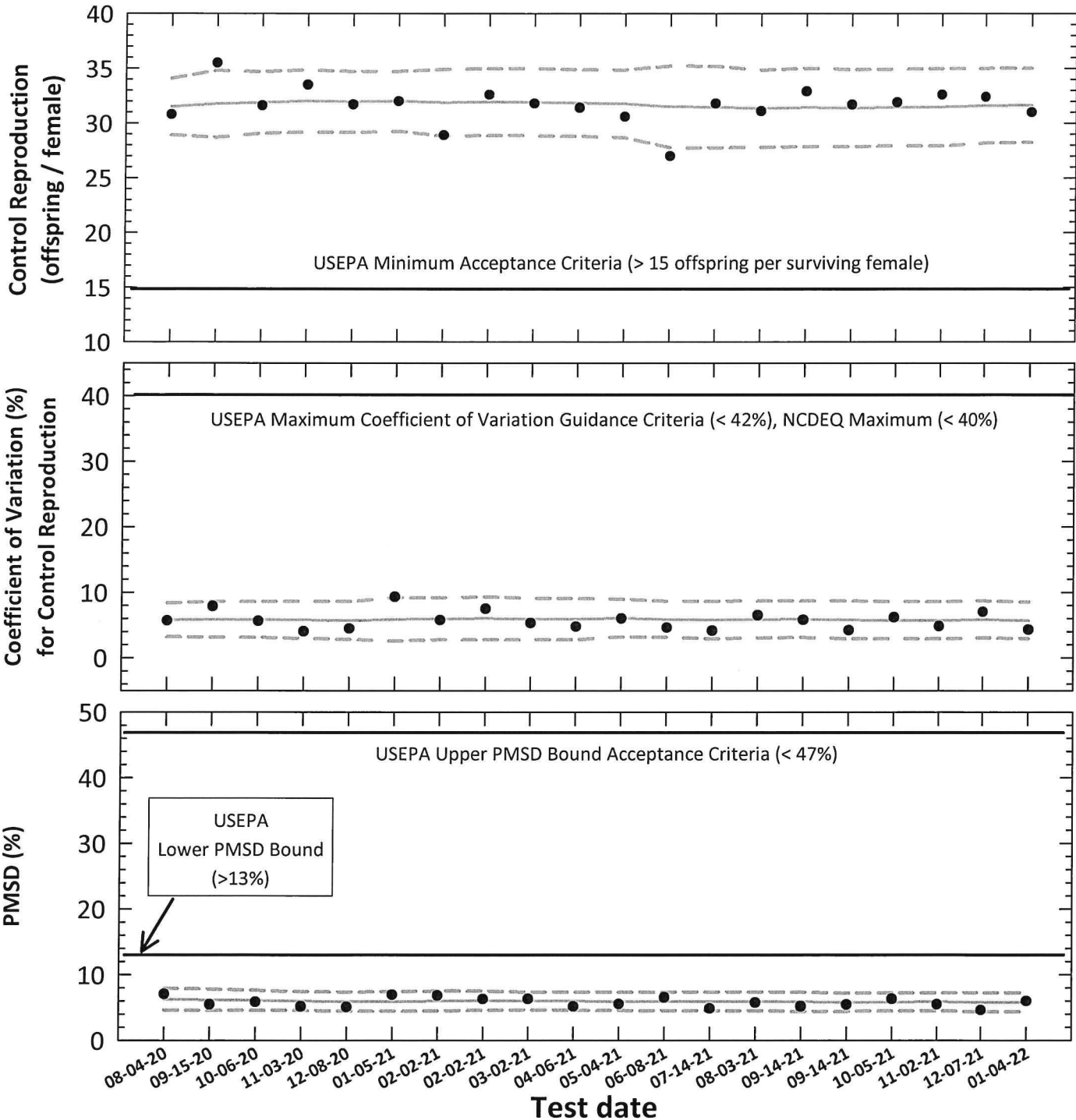
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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		Control Reproduction CV		Test PMSD (%)			
		Control Survival (%)	Control Reproduction		MSD	PMSD (%)	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 (%)									(offspring/female)	(%)	
1	08-04-20	100	30.8	5.7	2.175	7.1	31.5	28.9	34.1	5.8	3.2	8.4	6.3	4.6	7.9
2	09-15-20	100	35.5	7.9	1.941	5.5	31.8	28.7	34.8	5.9	3.1	8.6	6.2	4.5	7.8
3	10-06-20	100	31.6	5.6	1.847	5.8	31.9	29.1	34.7	5.8	3.1	8.6	6.1	4.6	7.6
4	11-03-20	100	33.5	4.0	1.731	5.2	32.0	29.2	34.9	5.8	2.9	8.6	6.0	4.5	7.4
5	12-08-20	100	31.7	4.5	1.613	5.1	32.0	29.2	34.7	5.7	2.8	8.6	5.9	4.5	7.4
6	01-05-21	100	32.0	9.3	2.224	7.0	32.0	29.3	34.7	5.9	2.6	9.2	5.9	4.4	7.4
7	02-02-21	100	28.9	5.8	1.975	6.8	31.9	28.8	34.9	6.0	2.8	9.2	6.0	4.5	7.5
8	02-02-21	100	32.6	7.5	2.045	6.3	31.9	28.9	35.0	6.0	2.8	9.3	6.0	4.6	7.5
9	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	9.1	6.0	4.7	7.3
10	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	6.0	4.6	7.4
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

CV = Coefficient of variation for control reproduction.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

Upper PMSD bound 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 #: 266

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2060</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>RED</u>
Date and times organisms were born between:	<u>01-04-22 0630 to 0908</u>										Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>12-18-21 A</u>											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	<u>3</u>	<u>4</u>	<u>5</u>	<u>9</u>	<u>13</u>	<u>17</u>	<u>18</u>	<u>23</u>	<u>28</u>	<u>32</u>		
Transfer vessel information:	pH (S.U.): <u>7.93</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	01-04-22	<u>0919</u>	<u>12-30-21</u>	<u>12-30-21</u>	<u>12-29-21 C</u>	<u>H</u>
1	01-05-22	<u>0720</u>			↓	<u>H</u>
2	01-06-22	<u>0720</u>			<u>12-29-21 D</u>	<u>H</u>
3	01-07-22	<u>0719</u>			↓	<u>H</u>
4	01-08-22	<u>0730</u>			<u>01-03-21 A</u>	<u>H</u>
5	01-09-22	<u>0720</u>			↓	<u>H</u>
6	01-10-22	<u>0719</u>	↓	↓	↓	<u>H</u>
7	01-11-22	<u>0720</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>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>21400</u>
% Adults having 3 rd Broods:	<u>1007.</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>31.0</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>4.37.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1101.7</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 266

CONTROL

Survival and Reproduction Data

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

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	31.0

600 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	31.4
% Reduction from Control:	-1.37.

Species: *Ceriodaphnia dubia*
800 mg NaCl/L

CdNaClCR #: 266

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	6	5	5	5	3	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	11	12	12	12	10	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	11	15	17	15	16	13	16	17	12	14
Total young produced		28	29	32	32	33	30	33	30	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:	30.9
% Reduction from Control:	0.37.

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	3	5	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	10	13	12	13	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	11	13	13	16	14	11	12	16	12	12
Total young produced		28	28	29	30	30	28	29	31	31	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:	29.3
% Reduction from Control:	5.57.

Species: Ceriodaphnia dubia

CdNaClCR #: 266

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	4	5	4	4	4	3	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	10	7	9	11	6	5	10	11	8
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	5	6	2	7	8	5	9	3	3
Total young produced		17	19	18	15	23	18	13	22	18	15
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	0%
Mean Offspring/Female:	17.7
% Reduction from Control:	42.9%

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	1	3	2	2	1	1	1	3	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		3	1	3	2	2	1	1	1	3	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:	0%
Mean Offspring/Female:	2.0
% Reduction from Control:	93.5%

Verification of Ceriodaphnia Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	6	5	4	6	6	5	6	6	54
5	12	10	13	13	11	10	12	12	12	13	118
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	12	14	16	13	14	12	15	12	138
Total	32	30	31	32	31	29	32	29	33	31	310

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	6	5	6	7	6	4	6	5	5	5	55
5	12	13	10	12	11	13	13	12	12	12	120
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	13	13	13	16	14	12	12	14	139
Total	33	35	29	32	30	33	33	29	29	31	314

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	6	5	5	5	3	6	4	48
5	12	10	10	11	12	12	12	10	13	13	115
6	0	0	0	0	0	0	0	0	0	0	0
7	11	15	17	15	16	13	16	17	12	14	146
Total	28	29	32	32	33	30	33	30	31	31	309

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	4	3	5	4	4	6	4	43
5	12	11	12	10	13	12	13	11	13	13	120
6	0	0	0	0	0	0	0	0	0	0	0
7	11	13	13	16	14	11	12	16	12	12	130
Total	28	28	29	30	30	28	29	31	31	29	293

1200 mg NaCl/L

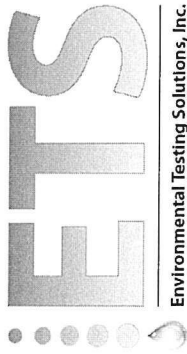
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	4	4	4	3	3	4	4	39
5	8	10	7	9	11	6	5	10	11	8	85
6	0	0	0	0	0	0	0	0	0	0	0
7	5	5	6	2	7	8	5	9	3	3	53
Total	17	19	18	15	22	18	13	22	18	15	177

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	1	3	2	2	1	1	1	3	3	20
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	3	1	3	2	2	1	1	1	3	3	20

Prepared and Reviewed 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 #266**
 Test dates: **January 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	32	30	31	32	31	29	32	29	33	31	100	31.0	4.3	Not applicable
600	33	35	29	32	30	33	33	29	29	31	100	31.4	6.7	-1.3
800	28	29	32	32	33	30	33	30	31	31	100	30.9	5.4	0.3
1000	28	28	29	30	30	28	29	31	31	29	100	29.3	4.0	5.5
1200	17	19	18	15	22	18	13	22	18	15	100	17.7	16.4	42.9
1400	3	1	3	2	2	1	1	1	3	3	100	2.0	47.1	93.5

Dunnett's MSD value: 1.854 MSD = Minimum Significant Difference
 PMSD: 6.0 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.



Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 1/4/2022	Test ID: CdNaClCR	Sample ID: REF-Ref Toxicant
End Date: 1/11/2022	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

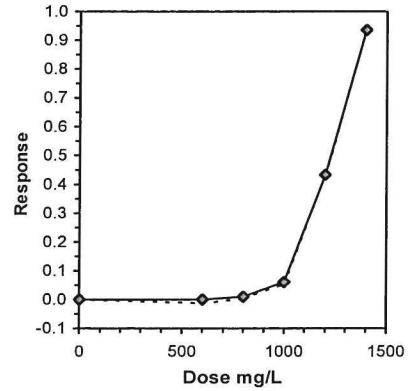
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	32.000	30.000	31.000	32.000	31.000	29.000	32.000	29.000	33.000	31.000
600	33.000	35.000	29.000	32.000	30.000	33.000	33.000	29.000	29.000	31.000
800	28.000	29.000	32.000	32.000	33.000	30.000	33.000	30.000	31.000	31.000
1000	28.000	28.000	29.000	30.000	30.000	28.000	29.000	31.000	31.000	29.000
1200	17.000	19.000	18.000	15.000	22.000	18.000	13.000	22.000	18.000	15.000
1400	3.000	1.000	3.000	2.000	2.000	1.000	1.000	1.000	3.000	3.000

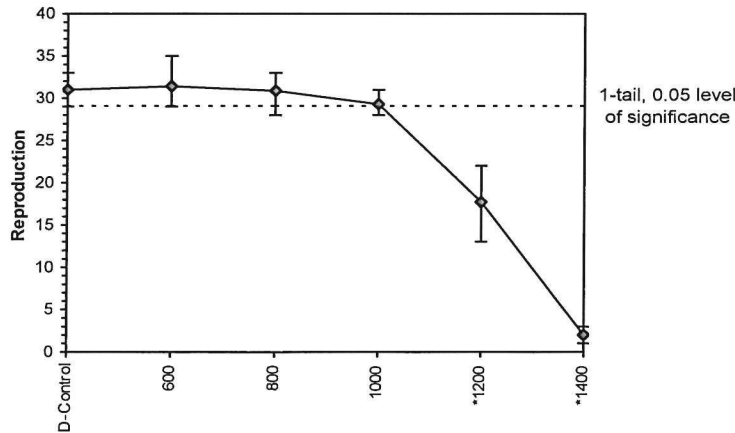
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	31.000	1.0000	31.000	29.000	33.000	4.301	10				31.200	1.0000	
600	31.400	1.0129	31.400	29.000	35.000	6.747	10	-0.493	2.287	1.854	31.200	1.0000	
800	30.900	0.9968	30.900	28.000	33.000	5.383	10	0.123	2.287	1.854	30.900	0.9904	
1000	29.300	0.9452	29.300	28.000	31.000	3.957	10	2.097	2.287	1.854	29.300	0.9391	
*1200	17.700	0.5710	17.700	13.000	22.000	16.428	10	16.403	2.287	1.854	17.700	0.5673	
*1400	2.000	0.0645	2.000	1.000	3.000	47.140	10	35.767	2.287	1.854	2.000	0.0641	

Auxiliary Tests		Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)		0.53252	1.035	0.06371	0.54172						
Bartlett's Test indicates equal variances (p = 0.01)		14.9448	15.0863								
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		1000	1200	1095.45		1.85404	0.05981	1405.34	3.28704	4.1E-42	5, 54
Treatments vs D-Control											

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	957.5	62.8053	776.053	1011.3	-0.9707
IC10	1021.03	10.2533	1003.97	1038.12	-1.4943
IC15	1047.93	8.5912	1032.1	1064.93	0.2192
IC20	1074.83	8.86076	1059.64	1092.46	0.2920
IC25	1101.72	9.62991	1083.83	1120.41	0.2753
IC40	1182.41	13.3721	1155.25	1204.74	-0.0574
IC50	1226.75	10.1001	1202.09	1240.7	-0.5551



Dose-Response Plot



Entered and Reviewed by
Jin Sumner

Species: Ceriodaphnia dubia

CdNaClCR #: 266

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	
		u	u	u	u	u	u
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.40	7.71	7.50	7.45	7.62	7.68
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.7	7.4	7.7
	Conductivity (µmhos/cm)	304		306		300	
	Alkalinity (mg CaCO ₃ /L)	61				60	
	Hardness (mg CaCO ₃ /L)	94				90	
	Temperature (°C)	24.9	25.2	24.8	25.1	25.0	25.2
600 mg NaCl/L	pH (S.U.)	7.48	7.81	7.70	7.80	7.83	7.76
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.7	7.4	7.6
	Conductivity (µmhos/cm)	1520		1470		1440	
	Temperature (°C)	25.0	24.9	24.9	24.9	24.9	24.9
800 mg NaCl/L	pH (S.U.)	7.71	7.81	7.79	7.82	7.84	7.78
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.4	7.6	7.6
	Conductivity (µmhos/cm)	1900		1830		1810	
	Temperature (°C)	25.0	24.9	24.9	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.75	7.83	7.80	7.82	7.86	7.78
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.6	7.6	7.6
	Conductivity (µmhos/cm)	2260		2190		2170	
	Temperature (°C)	24.9	25.1	25.0	25.2	25.0	25.0
1200 mg NaCl/L	pH (S.U.)	7.77	7.82	7.84	7.83	7.88	7.78
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.6	7.6	7.7
	Conductivity (µmhos/cm)	2630		2550		2540	
	Temperature (°C)	24.9	24.9	24.9	25.0	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	7.78	7.82	7.86	7.83	7.89	7.75
	Dissolved oxygen (mg/L)	7.6	7.8	8.0	7.7	7.7	7.7
	Conductivity (µmhos/cm)	3120		2930		2940	
	Temperature (°C)	24.9	24.9	24.9	25.0	25.0	25.1
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

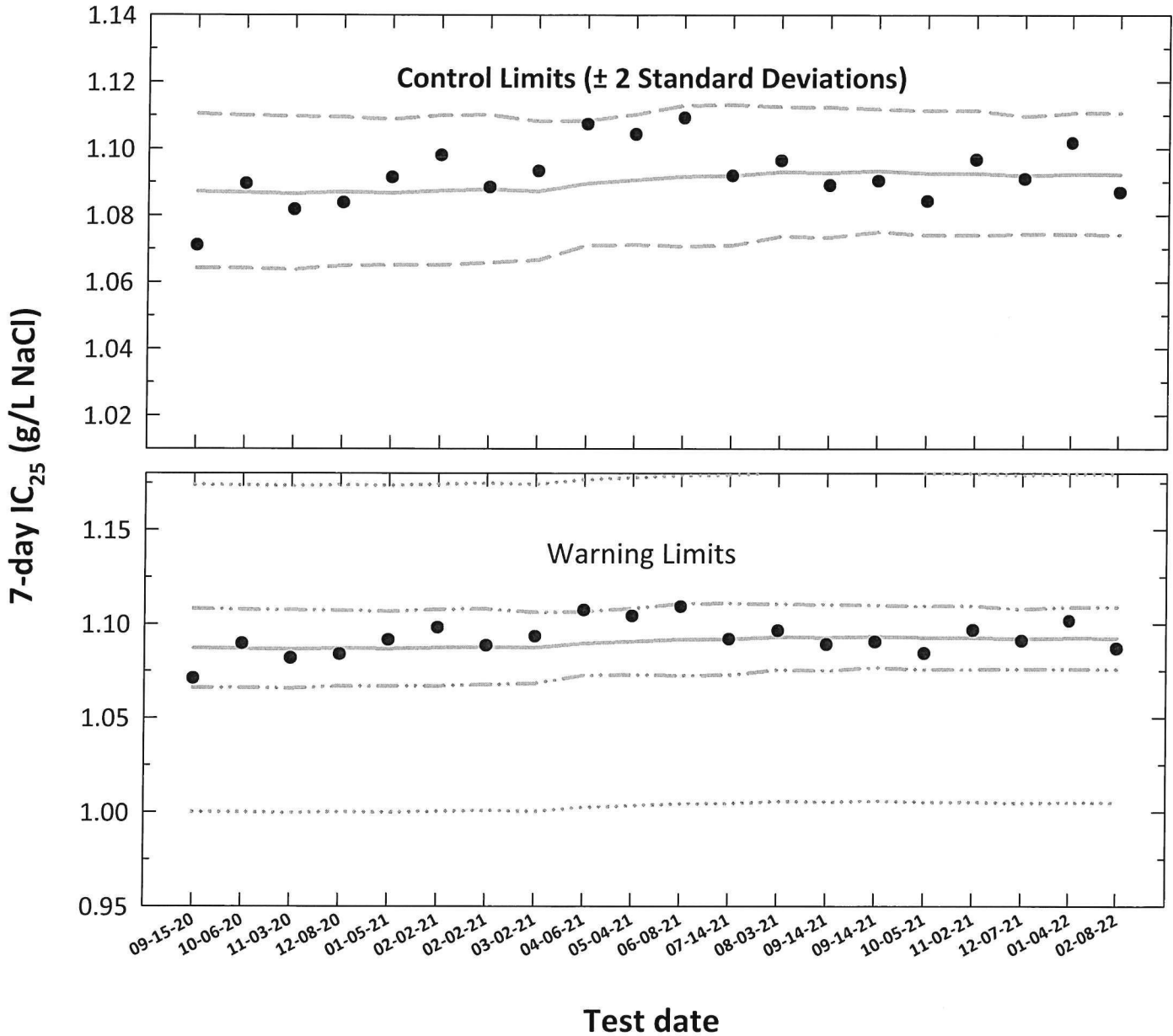
CdNaClCR #: 266

		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Analyst		KL	BSL	BSL	N	N	N	N	JW
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.48	7.39	7.38	7.70	7.66	7.73	7.61	7.70
	Dissolved oxygen (mg/L)	7.0	7.7	7.8	7.6	7.7	7.7	7.0	7.8
	Conductivity (µmhos/cm)	299		289		310		297	
	Alkalinity (mg CaCO ₃ /L)	101-0-22		59					
	Hardness (mg CaCO ₃ /L)	101-0-22		90					101-0-22
	Temperature (°C)	24.9	25.2	24.8	24.9	24.8	25.2	24.9	25.1
600 mg NaCl/L	pH (S.U.)	7.70	7.88	7.82	7.02	7.77	7.03	7.06	7.83
	Dissolved oxygen (mg/L)	7.0	7.7	7.6	7.6	7.9	7.7	7.0	7.8
	Conductivity (µmhos/cm)	1450		1400		1430		1410	
	Temperature (°C)	24.9	24.9	24.8	25.1	24.8	24.8	25.0	24.9
800 mg NaCl/L	pH (S.U.)	7.72	7.88	7.87	7.04	7.79	7.06	7.00	7.84
	Dissolved oxygen (mg/L)	7.9	7.7	7.7	7.7	7.9	7.0	7.9	7.8
	Conductivity (µmhos/cm)	1810		1740		1830		1870	
	Temperature (°C)	25.0	24.9	24.8	24.9	24.9	24.8	25.0	24.9
1000 mg NaCl/L	pH (S.U.)	7.73	7.89	7.87	7.06	7.02	7.06	7.00	7.85
	Dissolved oxygen (mg/L)	7.9	7.7	7.8	7.7	7.9	7.0	7.9	7.9
	Conductivity (µmhos/cm)	2210		2130		2200		2240	
	Temperature (°C)	25.0	25.0	24.9	24.9	24.9	25.0	24.9	25.0
1200 mg NaCl/L	pH (S.U.)	7.75	7.89	7.89	7.06	7.05	7.06	7.91	7.85
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.0	0.0	7.0	7.9	7.9
	Conductivity (µmhos/cm)	2530		2480		2580		2500	
	Temperature (°C)	25.0	25.0	24.9	25.0	25.0	25.0	24.9	25.1
1400 mg NaCl/L	pH (S.U.)	7.75	7.89	7.88	7.07	7.07	7.07	7.93	7.86
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	0.0	0.0	7.9	0.0	7.9
	Conductivity (µmhos/cm)	2940		2860		3000		2970	
	Temperature (°C)	25.1	25.2	24.9	25.0	25.0	25.1	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		Laboratory Calculated CV			
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	09-15-20	1.0710	0.0298	0.0363	0.0046	1.0871	1.0642	1.1104	1.0661	1.1081	1.0001	1.1741
2	10-06-20	1.0896	0.0373	0.0362	0.0046	1.0869	1.0642	1.1100	1.0660	1.1077	0.9999	1.1738
3	11-03-20	1.0818	0.0341	0.0360	0.0046	1.0865	1.0638	1.1097	1.0656	1.1074	0.9996	1.1734
4	12-08-20	1.0838	0.0349	0.0362	0.0044	1.0870	1.0650	1.1095	1.0668	1.1073	1.0001	1.1740
5	01-05-21	1.0914	0.0380	0.0361	0.0044	1.0867	1.0651	1.1088	1.0668	1.1067	0.9998	1.1737
6	02-02-21	1.0980	0.0406	0.0364	0.0045	1.0873	1.0651	1.1100	1.0669	1.1077	1.0003	1.1743
7	02-02-21	1.0884	0.0368	0.0365	0.0044	1.0878	1.0659	1.1101	1.0677	1.1079	1.0008	1.1748
8	03-02-21	1.0933	0.0387	0.0363	0.0041	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
9	04-06-21	1.1074	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
10	05-04-21	1.1043	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
11	06-08-21	1.1092	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
12	07-14-21	1.0919	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
13	08-03-21	1.0964	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
14	09-14-21	1.0890	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
15	09-14-21	1.0904	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
16	10-05-21	1.0843	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
17	11-02-21	1.0967	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
18	12-07-21	1.0909	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
19	01-04-22	1.1017	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
20	02-08-22	1.0869	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

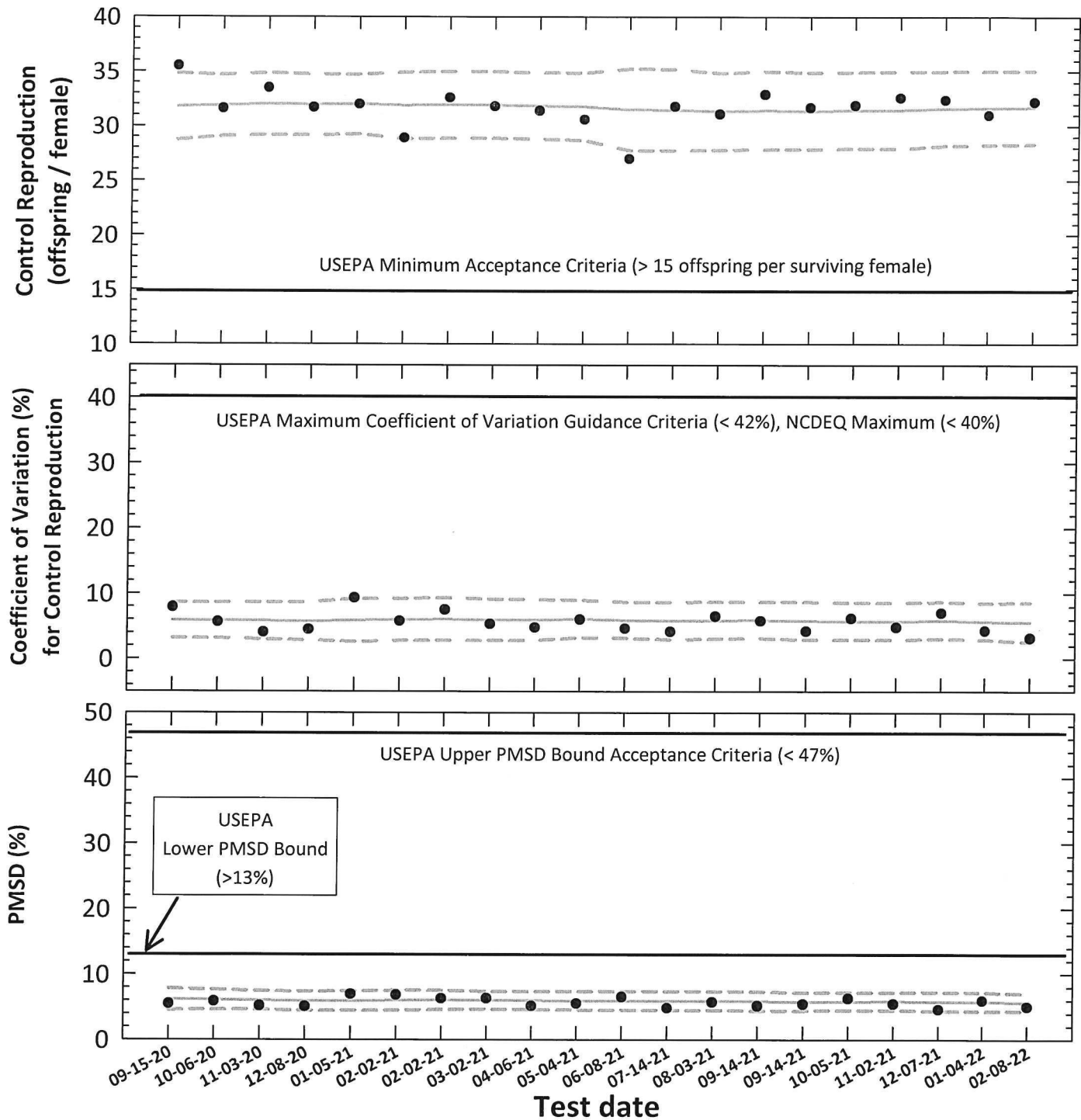
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Ceriodaphnia dubia

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



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

Entered and Reviewed by
Jim Sumner
JS

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

Test number	Test date	ToxCal Determination						Control Reproduction			Control Reproduction CV			Test PMSD	
		Control Survival (%)	Control Reproduction (offspring/female)		Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	09-15-20	100	35.5	7.9	1.941	5.5	31.8	28.7	34.8	5.9	3.1	8.6	6.2	4.5	7.8
2	10-06-20	100	31.6	5.6	1.847	5.8	31.9	29.1	34.7	5.8	3.1	8.6	6.1	4.6	7.6
3	11-03-20	100	33.5	4.0	1.731	5.2	32.0	29.2	34.9	5.8	2.9	8.6	6.0	4.5	7.4
4	12-08-20	100	31.7	4.5	1.613	5.1	32.0	29.2	34.7	5.7	2.8	8.6	5.9	4.5	7.4
5	01-05-21	100	32.0	9.3	2.224	7.0	32.0	29.3	34.7	5.9	2.6	9.2	5.9	4.4	7.4
6	02-02-21	100	28.9	5.8	1.975	6.8	31.9	28.8	34.9	6.0	2.8	9.2	6.0	4.5	7.5
7	02-02-21	100	32.6	7.5	2.045	6.3	31.9	28.9	35.0	6.0	2.8	9.3	6.0	4.6	7.5
8	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	9.1	6.0	4.7	7.3
9	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	6.0	4.6	7.4
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	8.6	5.8	4.5	7.2
18	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
19	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
20	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

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 #: 267

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2071</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>Gold</u>
Date and times organisms were born between:	<u>02-08-22 0620 to 0830</u>	Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>02-02-0201-22 A</u>		
Replicate number:	1 2 3 4 5 6 7 8 9 10		
Culture board cup number:	<u>3 5 8 9 10 13 23 31 32 38</u>		
Transfer vessel information:	pH (S.U.): <u>8.02</u> Temperature (°C): <u>25.1</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
			Selenastrum	YWT		
0	02-08-22	<u>0859</u>	<u>01-27-22</u>	<u>01-27-22</u>	<u>02-03-22B</u>	<u>JL</u>
1	02-09-22	<u>0813</u>	↓	↓	↓	<u>JL</u>
2	02-10-22	<u>0826</u>	↓	↓	<u>02-03-22C</u>	<u>JL</u>
3	02-11-22	<u>0832</u>	↓	↓	↓	<u>JL</u>
4	02-12-22	<u>0900</u>	↓	↓	<u>02-03-22D</u>	<u>JL</u>
5	02-13-22	<u>0830</u>	↓	↓	↓	<u>JL</u>
6	02-14-22	<u>0830</u>	↓	↓	↓	<u>JL</u>
7	02-15-22	<u>0814</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>1007.</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>32.2</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>3.27.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1086.9</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 267

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	4	5	5	3	6	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	13	12	12	14	15	11	13	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	13	16	15	15	17	15	13	15	15	15
Total young produced		32	31	32	32	34	32	34	32	32	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	32.2

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	5	5	4	5	5	3	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	12	12	11	13	11	14	12	13	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	15	14	17	16	14	17	14	15
Total young produced		33	36	32	30	34	32	33	32	32	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	32.7
% Reduction from Control:	-167.

Species: Ceriodaphnia dubia
 800 mg NaCl/L

CdNaClCR #: 267

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	4	6	5	5	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	11	11	13	12	13	10	10	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	12	17	15	13	16	16	14	15	17	17
Total young produced		28	33	30	30	34	33	32	29	32	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:	31.6
% Reduction from Control:	1.97.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	4	4	4	3	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	10	10	12	10	11	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	14	12	16	15	14	15	15	13	15	17
Total young produced		30	28	32	29	28	30	30	28	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:	29.9
% Reduction from Control:	7.17.

Species: *Ceriodaphnia dubia*
1200 mg NaCl/L

CdNaClCR #: 267

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07
Mean Offspring/Female:	17.1
% Reduction from Control:	46.97

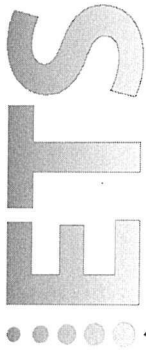
1400 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07
Mean Offspring/Female:	1.7
% Reduction from Control:	94.77



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	4	5	5	3	6	6	4	4	47
5	13	11	13	12	12	14	15	11	13	12	126
6	0	0	0	0	0	0	0	0	0	0	0
7	13	16	15	15	17	15	13	15	15	15	149
Total	32	31	32	32	34	32	34	32	32	31	322

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	5	4	5	5	3	5	6	48
5	14	12	12	11	13	11	14	12	13	12	124
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	15	14	17	16	14	17	14	15	155
Total	33	36	32	30	34	32	33	32	32	33	327

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	6	5	5	5	4	5	5	49
5	12	10	11	11	13	12	13	10	10	13	115
6	0	0	0	0	0	0	0	0	0	0	0
7	12	17	15	13	16	16	14	15	17	17	152
Total	28	33	30	30	34	33	32	29	32	35	316

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	4	4	4	3	5	4	5	5	43
5	12	11	12	10	10	12	10	11	11	11	110
6	0	0	0	0	0	0	0	0	0	0	0
7	14	12	16	15	14	15	15	13	15	17	146
Total	30	28	32	29	28	30	30	28	31	33	299

1200 mg NaCl/L

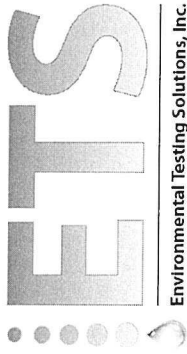
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	2	4	2	3	3	3	2	4	30
5	8	5	6	4	6	5	9	8	9	4	64
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	6	9	10	8	7	9	5	9	77
Total	16	18	14	17	18	16	19	20	16	17	171

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	1	2	2	1	3	1	1	1	1	15
5	0	0	0	0	0	0	1	0	0	1	2
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	1	2	2	1	3	2	1	1	2	17

Prepared and
Reviewed 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 #267
Test dates: February 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	32	31	32	32	34	32	34	32	32	31	100	32.2	3.2	Not applicable
600	33	36	32	30	34	32	33	32	32	33	100	32.7	4.8	-1.6
800	28	33	30	30	34	33	32	29	32	35	100	31.6	7.2	1.9
1000	30	28	32	29	28	30	30	28	31	33	100	29.9	5.8	7.1
1200	16	18	14	17	18	16	19	20	16	17	100	17.1	10.1	46.9
1400	2	1	2	2	1	3	2	1	1	2	100	1.7	39.7	94.7

Dunnett's MSD value: 1.623
PMSD: 5.0

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) = 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

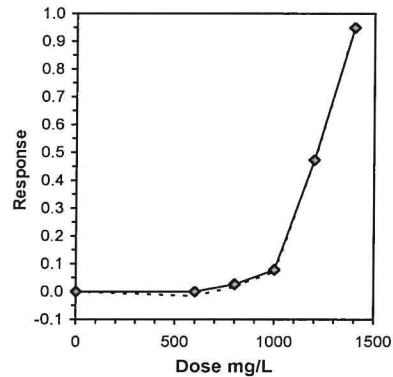
Start Date: 2/8/2022 Test ID: CdNaCICR Sample ID: REF-Ref Toxicant
 End Date: 2/15/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	32.000	31.000	32.000	32.000	34.000	32.000	34.000	32.000	32.000	31.000
600	33.000	36.000	32.000	30.000	34.000	32.000	33.000	32.000	32.000	33.000
800	28.000	33.000	30.000	30.000	34.000	33.000	32.000	29.000	32.000	35.000
1000	30.000	28.000	32.000	29.000	28.000	30.000	30.000	28.000	31.000	33.000
1200	16.000	18.000	14.000	17.000	18.000	16.000	19.000	20.000	16.000	17.000
1400	2.000	1.000	2.000	2.000	1.000	3.000	2.000	1.000	1.000	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	32.200	1.0000	32.200	31.000	34.000	3.207	10				32.450	1.0000
600	32.700	1.0155	32.700	30.000	36.000	4.792	10	-0.705	2.287	1.623	32.450	1.0000
800	31.600	0.9814	31.600	28.000	35.000	7.185	10	0.845	2.287	1.623	31.600	0.9738
*1000	29.900	0.9286	29.900	28.000	33.000	5.782	10	3.241	2.287	1.623	29.900	0.9214
*1200	17.100	0.5311	17.100	14.000	20.000	10.110	10	21.276	2.287	1.623	17.100	0.5270
*1400	1.700	0.0528	1.700	1.000	3.000	39.703	10	42.975	2.287	1.623	1.700	0.0524

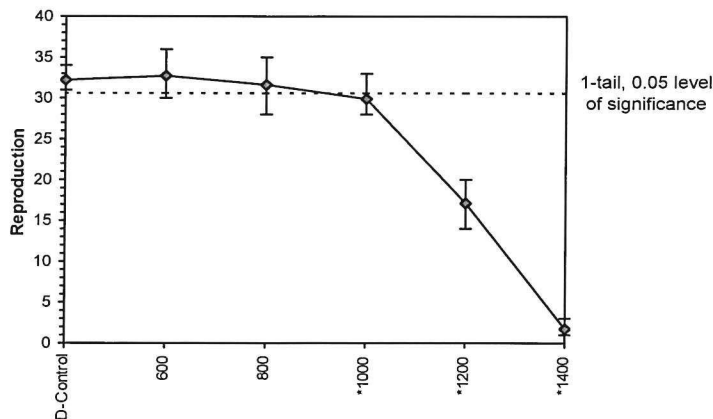
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ($p > 0.01$)	1.01548	1.035	0.15331	0.16334
Bartlett's Test indicates equal variances ($p = 0.02$)	13.247	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	800	1000	894.427	
Treatments vs D-Control				
	MSDu	MSDp	MSB	MSE
	1.6229	0.0504	1560.32	2.51852
			F-Prob	df
			0.0E+00	5, 54

Point	Linear Interpolation (200 Resamples)				
	mg/L	SD	95% CL	Skew	
IC05	890.882	74.7225	732.444	1007.16	-0.3112
IC10	1010.86	14.2241	972.357	1030.71	-2.1257
IC15	1036.21	8.02926	1023.25	1053.57	0.1644
IC20	1061.56	7.25826	1049.57	1078.05	0.1812
IC25	1086.91	6.75533	1075.18	1100.37	0.2077
IC40	1162.97	7.2749	1151.34	1181.76	0.3971
IC50	1211.36	6.47072	1197.51	1225.62	-0.0440



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 #: 267

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	UW	JW	JW	N	N
CONTROL, MHSW	pH (S.U.)	7.91	7.88	7.70	7.80	7.72	7.91
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.8	7.7	7.9
	Conductivity (µmhos/cm)	31836		301		292	
	Alkalinity (mg CaCO ₃ /L)	60				59	
	Hardness (mg CaCO ₃ /L)	87				87	
	Temperature (°C)	25.0	25.1	24.9	25.2	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.97	7.84	7.86	7.74	7.70	7.80
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1510		1430		1380	
	Temperature (°C)	24.9	24.9	25.0	25.0	25.0	24.9
800 mg NaCl/L	pH (S.U.)	7.97	7.83	7.86	7.73	7.80	7.78
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1870		1800		1760	
	Temperature (°C)	24.9	24.9	24.9	25.0	25.0	25.1
1000 mg NaCl/L	pH (S.U.)	7.98	7.83	7.89	7.73	7.83	7.78
	Dissolved oxygen (mg/L)	7.7	7.8	7.8	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2270		2170		2150	
	Temperature (°C)	25.1	24.9	24.9	24.9	24.8	25.1
1200 mg NaCl/L	pH (S.U.)	7.98	7.83	7.89	7.73	7.84	7.79
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2610		2580		2500	
	Temperature (°C)	25.1	25.2	24.9	25.1	24.8	25.1
1400 mg NaCl/L	pH (S.U.)	7.98	7.84	7.90	7.72	7.84	7.80
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.8	7.8	8.0
	Conductivity (µmhos/cm)	3010		2910		2870	
	Temperature (°C)	25.0	25.2	24.9	24.9	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

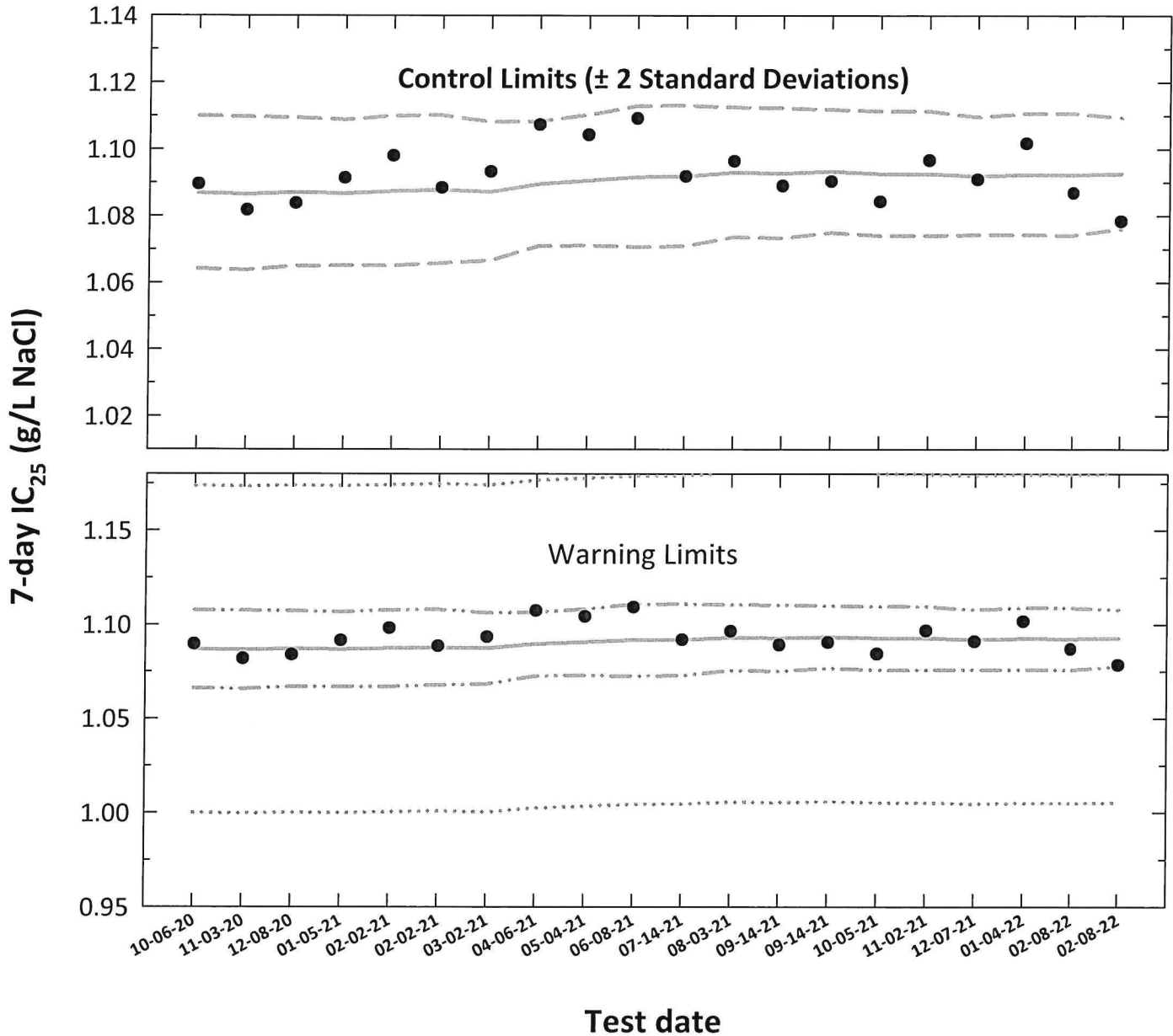
CdNaClCR #: 267

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		KL	BSL	BSL	KL	KL	JW	JW	JW
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.72	7.90	7.63	7.94	7.60	7.85	7.64	7.64
	Dissolved oxygen (mg/L)	7.7	7.9	7.7	7.8	7.77	7.9	7.8	7.87
	Conductivity (µmhos/cm)	298		289		299		321	
	Alkalinity (mg CaCO ₃ /L)			59					
	Hardness (mg CaCO ₃ /L)			85					
	Temperature (°C)	25.0	25.2	24.9	25.2	24.9	25.1	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.77	7.90	7.84	7.80	7.84	7.88	7.85	7.74
	Dissolved oxygen (mg/L)	7.8	7.9	7.8	7.8	7.7	7.9	7.8	7.7
	Conductivity (µmhos/cm)	1410		1380		1370		1440	
	Temperature (°C)	24.9	25.0	24.8	24.9	25.0	24.9	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.77	7.91	7.87	7.80	7.80	7.89	7.87	7.75
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.8	7.7	7.8	8.0	7.9
	Conductivity (µmhos/cm)	1770		1740		1770		1870	
	Temperature (°C)	24.9	25.3	24.8	25.2	25.0	24.9	24.8	25.2
1000 mg NaCl/L	pH (S.U.)	7.82	7.90	7.89	7.80	7.83	7.89	7.87	7.75
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.8	7.8	7.9	8.0	8.0
	Conductivity (µmhos/cm)	2100		2110		2130		2230	
	Temperature (°C)	25.0	25.1	24.8	25.2	25.0	25.0	24.8	25.1
1200 mg NaCl/L	pH (S.U.)	7.84	7.90	7.91	7.79	7.89	7.89	7.89	7.76
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.9	7.9	7.8	7.9	8.0
	Conductivity (µmhos/cm)	2470		2460		2500		2620	
	Temperature (°C)	25.0	25.1	24.9	25.2	25.0	25.0	24.7	24.9
1400 mg NaCl/L	pH (S.U.)	7.85	7.90	7.92	7.79	7.89	7.91	7.91	7.87
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	8.0	7.9	7.9	8.0	8.0
	Conductivity (µmhos/cm)	2820		2800		2840		2990	
	Temperature (°C)	25.0	25.1	24.9	25.0	25.0	25.1	24.7	24.9
		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		CT	Anti-logarithmic Values (g/L NaCl)					
			7-day IC ₂₅	CT		S	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}		
1	10-06-20	1.0896	0.0373	0.0362	1.0869	1.0642	1.1100	1.0660	1.1077	0.9999	1.1738
2	11-03-20	1.0818	0.0341	0.0360	1.0865	1.0638	1.1097	1.0656	1.1074	0.9996	1.1734
3	12-08-20	1.0838	0.0349	0.0362	1.0870	1.0650	1.1095	1.0668	1.1073	1.0001	1.1740
4	01-05-21	1.0914	0.0380	0.0361	1.0867	1.0651	1.1088	1.0668	1.1067	0.9998	1.1737
5	02-02-21	1.0980	0.0406	0.0364	1.0873	1.0651	1.1100	1.0669	1.1077	1.0003	1.1743
6	02-02-21	1.0884	0.0368	0.0365	1.0878	1.0659	1.1101	1.0677	1.1079	1.0008	1.1748
7	03-02-21	1.0933	0.0387	0.0363	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
8	04-06-21	1.1074	0.0443	0.0372	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
9	05-04-21	1.1043	0.0431	0.0376	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
10	06-08-21	1.1092	0.0450	0.0381	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
11	07-14-21	1.0919	0.0382	0.0382	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
12	08-03-21	1.0964	0.0400	0.0386	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
13	09-14-21	1.0890	0.0370	0.0385	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
14	09-14-21	1.0904	0.0376	0.0387	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
15	10-05-21	1.0843	0.0351	0.0385	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
16	11-02-21	1.0967	0.0401	0.0385	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
17	12-07-21	1.0909	0.0378	0.0382	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
18	01-04-22	1.1017	0.0421	0.0384	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
19	02-08-22	1.0869	0.0362	0.0383	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
20	02-08-22	1.0784	0.0328	0.0385	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801

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.

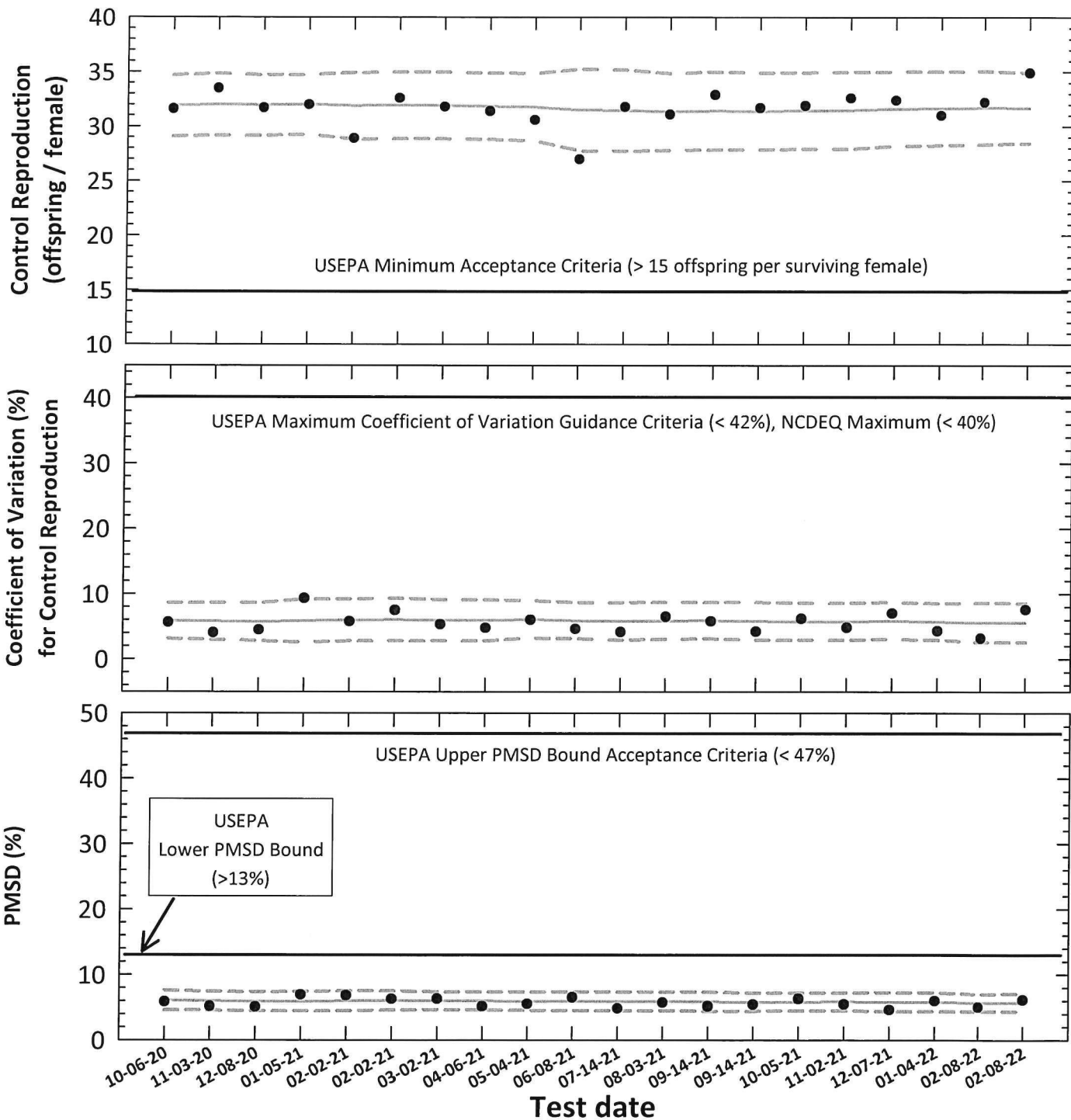
S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

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)

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

Test number	Test date	ToxCal Determination				Control Reproduction			Control Reproduction CV			Test PMSD				
		Control Survival (%)	Control Reproduction		PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval			
			Mean (offspring/female)	CV (%)			MSD	MSD		CT - 2S	CT + 2S		CT - 2S	CT + 2S	CT - 2S	CT + 2S
1	10-06-20	100	31.6	5.6	1.847	5.8	31.9	29.1	34.7	5.8	31.1	29.1	34.7	6.1	4.6	7.6
2	11-03-20	100	33.5	4.0	1.731	5.2	32.0	29.2	34.9	5.8	2.9	2.9	8.6	6.0	4.5	7.4
3	12-08-20	100	31.7	4.5	1.613	5.1	32.0	29.2	34.7	5.7	2.8	2.8	8.6	5.9	4.5	7.4
4	01-05-21	100	32.0	9.3	2.224	7.0	32.0	29.3	34.7	5.9	2.6	2.6	9.2	5.9	4.4	7.4
5	02-02-21	100	28.9	5.8	1.975	6.8	31.9	28.8	34.9	6.0	2.8	2.8	9.2	6.0	4.5	7.5
6	02-02-21	100	32.6	7.5	2.045	6.3	31.9	28.9	35.0	6.0	2.8	2.8	9.3	6.0	4.6	7.5
7	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	2.7	9.1	6.0	4.7	7.3
8	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	2.8	9.1	6.0	4.6	7.4
9	05-04-21	100	30.6	6.0	1.690	5.5	31.8	28.7	34.9	6.1	3.2	3.2	8.9	5.9	4.5	7.3
10	06-08-21	100	27.0	4.6	1.762	6.5	31.5	27.8	35.2	5.9	3.1	3.1	8.7	5.9	4.5	7.4
11	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	2.9	8.6	5.9	4.5	7.4
12	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	3.1	8.7	5.9	4.5	7.4
13	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	3.1	8.7	5.9	4.4	7.3
14	09-14-21	100	31.7	4.2	1.729	5.5	31.4	27.9	34.9	5.8	2.9	2.9	8.7	5.8	4.4	7.2
15	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	2.9	2.9	8.6	5.8	4.5	7.2
16	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	2.9	8.6	5.9	4.5	7.2
17	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	3.0	3.0	8.7	5.8	4.4	7.3
18	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	2.9	8.5	5.8	4.4	7.2
19	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	2.6	8.6	5.7	4.4	7.0
20	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	2.6	8.5	5.7	4.4	7.1

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 #: 268 - NEW AGAL

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2071</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>RED</u>
Date and times organisms were born between:	<u>02-08-22 0620 TO 0850</u>	Incubator number and shelf location:	<u>282</u>
Culture board:	<u>02-01-22 A</u>		
Replicate number:	1 2 3 4 5 6 7 8 9 10		
Culture board cup number:	<u>3 5 8 9 10 13 13 31 32 38</u>		
Transfer vessel information:	pH (S.U.): <u>8.02</u> Temperature (°C): <u>25.1</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	02-08-22	<u>0909</u>	<u>02-03-22 NEW</u>	<u>01-27-22</u>	<u>02-03-22 B</u>	<u>JL</u>
1	02-09-22	<u>0824</u>			↓	<u>JL</u>
2	02-10-22	<u>0836</u>			<u>02-03-22 C</u>	<u>JL</u>
3	02-11-22	<u>0843</u>			↓	<u>X</u>
4	02-12-22	<u>0910</u>			<u>02-03-22 D</u>	<u>JL</u>
5	02-13-22	<u>0840</u>			↓	<u>H</u>
6	02-14-22	<u>0840</u>			↓	<u>H</u>
7	02-15-22	<u>0832</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>1306646FS</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>1007.</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>34.9</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>7.67.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1078.4</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 268 - NEW ALGAE

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	4	5	5	5	6	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	14	12	12	11	14	12	11	13	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	18	15	19	16	20	17	17	19	18
Total young produced		33	38	31	36	32	39	35	33	37	35
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:	34.9

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	5	6	5	6	6	4	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	14	12	12	14	12	11	13	14	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	19	19	15	19	18	15	18	20	15
Total young produced		36	36	36	33	38	36	32	35	40	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:	35.5
% Reduction from Control:	-1.77.

Species: Ceriodaphnia dubia
800 mg NaCl/L

CdNaCICR #: 268 - NEW ALGAL

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	6	4	5	5	5	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	13	12	10	14	12	12	13	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	15	16	18	16	16	16	19	19
Total young produced		33	32	32	34	32	35	33	33	37	37
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	33.8
% Reduction from Control:	3.27.

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	4	5	4	4	4	5	5	5	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	14	10	10	14	11	13	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	19	15	14	17	16	15	15	18	15	18
Total young produced		33	32	32	31	30	34	31	36	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:	32.4
% Reduction from Control:	7.27.

Species: Ceriodaphnia dubia
1200 mg NaCl/L

CdNaCICR #: 268 - NEW ALGAE

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	3	5	4	4	4	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	7	9	7	6	10	8	5	9	6	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	9	4	10	7	5	3	3	5	6	5
Total young produced		19	17	20	18	19	15	12	17	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:	51.07.

1400 mg NaCl/L

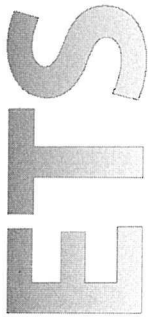
Survival and Reproduction Data

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	2	3	2	1	2	1	1	1	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	1	1	1	0	0	1	0	2	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		2	3	4	3	1	2	2	1	3	1
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	2.2
% Reduction from Control:	93.77.



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	6	4	5	5	6	5	5	5	5	50
5	12	14	12	11	14	12	11	13	12	12	123
6	0	0	0	0	0	0	0	0	0	0	0
7	17	18	15	19	16	20	17	17	19	18	176
Total	33	38	31	36	32	39	35	33	37	35	349

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	5	6	5	6	6	4	6	6	52
5	13	14	12	12	14	12	11	13	14	12	127
6	0	0	0	0	0	0	0	0	0	0	0
7	18	19	19	15	19	18	15	18	20	15	176
Total	36	36	36	33	38	36	32	35	40	33	355

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	6	4	5	5	5	5	6	49
5	13	10	13	12	10	14	12	12	13	12	121
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	15	16	18	16	16	16	19	19	168
Total	33	32	32	34	32	35	33	33	37	37	338

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	4	4	4	5	5	5	6	6	48
5	10	12	14	10	10	14	11	13	10	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	19	15	14	17	16	15	15	18	15	18	162
Total	33	32	32	31	30	34	31	36	31	34	324

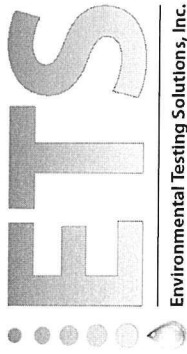
1200 mg NaCl/L

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	3	5	4	4	4	3	4	4	38
5	7	9	7	6	10	8	5	9	6	9	76
6	0	0	0	0	0	0	0	0	0	0	0
7	9	4	10	7	5	3	3	5	6	5	57
Total	19	17	20	18	19	15	12	17	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	2	3	2	1	2	1	1	1	1	16
5	0	1	1	1	0	0	1	0	2	0	6
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	4	3	1	2	2	1	3	1	22

Entered and Reviewed 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 #268 - New Algae
 Test dates: February 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	33	38	31	36	32	39	35	33	37	35	100	34.9	7.6	Not applicable
600	36	36	36	33	38	36	32	35	40	33	100	35.5	6.8	-1.7
800	33	32	32	34	32	35	33	33	37	37	100	33.8	5.7	3.2
1000	33	32	32	31	30	34	31	36	31	34	100	32.4	5.7	7.2
1200	19	17	20	18	19	15	12	17	16	18	100	17.1	13.6	51.0
1400	2	3	4	3	1	2	2	1	3	1	100	2.2	46.9	93.7

Dunnnett's MSD value: 2.146
 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

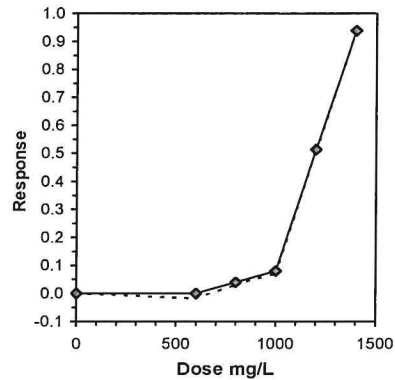
Start Date: 2/8/2022 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
 End Date: 2/15/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	38.000	31.000	36.000	32.000	39.000	35.000	33.000	37.000	35.000
600	36.000	36.000	36.000	33.000	38.000	36.000	32.000	35.000	40.000	33.000
800	33.000	32.000	32.000	34.000	32.000	35.000	33.000	33.000	37.000	37.000
1000	33.000	32.000	32.000	31.000	30.000	34.000	31.000	36.000	31.000	34.000
1200	19.000	17.000	20.000	18.000	19.000	15.000	12.000	17.000	16.000	18.000
1400	2.000	3.000	4.000	3.000	1.000	2.000	2.000	1.000	3.000	1.000

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	34.900	1.0000	34.900	31.000	39.000	7.575	10				35.200	1.0000
600	35.500	1.0172	35.500	32.000	40.000	6.803	10	-0.639	2.287	2.146	35.200	1.0000
800	33.800	0.9685	33.800	32.000	37.000	5.717	10	1.172	2.287	2.146	33.800	0.9602
*1000	32.400	0.9284	32.400	30.000	36.000	5.672	10	2.663	2.287	2.146	32.400	0.9205
*1200	17.100	0.4900	17.100	12.000	20.000	13.631	10	18.963	2.287	2.146	17.100	0.4858
*1400	2.200	0.0630	2.200	1.000	4.000	46.945	10	34.836	2.287	2.146	2.200	0.0625

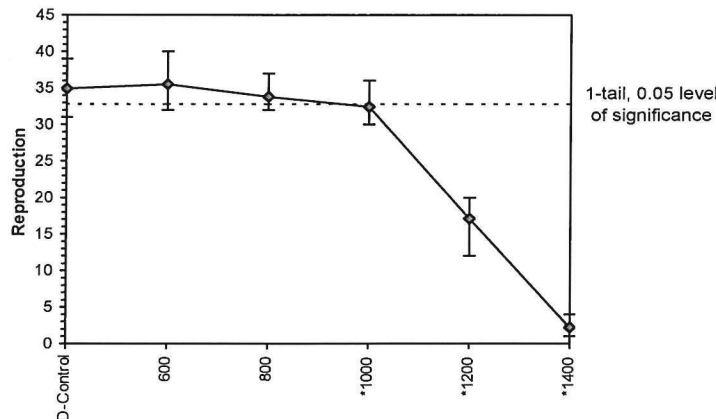
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.47339	1.035	0.05301	-0.0594						
Bartlett's Test indicates equal variances (p = 0.17)	7.74446	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		2.14644	0.0615	1833.82	4.40556	8.2E-42	5, 54

Point	Linear Interpolation (200 Resamples)				
	mg/L	SD	95% CL	Skew	
IC05	851.429	89.3891	656.275	1005.08	-0.1897
IC10	1009.41	38.2948	867.676	1028.04	-2.7028
IC15	1032.42	9.7353	1009.64	1049.79	-0.5428
IC20	1055.42	9.08579	1034.73	1071.05	-0.4444
IC25	1078.43	8.72782	1059.13	1093.76	-0.3911
IC40	1147.45	9.04978	1128.42	1163.43	-0.1310
IC50	1193.46	9.96448	1174.95	1210.34	-0.1513



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 #: 268 - NEW ALGAE

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		JK	JW	JW	JK	JK	JK
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.91	7.93	7.70	7.70	7.72	8.01
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.7	7.7	8.0
	Conductivity (µmhos/cm)	316		301		292	
	Alkalinity (mg CaCO ₃ /L)	60				59	
	Hardness (mg CaCO ₃ /L)	87				87	
	Temperature (°C)	24.9	25.1	24.9	25.0	24.8	24.8
600 mg NaCl/L	pH (S.U.)	7.97	7.87	7.86	7.69	7.70	7.97
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.7	7.8	8.0
	Conductivity (µmhos/cm)	1510		1430		1300	
	Temperature (°C)	25.0	24.9	24.9	25.2	24.9	24.9
800 mg NaCl/L	pH (S.U.)	7.97	7.85	7.86	7.85	7.80	7.93
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.8	7.8	8.0
	Conductivity (µmhos/cm)	1870		1800		1760	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.9	25.1
1000 mg NaCl/L	pH (S.U.)	7.98	7.85	7.89	7.84	7.83	7.89
	Dissolved oxygen (mg/L)	7.7	7.8	7.8	7.9	7.8	8.1
	Conductivity (µmhos/cm)	2270		2170		2150	
	Temperature (°C)	25.0	25.0	25.0	24.8	24.9	25.1
1200 mg NaCl/L	pH (S.U.)	7.98	7.84	7.89	7.81	7.84	7.85
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.9	7.8	8.1
	Conductivity (µmhos/cm)	2610		2580		2500	
	Temperature (°C)	24.9	25.2	25.0	25.2	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.98	7.85	7.90	7.79	7.84	7.82
	Dissolved oxygen (mg/L)	7.9	8.0	7.9	7.8	7.8	8.1
	Conductivity (µmhos/cm)	3010		2910		2870	
	Temperature (°C)	24.9	25.2	25.0	24.9	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

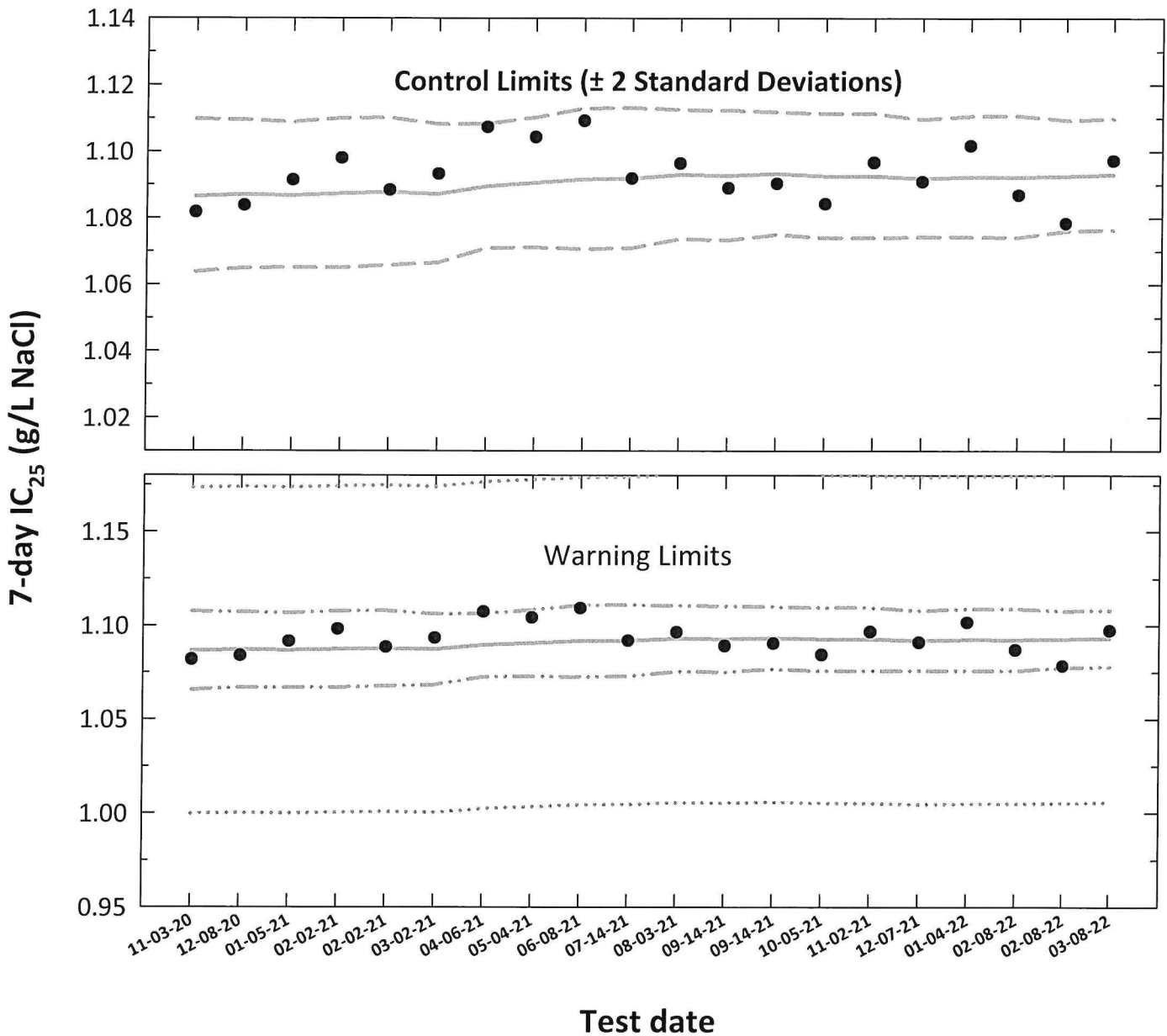
CdNaClCR #: 268 - NEW ALGAE

Concentration		Parameter		Day							
				(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
				3		4		5		6	
Analyst		KL	BSL	BSL	KL	KL	JW	JW	JW		
CONTROL, MHSW	pH (S.U.)	7.72	7.87	7.63	7.92	7.60	7.89	7.64	7.72		
	Dissolved oxygen (mg/L)	7.7	7.9	7.7	7.8	7.7	8.0	7.8	7.9		
	Conductivity (µmhos/cm)	298		289		299		321			
	Alkalinity (mg CaCO ₃ /L)			59							
	Hardness (mg CaCO ₃ /L)		2-13-14 K	85					0-13-14 K		
	Temperature (°C)	24.9	25.2	24.8	25.1	25.0	25.0	24.7	25.2		
600 mg NaCl/L	pH (S.U.)	7.77	7.88	7.84	7.82	7.81	7.85	7.85	7.81		
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.8	7.7	8.0	7.8	8.0		
	Conductivity (µmhos/cm)	1410		1380		1370		1440			
	Temperature (°C)	25.0	24.9	24.9	24.9	24.9	25.3	24.8	25.2		
800 mg NaCl/L	pH (S.U.)	7.77	7.89	7.87	7.82	7.86	7.87	7.87	7.79		
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.9	7.7	8.0	8.0	8.0		
	Conductivity (µmhos/cm)	1770		1740		1770		1870			
	Temperature (°C)	25.0	24.9	24.9	24.9	24.9	25.0	24.7	25.0		
1000 mg NaCl/L	pH (S.U.)	7.82	7.89	7.89	7.82	7.88	7.88	7.87	7.79		
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.9	7.8	8.0	8.0	8.0		
	Conductivity (µmhos/cm)	2100		2110		2130		2230			
	Temperature (°C)	25.0	24.9	24.9	25.1	25.0	25.1	24.7	24.9		
1200 mg NaCl/L	pH (S.U.)	7.84	7.90	7.91	7.83	7.89	7.89	7.89	7.87		
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	8.0	7.9	8.0	7.9	8.0		
	Conductivity (µmhos/cm)	2470		2460		2500		2620			
	Temperature (°C)	25.0	25.2	24.8	25.3	25.0	25.1	24.7	24.9		
1400 mg NaCl/L	pH (S.U.)	7.85	7.90	7.92	7.88	7.89	7.90	7.91	7.89		
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	8.1	7.9	8.0	8.0	8.0		
	Conductivity (µmhos/cm)	2820		2800		2840		2990			
	Temperature (°C)	24.9	24.9	24.8	25.1	25.0	25.1	24.9	24.9		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final		

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



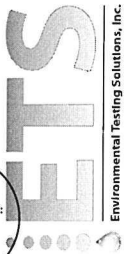
- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (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)

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 Warning Limits CT - S _{A,10} CT + S _{A,10}		
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV			
1	11-03-20	1.0818	0.0341	0.0360	0.0046	1.0865	1.1097	1.0656	1.1074	0.9996	1.1734
2	12-08-20	1.0838	0.0349	0.0362	0.0044	1.0870	1.1095	1.0668	1.1073	1.0001	1.1740
3	01-05-21	1.0914	0.0380	0.0361	0.0044	1.0867	1.1088	1.0668	1.1067	0.9998	1.1737
4	02-02-21	1.0980	0.0406	0.0364	0.0045	1.0873	1.1100	1.0669	1.1077	1.0003	1.1743
5	02-02-21	1.0884	0.0368	0.0365	0.0044	1.0878	1.1101	1.0677	1.1079	1.0008	1.1748
6	03-02-21	1.0933	0.0387	0.0363	0.0041	1.0872	1.1082	1.0683	1.1061	1.0002	1.1742
7	04-06-21	1.1074	0.0443	0.0372	0.0037	1.0895	1.1083	1.0725	1.1065	1.0024	1.1767
8	05-04-21	1.1043	0.0431	0.0376	0.0039	1.0906	1.1103	1.0728	1.1083	1.0033	1.1778
9	06-08-21	1.1092	0.0450	0.0381	0.0042	1.0917	1.1130	1.0725	1.1108	1.0043	1.1790
10	07-14-21	1.0919	0.0382	0.0382	0.0042	1.0919	1.1131	1.0728	1.1110	1.0045	1.1792
11	08-03-21	1.0964	0.0400	0.0386	0.0039	1.0930	1.1126	1.0754	1.1106	1.0056	1.1804
12	09-14-21	1.0890	0.0370	0.0385	0.0039	1.0927	1.1124	1.0751	1.1104	1.0053	1.1801
13	09-14-21	1.0904	0.0376	0.0387	0.0037	1.0933	1.1119	1.0766	1.1100	1.0058	1.1808
14	10-05-21	1.0843	0.0351	0.0385	0.0037	1.0926	1.1114	1.0757	1.1095	1.0052	1.1800
15	11-02-21	1.0967	0.0401	0.0385	0.0037	1.0926	1.1114	1.0757	1.1095	1.0052	1.1800
16	12-07-21	1.0909	0.0378	0.0382	0.0035	1.0919	1.1096	1.0758	1.1079	1.0045	1.1792
17	01-04-22	1.1017	0.0421	0.0384	0.0036	1.0924	1.1107	1.0759	1.1089	1.0050	1.1798
18	02-08-22	1.0869	0.0362	0.0383	0.0036	1.0923	1.1107	1.0757	1.1089	1.0049	1.1797
19	02-08-22	1.0784	0.0328	0.0385	0.0033	1.0927	1.1094	1.0775	1.1078	1.0052	1.1801
20	03-08-22	1.0972	0.0403	0.0386	0.0033	1.0930	1.1099	1.0779	1.1082	1.0056	1.1805

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).

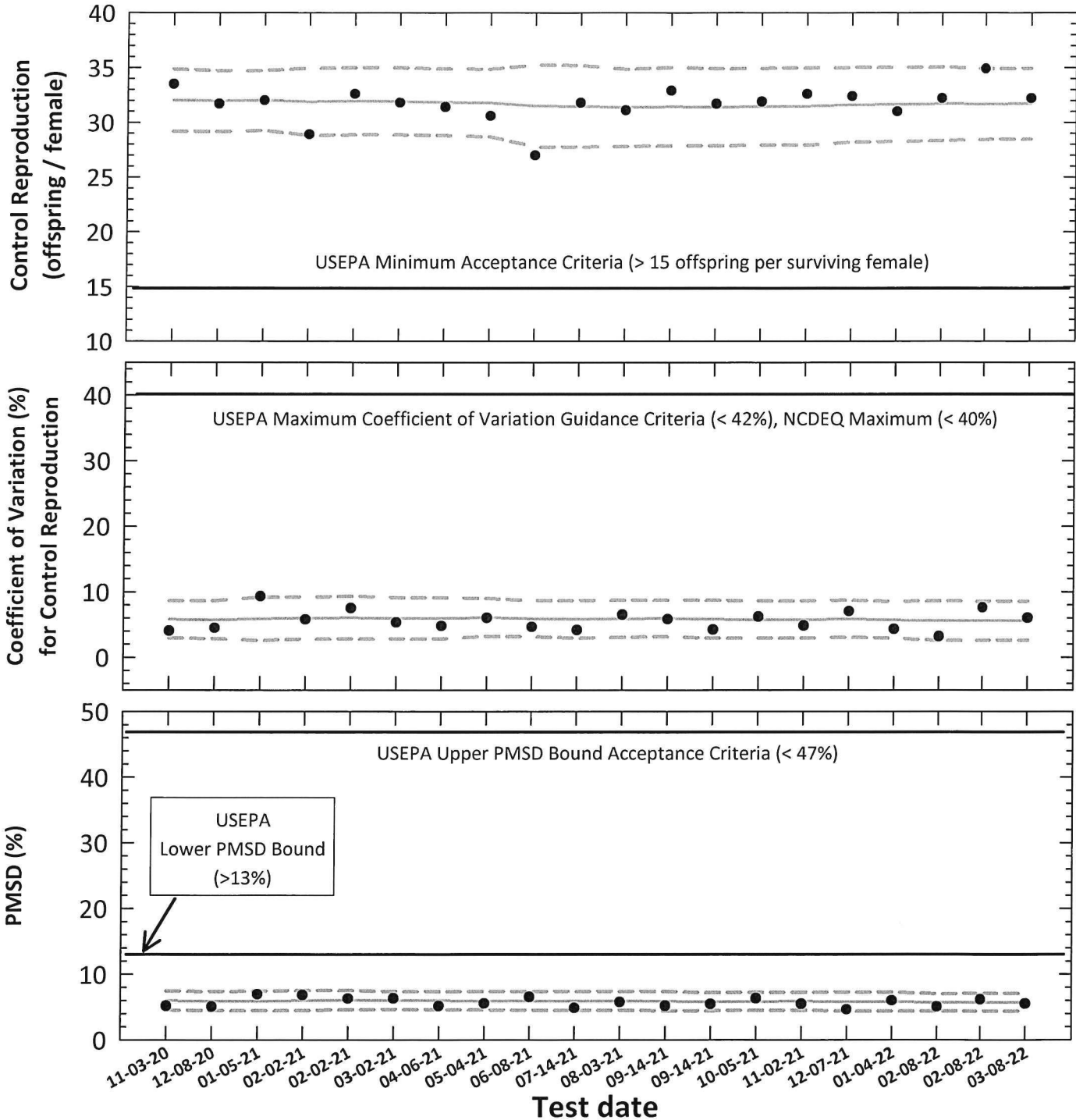
CV = Coefficient of variation.



Reviewed and
 Released by
 Jim Sumner

Ceriodaphnia dubia

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

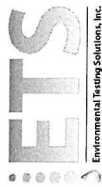


- **Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity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

● **Central Tendency** (mean Control Reproduction, CV or PMSD)

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





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

Ceriodaphnia dubia

Test number	Test date	ToxCal Determination				Control Reproduction			Control Reproduction CV			Test PMSD (%)			
		Control Survival (%)	Control Reproduction		Test MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (offspring/female)	CV (%)				(offspring/female)	CT - 2S		CT + 2S	(offspring/female)			CT - 2S
1	11-03-20	100	33.5	4.0	1.731	5.2	32.0	29.2	34.9	5.8	2.9	8.6	6.0	4.5	7.4
2	12-08-20	100	31.7	4.5	1.613	5.1	32.0	29.2	34.7	5.7	2.8	8.6	5.9	4.5	7.4
3	01-05-21	100	32.0	9.3	2.224	7.0	32.0	29.3	34.7	5.9	2.6	9.2	5.9	4.4	7.4
4	02-02-21	100	28.9	5.8	1.975	6.8	31.9	28.8	34.9	6.0	2.8	9.2	6.0	4.5	7.5
5	02-02-21	100	32.6	7.5	2.045	6.3	31.9	28.9	35.0	6.0	2.8	9.3	6.0	4.6	7.5
6	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	9.1	6.0	4.7	7.3
7	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	6.0	4.6	7.4
8	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
9	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
10	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
11	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
12	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	4.5	7.4
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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%.

MSTD = 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 represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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 #: 269

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2071</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>Blue</u>
Date and times organisms were born between:	<u>03-08-22 0545 to 0851</u>										Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>03-01-22 A</u>											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	<u>5</u>	<u>8</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>18</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>29</u>		
Transfer vessel information:	pH (S.U.): <u>7.94</u> Temperature (°C): <u>25.1</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	03-08-22	<u>0903</u>	<u>03-03-22</u>	<u>03-03-22</u>	<u>03-03-22B</u>	<u>J</u>
1	03-09-22	<u>0837</u>	↓	↓	↓	<u>J</u>
2	03-10-22	<u>0820</u>	↓	↓	<u>03-03-22C</u>	<u>J</u>
3	03-11-22	<u>0814</u>	↓	↓	↓	<u>J</u>
4	03-12-22	<u>0912</u>	↓	↓	<u>03-07-22</u>	<u>J</u>
5	03-13-22	<u>0836</u>	↓	↓	↓	<u>J</u>
6	03-14-22	<u>0841</u>	↓	↓	↓	<u>J</u>
7	03-15-22	<u>0804</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>1007.</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>32.2</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.4</u>
% CV:	<u>6.07.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1097.2</u>



Species: Ceriodaphnia dubia

CdNaClCR #: 269

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	4	5	4	5	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	14	12	13	12	10	10	13	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	16	17	15	18	17	13	17	17	15
Total young produced		33	34	33	33	34	32	29	34	31	29
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:	01.
Mean Offspring/Female:	32.2

600 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	01.
Mean Offspring/Female:	32.2
% Reduction from Control:	0.0



Species: Ceriodaphnia dubia
800 mg NaCl/L

CdNaClCR #: 269

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	4	6	5	5	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	13	10	12	12	10	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	15	17	19	16	15	15	16	15	18	15
Total young produced		33	32	38	30	31	33	31	31	35	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	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	4	3	3	4	4	4	3	4	4	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	12	10	12	12	12	10	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	18	14	15	15	16	13	17	12	15	15
Total young produced		31	29	28	31	32	29	32	26	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:	29.5
% Reduction from Control:	8.47.



Species: *Ceriodaphnia dubia*
1200 mg NaCl/L

CdNaClCR #: 269

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	4	2	2	1	4	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	8	8	7	10	6	10	7	9	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	7	7	9	8	6	11	5	9	9	10
Total young produced		19	17	20	19	18	19	16	20	21	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:	18.7
% Reduction from Control:	41.97

1400 mg NaCl/L

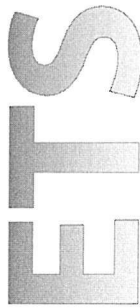
Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	0	0	0	0	0	0	0	2	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	1	2	1	3	2	0	0	2	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	1	0	0	1	1	0	0	0
Total young produced		0	1	3	1	3	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:	94.77





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	4	5	4	5	6	4	4	4	44
5	12	14	12	13	12	10	10	13	10	10	116
6	0	0	0	0	0	0	0	0	0	0	0
7	17	16	17	15	18	17	13	17	17	15	162
Total	33	34	33	33	34	32	29	34	31	29	322

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	3	5	4	5	5	6	4	4	6	5	47
5	11	14	12	12	12	10	11	14	10	10	116
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	15	17	13	16	18	14	16	17	159
Total	32	34	31	34	30	32	33	32	32	32	322

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	6	4	4	6	5	5	5	6	49
5	13	12	13	10	12	12	10	11	12	11	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	19	16	15	15	16	15	18	15	161
Total	33	32	38	30	31	33	31	31	35	32	326

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	3	4	4	4	4	5	4	4	38
5	9	12	10	12	12	12	10	10	10	10	107
6	0	0	0	0	0	0	0	0	0	0	0
7	18	14	15	15	16	13	17	12	15	15	150
Total	31	29	28	31	32	29	32	26	29	28	295

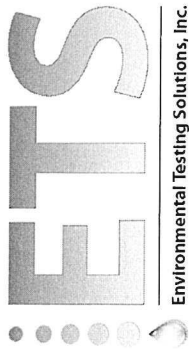
1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	2	3	4	2	2	1	4	3	3	27
5	9	8	8	7	10	6	10	7	9	5	79
6	0	0	0	0	0	0	0	0	0	0	0
7	7	7	9	8	6	11	5	9	9	10	81
Total	19	17	20	19	18	19	16	20	21	18	187

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	2	0	0	2
5	0	1	2	1	3	2	0	0	2	1	12
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	1	0	0	1	1	0	0	0	3
Total	0	1	3	1	3	3	1	2	2	1	17

Entered and Reviewed 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 #269
 Test dates: March 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	33	34	33	33	34	32	29	34	31	29	100	32.2	6.0	Not applicable
600	32	34	31	34	30	32	33	32	32	32	100	32.2	3.8	0.0
800	33	32	38	30	31	33	31	31	35	32	100	32.6	7.3	-1.2
1000	31	29	28	31	32	29	32	26	29	28	100	29.5	6.6	8.4
1200	19	17	20	19	18	19	16	20	21	18	100	18.7	8.0	41.9
1400	0	1	3	1	3	3	1	2	2	1	100	1.7	62.3	94.7

Dunnett's MSD value: 1.773 MSD = Minimum Significant Difference
 PMSD: 5.5 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

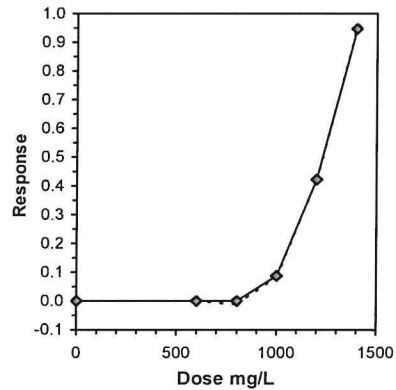
Start Date:	3/8/2022	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	34.000	33.000	33.000	34.000	32.000	29.000	34.000	31.000	29.000
600	32.000	34.000	31.000	34.000	30.000	32.000	33.000	32.000	32.000	32.000
800	33.000	32.000	38.000	30.000	31.000	33.000	31.000	31.000	35.000	32.000
1000	31.000	29.000	28.000	31.000	32.000	29.000	32.000	26.000	29.000	28.000
1200	19.000	17.000	20.000	19.000	18.000	19.000	16.000	20.000	21.000	18.000
1400	0.000	1.000	3.000	1.000	3.000	3.000	1.000	2.000	2.000	1.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	32.200	1.0000	32.200	29.000	34.000	6.001	10				32.333	1.0000	
600	32.200	1.0000	32.200	30.000	34.000	3.818	10	0.000	2.287	1.773	32.333	1.0000	
800	32.600	1.0124	32.600	30.000	38.000	7.259	10	-0.516	2.287	1.773	32.333	1.0000	
*1000	29.500	0.9161	29.500	26.000	32.000	6.637	10	3.482	2.287	1.773	29.500	0.9124	
*1200	18.700	0.5807	18.700	16.000	21.000	7.992	10	17.412	2.287	1.773	18.700	0.5784	
*1400	1.700	0.0528	1.700	0.000	3.000	62.315	10	39.339	2.287	1.773	1.700	0.0526	

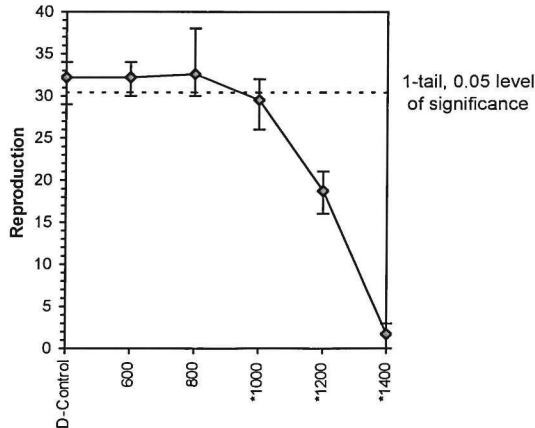
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution ($p > 0.01$)	0.67861	1.035	0.27808	0.81559						
Bartlett's Test indicates equal variances ($p = 0.18$)	7.65511	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		1.77288	0.05506	1525.34	3.00556	4.5E-44	5, 54
Treatments vs D-Control										

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	914.118	41.4332	823.629	997.347	-0.0218
IC10	1007.41	21.273	949.286	1025.98	-1.2909
IC15	1037.35	10.5744	1013.37	1053.74	-0.2752
IC20	1067.28	9.27569	1045.1	1081.61	-0.3235
IC25	1097.22	8.3363	1077.17	1109.58	-0.3764
IC40	1187.04	8.57663	1167.92	1200.43	-0.3078
IC50	1229.8	5.12523	1217.68	1236.6	-0.5455



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 #: 269

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		K	K	K	K	K	K
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.69	7.83	7.77	7.81	7.67	7.74
	Dissolved oxygen (mg/L)	7.6	7.6	7.6	7.6	7.6	7.5
	Conductivity (µmhos/cm)	300		299		300	
	Alkalinity (mg CaCO ₃ /L)	60				61	
	Hardness (mg CaCO ₃ /L)	80				80	
	Temperature (°C)	24.8	25.2	24.9	25.0	24.9	25.2
	600 mg NaCl/L	pH (S.U.)	7.88	7.76	7.82	7.79	7.84
Dissolved oxygen (mg/L)		7.8	7.5	7.6	7.6	7.6	7.6
Conductivity (µmhos/cm)		1420		1430		1410	
Temperature (°C)		24.8	25.0	25.0	25.1	24.9	24.9
800 mg NaCl/L	pH (S.U.)	7.89	7.80	7.86	7.79	7.86	7.79
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.6	7.6	7.6
	Conductivity (µmhos/cm)	1840		1800		1820	
	Temperature (°C)	24.9	25.2	25.0	25.0	25.0	24.9
1000 mg NaCl/L	pH (S.U.)	7.90	7.83	7.86	7.79	7.88	7.80
	Dissolved oxygen (mg/L)	7.8	7.4	7.7	7.6	7.7	7.6
	Conductivity (µmhos/cm)	2180		2150		2150	
	Temperature (°C)	24.9	25.2	24.9	24.9	24.8	25.1
1200 mg NaCl/L	pH (S.U.)	7.91	7.82	7.89	7.80	7.89	7.81
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.6	7.8	7.6
	Conductivity (µmhos/cm)	2560		2520		2540	
	Temperature (°C)	25.0	25.1	24.9	24.9	24.8	24.8
1400 mg NaCl/L	pH (S.U.)	7.94	7.86	7.94	7.81	7.91	7.81
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.6	7.8	7.6
	Conductivity (µmhos/cm)	2930		2920		2890	
	Temperature (°C)	25.0	25.3	24.9	24.9	24.8	25.2
		Initial	Final	Initial	Final	Initial	Final



Species: Ceriodaphnia dubia

CdNaClCR #: 269

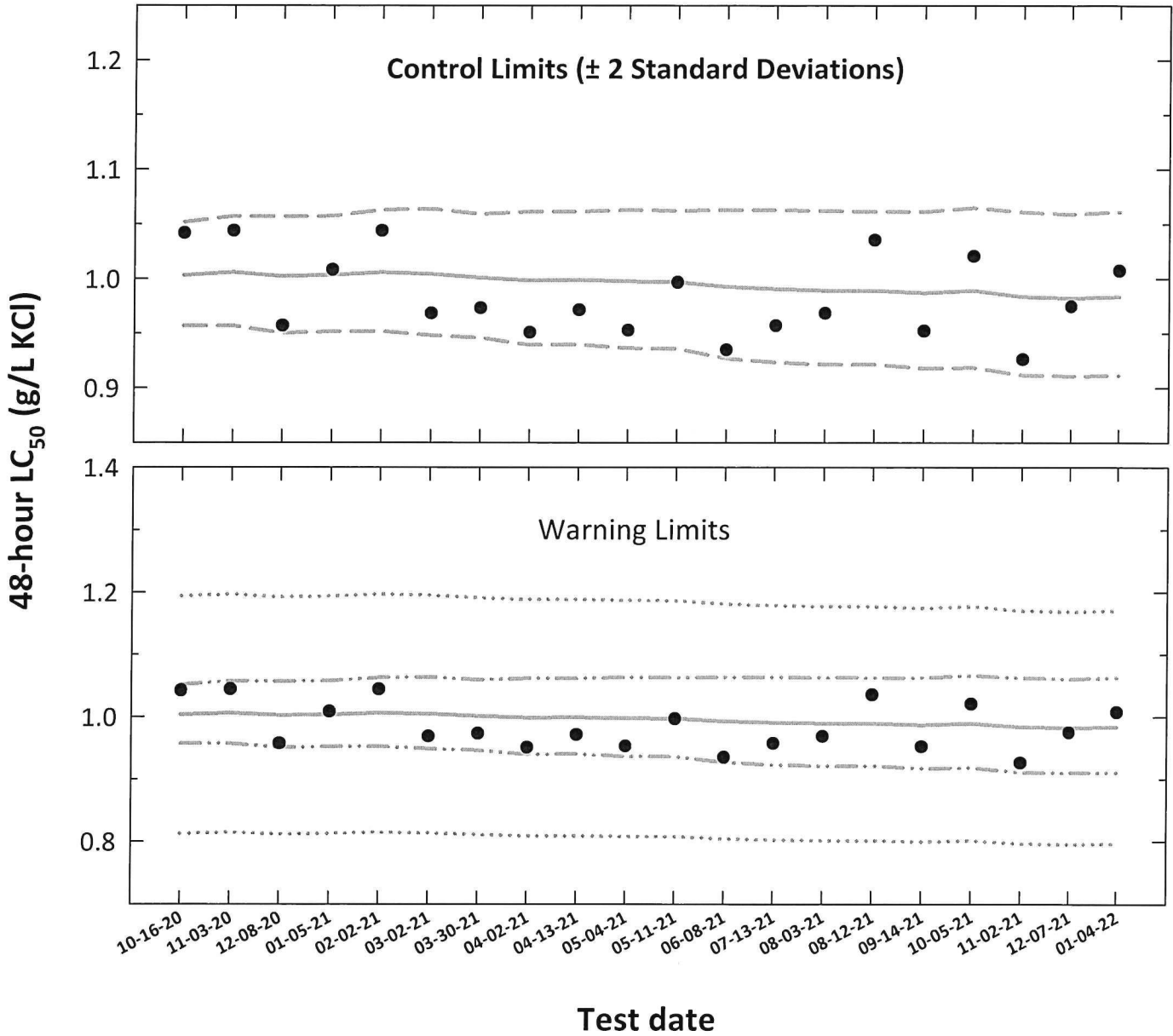
Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	N	BSL	BSL	N	N	N	N	JW
CONTROL, MHSW	pH (S.U.)	7.70	7.94	7.61	7.98	7.74	7.60	7.64	8.02
	Dissolved oxygen (mg/L)	7.7	7.6	7.9	7.8	7.7	7.7	7.7	7.8
	Conductivity (µmhos/cm)	311		309		291		303	
	Alkalinity (mg CaCO ₃ /L)			60					
	Hardness (mg CaCO ₃ /L)			84					
	Temperature (°C)	24.8	25.2	24.9	25.1	24.8	25.2	24.7	25.0
600 mg NaCl/L	pH (S.U.)	7.87	7.94	7.99	7.90	7.89	7.73	7.82	8.00
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.9	7.7	7.7	7.7	7.8
	Conductivity (µmhos/cm)	1450		1360		1430		1440	
	Temperature (°C)	24.9	25.0	24.9	25.3	24.9	24.8	24.7	24.9
800 mg NaCl/L	pH (S.U.)	7.89	7.96	8.00	7.89	7.90	7.74	7.84	7.98
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.9	7.7	7.6	7.7	7.7
	Conductivity (µmhos/cm)	1880		1750		1840		1870	
	Temperature (°C)	25.0	24.9	24.8	25.2	24.9	24.9	24.8	24.9
1000 mg NaCl/L	pH (S.U.)	7.89	7.97	7.99	7.89	7.92	7.74	7.85	8.00
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	8.0	7.8	7.6	7.7	7.7
	Conductivity (µmhos/cm)	2220		2100		2200		2190	
	Temperature (°C)	24.9	24.9	24.9	25.2	24.8	25.1	24.7	24.9
1200 mg NaCl/L	pH (S.U.)	7.92	7.96	7.99	7.88	7.93	7.77	7.86	7.99
	Dissolved oxygen (mg/L)	7.8	7.5	7.7	8.0	7.8	7.6	7.7	7.8
	Conductivity (µmhos/cm)	2610		2480		2580		2590	
	Temperature (°C)	24.9	25.1	25.0	24.9	25.0	24.8	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.98	7.96	8.00	7.89	7.94	7.79	7.88	7.99
	Dissolved oxygen (mg/L)	7.7	7.5	7.6	8.1	7.8	7.6	7.7	7.9
	Conductivity (µmhos/cm)	3030		2800		2960		2980	
	Temperature (°C)	25.0	24.8	24.9	24.9	24.8	24.8	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

N

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	10-16-20	1.0418	0.0178	0.0014	0.0103	1.0033	0.9570	1.0518	0.9572	1.0517	0.8127	1.1939
2	11-03-20	1.0440	0.0187	0.0026	0.0108	1.0060	0.9570	1.0575	0.9573	1.0572	0.8148	1.1971
3	12-08-20	0.9572	-0.0190	0.0010	0.0115	1.0023	0.9503	1.0570	0.9505	1.0569	0.8118	1.1927
4	01-05-21	1.0083	0.0036	0.0015	0.0114	1.0034	0.9519	1.0576	0.9521	1.0575	0.8128	1.1940
5	02-02-21	1.0440	0.0187	0.0026	0.0120	1.0060	0.9521	1.0631	0.9524	1.0627	0.8149	1.1972
6	03-02-21	0.9685	-0.0139	0.0020	0.0125	1.0046	0.9484	1.0641	0.9486	1.0639	0.8137	1.1954
7	03-30-21	0.9732	-0.0118	0.0005	0.0123	1.0011	0.9462	1.0593	0.9462	1.0592	0.8109	1.1913
8	04-02-21	0.9509	-0.0219	-0.0005	0.0132	0.9988	0.9397	1.0616	0.9396	1.0616	0.8090	1.1885
9	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9989	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887
10	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9978	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874
11	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
12	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 130

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 #: 2063

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Analyst	<i>K</i>	<i>K</i>	<i>K</i>
	pH (S.U.)	7.10	7.02	7.08
	Dissolved oxygen (mg/L)	7.7	7.0	7.0
	Conductivity (µmhos/cm)	304		
	Alkalinity (mg/L CaCO ₃)	61		
	Hardness (mg/L CaCO ₃)	94		
	Temperature (°C)	24.7	25.1	24.5
500 mg/L	pH (S.U.)	7.79	7.76	7.75
	Dissolved oxygen (mg/L)	7.9	7.0	7.7
	Conductivity (µmhos/cm)	1210		
	Temperature (°C)	24.8	24.9	24.8
750 mg/L	pH (S.U.)	7.79	7.03	7.77
	Dissolved oxygen (mg/L)	7.9	7.0	7.7
	Conductivity (µmhos/cm)	1030		
	Temperature (°C)	24.7	24.9	24.7
1000 mg/L	pH (S.U.)	7.01	7.05	7.77
	Dissolved oxygen (mg/L)	0.0	7.0	7.0
	Conductivity (µmhos/cm)	2070		
	Temperature (°C)	24.7	25.0	24.7
1250 mg/L	pH (S.U.)	7.03	7.06	7.01
	Dissolved oxygen (mg/L)	7.9	7.9	7.0
	Conductivity (µmhos/cm)	2520		
	Temperature (°C)	24.7	25.0	24.4
1500 mg/L	pH (S.U.)	7.05	7.06	7.05
	Dissolved oxygen (mg/L)	7.9	8.0	7.05
	Conductivity (µmhos/cm)	2940		7.05
	Temperature (°C)	24.7	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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 # 130

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-04-22	* 0610	JL	0810	JL	TF	GREEN	12-29-21C
24	01-05-22			0808	K			
48 Termination	01-06-22			0810	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:	12-23-21
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	12-28-21 1210 TO 12-29-21 0900
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.93
	Temperature (°C): 24.4°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	5 ^{sd}	6 ^{ud}	2 ^{sd}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	4 ^{1d}	5 ^{1d}	2	0	0	0
Mean Survival	100%		100%		100%		45%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	937.2
Upper 95% confidence limit (mg KCl/L)	1072.7
48-hour LC ₅₀ (mg KCl/L)	1007.5

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 1/4/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/6/2022	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	1.0000	1.0000
1000	0.5000	0.6000
1250	0.2000	0.0000
1500	0.0000	0.0000

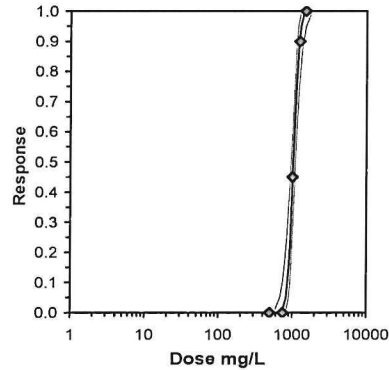
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20	
750	1.0000	1.0000	1.4120	1.4120	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	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		0.16274	0.16691	0.49067	0.01031	3.6E-04	4, 5

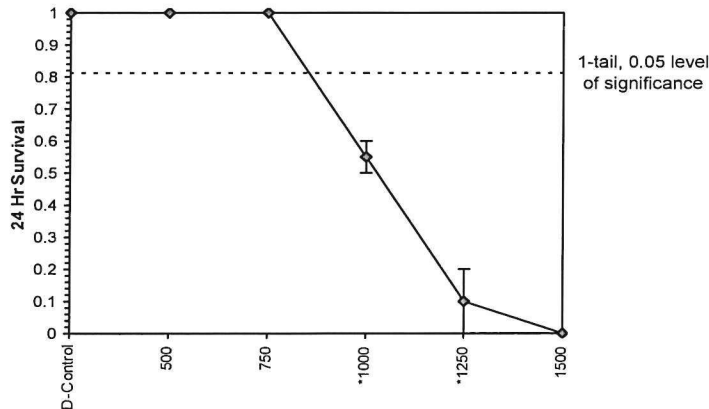
Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iiter		
Slope	17.071	3.53267	10.1469	23.995	0	0.52223	7.81472	0.91398	3.01329	0.05858	4
Intercept	-46.44	10.6862	-67.385	-25.495							

TSCR

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: 1/4/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/6/2022	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

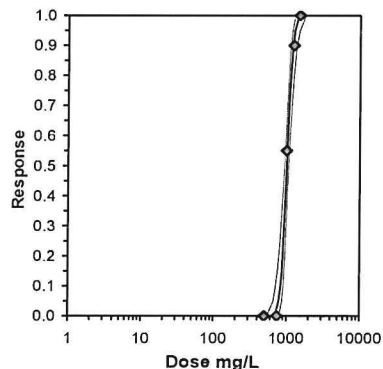
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.4000	0.5000
1250	0.2000	0.0000
1500	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	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	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	6.668	2.850	0.2894	11	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

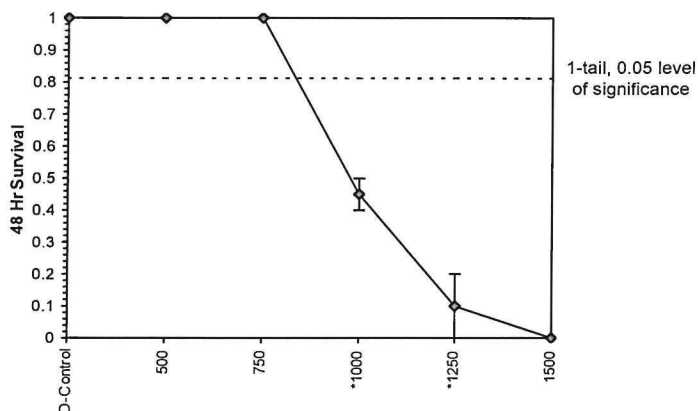
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	750	1000	866.025	0.16274
Treatments vs D-Control	MSDu	MSDp	MSB	MSE
	0.16691	0.51898	0.01031	3.1E-04
	F-Prob	df		
	4, 5			

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	16.2237	3.23436	9.88433	22.563	0	1.26017	7.81472	0.73861	3.00326	0.06164	5
Intercept	-43.724	9.75376	-62.841	-24.606							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	724.209	568.642	810.983
EC05	3.355	797.756	663.652	873.095
EC10	3.718	839.969	719.716	909.281
EC15	3.964	869.705	759.549	935.332
EC20	4.158	894.087	792.214	957.242
EC25	4.326	915.549	820.807	977.101
EC40	4.747	971.941	893.904	1033.15
EC50	5.000	1007.53	937.235	1072.66
EC60	5.253	1044.41	978.852	1118.03
EC75	5.674	1108.74	1042.98	1208.28
EC80	5.842	1135.36	1066.88	1249.24
EC85	6.036	1167.19	1093.95	1300.48
EC90	6.282	1208.51	1127.25	1370.07
EC95	6.645	1272.45	1175.95	1483.32
EC99	7.326	1401.68	1268.09	1728.32



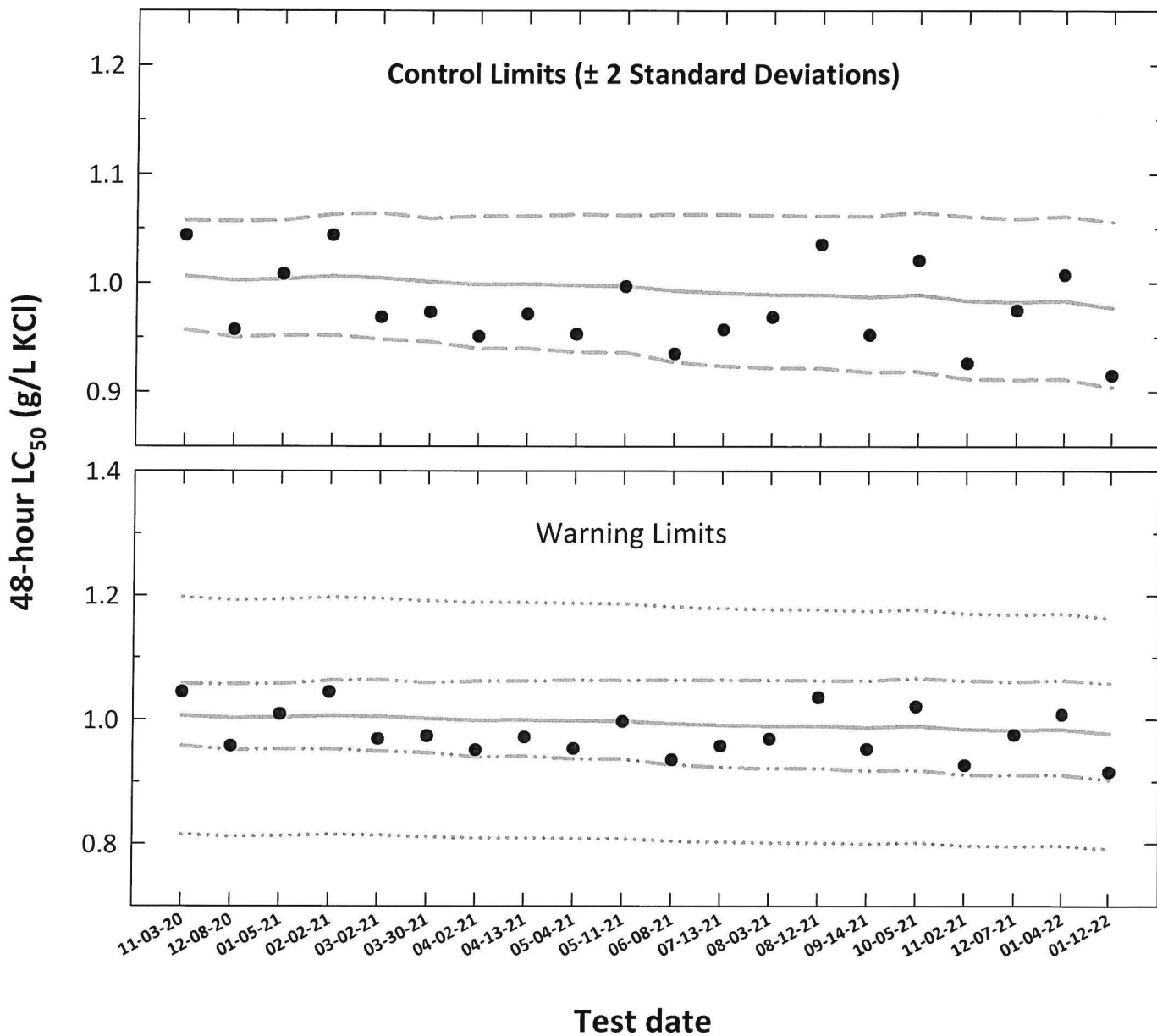
Dose-Response Plot



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	11-03-20	1.0440	0.0187	0.0026	0.0108	0.9570	1.0575	0.9573	1.0572	0.8148	1.1971		
2	12-08-20	0.9572	-0.0190	0.0010	0.0115	0.9503	1.0570	0.9505	1.0569	0.8118	1.1927		
3	01-05-21	1.0083	0.0036	0.0015	0.0114	0.9519	1.0576	0.9521	1.0575	0.8128	1.1940		
4	02-02-21	1.0440	0.0187	0.0026	0.0120	0.9521	1.0631	0.9524	1.0627	0.8149	1.1972		
5	03-02-21	0.9685	-0.0139	0.0020	0.0125	0.9484	1.0641	0.9486	1.0639	0.8137	1.1954		
6	03-30-21	0.9732	-0.0118	0.0005	0.0123	0.9462	1.0593	0.9462	1.0592	0.8109	1.1913		
7	04-02-21	0.9509	-0.0219	-0.0005	0.0132	0.9397	1.0616	0.9396	1.0616	0.8090	1.1885		
8	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887		
9	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874		
10	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867		
11	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815		
12	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791		
13	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774		
14	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773		
15	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746		
16	10-05-21	1.0207	0.0089	-0.0047	0.0160	0.9190	1.0650	0.9182	1.0659	0.8014	1.1773		
17	11-02-21	0.9262	-0.0333	-0.0071	0.0164	0.9120	1.0610	0.9108	1.0623	0.7968	1.1706		
18	12-07-21	0.9747	-0.0111	-0.0078	0.0163	0.9111	1.0590	0.9099	1.0604	0.7957	1.1689		
19	01-04-22	1.0075	0.0033	-0.0072	0.0165	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705		
20	01-12-22	0.9151	-0.0385	-0.0100	0.0168	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630		

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 # 131

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 #: 2063

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	JW 7.56	JW 7.56	K 7.06
	Dissolved oxygen (mg/L)	JW 7.28	JW 7.9	K 7.6
	Conductivity (µmhos/cm)	312		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	82		
	Temperature (°C)	24.2	25.2	25.4
500 mg/L	pH (S.U.)	7.75	7.68	7.07
	Dissolved oxygen (mg/L)	7.8	7.9	7.5
	Conductivity (µmhos/cm)	1200		
	Temperature (°C)	24.3	25.5	25.3
750 mg/L	pH (S.U.)	7.83	7.69	7.00
	Dissolved oxygen (mg/L)	7.8	8.0	7.5
	Conductivity (µmhos/cm)	1620		
	Temperature (°C)	24.3	25.3	25.2
1000 mg/L	pH (S.U.)	7.87	7.69	7.00
	Dissolved oxygen (mg/L)	7.7	7.7	7.5
	Conductivity (µmhos/cm)	2140		
	Temperature (°C)	24.4	25.5	25.2
1250 mg/L	pH (S.U.)	7.89	7.68	7.09
	Dissolved oxygen (mg/L)	7.8	7.7	7.6
	Conductivity (µmhos/cm)	2880		
	Temperature (°C)	24.1	25.4	25.5
1500 mg/L	pH (S.U.)	7.90	7.70	7.10
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	3060		
	Temperature (°C)	24.5	25.4	25.2

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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 # 131

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-12-22	*0900 1200 01-12-22 8:1	01-12-22 A.I	1200	A.I	6A	Orange	01-08-22
24	01-13-22			1200	A.I			
48 Termination	01-14-22			1205	A.I			

*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:	12-29-21
Age (1 to 14 days old):	8 d, 7 s 8-9 DAYS
Hatch date and times:	01-02-22 0920 1452 01-02-22 11 01-04-22 0608
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.93 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 ^{1d}	7 ^{2d 1/2} 9 ⁸ 01-13-22	2 ^{8d}	5 ^{5d}	1 ^{9d}	0 ^{10d}	0 ^{10d}	1 ^{9d}
48 Termination	10	10	10	10	8 ^{1d}	8 ⁸ 01-13-22	2	5	1	0	0	1
Mean Survival	100%		100%		80%		35%		5%		5%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	830.2
Upper 95% confidence limit (mg KCl/L)	994.5
48-hour LC ₅₀ (mg KCl/L)	915.1

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 1/4/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 1/6/2022 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	0.8000
1000	0.2000	0.5000
1250	0.1000	0.0000
1500	0.0000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.3435	0	20
750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	1.927	2.830	0.3435	3	20
*1000	0.3500	0.3500	0.6245	0.4636	0.7854	36.430	2	6.488	2.830	0.3435	13	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	9.654	2.830	0.3435	19	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	9.654	2.830	0.3435	19	20

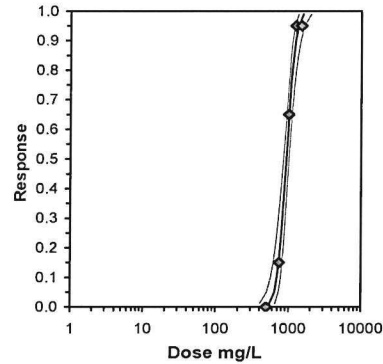
Auxiliary Tests

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

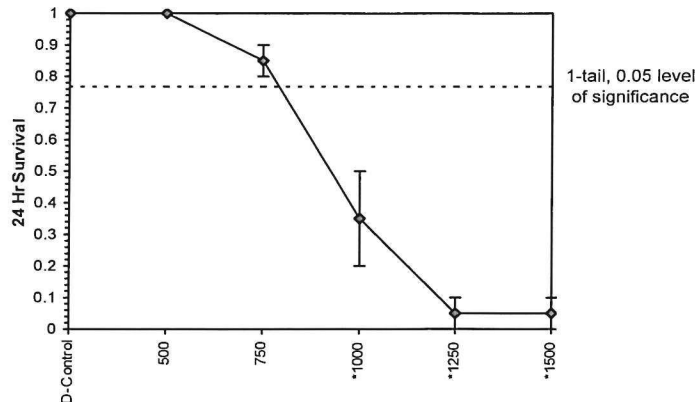
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	750	1000	866.025		0.20676	0.21206	0.6135	0.01473	1.4E-04	5, 6

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	10.28	1.78918	6.7732	13.7868	0	1.98208	7.81472	0.57613	2.96875	0.09728	3
Intercept	-25.519	5.35423	-36.013	-15.024							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	552.651	404.62	649.275
EC05	3.355	643.79	507.505	731.279
EC10	3.718	698.369	571.783	780.337
EC15	3.964	737.783	619.056	816.117
EC20	4.158	770.69	658.835	846.444
EC25	4.326	800.087	694.425	874.072
EC40	4.747	879.234	788.897	952.526
EC50	5.000	930.571	847.473	1008.21
EC60	5.253	984.904	905.693	1072.7
EC75	5.674	1082.33	999.305	1203.61
EC80	5.842	1123.62	1035.4	1264.36
EC85	6.036	1173.73	1077.11	1341.57
EC90	6.282	1239.98	1129.56	1448.55
EC95	6.645	1345.1	1208.46	1627.79
EC99	7.326	1566.92	1364.49	2036.62



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 1/4/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/6/2022	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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.8000	0.8000
1000	0.2000	0.5000
1250	0.1000	0.0000
1500	0.0000	0.1000

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	2.830	0.3233	0	20
750	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	2	2.668	2.830	0.3233	4	20
*1000	0.3500	0.3500	0.6245	0.4636	0.7854	36.430	2	6.893	2.830	0.3233	13	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	10.256	2.830	0.3233	19	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	10.256	2.830	0.3233	19	20

Auxiliary Tests

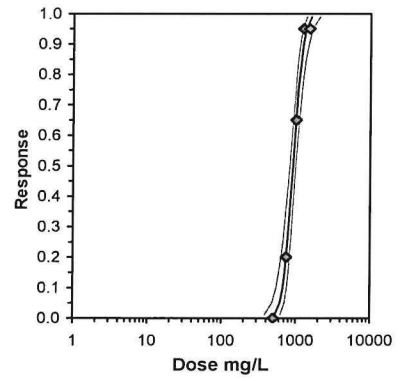
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.18997	0.19484	0.59663	0.01305	1.1E-04	5, 6

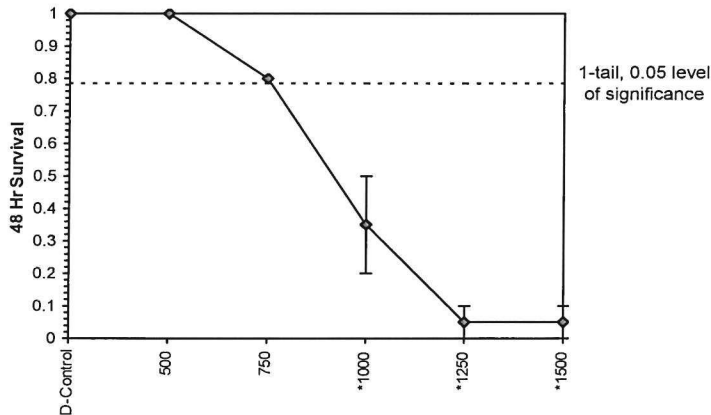
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.68096	1.66004	6.42728 12.9347	0	1.62955	7.81472	0.65271	2.96148	0.1033	3
Intercept	-23.67	4.95957	-33.391 -13.949							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	526.235	380.419 623.346
EC05	3.355	618.835	483.064 707.464
EC10	3.718	674.687	547.81 758.04
EC15	3.964	715.191	595.702 795.025
EC20	4.158	749.109	636.174 826.428
EC25	4.326	779.488	672.515 855.074
EC40	4.747	861.611	769.571 936.571
EC50	5.000	915.126	830.185 994.532
EC60	5.253	971.965	890.755 1061.79
EC75	5.674	1074.37	988.778 1198.9
EC80	5.842	1117.94	1026.77 1262.8
EC85	6.036	1170.95	1070.78 1344.24
EC90	6.282	1241.25	1126.3 1457.51
EC95	6.645	1353.28	1210.18 1648.27
EC99	7.326	1591.41	1377.15 2087.44



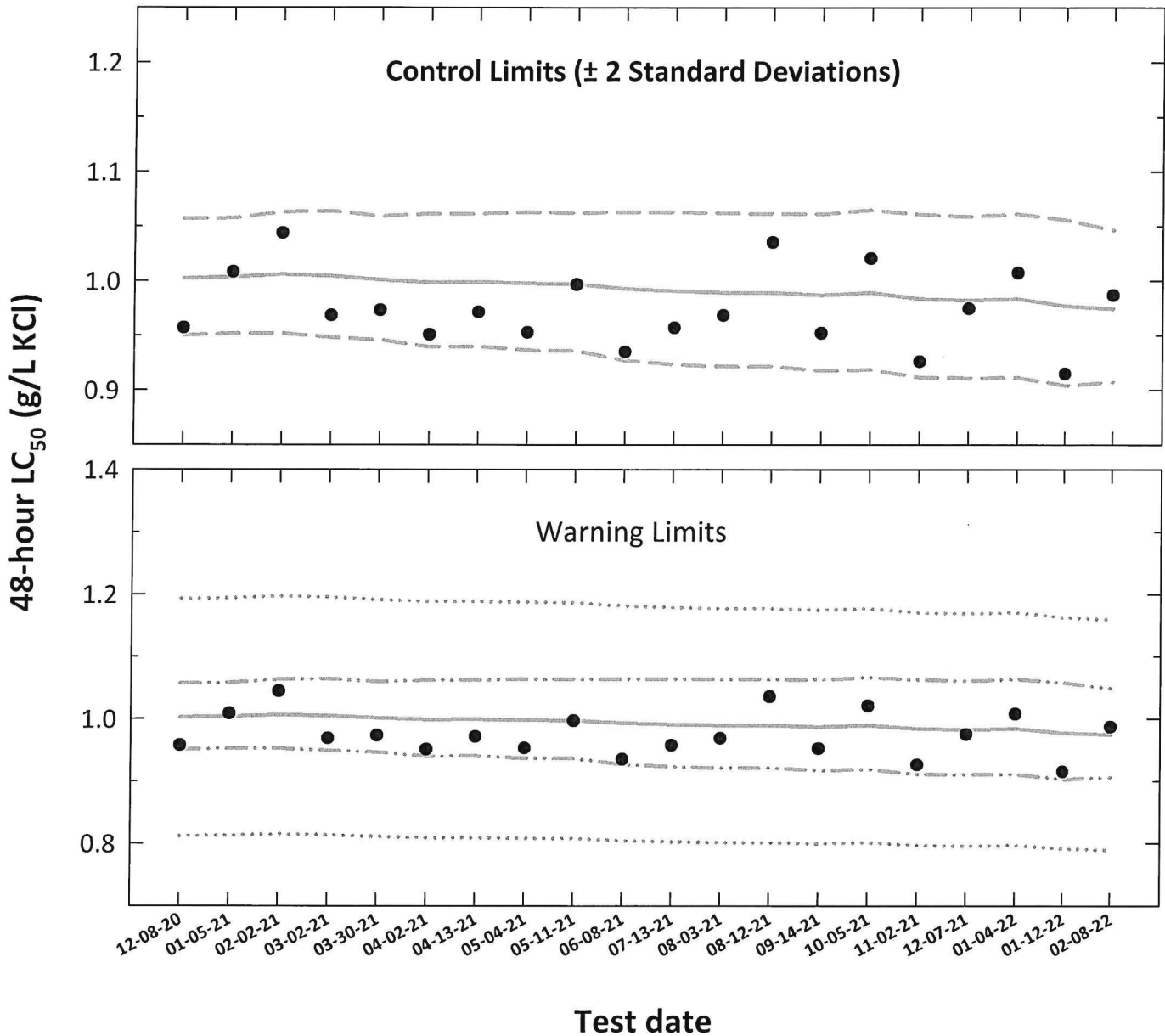
Dose-Response Plot



Pimephales promelas

Acute Reference Toxicant Control Chart

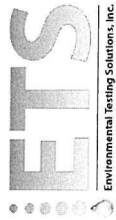
Source: In-house Culture



- **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)

Independent Review by
Kelley E. Keenan:

Entered and Reviewed by
Jim Sumner



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	12-08-20	0.9572	-0.0190	0.0010	0.0115	1.0023	0.9503	1.0570	0.9505	1.0569	0.8118	1.1927
2	01-05-21	1.0083	0.0036	0.0015	0.0114	1.0034	0.9519	1.0576	0.9521	1.0575	0.8128	1.1940
3	02-02-21	1.0440	0.0187	0.0026	0.0120	1.0060	0.9521	1.0631	0.9524	1.0627	0.8149	1.1972
4	03-02-21	0.9685	-0.0139	0.0020	0.0125	1.0046	0.9484	1.0641	0.9486	1.0639	0.8137	1.1954
5	03-30-21	0.9732	-0.0118	0.0005	0.0123	1.0011	0.9462	1.0593	0.9462	1.0592	0.8109	1.1913
6	04-02-21	0.9509	-0.0219	-0.0005	0.0132	0.9988	0.9397	1.0616	0.9396	1.0616	0.8090	1.1885
7	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9989	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887
8	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9978	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874
9	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
10	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 132

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 #: 2063

Chemical Analyses:

		Hours			
		0	24	48	
Control, MHSW	Concentration	Analyst	JW	JW	N
	pH (S.U.)		7.91	7.87	8.05
	Dissolved oxygen (mg/L)		7.7	7.8	7.0
	Conductivity (µmhos/cm)		316		
	Alkalinity (mg/L CaCO ₃)		60		
	Hardness (mg/L CaCO ₃)		87		
500 mg/L	Temperature (°C)		24.8	25.2	25.4
	pH (S.U.)		8.04	7.83	7.05
	Dissolved oxygen (mg/L)		7.9	7.9	7.0
	Conductivity (µmhos/cm)		1220		
750 mg/L	Temperature (°C)		24.9	25.3	25.2
	pH (S.U.)		8.01	7.84	7.01
	Dissolved oxygen (mg/L)		7.9	7.8	7.0
	Conductivity (µmhos/cm)		1670		
1000 mg/L	Temperature (°C)		24.9	25.2	25.4
	pH (S.U.)		8.01	7.84	7.70
	Dissolved oxygen (mg/L)		7.9	7.8	7.0
	Conductivity (µmhos/cm)		2110		
1250 mg/L	Temperature (°C)		24.8	25.4	25.4
	pH (S.U.)		8.01	7.85	7.00
	Dissolved oxygen (mg/L)		8.0	7.8	7.9
	Conductivity (µmhos/cm)		2570		
1500 mg/L	Temperature (°C)		24.8	25.4	25.5
	pH (S.U.)		8.01	7.84	7.00
	Dissolved oxygen (mg/L)		8.0	7.8	7.9
	Conductivity (µmhos/cm)		3010		
	Temperature (°C)		24.8	25.4	25.3

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on 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 # 132

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-08-22	* 0805	J	0805	J	7F	GREEN	02-08-22 B
24	02-08-22			0805	J			
48 Termination	02-10-22			0805	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:	01-26-22
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	02-01-22 1220 TO 02-02-22 0630
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.53
	Temperature (°C): 24.1

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):

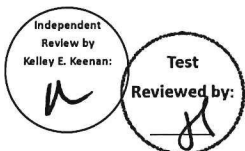
Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	8 ^{2d}	7 ^{3d}	2 ^{8d}	2 ^{8d}	1 ^{9d}	1 ^{9d}
48 Termination	10	10	10	10	10	9 ^{1d}	5 ^{3d}	4 ^{3d}	0 ^{2d}	1 ^{1d}	0 ^{1d}	1
Mean Survival	100%		100%		95%		45%		5%		5%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	909.1
Upper 95% confidence limit (mg KCl/L)	1060.6
48-hour LC ₅₀ (mg KCl/L)	986.9

Comments:



Acute Fathead Minnow Test-24 Hr Survival

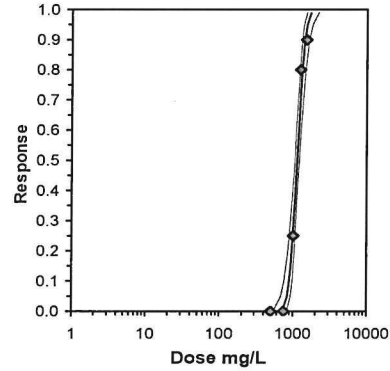
Start Date:	2/8/2022	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.8000	0.7000
1250	0.2000	0.2000
1500	0.1000	0.1000

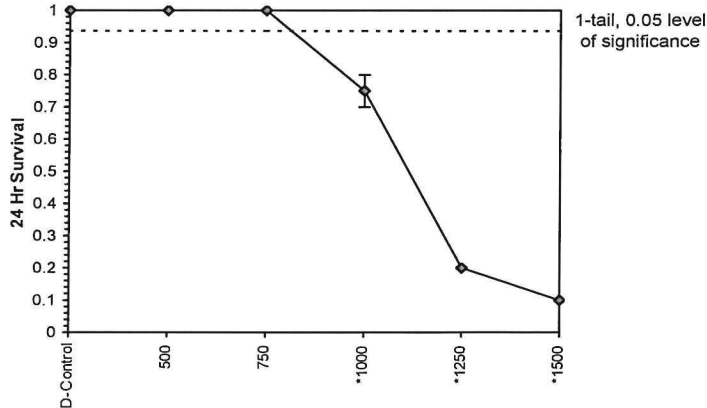
Conc-mg/L	Transform: Arcsin Square Root							1-Tailed			Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.0948	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.0948	0	20
*1000	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	10.837	2.830	0.0948	5	20
*1250	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	2	28.323	2.830	0.0948	16	20
*1500	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	32.561	2.830	0.0948	18	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		0.03792	0.03889	0.50342	0.00112	1.2E-07	5, 6
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	12.8252	2.39471	8.13158	17.5188	0	1.65503	7.81472	0.64698	3.05156	0.07797	4
Intercept	-34.137	7.33166	-48.507	-19.767							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	741.611	568.3	845.039							
EC05	3.355	838.133	686.141	928.431							
EC10	3.718	894.623	757.551	977.614							
EC15	3.964	934.873	809.073	1013.27							
EC20	4.158	968.149	851.781	1043.43							
EC25	4.326	997.64	889.474	1070.91							
EC40	4.747	1076	986.897	1149.33							
EC50	5.000	1126.07	1045.11	1205.51							
EC60	5.253	1178.47	1101.22	1270.79							
EC75	5.674	1271.03	1188.61	1401.98							
EC80	5.842	1309.75	1221.72	1461.86							
EC85	6.036	1356.37	1259.72	1537.02							
EC90	6.282	1417.39	1307.18	1639.66							
EC95	6.645	1512.92	1377.92	1808.34							
EC99	7.326	1709.83	1515.46	2181.07							



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 2/8/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 2/10/2022 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:

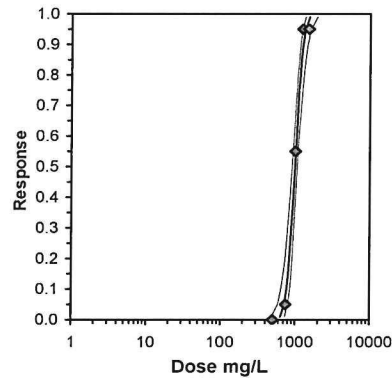
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.5000	0.4000
1250	0.0000	0.1000
1500	0.0000	0.1000

Conc-mg/L	Transform: Arcsin Square Root							N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2					0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2448		0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.942	2.830	0.2448		1	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	7.825	2.830	0.2448		11	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	13.544	2.830	0.2448		19	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	13.544	2.830	0.2448		19	20

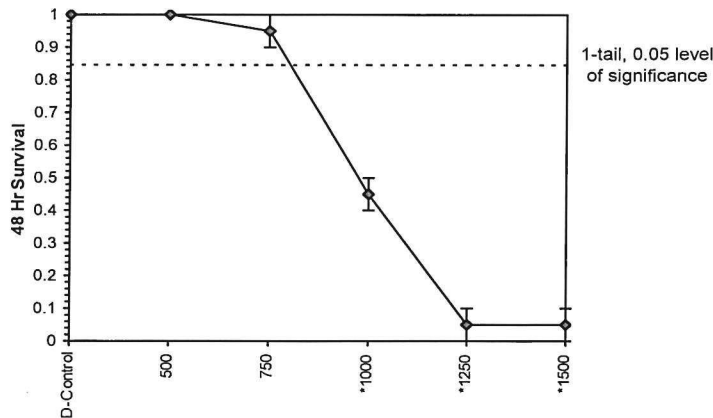
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12925	0.13256	0.64289	0.00748	1.7E-05	5, 6

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	12.1623	2.16764	7.91377	16.4109	0	2.86762	7.81472	0.41249	2.99427	0.08222	3
Intercept	-31.417	6.52744	-44.211	-18.624							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	635.325	484.759	729.321
EC05	3.355	722.819	588.234	806.374
EC10	3.718	774.284	651.204	851.951
EC15	3.964	811.062	696.783	885.002
EC20	4.158	841.534	734.682	912.915
EC25	4.326	868.567	768.247	938.279
EC40	4.747	940.677	855.821	1010.02
EC50	5.000	986.895	909.061	1060.64
EC60	5.253	1035.38	961.277	1118.83
EC75	5.674	1121.32	1044.13	1235.21
EC80	5.842	1157.36	1075.79	1288.46
EC85	6.036	1200.85	1112.18	1355.53
EC90	6.282	1257.89	1157.67	1447.47
EC95	6.645	1347.45	1225.48	1599.31
EC99	7.326	1533.01	1357.51	1937.03



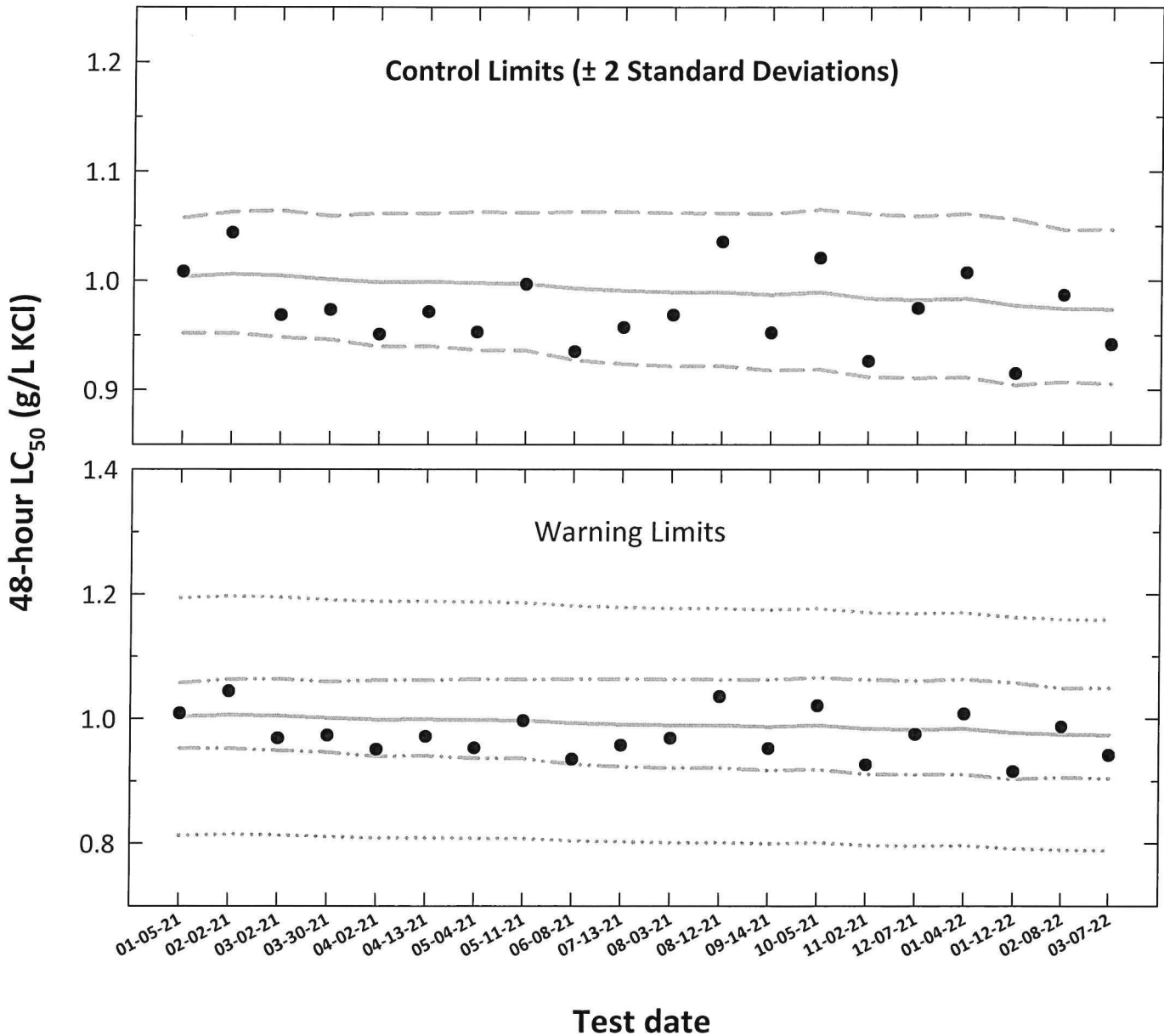
Dose-Response Plot



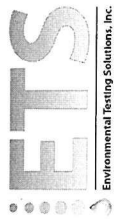
Pimephales promelas

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}
1	01-05-21	1.0083	0.0036	0.0015	0.0114	1.0034	0.9519	1.0576	0.9521	1.0575	0.8128	1.1940
2	02-02-21	1.0440	0.0187	0.0026	0.0120	1.0060	0.9521	1.0631	0.9524	1.0627	0.8149	1.1972
3	03-02-21	0.9685	-0.0139	0.0020	0.0125	1.0046	0.9484	1.0641	0.9486	1.0639	0.8137	1.1954
4	03-30-21	0.9732	-0.0118	0.0005	0.0123	1.0011	0.9462	1.0593	0.9462	1.0592	0.8109	1.1913
5	04-02-21	0.9509	-0.0219	-0.0005	0.0132	0.9988	0.9397	1.0616	0.9396	1.0616	0.8090	1.1885
6	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9989	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887
7	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9978	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874
8	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
9	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 133

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 #: 2063

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	JW 7.71	N 7.86	N 7.92
	Dissolved oxygen (mg/L)	7.7	7.6	7.7
	Conductivity (µmhos/cm)	310		
	Alkalinity (mg/L CaCO ₃)	59		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.8	24.7	24.9
500 mg/L	pH (S.U.)	7.88	7.73	7.77
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	Conductivity (µmhos/cm)	1370		
	Temperature (°C)	24.9	24.8	24.7
750 mg/L	pH (S.U.)	7.89	7.74	7.70
	Dissolved oxygen (mg/L)	7.9	7.6	7.7
	Conductivity (µmhos/cm)	1780		
	Temperature (°C)	24.9	25.0	24.7
1000 mg/L	pH (S.U.)	7.89	7.71	7.77
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	Conductivity (µmhos/cm)	2250		
	Temperature (°C)	24.9	24.7	24.8
1250 mg/L	pH (S.U.)	7.89	7.73	7.77
	Dissolved oxygen (mg/L)	7.9	7.6	7.8
	Conductivity (µmhos/cm)	2600		
	Temperature (°C)	24.8	24.7	24.8
1500 mg/L	pH (S.U.)	7.89	7.72	7.80
	Dissolved oxygen (mg/L)	8.0	7.5	7.8
	Conductivity (µmhos/cm)	3150		
	Temperature (°C)	24.9	25.0	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	150664685



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 # 133

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-07-22	* 0620	J	0821	J	7B	PINK	02-28-22
24	03-08-22			0825	J			
48 Termination	03-09-22			0820	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:	02-23-22
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	02-26-22 1210 TO 03-01-22 0630
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.91 Temperature (°C): 24.7

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

Survival Data (number of living organisms):

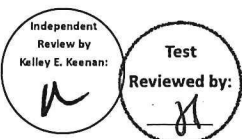
Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^{id}	5 ^{sd}	6 ^{sd}	2 ^{sd}	1 ^{sd}	0 ^{10d}	1 ^{sd}
48 Termination	10	10	10	10	9 ^{id}	8 ^{id}	5	3 ^{sd}	1 ^{id}	0 ^{id}	0	1
Mean Survival	100%		100%		85%		40%		5%		5%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	858.0
Upper 95% confidence limit (mg KCl/L)	1019.9
48-hour LC ₅₀ (mg KCl/L)	941.6

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 3/7/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/9/2022 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	0.6000
1250	0.2000	0.1000
1500	0.0000	0.1000

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	2.830	0.2359	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.977	2.830	0.2359	1	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	6.913	2.830	0.2359	9	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	12.228	2.830	0.2359	17	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	14.056	2.830	0.2359	19	20

Auxiliary Tests

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)

	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12286	0.12601	0.55924	0.00695	2.0E-05	5, 6

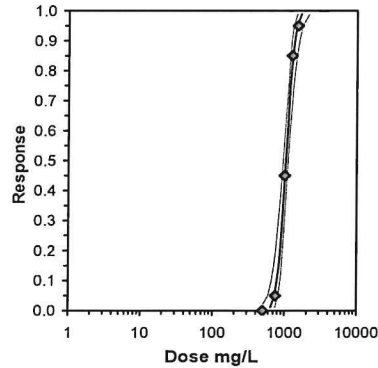
Treatments vs D-Control

Maximum Likelihood-Probit

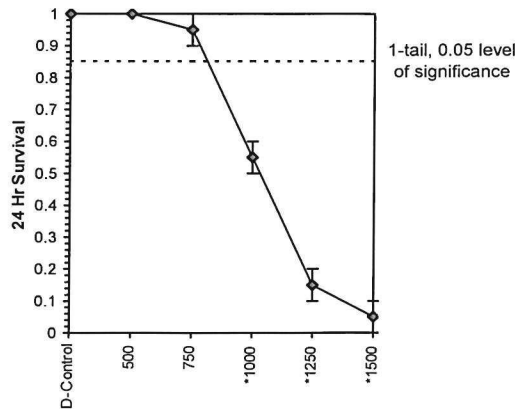
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.2752	1.99443	7.36607 15.1843	0	0.29183	7.81472	0.96156	3.01349	0.08869	3
Intercept	-28.978	6.04206	-40.82 -17.135							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	641.454	482.651 741.877
EC05	3.355	737.238	594.331 826.677
EC10	3.718	794.016	663.078 877.096
EC15	3.964	834.774	713.17 913.773
EC20	4.158	868.654	755.013 944.83
EC25	4.326	898.814	792.205 973.121
EC40	4.747	979.538	889.689 1053.6
EC50	5.000	1031.55	949.119 1110.89
EC60	5.253	1086.33	1007.41 1177.24
EC75	5.674	1183.89	1099.97 1310.99
EC80	5.842	1225	1135.46 1372.48
EC85	6.036	1274.71	1176.39 1450.11
EC90	6.282	1340.15	1227.79 1556.86
EC95	6.645	1443.36	1304.89 1733.99
EC99	7.326	1658.88	1456.42 2131.73



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 3/7/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 3/9/2022	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	0.9000	0.8000
1000	0.5000	0.3000
1250	0.1000	0.0000
1500	0.0000	0.1000

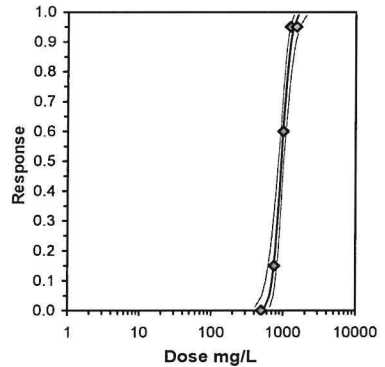
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2778	0	20
750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	2.383	2.830	0.2778	3	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	7.433	2.830	0.2778	12	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.939	2.830	0.2778	19	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.939	2.830	0.2778	19	20

Auxiliary Tests

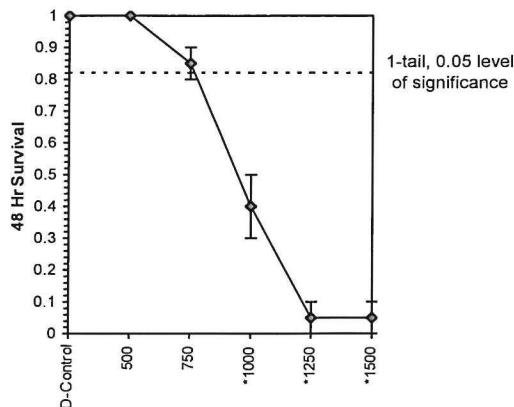
Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed									
Equality of variance cannot be confirmed									
Hypothesis Test (1-tail, 0.05)									
Dunnett's Test	750	1000	866.025	0.15376	0.1577	0.60411	0.00963	4.3E-05	5, 6

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	10.2559	1.78332	6.76055	13.7512	0	1.69951	7.81472	0.63704	2.97385	0.09751	3
Intercept	-25.499	5.34469	-35.975	-15.024							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	558.498	409.143	655.994
EC05	3.355	650.835	513.405	739.058
EC10	3.718	706.146	578.563	788.762
EC15	3.964	746.096	626.491	825.019
EC20	4.158	779.453	666.824	855.758
EC25	4.326	809.257	702.909	883.768
EC40	4.747	889.508	798.675	963.357
EC50	5.000	941.57	858.02	1019.9
EC60	5.253	996.679	916.964	1085.43
EC75	5.674	1095.52	1011.69	1218.5
EC80	5.842	1137.41	1048.23	1280.25
EC85	6.036	1188.26	1090.45	1358.71
EC90	6.282	1255.48	1143.57	1467.41
EC95	6.645	1362.18	1223.54	1649.51
EC99	7.326	1587.39	1381.78	2064.89



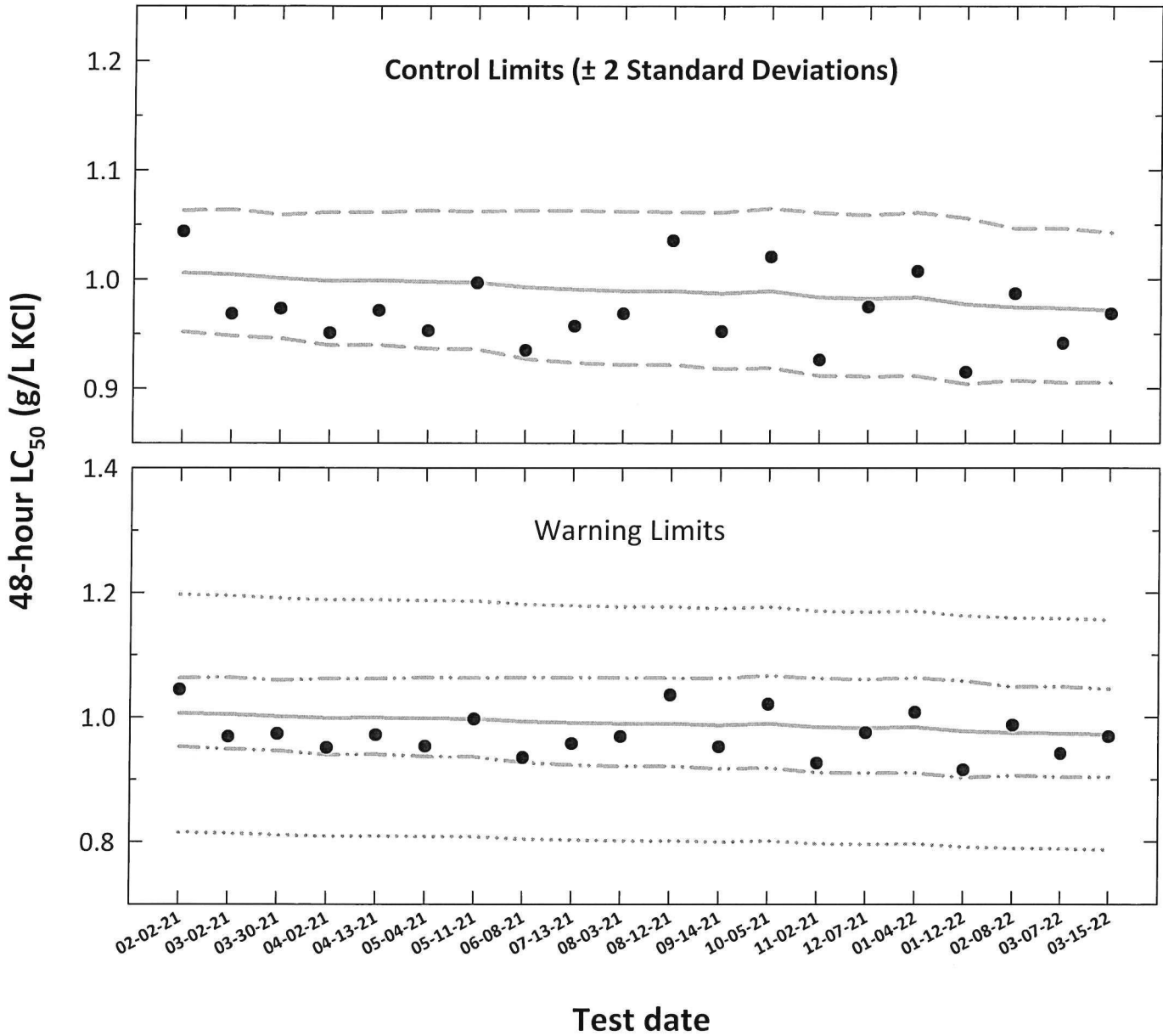
Dose-Response Plot



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 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	02-02-21	1.0440	0.0187	0.0026	0.0120	1.0060	0.9521	1.0631	0.9524	1.0627	0.8149	1.1972
2	03-02-21	0.9685	-0.0139	0.0020	0.0125	1.0046	0.9484	1.0641	0.9486	1.0639	0.8137	1.1954
3	03-30-21	0.9732	-0.0118	0.0005	0.0123	1.0011	0.9462	1.0593	0.9462	1.0592	0.8109	1.1913
4	04-02-21	0.9509	-0.0219	-0.0005	0.0132	0.9988	0.9397	1.0616	0.9396	1.0616	0.8090	1.1885
5	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9989	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887
6	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9978	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874
7	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
8	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 134

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 #: 2078

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	<u>7.60</u>	<u>7.74</u>	<u>7.53</u>
	Dissolved oxygen (mg/L)	<u>7.6</u>	<u>7.8</u>	<u>7.5</u>
	Conductivity (µmhos/cm)	<u>312</u>		
	Alkalinity (mg/L CaCO ₃)	<u>60</u>		
	Hardness (mg/L CaCO ₃)	<u>84</u>		
	Temperature (°C)	<u>24.3</u>	<u>25.0</u>	<u>25.0</u>
500 mg/L	pH (S.U.)	<u>7.74</u>	<u>7.71</u>	<u>7.53</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.7</u>	<u>7.6</u>
	Conductivity (µmhos/cm)	<u>1150</u>		
	Temperature (°C)	<u>24.3</u>	<u>25.0</u>	<u>24.8</u>
750 mg/L	pH (S.U.)	<u>7.76</u>	<u>7.71</u>	<u>7.52</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.7</u>	<u>7.6</u>
	Conductivity (µmhos/cm)	<u>1610</u>		
	Temperature (°C)	<u>24.5</u>	<u>25.2</u>	<u>24.8</u>
1000 mg/L	pH (S.U.)	<u>7.79</u>	<u>7.72</u>	<u>7.50</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.8</u>	<u>7.6</u>
	Conductivity (µmhos/cm)	<u>2090</u>		
	Temperature (°C)	<u>24.6</u>	<u>25.0</u>	<u>24.7</u>
1250 mg/L	pH (S.U.)	<u>7.80</u>	<u>7.72</u>	<u>7.52</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.8</u>	<u>7.6</u>
	Conductivity (µmhos/cm)	<u>2520</u>		
	Temperature (°C)	<u>24.7</u>	<u>24.9</u>	<u>24.7</u>
1500 mg/L	pH (S.U.)	<u>7.81</u>	<u>7.71</u>	
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.7</u>	
	Conductivity (µmhos/cm)	<u>2940</u>		
	Temperature (°C)	<u>24.7</u>	<u>25.1</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>150664685</u>

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 # 131

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-15-22	* 0800	A.C	1215	A.C	1B	pink	02-11-22C
24	03-16-22			1220	A.S			
48 Termination	03-17-22			1215	A.C			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	03-02-22
Age (1 to 14 days old):	7-8d-21
Hatch date and times:	03-07-22 1455 +0 03-08-22 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86
	Temperature (°C): 24.06

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 41 82	10	5 ps	5 ps	1 ps	1 ps	0 ps	0 ps
48 Termination	10	10	10	10	8	10	4 id	5	1	1	0	0
Mean Survival	100%		100%		90%		45%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	891.3
Upper 95% confidence limit (mg KCl/L)	1041.3
48-hour LC ₅₀ (mg KCl/L)	968.5

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 3/15/2022 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/17/2022 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	0.8000	1.0000
1000	0.5000	0.5000
1250	0.1000	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	0.2748	0	20	
750	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.581	2.850	0.2748	2	20	
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	6.500	2.850	0.2748	10	20	
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	11.309	2.850	0.2748	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)

Dunnett's Test NOEC LOEC ChV TU MSDu MSDp MSB MSE F-Prob df

Treatments vs D-Control 750 1000 866.025 0.15147 0.15536 0.45285 0.00929 3.4E-04 4, 5

Maximum Likelihood-Probit

Parameter Value SE 95% Fiducial Limits Control Chi-Sq Critical P-value Mu Sigma Iter

Slope 12.2001 2.17755 7.93208 16.4681 0 0.52701 7.81472 0.91292 2.99096 0.08197 3

Intercept -31.49 6.55042 -44.329 -18.651

TSCR

Point Probits mg/L 95% Fiducial Limits

EC01 2.674 631.358 481.687 724.713

EC05 3.355 718.019 584.235 801.02

EC10 3.718 768.979 646.617 846.147

EC15 3.964 805.389 691.761 878.868

EC20 4.158 835.553 729.292 906.5

EC25 4.326 862.33 762.529 931.605

EC40 4.747 933.669 849.239 1002.59

EC50 5.000 979.398 901.956 1052.67

EC60 5.253 1027.37 953.665 1110.22

EC75 5.674 1112.36 1035.71 1225.27

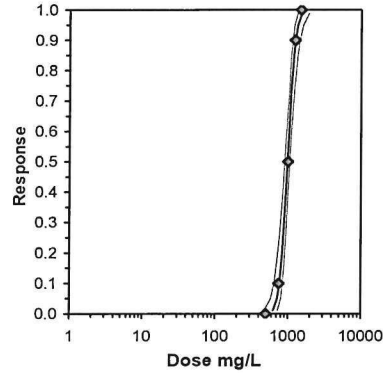
EC80 5.842 1148.01 1067.06 1277.91

EC85 6.036 1191 1103.09 1344.22

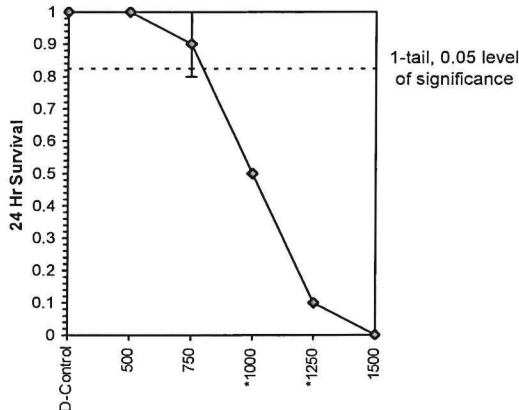
EC90 6.282 1247.39 1148.09 1435.12

EC95 6.645 1335.93 1215.17 1585.22

EC99 7.326 1519.3 1345.7 1919.01



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date:	3/15/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/17/2022	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	0.8000	1.0000
1000	0.4000	0.5000
1250	0.1000	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	0.2894	0	20	
750	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.501	2.850	0.2894	2	20	
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	6.668	2.850	0.2894	11	20	
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	10.739	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

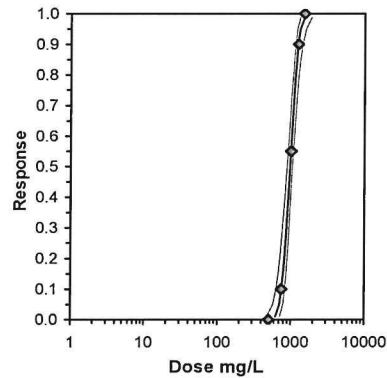
Equality of variance cannot be confirmed

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	0.46659	0.01031	4.1E-04	4, 5
Treatments vs D-Control										

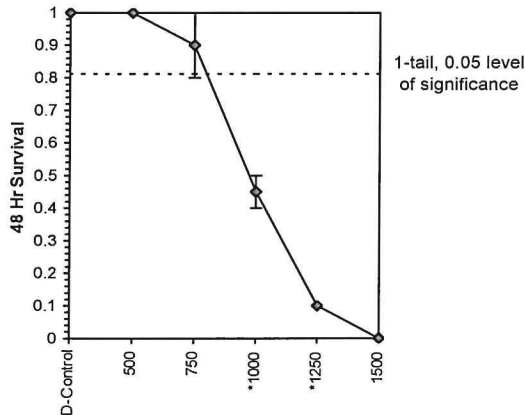
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.1787	2.1783	7.90923	16.4481	0	0.30493	7.81472	0.9591	2.98609	0.08211	3
Intercept	-31.367	6.54281	-44.191	-18.543							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	623.838	475.125	716.563
EC05	3.355	709.626	576.593	792.118
EC10	3.718	760.082	638.346	836.807
EC15	3.964	796.137	683.046	869.211
EC20	4.158	826.007	720.216	896.576
EC25	4.326	852.525	753.14	921.438
EC40	4.747	923.182	839.067	991.738
EC50	5.000	968.478	891.335	1041.33
EC60	5.253	1016	942.622	1098.31
EC75	5.674	1100.2	1024.02	1212.29
EC80	5.842	1135.52	1055.11	1264.47
EC85	6.036	1178.13	1090.84	1330.22
EC90	6.282	1234.01	1135.46	1420.38
EC95	6.645	1321.75	1201.95	1569.32
EC99	7.326	1503.51	1331.3	1900.71



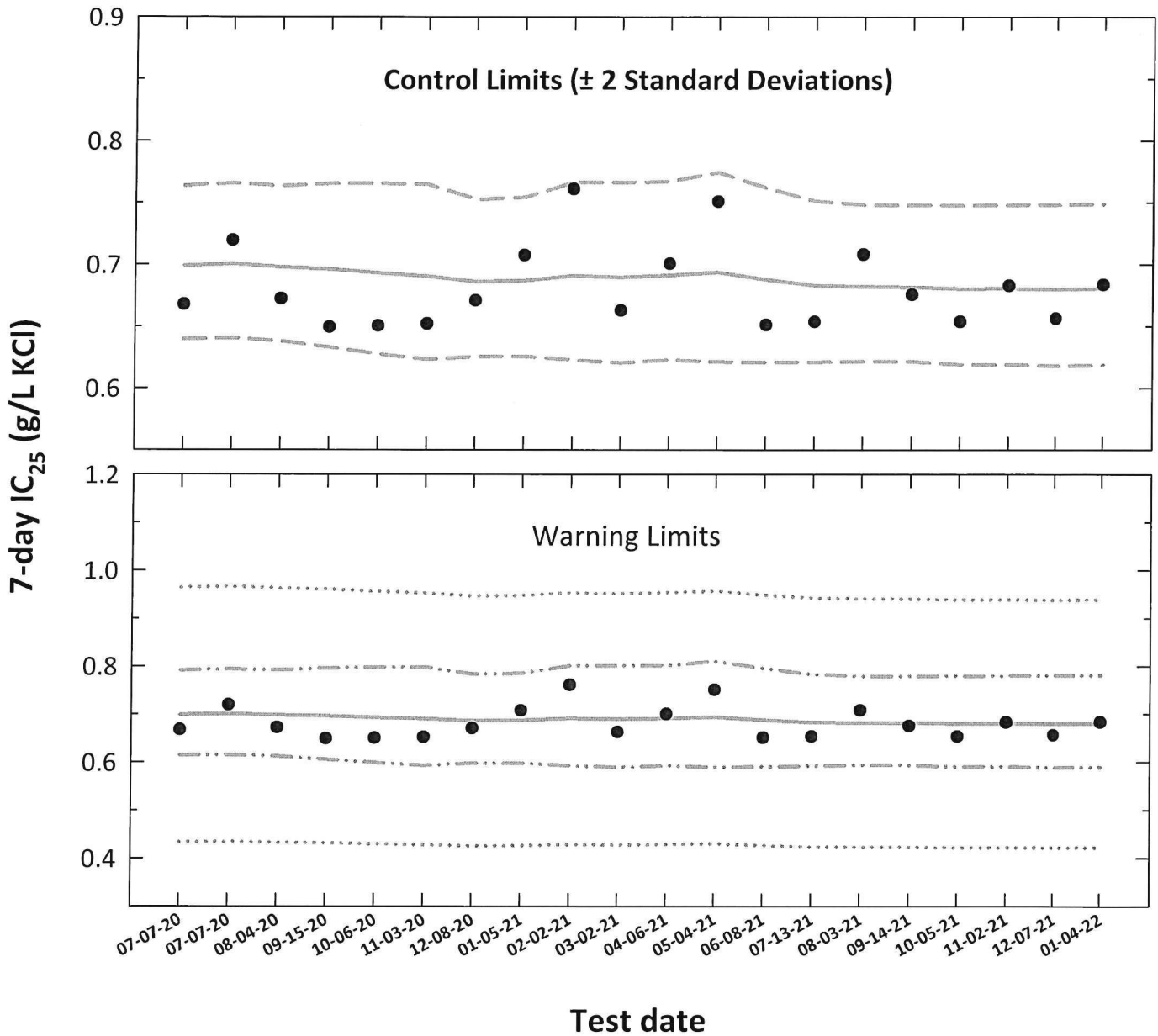
Dose-Response Plot



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 \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,75} converted to anti-logarithmic values,
S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}			
1	07-07-20	0.6677	-0.1754	-0.1556	0.0192	0.6990	0.6397	0.7637	0.6141	0.7916	0.4334	0.9646
2	07-07-20	0.7195	-0.1430	-0.1546	0.0194	0.7005	0.6407	0.7659	0.6152	0.7938	0.4343	0.9667
3	08-04-20	0.6723	-0.1725	-0.1562	0.0195	0.6980	0.6381	0.7635	0.6122	0.7918	0.4327	0.9632
4	09-15-20	0.6494	-0.1875	-0.1573	0.0206	0.6962	0.6331	0.7655	0.6055	0.7958	0.4316	0.9607
5	10-06-20	0.6504	-0.1868	-0.1592	0.0216	0.6932	0.6277	0.7655	0.5987	0.7975	0.4298	0.9566
6	11-03-20	0.6522	-0.1857	-0.1609	0.0222	0.6904	0.6232	0.7649	0.5930	0.7983	0.4280	0.9528
7	12-08-20	0.6708	-0.1734	-0.1636	0.0201	0.6861	0.6255	0.7526	0.5977	0.7830	0.4254	0.9468
8	01-05-21	0.7076	-0.1502	-0.1630	0.0203	0.6870	0.6257	0.7544	0.5977	0.7850	0.4259	0.9481
9	02-02-21	0.7611	-0.1186	-0.1606	0.0226	0.6909	0.6228	0.7666	0.5923	0.8004	0.4284	0.9535
10	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 ToxCal).
 CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

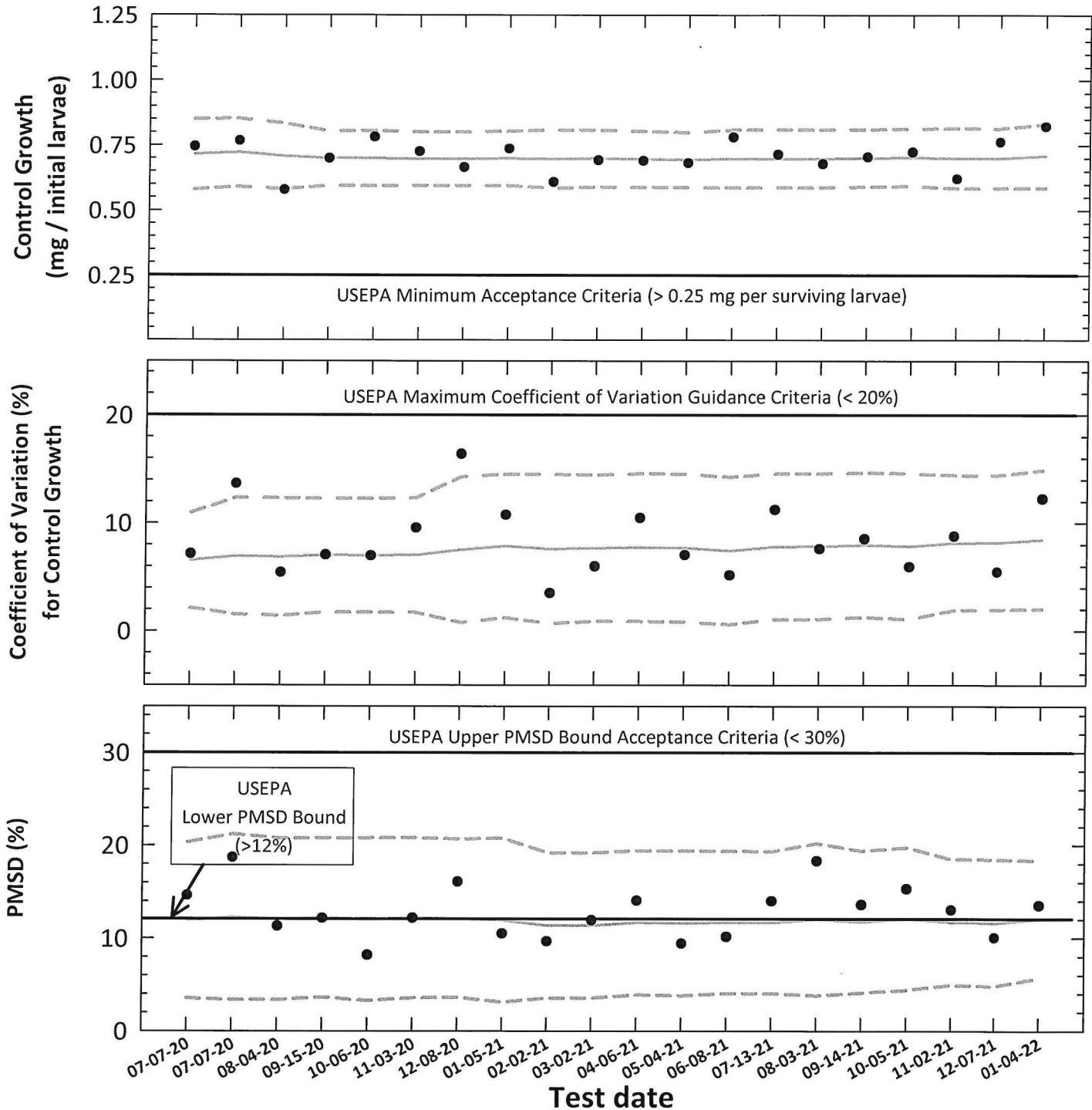
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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		Control Growth CV		Test PMSD		
		Control Survival (%)	Control Growth		MSD	PMSD (%)	CT	(mg/initial larvae)		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	07-07-20	100	0.745	7.2	0.1088	14.6	0.715	0.580	0.851	6.5	2.1	11.9	3.5	20.3	
2	07-07-20	100	0.767	13.7	0.1434	18.7	0.722	0.591	0.853	6.9	1.5	12.3	3.4	21.2	
3	08-04-20	100	0.579	5.4	0.0654	11.3	0.708	0.582	0.834	6.9	1.4	12.1	3.4	20.8	
4	09-15-20	100	0.700	7.1	0.0851	12.2	0.700	0.595	0.805	7.0	1.7	12.2	3.6	20.8	
5	10-06-20	100	0.782	7.0	0.0640	8.2	0.700	0.594	0.806	7.0	1.7	12.0	3.3	20.8	
6	11-03-20	97.5	0.727	9.6	0.0885	12.2	0.698	0.595	0.801	7.0	1.7	12.2	3.6	20.8	
7	12-08-20	100	0.666	16.4	0.1070	16.1	0.698	0.595	0.801	7.5	0.8	12.1	3.6	20.8	
8	01-05-21	100	0.737	10.8	0.0774	10.5	0.700	0.596	0.805	7.5	1.2	11.9	3.1	20.8	
9	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	0.809	7.6	0.7	11.4	3.5	19.2	
10	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.9	11.4	3.5	19.2	
11	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	11.7	3.9	19.4	
12	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.4	0.8	11.6	3.8	19.4	
13	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	11.7	4.0	19.4	
14	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	11.7	4.0	19.3	
15	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	12.0	3.8	20.2	
16	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	11.7	4.1	19.4	
17	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	12.1	4.4	19.7	
18	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	11.7	4.9	18.5	
19	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	11.6	4.8	18.5	
20	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	12.0	5.7	18.3	

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%.

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

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: 85

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2063</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>12-29-21</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>01-03-22 MS2 TO 01-04-22 0608</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.93</u> S.U. Temperature = <u>24.4</u> °C	Date / Time in oven:	<u>01-04-22 0800</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>01-11-22 0800</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	01-04-22	0610	<u>J</u>	1210	<u>J</u>	0855	<u>J</u>	<u>12-29-21 C</u>
1	01-05-22	0505	<u>J</u>	1105	<u>J</u>	0705	<u>J</u>	<u>↓</u>
2	01-06-22	0530	<u>J</u>	1230	<u>J</u>	0730	<u>J</u>	<u>12-29-21 D</u>
3	01-07-22	0600	<u>J</u>	1200	<u>J</u>	0800	<u>J</u>	<u>↓</u>
4	01-08-22	0700	<u>J</u>	1300	<u>J</u>	0900	<u>J</u>	<u>01-03-21 A</u>
5	01-09-22	0700	<u>J</u>	1300	<u>J</u>	0900	<u>J</u>	<u>↓</u>
6	01-10-22	0600	<u>J</u>	1200	<u>J</u>	0800	<u>J</u>	<u>↓</u>
7	01-11-22		<u>J</u>		<u>J</u>	0756	<u>J</u>	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>818.9</u>
Average weight per initial larvae:	<u>0.822</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.822</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>683.8</u>

Species: Pimephales promelas

PpKICR Test Number: 85

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 ^{lg}	10	10	10	10 ^{lg}	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>GREY</u> Analyst: <u>SW</u> Date: <u>12-20-21</u>	13.89	14.59	16.27	14.70	15.48	15.31	13.91	14.66	15.75	16.21	15.06	14.65
*B = Pan + Larvae weight (mg) Analyst: <u>JW</u> Date: <u>01-13-22</u>	20.81	23.79	24.24	23.50	23.69	24.68	22.92	22.43	23.34	23.84	23.68	22.78
C = Larvae weight (mg) = B - A Analyst: <u>J</u>	6.92	9.20	7.97	8.80	8.21	9.37	9.01	7.77	7.59	7.63	8.62	8.13
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>	0.692	0.920	0.797	0.880	0.821	0.937	0.901	0.777	0.759	0.763	0.862	0.813
Average weight per initial number of larvae (mg)	0.822				0.859		-4.57		0.799		2.87	
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: 85

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L						
	M	N	O	P	Q	R	S	T	U	V	W	X			
0	10	10	10	10	10	10	10	10	10	10	10	10			
1	10	10	10	10	10	10	10	10	8 ^{ud}	8 ^{ud}	8 ^{2d}	7 ^{3d}			
2	10	10	10	10	10	10	10	10	8	8	8	7			
3	10	10	10	10	10	10	10	10	8	8	8	7			
4	10	10	10	10	10	9 ^{ud}	9 ^{ud}	10	5 ^{3d}	5 ^{3d}	6 ^{4d}	6 ^{4d}			
5	10	10	10	10	8 ^{2d}	8 ^{1d}	8 ^{1d}	8 ^{2d}	3 ^{2d}	2 ^{3d}	3 ^{3d}	2 ^{4d}			
6	10	10	10	10	7 ^{1d}	8	8	7 ^{1d}	3	2	3	2			
7	10	10	10	10	7	6 ^{2d}	7 ^{1d}	7	3	2	3	2			
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>SW</u> Date: <u>12-20-21</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>JW</u> Date: <u>01-13-22</u>															
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.754		8.37		0.533		35.27		0.177		78.57	

*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: 85

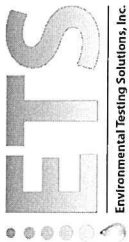
Survival and Growth Data

Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10	10	10	10	
1	6 ^{4d}	6 ^{4d}	5 ^{5d}	5 ^{5d}	
2	6	6	5	5	
3	6	6	5	5	
4	4 ^{2d}	3 ^{3d}	3 ^{2d}	4 ^{4d}	
5	1 ^{3d}	2 ^{1d}	1 ^{2d}	2 ^{2d}	
6	1	1 ^{1d}	1	1 ^{1d}	
7	1	1	0 ^{1d}	1 ^{lg}	
*A = Pan weight (mg) Tray color code: <u>grey</u> Analyst: <u>SWD</u> Date: <u>12-20-21</u>		13.90	13.26	15.47	13.81
*B = Pan + Larvae weight (mg) Analyst: <u>JW</u> Date: <u>01-13-22</u>		14.57	13.92	15.47	15.01
C = Larvae weight (mg) = B - A Analyst: <u>J</u>		0.67	0.66	0 ⁰¹⁻¹³⁻²²	1.20
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>		0.067	0.066	0	0.120
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.063	92.37.

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

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

Comments:



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

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

85
12-14-2021

Test number: _____
Test dates: **JAN 04-11, 2021**

Concentration (mg/L/KG)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	13.89	20.81	6.92	0.692							
	B	10	10	14.59	23.79	9.20	0.920				100.0	0.822	12.3	Not applicable
	C	10	10	16.27	24.24	7.97	0.797	0.822	12.3					
	D	10	10	14.70	23.50	8.80	0.880							
300	E	10	10	15.48	23.69	8.21	0.821							
	F	10	10	15.31	24.68	9.37	0.937	0.859	8.5		100.0	0.859	8.5	-4.5
	G	10	10	13.91	22.92	9.01	0.901							
	H	10	10	14.66	22.43	7.77	0.777							
450	I	10	10	15.75	23.34	7.59	0.759							
	J	10	10	16.21	23.84	7.63	0.763	0.799	6.1		100.0	0.799	6.1	2.8
	K	10	10	15.06	23.68	8.62	0.862							
	L	10	10	14.65	22.78	8.13	0.813							
600	M	10	10	16.07	23.15	7.08	0.708							
	N	10	10	13.41	21.22	7.81	0.781	0.754	4.2		100.0	0.754	4.2	8.3
	O	10	10	14.77	22.39	7.62	0.762							
	P	10	10	13.30	20.95	7.65	0.765							
750	Q	10	7	14.26	19.84	5.58	0.797							
	R	10	6	15.36	20.57	5.21	0.868	0.792	7.0		67.5	0.533	3.2	35.2
	S	10	7	15.30	20.58	5.28	0.754							
	T	10	7	14.10	19.34	5.24	0.749							
900	U	10	3	13.93	15.87	1.94	0.647							
	V	10	2	14.11	15.71	1.60	0.800	0.707	13.5		25.0	0.177	27.0	78.5
	W	10	3	15.04	17.37	2.33	0.777							
	X	10	2	15.10	16.31	1.21	0.605							
1050	Y	10	1	13.90	14.57	0.670	0.670							
	Z	10	1	13.26	13.92	0.66	0.660	0.843	36.6		7.5	0.063	0.0	92.3
	AA	10	0	0.00	0.00	0.00	0.000							
	BB	10	1	13.81	15.01	1.20	1.200							

Dunnett's MSD value: 0.1112
PMSD: 13.5

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.

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



Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 1/4/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 1/11/2022	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	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.7000	0.6000	0.7000	0.7000
900	0.3000	0.2000	0.3000	0.2000
1050	0.1000	0.1000	0.0000	0.1000

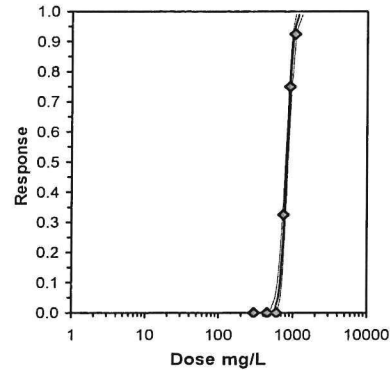
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40
*900	0.2500	0.2500	0.5216	0.4636	0.5796	12.838	4	10.00	10.00	30	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 ($p \leq 0.01$)	0.80734	0.896	-1.3548	2.79204

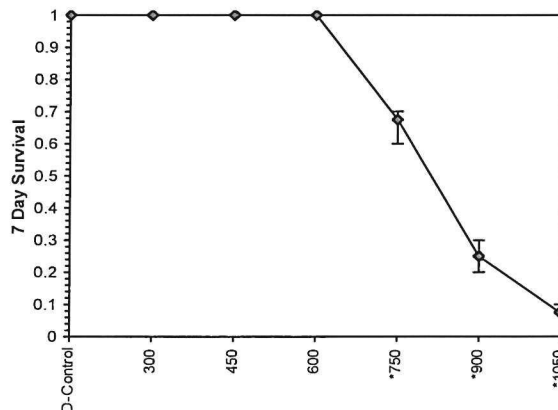
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.0924	1.93861	11.2927 18.8921	0	1.72126	9.48773	0.78685	2.91324	0.06626	4
Intercept	-38.968	5.66111	-50.064 -27.872							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	574.252	503.204	623.396
EC05	3.355	637.173	576.641	679.242
EC10	3.718	673.487	619.54	711.643
EC15	3.964	699.15	649.889	734.805
EC20	4.158	720.242	674.731	754.126
EC25	4.326	738.843	696.466	771.473
EC40	4.747	787.873	752.216	819.339
EC50	5.000	818.922	785.674	851.94
EC60	5.253	851.195	818.487	888.146
EC75	5.674	907.681	871.368	956.928
EC80	5.842	931.123	891.99	987.115
EC85	6.036	959.213	915.971	1024.26
EC90	6.282	995.764	946.275	1073.87
EC95	6.645	1052.51	991.907	1153.19
EC99	7.326	1167.84	1081.28	1320.85



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 1/4/2022	Test ID: PpKICR	Sample ID: REF-Ref Toxicant
End Date: 1/11/2022	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

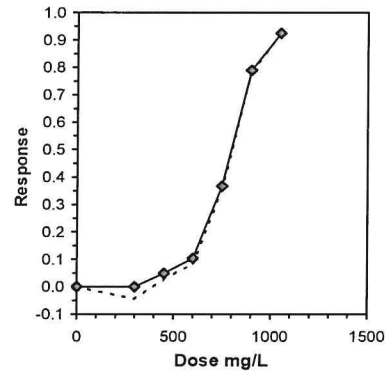
Conc-mg/L	1	2	3	4
D-Control	0.6920	0.9200	0.7970	0.8800
300	0.8210	0.9370	0.9010	0.7770
450	0.7590	0.7630	0.8620	0.8130
600	0.7080	0.7810	0.7620	0.7650
750	0.5580	0.5210	0.5280	0.5240
900	0.1940	0.1600	0.2330	0.1210
1050	0.0670	0.0660	0.0000	0.1200

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	0.8223	1.0000	0.8223	0.6920	0.9200	12.261	4				0.8406	1.0000
300	0.8590	1.0447	0.8590	0.7770	0.9370	8.506	4	-0.757	2.290	0.1112	0.8406	1.0000
450	0.7993	0.9720	0.7993	0.7590	0.8620	6.070	4	0.474	2.290	0.1112	0.7993	0.9508
600	0.7540	0.9170	0.7540	0.7080	0.7810	4.215	4	1.405	2.290	0.1112	0.7540	0.8970
750	0.5328	0.6479	0.5328	0.5210	0.5580	3.205	4				0.5328	0.6338
900	0.1770	0.2153	0.1770	0.1210	0.2330	26.997	4				0.1770	0.2106
1050	0.0633	0.0769	0.0633	0.0000	0.1200	77.684	4				0.0633	0.0752

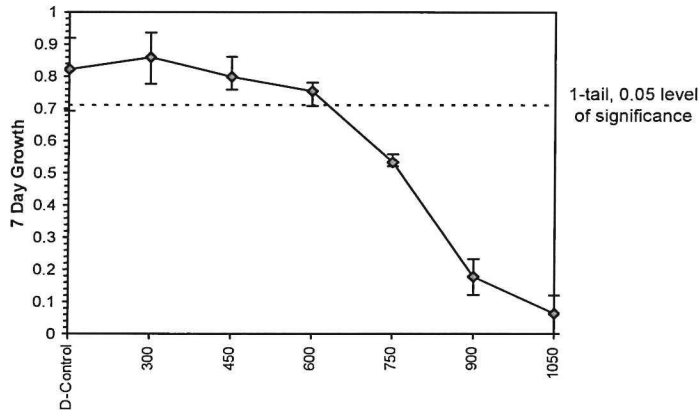
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97272	0.844	-0.3602	-0.189
Bartlett's Test indicates equal variances (p = 0.32)	3.54196	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.11121	0.13525	0.00773	0.00472	0.23275	3, 12

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	452.18	97.42	67.30 697.62	-0.4243
IC10	591.51	73.15	250.97 654.82	-1.1048
IC15	626.76	32.07	481.11 676.05	-2.5546
IC20	655.25	17.12	599.82 701.12	-0.1287
IC25	683.75	15.82	631.94 726.10	-0.0641
IC40	761.96	8.43	731.69 783.37	-0.2235
IC50	797.41	7.14	774.69 817.45	0.0384



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Multiple weigh of final pans for growth determinations in chronic toxicity tests.

Test type: P. promelas Chronic Reference Toxicant Test
 Test dates: JANUARY 04-11, 2022
 Associated test: PKCICR # 85

1st Weight = Pans were dried for 24-hours at $60 \pm 2^\circ\text{C}$, desiccated, and weighed following standard operating procedures.

2nd Weight = The same pans used for determining the 1st weight measurements were dried for an additional 24-hours at $60 \pm 2^\circ\text{C}$, desiccated, and weighed following standard operating procedures.

	1st Weight	2nd Weight	Difference (mg)	Percent Difference from 1st Weight (%)
Analyst:		JL		
Tray color code:		GREEN		
Date:		02-15-22		
A		20.78		
B		23.76		
C		21.20		
D		23.44		
E		23.64		
F		21.64		
G		22.86		
H		22.37		
I		23.28		
J		23.80		
K		23.61		
L		22.70		
M		23.13		
N		21.15		
O		22.33		
P		20.89		
Q		19.79		
R		20.52		
S		20.52		
T		19.30		
U		15.86		
V		15.68		
W		17.34		
X		16.29		
Y		14.56		
Z		13.91		
AA		15.48		
BB		15.00		

Average

Multiple weigh of final pans for growth determinations in chronic toxicity tests.

Test type: P. promelas Chronic Reference Toxicant Test
 Test dates: January 04-11, 2022
 Associated test: PpKCICR # 85

1st Weight = Pans were dried for 24-hours at $60 \pm 2^\circ\text{C}$, desiccated, and weighed following standard operating procedures.

2nd Weight = The same pans used for determining the 1st weight measurements were dried for an additional 24-hours at $60 \pm 2^\circ\text{C}$, desiccated, and weighed following standard operating procedures.

	1st Weight	2nd Weight		
Analyst:	JW	JS	Difference (mg)	Percent Difference from 1st Weight (%)
Tray color code:	Grey	Grey		
Date:	01-13-22	02-15-22		
A	20.81	20.78	-0.03	-0.14
B	23.79	23.76	-0.03	-0.13
C	24.24	24.20	-0.04	-0.17
D	23.50	23.44	-0.06	-0.26
E	23.69	23.64	-0.05	-0.21
F	24.68	24.64	-0.04	-0.16
G	22.92	22.86	-0.06	-0.26
H	22.43	22.37	-0.06	-0.27
I	23.34	23.28	-0.06	-0.26
J	23.84	23.80	-0.04	-0.17
K	23.68	23.61	-0.07	-0.30
L	22.78	22.70	-0.08	-0.35
M	23.15	23.13	-0.02	-0.09
N	21.22	21.15	-0.07	-0.33
O	22.39	22.33	-0.06	-0.27
P	20.95	20.89	-0.06	-0.29
Q	19.84	19.79	-0.05	-0.25
R	20.57	20.52	-0.05	-0.24
S	20.58	20.52	-0.06	-0.29
T	19.34	19.30	-0.04	-0.21
U	15.87	15.86	-0.01	-0.06
V	15.71	15.68	-0.03	-0.19
W	17.37	17.34	-0.03	-0.17
X	16.31	16.29	-0.02	-0.12
Y	14.57	14.56	-0.01	-0.07
Z	13.92	13.91	-0.01	-0.07
AA	15.47	15.48	0.01	0.06
BB	15.01	15.00	-0.01	-0.07
	Average		-0.04	-0.19

Species: Pimephales promelas

PpKICR Test Number: 85

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	
Concentration	Parameter	u	u	u	u	u	u
CONTROL, MHSW	pH (S.U.)	7.40	7.08	7.50	7.45	7.02	7.64
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.4	7.6	7.4
	Conductivity (µmhos/cm)	304		306		300	
	Alkalinity (mg CaCO ₃ /L)	61				60	
	Hardness (mg CaCO ₃ /L)	94				90	
	Temperature (°C)	24.7	24.7	24.7	24.6	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.72	7.70	7.03	7.52	7.75	7.69
	Dissolved oxygen (mg/L)	7.9	7.7	0.0	7.1	7.9	7.2
	Conductivity (µmhos/cm)	008		041		046	
	Temperature (°C)	24.6	24.9	24.6	24.5	24.8	24.6
450 mg KCl/L	pH (S.U.)	7.73	7.70	7.04	7.50	7.77	7.69
	Dissolved oxygen (mg/L)	7.9	7.0	0.0	7.4	7.9	7.3
	Conductivity (µmhos/cm)	1130		1110		1110	
	Temperature (°C)	24.6	24.6	24.6	24.7	24.7	24.7
600 mg KCl/L	pH (S.U.)	7.75	7.70	7.06	7.58	7.02	7.00
	Dissolved oxygen (mg/L)	7.0	7.0	0.0	7.4	7.9	7.3
	Conductivity (µmhos/cm)	1390		1370		1360	
	Temperature (°C)	24.8	24.6	24.7	24.7	24.8	24.7
750 mg KCl/L	pH (S.U.)	7.77	7.79	7.90	7.00	7.04	7.69
	Dissolved oxygen (mg/L)	7.0	7.0	0.1	7.4	0.0	7.3
	Conductivity (µmhos/cm)	1640		1620		1610	
	Temperature (°C)	24.8	24.9	24.7	24.6	24.8	24.5
900 mg KCl/L	pH (S.U.)	7.00	7.00	7.93	7.01	7.06	7.60
	Dissolved oxygen (mg/L)	7.0	7.0	0.1	7.5	0.0	7.4
	Conductivity (µmhos/cm)	1900		1870		1800	
	Temperature (°C)	24.7	24.9	24.7	24.3	24.7	24.7
1050 mg KCl/L	pH (S.U.)	7.02	7.03	7.95	7.04	7.07	7.60
	Dissolved oxygen (mg/L)	7.0	7.0	0.2	7.6	0.0	7.5
	Conductivity (µmhos/cm)	2170		2150		2100	
	Temperature (°C)	24.8	24.8	24.7	24.6	24.8	24.7
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

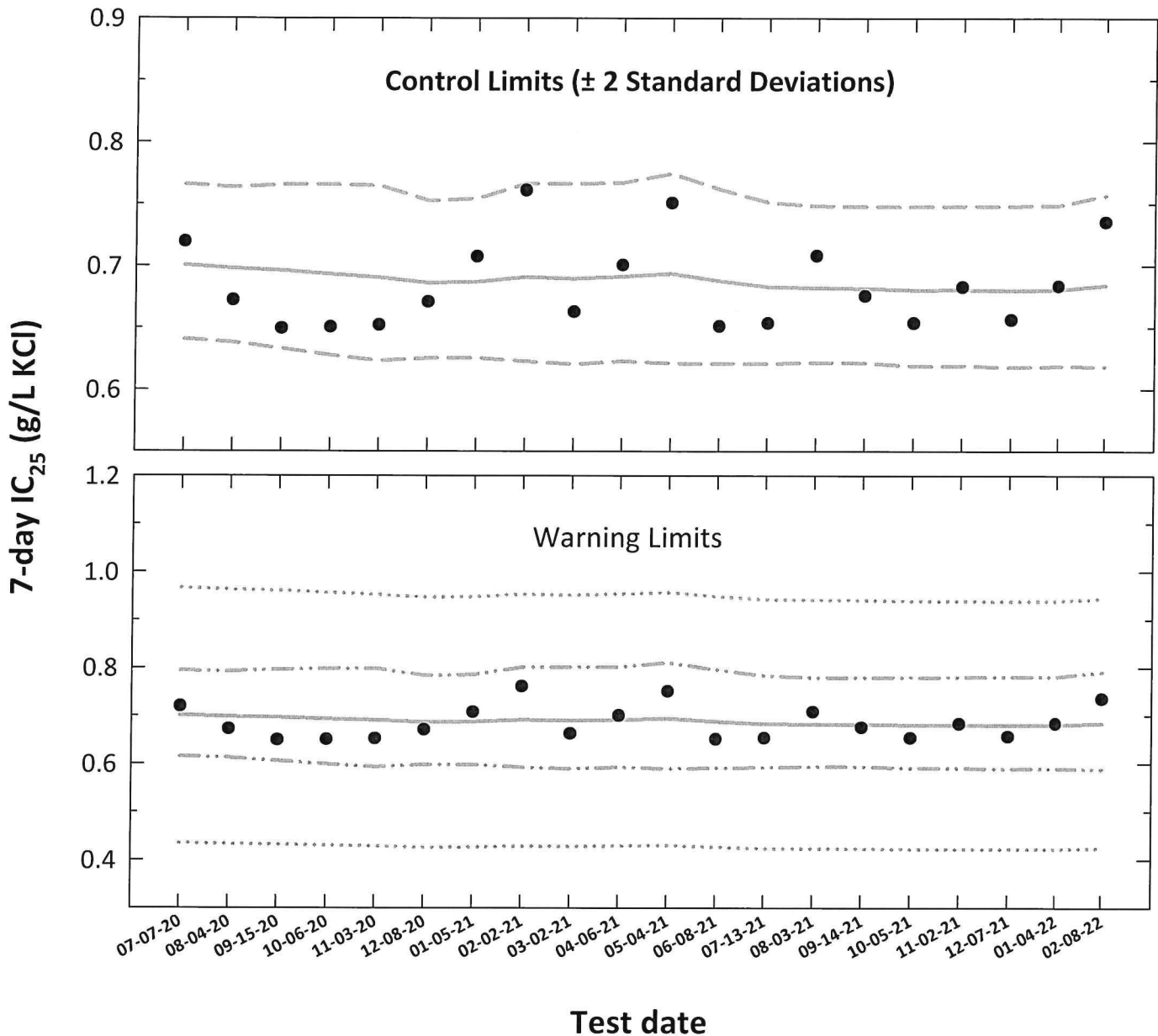
PpKCICR Test Number: 85

Concentration		Parameter		Day							
				(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
				3		4		5		6	
Analyst		U	BSC	BSC	U	U	U	U	JW		
CONTROL, MHSW	pH (S.U.)	7.40	7.42	7.38	7.55	7.66	7.60	7.61	7.28		
	Dissolved oxygen (mg/L)	7.0	7.1	7.8	7.1	7.7	7.6	7.0	7.6		
	Conductivity (µmhos/cm)	299		289		310		297			
	Alkalinity (mg CaCO ₃ /L)			59							
	Hardness (mg CaCO ₃ /L)			90							
	Temperature (°C)	24.8	24.6	24.7	24.7	24.8	24.6	24.8	24.7		
300 mg KCl/L	pH (S.U.)	7.79	7.54	7.73	7.61	7.80	7.66	7.03	7.46		
	Dissolved oxygen (mg/L)	7.9	7.1	7.8	7.1	7.7	7.6	8.1	7.4		
	Conductivity (µmhos/cm)	846		811		843		825			
	Temperature (°C)	24.9	24.7	24.7	24.7	24.8	24.4	24.8	24.3		
450 mg KCl/L	pH (S.U.)	7.81	7.56	7.81	7.84	7.84	7.68	7.84	7.52		
	Dissolved oxygen (mg/L)	7.9	7.0	7.8	7.4	7.7	7.6	8.1	7.4		
	Conductivity (µmhos/cm)	1090		1060		1110		1090			
	Temperature (°C)	24.9	24.7	24.8	24.9	24.7	24.4	24.9	24.5		
600 mg KCl/L	pH (S.U.)	7.84	7.58	7.87	7.79	7.86	7.68	7.86	7.86		
	Dissolved oxygen (mg/L)	7.9	7.0	7.9	7.4	7.8	7.6	8.1	7.4		
	Conductivity (µmhos/cm)	1370		1310		1370		1330			
	Temperature (°C)	25.0	24.8	24.8	24.8	24.8	24.7	24.8	24.5		
750 mg KCl/L	pH (S.U.)	7.85	7.62	7.90	7.78	7.89	7.69	7.89	7.57		
	Dissolved oxygen (mg/L)	7.9	7.0	7.9	7.3	7.8	7.6	8.1	7.5		
	Conductivity (µmhos/cm)	1600		1550		1650		1600			
	Temperature (°C)	25.0	24.8	24.7	24.6	24.8	24.6	24.9	24.5		
900 mg KCl/L	pH (S.U.)	7.88	7.61	7.93	7.76	7.91	7.71	7.91	7.58		
	Dissolved oxygen (mg/L)	8.0	7.1	7.8	7.4	7.8	7.6	8.2	7.6		
	Conductivity (µmhos/cm)	1880		1850		1930		1860			
	Temperature (°C)	24.9	24.6	24.7	24.6	24.9	24.6	24.9	24.6		
1050 mg KCl/L	pH (S.U.)	7.89	7.61	7.95	7.75	7.94	7.72	7.92	7.63		
	Dissolved oxygen (mg/L)	8.1	7.0	7.8	7.3	7.9	7.8	8.2	7.7		
	Conductivity (µmhos/cm)	2140		2070		2130		2130			
	Temperature (°C)	24.9	24.6	24.7	24.8	24.9	24.6	24.9	24.4		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final		

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}			
1	07-07-20	0.7195	-0.1430	-0.1546	0.0194	0.7005	0.6407	0.7659	0.6152	0.7938	0.4343	0.9667
2	08-04-20	0.6723	-0.1725	-0.1562	0.0195	0.6980	0.6381	0.7635	0.6122	0.7918	0.4327	0.9632
3	09-15-20	0.6494	-0.1875	-0.1573	0.0206	0.6962	0.6331	0.7655	0.6055	0.7958	0.4316	0.9607
4	10-06-20	0.6504	-0.1868	-0.1592	0.0216	0.6932	0.6277	0.7655	0.5987	0.7975	0.4298	0.9566
5	11-03-20	0.6522	-0.1857	-0.1609	0.0222	0.6904	0.6232	0.7649	0.5930	0.7983	0.4280	0.9528
6	12-08-20	0.6708	-0.1734	-0.1636	0.0201	0.6861	0.6255	0.7526	0.5977	0.7830	0.4254	0.9468
7	01-05-21	0.7076	-0.1502	-0.1630	0.0203	0.6870	0.6257	0.7544	0.5977	0.7850	0.4259	0.9481
8	02-02-21	0.7611	-0.1186	-0.1606	0.0226	0.6909	0.6228	0.7666	0.5923	0.8004	0.4284	0.9535
9	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 ToxCaIc).
 CT = Central tendency of the IC₂₅ values.

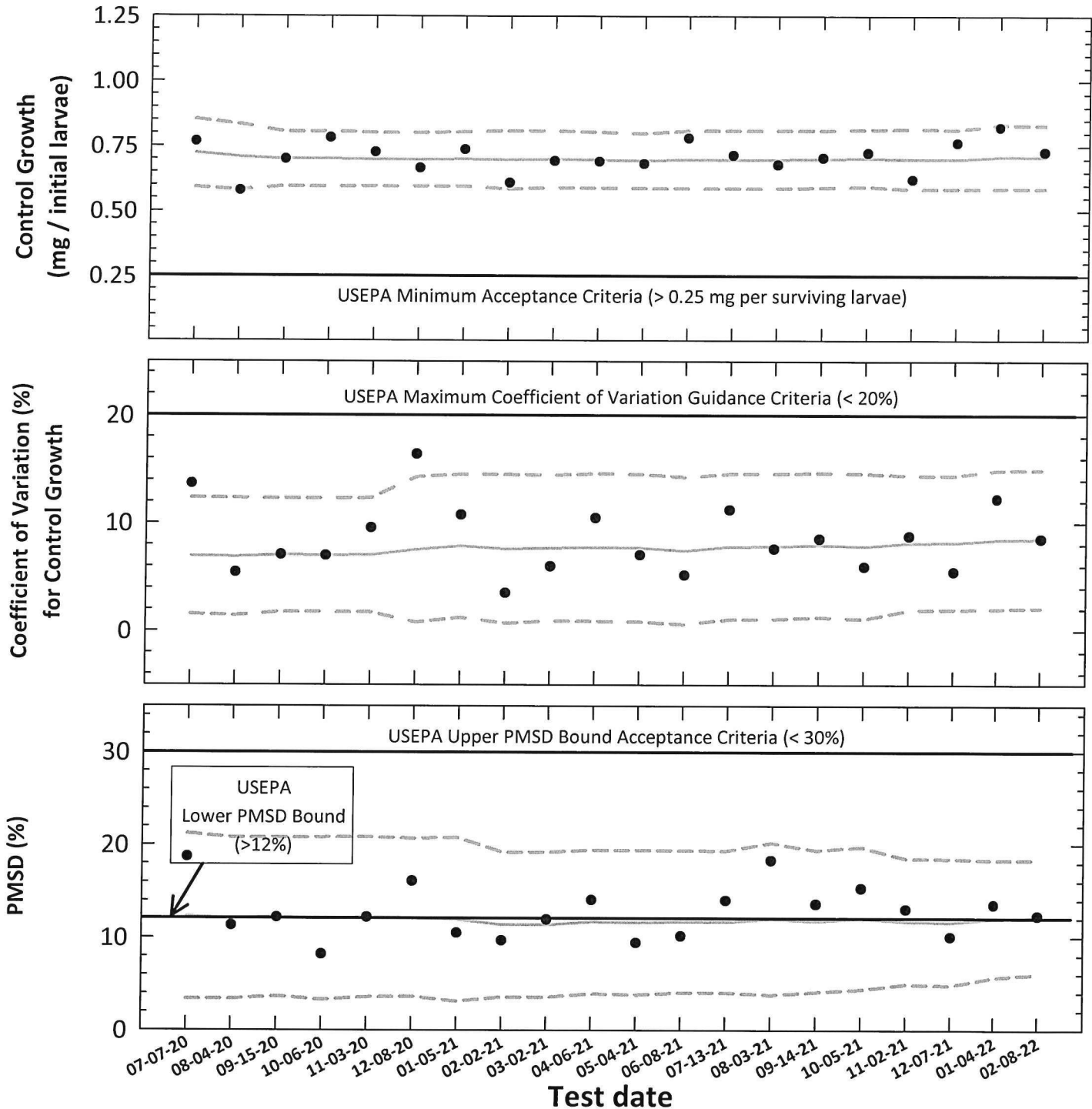
S = Standard deviation of the IC₂₅ values.
 Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.
 S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).
 CV = Coefficient of variation.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

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	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	07-07-20	100	0.767	13.7	0.1434	18.7	0.722	0.591	0.853	6.9	1.5	12.4	12.3	3.4	21.2
2	08-04-20	100	0.579	5.4	0.0654	11.3	0.708	0.582	0.834	6.9	1.4	12.3	12.1	3.4	20.8
3	09-15-20	100	0.700	7.1	0.0851	12.2	0.700	0.595	0.805	7.0	1.7	12.3	12.2	3.6	20.8
4	10-06-20	100	0.782	7.0	0.0640	8.2	0.700	0.594	0.806	7.0	1.7	12.3	12.0	3.3	20.8
5	11-03-20	97.5	0.727	9.6	0.0885	12.2	0.698	0.595	0.801	7.0	1.7	12.3	12.2	3.6	20.8
6	12-08-20	100	0.666	16.4	0.1070	16.1	0.698	0.595	0.801	7.5	0.8	14.3	12.1	3.6	20.7
7	01-05-21	100	0.737	10.8	0.0774	10.5	0.700	0.596	0.805	7.9	1.2	14.5	11.9	3.1	20.8
8	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	0.809	7.6	0.7	14.5	11.4	3.5	19.2
9	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.9	14.5	11.4	3.5	19.2
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.

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

Species: *Pimephales promelas*

PpKICR Test Number: 86

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2063</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>02-01-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>02-02-22 MYS TO 02-08-22 0600</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>8.03</u> S.U. Temperature = <u>24.1</u> °C	Date / Time in oven:	<u>02-15-22 0745</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>02-16-22 0745</u>
		*Final oven temperature:	<u>60 °C</u>
		Total drying time:	<u>24-Hours</u>

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	02-08-22	0605	JL	1300	JL	0815	JL	02-03-22 B
1	02-09-22	0530	JL	1130	JL	0730	JL	↓
2	02-10-22	0600	JL	1200	JL	0800	JL	02-05-22 C
3	02-11-22	0600	JL	1200	JL	0800	JL	↓
4	02-12-22	0600	JL	1200	JL	0920	JL	02-03-22 D
5	02-13-22	0600	JL	1200	JL	0800	JL	↓
6	02-14-22	0600	JL	1200	JL	0800	JL	↓
7	02-15-22		JL		JL	0716	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>0.1</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>803.1</u>
Average weight per initial larvae:	<u>0.728</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.728</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

PpKCICR Test Number: 86

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	²⁶ 10	²⁶ 10	¹⁶ 10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>LIGHT BLUE</u> Analyst: <u>JL TG</u> Date: <u>01-26-22</u>	14.91	15.30	16.65	15.05	15.85	15.13	13.47	14.46	13.88	11.83	15.72	14.67
*B = Pan + Larvae weight (mg) Analyst: <u>A-S</u> Date: <u>02-17-22</u>	21.64	22.38	24.82	22.17	25.56	24.87	22.55	22.78	21.43	20.11	23.60	23.18
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	6.73	7.08	8.17	7.12	9.71	9.74	9.08	8.32	7.55	8.28	7.88	8.51
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0.673	0.708	0.817	0.712	0.971	0.974	0.908	0.832	0.755	0.828	0.788	0.851
Average weight per initial number of larvae (mg)	0.728				0.921		-26.67		0.806		-10.77	
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: 86

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L						
	M	N	O	P	Q	R	S	T	U	V	W	X			
0	10	10	10	10	10	10	10	10	10	10	10	10			
1	10	10	10	10	10	10	10	10	5 ^{sd}	5 ^{sd}	6 ^{sd}	6 ^{sd}			
2	10	10	10	10	10	9 ^{id}	10	9 ^{id}	5	5	5 ^{id}	5 ^{id}			
3	10	10	10	10	9 ^{id}	9	9 ^{id}	9	3 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}			
4	10	10	10	10	8 ^{id}	9	8 ^{id}	9	2 ^{id}	1 ^{sd}	2 ^{id}	2 ^{id}			
5	10	10	10	10	7 ^{id}	8 ^{id}	7 ^{id}	8 ^{id}	2	1	2	2			
6	10	10	10	10	7	8	7	8	2	1	1 ^{id}	1 ^{id}			
7	10	9 ^{id}	10	10	7	8	7	8	2 ^{lg}	1 ^{lg}	1 ^{lg}	1 ^{lg}			
*A = Pan weight (mg) Tray color code: <u>Light blue</u> Analyst: <u>TG</u> Date: <u>01-26-22</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>AS</u> Date: <u>02-17-22</u>															
C = Larvae weight (mg) = B - A Analyst: <u>JK</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JK</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.801		-10.17		0.599		17.77		0.138		81.17	

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

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

Comments:

Species: Pimephales promelas

PpKCICR Test Number: 86

Survival and Growth Data

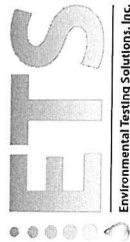
Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10 4 ^{hd}	10 4 ^{hd}	10 3 ^{sd}	10 4 ^{hd}	
1	15.15	13.20	14.59	13.38	
2	2 ^{sd}	2 ^{sd}	2 ^{sd}	2 ^{sd}	
3	1 ^{sd}	2	2	2	
4	1	1 ^{sd}	1 ^{sd}	1 ^{sd}	
5	0 ^{sd}	1	1	0 ^{sd}	
6	0	1	1	0	
7	0	1	1	0	
*A = Pan weight (mg) Tray color code: <u>Light Blue</u> Analyst: <u>TG</u> Date: <u>01-26-22</u>		15.15	13.20	14.59	13.38
*B = Pan + Larvae weight (mg) Analyst: <u>AS</u> Date: <u>02-17-22</u>		15.16	14.07	15.74	13.33
C = Larvae weight (mg) = B - A Analyst: <u>AS</u>		0	0.87	1.15	0
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>AS</u>		0	0.087	0.115	0
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	0.051		93.17.	

TG 01-26-22

*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: 86
 Test dates: February 08-15, 2022

Concentration (mg/L/KC)	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	14.91	21.64	6.73	0.673	0.728	8.5	0.673	100.0	0.728	8.5	Not applicable
	B	10	10	15.30	22.38	7.08	0.708			0.708				
	C	10	10	16.65	24.82	8.17	0.817			0.817				
	D	10	10	15.05	22.17	7.12	0.712			0.712				
300	E	10	10	15.85	25.56	9.71	0.971	0.921	7.3	0.971	100.0	0.921	7.3	-26.6
	F	10	10	15.13	24.87	9.74	0.974			0.974				
	G	10	10	13.47	22.55	9.08	0.908			0.908				
	H	10	10	14.46	22.78	8.32	0.832			0.832				
450	I	10	10	13.88	21.43	7.55	0.755	0.806	5.3	0.755	100.0	0.806	5.3	-10.7
	J	10	10	11.83	20.11	8.28	0.828			0.828				
	K	10	10	15.72	23.60	7.88	0.788			0.788				
	L	10	10	14.67	23.18	8.51	0.851			0.851				
600	M	10	10	13.78	21.65	7.87	0.787	0.822	4.3	0.787	97.5	0.822	4.3	-10.1
	N	10	9	13.70	21.23	7.53	0.837			0.753				
	O	10	10	15.28	23.92	8.64	0.864			0.864				
	P	10	10	15.91	23.91	8.00	0.800			0.800				
750	Q	10	7	14.49	20.06	5.57	0.796	0.797	5.0	0.557	75.0	0.599	10.3	17.7
	R	10	8	14.95	21.03	6.08	0.760			0.608				
	S	10	7	14.28	19.75	5.47	0.781			0.547				
	T	10	8	15.24	22.06	6.82	0.853			0.682				
900	U	10	2	13.63	15.58	1.95	0.975	1.131	16.4	0.195	12.5	0.138	30.0	81.1
	V	10	1	14.97	16.03	1.06	1.060			0.106				
	W	10	1	15.73	16.82	1.09	1.090			0.109				
	X	10	1	14.84	16.24	1.40	1.400			0.140				
1050	Y	10	0	0.00	0.00	0.00	0.000	1.010	19.6	0.000	5.0	0.051	0.0	93.1
	Z	10	1	13.20	14.07	0.87	0.870			0.087				
	AA	10	1	14.59	15.74	1.15	1.150			0.115				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.0898
 PMSD: 12.3
 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.

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



Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 2/8/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 2/15/2022	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

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	1.0000
750	0.7000	0.8000	0.7000	0.8000
900	0.2000	0.1000	0.1000	0.1000
1050	0.0000	0.1000	0.1000	0.0000

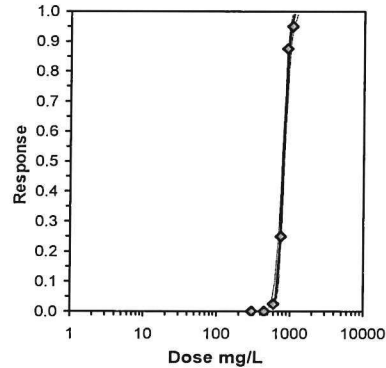
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	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	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
*1050	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94747	0.896	-0.1502	0.14655

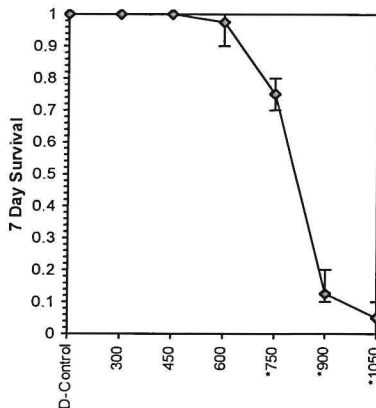
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	16.812	2.18429	12.5308	21.0932	0	3.32334	9.48773	0.50524	2.90475	0.05948	3
Intercept	-43.835	6.36317	-56.306	-31.363							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	583.947	516.578	630.011
EC05	3.355	641.076	583.979	680.426
EC10	3.718	673.781	622.943	709.49
EC15	3.964	696.785	650.345	730.185
EC20	4.158	715.627	672.683	747.392
EC25	4.326	732.197	692.169	762.795
EC40	4.747	775.671	741.959	805.034
EC50	5.000	803.058	771.763	833.564
EC60	5.253	831.413	800.961	865.048
EC75	5.674	880.778	847.851	924.499
EC80	5.842	901.172	866.039	950.494
EC85	6.036	925.541	887.108	982.411
EC90	6.282	957.14	913.606	1024.93
EC95	6.645	1005.97	953.259	1092.59
EC99	7.326	1104.39	1030.21	1234.35



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Larval Fish Growth and Survival Test-7 Day Growth

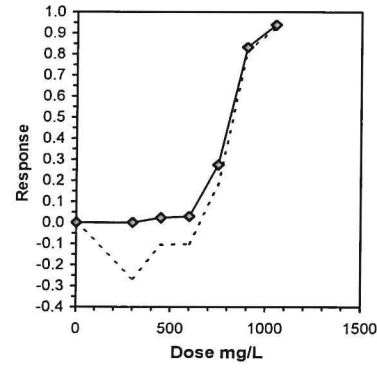
Start Date: 2/8/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 2/15/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	0.6730	0.7080	0.8170	0.7120
300	0.9710	0.9740	0.9080	0.8320
450	0.7550	0.8280	0.7880	0.8510
600	0.7870	0.7530	0.8640	0.8000
750	0.5570	0.6080	0.5470	0.6820
900	0.1950	0.1060	0.1090	0.1400
1050	0.0000	0.0870	0.1150	0.0000

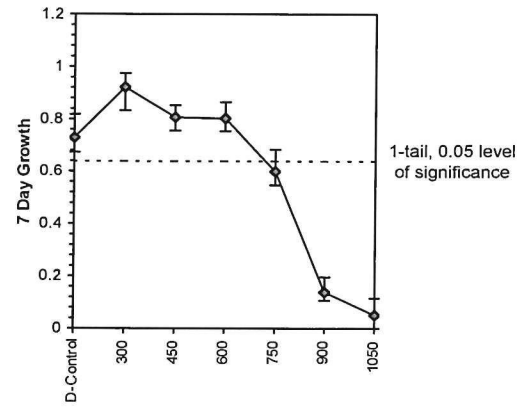
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	t-Stat		Critical	MSD	Mean	N-Mean	
D-Control	0.7275	1.0000	0.7275	0.6730	0.8170	8.548	4				0.8244	1.0000	
300	0.9213	1.2663	0.9213	0.8320	0.9740	7.254	4	-4.941	2.290	0.0898	0.8244	1.0000	
450	0.8055	1.1072	0.8055	0.7550	0.8510	5.283	4	-1.989	2.290	0.0898	0.8055	0.9771	
600	0.8010	1.1010	0.8010	0.7530	0.8640	5.798	4	-1.874	2.290	0.0898	0.8010	0.9716	
750	0.5985	0.8227	0.5985	0.5470	0.6820	10.316	4				0.5985	0.7260	
900	0.1375	0.1890	0.1375	0.1060	0.1950	30.036	4				0.1375	0.1668	
1050	0.0505	0.0694	0.0505	0.0000	0.1150	117.668	4				0.0505	0.0613	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.96099	0.844	0.13032	-0.7						
Bartlett's Test indicates equal variances ($p = 0.86$)	0.74295	11.3449								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.0898	0.12343	0.02563	0.00308	0.0029	3, 12

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL(Exp)	Skew	
IC05	613.22	75.95	275.25	649.13	-1.4558
IC10	643.75	22.10	585.03	692.10	-2.8703
IC15	674.28	18.85	621.64	741.54	0.2750
IC20	704.81	20.60	652.55	780.59	0.4641
IC25	735.35	17.67	681.43	780.75	-0.1109
IC40	783.80	7.99	759.94	809.44	-0.0088
IC50	810.62	6.57	790.30	831.08	-0.0041



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: *Pimephales promelas*

PpKCICR Test Number: 86

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	
Concentration	Parameter	JW	JW	JW	N	N	N
CONTROL, MHSW	pH (S.U.)	7.91	7.83	7.70	7.62	7.72	7.63
	Dissolved oxygen (mg/L)	7.7	7.8	7.8	7.6	7.72 0.700	7.4
	Conductivity (µmhos/cm)	316		301		292	
	Alkalinity (mg CaCO ₃ /L)	60				59	
	Hardness (mg CaCO ₃ /L)	87				87	
	Temperature (°C)	24.9	25.2	24.9	24.9	24.8	25.0
300 mg KCl/L	pH (S.U.)	7.96	7.72	7.94	7.42	7.91	7.53
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.6	7.9	7.3
	Conductivity (µmhos/cm)	896		870		836	
	Temperature (°C)	24.8	25.0	25.0	25.2	24.9	25.1
450 mg KCl/L	pH (S.U.)	7.94	7.70	7.90	7.40	7.90	7.50
	Dissolved oxygen (mg/L)	7.8	7.6	8.0	7.4	7.9	7.3
	Conductivity (µmhos/cm)	1160		1100		1080	
	Temperature (°C)	24.8	25.0	24.9	25.2	24.9	24.9
600 mg KCl/L	pH (S.U.)	7.94	7.76	7.90	7.46	7.88	7.53
	Dissolved oxygen (mg/L)	7.8	7.6	7.9	7.3	8.0	7.3
	Conductivity (µmhos/cm)	1440		1380		1340	
	Temperature (°C)	24.8	24.8	24.9	25.0	25.0	24.9
750 mg KCl/L	pH (S.U.)	7.95	7.75	7.89	7.51	7.88	7.53
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.3	8.0	7.2
	Conductivity (µmhos/cm)	1680		1630		1590	
	Temperature (°C)	24.9	24.8	24.8	25.0	25.0	25.0
900 mg KCl/L	pH (S.U.)	7.96	7.76	7.88	7.49	7.88	7.53
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.3	8.1	7.3
	Conductivity (µmhos/cm)	1930		1920		1870	
	Temperature (°C)	24.9	25.3	24.8	25.0	25.0	25.0
1050 mg KCl/L	pH (S.U.)	7.97	7.80	7.92	7.50	7.88	7.62
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	7.3	8.1	7.3
	Conductivity (µmhos/cm)	2200		2190		2130	
	Temperature (°C)	24.9	25.1	24.8	25.2	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

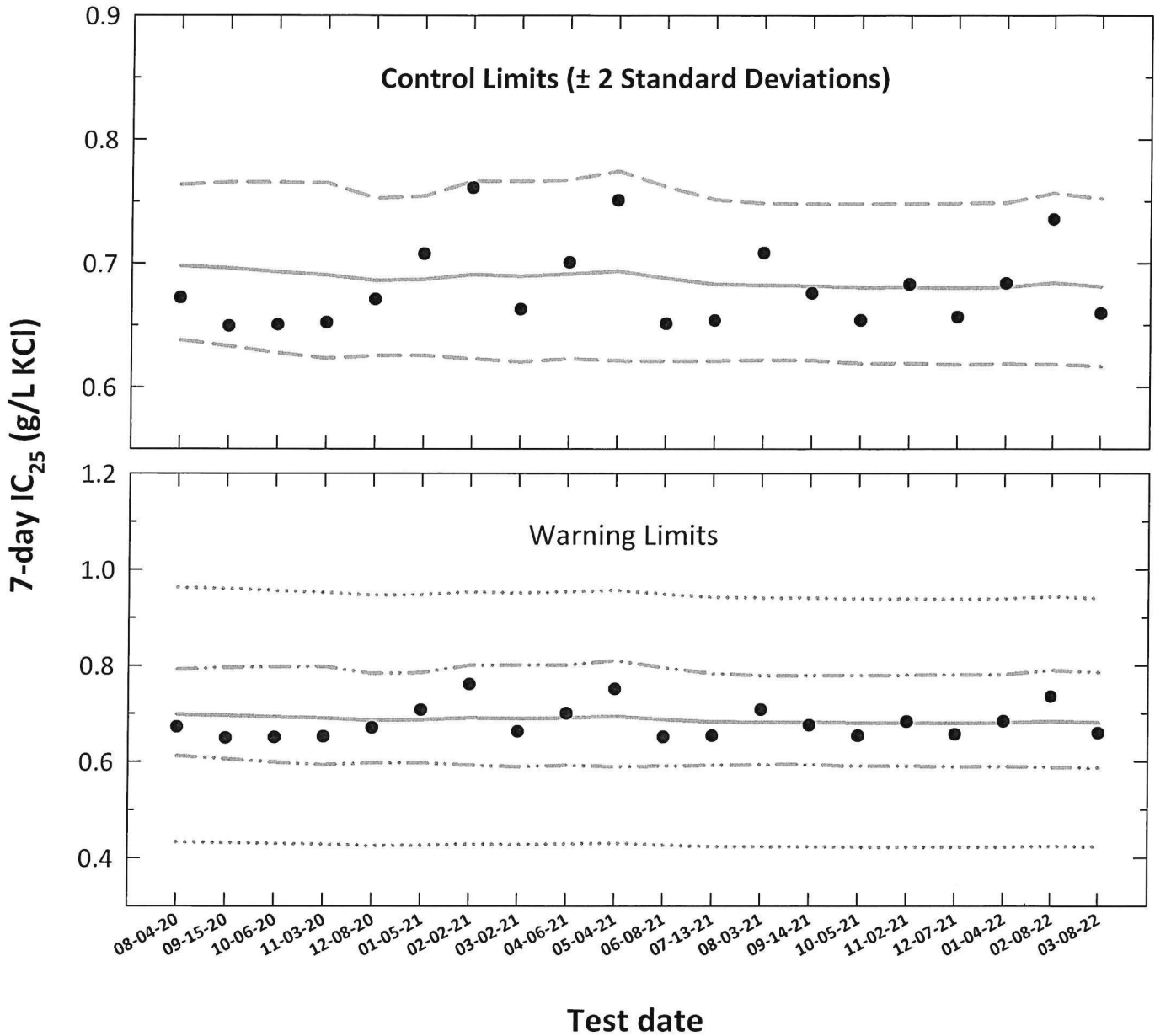
PpKICR Test Number: 86

Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	K	BSL	BSL	N	N	JW	JW	OW
CONTROL, MHSW	pH (S.U.)	7.72	7.69	7.63	7.40	7.60	7.84	7.64	7.66
	Dissolved oxygen (mg/L)	7.7	7.5	7.7	7.3	7.7	7.6	8.178	7.5
	Conductivity (µmhos/cm)	290		289		299		321	
	Alkalinity (mg CaCO ₃ /L)			59					
	Hardness (mg CaCO ₃ /L)			85					
	Temperature (°C)	24.9	25.0	24.9	24.9	24.9	24.8	24.9	24.7
300 mg KCl/L	pH (S.U.)	7.89	7.66	7.98	7.40	7.75	7.82	8.00	7.66
	Dissolved oxygen (mg/L)	8.0	7.5	8.0	7.3	7.7	7.6	8.1	7.4
	Conductivity (µmhos/cm)	827		812		850		903	
	Temperature (°C)	25.0	24.9	24.8	25.1	25.0	25.0	24.7	24.9
450 mg KCl/L	pH (S.U.)	7.89	7.59	7.95	7.43	7.82	7.85	7.97	7.67
	Dissolved oxygen (mg/L)	8.0	7.3	8.0	7.4	7.8	7.6	8.1	7.4
	Conductivity (µmhos/cm)	1090		1070		1110		1130	
	Temperature (°C)	25.0	24.9	24.8	25.0	24.8	24.8	24.7	24.9
600 mg KCl/L	pH (S.U.)	7.88	7.59	7.92	7.43	7.85	7.82	7.96	7.68
	Dissolved oxygen (mg/L)	8.0	7.3	8.0	7.4	7.8	7.6	8.1	7.5
	Conductivity (µmhos/cm)	1360		1320		1370		1400	
	Temperature (°C)	25.0	25.1	24.8	25.2	24.8	24.8	24.9	24.7
750 mg KCl/L	pH (S.U.)	7.88	7.66	7.93	7.44	7.87	7.81	7.95	7.69
	Dissolved oxygen (mg/L)	8.1	7.3	8.0	7.4	7.8	7.7	8.1	7.5
	Conductivity (µmhos/cm)	1400		1570		1620		1670	
	Temperature (°C)	25.0	25.1	24.8	25.2	24.8	24.8	24.9	24.7
900 mg KCl/L	pH (S.U.)	7.88	7.67	7.92	7.43	7.88	7.84	7.95	7.74
	Dissolved oxygen (mg/L)	8.1	7.3	8.0	7.3	7.8	7.6	8.1	7.5
	Conductivity (µmhos/cm)	1880		1810		1890		1930	
	Temperature (°C)	24.9	25.2	24.9	25.2	24.8	24.9	24.9	24.7
1050 mg KCl/L	pH (S.U.)	7.89	7.69	7.93	7.52	7.88	7.84	7.95	7.77
	Dissolved oxygen (mg/L)	8.1	7.4	8.1	7.2	7.8	7.5	8.1	7.4
	Conductivity (µmhos/cm)	2140		2100		2130		2200	
	Temperature (°C)	24.9	25.0	24.8	24.9	25.0	24.9	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

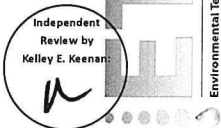
Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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



Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)													
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV										
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	75th Percentile CV Warning Limits								
1	08-04-20	0.6723	-0.1725	-0.1562	0.0195	0.6980	0.6381	0.7635	0.6122	0.7918	0.4327	0.9632							
2	09-15-20	0.6494	-0.1875	-0.1573	0.0206	0.6962	0.6331	0.7655	0.6055	0.7958	0.4316	0.9607							
3	10-06-20	0.6504	-0.1868	-0.1592	0.0216	0.6932	0.6277	0.7655	0.5987	0.7975	0.4298	0.9566							
4	11-03-20	0.6522	-0.1857	-0.1609	0.0222	0.6904	0.6232	0.7649	0.5930	0.7983	0.4280	0.9528							
5	12-08-20	0.6708	-0.1734	-0.1636	0.0201	0.6861	0.6255	0.7526	0.5977	0.7830	0.4254	0.9468							
6	01-05-21	0.7076	-0.1502	-0.1630	0.0203	0.6870	0.6257	0.7544	0.5977	0.7850	0.4259	0.9481							
7	02-02-21	0.7611	-0.1186	-0.1606	0.0226	0.6909	0.6228	0.7666	0.5923	0.8004	0.4284	0.9535							
8	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515							
9	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							
10	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							
11	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							
12	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							
13	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							
14	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							
15	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							
16	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							
17	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							
18	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							
19	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							
20	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							

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 ToxCal(c)).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

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

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

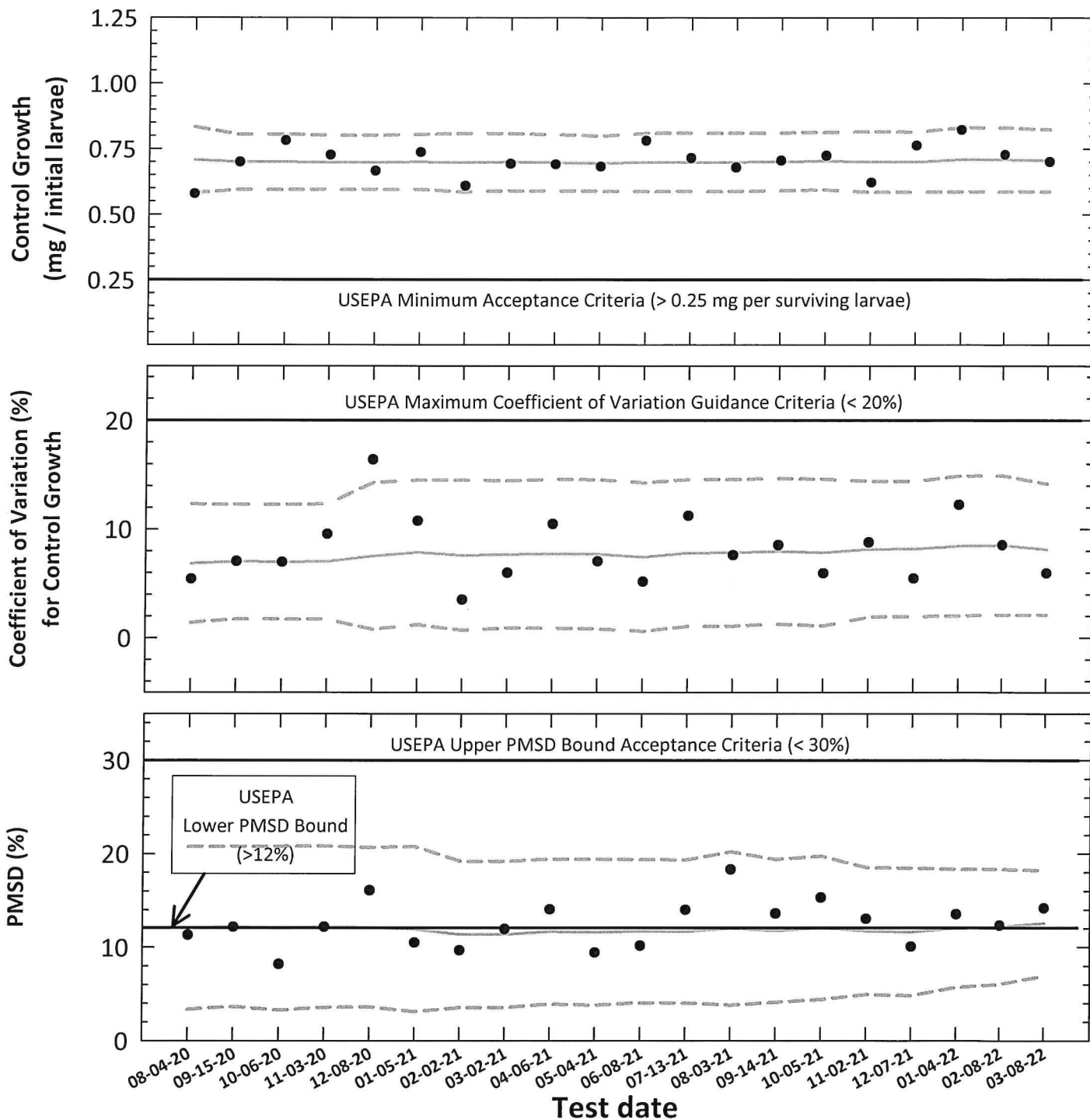
CV = Coefficient of variation.



Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture

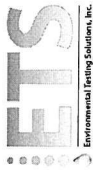


- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity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

● **Central Tendency** (mean Control Growth, CV or PMSD)

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





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

Test number	Test date	ToxCal Determination				Control Growth		Test		Control Growth			Control Growth CV		Test PMSD (%)			
		Control Survival (%)	Control Growth		CV (%)	CT	PMSD (%)	MSD	CT	(mg/initial larvae)		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	08-04-20	100	0.579	5.4	0.0654	11.3	0.708	0.582	0.834	6.9	0.582	0.834	6.9	1.4	12.3	12.1	3.4	20.8
2	09-15-20	100	0.700	7.1	0.0851	12.2	0.700	0.595	0.805	7.0	0.595	0.805	7.0	1.7	12.3	12.2	3.6	20.8
3	10-06-20	100	0.782	7.0	0.0640	8.2	0.700	0.594	0.806	7.0	0.594	0.806	7.0	1.7	12.3	12.0	3.3	20.8
4	11-03-20	97.5	0.727	9.6	0.0885	12.2	0.698	0.595	0.801	7.0	0.595	0.801	7.0	1.7	12.3	12.2	3.6	20.8
5	12-08-20	100	0.666	16.4	0.1070	16.1	0.698	0.595	0.801	7.5	0.595	0.801	7.5	0.8	14.3	12.1	3.6	20.7
6	01-05-21	100	0.737	10.8	0.0774	10.5	0.700	0.596	0.805	7.9	0.596	0.805	7.9	1.2	14.5	11.9	3.1	20.8
7	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	0.809	7.6	0.585	0.809	7.6	0.7	14.5	11.4	3.5	19.2
8	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.590	0.809	7.7	0.9	14.5	11.4	3.5	19.2
9	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4
10	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.589	0.799	7.7	0.8	14.6	11.6	3.8	19.4
11	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
12	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
13	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
14	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
15	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
16	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
17	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
18	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
19	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
20	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2

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.

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) > 12%.

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

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

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



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

Species: Pimephales promelas

PpKClCR Test Number: 87

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2063</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>03-02-22</u>	Artemia CHM number:	<u>CHM1149</u>
Hatch dates and times:	<u>03-07-22 1455 TO 03-08-22 0520</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.91</u> S.U. Temperature = <u>24.3</u> °C	Date / Time in oven:	<u>03-15-22 0742</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>03-16-22 0745</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	03-08-22	<u>0530</u>	<u>JL</u>	<u>1130</u>	<u>JL</u>	<u>0755</u>	<u>JL</u>	<u>03-03-22 B</u>
1	03-09-22	<u>0530</u>	<u>JL</u>	<u>1130</u>	<u>JL</u>	<u>0738</u>	<u>JL</u>	<u>↓</u>
2	03-10-22	<u>0530</u>	<u>JL</u>	<u>1130</u>	<u>JL</u>	<u>0730</u>	<u>JL</u>	<u>03-03-22 C</u>
3	03-11-22	<u>0530</u>	<u>JL</u>	<u>1130</u>	<u>JL</u>	<u>0730</u>	<u>JL</u>	<u>↓</u>
4	03-12-22	<u>0630</u>	<u>JL</u>	<u>1230</u>	<u>JL</u>	<u>0830</u>	<u>JL</u>	<u>03-03-22 03-07-22</u>
5	03-13-22	<u>0555</u>	<u>JL</u>	<u>1155</u>	<u>JL</u>	<u>0755</u>	<u>JL</u>	<u>↓</u>
6	03-14-22	<u>0600</u>	<u>JL</u>	<u>1200</u>	<u>JL</u>	<u>0800</u>	<u>JL</u>	<u>↓</u>
7	03-15-22					<u>0655</u>	<u>JL</u>	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	<u>13066465</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>777.3</u>
Average weight per initial larvae:	<u>0.701</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.701</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>659.4</u>



Species: Pimephales promelas

PpKICR Test Number: 87

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	26 10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>mgentz</u> Analyst: <u>A.S.</u> Date: <u>02-22-22</u> 02-23-22	14.74	14.41	12.77	14.73	15.27	15.81	14.97	14.68	13.39	15.06	13.85	14.47
*B = Pan + Larvae weight (mg) Analyst: <u>A.S.</u> Date: <u>02-16-22</u>	21.31	21.15	20.03	22.18	23.95	23.15	23.28	24.17	20.55	21.38	20.50	21.83
C = Larvae weight (mg) = B - A Analyst: <u>JA</u>	6.57	6.74	7.26	7.45	8.68	7.34	8.31	9.49	7.16	6.32	6.65	7.36
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JA</u>	0.657	0.674	0.726	0.745	0.868	0.734	0.831	0.949	0.716	0.632	0.665	0.736
Average weight per initial number of larvae (mg)	0.701				0.846		-20.77		0.687		1.97	
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: 87

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	6 ^{4d}	6 ^{4d}	7 ^{3d}	6 ^{4d}
2	10	10	10	10	10	10	10	10	6	5 ^{1d}	6 ^{1d}	6
3	10	10	10	10	10	10	10	10	5 ^{1d}	5	5 ^{1d}	6
4	10	10	10	10	9 ^{1d}	9 ^{1d}	9 ^{1d}	9 ^{1d}	4 ^{1d}	3 ^{2d}	3 ^{2d}	3 ^{3d}
5	10	10	10	10	7 ^{2d}	6 ^{3d}	7 ^{2d}	7 ^{2d}	1 ^{3d}	2 ^{1d}	1 ^{2d}	2 ^{1d}
6	9 ^{1d}	9 ^{1d}	10	10	7	6	7	6 ^{1d}	1	1 ^{1d}	1	2
7	9	9	10	9 ^{1d}	6 ^{1d}	6	7	6	1 ^{1d}	1 ^{1d}	1 ^{1d}	2
*A = Pan weight (mg) Tray color code: <u>M-9ent</u> Analyst: <u>A.F</u> Date: <u>02-23-22</u>	16.30	14.47	14.06	14.85	15.19	14.83	16.00 ^{15.95}	14.69	14.90	13.47	14.58	15.43
*B = Pan + Larvae weight (mg) Analyst: <u>A.F</u> Date: <u>02-16-22</u>	22.32	21.32	20.89	20.61	19.90	19.36	21.59	19.59	15.96	14.87	15.77	16.97
C = Larvae weight (mg) = B - A Analyst: <u>JK</u>	6.02	6.85	6.83	5.76	4.71	4.53	5.59	4.90	1.06	1.40	1.19	1.54
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JK</u>	0.669	0.761	0.683	0.640	0.785	0.755	0.799	0.817	1.060	1.400	1.190	0.770
Average weight per initial number of larvae (mg)	0.637		0.637		0.493		0.493		0.130		0.130	
Percent reduction from control (%)	9.17		9.17		29.67		29.67		81.57		81.57	

*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*

PpKICR Test Number: 87

Survival and Growth Data

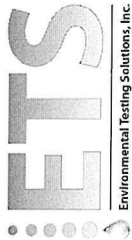
Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10	10	10	10	
1	4 ^{6d}	4 ^{6d}	4 ^{6d}	3 ^{7d}	
2	3 ^{1d}	4	3 ^{1d}	3	
3	3	4	3	3	
4	2 ^{1d}	2 ^{2d}	3	3	
5	0 ^{2d}	1 ^{1d}	1 ^{2d}	1 ^{2d}	
6	0	1	1	1	
7	0	1 ^{1d}	1 ^{1d}	1	
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>A.S</u> Date: <u>02-23-22</u>		15.06	14.66	15.03 15.02	14.21
*B = Pan + Larvae weight (mg) Analyst: <u>A.S</u> Date: <u>02-16-22</u>		15.06	15.68	16.20	15.09
C = Larvae weight (mg) = B - A Analyst: <u>JK</u>		0.00 → 1.02	1.18	0.88	
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JK</u>		0	0.102	0.118	0.088
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	0.077		89.07.	

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

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

Comments:





***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: 87
 Test dates: March 08-15, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.74	21.31	6.57	0.657	0.701	6.0	0.657	100.0	0.701	6.0	Not applicable
	B	10	10	14.41	21.15	6.74	0.674			0.674				
	C	10	10	12.77	20.03	7.26	0.726			0.726				
	D	10	10	14.73	22.18	7.45	0.745			0.745				
300	E	10	10	15.27	23.95	8.68	0.868	0.846	10.5	0.868	100.0	0.846	10.5	-20.7
	F	10	10	15.81	23.15	7.34	0.734			0.734				
	G	10	10	14.97	23.28	8.31	0.831			0.831				
	H	10	10	14.68	24.17	9.49	0.949			0.949				
450	I	10	10	13.39	20.55	7.16	0.716	0.687	6.9	0.716	100.0	0.687	6.9	1.9
	J	10	10	15.06	21.38	6.32	0.632			0.632				
	K	10	10	13.85	20.50	6.65	0.665			0.665				
	L	10	10	14.47	21.83	7.36	0.736			0.736				
600	M	10	9	16.30	22.32	6.02	0.669	0.688	7.5	0.602	92.5	0.637	8.8	9.1
	N	10	9	14.47	21.32	6.85	0.761			0.685				
	O	10	10	14.06	20.89	6.83	0.683			0.683				
	P	10	9	14.85	20.61	5.76	0.640			0.576				
750	Q	10	6	15.19	19.90	4.71	0.785	0.789	3.3	0.471	62.5	0.493	9.4	29.6
	R	10	6	14.83	19.36	4.53	0.755			0.453				
	S	10	7	16.00	21.59	5.59	0.799			0.559				
	T	10	6	14.69	19.59	4.90	0.817			0.490				
900	U	10	1	14.90	15.96	1.06	1.060	1.105	23.9	0.106	12.5	0.130	16.5	81.5
	V	10	1	13.47	14.87	1.40	1.400			0.140				
	W	10	1	14.58	15.77	1.19	1.190			0.119				
	X	10	2	15.43	16.97	1.54	0.770			0.154				
1050	Y	10	0	0.00	0.00	0.00	0.000	1.027	14.6	0.000	7.5	0.077	68.5	89.0
	Z	10	1	14.66	15.68	1.02	1.020			0.102				
	AA	10	1	15.02	16.20	1.18	1.180			0.118				
	BB	10	1	14.21	15.09	0.88	0.880			0.088				

Dunnett's MSD value: 0.0994 MSD = _____
 PMSD: 14.2 PMSD = _____

Minimum Significant Difference
 Percent Minimum Significant Difference

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

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-R-01-004 and EPA-821-R-01-005. US Environmental Protection Agency, Cincinnati, OH.



Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 3/8/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 3/15/2022	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

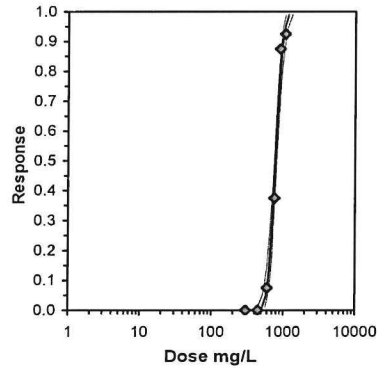
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	0.9000	1.0000	0.9000
750	0.6000	0.6000	0.7000	0.6000
900	0.1000	0.1000	0.1000	0.2000
1050	0.0000	0.1000	0.1000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40	
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40	
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40	
600	0.9250	0.9250	1.2898	1.2490	1.4120	6.318	4	12.00	10.00	3	40	
*750	0.6250	0.6250	0.9123	0.8861	0.9912	5.759	4	10.00	10.00	15	40	
*900	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40	
*1050	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40	

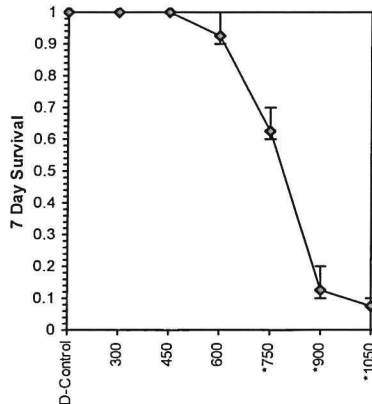
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$) Equality of variance cannot be confirmed	0.88262	0.896	0.53032	2.00199
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	13.1273	1.61551	9.9609	16.2937	0	2.81114	9.48773	0.58991	2.8906	0.07618	3
Intercept	-32.946	4.68602	-42.13	-23.761							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	516.874	447.173	566.611
EC05	3.355	582.504	521.939	625.725
EC10	3.718	620.832	566.261	660.323
EC15	3.964	648.107	597.9	685.166
EC20	4.158	670.636	623.975	705.947
EC25	4.326	690.587	646.923	724.64
EC40	4.747	743.533	706.403	776.335
EC50	5.000	777.319	742.559	811.621
EC60	5.253	812.641	778.361	850.915
EC75	5.674	874.943	836.695	926.033
EC80	5.842	900.973	859.627	959.224
EC85	6.036	932.292	886.41	1000.25
EC90	6.282	973.249	920.428	1055.37
EC95	6.645	1037.29	971.995	1144.2
EC99	7.326	1169	1074.12	1334.61



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS



Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	3/8/2022	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/15/2022	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

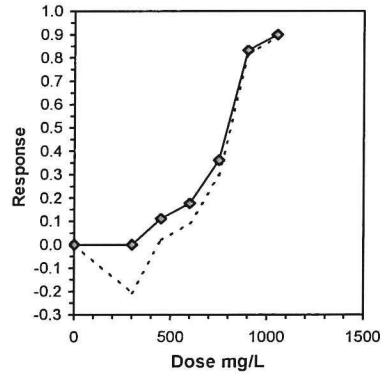
Conc-mg/L	1	2	3	4
D-Control	0.6570	0.6740	0.7260	0.7450
300	0.8680	0.7340	0.8310	0.9490
450	0.7160	0.6320	0.6650	0.7360
600	0.6020	0.6850	0.6830	0.5760
750	0.4710	0.4530	0.5590	0.4900
900	0.1060	0.1400	0.1190	0.1540
1050	0.0000	0.1020	0.1180	0.0880

Conc-mg/L	Transform: Untransformed						1-Tailed			Isotonic		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7005	1.0000	0.7005	0.6570	0.7450	5.958	4				0.7730	1.0000
300	0.8455	1.2070	0.8455	0.7340	0.9490	10.548	4	-3.341	2.290	0.0994	0.7730	1.0000
450	0.6873	0.9811	0.6873	0.6320	0.7360	6.902	4	0.305	2.290	0.0994	0.6873	0.8891
600	0.6365	0.9086	0.6365	0.5760	0.6850	8.778	4	1.475	2.290	0.0994	0.6365	0.8234
750	0.4933	0.7041	0.4933	0.4530	0.5590	9.400	4				0.4933	0.6381
900	0.1298	0.1852	0.1298	0.1060	0.1540	16.486	4				0.1298	0.1679
1050	0.0770	0.1099	0.0770	0.0000	0.1180	68.541	4				0.0770	0.0996

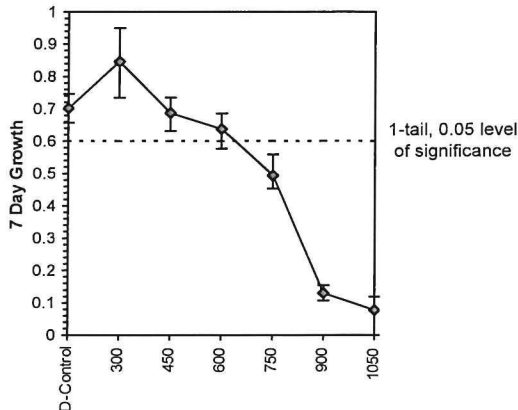
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96446	0.844	-0.1627	-0.2093
Bartlett's Test indicates equal variances (p = 0.59)	1.91511	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.09938	0.14187	0.0322	0.00377	0.00262	3, 12

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	367.61	29.26	328.86	507.68	1.9694
IC10	435.22	44.96	357.72	622.91	1.1639
IC15	539.26	58.84	364.72	677.17	-0.2351
IC20	618.95	32.80	484.99	692.40	-0.9526
IC25	659.42	24.34	582.73	735.96	0.0438
IC40	762.15	10.01	728.93	790.43	-0.0553
IC50	794.05	7.43	772.08	817.67	0.2435



Dose-Response Plot



Entered and Reviewed by Jim Sumner



Species: Pimephales promelas

PpKCICR Test Number: 87

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		u	n	n	u	u	u		
CONTROL, MHSW	pH (S.U.)	7.69	7.70	7.77	7.55	7.67	7.48		
	Dissolved oxygen (mg/L)	7.6	7.6	7.6	7.3	7.6	6.9		
	Conductivity (µmhos/cm)	300		299		300			
	Alkalinity (mg CaCO ₃ /L)	60				61			
	Hardness (mg CaCO ₃ /L)	80				80			
	Temperature (°C)	24.8	25.0	24.9	25.1	24.9	24.8		
300 mg KCl/L	pH (S.U.)	7.99	7.65	8.00	7.54	7.95	7.45		
	Dissolved oxygen (mg/L)	7.7	7.6	7.7	7.2	7.6	6.9		
	Conductivity (µmhos/cm)	846		838		832			
	Temperature (°C)	24.9	24.9	25.0	24.8	24.8	25.0		
450 mg KCl/L	pH (S.U.)	7.98	7.65	7.98	7.54	7.95	7.46		
	Dissolved oxygen (mg/L)	7.7	7.6	7.7	7.3	7.6	7.0		
	Conductivity (µmhos/cm)	1110		1110		1110			
	Temperature (°C)	25.0	24.9	24.9	24.8	24.8	24.9		
600 mg KCl/L	pH (S.U.)	7.97	7.66	7.97	7.52	7.93	7.45		
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.4	7.6	6.8		
	Conductivity (µmhos/cm)	1380		1370		1360			
	Temperature (°C)	25.0	25.1	24.9	25.0	24.8	24.9		
750 mg KCl/L	pH (S.U.)	7.96	7.64	7.96	7.57	7.94	7.46		
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.4	7.8	6.6		
	Conductivity (µmhos/cm)	1640		1620		1610			
	Temperature (°C)	25.0	24.9	24.9	25.0	24.8	25.0		
900 mg KCl/L	pH (S.U.)	7.96	7.62	7.96	7.58	7.94	7.46		
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.3	7.8	7.1		
	Conductivity (µmhos/cm)	1910		1870		1860			
	Temperature (°C)	25.0	24.8	24.9	25.1	24.8	24.8		
1050 mg KCl/L	pH (S.U.)	8.00	7.61	7.96	7.03	7.95	7.54		
	Dissolved oxygen (mg/L)	7.8	7.7	8.0	7.2	7.9	7.2		
	Conductivity (µmhos/cm)	2200		2170		2150			
	Temperature (°C)	25.0	24.8	24.9	24.8	24.9	25.0		
		Initial	Final	Initial	Final	Initial	Final		



Species: Pimephales promelas

PpKICR Test Number: 87

Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	N	BSL	BSL	N	N	N	N	JW
CONTROL, MHSW	pH (S.U.)	7.70	7.66	7.61	7.37	7.74	7.48	7.64	7.39
	Dissolved oxygen (mg/L)	7.7	7.5	7.9	7.2	7.6	7.4	7.7	7.1
	Conductivity (µmhos/cm)	311		309		291		303	
	Alkalinity (mg CaCO ₃ /L)			60					
	Hardness (mg CaCO ₃ /L)			84					
	Temperature (°C)	24.8	24.9	24.7	25.0	24.8	24.9	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.96	7.62	8.03	7.40	7.77	7.40	7.85	7.46
	Dissolved oxygen (mg/L)	7.7	7.5	8.0	7.2	7.7	7.3	7.8	7.1
	Conductivity (µmhos/cm)	846		846		825		843	
	Temperature (°C)	24.7	25.0	24.7	24.8	24.9	24.9	24.8	24.7
450 mg KCl/L	pH (S.U.)	7.95	7.63	8.06	7.41	7.82	7.49	7.84	7.47
	Dissolved oxygen (mg/L)	7.8	7.5	8.0	7.2	7.7	7.3	7.8	7.2
	Conductivity (µmhos/cm)	1030		1130		1110		1080	
	Temperature (°C)	24.7	24.8	24.8	24.8	24.9	24.7	24.9	24.8
600 mg KCl/L	pH (S.U.)	7.95	7.62	8.06	7.41	7.88	7.51	7.85	7.52
	Dissolved oxygen (mg/L)	7.9	7.4	8.0	7.2	7.8	7.4	7.9	7.2
	Conductivity (µmhos/cm)	1370		1370		1370		1370	
	Temperature (°C)	24.7	24.8	24.8	24.8	24.9	24.7	24.9	24.8
750 mg KCl/L	pH (S.U.)	7.94	7.63	8.07	7.43	7.90	7.53	7.86	7.59
	Dissolved oxygen (mg/L)	8.0	7.4	8.0	7.2	7.3	7.5	7.9	7.2
	Conductivity (µmhos/cm)	1640		1650		1630		1640	
	Temperature (°C)	24.7	24.8	24.8	25.1	24.8	24.6	24.8	24.8
900 mg KCl/L	pH (S.U.)	7.95	7.74	8.07	7.54	7.93	7.52	7.87	7.62
	Dissolved oxygen (mg/L)	8.0	7.3	8.0	7.6	7.8	7.5	8.0	7.8
	Conductivity (µmhos/cm)	1910		1940		1940		1890	
	Temperature (°C)	24.8	25.0	24.8	24.9	24.8	24.9	24.8	24.6
1050 mg KCl/L	pH (S.U.)	8.00	7.71	8.06	7.55	7.96	7.58	7.90	7.66
	Dissolved oxygen (mg/L)	8.0	7.3	7.9	7.4	7.7	7.7	8.0	7.4
	Conductivity (µmhos/cm)	2200		2180		2120		2160	
	Temperature (°C)	24.7	24.7	24.9	25.1	24.8	24.8	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

