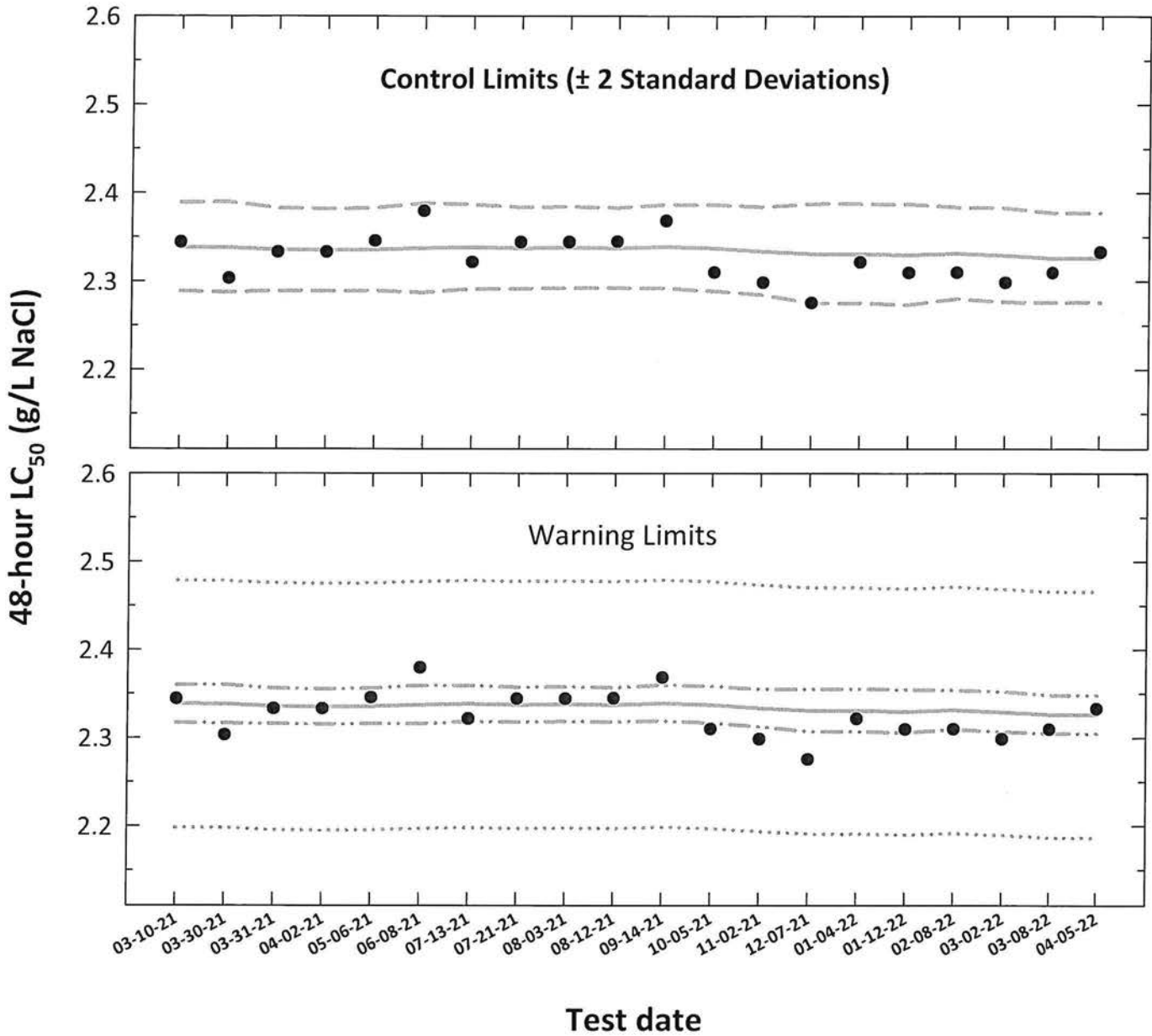


Ceriodaphnia dubia

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	03-08-22	2.3096	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
20	04-05-22	2.3330	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658

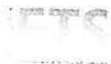
Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
 CT = Central tendency of the LC₅₀ values.
 S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant TestCdNaClAC # 319

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:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	JW	JW	W	
	pH (S.U.)	7.5	7.68	7.63
	Dissolved oxygen (mg/L)	7.7	7.7	8.0
	Conductivity (µmhos/cm)	324		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	90		
1750 mg/L				
	pH (S.U.)	7.86	7.81	7.72
	Dissolved oxygen (mg/L)	7.9	7.8	8.0
	Conductivity (µmhos/cm)	3490		
2000 mg/L				
	pH (S.U.)	7.86	7.81	7.77
	Dissolved oxygen (mg/L)	7.9	7.9	8.0
	Conductivity (µmhos/cm)	3800		
2250 mg/L				
	pH (S.U.)	7.86	7.80	7.85
	Dissolved oxygen (mg/L)	7.9	7.9	8.1
	Conductivity (µmhos/cm)	4310		
2500 mg/L				
	pH (S.U.)	7.86	7.82	7.87
	Dissolved oxygen (mg/L)	7.9	7.9	8.1
	Conductivity (µmhos/cm)	4740		
2750 mg/L				
	pH (S.U.)	7.86	7.83	7.87
	Dissolved oxygen (mg/L)	8.0	8.0	8.1
	Conductivity (µmhos/cm)	5290		
	Temperature (°C)	25.1	24.8	24.9

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

Chemical analyses:

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

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

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 379

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-05-22	0530	JL	0750	JL	2B3	RES	0330-21A
24	04-06-22			0749	JL			
48 Termination	04-07-22			0749	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	03-29-22 A
Age:	< 24-hours old
Date and time organisms were born between:	04-04-22 1504 to 04-05-22 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.71
	Temperature (°C): 25.1

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	3 ^{2d}	5	4 ^{2d}	4 ^{1d}	3 ^{2d}	3 ^{2d}	4 ^{1d}	4 ^{1d}
48 Termination	4 ^{1d}	3 ^{2d}	2 ^{3d}	3 ^{2d}	1 ^{2d}	2 ^{3d}	1 ^{2d}	1 ^{3d}	0 ^{2d}	0 ^{3d}	0 ^{4d}	0 ^{4d}
Mean Survival	60%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT	Comments:
Lower 95% confidence limit (mg NaCl/L)	2258.7	
Upper 95% confidence limit (mg NaCl/L)	2405.4	
48-hour LC ₅₀ (mg NaCl/L)	2333.0	

2333.0



Acute Daphnid Test-48 Hr Survival

Start Date: 4/5/2022	Test ID: CdNaClAC	Sample ID: REF-Ref Toxicant
End Date: 4/7/2022	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	0.6000
2500	0.2000	0.4000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

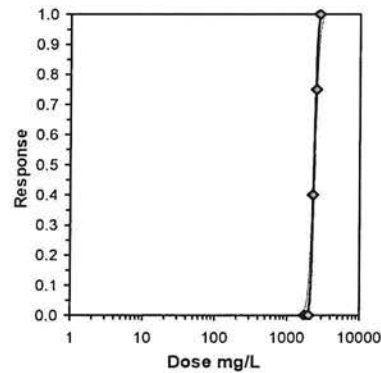
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.6000	0.6000	0.8910	0.6847	1.1071	19.366	4	10.00	10.00	8	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.70565	0.868	0.58177	4.311
Equality of variance cannot be confirmed				

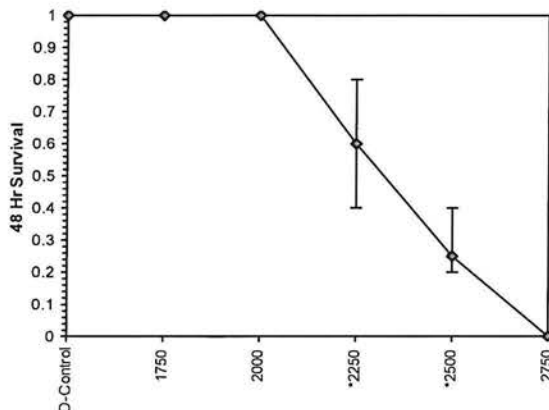
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	30.6363	5.60404	19.6524	41.6203	0	2.06471	7.81472	0.55909	3.36791	0.03264	5
Intercept	-98.18	18.8894	-135.2	-61.157							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1958.73	1759.29	2067.52
EC05	3.355	2061.67	1901.34	2151.69
EC10	3.718	2118.74	1980.33	2199.46
EC15	3.964	2158.14	2034.52	2233.34
EC20	4.158	2189.97	2077.83	2261.52
EC25	4.326	2217.65	2114.93	2286.83
EC40	4.747	2288.97	2206.51	2357.07
EC50	5.000	2332.97	2258.74	2405.42
EC60	5.253	2377.82	2307.79	2459.45
EC75	5.674	2454.28	2382.3	2562.04
EC80	5.842	2485.31	2409.97	2606.71
EC85	6.036	2521.97	2441.29	2661.21
EC90	6.282	2568.86	2479.73	2733.1
EC95	6.645	2639.97	2535.62	2845.71
EC99	7.326	2778.71	2639.69	3074.49



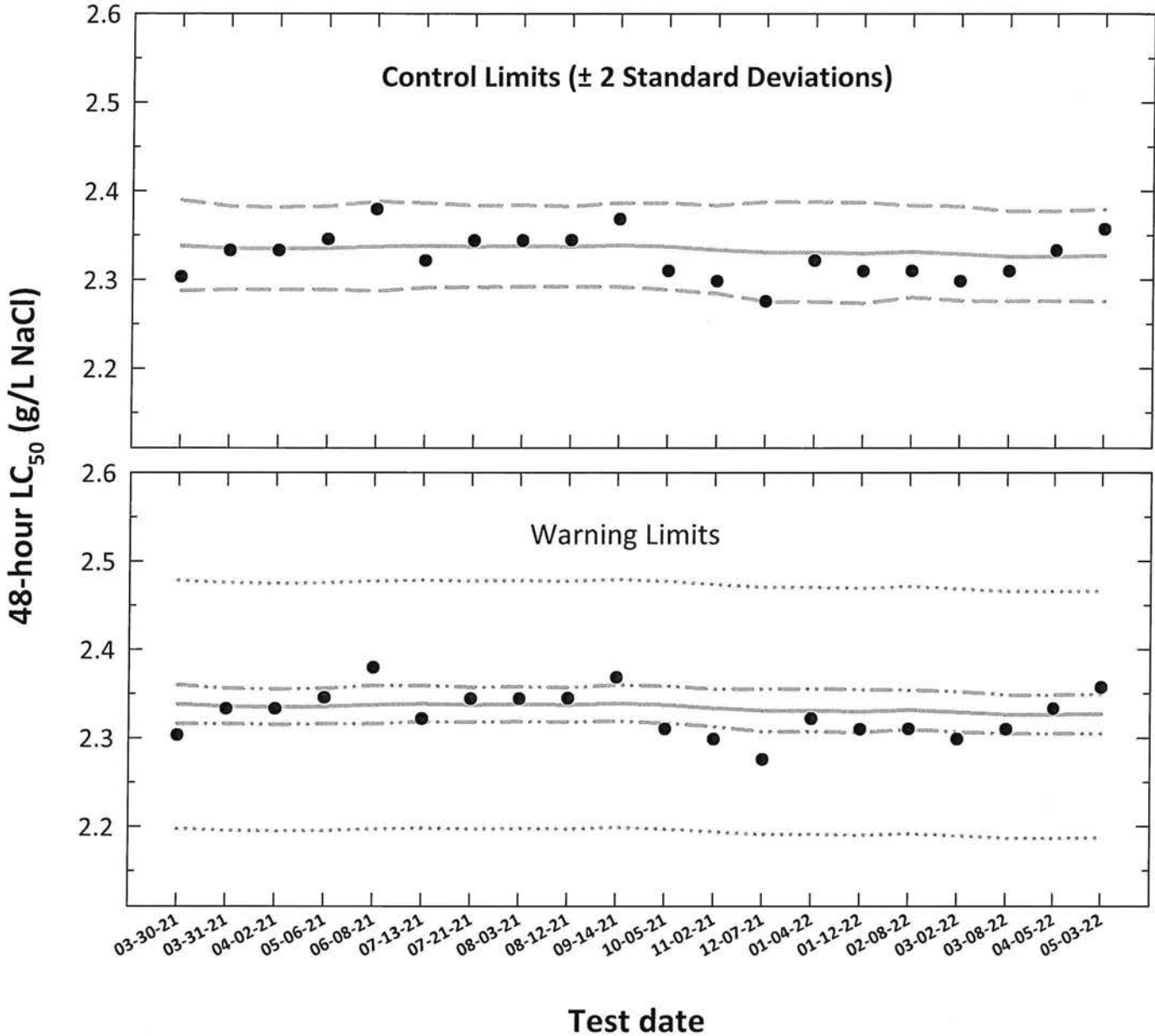
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	03-08-22	2.3096	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
19	04-05-22	2.3330	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
20	05-03-22	2.3569	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665

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 # 380

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 #: 2098

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	JW	JW	KL	
	pH (S.U.)	7.80	7.73	7.86
	Dissolved oxygen (mg/L)	7.8	8.0	7.8
	Conductivity (µmhos/cm)	310		
	Alkalinity (mg/L CaCO ₃)	62		
	Hardness (mg/L CaCO ₃)	88		
Temperature (°C)	24.8	24.9	25.2	
1750 mg/L	pH (S.U.)	8.04	7.86	7.89
	Dissolved oxygen (mg/L)	8.0	8.0	7.8
	Conductivity (µmhos/cm)	3660		
	Temperature (°C)	24.9	25.1	24.9
2000 mg/L	pH (S.U.)	8.03	7.85	7.90
	Dissolved oxygen (mg/L)	8.0	8.1	7.8
	Conductivity (µmhos/cm)	4190		
	Temperature (°C)	24.9	24.8	24.9
2250 mg/L	pH (S.U.)	8.03	7.86	7.90
	Dissolved oxygen (mg/L)	8.0	8.0	7.8
	Conductivity (µmhos/cm)	4500		
	Temperature (°C)	25.0	25.0	25.1
2500 mg/L	pH (S.U.)	8.02	7.87	7.89
	Dissolved oxygen (mg/L)	8.1	8.1	7.9
	Conductivity (µmhos/cm)	5010		
	Temperature (°C)	25.0	25.0	24.9
2750 mg/L	pH (S.U.)	8.01	7.87	7.89
	Dissolved oxygen (mg/L)	8.2	8.1	7.9
	Conductivity (µmhos/cm)	5480		
	Temperature (°C)	25.0	25.0	24.9

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13064685

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 # 380

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-03-22	0520	JL	0810	H	2C1	RED	022-22B
24	05-04-22			0802	H			
48 Termination	05-05-22			0805	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	04-26-22 D
Age:	< 24-hours old
Date and time organisms were born between:	05-02-22 1452 TO 05-03-22 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.98
	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	4 ^u	3 ^u
48 Termination	3 ^u	3 ^u	4 ^u	4 ^u	2 ^u	2 ^u	0 ^u	1 ^u	0 ^u	0 ^u	0 ^u	0 ^u
Mean Survival	70%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2284.9
Upper 95% confidence limit (mg NaCl/L)	2421.1
48-hour LC ₅₀ (mg NaCl/L)	2356.9

Comments:

Test Reviewed by: JL

Acute Daphnid Test-48 Hr Survival

Start Date: 5/3/2022	Test ID: CdNaClAC	Sample ID: REF-Ref Toxicant
End Date: 5/5/2022	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

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	0.8000	0.8000
2500	0.4000	0.4000	0.0000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

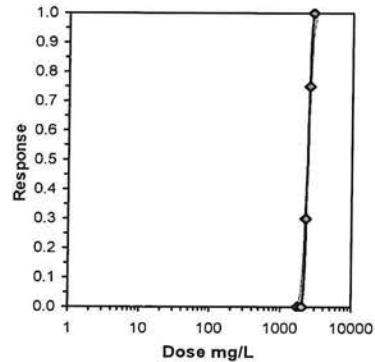
Conc-mg/L	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number	
	Mean	N-Mean	Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4		0	20	
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.7000	0.7000	0.9966	0.8861	1.1071	12.807	4	10.00	10.00	6	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)	0.82057	0.868	-0.8268	2.95332

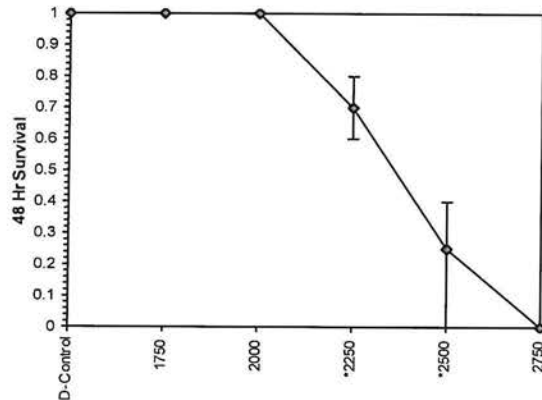
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	33.2273	6.2953	20.8885	45.5661	0	1.04116	7.81472	0.79129	3.37235	0.0301	5
Intercept	-107.05	21.2455	-148.7	-65.413							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2006.02	1807.09	2111.32
EC05	3.355	2103.03	1943.97	2189.9
EC10	3.718	2156.65	2019.79	2234.47
EC15	3.964	2193.59	2071.67	2266.06
EC20	4.158	2223.41	2113.06	2292.36
EC25	4.326	2249.31	2148.46	2315.99
EC40	4.747	2315.92	2235.5	2381.73
EC50	5.000	2356.94	2284.88	2427.13
EC60	5.253	2398.68	2331.03	2477.98
EC75	5.674	2469.72	2400.63	2574.68
EC80	5.842	2498.49	2426.33	2616.79
EC85	6.036	2532.45	2455.34	2668.13
EC90	6.282	2575.83	2490.85	2735.8
EC95	6.645	2641.5	2542.32	2841.64
EC99	7.326	2769.25	2637.74	3055.97



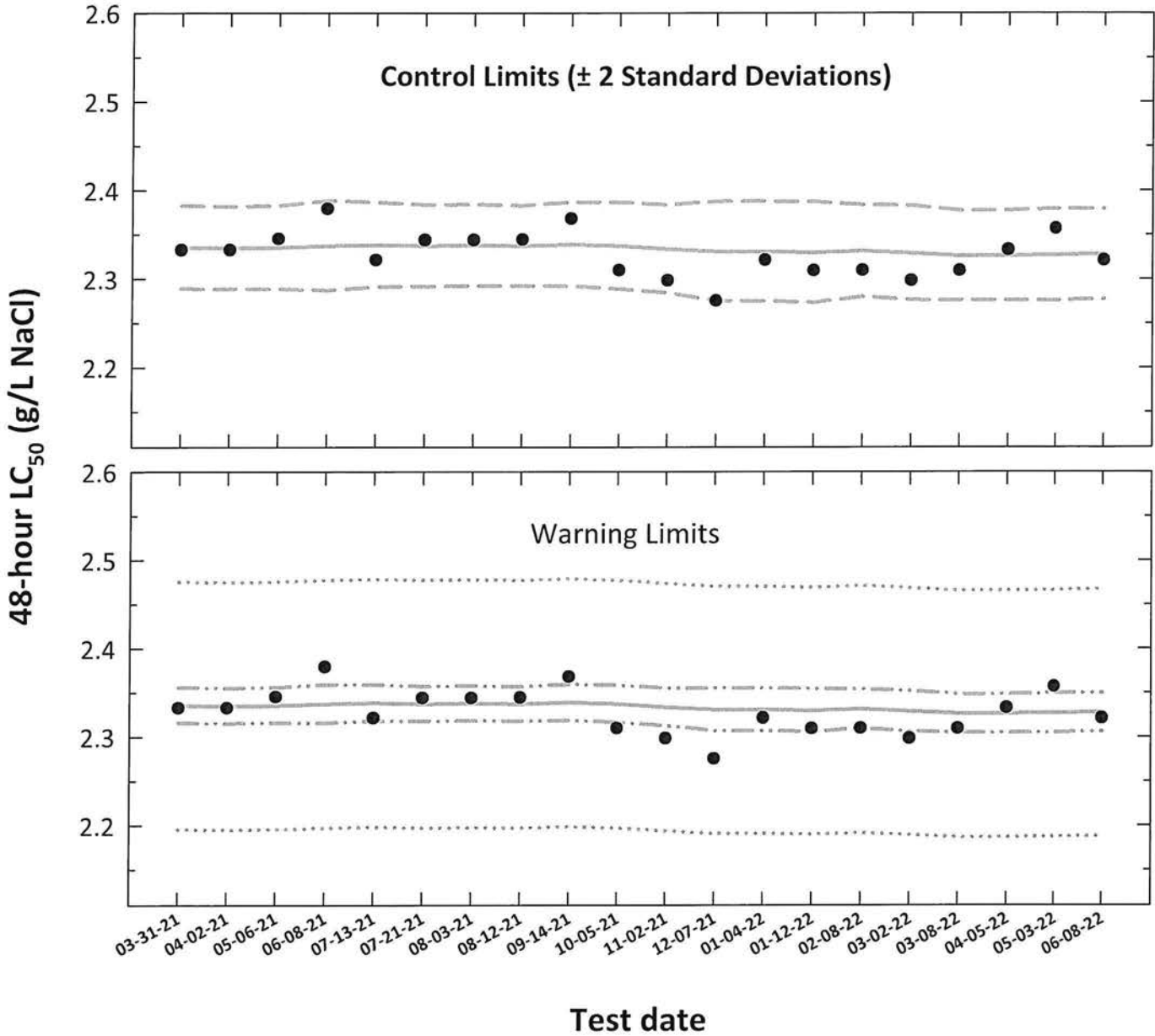
Dose-Response Plot



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		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	03-31-21	2.3330	0.3679	0.3684	2.3356	2.2893	2.3829	2.3158	2.3558	2.1955	2.4757
2	04-02-21	2.3330	0.3679	0.3683	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
3	05-06-21	2.3455	0.3702	0.3684	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
4	06-08-21	2.3795	0.3765	0.3687	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
5	07-13-21	2.3217	0.3658	0.3689	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
6	07-21-21	2.3442	0.3700	0.3687	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
7	08-03-21	2.3442	0.3700	0.3688	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
8	08-12-21	2.3449	0.3701	0.3687	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
9	09-14-21	2.3682	0.3744	0.3690	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
10	10-05-21	2.3099	0.3636	0.3687	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
11	11-02-21	2.2985	0.3614	0.3681	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
12	12-07-21	2.2755	0.3571	0.3675	2.3309	2.2754	2.3877	2.3071	2.3553	2.1910	2.4707
13	01-04-22	2.3217	0.3658	0.3675	2.3309	2.2754	2.3877	2.3071	2.3553	2.1911	2.4708
14	01-12-22	2.3096	0.3635	0.3673	2.3297	2.2735	2.3873	2.3056	2.3544	2.1899	2.4695
15	02-08-22	2.3099	0.3636	0.3676	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
16	03-02-22	2.2985	0.3614	0.3672	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
17	03-08-22	2.3096	0.3635	0.3667	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
18	04-05-22	2.3330	0.3679	0.3667	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
19	05-03-22	2.3569	0.3723	0.3668	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
20	06-08-22	2.3212	0.3657	0.3669	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675

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 # 381

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 #: 2098

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JW	N	JW
Control, MHSW	pH (S.U.)	7.59	7.71	7.75
	Dissolved oxygen (mg/L)	7.7	7.8	7.9
	Conductivity (µmhos/cm)	296		
	Alkalinity (mg/L CaCO ₃)	64		
	Hardness (mg/L CaCO ₃)	87		
	Temperature (°C)	24.9	25.2	25.2
1750 mg/L	pH (S.U.)	7.82	7.71	7.87
	Dissolved oxygen (mg/L)	7.9	7.8	7.9
	Conductivity (µmhos/cm)	3510		
	Temperature (°C)	25.0	25.0	25.2
2000 mg/L	pH (S.U.)	7.85	7.79	7.87
	Dissolved oxygen (mg/L)	7.9	7.8	8.0
	Conductivity (µmhos/cm)	4040		
	Temperature (°C)	25.0	25.0	25.0
2250 mg/L	pH (S.U.)	7.85	7.83	7.87
	Dissolved oxygen (mg/L)	7.8	7.9	8.0
	Conductivity (µmhos/cm)	4480		
	Temperature (°C)	24.9	25.0	25.0
2500 mg/L	pH (S.U.)	7.85	7.84	7.87
	Dissolved oxygen (mg/L)	7.8	7.9	8.0
	Conductivity (µmhos/cm)	4980		
	Temperature (°C)	24.9	25.0	25.1
2750 mg/L	pH (S.U.)	7.85	7.85	7.89
	Dissolved oxygen (mg/L)	7.7	7.9	8.1
	Conductivity (µmhos/cm)	5400		
	Temperature (°C)	24.9	25.2	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 # 381

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	06-08-22	0405	JK	0605	JK	2D3	RED	06-01-22
24	06-09-22			0603	JK			
48 Termination	06-10-22			0600	JK			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	05-31-22 D
Age:	< 24-hours old
Date and time organisms were born between:	06-07-22 1616 to 06-08-22 0405
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.90
	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	4 ^{1d}	4 ^{1d}	4 ^{1d}	3 ^{2d}
48 Termination	3 ^{2d}	3 ^{2d}	3 ^{2d}	2 ^{3d}	1 ^{4d}	2 ^{3d}	1 ^{4d}	1 ^{4d}	0 ^{4d}	0 ^{4d}	0 ^{4d}	0 ^{3d}
Mean Survival	55.7%				25.7%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2246.1
Upper 95% confidence limit (mg NaCl/L)	2344.5
48-hour LC ₅₀ (mg NaCl/L)	2321.2

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 6/8/2022	Test ID: CdNaCIAC	Sample ID: REF-Ref Toxicant
End Date: 6/10/2022	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

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	0.4000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

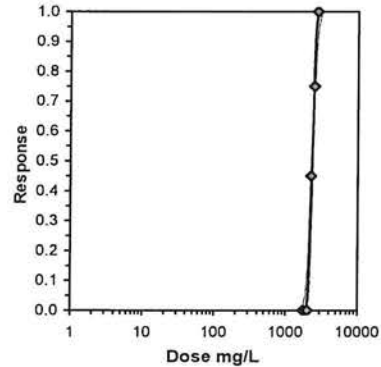
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8357	0.6847	0.8861	12.047	4	10.00	10.00	9	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.80953	0.868	0.27612	4.13297

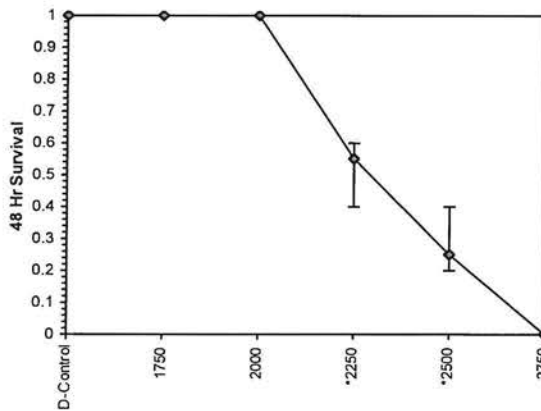
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	29.6833	5.36193	19.1739 40.1927	0	2.77937	7.81472	0.42691	3.36571	0.03369	6
Intercept	-94.906	18.0622	-130.31 -59.504							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1937.95	1738.22 2048.06
EC05	3.355	2043.15	1882.24 2134.37
EC10	3.718	2101.55	1962.46 2183.38
EC15	3.964	2141.89	2017.55 2218.14
EC20	4.158	2174.51	2061.62 2247.05
EC25	4.326	2202.88	2099.4 2273.01
EC40	4.747	2276.03	2192.77 2345.02
EC50	5.000	2321.21	2246.13 2394.53
EC60	5.253	2367.28	2296.33 2449.83
EC75	5.674	2445.89	2372.79 2554.79
EC80	5.842	2477.81	2401.22 2600.5
EC85	6.036	2515.53	2433.45 2656.27
EC90	6.282	2563.82	2473.06 2729.88
EC95	6.645	2637.1	2530.71 2845.27
EC99	7.326	2780.26	2638.23 3080



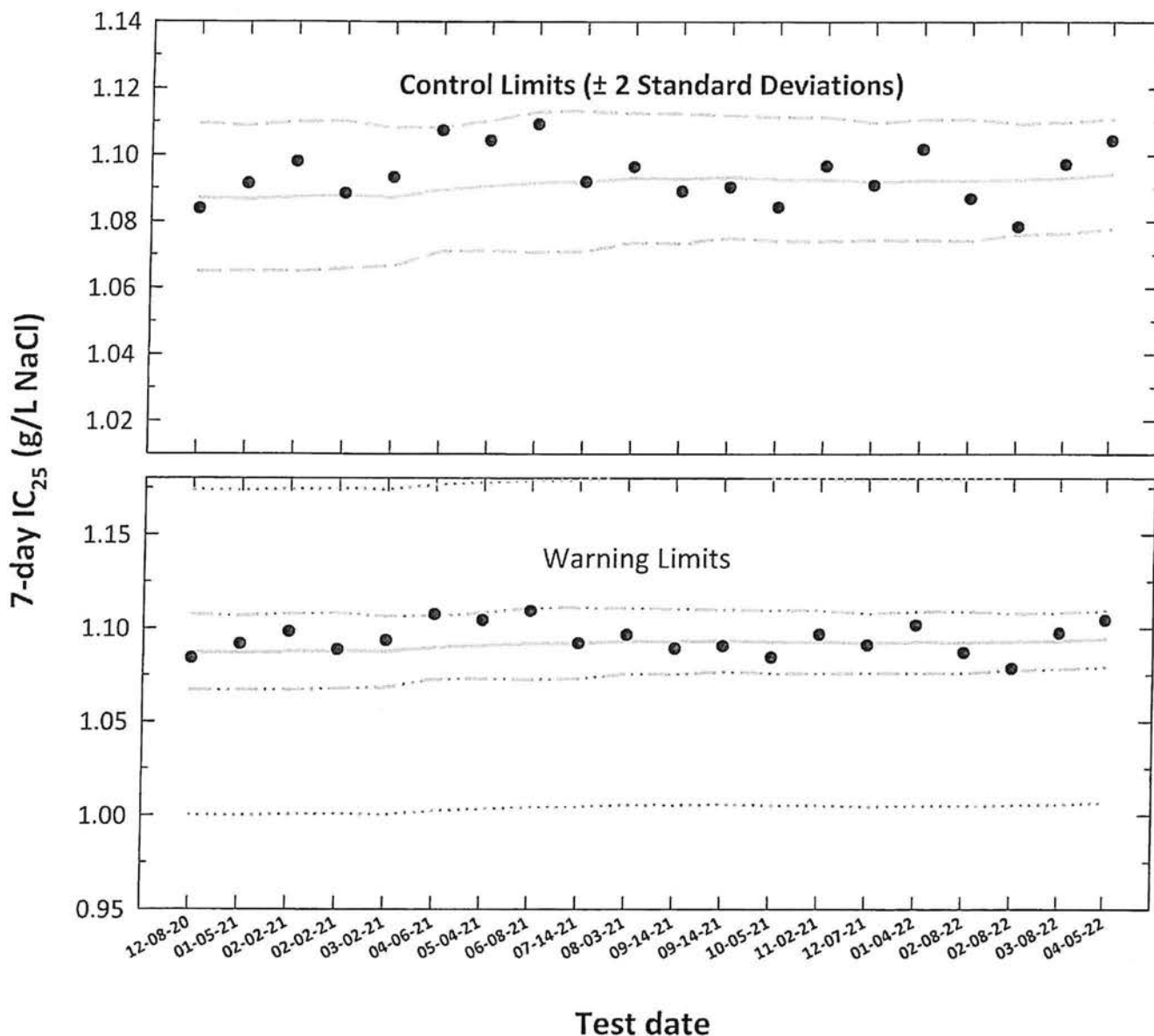
Dose-Response Plot



Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



Independent Review by
Kelley E. Keenan

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	Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	02-08-22	1.0784	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
19	03-08-22	1.0972	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
20	04-05-22	1.1043	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817

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.

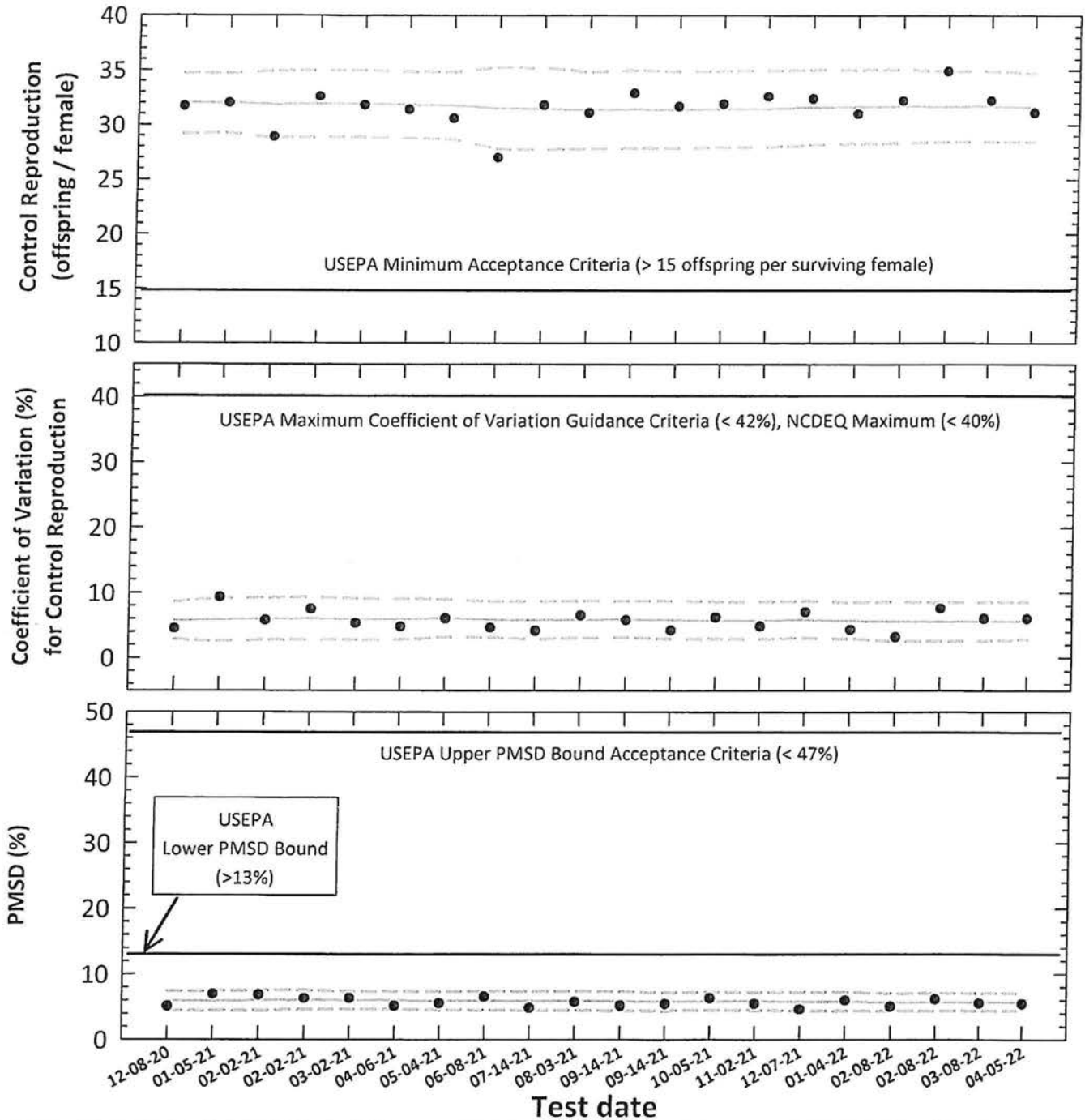
S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).
 CV = Coefficient of variation.



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)**

Independent Review by
Kelley E. Keenan:

Entered and Reviewed by
Jim Sumner



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

Ceriodaphnia dubia

Test number	Test date	ToxCal Determination				Control Reproduction				Test				Control Reproduction (offspring/female)				Control Reproduction CV (%)				Test PMSD (%)				
		Control Survival (%)		Control Reproduction (offspring/female)		CV (%)		Mean (offspring/female)		MSD		PMSD (%)		CT		95% Confidence Interval CT - 2S CT + 2S		CT		95% Confidence Interval CT - 2S CT + 2S		CT		95% Confidence Interval CT - 2S CT + 2S		
1	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	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
2	01-05-21	100	32.0	9.3	2.224	7.0	32.0	29.3	34.7	5.9	2.6	8.6	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
3	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	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
4	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	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
5	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	9.1	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
6	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
7	05-04-21	100	30.6	6.0	1.690	5.5	31.8	28.7	34.9	6.1	3.2	8.9	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
8	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	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
9	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
10	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	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
11	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	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
12	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	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
13	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	2.9	8.6	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
14	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	8.6	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
15	12-07-21	100	32.4	7.0	1.499	4.6	31.5	27.9	35.0	5.7	2.9	8.6	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
16	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.2	35.0	5.9	3.0	8.7	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
17	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
18	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
19	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
20	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7

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 #: 270

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS 2071					
Stock preparation:	100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.					
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	04-05-22 0530 TO 0820									
Culture board:	03-29-22 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	10	12	17	19	22	24	25	29
Transfer vessel information:	pH (S.U.): 7.71 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

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	04-05-22	0832	03-31-22	03-31-22	03-30-22A	JL
1	04-06-22	0712	↓	↓	↓	JL
2	04-07-22	0812			03-30-22C	JL
3	04-08-22	0743			↓	JL
4	04-09-22	0831			04-06-22A	JL
5	04-10-22	0830			↓	JL
6	04-11-22	0732			↓	JL
7	04-12-22	0753			↓	JL

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

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	07.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	>1400
% Adults having 3 rd Broods:	1007.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	07.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	31.1	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.5
% CV:	6.07.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1104.3

Species: *Ceriodaphnia dubia*

CdNaClCR #: 270

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	4	5	4	4	4	6	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	10	10	12	10	12	11	13	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	14	17	13	16	16	15	16	14	15
Total young produced		33	30	32	27	33	36	31	31	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:	31.1

600 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	31.5
% Reduction from Control:	-1,32.



Species: *Ceriodaphnia dubia*

CdNaClCR #: 270

800 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	5	6	5	4	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	12	11	11	10	13	10	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	18	16	14	14	18	15	16	15	13
Total young produced		29	34	32	30	31	33	32	30	29	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:	0%
Mean Offspring/Female:	30.9
% Reduction from Control:	0.6%

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	3	5	5	4	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	11	12	11	13	13	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	16	15	13	15	16	12	14	14	14
Total young produced		31	30	28	29	32	31	29	32	29	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.1
% Reduction from Control:	3.2%

Independent Review by Kelley E. Keenan:

Species: Ceriodaphnia dubia

CdNaClCR #: 270

1200 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	5	3	3	4	4	3	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	10	7	11	8	7	11	9	7	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	3	4	9	6	4	8	3	5	4	7
Total young produced		14	19	19	20	16	19	17	18	15	17
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	17.4
% Reduction from Control:	44.17.

1400 mg NaCl/L

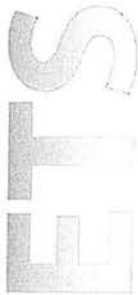
Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	2.3
% Reduction from Control:	92.67.





Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	4	5	4	4	4	6	5	45
5	11	13	10	10	12	10	12	11	13	11	113
6	0	0	0	0	0	0	0	0	0	0	0
7	17	14	17	13	16	16	15	16	14	15	153
Total	33	30	32	27	33	30	31	31	33	31	311

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	5	3	6	4	4	5	4	45
5	12	13	10	11	10	12	12	11	10	11	112
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	16	15	17	14	16	16	16	15	158
Total	35	33	30	31	30	32	32	31	31	30	315

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	5	6	5	4	4	4	5	46
5	10	12	12	11	11	10	13	10	10	11	110
6	0	0	0	0	0	0	0	0	0	0	0
7	14	18	16	14	14	18	15	16	15	13	153
Total	29	34	32	30	31	33	32	30	29	29	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	3	5	5	4	4	5	5	5	45
5	12	10	10	11	12	11	13	13	10	11	113
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	15	13	15	16	12	14	14	14	143
Total	31	30	28	29	32	31	29	32	29	30	301

1200 mg NaCl/L

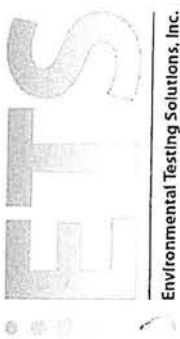
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	5	3	3	4	4	3	4	4	4	37
5	8	10	7	11	8	7	11	9	7	6	84
6	0	0	0	0	0	0	0	0	0	0	0
7	3	4	9	6	4	8	3	5	4	7	53
Total	14	19	19	20	16	19	17	18	15	17	174

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	3	1	2	2	1	1	1	3	17
5	0	0	0	0	2	2	0	2	0	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	1	3	1	4	4	1	3	1	3	23

Control and
Replicates by
Investigator





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

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

Test number: CdNaClCR #270
 Test dates: April 05-12, 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	30	32	27	33	30	31	31	33	31	100	31.1	6.0	Not applicable
600	35	33	30	31	30	32	32	31	31	30	100	31.5	5.0	-1.3
800	29	34	32	30	31	33	32	30	29	29	100	30.9	5.8	0.6
1000	31	30	28	29	32	31	29	32	29	30	100	30.1	4.6	3.2
1200	14	19	19	20	16	19	17	18	15	17	100	17.4	11.2	44.1
1400	2	1	3	1	4	4	1	3	1	3	100	2.3	54.4	92.6

Dunnett's MSD value: $\frac{1.691}{5.4}$ MSD = Minimum Significant Difference
 PMSD: $\frac{5.4}{5.4}$ 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

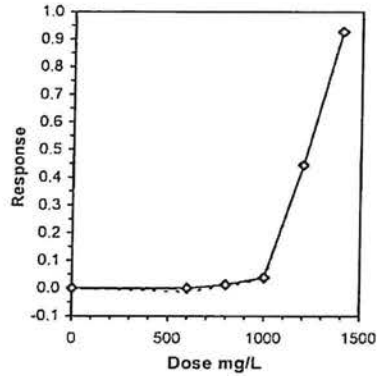
Start Date: 4/5/2022 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
 End Date: 4/12/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	30.000	32.000	27.000	33.000	30.000	31.000	31.000	33.000	31.000
600	35.000	33.000	30.000	31.000	30.000	32.000	32.000	31.000	31.000	30.000
800	29.000	34.000	32.000	30.000	31.000	33.000	32.000	30.000	29.000	29.000
1000	31.000	30.000	28.000	29.000	32.000	31.000	29.000	32.000	29.000	30.000
1200	14.000	19.000	19.000	20.000	16.000	19.000	17.000	18.000	15.000	17.000
1400	2.000	1.000	3.000	1.000	4.000	4.000	1.000	3.000	1.000	3.000

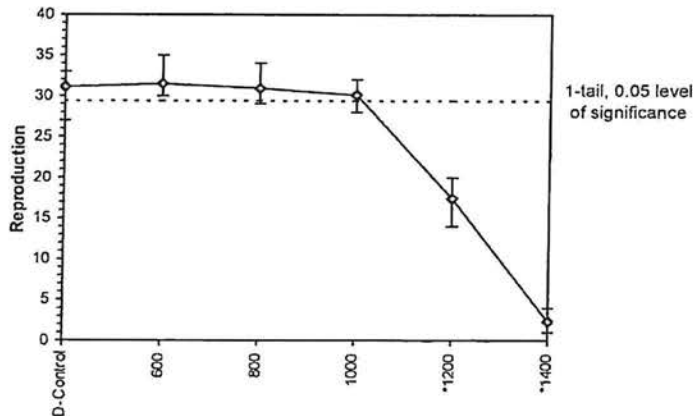
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	31.100	1.0000	31.100	27.000	33.000	5.958	10				31.300	1.0000
600	31.500	1.0129	31.500	30.000	35.000	5.019	10	-0.541	2.287	1.691	31.300	1.0000
800	30.900	0.9936	30.900	29.000	34.000	5.799	10	0.270	2.287	1.691	30.900	0.9872
1000	30.100	0.9678	30.100	28.000	32.000	4.553	10	1.352	2.287	1.691	30.100	0.9617
*1200	17.400	0.5595	17.400	14.000	20.000	11.236	10	18.523	2.287	1.691	17.400	0.5559
*1400	2.300	0.0740	2.300	1.000	4.000	54.420	10	38.939	2.287	1.691	2.300	0.0735

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ($p > 0.01$)					0.70515	1.035	-0.0733	-0.2626
Bartlett's Test indicates equal variances ($p = 0.76$)					2.59579	15.0863		
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Dunnett's Test					1000	1200	1095.45	
Treatments vs D-Control					1.69126	0.05438	1411.7	2.73519
					2.8E-44	5.54		

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	1005.75	65.8103	785.446	1020.49	-2.5399
IC10	1030.39	8.2892	1010.31	1043.38	-0.3248
IC15	1055.04	7.83	1036.8	1067.28	-0.3096
IC20	1079.69	7.62159	1062.59	1091.14	-0.2781
IC25	1104.33	7.68439	1087.21	1115.71	-0.2239
IC40	1178.27	9.30095	1159.7	1194.07	-0.0138
IC50	1223.18	7.76269	1209.21	1235.74	-0.4638



Dose-Response Plot



Checked and Reviewed by
Jim Sumner
JK

JK

Environmental Testing Solutions, Inc.

Species: Ceriodaphnia dubia

CdNaClCR #: 270

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	JW	JW	JW	K	K	K
Concentration	Parameter							
CONTROL, MHSW	pH (S.U.)	7.75	7.44	7.76	7.68	7.61	7.47	7.66
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.7	7.8	7.9	7.7
	Conductivity (µmhos/cm)	324		316			369	
	Alkalinity (mg CaCO ₃ /L)	63					63	
	Hardness (mg CaCO ₃ /L)	90					86	
	Temperature (°C)	24.8	25.2	24.8	25.0	24.9	25.2	
	600 mg NaCl/L	pH (S.U.)	7.74	7.78	7.84	7.74	7.74	7.70
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.8	7.9	7.6	
	Conductivity (µmhos/cm)	1470		1470		1430		
	Temperature (°C)	24.9	25.0	24.9	25.2	25.0	25.0	
800 mg NaCl/L	pH (S.U.)	7.77	7.79	7.85	7.74	7.76	7.77	
	Dissolved oxygen (mg/L)	7.7	7.7	7.6	7.8	8.0	7.7	
	Conductivity (µmhos/cm)	1840		1860		1810		
	Temperature (°C)	24.9	25.3	24.9	24.9	24.9	25.1	
1000 mg NaCl/L	pH (S.U.)	7.80	7.80	7.87	7.74	7.81	7.70	
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.8	8.0	7.7	
	Conductivity (µmhos/cm)	2240		2260		2210		
	Temperature (°C)	25.0	25.2	24.9	24.9	24.9	25.1	
1200 mg NaCl/L	pH (S.U.)	7.80	7.81	7.87	7.75	7.84	7.80	
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.8	8.0	7.8	
	Conductivity (µmhos/cm)	2610		2640		2580		
	Temperature (°C)	24.9	25.2	25.0	25.1	24.9	25.0	
1400 mg NaCl/L	pH (S.U.)	7.82	7.81	7.87	7.75	7.85	7.82	
	Dissolved oxygen (mg/L)	7.9	8.0	7.8	7.9	8.0	7.9	
	Conductivity (µmhos/cm)	2990		3050		2930		
	Temperature (°C)	24.9	25.1	25.0	24.8	25.0	25.0	
		Initial	Final	Initial	Final	Initial	Final	



Species: Ceriodaphnia dubia

CdNaClCR #: 270

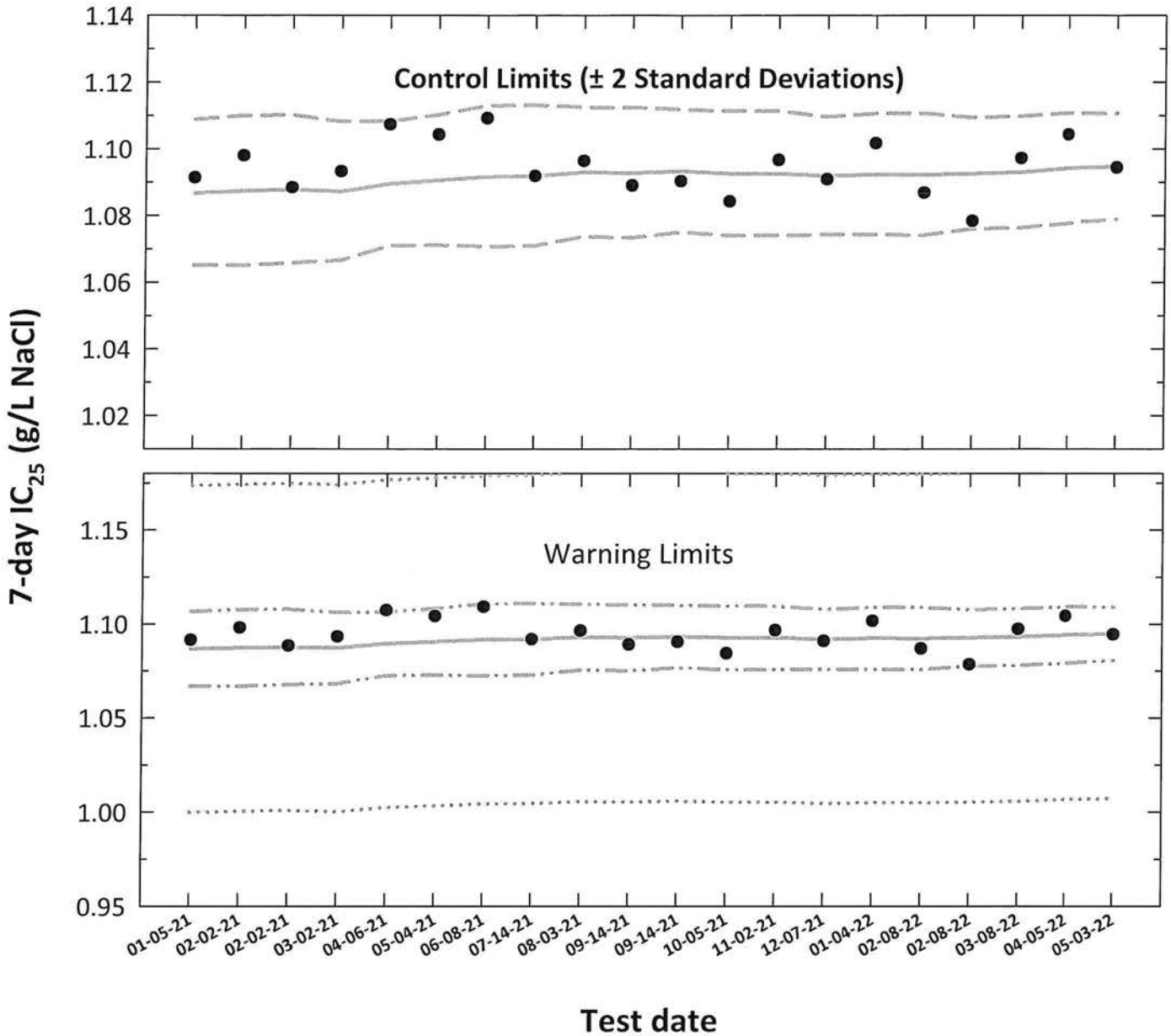
Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
Analyst		N	BSL	BSL	N	N	N	N	N	
CONTROL, MHSW	pH (S.U.)	7.67	7.93	7.53	7.04	7.66	7.65	7.67	7.95	
	Dissolved oxygen (mg/L)	7.0	7.7	7.7	7.7	7.9	7.7	7.0	7.0	
	Conductivity (µmhos/cm)	305		295		314		303		
	Alkalinity (mg CaCO ₃ /L)			58						
	Hardness (mg CaCO ₃ /L)			86						
	Temperature (°C)	24.8	25.2	24.9	25.0	24.8	25.0	24.9	25.2	
			K ⁰⁴⁻¹⁰⁻¹¹ N						K ⁰⁴⁻¹⁰⁻¹² K	
600 mg NaCl/L	pH (S.U.)	7.00	7.93	7.94	7.03	7.09	7.70	7.05	7.97	
	Dissolved oxygen (mg/L)	7.0	7.6	7.7	7.7	7.7	7.7	7.0	7.0	
	Conductivity (µmhos/cm)	1410		1440		1440		1410		
	Temperature (°C)	24.9	25.0	25.0	24.8	24.8	24.7	24.8	25.4	
800 mg NaCl/L	pH (S.U.)	7.00	7.93	7.96	7.00	7.90	7.00	7.00	7.90	
	Dissolved oxygen (mg/L)	7.0	7.6	7.7	7.0	7.0	7.6	7.0	7.0	
	Conductivity (µmhos/cm)	1700		1760		1800		1830		
	Temperature (°C)	24.9	25.0	24.8	24.8	24.8	24.9	24.9	25.2	
1000 mg NaCl/L	pH (S.U.)	7.91	7.93	7.97	7.05	7.93	7.01	7.90	7.90	
	Dissolved oxygen (mg/L)	7.0	7.6	7.7	7.9	7.9	7.7	7.0	7.0	
	Conductivity (µmhos/cm)	2170		2160		2190		2210		
	Temperature (°C)	24.9	25.1	24.8	24.9	24.8	24.9	24.9	25.3	
1200 mg NaCl/L	pH (S.U.)	7.92	7.92	7.97	7.04	7.93	7.03	7.91	7.97	
	Dissolved oxygen (mg/L)	7.9	7.5	7.7	7.0	0.0	7.0	7.0	7.0	
	Conductivity (µmhos/cm)	2500		2560		2620		2640		
	Temperature (°C)	25.0	25.1	25.0	25.1	24.9	24.8	24.9	25.3	
1400 mg NaCl/L	pH (S.U.)	7.93	7.93	7.96	7.05	7.93	7.04	7.92	7.97	
	Dissolved oxygen (mg/L)	7.9	7.4	7.7	7.0	0.0	7.0	7.0	7.0	
	Conductivity (µmhos/cm)	2910		2870		2980		3000		
	Temperature (°C)	24.9	25.1	25.0	24.8	24.9	25.0	24.8	25.1	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	



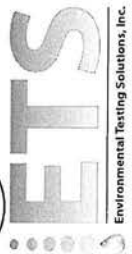
Ceriodaphnia dubia

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	10th Percentile CV	Warning Limits
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	02-08-22	1.0784	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
18	03-08-22	1.0972	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
19	04-05-22	1.1043	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
20	05-03-22	1.0944	0.0392	0.0393	0.0031	1.0947	1.0790	1.1106	1.0804	1.1090	1.0071	1.1823

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

S = Standard deviation of the IC₂₅ values.

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

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

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

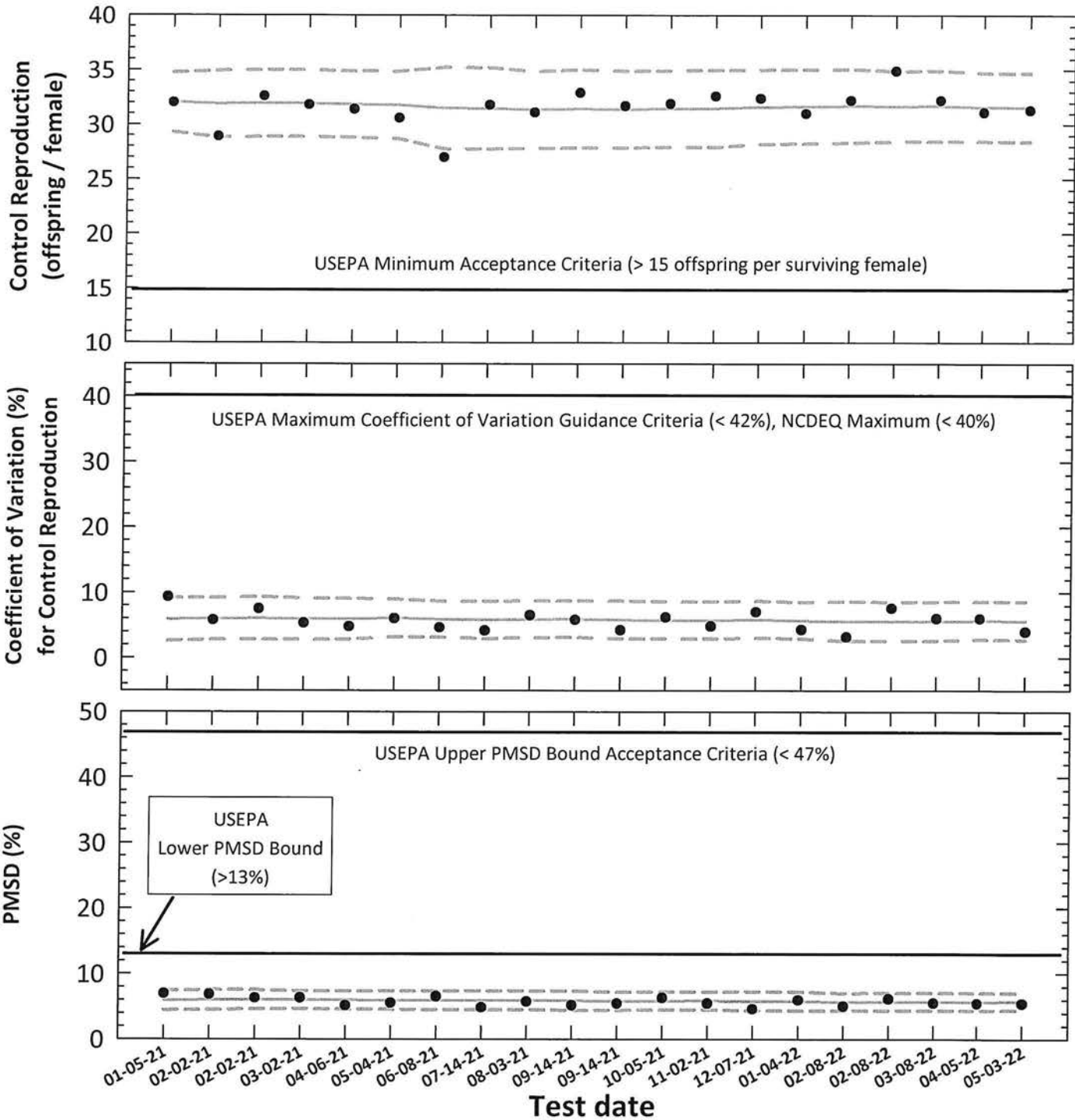
CV = Coefficient of variation.



Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

Independent Review by
Kalley E. Keenan:

Entered and Reviewed by
Jim Sumner

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Source: In-house Culture

Test number	Test date	ToxCal Determination				Control Reproduction		Test		Control Reproduction (offspring/female)			Control Reproduction CV (%)		Test PMSD (%)	
		Control Survival (%)	Control Reproduction (offspring/female)		CV (%)	MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean	CV												
1	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	
2	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	
3	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	
4	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	
5	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	
6	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	
7	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	
8	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	
9	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	
10	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	
11	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	
12	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	
13	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	
14	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	
15	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	
16	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	
17	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	
18	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	
19	04-05-22	100	31.1	6.0	1,691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0	
20	05-03-22	100	31.3	4.0	1,707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0	

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 #: 271

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2098</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>ORANGE</u>
Date and times organisms were born between:	<u>05-03-22 0520 TO 0900</u>										Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>04-26-22 A</u>											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>14</u>	<u>15</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>25</u>		
Transfer vessel information:	pH (S.U.): <u>7.98</u> Temperature (°C): <u>25.0</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	05-03-22	<u>0911</u>	<u>04-29-22</u>	<u>04-28-22</u>	<u>04-27-22B</u>	<u>H</u>
1	05-04-22	<u>0812</u>			↓	<u>H</u>
2	05-05-22	<u>0814</u>			<u>04-27-22D</u>	<u>H</u>
3	05-06-22	<u>0811</u>			↓	<u>H</u>
4	05-07-22	<u>0900</u>			<u>05-04-22A</u>	<u>H</u>
5	05-08-22	<u>0800</u>			↓	<u>H</u>
6	05-09-22	<u>0800</u>	↓	↓	<u>05-04-22B</u>	<u>H</u>
7	05-10-22	<u>0811</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>>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>31.3</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>4.07.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1094.4</u>



Species: *Ceriodaphnia dubia*

CdNaClCR #: 271

CONTROL

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	31.3

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	5	5	5	5	4	4	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	12	13	11	10	12	13	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	14	16	15	19	15	16	14	17	13	17
Total young produced		33	32	33	35	30	33	31	33	30	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:	-35.7.





Species: Ceriodaphnia dubia
800 mg NaCl/L

CdNaClCR #: 271

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	4	5	3	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	13	11	13	10	10	13	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	19	16	14	14	17	15	13	16	17
Total young produced		30	33	33	29	32	30	36	30	30	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	31.1
% Reduction from Control:	0.67.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	4	5	5	4	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	10	11	10	12	12	11	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	13	16	16	16	14	12	15	14	14
Total young produced		32	26	30	31	31	31	28	30	28	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	29.6
% Reduction from Control:	5.47.



Species: Ceriodaphnia dubia
1200 mg NaCl/L

CdNaClCR #: 271

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	6	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	4	2	4	3	3	5	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	6	9	5	10	7	10	8	7	9	8
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	6	5	11	9	4	4	3	7	6	6
Total young produced		15	18	20	21	15	17	14	19	18	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:	17.5
% Reduction from Control:	44.17.

1400 mg NaCl/L

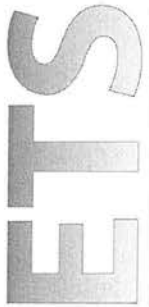
Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	1.7
% Reduction from Control:	94.67.





Verification of *Ceriodaphnia* Reproduction Totals

Environmental Testing Solutions, Inc.

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	3	5	4	5	5	4	5	4	45
5	10	11	13	10	10	13	10	12	12	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	15	18	15	14	17	15	14	15	155
Total	30	32	31	33	29	32	32	32	30	32	313

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	5	5	4	4	4	5	4	46
5	14	12	13	11	10	12	13	12	12	13	122
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	15	19	15	16	14	17	13	17	156
Total	33	32	33	35	30	33	31	33	30	34	324

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	3	4	4	5	3	5	4	4	4	40
5	10	13	13	11	13	10	10	13	10	11	114
6	0	0	0	0	0	0	0	0	0	0	0
7	16	19	16	14	14	17	15	13	16	17	157
Total	30	35	33	29	32	30	30	30	30	32	311

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	4	4	5	5	4	4	4	5	42
5	13	10	10	11	10	12	12	11	10	10	109
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	16	16	16	14	12	15	14	14	145
Total	32	26	30	31	31	31	28	30	28	29	296

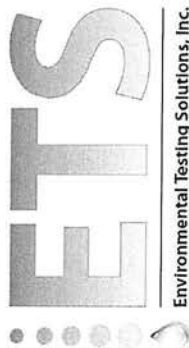
1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	4	2	4	3	3	5	3	4	35
5	6	9	5	10	7	10	8	7	9	8	79
6	0	0	0	0	0	0	0	0	0	0	0
7	6	5	11	9	4	4	3	7	6	6	61
Total	15	18	20	21	15	17	14	19	18	18	175

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	3	2	1	1	1	2	2	2	17
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	2	1	3	2	1	1	1	2	2	2	17

Accepted and Reviewed by Jim Sumner



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

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

Test number: CdNaClCR #271
 Test dates: May 03-10, 2022

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	30	32	31	33	29	32	32	32	30	32	100	31.3	4.0	Not applicable
600	33	32	33	35	30	33	31	33	30	34	100	32.4	5.1	-3.5
800	30	35	33	29	32	30	30	30	30	32	100	31.1	6.0	0.6
1000	32	26	30	31	31	31	28	30	28	29	100	29.6	6.2	5.4
1200	15	18	20	21	15	17	14	19	18	18	100	17.5	13.0	44.1
1400	2	1	3	2	1	1	1	2	2	2	100	1.7	39.7	94.6

Dunnett's MSD value: 1.707 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

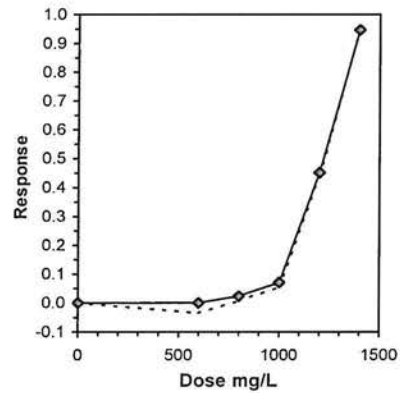
Start Date: 5/3/2022	Test ID: CdNaCICR	Sample ID: REF-Ref Toxicant
End Date: 5/10/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: NaCl-Sodium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: CD-Ceriodaphnia dubia
Comments:		

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	32.000	31.000	33.000	29.000	32.000	32.000	32.000	30.000	32.000
600	33.000	32.000	33.000	35.000	30.000	33.000	31.000	33.000	30.000	34.000
800	30.000	35.000	33.000	29.000	32.000	30.000	30.000	30.000	30.000	32.000
1000	32.000	26.000	30.000	31.000	31.000	31.000	28.000	30.000	28.000	29.000
1200	15.000	18.000	20.000	21.000	15.000	17.000	14.000	19.000	18.000	18.000
1400	2.000	1.000	3.000	2.000	1.000	1.000	1.000	2.000	2.000	2.000

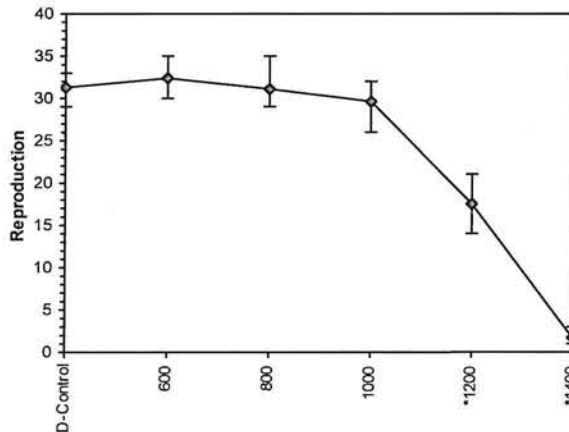
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
D-Control	31.300	1.0000	31.300	29.000	33.000	3.999	10			31.850	1.0000
600	32.400	1.0351	32.400	30.000	35.000	5.082	10	127.00	75.00	31.850	1.0000
800	31.100	0.9936	31.100	29.000	35.000	5.958	10	98.00	75.00	31.100	0.9765
1000	29.600	0.9457	29.600	26.000	32.000	6.209	10	76.50	75.00	29.600	0.9294
*1200	17.500	0.5591	17.500	14.000	21.000	12.989	10	55.00	75.00	17.500	0.5495
*1400	1.700	0.0543	1.700	1.000	3.000	39.703	10	55.00	75.00	1.700	0.0534

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.10772	1.035	-0.0434	-0.008
Bartlett's Test indicates equal variances (p = 0.03)	12.0163	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	912.333	71.3963	763.5	1006.52	-0.1553
IC10	1015.45	13.7684	972.528	1030.3	-1.7963
IC15	1041.78	8.75873	1023.46	1055.18	-0.2587
IC20	1068.1	8.03862	1052.76	1081.36	0.0047
IC25	1094.42	7.83682	1080.58	1110.35	0.2519
IC40	1173.39	10.2058	1154.96	1193.19	0.2866
IC50	1219.94	8.09688	1203.06	1233.35	-0.3100



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS



Statistical Analyses

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

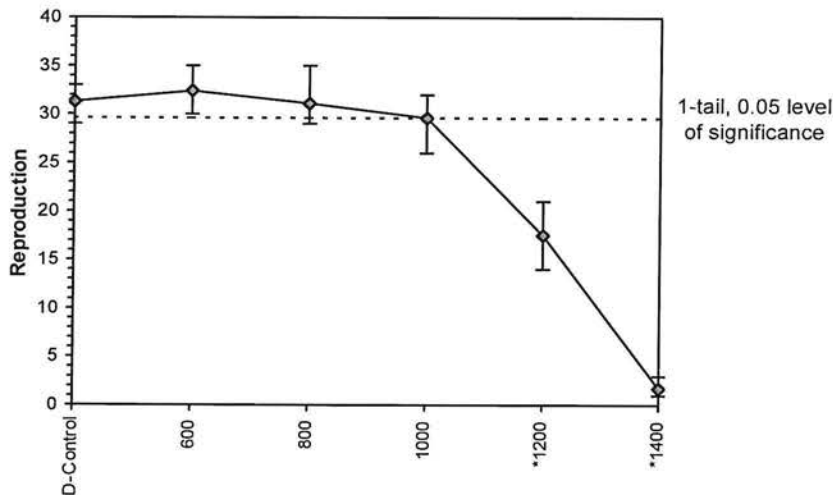
Start Date: 5/3/2022 Test ID: CdNaCICR Sample ID: REF-Ref Toxicant
 End Date: 5/10/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	32.000	31.000	33.000	29.000	32.000	32.000	32.000	30.000	32.000
600	33.000	32.000	33.000	35.000	30.000	33.000	31.000	33.000	30.000	34.000
800	30.000	35.000	33.000	29.000	32.000	30.000	30.000	30.000	30.000	32.000
1000	32.000	26.000	30.000	31.000	31.000	31.000	28.000	30.000	28.000	29.000
1200	15.000	18.000	20.000	21.000	15.000	17.000	14.000	19.000	18.000	18.000
1400	2.000	1.000	3.000	2.000	1.000	1.000	1.000	2.000	2.000	2.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%				
D-Control	31.300	1.0000	31.300	29.000	33.000	3.999	10			
600	32.400	1.0351	32.400	30.000	35.000	5.082	10	-1.474	2.287	1.707
800	31.100	0.9936	31.100	29.000	35.000	5.958	10	0.268	2.287	1.707
1000	29.600	0.9457	29.600	26.000	32.000	6.209	10	2.278	2.287	1.707
*1200	17.500	0.5591	17.500	14.000	21.000	12.989	10	18.490	2.287	1.707
*1400	1.700	0.0543	1.700	1.000	3.000	39.703	10	39.660	2.287	1.707

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.10772	1.035	-0.0434	-0.008						
Bartlett's Test indicates equal variances (p = 0.03)	12.0163	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	1000	1200	1095.45		1.70665	0.05453	1490.27	2.78519	1.1E-44	5, 54
Treatments vs D-Control										

Dose-Response Plot



Entered and Reviewed by
 Jim Sumner
JS



Species: Ceriodaphnia dubia

CdNaClCR #: 271

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		JW	JW	JW	N	N	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.80	7.79	7.73	7.81	7.77	7.91
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.8	7.7	7.7
	Conductivity (µmhos/cm)	310		308		188	
	Alkalinity (mg CaCO ₃ /L)	62				63	
	Hardness (mg CaCO ₃ /L)	88				88	
	Temperature (°C)	24.8	24.9	24.9	25.1	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.99	7.84	7.86	7.86	7.90	7.81
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.8	7.6	7.5
	Conductivity (µmhos/cm)	1510		1450		1370	
	Temperature (°C)	24.9	25.1	24.9	24.9	24.8	24.9
800 mg NaCl/L	pH (S.U.)	7.98	7.85	7.87	7.87	7.92	7.81
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.8	7.6	7.6
	Conductivity (µmhos/cm)	1870		1820		1730	
	Temperature (°C)	25.0	25.1	25.0	24.9	24.8	25.1
1000 mg NaCl/L	pH (S.U.)	7.99	7.85	7.87	7.86	7.91	7.81
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.8	7.7	7.8
	Conductivity (µmhos/cm)	2230		2220		2090	
	Temperature (°C)	25.0	25.0	25.0	25.2	24.9	24.8
1200 mg NaCl/L	pH (S.U.)	8.01	7.85	7.88	7.86	7.92	7.80
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.8	7.7	7.8
	Conductivity (µmhos/cm)	2640		2600		2470	
	Temperature (°C)	25.0	25.2	25.0	24.9	24.9	24.8
1400 mg NaCl/L	pH (S.U.)	8.01	7.85	7.88	7.88	7.92	7.80
	Dissolved oxygen (mg/L)	7.8	7.9	8.0	7.8	7.8	7.6
	Conductivity (µmhos/cm)	3030		2960		2770	
	Temperature (°C)	24.9	24.9	25.0	24.9	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final

Independent Review by Kelley E. Keenan:

Species: Ceriodaphnia dubia

CdNaClCR #: 271

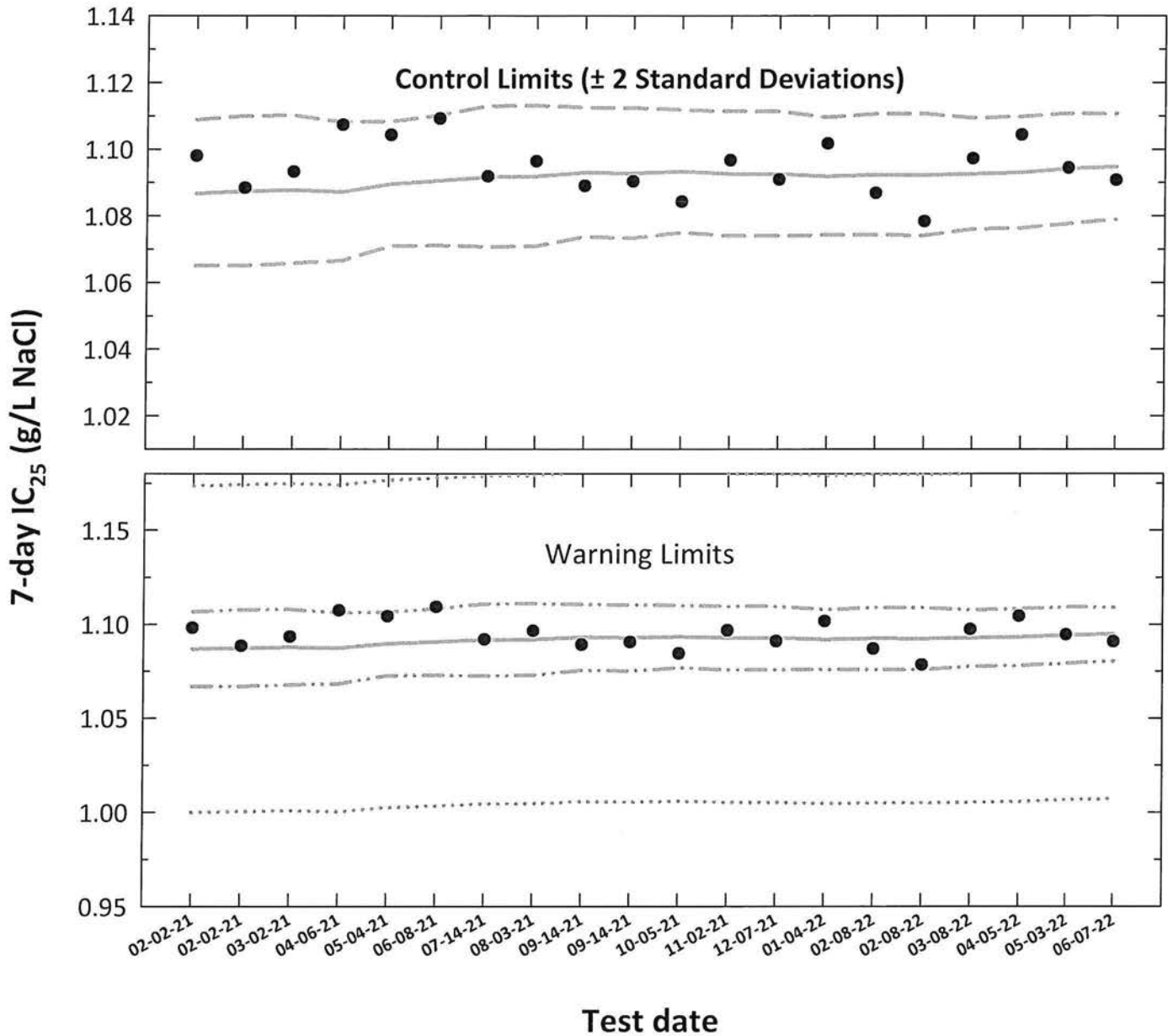
Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	N	BSL	BSL	BSL	BSL	N	N	N
CONTROL, MHSW	pH (S.U.)	7.78	7.82	7.86	7.83	7.71	7.81	7.69	7.92
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.8	7.7	7.7	7.8	7.7
	Conductivity (µmhos/cm)	295		297		300		302	
	Alkalinity (mg CaCO ₃ /L)			60				63	
	Hardness (mg CaCO ₃ /L)			86				86	
	Temperature (°C)	24.9	25.1	24.8	25.2	24.8	25.1	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.85	7.86	7.84	7.87	7.86	7.79	7.83	7.91
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.8	7.7	7.7	7.8	7.7
	Conductivity (µmhos/cm)	1300		1430		1460		1440	
	Temperature (°C)	24.8	24.8	24.9	25.2	24.8	24.9	24.8	25.2
800 mg NaCl/L	pH (S.U.)	7.85	7.86	7.88	7.87	7.90	7.79	7.84	7.91
	Dissolved oxygen (mg/L)	7.7	7.8	7.7	7.8	7.8	7.7	7.8	7.7
	Conductivity (µmhos/cm)	1810		1800		1870		1850	
	Temperature (°C)	24.9	24.8	24.9	25.0	24.9	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.83	7.85	7.89	7.86	7.91	7.79	7.86	7.92
	Dissolved oxygen (mg/L)	7.7	7.8	7.8	7.8	7.9	7.7	7.8	7.7
	Conductivity (µmhos/cm)	2120		2130		2190		2190	
	Temperature (°C)	24.9	25.2	25.0	25.2	24.8	25.2	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.87	7.87	7.88	7.86	7.91	7.80	7.86	7.93
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.8	7.9	7.7	7.9	7.8
	Conductivity (µmhos/cm)	2530		2510		2600		2590	
	Temperature (°C)	25.0	24.8	25.0	25.1	24.8	25.0	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.89	7.86	7.91	7.85	7.90	7.81	7.89	7.93
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.7	7.9	7.7	7.9	7.8
	Conductivity (µmhos/cm)	2800		2900		2970		2990	
	Temperature (°C)	24.8	24.9	25.0	25.0	24.8	24.9	25.0	25.1
		Initial	Final	Initial	Final	Initial	Final	Initial	Final



Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		CT	Anti-logarithmic Values (g/L NaCl)						
			7-day IC ₂₅	CT		S	Control Limits		Warning Limits			
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		
					CT	10th Percentile CV Warning Limits		CT - S _{A,10} CT + S _{A,10}				
1	02-02-21	1.0980	0.0380	0.0361	0.0044	1.0867	1.0651	1.1088	1.0668	1.1067	0.9998	1.1737
2	02-02-21	1.0884	0.0406	0.0364	0.0045	1.0873	1.0651	1.1100	1.0669	1.1077	1.0003	1.1743
3	03-02-21	1.0933	0.0368	0.0365	0.0044	1.0878	1.0659	1.1101	1.0677	1.1079	1.0008	1.1748
4	04-06-21	1.1074	0.0387	0.0363	0.0041	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
5	05-04-21	1.1043	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
6	06-08-21	1.1092	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
7	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
8	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
9	09-14-21	1.0890	0.0400	0.0386	0.0039	1.0930	1.0738	1.1126	1.0754	1.1106	1.0056	1.1804
10	09-14-21	1.0904	0.0370	0.0385	0.0039	1.0927	1.0734	1.1124	1.0751	1.1104	1.0053	1.1801
11	10-05-21	1.0843	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
12	11-02-21	1.0967	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
13	12-07-21	1.0909	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
14	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
15	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
16	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
17	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
18	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
19	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
20	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

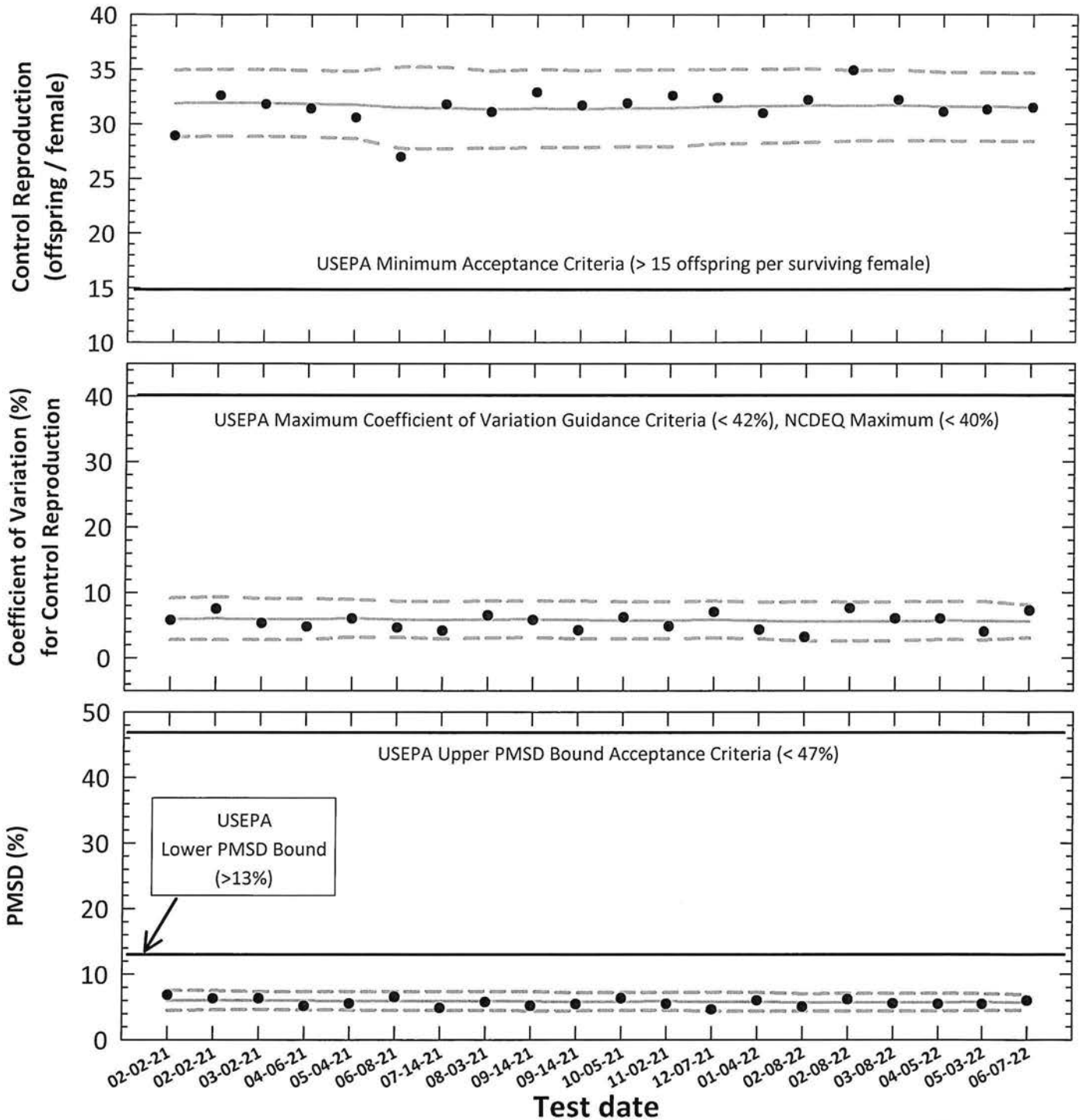
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Ceriodaphnia dubia

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



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

Entered and Reviewed by
 Jim Sumner

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Source: In-house Culture

Test number	Test date	ToxCal Determination				Control Reproduction		Test		Control Reproduction (offspring/female)			Control Reproduction CV			Test PMSD (%)		
		Control Survival (%)	Control Reproduction		CV (%)	MSD	PMSD (%)	CT	95% Confidence Interval	CT - 2S	CT + 2S	CT	95% Confidence Interval	CT - 2S	CT + 2S	CT	95% Confidence Interval	
			Mean (offspring/female)	CV (%)														CT
1	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	2.8	9.2	6.0	4.5	7.5
2	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	2.8	9.3	6.0	4.6	7.5
3	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	2.7	9.1	6.0	4.7	7.3
4	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	2.8	9.1	6.0	4.6	7.4
5	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	3.2	8.9	5.9	4.5	7.3
6	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	3.1	8.7	5.9	4.5	7.4
7	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	2.9	8.6	5.9	4.5	7.4
8	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	3.1	8.7	5.9	4.5	7.4
9	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	3.1	8.7	5.9	4.4	7.3
10	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	2.9	8.7	5.8	4.4	7.2
11	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	2.9	8.6	5.8	4.5	7.2
12	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	2.9	8.6	5.9	4.5	7.2
13	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	3.0	8.7	5.8	4.4	7.3
14	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	2.9	8.5	5.8	4.4	7.2
15	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	2.6	8.6	5.7	4.4	7.0
16	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	2.6	8.5	5.7	4.4	7.1
17	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	2.6	8.6	5.7	4.4	7.1
18	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	2.8	8.6	5.7	4.4	7.0
19	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	2.7	8.6	5.7	4.4	7.0
20	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	3.0	8.1	5.7	4.5	6.9

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 #: 272

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2088</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>06-07-22 0518 TO 0900</u>										Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>05-31-22 A</u>											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	<u>1</u>	<u>2</u>	<u>3</u>	<u>5</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>19</u>	<u>24</u>		
Transfer vessel information:	pH (S.U.): <u>7.90</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	06-07-22	<u>0909</u>	<u>06-07-22</u>	<u>06-07-22</u>	<u>06-01-22C</u>	<u>JL</u>
1	06-08-22	<u>06-08-22 0819</u>	↓	↓	↓	<u>JL</u>
2	06-09-22	<u>0818</u>	↓	↓	<u>06-06-22B</u>	<u>JL</u>
3	06-10-22	<u>0819</u>	↓	↓	↓	<u>JL</u>
4	06-11-22	<u>0835</u>	↓	↓	<u>06-06-22C</u>	<u>JL</u>
5	06-12-22	<u>0819</u>	↓	↓	↓	<u>JL</u>
6	06-13-22	<u>0816</u>	↓	↓	<u>06-09-22B</u>	<u>JL</u>
7	06-14-22	<u>0812</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>13064685</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>31.5</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>7.27.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1090.8</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 272

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	5	3	4	3	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	9	12	10	10	10	12	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	17	15	16	15	15	17	15	18	19	18
Total young produced		33	29	33	28	29	30	32	34	34	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	07
Mean Offspring/Female:	31.5

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	4	6	4	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	10	10	11	10	12	10	11	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	15	19	15	19	17	18	18	19	17
Total young produced		35	31	34	29	36	31	35	33	35	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07
Mean Offspring/Female:	33.1
% Reduction from Control:	-5.17

Species: *Ceriodaphnia dubia*
800 mg NaCl/L

CdNaClCR #: 272

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	3	5	4	4	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	10	10	13	11	12	12	13	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	19	18	18	15	18	16	15	18	17
Total young produced		34	36	33	31	33	33	32	32	35	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	33.0
% Reduction from Control:	-4.87.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	3	4	3	4	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	10	11	10	12	9	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	19	15	15	15	17	16	15	16	14
Total young produced		34	32	28	29	29	31	32	28	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:	30.3
% Reduction from Control:	3.87.

Species: Ceriodaphnia dubia
1200 mg NaCl/L

CdNaClCR #: 272

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	3	4	4	2	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	6	9	10	5	9	8	11	6	4	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	4	4	5	9	6	4	5	9	9	6
Total young produced		14	17	19	17	19	16	18	19	17	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:	17.3
% Reduction from Control:	45.17.

1400 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	2	3	1	3	3	2	2	2	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	1	0	0	0
Total young produced		3	2	3	1	3	3	3	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:	2.3
% Reduction from Control:	92.72.

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	5	3	4	3	5	5	4	4	43
5	11	9	12	10	10	10	12	11	11	11	107
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	16	15	15	17	15	18	19	18	165
Total	33	29	33	28	29	30	32	34	34	33	315

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	4	5	4	6	4	5	5	5	4	46
5	13	12	10	11	10	12	10	11	11	11	110
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	19	15	19	17	18	18	19	17	175
Total	35	31	34	29	36	31	35	33	35	32	331

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	3	5	4	4	5	4	4	43
5	10	13	10	10	13	11	12	12	13	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	19	19	18	18	15	18	16	15	18	17	173
Total	34	36	33	31	33	33	32	32	35	31	330

1000 mg NaCl/L

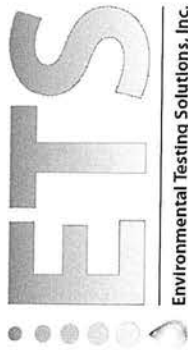
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	3	4	3	4	4	4	5	5	40
5	12	10	10	10	11	10	12	9	10	10	104
6	0	0	0	0	0	0	0	0	0	0	0
7	17	19	15	15	15	17	16	15	16	14	159
Total	34	32	28	29	29	31	32	28	31	29	303

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	4	4	3	4	4	2	4	4	37
5	6	9	10	5	9	8	11	6	4	7	75
6	0	0	0	0	0	0	0	0	0	0	0
7	4	4	4	5	9	6	4	5	9	6	61
Total	14	17	19	17	19	16	18	19	17	17	173

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	2	3	1	3	3	2	2	2	1	22
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	1	0	0	1
Total	3	2	3	1	3	3	3	2	2	1	23



***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 #272**
 Test dates: **June 07-14, 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	29	33	28	29	30	32	34	34	33	100	31.5	7.2	Not applicable
600	35	31	34	29	36	31	35	33	35	32	100	33.1	6.9	-5.1
800	34	36	33	31	33	33	32	32	35	31	100	33.0	4.9	-4.8
1000	34	32	28	29	29	31	32	28	31	29	100	30.3	6.6	3.8
1200	14	17	19	17	19	16	18	19	17	17	100	17.3	9.1	45.1
1400	3	2	3	1	3	3	3	2	2	1	100	2.3	35.8	92.7

Dunnnett's MSD value: 1.876
 PMSD: 6.0

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

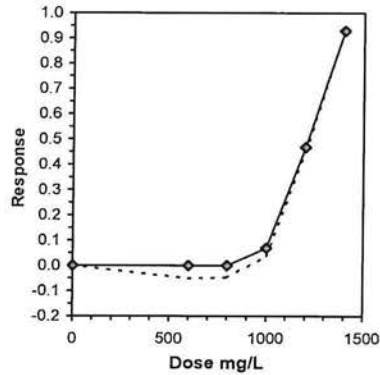
Start Date: 6/7/2022 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
 End Date: 6/14/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	29.000	33.000	28.000	29.000	30.000	32.000	34.000	34.000	33.000
600	35.000	31.000	34.000	29.000	36.000	31.000	35.000	33.000	35.000	32.000
800	34.000	36.000	33.000	31.000	33.000	33.000	32.000	32.000	35.000	31.000
1000	34.000	32.000	28.000	29.000	29.000	31.000	32.000	28.000	31.000	29.000
1200	14.000	17.000	19.000	17.000	19.000	16.000	18.000	19.000	17.000	17.000
1400	3.000	2.000	3.000	1.000	3.000	3.000	3.000	2.000	2.000	1.000

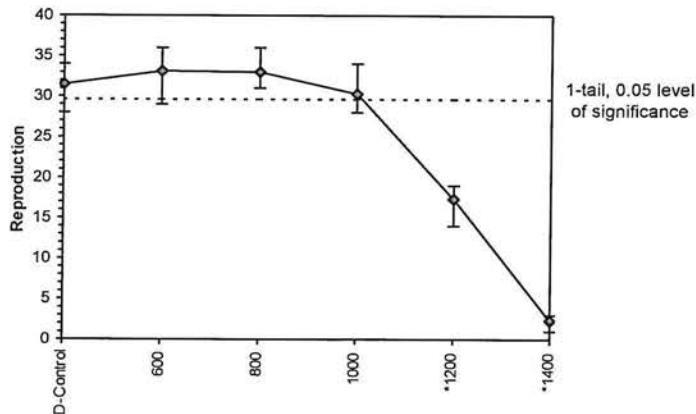
Conc-mg/L	Transform: Untransformed							1-Tailed		Isotonic		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.500	1.0000	31.500	28.000	34.000	7.216	10				32.533	1.0000
600	33.100	1.0508	33.100	29.000	36.000	6.897	10	-1.950	2.287	1.876	32.533	1.0000
800	33.000	1.0476	33.000	31.000	36.000	4.948	10	-1.829	2.287	1.876	32.533	1.0000
1000	30.300	0.9619	30.300	28.000	34.000	6.610	10	1.463	2.287	1.876	30.300	0.9314
*1200	17.300	0.5492	17.300	14.000	19.000	9.058	10	17.310	2.287	1.876	17.300	0.5318
*1400	2.300	0.0730	2.300	1.000	3.000	35.794	10	35.595	2.287	1.876	2.300	0.0707

Auxiliary Tests				Statistic	Critical	Skew	Kurt			
Kolmogorov D Test indicates normal distribution ($p > 0.01$)				0.82388	1.035	-0.1841	-0.5574			
Bartlett's Test indicates equal variances ($p = 0.09$)				9.55811	15.0863					
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	1000	1200	1095.45		1.87585	0.05955	1546.98	3.36481	6.0E-43	5, 54

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	945.672	38.1013	878.796	1011.87	0.2386
IC10	1015.69	11.1706	992.257	1035.23	-0.8827
IC15	1040.72	8.77146	1024.92	1058.61	0.0627
IC20	1065.74	7.96577	1049.34	1081.84	-0.0148
IC25	1090.77	7.36794	1075.15	1104.69	-0.1176
IC40	1165.85	7.25908	1150.23	1179.34	-0.2090
IC50	1213.78	6.46176	1199.97	1224.74	-0.4765



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Ceriodaphnia dubia

CdNaClCR #: 272

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	JK	JK	JK	JK	JW
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.72	7.72	7.59	7.82	7.78	7.75
	Dissolved oxygen (mg/L)	7.7	7.8	7.7	7.7	7.78	7.6
	Conductivity (µmhos/cm)	298		296		301	
	Alkalinity (mg CaCO ₃ /L)	64		64		63	
	Hardness (mg CaCO ₃ /L)	87				87	
	Temperature (°C)	24.9	25.1	24.9	25.2	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.80	7.73	7.75	7.84	7.88	7.78
	Dissolved oxygen (mg/L)	7.6	7.7	7.7	7.6	7.8	7.7
	Conductivity (µmhos/cm)	1480		1410		1440	
	Temperature (°C)	25.0	24.8	24.9	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.82	7.75	7.77	7.84	7.89	7.80
	Dissolved oxygen (mg/L)	7.6	7.7	7.7	7.6	7.8	7.7
	Conductivity (µmhos/cm)	1840		1770		1790	
	Temperature (°C)	25.0	24.8	25.0	25.2	25.0	25.0
1000 mg NaCl/L	pH (S.U.)	7.84	7.75	7.77	7.85	7.90	7.80
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.6	7.9	7.7
	Conductivity (µmhos/cm)	2240		2140		2180	
	Temperature (°C)	25.1	25.0	25.0	25.1	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.87	7.76	7.79	7.85	7.90	7.80
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.6	7.9	7.7
	Conductivity (µmhos/cm)	2600		2500		2540	
	Temperature (°C)	24.9	25.0	25.0	25.1	24.9	25.1
1400 mg NaCl/L	pH (S.U.)	7.90	7.76	7.83	7.85	7.91	7.80
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.6	7.9	7.7
	Conductivity (µmhos/cm)	3000		2870		2920	
	Temperature (°C)	24.9	25.1	25.0	24.9	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

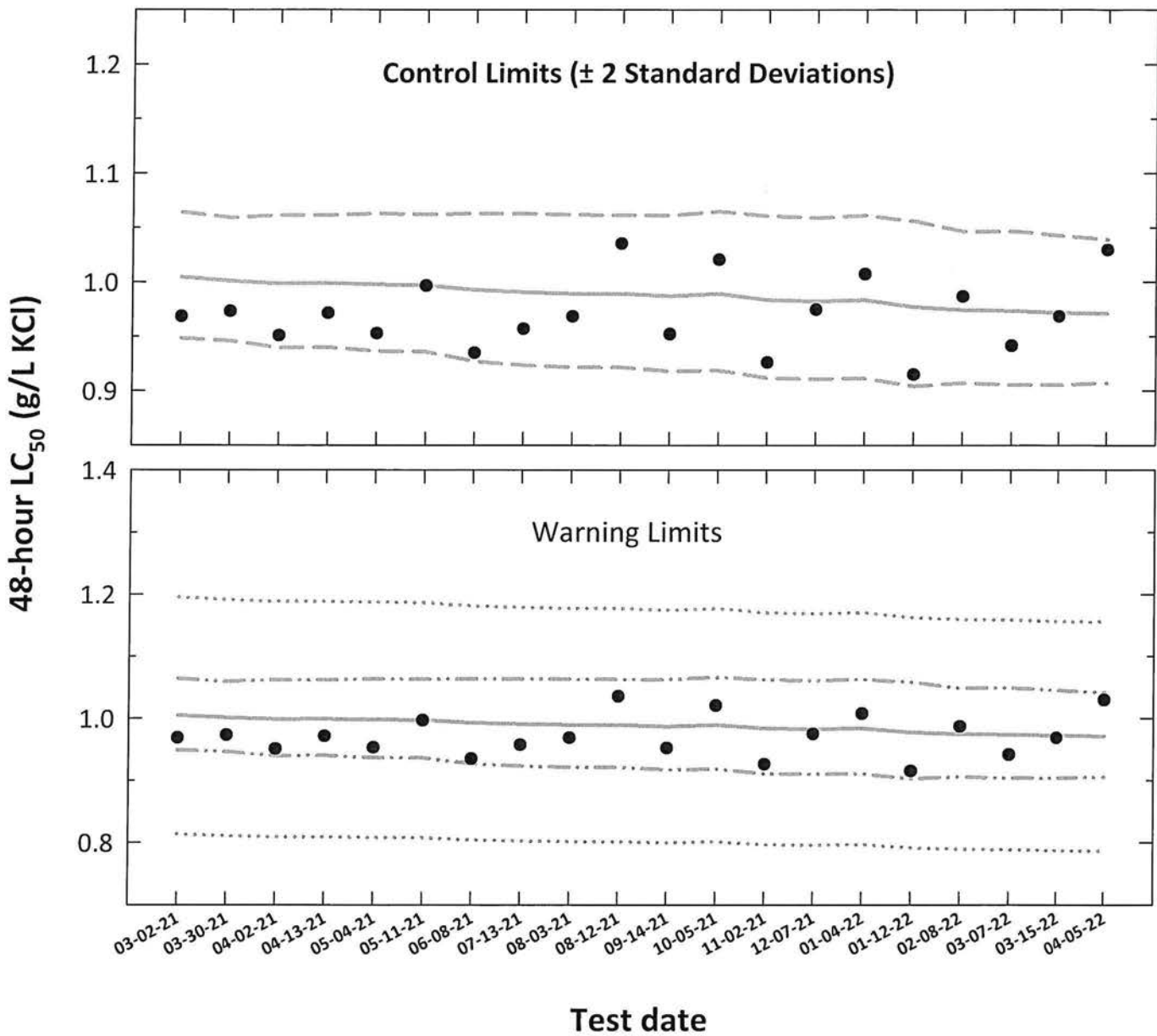
CdNaClCR #: 272

Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
Analyst		TW	BSC	BSC	BSC	BSC	W	W	JW	
CONTROL, MHSW	pH (S.U.)	7.68	7.75	7.64	7.73	7.62	7.73	7.57	7.69	
	Dissolved oxygen (mg/L)	7.7	8.0	7.7	7.6	7.7	7.6	7.6	7.6	
	Conductivity (µmhos/cm)	305		304		300		303		
	Alkalinity (mg CaCO ₃ /L)	7.06-14.22		62		7.06-14.22		60		
	Hardness (mg CaCO ₃ /L)			85				85		
	Temperature (°C)	24.7	25.1	24.8	25.2	24.7	25.1	24.9	25.2	
600 mg NaCl/L	pH (S.U.)	7.83	7.84	7.83	7.80	7.81	7.74	7.81	7.82	
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.6	7.8	7.6	7.6	7.4	
	Conductivity (µmhos/cm)	1450		1400		1440		1410		
	Temperature (°C)	24.8	24.9	24.9	24.9	24.8	25.0	25.0	25.0	
800 mg NaCl/L	pH (S.U.)	7.84	7.85	7.87	7.81	7.85	7.81	7.82	7.83	
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.8	7.5	7.7	7.6	
	Conductivity (µmhos/cm)	1840		1830		1860		1830		
	Temperature (°C)	24.8	25.0	24.9	24.9	24.8	25.0	24.9	25.0	
1000 mg NaCl/L	pH (S.U.)	7.85	7.84	7.86	7.80	7.85	7.83	7.85	7.83	
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.7	7.8	7.5	7.7	7.6	
	Conductivity (µmhos/cm)	2240		2140		2240		2170		
	Temperature (°C)	24.8	25.0	25.0	24.8	24.8	25.0	24.9	25.2	
1200 mg NaCl/L	pH (S.U.)	7.86	7.84	7.86	7.83	7.85	7.83	7.85	7.86	
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	7.7	7.8	7.6	7.8	7.7	
	Conductivity (µmhos/cm)	2580		2520		2610		2550		
	Temperature (°C)	24.9	25.0	25.0	24.8	24.9	24.9	25.1	24.9	
1400 mg NaCl/L	pH (S.U.)	7.86	7.84	7.87	7.83	7.86	7.84	7.88	7.86	
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.7	7.9	7.4	7.8	7.6	
	Conductivity (µmhos/cm)	2960		2910		2970		2910		
	Temperature (°C)	24.8	25.2	25.0	25.0	24.9	24.9	25.0	24.9	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	

Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ \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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556

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 # 135

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.)	JW	JW	JW	
	Dissolved oxygen (mg/L)	7.75	7.44	7.71	7.61
	Conductivity (µmhos/cm)	324			
	Alkalinity (mg/L CaCO ₃)	63			
	Hardness (mg/L CaCO ₃)	90			
	Temperature (°C)	24.9	24.7	24.7	
500 mg/L	pH (S.U.)	7.91	7.75	7.64	
	Dissolved oxygen (mg/L)	8.0	8.1	7.9	
	Conductivity (µmhos/cm)	1190			
	Temperature (°C)	25.0	24.5	24.5	
750 mg/L	pH (S.U.)	7.91	7.77	7.60	
	Dissolved oxygen (mg/L)	8.0	8.1	7.9	
	Conductivity (µmhos/cm)	1590			
	Temperature (°C)	24.9	24.6	24.6	
1000 mg/L	pH (S.U.)	7.90	7.77	7.72	
	Dissolved oxygen (mg/L)	8.1	8.2	7.9	
	Conductivity (µmhos/cm)	2020			
	Temperature (°C)	24.9	24.6	24.5	
1250 mg/L	pH (S.U.)	7.89	7.77	7.74	
	Dissolved oxygen (mg/L)	8.1	8.2	7.9	
	Conductivity (µmhos/cm)	2420			
	Temperature (°C)	24.8	24.8	24.5	
1500 mg/L	pH (S.U.)	7.89	7.76	7.77	
	Dissolved oxygen (mg/L)	8.1	8.2	8.0	
	Conductivity (µmhos/cm)	2920			
	Temperature (°C)	24.8	24.6	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	130464685

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 # 135

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-05-22	0510	JL	0710	JL	6E	LIGHT BLUE	03-30-22A
24	04-06-22			0708	JL			
48 Termination	04-07-22			0711	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:	03-24-22
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	03-29-22 1210 TO 03-30-22 0910
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86 Temperature (°C): 24.0°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 ^u	6 ^u	1 ^u	2 ^u	1 ^u	0 ^u
48 Termination	10	10	10	10	10	10	4 ^u	6	1	2	0 ^u	0
Mean Survival	100%		100%		100%		50%		15%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	957.0
Upper 95% confidence limit (mg KCl/L)	1097.6
48-hour LC ₅₀ (mg KCl/L)	1029.7

Comments:



Acute Fathead Minnow Test-24 Hr Survival

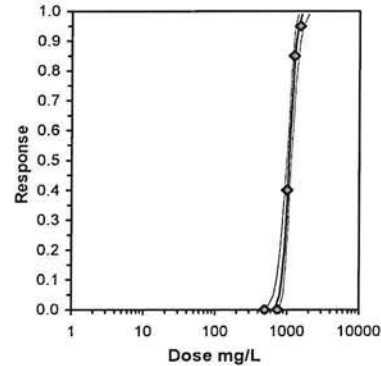
Start Date: 4/5/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 4/7/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: PP-Pimephales promelas
Comments:		

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.6000
1250	0.1000	0.2000
1500	0.1000	0.0000

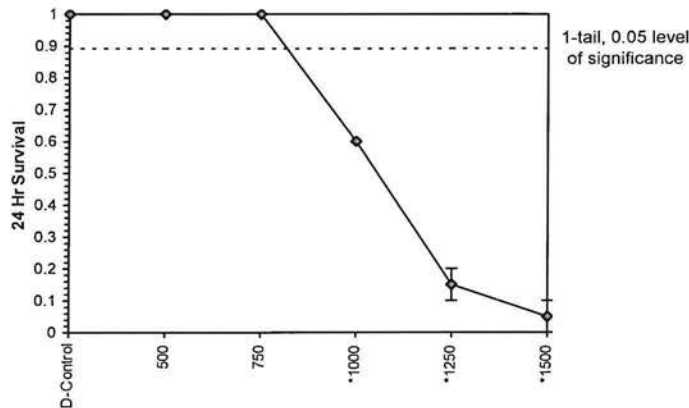
Conc-mg/L	Transform: Arcsin Square Root							1-Tailed t-Stat	Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1765	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1765	0	20
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	8.431	2.830	0.1765	8	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	16.341	2.830	0.1765	17	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	18.784	2.830	0.1765	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		0.08328	0.08542	0.58331	0.00389	3.3E-06	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	13.3184	2.44005	8.53591	18.1009	0	1.28086	7.81472	0.73368	3.02727	0.07508	4
Intercept	-35.318	7.41978	-49.861	-20.776							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	712.191	551.927	808.893							
EC05	3.355	801.246	660.332	886.131							
EC10	3.718	853.187	725.578	931.556							
EC15	3.964	890.12	772.487	964.405							
EC20	4.158	920.611	811.291	992.109							
EC25	4.326	947.6	845.504	1017.26							
EC40	4.747	1019.17	934.013	1088.43							
EC50	5.000	1064.8	987.208	1138.73							
EC60	5.253	1112.48	1038.87	1196.59							
EC75	5.674	1196.5	1119.9	1311.99							
EC80	5.842	1231.57	1150.62	1364.55							
EC85	6.036	1273.76	1185.82	1430.51							
EC90	6.282	1328.9	1229.65	1520.5							
EC95	6.645	1415.05	1294.71	1668.12							
EC99	7.326	1591.99	1420.49	1992.74							



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 4/5/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 4/7/2022	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

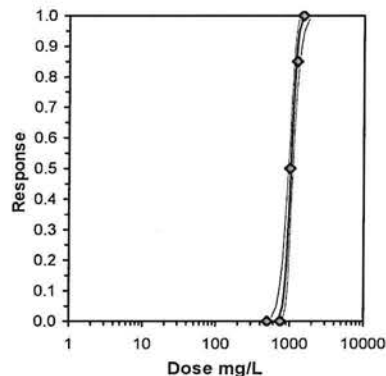
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.4000	0.6000
1250	0.1000	0.2000
1500	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2220	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2220	0	20
*1000	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	8.044	2.850	0.2220	10	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	13.085	2.850	0.2220	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

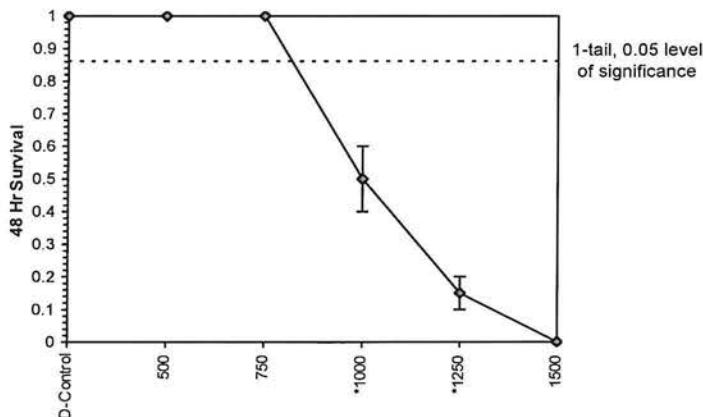
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	750	1000	866.025	0.11312
Treatments vs D-Control	MSDu	MSDp	MSB	MSE
	0.11603	0.44492	0.00607	1.3E-04
	F-Prob	df		
	4, 5			

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	15.1232	2.89765	9.44383	20.8026	0	1.50888	7.81472	0.68022	3.0127	0.06612	5
Intercept	-40.562	8.76793	-57.747	-23.377							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	722.56	566.603	812.668
EC05	3.355	801.562	666.136	880.141
EC10	3.718	847.149	725.218	919.56
EC15	3.964	879.362	767.35	947.968
EC20	4.158	905.836	801.996	971.87
EC25	4.326	929.182	832.395	993.534
EC40	4.747	990.714	910.448	1054.62
EC50	5.000	1029.68	956.986	1097.62
EC60	5.253	1070.17	1001.92	1146.9
EC75	5.674	1141.04	1071.75	1244.82
EC80	5.842	1170.45	1097.97	1289.27
EC85	6.036	1205.68	1127.8	1344.91
EC90	6.282	1251.53	1164.67	1420.56
EC95	6.645	1322.71	1218.88	1543.96
EC99	7.326	1467.33	1322.25	1812.21



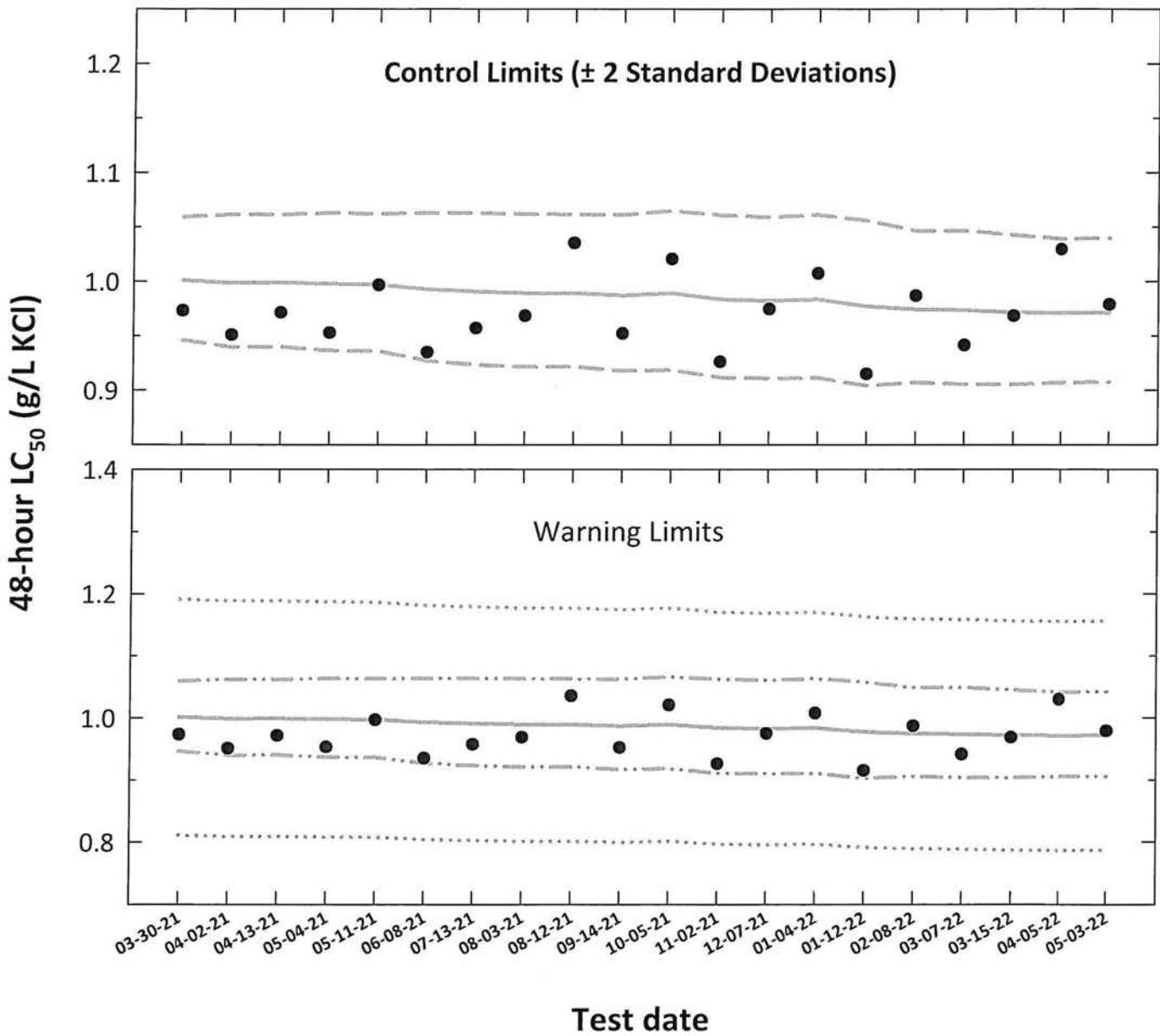
Dose-Response Plot



Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
20	05-03-22	0.9788	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562

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 # 136

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:

		Hours		
		0	24	48
Concentration	Analyst	JW	JW	U
Control, MHSW	pH (S.U.)	7.80	7.79	7.72
	Dissolved oxygen (mg/L)	7.8	8.0	7.7
	Conductivity (µmhos/cm)	310		
	Alkalinity (mg/L CaCO ₃)	62		
	Hardness (mg/L CaCO ₃)	88		
	Temperature (°C)	24.9	24.7	24.6
500 mg/L	pH (S.U.)	8.18	7.81	7.70
	Dissolved oxygen (mg/L)	8.0	8.0	7.7
	Conductivity (µmhos/cm)	1240		
	Temperature (°C)	25.0	24.8	24.9
	750 mg/L	pH (S.U.)	8.14	7.83
Dissolved oxygen (mg/L)		8.0	8.0	7.8
Conductivity (µmhos/cm)		1640		
Temperature (°C)		25.0	24.8	24.9
1000 mg/L	pH (S.U.)	8.11	7.84	7.75
	Dissolved oxygen (mg/L)	8.0	8.0	7.9
	Conductivity (µmhos/cm)	2090		
	Temperature (°C)	25.0	24.6	24.9
1250 mg/L	pH (S.U.)	8.09	7.83	7.70
	Dissolved oxygen (mg/L)	8.1	8.0	7.9
	Conductivity (µmhos/cm)	2570		
	Temperature (°C)	25.0	24.9	24.8
1500 mg/L	pH (S.U.)	8.08	7.84	05 Oct 12 U
	Dissolved oxygen (mg/L)	8.2	8.1	
	Conductivity (µmhos/cm)	3170		
	Temperature (°C)	24.9	24.7	

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

Chemical analyses:

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

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

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 136

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-03-22*	0500	X	0900	X	1D	WHIRE	04-21-22B
24	05-04-22			0655	J			
48 Termination	05-05-22			0658	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
Spawning date:	04-21-22
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	04-26-22 1200 TO 04-27-22 0900
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.08
	Temperature (°C): 24.0°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	9 ^{ud}	6 ^{ud}	6 ^{ud}	1 ^{ud}	1 ^{ud}	0 ^{ud}	0 ^{ud}
48 Termination	10	10	10	10	9 ^{ud}	9	6	5 ^{id}	1	0 ^{id}	0	0
Mean Survival	100%		100%		90%		55%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	904.4
Upper 95% confidence limit (mg KCl/L)	1102.7 ⁰⁵⁻¹²⁻²² 1049.4
48-hour LC ₅₀ (mg KCl/L)	978.8

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 5/3/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 5/5/2022	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	0.9000
1000	0.6000	0.6000
1250	0.1000	0.1000
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	0.1469	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	10.205	2.850	0.1469	8	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	21.156	2.850	0.1469	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

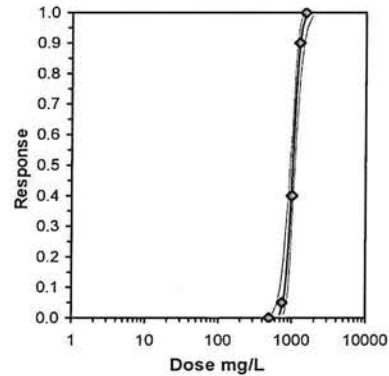
Auxiliary Tests

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
Dunnnett's Test	750	1000	866.025
Treatments vs D-Control	MSDu	MSDp	MSB
	0.06555	0.06723	0.44775
	MSE	F-Prob	df
	0.00266	1.6E-05	4, 5

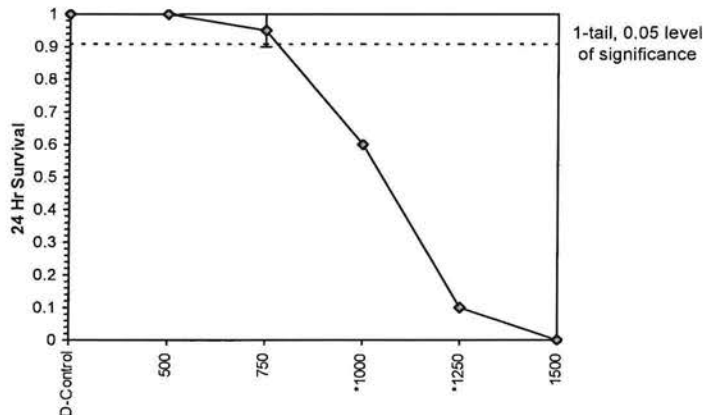
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.0557	2.60194	8.95589 19.1555	0	0.68123	7.81472	0.87761	3.00847	0.07115	3
Intercept	-37.286	7.86458	-52.701 -21.871							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	696.557	543.644 787.844
EC05	3.355	778.829	644.836 858.967
EC10	3.718	826.589	705.319 900.683
EC15	3.964	860.456	748.627 930.803
EC20	4.158	888.359	784.35 956.172
EC25	4.326	913.018	815.776 979.178
EC40	4.747	978.232	896.844 1044.06
EC50	5.000	1019.69	945.488 1089.68
EC60	5.253	1062.9	992.739 1141.92
EC75	5.674	1138.82	1066.82 1245.63
EC80	5.842	1170.43	1094.85 1292.74
EC85	6.036	1208.38	1126.88 1351.8
EC90	6.282	1257.89	1166.64 1432.25
EC95	6.645	1335.03	1225.41 1563.89
EC99	7.326	1492.71	1338.28 1851.86



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 5/3/2022	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 5/5/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: PP-Pimephales promelas
Comments:		

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.6000	0.5000
1250	0.1000	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	0.1726	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.690	2.850	0.1726	2	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.343	2.850	0.1726	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

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		0.08088	0.08295	0.50064	0.00367	2.7E-05	4, 5

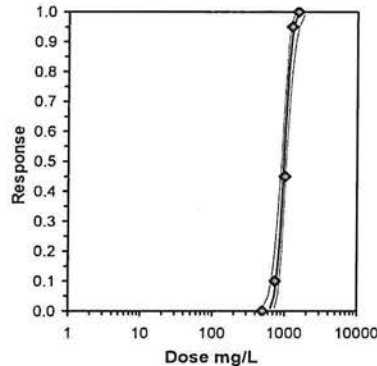
Treatments vs D-Control

Maximum Likelihood-Probit

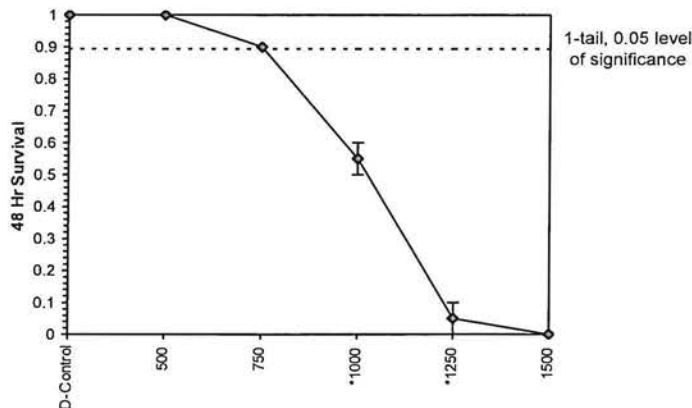
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2013	2.39931	8.49862 17.9039	0	1.63607	7.81472	0.65124	2.99071	0.07575	3
Intercept	-34.481	7.21256	-48.618 -20.345							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	652.364	504.505 742.597
EC05	3.355	734.706	603.946 814.467
EC10	3.718	782.769	663.804 856.785
EC15	3.964	816.962	706.844 887.404
EC20	4.158	845.199	742.456 913.226
EC25	4.326	870.2	773.866 936.663
EC40	4.747	936.528	855.269 1002.82
EC50	5.000	978.84	904.4 1049.36
EC60	5.253	1023.06	952.365 1102.67
EC75	5.674	1101.04	1028.07 1208.65
EC80	5.842	1133.61	1056.88 1256.9
EC85	6.036	1172.79	1089.88 1317.5
EC90	6.282	1224.02	1130.97 1400.26
EC95	6.645	1304.1	1191.93 1536.21
EC99	7.326	1468.7	1309.64 1835.7



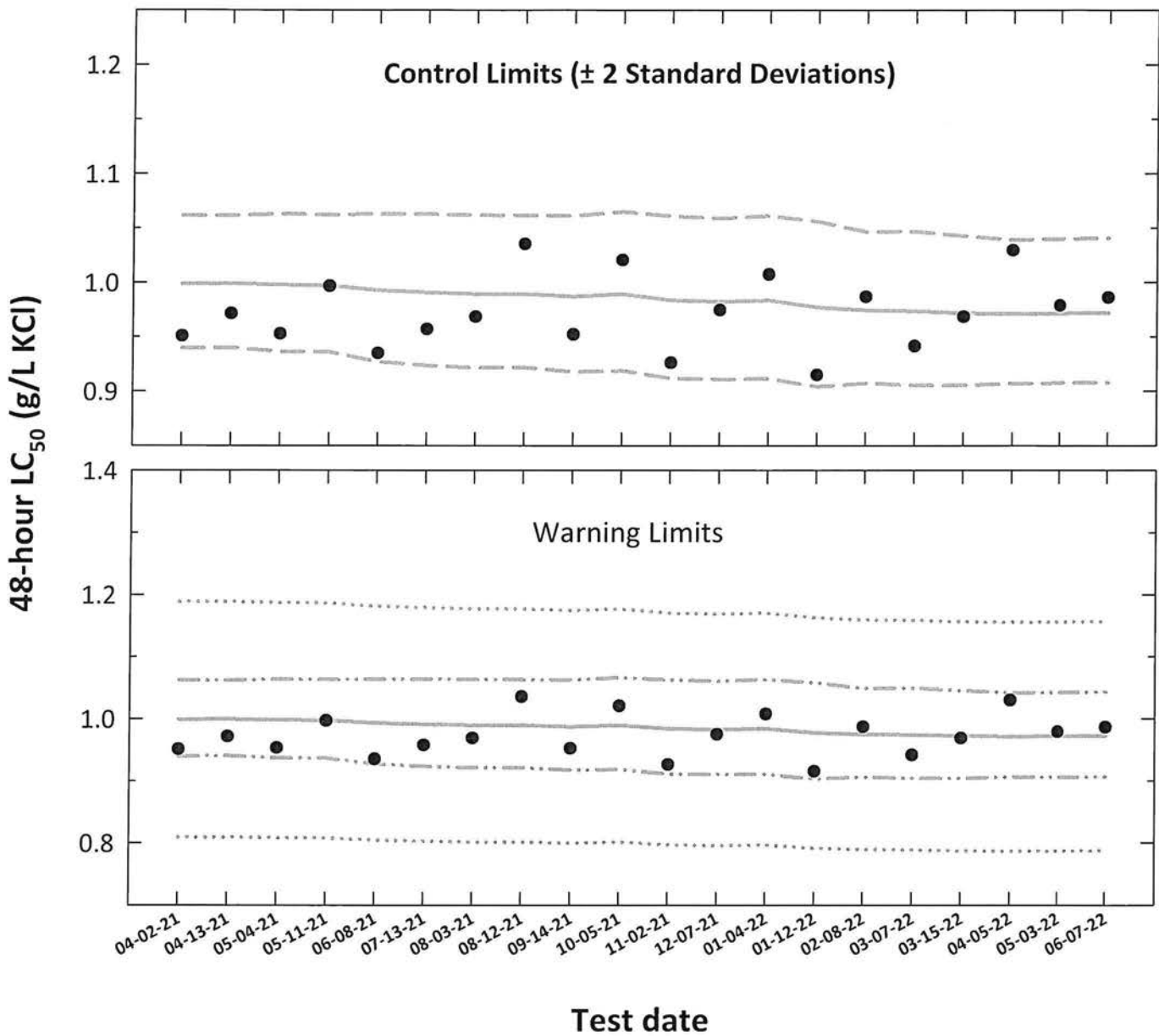
Dose-Response Plot



Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.75}$ converted to anti-logarithmic values,
 $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			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}	Warning Limits	Warning Limits
1	04-02-21	0.9509	-0.0219	-0.0005	0.0132	0.9397	1.0616	0.9396	1.0616	0.8090	1.1885	0.8090	1.1885
2	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887	0.8091	1.1887
3	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874	0.8082	1.1874
4	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867	0.8078	1.1867
5	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815	0.8042	1.1815
6	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791	0.8026	1.1791
7	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774	0.8014	1.1774
8	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773	0.8014	1.1773
9	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746	0.7995	1.1746
10	10-05-21	1.0207	0.0089	-0.0047	0.0160	0.9190	1.0650	0.9182	1.0659	0.8014	1.1773	0.8014	1.1773
11	11-02-21	0.9262	-0.0333	-0.0071	0.0164	0.9120	1.0610	0.9108	1.0623	0.7968	1.1706	0.7968	1.1706
12	12-07-21	0.9747	-0.0111	-0.0078	0.0163	0.9111	1.0590	0.9099	1.0604	0.7957	1.1689	0.7957	1.1689
13	01-04-22	1.0075	0.0033	-0.0072	0.0165	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705	0.7967	1.1705
14	01-12-22	0.9151	-0.0385	-0.0100	0.0168	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630	0.7916	1.1630
15	02-08-22	0.9869	-0.0057	-0.0112	0.0155	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597	0.7894	1.1597
16	03-07-22	0.9416	-0.0261	-0.0116	0.0157	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587	0.7887	1.1587
17	03-15-22	0.9685	-0.0139	-0.0124	0.0153	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564	0.7871	1.1564
18	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556	0.7866	1.1556
19	05-03-22	0.9788	-0.0093	-0.0125	0.0148	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562	0.7870	1.1562
20	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570	0.7875	1.1570

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 # 137

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 #: 2101

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Analyst	<u>KL</u>	<u>KL</u>	<u>KL</u>
	pH (S.U.)	<u>7.72</u>	<u>7.53</u>	<u>7.72</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.7</u>	<u>7.6</u>
	Conductivity (µmhos/cm)	<u>298</u>		
	Alkalinity (mg/L CaCO ₃)	<u>64</u>		
	Hardness (mg/L CaCO ₃)	<u>87</u>		
	Temperature (°C)	<u>24.8</u>	<u>24.8</u>	<u>24.5</u>
500 mg/L	pH (S.U.)	<u>7.73</u>	<u>7.50</u>	<u>7.74</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.6</u>	<u>7.0</u>
	Conductivity (µmhos/cm)	<u>1160</u>		
	Temperature (°C)	<u>24.7</u>	<u>24.7</u>	<u>24.7</u>
	750 mg/L	pH (S.U.)	<u>7.79</u>	<u>7.61</u>
Dissolved oxygen (mg/L)		<u>7.7</u>	<u>7.6</u>	<u>7.6</u>
Conductivity (µmhos/cm)		<u>1560</u>		
Temperature (°C)		<u>24.9</u>	<u>24.7</u>	<u>24.6</u>
1000 mg/L		pH (S.U.)	<u>7.01</u>	<u>7.62</u>
	Dissolved oxygen (mg/L)	<u>7.0</u>	<u>7.6</u>	<u>7.0</u>
	Conductivity (µmhos/cm)	<u>2010</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.0</u>	<u>24.6</u>
	1250 mg/L	pH (S.U.)	<u>7.03</u>	<u>7.62</u>
Dissolved oxygen (mg/L)		<u>7.0</u>	<u>7.6</u>	<u>7.6</u>
Conductivity (µmhos/cm)		<u>2440</u>		
Temperature (°C)		<u>24.9</u>	<u>24.8</u>	<u>24.6</u>
1500 mg/L		pH (S.U.)	<u>7.05</u>	<u>7.66</u>
	Dissolved oxygen (mg/L)	<u>7.0</u>	<u>7.0</u>	
	Conductivity (µmhos/cm)	<u>1070</u>		
	Temperature (°C)	<u>24.7</u>	<u>24.8</u>	
				<u>0.01-24.8</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: 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	06-07-22	0455	J	0655	J	7A	Yellow	06-01-22C
24	06-08-22			0650	J			
48 Termination	06-09-22			0652	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:	05-16-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	06-01-22 1130 TO 06-02-22 0850
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.83
	Temperature (°C): 24.0°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	4 ^d 6	7 ^d	9 ^d 1	2 ^d	10 ^d 0	10 ^d 0
48 Termination	10	10	10	10	9 ^u	10	4 ^u	5 ^u	0 ^u	2	0	0
Mean Survival	100%		100%		95%		45%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	912.5
Upper 95% confidence limit (mg KCl/L)	1055.9
48-hour LC ₅₀ (mg KCl/L)	986.2

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date:	6/7/2022	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2022	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:					

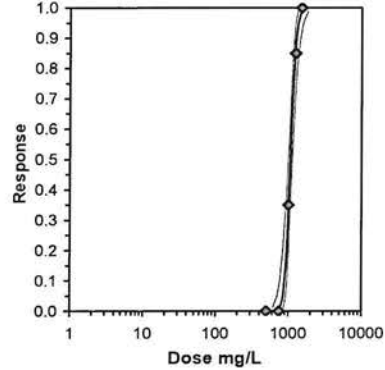
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.7000
1250	0.1000	0.2000
1500	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1591	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1591	0	20
*1000	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	8.478	2.850	0.1591	7	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	18.256	2.850	0.1591	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

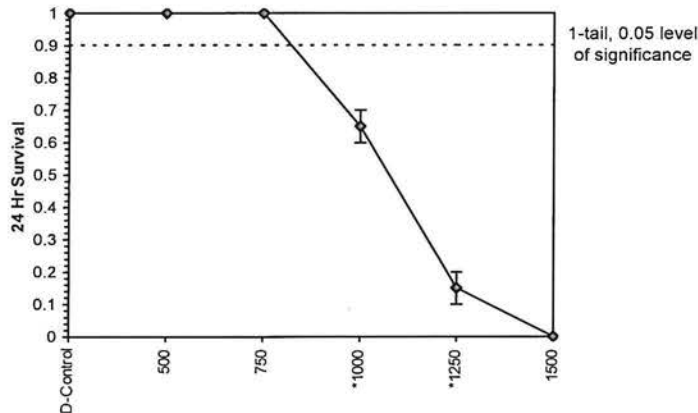
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.07271	0.07457	0.40874	0.00312	3.0E-05	4, 5

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.8087	3.41655	10.1123	23.5052	0	0.45103	7.81472	0.92951	3.02777	0.05949	4
Intercept	-45.893	10.3825	-66.243	-25.543							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	775.112	610.381	864.895
EC05	3.355	850.959	709.996	928.314
EC10	3.718	894.381	768.646	965.178
EC15	3.964	924.923	810.262	991.691
EC20	4.158	949.938	844.353	1013.98
EC25	4.326	971.938	874.161	1034.19
EC40	4.747	1029.66	950.156	1091.34
EC50	5.000	1066.02	994.957	1131.84
EC60	5.253	1103.67	1037.71	1178.54
EC75	5.674	1169.21	1102.98	1271.8
EC80	5.842	1196.29	1127.15	1314.14
EC85	6.036	1228.64	1154.48	1367.07
EC90	6.282	1270.6	1188.04	1438.83
EC95	6.645	1335.44	1237.06	1555.37
EC99	7.326	1466.11	1329.68	1806.62



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date:	6/7/2022	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2022	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

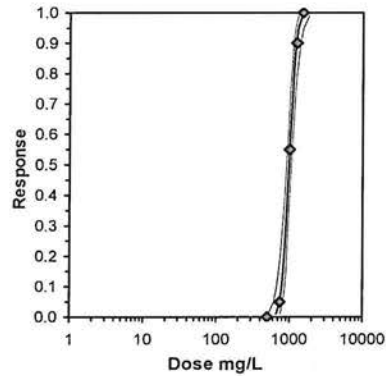
Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	1.0000
1000	0.4000	0.5000
1250	0.0000	0.2000
1500	0.0000	0.0000

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	0.3245	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.716	2.850	0.3245	1	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	5.946	2.850	0.3245	11	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	9.668	2.850	0.3245	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

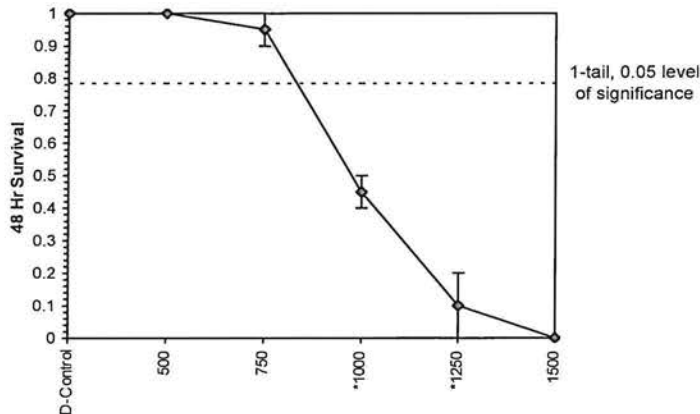
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	750	1000	866.025	0.19093
Treatments vs D-Control	MSDu	MSDp	MSB	MSE
	0.19583	0.49266	0.01296	6.2E-04
	F-Prob	df		
	4, 5			

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	13.6436	2.50045	8.74277	18.5445	0	0.26487	7.81472	0.96649	2.99395	0.07329	3
Intercept	-35.848	7.5229	-50.593	-21.103							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	665.946	517.712	755.519
EC05	3.355	747.117	616.608	826.082
EC10	3.718	794.358	675.895	867.557
EC15	3.964	827.908	718.421	897.538
EC20	4.158	855.58	753.543	922.809
EC25	4.326	880.056	784.474	945.737
EC40	4.747	944.882	864.425	1010.42
EC50	5.000	986.158	912.536	1055.89
EC60	5.253	1029.24	959.404	1107.91
EC75	5.674	1105.05	1033.19	1211.17
EC80	5.842	1136.67	1061.2	1258.11
EC85	6.036	1174.66	1093.26	1316.98
EC90	6.282	1224.27	1133.12	1397.28
EC95	6.645	1301.68	1192.14	1528.9
EC99	7.326	1460.34	1305.76	1817.78



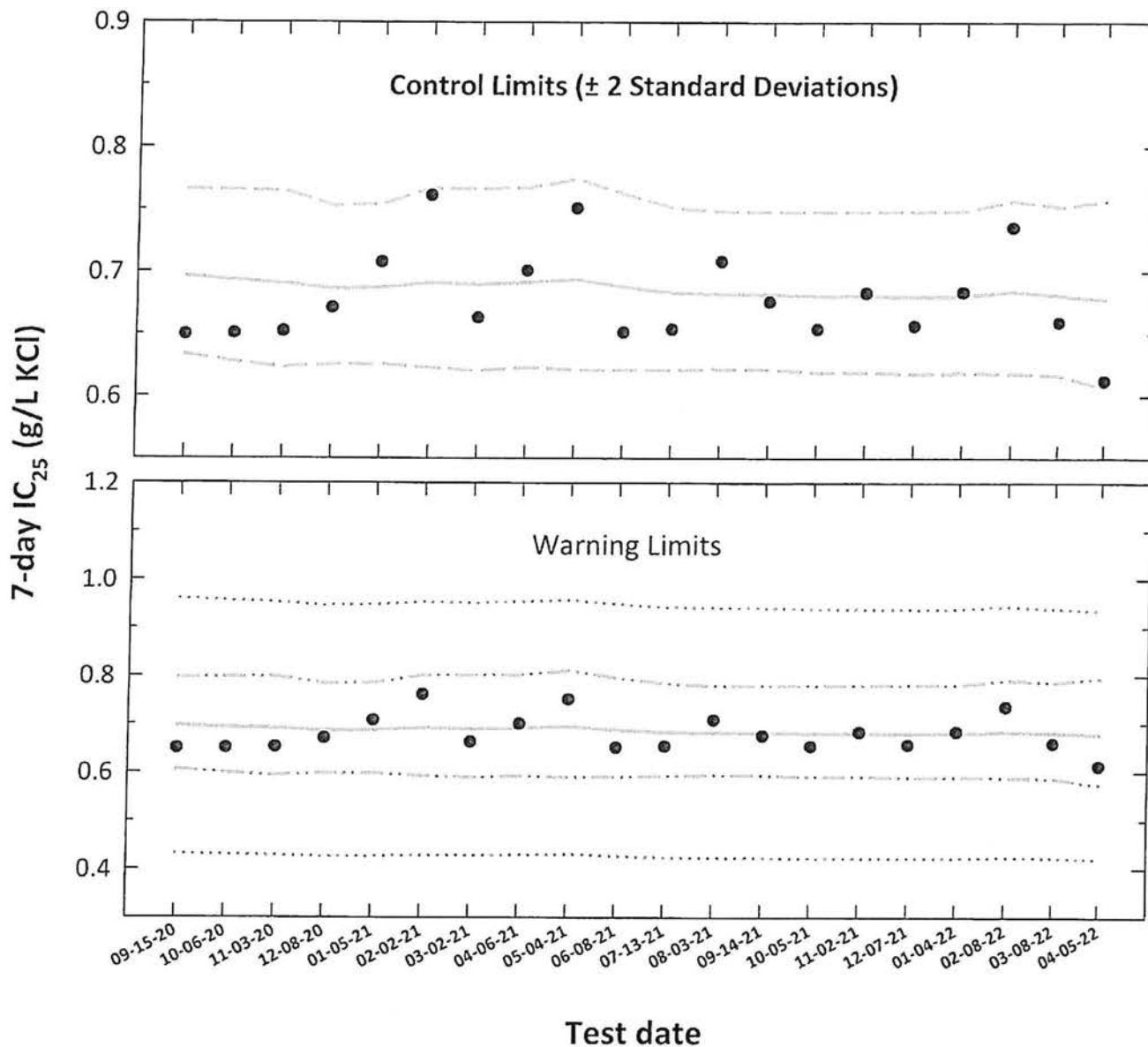
Dose-Response Plot



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		CT	S	CT	Anti-logarithmic Values (g/L KCl)				
			7-day IC ₂₅	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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	04-05-22	0.6124	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356

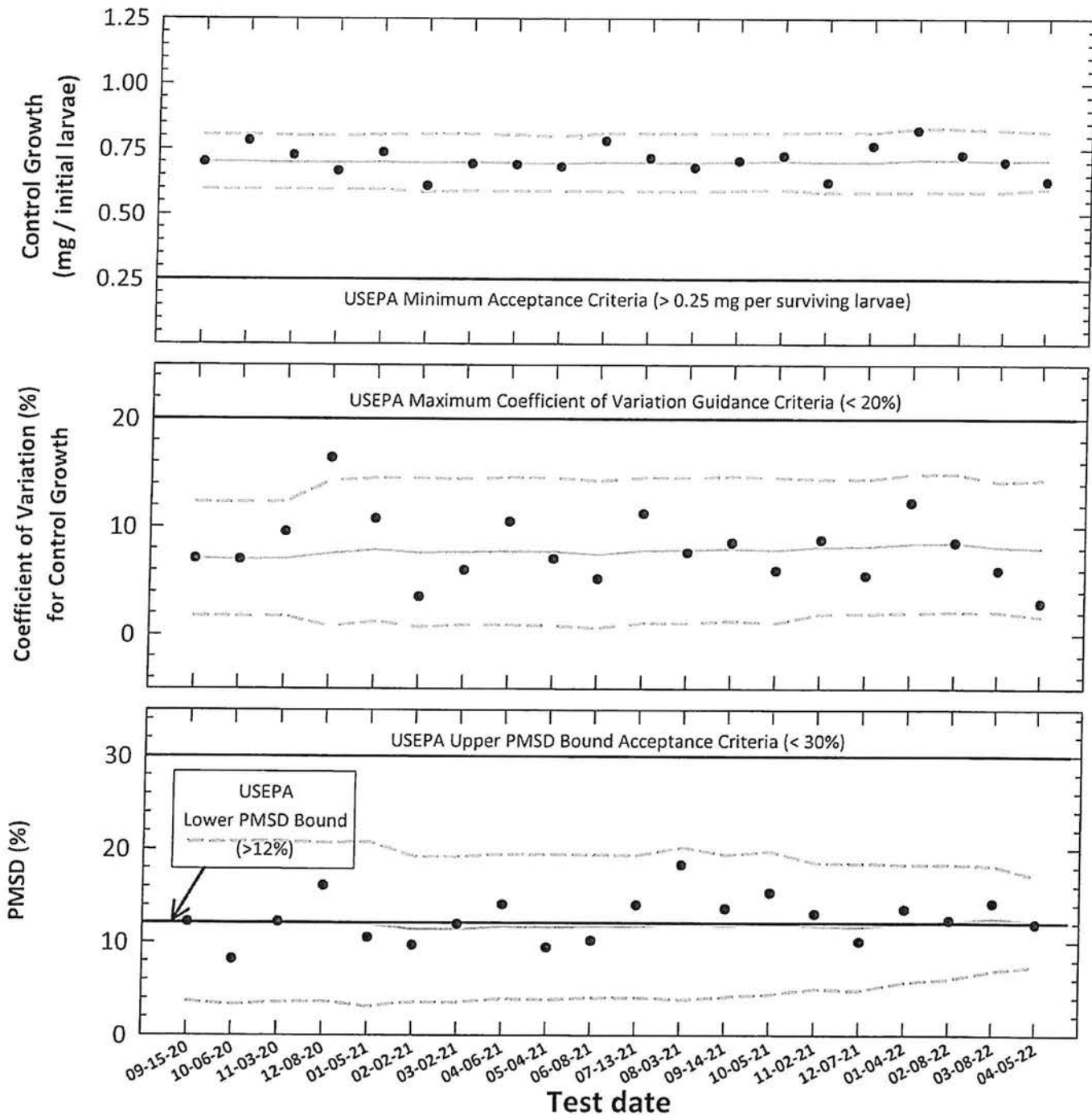
Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
 CT = Central tendency of the IC₂₅ values.
 S = Standard deviation of the IC₂₅ values.

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

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



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



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

Independent Review By
 Kelley E. Keenan

Entered and Reviewed by
 Jim Sumner



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

Test number	Test date	ToxCal Determination				Control Growth		Test		Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		CV (%)	CT	PMSD (%)	MSD	CT	Control Growth (mg/initial larvae)		CT	95% Confidence Interval		CT	95% Confidence Interval		
			Mean (mg/initial larvae)	Mean (mg/initial larvae)						CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S	
1	09-15-20	100	0.700	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		
2	10-06-20	100	0.782	0.6540	7.0	0.0885	12.2	0.700	0.594	0.806	7.0	1.7	12.3	12.0	3.3	20.8		
3	11-03-20	97.5	0.727	0.0885	9.6	0.1070	16.1	0.698	0.595	0.801	7.0	1.7	12.3	12.2	3.6	20.8		
4	12-08-20	100	0.666	0.0774	16.4	0.0774	10.5	0.700	0.596	0.805	7.5	0.8	14.3	12.1	3.6	20.7		
5	01-05-21	100	0.737	0.0588	10.8	0.0588	9.7	0.697	0.585	0.809	7.9	1.2	14.5	11.9	3.1	20.8		
6	02-02-21	100	0.609	0.0827	3.5	0.0827	11.9	0.699	0.590	0.809	7.6	0.7	14.5	11.4	3.5	19.2		
7	03-02-21	100	0.693	0.0970	6.0	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	14.5	11.4	3.5	19.2		
8	04-06-21	100	0.691	0.0643	10.5	0.0643	9.4	0.697	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4		
9	05-04-21	100	0.682	0.0794	7.1	0.0794	10.2	0.694	0.589	0.805	7.7	0.8	14.6	11.6	3.8	19.4		
10	06-08-21	100	0.781	0.1000	5.2	0.1000	14.0	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4		
11	07-13-21	100	0.715	0.0960	11.2	0.0960	18.3	0.699	0.588	0.810	7.8	1.1	14.6	11.7	4.0	19.3		
12	08-03-21	100	0.678	0.0810	7.6	0.0810	13.6	0.699	0.591	0.811	8.0	1.3	14.7	12.0	3.8	20.2		
13	09-14-21	100	0.706	0.1109	8.6	0.1109	15.3	0.701	0.594	0.813	8.0	1.1	14.6	11.7	4.1	19.4		
14	10-05-21	100	0.724	0.0810	6.0	0.0810	13.0	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7		
15	11-02-21	100	0.621	0.0767	8.8	0.0767	10.1	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5		
16	12-07-21	100	0.763	0.1112	5.5	0.1112	13.5	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5		
17	01-04-22	100	0.822	0.0898	12.3	0.0898	12.3	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3		
18	02-08-22	100	0.728	0.0994	8.5	0.0994	14.2	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3		
19	03-08-22	100	0.701	0.0747	6.0	0.0747	11.9	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2		
20	04-05-22	100	0.626	0.0747	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0		

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



Environmental Testing Solutions, Inc.

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

PpKCICR Test Number: 88

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2078</u>					
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.					
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7 B</u>
Spawn date:	<u>03-30-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>04-04-22 1458 to 04-05-22 0501</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.86</u> S.U. Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>04-12-22 0105</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>04-13-22 0105</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	04-05-22	0510	<u>JL</u>	1110	<u>JK</u>	0721	<u>JK</u>	03-30-22 A
1	04-06-22	0510	<u>JK</u>	1110	<u>JK</u>	0710	<u>JK</u>	↓
2	04-07-22	0530	<u>JK</u>	1130	<u>JK</u>	0730	<u>JK</u>	03-30-22 C
3	04-08-22	0500	<u>JK</u>	1100	<u>JK</u>	0702	<u>JK</u>	↓
4	04-09-22	0600	<u>JK</u>	1200	<u>JK</u>	0800	<u>JK</u>	04-06-30 A
5	04-10-22	0600	<u>JK</u>	1200	<u>JK</u>	0800	<u>JK</u>	↓
6	04-11-22	0500	<u>JK</u>	1100	<u>JK</u>	0700	<u>JK</u>	↓
7	04-12-22					0622	<u>JK</u>	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>756.5</u>
Average weight per initial larvae:	<u>0.626</u>		NOEC (mg/L KCl)	<u>450</u>
Average weight per surviving larvae:	<u>0.626</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>600</u>
			ChV (mg/L KCl)	<u>519.6</u>
			IC ₂₅ (mg/L KCl)	<u>612.4</u>

Independent
Review by
Kalley E. Keenan:K

Environmental Testing Solutions, Inc.

Species: Pimephales promelas

PpKCICR Test Number: 88

Survival and Growth Data

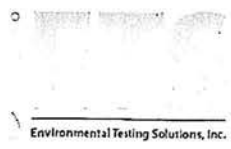
Day	Control				300 mg KCl/L				450 mg KCl/L				
	A	B	C	D	E	F	G	H	I	J	K	L	
0	10	10	10	10	10	10	10	10	10	10	10	10	
1	10	10	10	10	10	10	10	10	10	10	10	10	
2	10	10	10	10	10	10	10	10	10	10	10	10	
3	10	10	10	10	10	10	10	10	10	10	10	10	
4	10	10	10	10	10	10	10	10	10	10	10	10	
5	10	10	10	10	10	10	10	10	10	10	10	10	
6	10	10	10	10	10	10	10	10	10	10	10	10	
7	10	10	10	10	10 ^{lg}	10	10	10	10	10	10	10	
*A = Pan weight (mg) Tray color code: <u>Rv67</u> Analyst: <u>A.J.</u> Date: <u>03-26-22</u>													
*B = Pan + Larvae weight (mg) Analyst: <u>A.J.</u> Date: <u>04-15-22</u>													
C = Larvae weight (mg) = B - A Analyst: <u>J</u>													
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>													
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.626		0.845		-35.0%		0.735		-17.5%	

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

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

Comments:





Species: Pimephales promelas

PpKCICR Test Number: 88

Survival and Growth Data

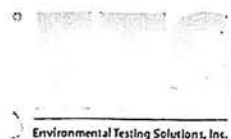
Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L						
	M	N	O	P	Q	R	S	T	U	V	W	X			
0	10	10	10	10	10	10	10	10	10	10	10	10			
1	10	10	10	10	10	10	10	10	8 ^{2d}	9 ^{1d}	9 ^{1d}	9 ^{1d}			
2	10	10	10	10	10	10	10	10	8	9	9	9			
3	10	10	10	10	10	10	10	10	8	9	9	9			
4	10	10	10	10	8 ^{2d}	8 ^{2d}	8 ^{2d}	8 ^{2d}	6 ^{2d}	5 ^{1d}	5 ^{4d}	5 ^{4d}			
5	7 ^{2d}	8 ^{2d}	8 ^{2d}	8 ^{2d}	6 ^{2d}	7 ^{1d}	6 ^{2d}	6 ^{2d}	3 ^{2d}	4 ^{1d}	3 ^{2d}	3 ^{2d}			
6	7	8	8	8	6	7	6	6	2 ^{2d}	2 ^{2d}	2 ^{1d}	2 ^{1d}			
7	7	8	8	8	6	7	5 ^{1d}	6	2 ^{1d}	2	2 ^{1d}	1 ^{1d}			
*A = Pan weight (mg) Tray color code: <u>Avbg</u> Analyst: <u>A.S</u> Date: <u>03-26-77</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>A.I</u> Date: <u>04-15-77</u>															
C = Larvae weight (mg) = B - A Analyst: <u>J</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.560		10.57.		0.459		26.77.		0.141		77.57.	

*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: 88

Survival and Growth Data

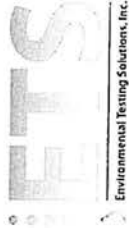
Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	9 ^{id}	8 ^{2d}	8 ^{2d}	8 ^{2d}
2	7 ^{2d}	7 ^{1d}	6 ^{2d}	6 ^{2d}
3	6 ^{1d}	5 ^{2d}	5 ^{1d}	5 ^{1d}
4	4 ^{2d}	4 ^{1d}	4 ^{1d}	4 ^{1d}
5	2 ^{2d}	3 ^{1d}	2 ^{2d}	2 ^{2d}
6	1 ^{1d}	1 ^{2d}	2	1 ^{1d}
7	1 ^{1d}	0 ^{1d}	2 ^{1d}	1 ^{1d}
*A = Pan weight (mg) Tray color code: <u>A667</u> Analyst: <u>A-J</u> Date: <u>07-26-77</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>A-J</u> Date: <u>04-15-77</u>				
C = Larvae weight (mg) = B - A Analyst: <u>K</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>K</u>				
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		
0.088		85.97		

*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: **88**
Test dates: **April 05-12, 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 (Mass weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	13.06	19.29	6.23	0.623			0.623				
	B	10	10	15.42	21.43	6.01	0.601			0.601				
	C	10	10	16.34	22.73	6.39	0.639	0.626	2.9	0.639	100.0	0.626	2.9	Not applicable
	D	10	10	15.38	21.78	6.40	0.640			0.640				
300	E	10	10	13.87	23.07	9.20	0.920			0.920				
	F	10	10	14.76	22.99	8.23	0.823	0.845	5.9	0.823	100.0	0.845	5.9	-35.0
	G	10	10	14.99	23.15	8.16	0.816			0.816				
	H	10	10	15.03	23.23	8.20	0.820			0.820				
450	I	10	10	14.00	20.60	6.60	0.660			0.660				
	J	10	10	15.35	21.10	7.75	0.775	0.735	8.8	0.775	100.0	0.735	8.8	-17.5
	K	10	10	14.86	21.90	7.04	0.704			0.704				
	L	10	10	14.00	22.01	8.01	0.801			0.801				
600	M	10	7	14.82	19.29	4.47	0.639			0.447				
	N	10	8	15.87	21.23	5.36	0.670	0.720	10.8	0.536	77.5	0.560	15.7	10.5
	O	10	8	14.53	20.96	6.43	0.804			0.643				
	P	10	8	15.26	21.39	6.13	0.766			0.613				
750	Q	10	6	13.67	18.31	4.64	0.773			0.464				
	R	10	7	15.34	21.10	5.76	0.823	0.765	11.4	0.576	60.0	0.459	18.5	26.7
	S	10	5	15.39	19.51	4.12	0.824			0.412				
	T	10	6	13.69	17.52	3.83	0.638			0.383				
900	U	10	2	15.89	17.69	1.80	0.900			0.180				
	V	10	2	13.08	14.28	1.20	0.600	0.805	17.7	0.120	17.5	0.141	34.4	77.5
	W	10	2	14.16	15.97	1.81	0.905			0.181				
	X	10	1	14.09	14.91	0.82	0.820			0.082				
1050	Y	10	1	13.36	14.26	0.90	0.900			0.090				
	Z	10	0	0.00	0.00	0.00	0.000	0.890	4.1	0.000	10.0	0.088	79.0	85.9
	AA	10	2	15.13	16.83	1.70	0.850			0.170				
	BB	10	1	14.75	15.67	0.92	0.920			0.092				

Dunnett's MSD value: **0.0747**
PMSD: **11.9**
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, 2003b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

USEPA, 2001a, 2003b. 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: 4/5/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant	
End Date: 4/12/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: FWCHR-EPA-B21-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	0.7000	0.8000	0.8000	0.8000
750	0.6000	0.7000	0.5000	0.6000
900	0.2000	0.2000	0.2000	0.1000
1050	0.1000	0.0000	0.2000	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	0.7750	0.7750	1.0762	0.9912	1.1071	5.379	4	10.00	10.00	9	40
*750	0.6000	0.6000	0.8872	0.7854	0.9912	9.469	4	10.00	10.00	16	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	40
*1050	0.1000	0.1000	0.3165	0.1588	0.4636	39.374	4	10.00	10.00	36	40

Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	Statistic	Critical	Skow	Kurt
Equality of variance cannot be confirmed	0.817	0.896	-0.4584	2.50798

Hypothesis Test (1-tail, 0.05)

NOEC	LOEC	ChV	TU
450	600	519.615	

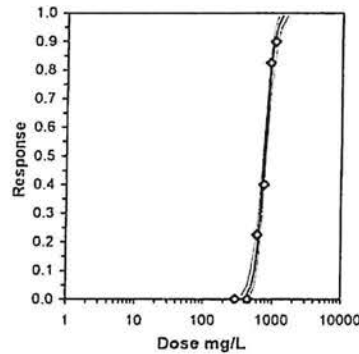
Steel's Many-One Rank Test
Treatments vs D-Control

Maximum Likelihood-Probit

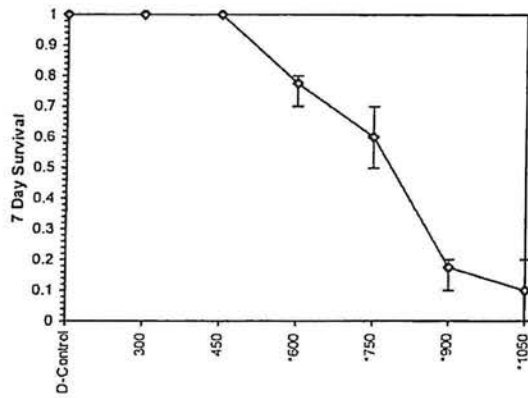
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Itr
Slope	9.73489	1.16428	7.4529	12.0169	0	3.71177	9.48773	0.44642	2.8788	0.10272	3
Intercept	-23.025	3.3621	-29.615	-16.435							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	436.348	363.239	490.719
EC05	3.355	512.67	446.811	561.117
EC10	3.718	558.673	498.39	603.375
EC15	3.964	592.021	536.083	634.179
EC20	4.158	619.938	567.668	660.236
EC25	4.326	644.936	595.839	683.908
EC40	4.747	712.489	670.307	750.596
EC50	5.000	756.489	716.43	797.228
EC60	5.253	803.207	762.642	850.18
EC75	5.674	887.337	839.298	953.825
EC80	5.842	923.117	869.996	1000.46
EC85	6.036	966.648	906.292	1058.77
EC90	6.282	1024.35	953.068	1138.23
EC95	6.645	1116.27	1025.35	1269
EC99	7.326	1311.51	1173	1560.23



Dose-Response Plot



Entered and Reviewed by
JMS/Summer
dt



Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 4/5/2022 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
 End Date: 4/12/2022 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

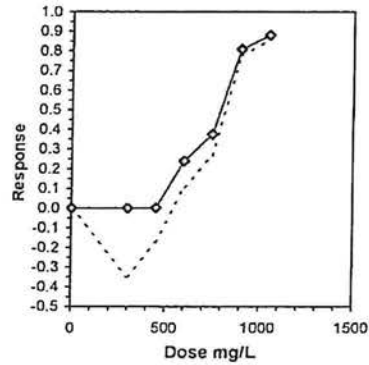
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.6230	0.6010	0.6390	0.6400
300	0.9200	0.8230	0.8160	0.8200
450	0.6600	0.7750	0.7040	0.8010
600	0.4470	0.5360	0.6430	0.6130
750	0.4640	0.5760	0.4120	0.3830
900	0.1800	0.1200	0.1810	0.0820
1050	0.0900	0.0000	0.1700	0.0920

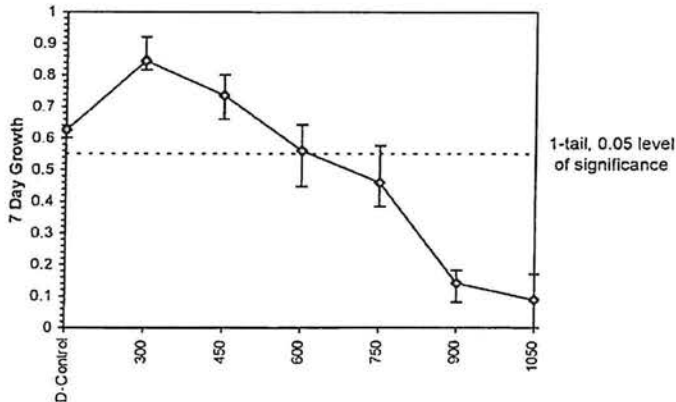
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	t-Stat		Critical	MSD	Mean	N-Mean	
D-Control	0.6258	1.0000	0.6258	0.6010	0.6400	2.916	4				0.7353	1.0000	
300	0.8448	1.3500	0.8448	0.8160	0.9200	5.948	4	-6.394	2.180	0.0747	0.7353	1.0000	
450	0.7350	1.1746	0.7350	0.6600	0.8010	8.797	4	-3.190	2.180	0.0747	0.7350	0.9997	
600	0.5598	0.8945	0.5598	0.4470	0.6430	15.657	4				0.5598	0.7613	
750	0.4588	0.7331	0.4588	0.3830	0.5760	18.539	4				0.4588	0.6239	
900	0.1408	0.2249	0.1408	0.0820	0.1810	34.424	4				0.1408	0.1914	
1050	0.0880	0.1406	0.0880	0.0000	0.1700	78.970	4				0.0880	0.1197	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.9404	0.805	0.34602	-0.2747
Bartlett's Test indicates equal variances ($p = 0.18$)	3.40078	9.21035		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnnett's Test	450	>450		
Treatments vs D-Control	MSDu	MSDp	MSB	MSE
	0.07467	0.11932	0.04796	0.00235
	F-Prob	df		
	4.5E-04	2, 9		

Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	481.25	26.16	339.24	504.04	-1.8165
IC10	512.72	19.01	432.96	560.93	-0.3114
IC15	544.18	24.63	463.86	620.95	0.4513
IC20	575.65	30.32	496.16	682.66	0.5345
IC25	612.35	38.63	521.15	733.55	0.6199
IC40	758.30	26.82	659.05	799.48	-1.5187
IC50	792.98	13.29	752.06	825.37	-0.1253



Dose-Response Plot



Entered and Reviewed by Jim Sumner



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*

PpKCICR Test Number: 88

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	JW	JW	JW	K	K
CONTROL, MHSW	pH (S.U.)	7.75	7.44 7.63	7.63	7.68	7.57	7.47	7.41
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.7	7.8	7.9	7.2
	Conductivity (µmhos/cm)	324		316			309	
	Alkalinity (mg CaCO ₃ /L)	63					63	
	Hardness (mg CaCO ₃ /L)	90					86	
	Temperature (°C)	24.8	24.8	24.7	24.9	24.7	24.8	
300 mg KCl/L	pH (S.U.)	7.80	7.64	7.85	7.59	7.80	7.43	
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.7	7.8	7.2	
	Conductivity (µmhos/cm)	869		871		860		
	Temperature (°C)	24.9	24.6	24.7	24.7	24.7	24.5	
450 mg KCl/L	pH (S.U.)	7.80	7.64	7.85	7.59	7.81	7.47	
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.7	7.8	7.2	
	Conductivity (µmhos/cm)	1110		1150		1120		
	Temperature (°C)	24.9	24.8	24.8	24.7	24.8	24.8	
600 mg KCl/L	pH (S.U.)	7.79	7.68	7.85	7.60	7.82	7.49	
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.7	7.9	7.3	
	Conductivity (µmhos/cm)	1370		1410		1370		
	Temperature (°C)	24.9	24.7	24.8	24.8	24.7	24.9	
750 mg KCl/L	pH (S.U.)	7.81	7.68	7.85	7.61	7.85	7.50	
	Dissolved oxygen (mg/L)	7.9	7.7	8.0	7.8	7.9	7.4	
	Conductivity (µmhos/cm)	1630		1680		1630		
	Temperature (°C)	25.0	24.7	24.8	24.8	24.8	24.7	
900 mg KCl/L	pH (S.U.)	7.82	7.68	7.85	7.63	7.86	7.51	
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	7.8	7.9	7.4	
	Conductivity (µmhos/cm)	1880		1940		1900		
	Temperature (°C)	24.8	24.6	24.7	24.9	24.8	24.7	
1050 mg KCl/L	pH (S.U.)	7.83	7.69	7.86	7.58	7.88	7.58	
	Dissolved oxygen (mg/L)	8.0	8.0	8.1	7.9	8.0	7.6	
	Conductivity (µmhos/cm)	2170		2220		2150		
	Temperature (°C)	24.8	24.7	24.7	24.9	24.8	24.7	
		Initial	Final	Initial	Final	Initial	Final	



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*

PpKCICR Test Number: 88

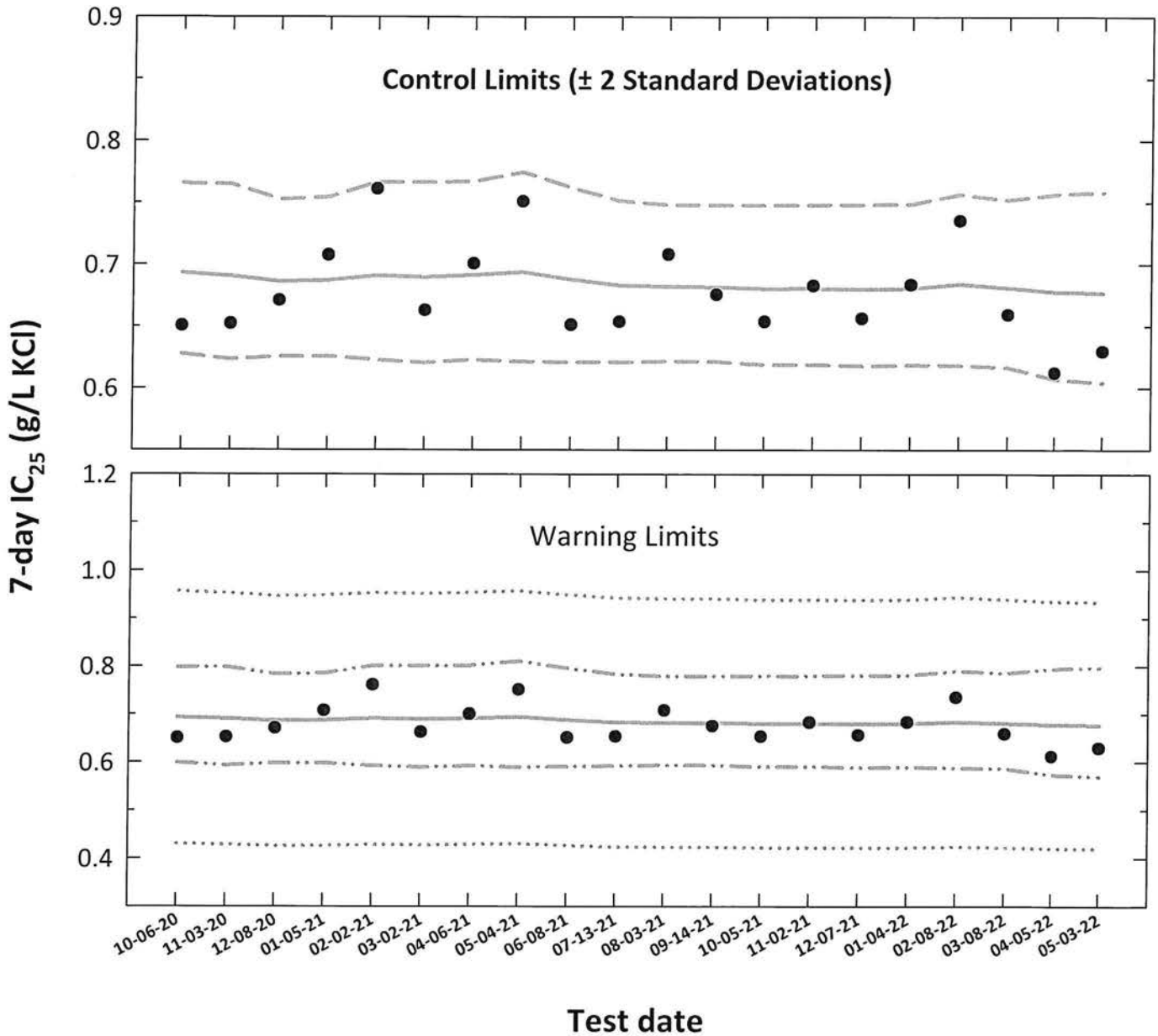
Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	U	BSU	BSL	U	U	U	U	U
CONTROL, MHSW	pH (S.U.)	7.67	7.50	7.53	7.76	7.66	7.45	7.67	7.13
	Dissolved oxygen (mg/L)	7.0	7.6	7.7	6.7	7.9	7.5	7.8	6.4
	Conductivity (µmhos/cm)	305		295		314		303	
	Alkalinity (mg CaCO ₃ /L)			58					
	Hardness (mg CaCO ₃ /L)			86					
	Temperature (°C)	24.8	24.7	24.9	24.9	24.8	24.8	24.9	24.8
300 mg KCl/L	pH (S.U.)	7.83	7.56	7.81	7.42	7.76	7.51	7.79	7.24
	Dissolved oxygen (mg/L)	7.9	7.5	7.7	6.6	7.8	7.4	7.0	6.6
	Conductivity (µmhos/cm)	834		836		843		836	
	Temperature (°C)	24.8	24.5	25.0	24.7	24.8	24.7	24.8	24.7
450 mg KCl/L	pH (S.U.)	7.84	7.63	7.87	7.45	7.80	7.56	7.81	7.26
	Dissolved oxygen (mg/L)	7.9	7.5	7.7	6.7	7.8	7.5	7.9	6.6
	Conductivity (µmhos/cm)	1080		1090		1110		1080	
	Temperature (°C)	24.9	24.5	24.9	24.7	24.9	24.7	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.86	7.62	7.90	7.45	7.84	7.57	7.83	7.30
	Dissolved oxygen (mg/L)	8.0	7.5	7.7	6.7	7.9	7.6	7.9	6.6
	Conductivity (µmhos/cm)	1350		1370		1360		1340	
	Temperature (°C)	24.8	24.7	24.9	24.9	24.9	24.9	24.8	24.7
750 mg KCl/L	pH (S.U.)	7.87	7.62	7.91	7.46	7.87	7.60	7.84	7.34
	Dissolved oxygen (mg/L)	8.0	7.4	7.7	6.6	7.9	7.6	8.0	6.5
	Conductivity (µmhos/cm)	1410		1650		1630		1590	
	Temperature (°C)	24.8	24.8	24.8	24.7	25.0	24.7	24.8	24.9
900 mg KCl/L	pH (S.U.)	7.88	7.62	7.95	7.44	7.89	7.63	7.85	7.39
	Dissolved oxygen (mg/L)	8.1	7.3	7.7	6.1	7.9	7.7	8.0	6.5
	Conductivity (µmhos/cm)	1860		1870		1910		1880	
	Temperature (°C)	24.9	24.8	25.0	24.9	25.0	24.8	24.9	24.9
1050 mg KCl/L	pH (S.U.)	7.92	7.68	7.98	7.46	7.92	7.63	7.86	7.43
	Dissolved oxygen (mg/L)	8.1	7.3	7.7	6.3	8.0	7.7	8.0	6.5
	Conductivity (µmhos/cm)	2120		2110		2130		2160	
	Temperature (°C)	24.9	24.6	25.0	24.8	24.9	24.8	24.9	24.7
		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)

Independent Review by
 Kelley E. Keenan:

Entered and Reviewed by
 Jim Sumner



Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	04-05-22	0.6124	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
20	05-03-22	0.6299	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341

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

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

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

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

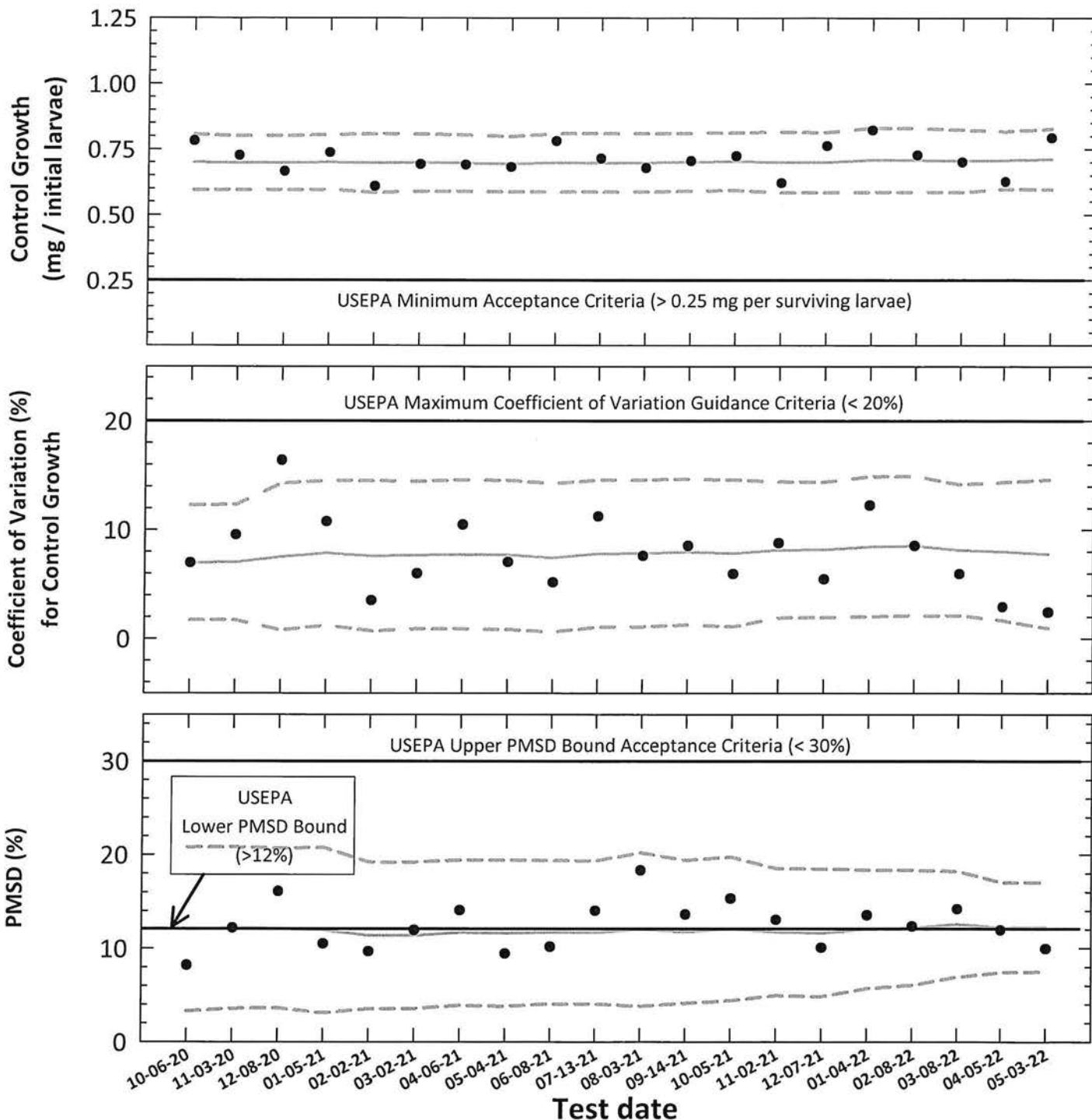
CV = Coefficient of variation.



Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture

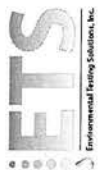


- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity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

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

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





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

Test number	Test date	ToxCal Determination					Control Growth		Control Growth CV		Test PMSD			
		Control Survival (%)	Control Growth		MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT + 2S		
			Mean (mg/initial larvae)	CV (%)									(mg/initial larvae)	(%)
1	10-06-20	100	0.782	7.0	0.0640	8.2	0.700	0.594	7.0	1.7	12.3	12.0	3.3	20.8
2	11-03-20	97.5	0.727	9.6	0.0885	12.2	0.698	0.595	7.0	1.7	12.3	12.2	3.6	20.8
3	12-08-20	100	0.666	16.4	0.1070	16.1	0.698	0.595	7.5	0.8	14.3	12.1	3.6	20.7
4	01-05-21	100	0.737	10.8	0.0774	10.5	0.700	0.596	7.9	1.2	14.5	11.9	3.1	20.8
5	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	7.6	0.7	14.5	11.4	3.5	19.2
6	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	7.7	0.9	14.5	11.4	3.5	19.2
7	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	7.7	0.9	14.6	11.7	3.9	19.4
8	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	7.7	0.8	14.6	11.6	3.8	19.4
9	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	7.4	0.6	14.3	11.7	4.0	19.4
10	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	7.8	1.1	14.6	11.7	4.0	19.3
11	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	7.8	1.1	14.6	12.0	3.8	20.2
12	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	8.0	1.3	14.7	11.7	4.1	19.4
13	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	7.9	1.1	14.6	12.1	4.4	19.7
14	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	8.2	1.9	14.4	11.7	4.9	18.5
15	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	8.2	2.0	14.4	11.6	4.8	18.5
16	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	8.5	2.0	14.9	12.0	5.7	18.3
17	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	8.5	2.1	14.9	12.2	6.0	18.3
18	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	8.1	2.1	14.2	12.6	6.9	18.2
19	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	8.0	1.6	14.4	12.2	7.4	17.0
20	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	7.8	0.9	14.6	12.3	7.5	17.0

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



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

Species: *Pimephales promelas*

PpKCICR Test Number: 89

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2078</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)	1989	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>04-27-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>05-02-22 1505 TO 04-22 0456</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>8.08</u> S.U. Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>05-10-22 0725</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>05-11-22 0730</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	05-03-22	0500	<u>H</u>	1100	<u>K</u>	0710	<u>H</u>	<u>04-27-22 B</u>
1	05-04-22	0500	<u>H</u>	1100	<u>K</u>	0700	<u>H</u>	<u>↓</u>
2	05-05-22	0500	<u>H</u>	1100	<u>H</u>	0700	<u>H</u>	<u>04-27-22 D</u>
3	05-06-22	0500	<u>H</u>	1100	<u>K</u>	0700	<u>H</u>	<u>↓</u>
4	05-07-22	0600	<u>H</u>	1200	<u>H</u>	0800	<u>H</u>	<u>05-04-22 A</u>
5	05-08-22	0500	<u>H</u>	1100	<u>H</u>	0700	<u>H</u>	<u>↓</u>
6	05-09-22	0500	<u>H</u>	1100	<u>H</u>	0700	<u>H</u>	<u>05-04-22 B</u>
7	05-10-22					0610	<u>H</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>759.5</u>
Average weight per initial larvae:	<u>0.793</u>		NOEC (mg/L KCl)	<u>450</u>
Average weight per surviving larvae:	<u>0.793</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>600</u>
			ChV (mg/L KCl)	<u>519.6</u>
			IC ₂₅ (mg/L KCl)	<u>629.9</u>



Species: Pimephales promelas

PpKICR Test Number: 89

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>Forest green</u> Analyst: <u>A.S</u> Date: <u>04-23-22</u>	15.41	14.67	14.46	15.90	14.63	14.42	15.18	15.15	15.03	14.80	14.05	14.74
*B = Pan + Larvae weight (mg) Analyst: <u>FG</u> Date: <u>05-16-22</u>	23.10	22.66	22.36	24.05	22.82	22.28	24.18	23.18	23.11	23.67	23.23	22.39
C = Larvae weight (mg) = B - A Analyst: <u>JK</u>	7.69	7.99	7.90	8.15	8.19	7.86	9.00	8.03	8.08	8.87	9.18	7.65
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JK</u>	0.769	0.799	0.790	0.815	0.819	0.786	0.900	0.803	0.808	0.887	0.918	0.765
Average weight per initial number of larvae (mg)	0.793				0.827			-4.37		0.845		-6.57
Percent reduction from control (%)												

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

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

Comments:



Species: Pimephales promelas

PpKCICR Test Number: 89

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	10 ^{9th}	10 ^{8th}	10 ^{8th}	10 ^{9th}			
2	10	10	10	10	10	10	10	10	9	7 ^d	8	9			
3	10	10	10	10	9 th	9 th	10	9 th	9	7	8	9			
4	10	9 th	10	9 th	8 th	8 th	7 th	7 th	6 th	6 th	6 th	6 th			
5	10	9	10	9	5 th	6 th	6 th	6 th	3 th	2 th	3 th	2 th			
6	9 th	9	9 th	9	5	6	6	6	2 th	2	2 th	2			
7	8 th	9	9	9	5	6 ¹⁶	6	6 ^{15M}	2	2	1 th	2			
*A = Pan weight (mg) Tray color code: <u>Forest green</u> Analyst: <u>A.J.</u> Date: <u>04-22-22</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>TG</u> Date: <u>05-16-22</u>															
C = Larvae weight (mg) = B - A Analyst: <u>J</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.663		16.57		0.430		45.87		0.107		86.57	

*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: 89

Survival and Growth Data

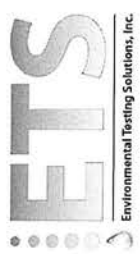
Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10	10	10	10	
1	4^{hd} 10	4^{hd} 10	3rd 10	3rd 10 <i>05-01-22 H</i>	
2	4	4	3	2 ^{id}	
3	2 rd 2	4	2 ^{id} 2	2	
4	1 ^{id}	2 ^{id}	1 ^{id}	1 ^{id}	
5	1	0 rd	0 ^{id}	0 ^{id}	
6	1	0	0	0	
7	SM 1	0	0	0	
*A = Pan weight (mg) Tray color code: <u>Forest green</u> Analyst: <u>A.S</u> Date: <u>04-22-22</u>		14.04	13.94	16.02	13.91 <i>05-17-22 H</i>
*B = Pan + Larvae weight (mg) Analyst: <u>JG</u> Date: 04-22-22 <u>05-16-22</u>		14.32	13.94	16.02	13.91 <i>05-17-22 H</i>
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>		0.28			<i>05-17-22 H</i>
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>		0.028	0	0	0
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.007	99.17

*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:





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: 89
 Test dates: May 03-10, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pen weight (mg)	B = Pen + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (from weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	15.41	23.10	7.69	0.769	0.769		0.769		0.769		
	B	10	10	14.67	22.66	7.99	0.799	0.799	2.4	0.799	100.0	0.793	2.4	Not applicable
	C	10	10	14.46	22.36	7.90	0.790	0.790		0.790		0.790		
	D	10	10	15.90	24.05	8.15	0.815	0.815		0.815		0.815		
300	E	10	10	14.63	22.82	8.19	0.819	0.819		0.819		0.819		
	F	10	10	14.42	22.28	7.86	0.786	0.786	6.1	0.786	100.0	0.827	6.1	-4.3
	G	10	10	15.18	24.18	9.00	0.900	0.900		0.900		0.900		
	H	10	10	15.15	23.18	8.03	0.803	0.803		0.803		0.803		
450	I	10	10	15.03	23.11	8.08	0.808	0.808		0.808		0.808		
	J	10	10	14.80	23.67	8.87	0.887	0.887	8.3	0.887	100.0	0.845	8.3	-6.5
	K	10	10	14.05	23.23	9.18	0.918	0.918		0.918		0.918		
	L	10	10	14.74	22.39	7.65	0.765	0.765		0.765		0.765		
600	M	10	8	13.68	20.29	6.61	0.826	0.826		0.661		0.661		
	N	10	9	14.89	21.22	6.33	0.703	0.703	6.7	0.633	87.5	0.663	3.4	16.5
	O	10	9	12.83	19.53	6.70	0.744	0.744		0.670		0.670		
	P	10	9	15.50	22.36	6.86	0.762	0.762		0.686		0.686		
750	Q	10	5	13.21	16.72	3.51	0.702	0.702		0.351		0.351		
	R	10	6	15.11	21.46	6.35	1.058	1.058		0.635	57.5	0.430	35.9	45.8
	S	10	6	14.07	18.63	4.56	0.760	0.760	32.7	0.456		0.456		
	T	10	6	15.87	18.66	2.79	0.465	0.465		0.279		0.279		
900	U	10	2	12.61	14.04	1.43	0.715	0.715		0.143		0.143		
	V	10	2	16.35	17.53	1.18	0.590	0.590	12.9	0.118	17.5	0.107	31.7	86.5
	W	10	1	14.60	15.22	0.62	0.620	0.620		0.062		0.062		
	X	10	2	12.65	13.70	1.05	0.525	0.525		0.105		0.105		
1050	Y	10	1	14.04	14.32	0.28	0.280	0.280		0.028		0.028		
	Z	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	2.5	0.007	200.0	99.1
	AA	10	0	0.00	0.00	0.00	0.000	0.000		0.000		0.000		
	BB	10	0	0.00	0.00	0.00	0.000	0.000		0.000		0.000		

Dunnett's MSD value: 0.0790
 PMSD: 10.0
 MSD = Minimum Significant Difference
 PMSD = Percent Minimum Significant Difference

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



Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 5/3/2022	Test ID: PpKClCR	Sample ID: REF-Ref Toxicant
End Date: 5/10/2022	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	0.8000	0.9000	0.9000	0.9000
750	0.5000	0.6000	0.6000	0.6000
900	0.2000	0.2000	0.1000	0.2000
1050	0.1000	0.0000	0.0000	0.0000

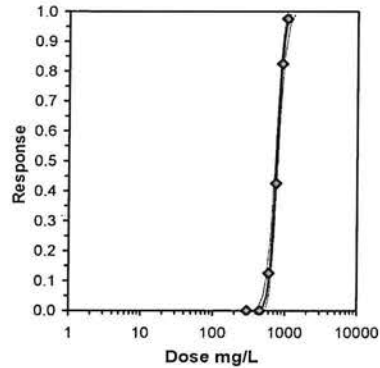
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*600	0.8750	0.8750	1.2136	1.1071	1.2490	5.846	4	10.00	10.00	5	40
*750	0.5750	0.5750	0.8609	0.7854	0.8861	5.847	4	10.00	10.00	17	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.87081	0.896	-0.3641	2.01277
Equality of variance cannot be confirmed				

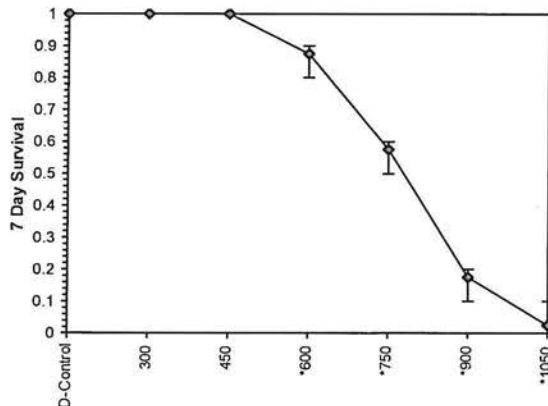
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	450	600	519.615	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	12.694	1.542	9.67171	15.7164	0	0.96317	9.48773	0.91533	2.88052	0.07878	3
Intercept	-31.565	4.45987	-40.307	-22.824							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	498.029	429.401	547.448
EC05	3.355	563.561	503.538	606.734
EC10	3.718	601.951	547.656	641.509
EC15	3.964	629.319	579.224	666.506
EC20	4.158	651.955	605.288	687.426
EC25	4.326	672.022	628.268	706.25
EC40	4.747	725.371	688.021	758.301
EC50	5.000	759.483	724.5	793.815
EC60	5.253	795.199	760.743	833.364
EC75	5.674	858.326	819.997	909.076
EC80	5.842	884.746	843.337	942.597
EC85	6.036	916.569	870.624	984.09
EC90	6.282	958.242	905.317	1039.93
EC95	6.645	1023.52	957.985	1130.12
EC99	7.326	1158.19	1062.56	1324.2



Dose-Response Plot



Entered and Reviewed by
Jan Sumner
JS

KK

Larval Fish Growth and Survival Test-7 Day Growth

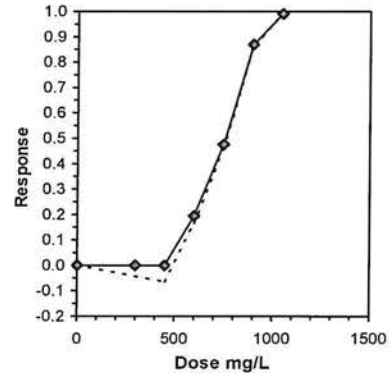
Start Date: 5/3/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 5/10/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	0.7690	0.7990	0.7900	0.8150
300	0.8190	0.7860	0.9000	0.8030
450	0.8080	0.8870	0.9180	0.7650
600	0.6610	0.6330	0.6700	0.6860
750	0.3510	0.6350	0.4560	0.2790
900	0.1430	0.1180	0.0620	0.1050
1050	0.0280	0.0000	0.0000	0.0000

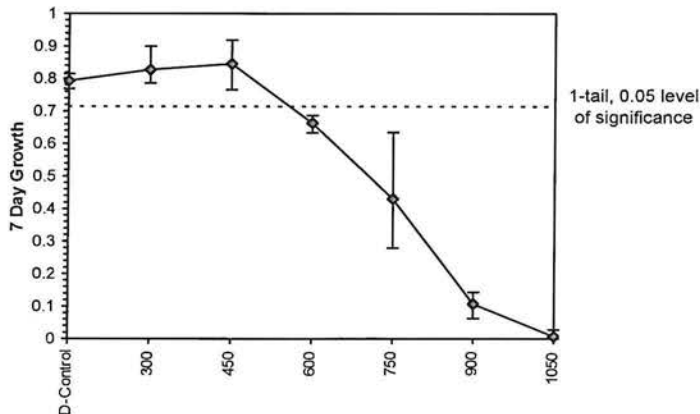
Conc-mg/L	Transform: Untransformed							1-Tailed		Isotonic		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7933	1.0000	0.7933	0.7690	0.8150	2.419	4				0.8216	1.0000
300	0.8270	1.0425	0.8270	0.7860	0.9000	6.106	4	-0.932	2.180	0.0790	0.8216	1.0000
450	0.8445	1.0646	0.8445	0.7650	0.9180	8.334	4	-1.415	2.180	0.0790	0.8216	1.0000
600	0.6625	0.8352	0.6625	0.6330	0.6860	3.354	4				0.6625	0.8064
750	0.4303	0.5424	0.4303	0.2790	0.6350	35.942	4				0.4303	0.5237
900	0.1070	0.1349	0.1070	0.0620	0.1430	31.675	4				0.1070	0.1302
1050	0.0070	0.0088	0.0070	0.0000	0.0280	200.000	4				0.0070	0.0085

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95779	0.805	0.24641	-0.3992
Bartlett's Test indicates equal variances ($p = 0.17$)	3.54529	9.21035		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	450	>450		
Treatments vs D-Control	MSDu	MSDp	MSB	MSE
	0.07896	0.09954	0.00271	0.00262
	F-Prob	df		
	0.39409	2, 9		

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL(Exp)	Skew	
IC05	488.73	9.47	458.28	499.69	-5.7116
IC10	527.47	8.44	503.66	550.93	-0.7143
IC15	566.20	11.06	535.48	602.78	0.1681
IC20	603.38	11.28	564.90	638.31	-0.2090
IC25	629.91	17.82	596.98	708.01	2.5785
IC40	709.50	29.27	647.10	812.63	0.4212
IC50	759.03	26.10	676.38	824.89	-0.2375



Dose-Response Plot



Entered and Reviewed by Jim Sumner
JS

Species: *Pimephales promelas*

PpKCICR Test Number: 89

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	
Concentration	Analyst	JLW	JW	JW	W	W	W
Parameter							
CONTROL, MHSW	pH (S.U.)	7.80	7.73	7.73	7.69	7.77	7.50
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.6	7.7	7.0
	Conductivity (µmhos/cm)	310		306		280	
	Alkalinity (mg CaCO ₃ /L)	62				63	
	Hardness (mg CaCO ₃ /L)	88				88	
	Temperature (°C)	24.7	24.9	24.8	24.6	24.7	24.8
300 mg KCl/L	pH (S.U.)	8.12	7.74	7.88	7.63	7.69	7.52
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.6	7.7	7.0
	Conductivity (µmhos/cm)	897		868		812	
	Temperature (°C)	24.8	24.7	24.8	24.8	24.8	24.7
450 mg KCl/L	pH (S.U.)	8.08	7.72	7.86	7.64	7.65	7.52
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.6	7.7	6.9
	Conductivity (µmhos/cm)	1160		1130		1070	
	Temperature (°C)	24.8	24.7	24.8	24.7	24.8	24.7
600 mg KCl/L	pH (S.U.)	8.06	7.73	7.85	7.62	7.64	7.53
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.6	7.6	6.8
	Conductivity (µmhos/cm)	1430		1380		1320	
	Temperature (°C)	24.9	24.6	24.9	24.7	24.9	24.6
750 mg KCl/L	pH (S.U.)	8.05	7.73	7.85	7.63	7.67	7.52
	Dissolved oxygen (mg/L)	8.0	8.0	7.9	7.6	7.6	6.9
	Conductivity (µmhos/cm)	1690		1650		1560	
	Temperature (°C)	24.9	24.9	24.9	24.9	24.8	24.8
900 mg KCl/L	pH (S.U.)	8.04	7.73	7.89	7.62	7.66	7.52
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	7.6	7.9	6.9
	Conductivity (µmhos/cm)	1970		1930		1830	
	Temperature (°C)	24.7	24.9	24.8	24.6	24.9	24.6
1050 mg KCl/L	pH (S.U.)	8.04	7.80	7.89	7.63	7.69	7.50
	Dissolved oxygen (mg/L)	8.0	8.0	8.1	7.4	8.0	7.1
	Conductivity (µmhos/cm)	2240		2180		2000	
	Temperature (°C)	24.9	24.8	24.8	24.9	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final



Species: *Pimephales promelas*

PpKCICR Test Number: 89

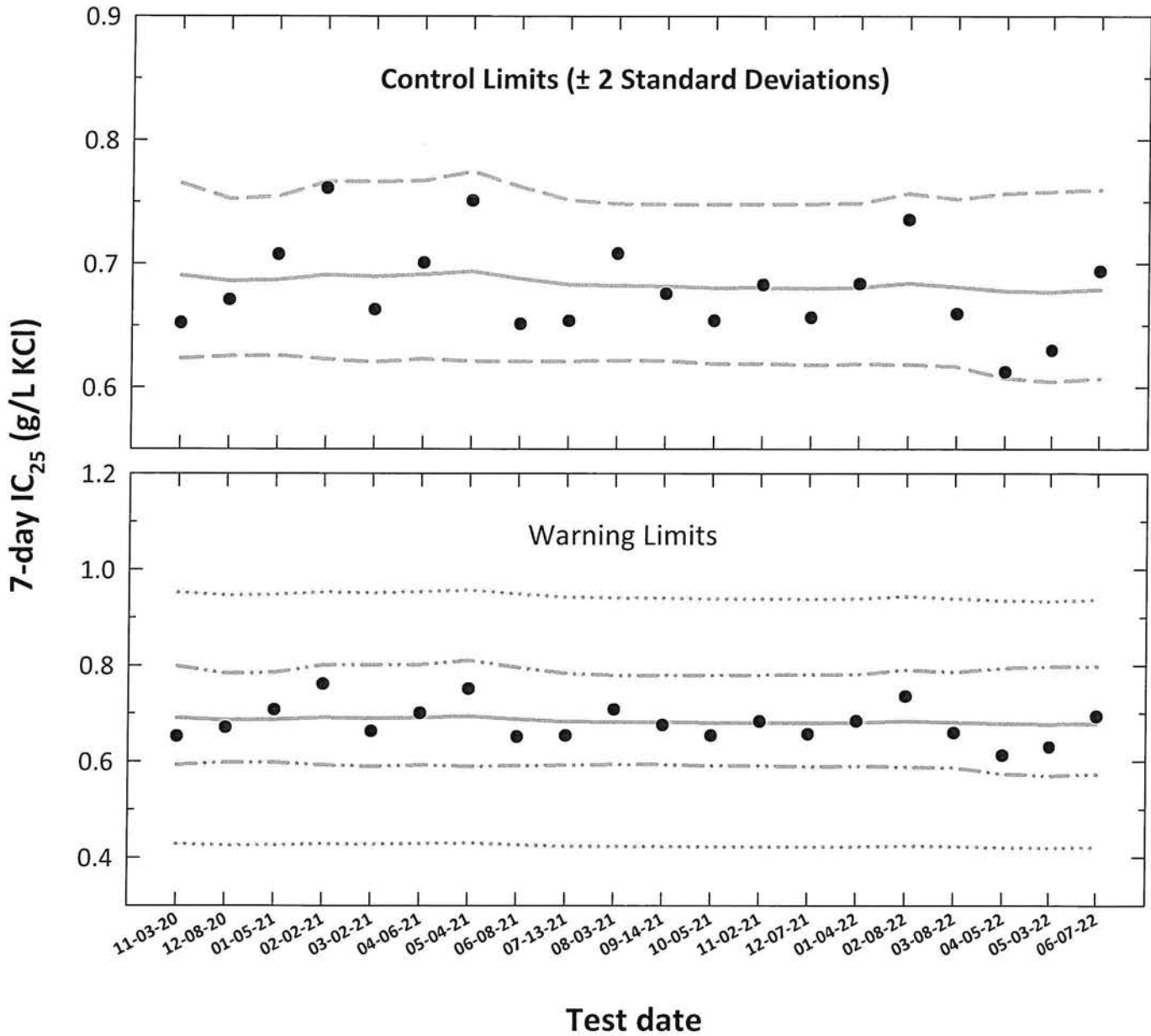
Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
Analyst		W	BSL	BSL	BSL	BSL	W	W	W	
CONTROL, MHSW	pH (S.U.)	7.78	7.63	7.66	7.58	7.71	7.50	7.69	7.26	
	Dissolved oxygen (mg/L)	7.7	7.4	7.7	7.4	7.7	7.1	7.0	5.9	
	Conductivity (µmhos/cm)	295		297		300		302		
	Alkalinity (mg CaCO ₃ /L)			60				63		
	Hardness (mg CaCO ₃ /L)			86				86		
	Temperature (°C)	24.8	24.9	24.7	25.2	24.8	24.8	24.7	24.8	
300 mg KCl/L	pH (S.U.)	7.88	7.61	7.82	7.58	7.83	7.53	7.91	7.34	
	Dissolved oxygen (mg/L)	7.9	7.4	7.7	7.4	7.8	7.1	8.6	5.9	
	Conductivity (µmhos/cm)	820		827		842		847		
	Temperature (°C)	24.7	24.8	24.7	25.0	24.9	24.8	24.7	24.8	
450 mg KCl/L	pH (S.U.)	7.86	7.61	7.85	7.58	7.87	7.53	7.90	7.34	
	Dissolved oxygen (mg/L)	7.9	7.4	7.8	7.3	7.8	7.1	8.0	5.7	
	Conductivity (µmhos/cm)	1060		1080		1100		1100		
	Temperature (°C)	24.7	24.8	24.7	25.0	24.9	24.9	24.7	24.7	
600 mg KCl/L	pH (S.U.)	7.86	7.60	7.87	7.58	7.89	7.52	7.89	7.37	
	Dissolved oxygen (mg/L)	8.0	7.4	7.8	7.3	7.8	7.1	8.0	5.6	
	Conductivity (µmhos/cm)	1320		1340		1360		1360		
	Temperature (°C)	24.8	24.8	24.7	24.9	24.9	24.7	24.8	24.8	
750 mg KCl/L	pH (S.U.)	7.86	7.63	7.87	7.57	7.90	7.54	7.89	7.46	
	Dissolved oxygen (mg/L)	8.0	7.4	7.9	7.2	7.8	6.9	8.0	5.7	
	Conductivity (µmhos/cm)	1570		1550		1620		1610		
	Temperature (°C)	24.8	24.9	24.7	24.9	24.8	24.9	24.8	24.6	
900 mg KCl/L	pH (S.U.)	7.87	7.63	7.89	7.61	7.92	7.60	7.89	7.44	
	Dissolved oxygen (mg/L)	8.0	7.4	8.0	7.2	7.9	6.9	8.0	5.5	
	Conductivity (µmhos/cm)	1810		1870		1900		1890		
	Temperature (°C)	24.8	25.0	24.6	24.9	24.8	24.7	24.8	24.6	
1050 mg KCl/L	pH (S.U.)	7.88	7.68	7.90	7.62	7.93	7.62	7.92	7.43	
	Dissolved oxygen (mg/L)	8.1	7.4	8.0	7.3	7.9	7.4	8.0	5.5	
	Conductivity (µmhos/cm)	2090		2120		2110		2130		
	Temperature (°C)	24.8	24.8	24.6	25.1	24.9	24.7	24.8	24.6	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	



Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	04-05-22	0.6124	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
19	05-03-22	0.6299	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
20	06-07-22	0.6939	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372

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

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

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

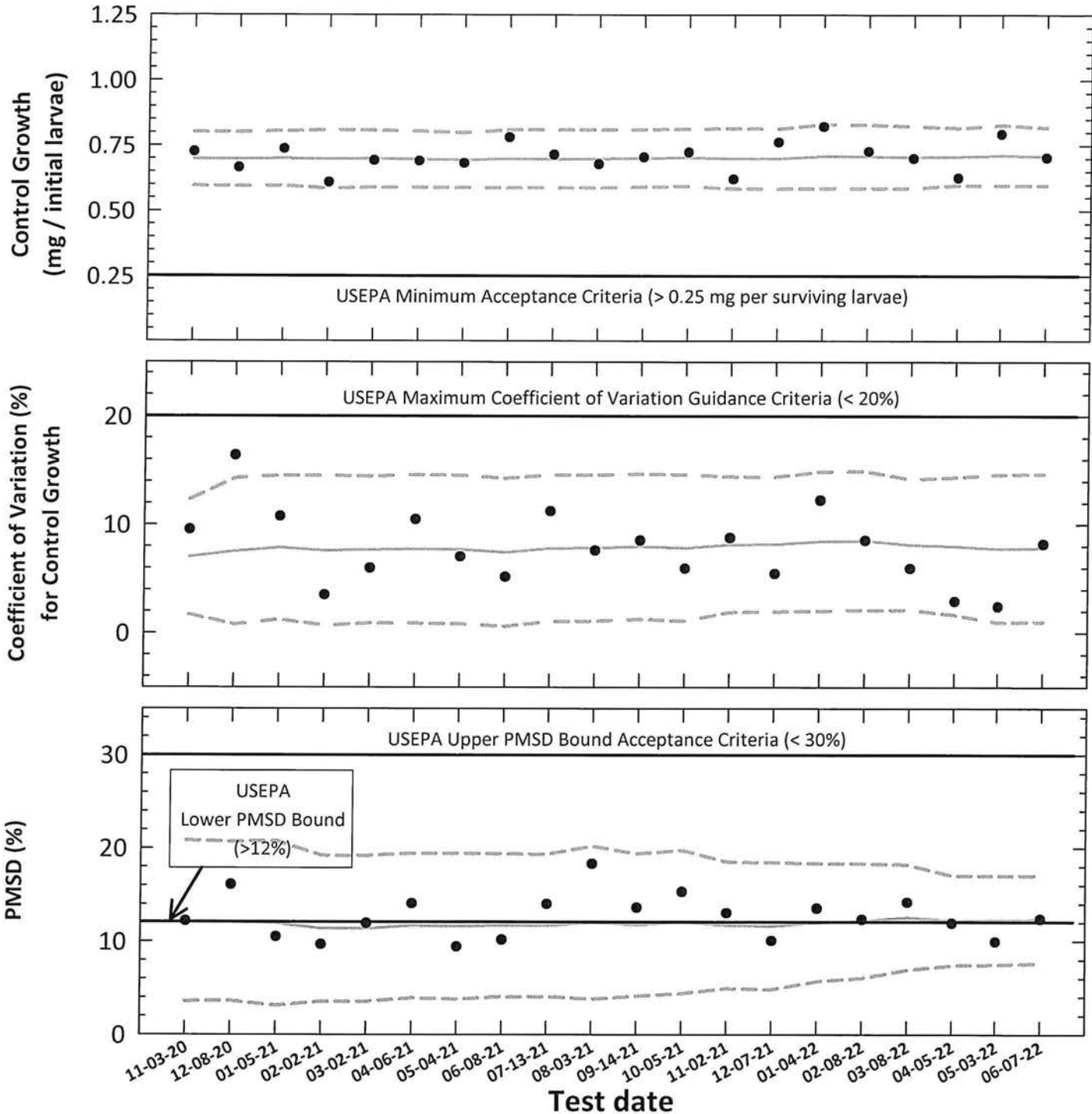
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

CV = Coefficient of variation.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

Entered and Reviewed by
Jim Sumner
JS

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		CV (%)	MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)	Mean (mg/initial larvae)					CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	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	
2	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	
3	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	
4	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	
5	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	
6	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	
7	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	
8	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	
9	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.588	0.810	7.8	1.1	14.6	11.7	4.0	19.3	
10	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.587	0.810	7.8	1.1	14.6	12.0	3.8	20.2	
11	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	
12	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	
13	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	
14	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	
15	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	
16	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	
17	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2	
18	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0	
19	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0	
20	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0	

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
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: 90

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2101</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>05-31-22</u>	Artemia CHM number:	CHM1149
Hatch dates and times:	<u>06-01-22 1500 to 06-07-22 0450</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.83</u> S.U. Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>06-14-22 0200</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>06-15-22 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	06-07-22	0455	JL	1100	JL	0705	JL	06-01-22 C
1	06-08-22	0500	JL	1100	JL	0700	JL	↓
2	06-09-22	0500	JL	1100	JL	0700	JL	06-06-22 B
3	06-10-22	0500	JL	1100	JL	0700	JL	↓
4	06-11-22	0530	JL	1215	JL	0730	JL	06-06-22 C
5	06-12-22	0500	JL	1100	JL	0700	JL	↓
6	06-13-22	0500	JL	1100	JL	0700	JL	06-09-22 B
7	06-14-22					0605	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>13064685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>806.5</u>
Average weight per initial larvae:	<u>0.704</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.704</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>643.9</u>

Species: Pimephales promelas

PpKICR Test Number: 90

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>TG</u> Date: <u>05-22-22</u>	14.47	15.86	14.83	15.74	14.59	13.86	14.50	13.64	15.11	14.77	15.09	13.99
*B = Pan + Larvae weight (mg) Analyst: <u>TG</u> Date: <u>06-14-22</u>	20.75	23.30	22.36	22.63	23.39	^{TG} 23.77 <u>22.88</u>	23.35	22.99	22.55	21.54	23.04	20.84
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	6.28	7.44	7.53	6.89	8.80	9.02	8.85	9.25	7.44	6.77	7.95	6.85
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0.628	0.744	0.753	0.689	0.880	0.902	0.885	0.925	0.744	0.677	0.795	0.685
Average weight per initial number of larvae (mg)	0.704				0.898				0.725			
Percent reduction from control (%)									-27.67			
									-3.17			

*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: 90

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	g ^{2d}	g ^{2d}	g ^{2d}	g ^{2d}			
2	10	10	10	10	10	10	10	10	8	8	7 ^{1d}	8			
3	10	10	10	10	10	10	10	10	8	8	7	8			
4	10	10	10	10	10	9 ^{1d}	8 ^{2d}	10	6 ^{2d}	6 ^{2d}	6 ^{1d}	7			
5	10	10	10	10	9 ^{1d}	9	8	8 ^{2d}	2 ^{4d}	2 ^{4d}	2 ^{4d}	2 ^{5d}			
6	10	10	10	10	9	9	8	7 ^{1d}	1 ^{1d}	2	1 ^{1d}	1 ^{1d}			
7	10	10	10	10	8 ^{1d}	8 ^{1d}	7 ^{1d}	7	1 ¹⁶	2	1	1 ¹⁶			
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>TL</u> Date: <u>05-22-22</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>TL</u> Date: <u>06-19-22</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 (%)		0.659		2.07		0.548		22.27		0.132		81.37	

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

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

Comments:

Species: Pimephales promelas

PpKCICR Test Number: 90

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	6 ^{4d}	5 ^{5d}	5 ^{5d}	5 ^{5d}
2	5 ^{1d}	5	5	5
3	5	5	4 ^{1d}	5
4	3 ^{2d}	4 ^{1d}	4	4 ^{1d}
5	2 ^{1d}	1 ^{2d}	1 ^{2d}	1 ^{2d}
6	1 ^{1d}	0 ^{1d}	0 ^{1d}	0 ^{1d}
7	1	0	0	0
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>TG</u> Date: <u>05-22-22</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>TG</u> Date: <u>06-19-22</u>				
C = Larvae weight (mg) = B - A Analyst: <u>Y</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>Y</u>				
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		
0.018		97.57		

*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:

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: 90
Test dates: June 07-14, 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	14.47	20.75	6.28	0.628	0.704	8.2	0.628	100.0	0.704	8.2	Not applicable
	B	10	10	15.86	23.30	7.44	0.744			0.744				
	C	10	10	14.83	22.36	7.53	0.753			0.753				
	D	10	10	15.74	22.63	6.89	0.689			0.689				
300	E	10	10	14.59	23.39	8.80	0.880	0.898	2.3	0.880	100.0	0.898	2.3	-27.6
	F	10	10	13.86	22.88	9.02	0.902			0.902				
	G	10	10	14.50	23.35	8.85	0.885			0.885				
	H	10	10	13.64	22.89	9.25	0.925			0.925				
450	I	10	10	15.11	22.55	7.44	0.744	0.725	7.6	0.744	100.0	0.725	7.6	-3.1
	J	10	10	14.77	21.54	6.77	0.677			0.677				
	K	10	10	15.09	23.04	7.95	0.795			0.795				
	L	10	10	13.99	20.84	6.85	0.685			0.685				
600	M	10	10	14.70	22.09	7.39	0.739	0.689	10.0	0.739	100.0	0.689	10.0	2.0
	N	10	10	13.71	20.37	6.66	0.666			0.666				
	O	10	10	14.41	20.43	6.02	0.602			0.602				
	P	10	10	15.71	23.21	7.50	0.750			0.750				
750	Q	10	8	13.38	19.82	6.44	0.805	0.730	11.8	0.644	75.0	0.548	14.4	22.2
	R	10	8	14.08	19.39	5.31	0.664			0.531				
	S	10	7	13.73	19.35	5.62	0.803			0.562				
	T	10	7	13.43	17.96	4.53	0.647			0.453				
900	U	10	1	14.59	15.75	1.16	1.160	1.094	21.6	0.116	12.5	0.132	28.2	81.3
	V	10	2	14.23	16.02	1.79	0.895			0.179				
	W	10	1	14.37	15.29	0.92	0.920			0.092				
	X	10	1	13.48	14.88	1.40	1.400			0.140				
1050	Y	10	1	13.91	14.62	0.71	0.710	0.710	0.0	0.071	2.5	0.018	200.0	97.5
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.0871
PMSD: 12.4

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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



Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	6/7/2022	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	6/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.8000	0.8000	0.7000	0.7000
900	0.1000	0.2000	0.1000	0.1000
1050	0.1000	0.0000	0.0000	0.0000

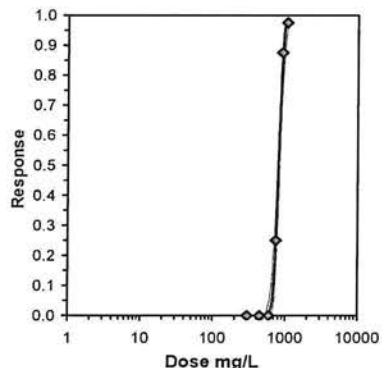
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	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	0.7500	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	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.78807	0.896	1.41279	2.51678
Equality of variance cannot be confirmed				

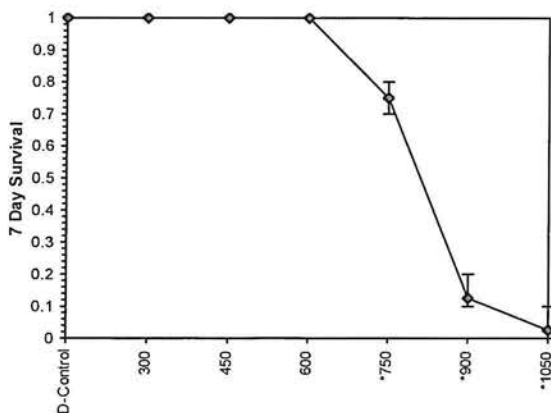
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	20.7018	2.91835	14.9818	26.4218	0	1.75953	9.48773	0.77988	2.90663	0.0483	3
Intercept	-55.172	8.50258	-71.838	-38.507							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	622.663	557.053	665.236
EC05	3.355	671.695	617.119	707.594
EC10	3.718	699.394	651.282	731.786
EC15	3.964	718.724	675.072	748.929
EC20	4.158	734.467	694.325	763.141
EC25	4.326	748.248	711.013	775.837
EC40	4.747	784.132	753.197	810.567
EC50	5.000	806.542	778.113	833.977
EC60	5.253	829.593	802.254	859.772
EC75	5.674	869.377	840.444	908.314
EC80	5.842	885.689	855.069	929.448
EC85	6.036	905.09	871.887	955.305
EC90	6.282	930.105	892.869	989.585
EC95	6.645	968.46	923.954	1043.73
EC99	7.326	1044.72	983.37	1155.59



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

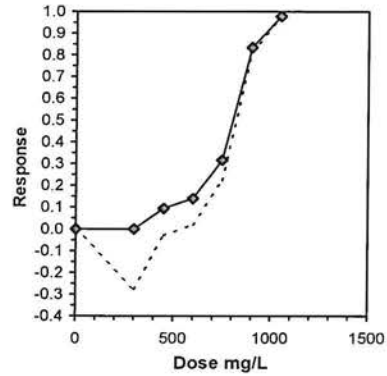
Start Date: 6/7/2022	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 6/14/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	0.6280	0.7440	0.7530	0.6890
300	0.8800	0.9020	0.8850	0.9250
450	0.7440	0.6770	0.7950	0.6850
600	0.7390	0.6660	0.6020	0.7500
750	0.6440	0.5310	0.5620	0.4530
900	0.1160	0.1790	0.0920	0.1400
1050	0.0710	0.0000	0.0000	0.0000

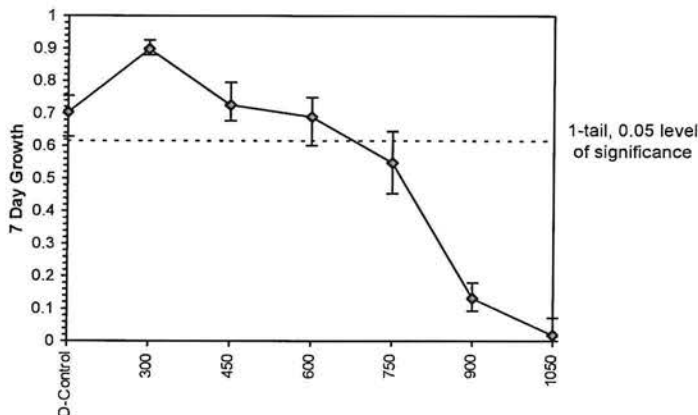
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7035	1.0000	0.7035	0.6280	0.7530	8.207	4				0.8008	1.0000
300	0.8980	1.2765	0.8980	0.8800	0.9250	2.262	4	-5.114	2.290	0.0871	0.8008	1.0000
450	0.7253	1.0309	0.7253	0.6770	0.7950	7.621	4	-0.572	2.290	0.0871	0.7253	0.9057
600	0.6893	0.9797	0.6893	0.6020	0.7500	10.023	4	0.375	2.290	0.0871	0.6893	0.8608
750	0.5475	0.7783	0.5475	0.4530	0.6440	14.430	4				0.5475	0.6837
900	0.1318	0.1873	0.1318	0.0920	0.1790	28.158	4				0.1318	0.1645
1050	0.0178	0.0252	0.0178	0.0000	0.0710	200.000	4				0.0178	0.0222

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.9578	0.844	-0.2702	-0.8877						
Bartlett's Test indicates equal variances ($p = 0.35$)	3.29599	11.3449								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	600	>600			0.0871	0.12381	0.03774	0.00289	4.4E-04	3, 12

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL(Exp)	Skew	
IC05	379.54	37.51	329.31	563.10	1.3917
IC10	469.06	67.64	352.63	698.02	0.5012
IC15	609.11	53.72	345.63	675.05	-1.1258
IC20	651.48	31.86	541.61	743.21	-0.1814
IC25	693.85	30.33	602.97	790.13	0.1015
IC40	774.19	12.57	722.87	804.83	-0.6297
IC50	803.08	9.73	769.71	827.80	-0.3774



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Pimephales promelas

PpKCICR Test Number: 90

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					
CONTROL, MHSW	pH (S.U.)	7.72	7.64	7.59	7.67	7.70	7.66	
	Dissolved oxygen (mg/L)	7.7	7.0	7.7	7.0	7.0	7.0	
	Conductivity (µmhos/cm)	298		296		301		
	Alkalinity (mg CaCO ₃ /L)	64		7.6-11.2		63		
	Hardness (mg CaCO ₃ /L)	87				87		
	Temperature (°C)	24.8	24.7	24.9	24.8	24.8	24.4	
	300 mg KCl/L	pH (S.U.)	7.07	7.03	7.01	7.65	7.08	7.62
Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.1	7.7	7.1		
Conductivity (µmhos/cm)	843		819		831			
Temperature (°C)	24.9	24.7	24.7	24.6	24.9	24.6		
450 mg KCl/L	pH (S.U.)	7.06	7.62	7.01	7.03	7.07	7.61	
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.0	7.7	6.7	
	Conductivity (µmhos/cm)	1090		1060		1080		
	Temperature (°C)	24.9	24.7	24.7	24.6	24.9	24.6	
600 mg KCl/L	pH (S.U.)	7.06	7.61	7.00	7.62	7.07	7.61	
	Dissolved oxygen (mg/L)	7.9	7.4	7.9	6.9	7.7	6.8	
	Conductivity (µmhos/cm)	1360		1310		1340		
	Temperature (°C)	25.0	24.9	24.8	24.4	24.9	24.6	
750 mg KCl/L	pH (S.U.)	7.07	7.62	7.01	7.63	7.08	7.62	
	Dissolved oxygen (mg/L)	7.9	7.6	8.0	6.9	7.8	7.0	
	Conductivity (µmhos/cm)	1600		1550		1580		
	Temperature (°C)	24.8	24.6	24.8	24.7	25.0	24.7	
900 mg KCl/L	pH (S.U.)	7.08	7.62	7.02	7.64	7.08	7.66	
	Dissolved oxygen (mg/L)	8.0	7.7	8.1	7.1	7.8	7.0	
	Conductivity (µmhos/cm)	1860		1800		1830		
	Temperature (°C)	24.8	24.9	24.9	24.7	25.0	24.5	
1050 mg KCl/L	pH (S.U.)	7.91	7.64	7.02	7.64	7.09	7.66	
	Dissolved oxygen (mg/L)	8.0	7.8	8.2	7.2	7.9	7.4	
	Conductivity (µmhos/cm)	2130		2060		2100		
	Temperature (°C)	24.9	24.9	24.9	24.8	25.0	24.7	
		Initial	Final	Initial	Final	Initial	Final	

Species: Pimephales promelas

PpKCICR Test Number: 90

Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
Analyst		JW	BSL	BSL	BSL	BSL	N	N	N	
CONTROL, MHSW	pH (S.U.)	7.68	7.52	7.44	7.62	7.62	7.62	7.57	7.34	
	Dissolved oxygen (mg/L)	7.7	7.5	7.7	7.8	7.7	7.6	7.6	6.7	
	Conductivity (µmhos/cm)	305		304		300		303		
	Alkalinity (mg CaCO ₃ /L)	7.06-11-22		62		7.06-11-22		60		
	Hardness (mg CaCO ₃ /L)			85				85		
	Temperature (°C)	24.8	24.8	24.8	25.0	24.7	24.6	24.7	24.3	
300 mg KCl/L	pH (S.U.)	7.86	7.59	7.83	7.60	7.76	7.58	7.66	7.35	
	Dissolved oxygen (mg/L)	7.9	7.5	7.9	7.3	7.7	7.0	7.7	6.7	
	Conductivity (µmhos/cm)	866		844		825		842		
	Temperature (°C)	24.9	24.6	24.9	24.7	24.8	24.6	24.8	24.5	
450 mg KCl/L	pH (S.U.)	7.82	7.59	7.87	7.59	7.81	7.57	7.05	7.34	
	Dissolved oxygen (mg/L)	8.1 7.06-11-22	7.4	7.9	7.4	7.8	6.7	7.7	6.1	
	Conductivity (µmhos/cm)	765 1120		1080		1090		1080		
	Temperature (°C)	24.8	24.8	24.9	24.7	24.8	24.8	24.7	24.5	
600 mg KCl/L	pH (S.U.)	7.86	7.61	7.87	7.59	7.84	7.55	7.05	7.35	
	Dissolved oxygen (mg/L)	7.7	7.3	7.9	7.4	7.9	6.6	7.7	6.1	
	Conductivity (µmhos/cm)	1360		1350		1330		1330		
	Temperature (°C)	24.8	24.7	24.9	24.6	24.8	24.5	24.7	24.2	
750 mg KCl/L	pH (S.U.)	7.85	7.61	7.92	7.58	7.86	7.55	7.06	7.40	
	Dissolved oxygen (mg/L)	7.8	7.2	8.0	7.3	7.9	6.6	7.8	6.1	
	Conductivity (µmhos/cm)	1610		1610		1560		1570		
	Temperature (°C)	24.9	24.7	25.0	24.6	24.7	24.8	24.7	24.6	
900 mg KCl/L	pH (S.U.)	7.89	7.67	7.92	7.58	7.86	7.50	7.08	7.50	
	Dissolved oxygen (mg/L)	7.8	7.2	8.0	7.2	8.0	7.3	7.9	7.3	
	Conductivity (µmhos/cm)	1860		1860		1840		1830		
	Temperature (°C)	24.9	24.7	25.0	24.6	24.7	24.6	24.7	24.3	
1050 mg KCl/L	pH (S.U.)	7.89	7.67	7.94	7.65	7.86	7.57	7.09	7.52	
	Dissolved oxygen (mg/L)	7.6	7.3	8.0	7.2	8.0	6.9	8.0	7.4	
	Conductivity (µmhos/cm)	2150		2090		2110		2110		
	Temperature (°C)	24.9	24.6	25.0	24.6	24.7	24.7	24.7	24.4	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	